

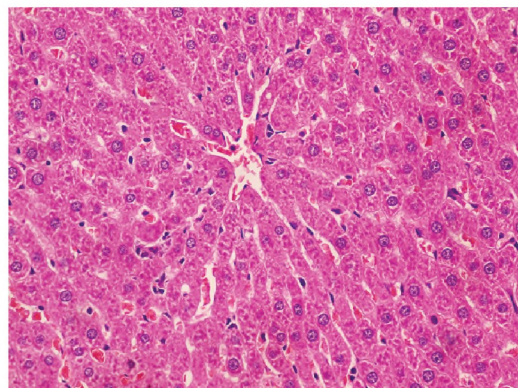


Vietnam Ministry of Science and Technology

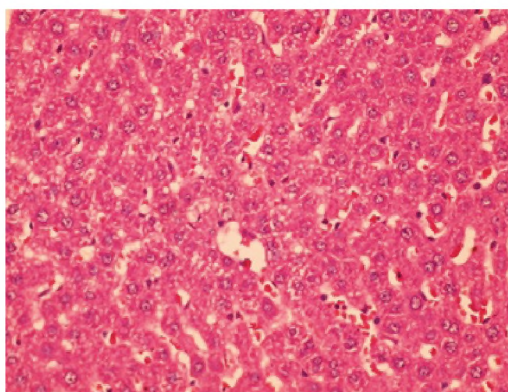
P-ISSN 2525-2461 • E-ISSN 2615-9937

Vietnam Journal of Science, Technology and Engineering

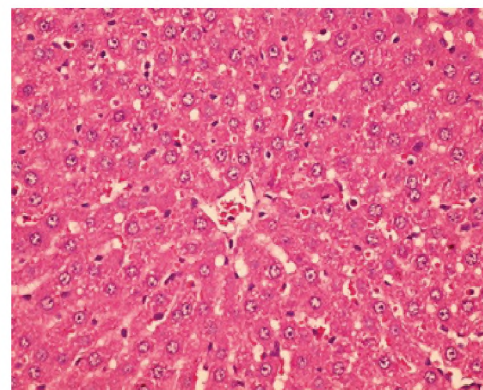
SEPTEMBER 2024 • VOLUME 66 NUMBER 3



Group 1



Group 2



Group 3

Investigation for subchronic oral toxicity and evaluation of analgesic activities of
Stephania tetrandra S. Moore liquid extract in experimental animals

Vietnam Journal of Science, Technology and Engineering (VJSTE) is a quarterly double-blind peer-reviewed academic journal. VJSTE publishes research articles and review articles in all areas of science, technology and engineering with the focus on the physical sciences, life sciences and environmental sciences including the following topics:

- Physical Sciences
- Agriculture
- Medicine
- Pharmacology
- Biology
- Biotechnology
- Biomedical Applications
- Ecology
- Climatology
- Environmental Science.

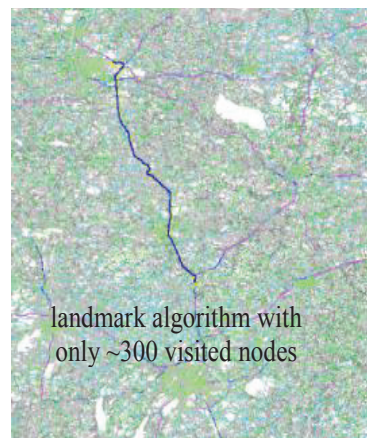
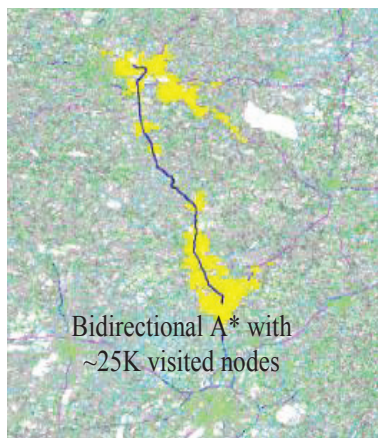
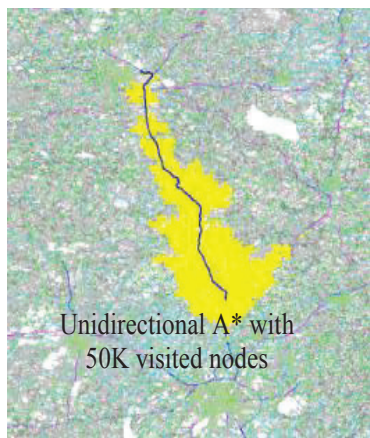
CLASSIFICATION SCHEME

1. Mathematics and Computer Science
 - 1.1. Mathematics
 - 1.2. Computer Science
 - 1.3. Computational Science
2. Physical Sciences
 - 2.1. Physics
 - 2.2. Chemistry
 - 2.3. Engineering
3. Life Sciences
 - 3.1. Agriculture
 - 3.2. Medicine
 - 3.3. Pharmacology
 - 3.4. Biology
 - 3.5. Biotechnology
 - 3.6. Biomedical Applications
4. Earth Sciences
 - 4.1. Geography
 - 4.2. Geology
 - 4.3. Geophysics
 - 4.4. Oceanography
5. Environmental Sciences
 - 5.1. Ecology
 - 5.2. Climatology
 - 5.3. Environmental Science

CONTENTS

SEPTEMBER 2024 • VOLUME 66 NUMBER 3

16



MATHEMATICS AND COMPUTER SCIENCE

3 Analysing the impact of field conditions, pitch features, and opponent strength on cricket performance: A machine learning approach.

Rameshwari Lokhande, Rawal Awale, Rahul Ingle

15 Researching open source WebGIS technology in building software to support optimal search and rescue equipment.

Yen Phan Quoc

24 Hydration number and allied parameters of aqueous solutions of ammonium bromide through ultrasonic study.

J.H. Rakini Chandrasekaran, S. Nithiyanantham

PHYSICAL SCIENCES

32 Analysis of a novel 3C-SiC thin layer on silicon diaphragms for enhanced stress amplification in MEMS piezoresistive pressure sensors.

Nguyen Chi Cuong, Trinh Xuan Thang, Lam Minh Thinh, Vuong Dinh Duy Phuc, Truong Huu Ly, Ngo Vo Ke Thanh, Le Quoc Cuong

39 Determination of phosphorus additives in instant noodle samples by Phosphorus-31 nuclear magnetic resonance (^{31}P -NMR).

Trung Quang Pham, Lan Anh Thi Nguyen, Nhung Thi Dao, Thao Thi Ta

46 Optimisation of calcinothemic reduction for metallic dysprosium preparation.

Nguyen Trong Hung, Nguyen Thanh Thuy, Le Ba Thuan

55 Preliminary study on simultaneous adsorption of nitrate and phosphate using CaFe_2O_4 -modified biochar originated from lychee peels.

Lan Huong Nguyen, Tuan Hiep Tran

63 Analysis of welding hazards from an occupational safety perspective.

S. Senthil Murugan, P. Sathiya

LIFE SCIENCES

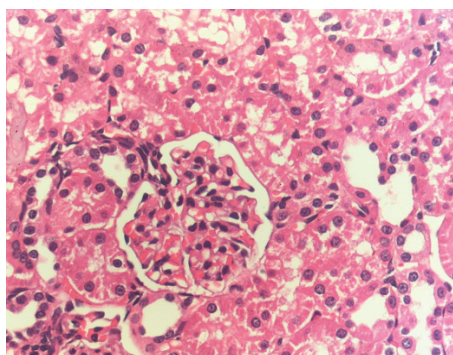
75 Investigation for subchronic oral toxicity and evaluation of analgesic activities of *Stephania tetrandra* S. Moore liquid extract in experimental animals.

Thanh Mai Phuong, Trang Tran Thi Thu, Khanh Do Thi Hong, Ngoc Tran Thi Hong, Dat Tran Tien, Trang Nguyen Thi Mai, Minh Phan Hong

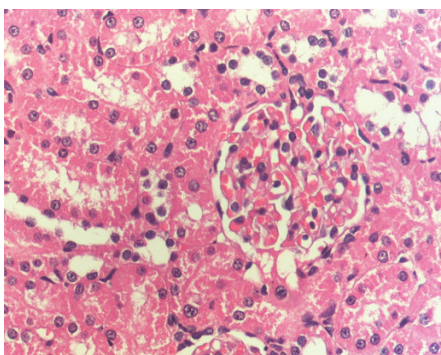
CONTENTS

SEPTEMBER 2024 • VOLUME 66 NUMBER 3

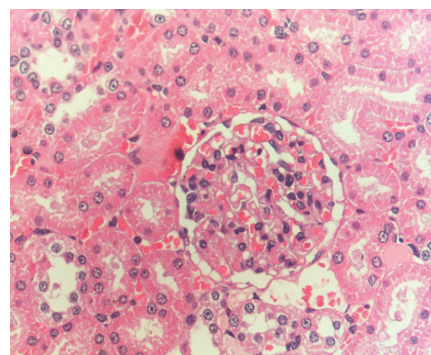
79



Group 1



Group 2



Group 3

83 Studies on the growth and essential oil accumulation of *Elsholtzia ciliate* (Thunb.) Hyland and the effect of gibberellic acid treatment on growth and essential oil accumulation.

Luong Thi Le Tho, Luu Tang Phuc Khang, Nguyen Xuan Hieu, Dinh Thi Bich Thuy, Vo Ngoc Khoi Nguyen, Chau Minh Hai Dang, Tran Thi Phuong Dung

91 Evaluating antioxidant and anti-inflammatory activities of *Dracaena cambodiana* Pierre ex Gagne extracts on RAW 264.7 and SW 1353 cell lines.

Huynh Cong Duy, Nguyen Trung Quynh Nhu, Mai Kieu Tien, Nguyen Phuc Thinh, Nguyen Thi Le Thuy, Le Thi Truc Linh, Tran Thi Van Anh, Pham Thi Kim Tram

97 Impact of pasteurisation and storage conditions on the physicochemical properties of acerola jelly.

Binh Hoang Quang, Uyen Tran Phuong Ho, Diep Ngoc Thi Duong

105 Assessing the effect of the Trier social stress test (TSST) on psychological and physiological measurements of Vietnamese students.

Quang Lam Nguyen, Ngan Huu Phuc Nguyen, Anh Phuc Hoang Le, Bao Hoai Pham, Vy Kim Huynh, Thien Hoang Minh Cao, Dong An Bui, Khon Huynh, Huong Thi Thanh Ha

EARTH SCIENCES

114 Geotechnical properties of cement-stabilised fill material: Developing a linear regression model for predicting unconfined compression strength and undrained shear stress.

Muntasir Ahmed, Mostakim Ahmed, Lubaba Afzal

120 The role of groundwater recharge in groundwater exploitation of the Red river delta plain.

Viet Hung Le, Quy Nhan Pham, Thi Linh Phung, Van Canh Doan, Quoc Cuong Tran, Tran Trung Dang

Analysing the impact of field conditions, pitch features, and opponent strength on cricket performance: A machine learning approach

Rameshwari Lokhande*, Rawal Awale, Rahul Ingle

Electrical Engineering Department, Veermata Jijabai Technological Institute, Hanamant Ramchandra Mahajani Road, Matunga East, Mumbai, Maharashtra 400019, India

Received 8 April 2024; revised 25 April 2024; accepted 4 June 2024

Abstract:

Cricket, a sport that is beloved worldwide, requires a combination of expertise, and strategic intelligence. This exposition explores the study of cricket performance, specifically examining how factors such as playing circumstances, pitch dynamics, and the qualities of opponents affect the effectiveness of bowlers and the skill of hitters. The study tries to discover underlying patterns and relationships between these characteristics and player success by using rigorous statistical analysis and other machine learning techniques. Assessment criteria, including accuracy, mean absolute error (MAE), root mean square error (RMSE), and R2 scores, are used to measure the prediction effectiveness of the models. The findings highlight the significant influence of the quality of opponents, the features of the pitches, and the circumstances of the field on the performance of players. In addition, the analysis clarifies the predictive ability of several machine learning algorithms, highlighting Random Forest, XGBoost, and LightGBM as the most precise models. These discoveries provide useful knowledge for academics, educators, and cricket enthusiasts, enabling a better understanding of the various factors that influence player performance and promoting informed strategy discussions.

Keywords: cricket analytics, machine learning, player performance analysis, prediction analysis, statistical modelling.

Classification numbers: 1.2, 1.3

1. Introduction

Cricket, often referred to as a gentleman's game, has captivated millions of fans worldwide through its unique blend of skill, strategy, and tradition. Originating in the 13th century, cricket's evolution from a rustic pastime to a global spectacle mirrors the broader growth of modern sports [1-3]. The transformation of cricket from a quaint English activity to an international phenomenon occurred over centuries, marked by significant milestones and innovations. A pivotal moment in this evolution was the formalisation of cricket's rules and regulations, which began in the 17th century and culminated in the establishment of the Marylebone cricket club (MCC) in 1787. The MCC's codification of cricket laws provided a standardised framework for the governance of the game, laying the foundation for its widespread popularity [4].

The 19th century saw the proliferation of cricket across the British Empire, as colonial administrators and military personnel introduced the sport to distant regions. Cricket became a symbol of British cultural influence, serving as a means of social integration and colonial identity. Its spread to countries such as India, Australia, and the West Indies facilitated cultural exchange and paved the

way for the emergence of new cricketing powerhouses [1-3]. The dawn of the 20th century ushered in a new era for cricket, characterised by technological advancements, commercialisation, and globalisation. The advent of radio broadcasts, followed by television coverage, brought cricket into the homes of millions, transforming it from a local pastime into a mass entertainment spectacle. The inaugural Cricket World Cup in 1975 marked a watershed moment in the sport's history, signalling its transition into a global sporting phenomenon [1-3].

As cricket's popularity grew, so too did the scrutiny of player performance and team strategies. Analysts and statisticians began examining every aspect of the game, from batting averages to bowling strike rates, in search of patterns and insights. The advent of computer technology revolutionised cricket analysis, enabling researchers to process vast amounts of data and uncover hidden trends [5]. Of particular interest in cricket analysis is the prediction of player performance, which involves assessing various factors that influence individual and team outcomes. Elements such as pitch conditions, weather, opposition strength, and player form play crucial roles in determining match results. Historically, cricket pundits relied on intuition and experience to forecast outcomes, but the introduction of

*Corresponding author: Email: rameshwarilokhande@gmail.com

statistical modelling and machine learning has transformed predictive methodologies [6, 7].

Statistical tests, including hypothesis testing and regression analysis, form the foundation of cricket performance prediction. Researchers utilise historical data to identify correlations between different variables and develop predictive models capable of forecasting match results with a high degree of accuracy. Machine learning algorithms, such as random forests, support vector machines, and neural networks, have emerged as powerful tools in cricket prediction, capable of processing complex data sets and generating accurate forecasts [8-10].

This exposition explores the intricate domain of cricket performance to identify the numerous factors that contribute to player success on the field. By employing advanced machine learning methods and rigorous statistical analysis, we examine various aspects of player performance to gain a deeper understanding of their impact on the game. Our focus includes, but is not limited to, batting averages, bowling strike rates, fielding ability, pitch conditions, weather, match context, and opposition strength. Through a comprehensive analysis of these elements, we aim to elucidate the complex relationship between match dynamics, environmental factors, and individual capabilities.

Our study seeks to contribute to the ongoing discourse on cricket performance evaluation by offering an enhanced understanding of player effectiveness. Machine learning and rigorous statistical analysis have the potential to uncover complex patterns and relationships within large data sets, making them invaluable tools in cricket research. By systematically analysing player performance, pitch dynamics, and playing conditions, these analytical approaches enable researchers to identify key factors and their influence on match outcomes. Statistical techniques allow for the measurement of relationships between variables such as pitch conditions, weather, and opposition strength, revealing the intricate interactions at play.

Machine learning models, by leveraging historical data, provide powerful methods for modelling and predicting player success. These models can account for complex interdependencies among variables and incorporate nonlinear interactions, resulting in more accurate and robust performance forecasts. Overall, the integration of machine learning and statistical analysis in cricket research enhances our understanding of the multiple factors influencing player performance and match outcomes.

The prediction efficacy of the models created in this study is evaluated using several criteria, including R-squared (R^2) score analysis, mean absolute error (MAE), root mean squared error (RMSE), and mean squared error (MSE). RMSE and MAE provide insight into the accuracy of the model by measuring the average difference between

predicted and actual values. MSE, by averaging the squares of the errors, reflects the overall variability in the prediction errors. Additionally, R^2 score analysis assesses the proportion of variance in the dependent variable explained by the independent variables. These evaluation criteria are considered significant as they provide comprehensive metrics for assessing the performance of predictive models, taking into account various aspects of predictive accuracy and reliability.

Through a detailed evaluation, researchers can determine how well their models capture performance variations based on different factors, allowing for informed decisions regarding model selection and improvement. Our study presents several new insights into the influence of factors such as opponents, pitch characteristics, and ground conditions. Specifically, we demonstrate that the calibre of opponents significantly impacts player performance, with stronger teams posing greater challenges to individual players. It is crucial to analyse a batsman's performance variation across different pitches, venues, and against varying opposition teams.

This study utilises data from Indian players, incorporating pitch data available from the pitch report. Variables such as the quality of opposition, venue, and pitch characteristics significantly influence player performance. Cricket matches are played on a wide range of surfaces, including grassy, flat, hard, plain, and soft pitches, each presenting different opportunities and challenges for players [11]. The various types of pitches are described below:

Grassy pitch: A pitch with substantial grass cover, benefiting fast bowlers as the ball tends to bounce and seam more on such surfaces.

Flat pitch: A level pitch with minimal grass or cracks, typically offering favourable conditions for batting with little assistance to bowlers.

Hard pitch: A compacted surface that increases ball speed and bounce, enabling batsmen to play with greater power and precision.

Plain pitch: A surface devoid of significant variations, presenting a neutral playing field.

Soft pitch: A softer, less compacted surface where the ball tends to slow down and lose speed, making shot-making more difficult.

By analysing how batsmen perform on different types of surfaces, we gain valuable insights into their versatility, shot-making abilities, and capacity for game-changing adjustments. This understanding is essential for enhancing player selection, game strategy, and training methods. The integration of statistical analysis with knowledge of pitch properties provides a deeper comprehension of cricket, facilitating more informed decision-making.

Cricket player performance is influenced by a myriad of factors, both intrinsic and extrinsic. In addition to technical skills and athleticism, psychological factors such as confidence, concentration, and temperament play a significant role in determining performance. Moreover, external factors, including pitch conditions, weather, and match context, profoundly impact player outcomes [12, 13]. One-day internationals (ODIs), test matches, and twenty-20 internationals (T20Is) represent the three primary formats of modern cricket, each characterised by unique challenges. ODIs are known for limited overs and high-scoring matches, while Test cricket is renowned for its strategic depth and endurance. T20 cricket, on the other hand, is fast-paced and explosive, appealing to a younger, more dynamic audience [14-16].

In recent years, cricket analytics has undergone a paradigm shift with the advent of advanced metrics and data-driven decision-making. Analysts now employ sophisticated statistical models and machine learning algorithms to assess player performance, optimise team strategies, and predict match outcomes. These tools enable coaches, players, and administrators to make informed decisions based on objective data and empirical evidence [17, 18]. The application of predictive analytics in cricket extends beyond match outcomes to player selection, talent identification, and performance evaluation. By leveraging historical data and advanced modelling techniques, teams can identify emerging talent, optimise player combinations, and maximise their chances of success on the field. Predictive analytics also allows teams to adapt their strategies in real-time, responding to evolving match conditions and opponent tactics [19-21].

The future of cricket analytics holds immense promise, with ongoing advancements in technology and data science driving innovation and insight. As cricket becomes increasingly globalised and commercialised, the demand for sophisticated analytical solutions will continue to grow, presenting new opportunities and challenges for researchers, analysts, and practitioners [22, 23]. Cricket analytics represents a fascinating intersection of sport, statistics, and technology, with the potential to revolutionise the way the game is played, coached, and experienced. By harnessing the power of data and analytics, cricket stakeholders can unlock new insights, optimise performance, and enhance the overall quality of the sport. As the game continues to evolve in the 21st century, analytics will play an increasingly integral role in shaping its future trajectory and success [18, 24, 25].

Additionally, machine learning models have been used to forecast the performance of Indian players, considering variables such as field conditions, pitch characteristics, and opponent strengths [12, 13]. Performance metrics such as precision, accuracy, F1 score, and recall have been employed

to evaluate the models' effectiveness in capturing performance differences based on these variables. This study investigates the influence of grounds, pitches, and opposition on the variability of bowler and batsman performance. The results reveal significant variations in wicket-taking performance based on these factors, supporting the alternative hypothesis. Furthermore, machine learning models demonstrate varying levels of accuracy and predictive power in forecasting player performance. Random forest, XGBoost, and LightGBM exhibit exceptional performance, while SVR and Decision Tree models show comparatively lower effectiveness. These findings underscore the importance of considering unique playing surfaces, pitch conditions, and opponent factors when evaluating and projecting player performance in cricket.

Machine learning and artificial intelligence have gained significant prominence in the railway transportation industry in recent years, particularly in predicting train delays. Several studies have attempted to solve this forecasting problem using different machine learning models. For example, on Serbian railways, support vector regression (SVR) was used to analyse train arrival delays and outperformed artificial neural networks (ANN) [26]. Statistical analysis has been utilised to predict running and dwell times [27], employing global prediction models such as LTS robust linear regression, regression trees (RT), and random forests (RF). The k-nearest neighbour (K-NN) and linear regression (LR) models have been used to determine off-peak and peak-hour dwell times, respectively [28]. Random forests (RF) have consistently outperformed extreme learning machines (ELM) and kernel. Regularised least squares (KRLS) in predicting train delays [29]. A deep extreme learning machine-based method for predicting train delays using real-time data has been presented [30]. Furthermore, methods based on neural networks such as backpropagation neural network (BPNN), wavelet neural network (WNN), and genetic algorithms (GA-BPNN and GA-WNN) have been employed to predict train arrival times [31]. Researchers have devised methods to predict running time, dwell time, train delays, and penalty costs by combining decision trees (DT) and random forest regression (RF) [32]. In other studies [33-35], Random forests have also demonstrated superior performance compared to other methods. Ensemble methods have been used to predict train delays up to 24 hours in advance [36]. Long short-term memory (LSTM) networks have shown improved performance over random forests and neural networks (NN) in predicting train arrival delays [37]. Gradient boosting regression trees (GBRT) have outperformed SVR and RF in predicting train delays [38]. A deep learning method called CLF-Net was developed for estimating train delays on China's high-speed rail lines by integrating 3D convolutional neural networks (CNN), LSTM, and fully connected neural network (FCNN) structures [39].

These machine learning methods demonstrate significant promise in improving the accuracy and ease of predicting train delays.

Significant progress has also been made in predicting air quality using machine learning techniques over the past few years. Researchers have investigated various forecasting algorithms for air pollutants, particularly PM2.5, in cities around the globe. J. Ma, et al. (2020) [40] predicted PM2.5 at the Beijing railway station using a spatial ensemble method. M. Asgari, et al. (2017) [41] employed a Hadoop architecture with multinomial naive Bayes and logistic regression to predict Tehran's air pollution, whereas M.Z. Joharestani, et al. (2019) [42] identified XGBoost as the most effective algorithm for predicting Tehran's air quality using geographical and satellite data. H. Karimian, et al. (2019) [43] utilised the Variance Inflation Factor (VIF) method and LSTM to forecast PM2.5 in Tehran, yielding promising results. In addition, other studies have investigated various machine learning models, such as random forest, support vector machine (SVM), ensemble methods, and deep learning approaches, to predict air pollutants in cities around the world. The objective of these efforts is to improve air quality forecasting, thereby enhancing public health and environmental management. As researchers continue to investigate and refine these AI-based techniques, the potential for more precise and effective air quality forecasts grows. These developments hold promise for addressing the challenges posed by air pollution and improving the overall quality of life in communities affected by poor air quality [40, 44-48].

2. Methods

2.1. Data wrangling

Data wrangling is a systematic method used to acquire, cleanse, and organise raw data relevant to cricket player performance. Its purpose is to forecast player success in the sport by extracting pertinent information from various sources, including match histories, player statistics, and match commentary. This acquired data is then cleaned, processed, and standardised to remove discrepancies and manage missing values. The resulting wrangled data is organised and prepared for further analysis, enabling the creation of prediction models to forecast player performance in cricket matches. The data for this study were collected from www.cricinfo.com. Batting contests from 14 January 2005 to 10 July 2017 were considered. Since Sachin Ramesh Tendulkar was the oldest player at that time, a record of each batsman's performance, innings by innings, was compiled, starting from his debut in an ODI on 18 December 1989. Bowling matches from 2 January 2000 to 10 July 2017 were also considered.

The batting parameters encompass several important components that shed light on a player's batting performance.

The "average venue" field provides information about a player's performance under various playing conditions by displaying the average runs the player has scored at a particular ground or venue. The "ground" feature identifies the location or stadium where the innings were played. "Day," "month," and "year" provide a chronological reference by indicating the precise date of the innings. The field "Oppositionteam" displays the team against which the player competed, offering insight into their performance against different opponents. Additional batting characteristics include "innings runs score," which shows the player's overall contribution to the team's run total over the course of an innings. "Strike rate venue" calculates a player's scoring rate at a particular location and displays the number of runs scored per 100 balls faced. "Average opposition" showcases a player's batting average against a specific opposition team, highlighting their performance against different adversaries. "Innings balls faced" indicates the number of balls faced, representing the player's participation in the game, while "50s" denotes the number of times the player scored between 50 and 99 runs in an innings. The variables "average yearly" and "strike rate yearly" provide details on the player's average runs scored and batting strike rate per year, respectively, offering an overview of their consistency over time. The player's strike rate against a specific opposition team is displayed in the "strike rate opposition" field. The term "innings player" simply denotes the participant in the inning. Finally, "100s" shows how many times the player scored 100 or more runs in an inning, demonstrating their ability to convert promising starts into noteworthy results.

The bowling parameters, on the other hand, provide insightful data regarding a player's performance with the ball. The term "average venue" refers to a player's bowling average at a particular location, demonstrating their effectiveness in taking wickets at various grounds. "Balls bowled" refers to the total number of balls a player has bowled during an innings, providing information on their workload and participation in the match. To evaluate a player's performance in limiting the opposition's scoring, "runs conceded" displays the total number of runs the player has allowed. The "average opposition" option displays the player's bowling average against a certain opposition team, providing information about how they performed against different foes. The "4 wickets" statistic counts how many times they took four wickets during an innings, showcasing their ability to take multiple wickets in a single bowling attempt. The statistic "average yearly" shows the player's average annual wicket total, emphasising their consistency over time. Similar to the batting parameters, "ground" denotes the location or stadium where the bowling took place, while "year," "month," and "day" provide the precise date of the bowling performance. The "country" field indicates the player's country of birth, while the "bowlingstyle" field indicates their preferred bowling technique, such as fast or

spin. “Maidens bowled” displays how many maiden overs a player has bowled, demonstrating their capacity to apply pressure consistently and limit runs. The “FF” element denotes the player’s economy rate, which is computed as runs allowed per over, providing information about how well they can limit the opposition’s scoring. “Form” denotes the bowler’s current performance level, while “5 wickets” lists the number of innings in which the player claimed five or more wickets, illustrating their propensity for significant bowling milestones. Indicating the player’s participation in the game, “innings number” displays the number of the innings in which they bowled, and “wickets taken” displays the total number of wickets they claimed during that innings. Together, these batting and bowling metrics offer in-depth statistical data regarding a player’s performance, strengths and weaknesses, consistency over time, and capacity to compete against different teams and in varied settings.

The proposed approach for predicting player performance in cricket using machine learning, as shown in Fig. 1, involves gathering comprehensive data from reliable databases using data preprocessing techniques to ensure consistency and quality.

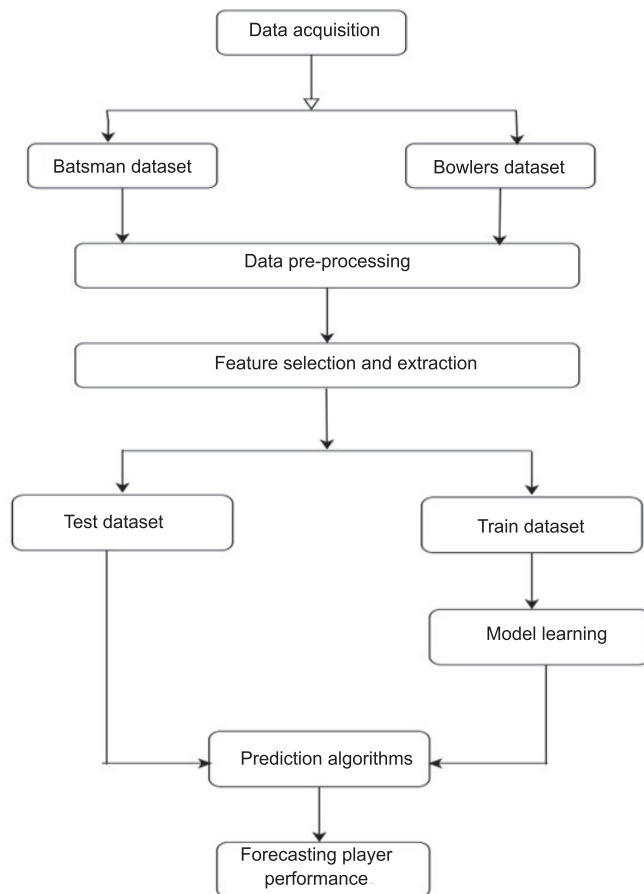


Fig. 1. Flow chart for player performance prediction in cricket.

In this work, machine learning methods such as random forest, decision tree, support vector machine (SVM), XGBoost, AdaBoost, and lightGBM have been demonstrated to be the most accurate models for forecasting cricket performance. These algorithms are distinguished by their ability to handle intricate, nonlinear relationships in the data and to forecast with high accuracy using past performance indicators. Random forest is renowned for its ensemble learning method, combining predictions from several decision trees to increase accuracy and reduce overfitting. Decision tree models are valuable for deciphering underlying trends in cricket performance statistics due to their ease of interpretation and visualisation. SVMs effectively capture nonlinear relationships and partition data points into discrete groups. XGBoost and AdaBoost employ boosting approaches, iteratively optimising prediction performance; lightGBM is particularly well-suited for real-time prediction tasks because of its efficient training speed and memory usage. These algorithms outperform conventional models by capturing complex patterns and relationships within cricket performance data, ultimately producing more precise forecasts.

Coaches, players, and analysts can also practically apply this information to optimise training plans, squad compositions, and game strategies, ultimately enhancing performance and competitiveness on the field. A better understanding of the various factors affecting cricket player performance can lead to more informed strategic discussions among players, coaches, and analysts. By identifying key success factors such as pitch conditions, opponent strength, and match context, stakeholders can tailor their plans to optimise performance outcomes. Training regimens designed by coaches can focus on specific skill sets and prepare players for a variety of playing environments. Players can utilise insights gained from data analysis to adjust their game plans and strategies, thereby improving their adaptability and efficiency on the field. Analysts can assist management and coaching staff in making informed decisions and recommendations that will guide team selections and strategic choices. Ultimately, teams of all skill levels benefit from a collaborative and data-driven approach to cricket strategy, fostered by a thorough understanding of player performance factors.

This research also involved conducting a thorough feature importance analysis to identify the salient player characteristics dictating performance in cricket. This entailed initially aggregating all pertinent player metrics, encompassing both batting and bowling parameters, for subsequent analysis. Machine learning algorithms such as random forest, decision trees, and gradient boosting were then trained using the compiled dataset. Following training, feature importance scores were computed to quantify

the contribution of each feature to the model's predictive efficacy. These scores were subsequently ranked, with higher values indicating a greater influence on player performance prediction. The findings of the feature importance analysis were then visualised using appropriate data visualisation techniques to facilitate comprehension and decision-making. This rigorous analysis provides crucial insights into the pivotal player characteristics driving performance in cricket, thereby informing feature selection for model refinement and directing efforts towards enhancing predictive precision.

2.2. Non-parametric models for predictive modelling

Non-parametric regression techniques such as support vector regression (SVR), random forest regressor, decision tree regressor, AdaBoost regressor, extreme gradient boosting (XGBoost), and light gradient boosting machine (lightGBM) were employed for predictive modelling. These models utilise various hyperparameters to optimise performance and accuracy. Hyperparameter optimisation techniques were applied to fine-tune model parameters and enhance predictive capabilities. The performance of each algorithm was evaluated based on metrics such as accuracy, root mean square error (RMSE), mean absolute error (MAE), and coefficient of determination (R^2).

Support vector regression (SVR): SVR is a powerful regression approach that utilises support vector machines to forecast continuous outcomes. In the context of cricket player performance, SVR is used to model and predict various performance measures [20, 49, 50]. SVR functions by mapping the input features to a higher-dimensional space and finding a hyperplane that minimises the difference between expected and actual values. This hyperplane is adjusted by tuning hyperparameters such as 'C' for regularisation, 'kernel' for the type of kernel function used, and 'epsilon' for the error margin. For our study, we set C to 1.0, kernel to 'rbf' (Radial Basis Function), and epsilon to 0.1.

Random forest regressor: Random forest regressor is an ensemble learning technique that integrates multiple decision trees to produce precise predictions. The final prediction is derived by averaging the outputs of all the decision trees, each constructed using a different random subset of the data [51-53]. In the context of predicting player performance, the random forest regressor captures intricate correlations between features and performance measures. The hyperparameters we adjusted for the random forest regressor include `n_estimators` for the number of trees in the forest, `max_depth` for the maximum depth of each tree, `min_samples_split` for the minimum number of samples required to split an internal node, and `min_samples_leaf` for the minimum number of samples required to be at a leaf node. For our analysis, we specified the following parameters:

`n_estimators=100`, `max_depth=10`, `min_samples_split=2`, and `min_samples_leaf=1`.

Decision tree regressor: Decision tree regressor is a non-parametric regression technique that creates a tree-like representation of decisions and their outcomes. Each leaf node of the tree indicates a predicted outcome, and each internal node represents a decision based on a particular attribute [54, 55]. In predicting cricket player performance, the decision tree regressor considers various characteristics. For the decision tree regressor, we set `max_depth` for the maximum depth of the tree, `min_samples_split` for the minimum number of samples required to split an internal node, and `min_samples_leaf` for the minimum number of samples required to be at a leaf node. For our study, we set the `max_depth` parameter to 5, `min_samples_split` to 2, and `min_samples_leaf` to 1.

AdaBoost regressor: AdaBoost (adaptive boosting) is an ensemble learning method that combines multiple weak learners to produce a strong predictive model. In the context of cricket player performance, AdaBoost is used to enhance the performance of individual weak models and increase forecast precision [51, 53, 56]. We adjusted two hyperparameters for AdaBoost: `n_estimators` for the number of weak learners (decision trees) in the ensemble, and `learning_rate` for the contribution of each weak learner to the outcome of the prediction. To determine the best settings for our study, we experimented with a range of `n_estimators` values (50, 100, and 200) and `learning_rate` values (0.1, 0.5, and 1.0).

Extreme gradient boosting (XGBoost): Extreme gradient boosting, or XGBoost, is another ensemble learning technique that combines multiple weak models to produce a powerful prediction model. XGBoost is renowned for handling large datasets and intricate relationships with accuracy and efficiency [42, 51, 54, 57]. In the context of predicting player performance, XGBoost captures complex patterns and interactions within the data. The hyperparameters `n_estimators` (number of weak models in the ensemble), `learning_rate` (contribution of each weak model), and `max_depth` (maximum depth of each weak model) were adjusted. To find the optimal configuration for our investigation, we experimented with various values of `n_estimators` (50, 100, 200), `learning_rate` (0.1, 0.5, 1.0), and `max_depth` (3, 6, 9).

Light gradient boosting machine (Light GMB): LightGBM is a gradient-boosting framework that employs a tree-based learning technique. It is designed to be efficient in terms of memory and training speed, making it suitable for large datasets. In the context of cricket player performance, LightGBM effectively captures complex relationships between features and performance measures [49, 51, 58, 59]. We modified the following hyperparameters for LightGBM: `num_leaves` for the maximum number of leaves

in a tree, `learning_rate` for the contribution of each tree, and `n_estimators` for the total number of trees in the ensemble. To determine the best parameters for our study, we tried a variety of `num_leaves` (31, 50, 100), `learning_rate` (0.05, 0.1, 0.2), and `n_estimators` (100, 200, 300) values.

By adjusting these hyperparameters, we aimed to identify the optimal configuration for each algorithm, resulting in better performance and more precise forecasts of cricket player performance. The chosen hyperparameter values were based on experimentation and empirical data from prior research, considering the trade-off between model complexity and performance.

The aggregation of comprehensive cricket player data from reliable sources included match statistics, player profiles, and performance metrics. This raw data underwent meticulous preprocessing steps to ensure consistency, integrity, and suitability for analysis. Feature engineering techniques were then applied to extract relevant information from the dataset, transforming raw attributes into meaningful predictors of player performance. Subsequently, machine learning models-including random forest, decision trees, and gradient boosting-were trained using the pre-processed dataset to predict player performance. Model performance was rigorously evaluated using appropriate metrics. Moreover, cross-validation techniques were employed to assess model generalisation and mitigate overfitting. The predictive models were then fine-tuned through hyperparameter optimisation to enhance their predictive efficacy. Finally, the performance of the optimised models was assessed on unseen data to gauge their real-world applicability and effectiveness. This systematic approach ensures the development of accurate predictive models capable of providing valuable insights into cricket player performance for stakeholders such as team management, selectors, and strategists.

3. Results

An analysis of batsman and bowlers performance variability: ground, pitch, and opposition. In this study, we aim to investigate the variability in the performance of both batsmen and bowlers concerning different grounds, pitches, and opposition teams. The null hypothesis suggests that there is no significant difference in the performance metrics across various conditions, while the alternative hypothesis argues for the presence of substantial disparities based on these factors. Statistical tests were conducted, yielding p-values to assess the significance of these differences. A p-value less than 0.05 indicates rejection of the null hypothesis, implying significant variations in performance. Figs. 2 and 3 illustrate the statistical test results for both bowler and batsman performance across different conditions, respectively.

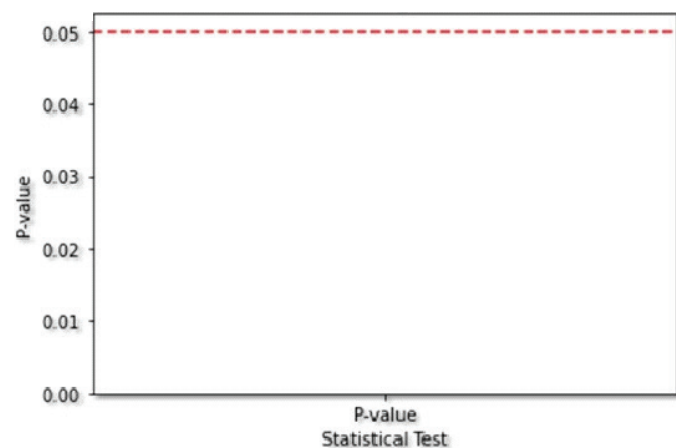


Fig. 2. Statistical test for bowler performance on different grounds, pitches, and against different opposition.

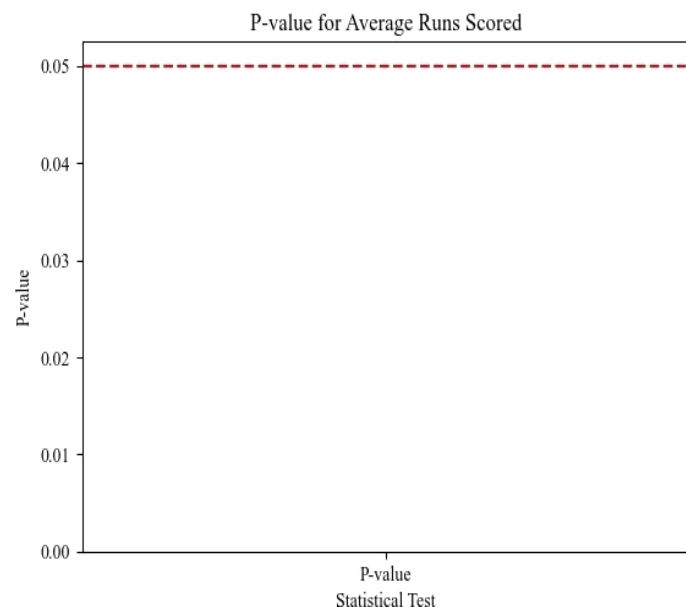


Fig. 3. Statistical test for batsman performance on different grounds, pitches, and against different opposition.

Table 1 presents the performance metrics of various machine learning algorithms used for forecasting bowler performance. These algorithms include decision tree, random forest, SVR (support vector regression), AdaBoost, LightGBM, and XGB (extreme gradient boosting). Each algorithm's accuracy, RMSE, MAE, and R^2 score are provided, offering insights into their effectiveness in predicting player performance.

The findings indicate that while decision tree and random forest algorithms provide reasonable accuracy in predicting bowling performance, random forest outperforms the decision tree in terms of RMSE, MAE, and R^2 score. Conversely, SVR's negative R^2 score suggests it fails to capture the underlying patterns in the data, resulting in

subpar performance. AdaBoost, LightGBM, and XGB demonstrate superior predictive power with higher accuracies and R^2 scores, making them more suitable for accurately forecasting bowling performance.

Table 1. Model performance metrics for bowler.

Model	Accuracy	RMSE	MAE	R^2
Decision tree	0.852075	0.305931	0.147925	0.542835
Random forest	0.919707	0.187015	0.080293	0.829165
SVR	0.738119	0.459590	0.261881	-0.031732
Adaboost	0.821608	0.318849	0.178392	0.503412
LightGBM	0.984394	0.067300	0.015606	0.977876
XGB	0.995257	0.018557	0.004743	0.998332

Similarly, Table 2 presents the performance metrics of machine learning algorithms for forecasting batsman performance. The algorithms and metrics are consistent with those used for bowler performance analysis.

Table 2. Model performance metrics for batsman.

Algorithm	Accuracy	RMSE	MAE	R^2 Score
Decision tree	0.735	0.473	0.265	0.831
Random forest	0.813	0.342	0.187	0.912
SVR	0.278	1.146	0.722	0.011
Adaboost	0.706	0.502	0.294	0.810
XGB	0.953	0.178	0.047	0.976
LightGBM	0.931	0.193	0.069	0.972

The results, as shown in Table 1 that the decision tree algorithm achieves an accuracy of 85.2%, with an RMSE of 0.3059 and an MAE of 0.1479. However, its R^2 score indicates that it can only explain 54.3% of the variance in bowling performance. In contrast, the Random Forest algorithm performs better in terms of accuracy (91.9%), RMSE (0.1870), MAE (0.0803), and R^2 score (82.9%). The SVR algorithm demonstrates poorer performance with an accuracy of 27.8% and a very low R^2 score, suggesting a lack of fit to the data. Adaboost, lightGBM, and XGB exhibit even higher accuracy and better predictive power, with XGB performing exceptionally well with an accuracy of 93.1% and an outstanding R^2 score of 97.2.

For further analysis, we utilised various machine learning models to forecast batsman and bowler performance, considering factors such as field conditions, pitch type, and opposition team. By evaluating the predictive power of these models using performance metrics such as accuracy, precision, recall, and F1 score, we could determine how effectively they represent differences in performance based on these variables. The results supported the alternative hypothesis, indicating significant differences in performance across different conditions.

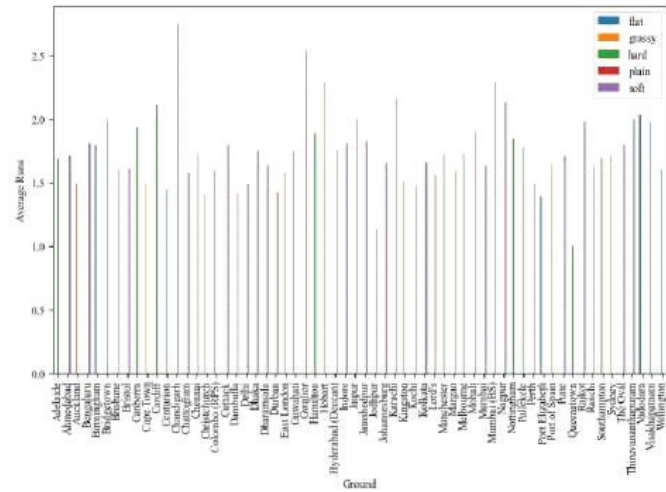


Fig. 4. Runs scored by batsman on different grounds and pitches.

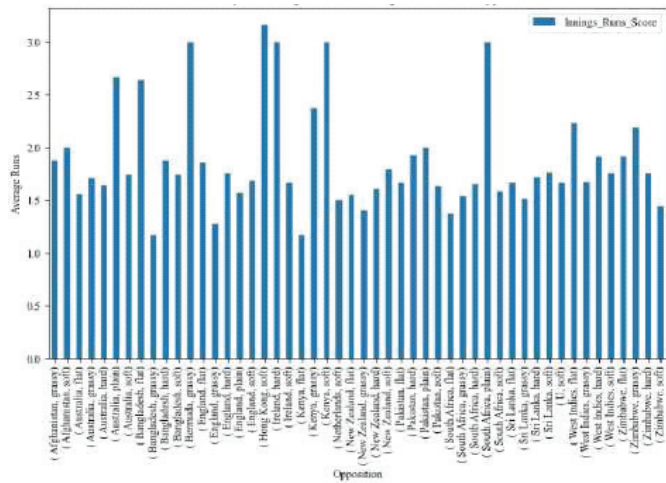


Fig. 5. Runs scored by batsman against different opposition and on different pitches.

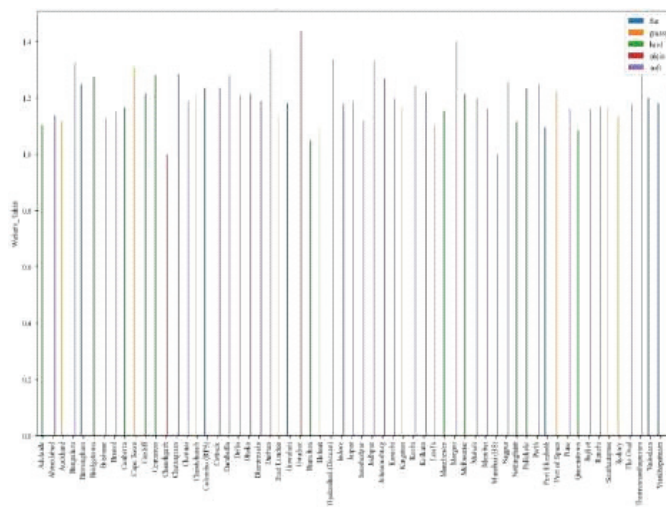


Fig. 6. Wickets taken by bowlers on different grounds and pitches.

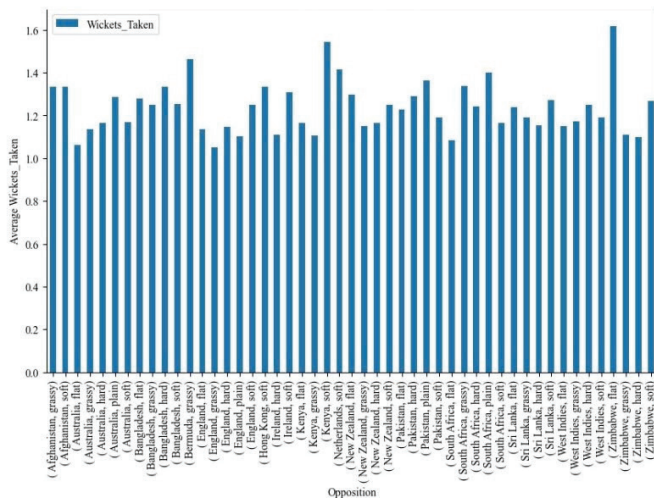


Fig. 7. Wickets taken by bowlers against different opposition and pitches.

Figures 4 and 5 display the runs scored by batsmen on different grounds and pitches, and against different opposition teams and on various pitches, respectively. Similarly, Figs. 6 and 7 illustrate the wickets taken by bowlers on different grounds and pitches, and against different opposition teams and on various pitches, respectively. These figures further

emphasise the impact of ground conditions, pitch types, and opposition teams on batsman and bowler performance.

In summary, our analysis demonstrates significant variability in player performance concerning different ground conditions, pitch types, and opposition teams. Machine learning algorithms, particularly random forest, XGB, and lightGBM, prove to be effective tools for forecasting player performance under diverse conditions. Understanding and accounting for these factors are essential for comprehensive player performance analysis and prediction.

4. Discussion

The investigation focused on evaluating the efficacy of various machine learning algorithms in predicting batter performance based on factors such as ground conditions, pitch characteristics, and opposition teams. The outcomes of both statistical analyses and machine learning models offer valuable insights into the significance of these variables and the accuracy of the algorithms employed. The results of the statistical tests revealed significant variations in the average runs scored on different grounds and pitches, indicating the substantial influence of venue and pitch properties on a batsman's scoring potential. Figs. 8 and 9 illustrate the actual and predicted runs and wickets for different algorithms,

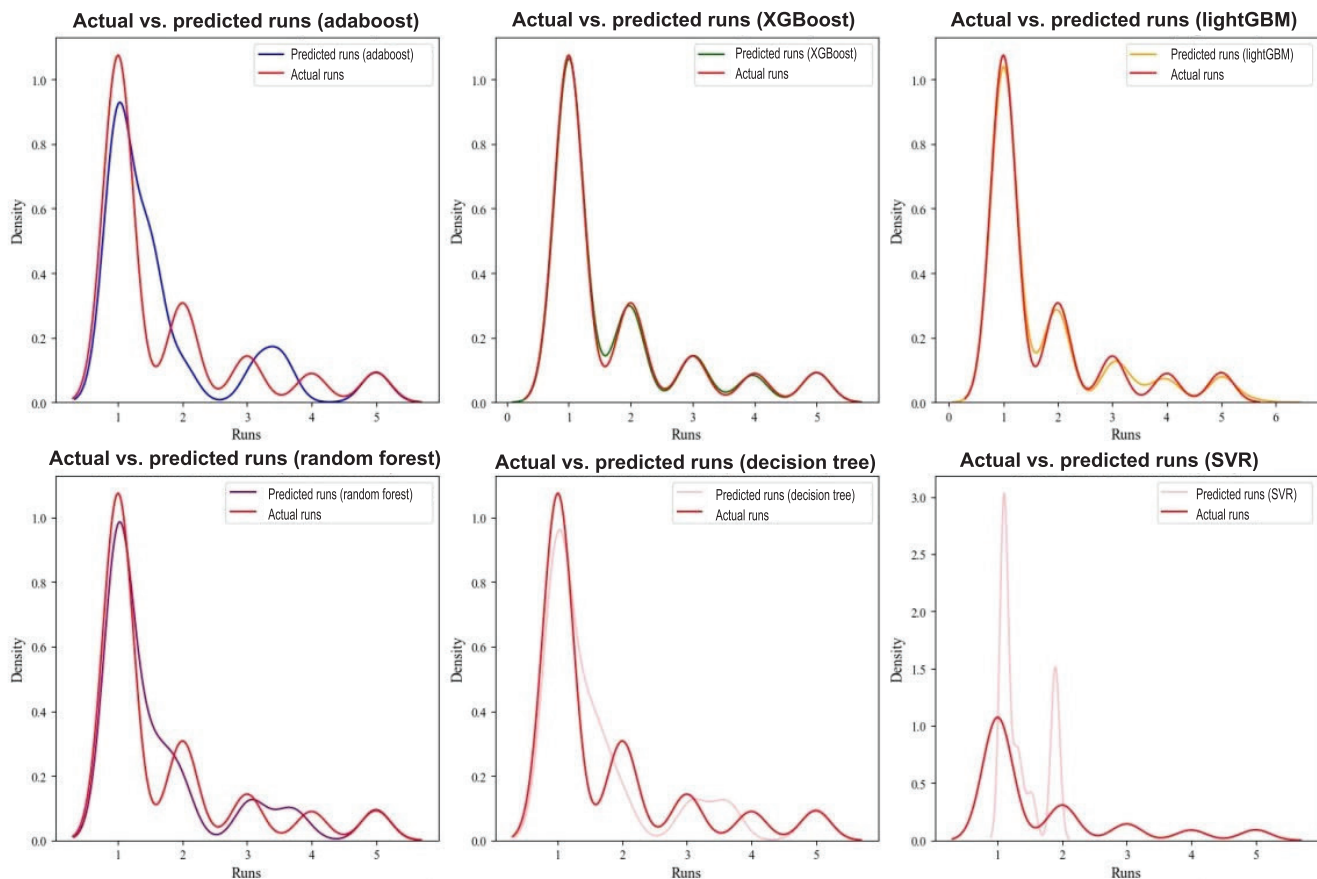


Fig. 8. Actual vs. predicted runs for different algorithms.

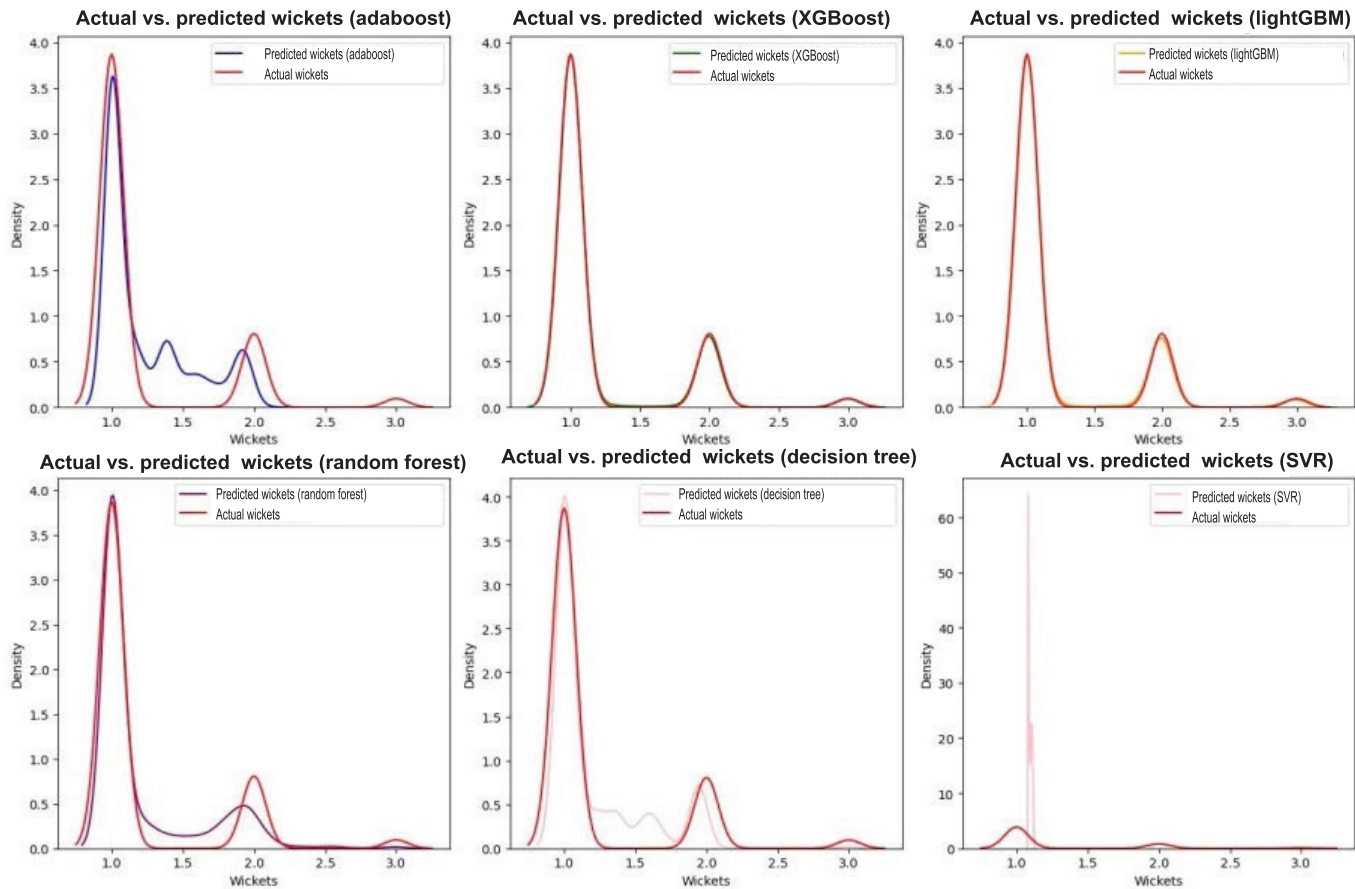


Fig. 9. Actual vs. predicted wickets for different algorithms.

underscoring the importance of considering these variables when assessing and forecasting batter performance.

Several machine learning techniques, including decision tree, random forest, support vector regressor (SVR), AdaBoost, XGBoost, and lightGBM, were employed to further analyse and validate these findings. The performance of these algorithms as predictors was evaluated based on metrics such as accuracy, root mean square error (RMSE), mean absolute error (MAE), and R-squared (R^2) scores. The decision tree algorithm achieved an accuracy score of 73.5%, indicating its ability to accurately forecast the outcome of batsman performance in 73.5% of cases. However, the RMSE and MAE values were relatively high, suggesting a significant level of prediction error. The decision tree method accounted for 83.1% of the variance in batting performance, as evidenced by an R^2 score of 0.831. In comparison, the random forest demonstrated superior performance with an accuracy of 81.3% and lower RMSE and MAE values than the decision tree method. Notably, the random forest algorithm explained approximately 91.2% of the variance in batting performance, as indicated by an R^2 score of 0.912. These results highlight the superior predictive accuracy of the random forest algorithm compared to the decision tree method.

The selection of machine learning algorithms for this study was based on their proven success in handling regression tasks and their ability to capture complex relationships in data. The random forest regressor, known for its ensemble approach, was expected to perform well due to its capability to capture intricate interactions between features. Conversely, the decision tree regressor, despite its simpler model structure, was anticipated to excel in predicting performance based on various factors. The SVR algorithm, specifically designed for regression tasks, was evaluated based on its ability to handle non-linear relationships effectively. Similarly, AdaBoost, XGBoost, and lightGBM, renowned for their boosting techniques, were expected to exhibit comparable accuracy and predictive capabilities. Through statistical analysis and comparison of performance metrics, including accuracy, RMSE, MAE, and R^2 scores, this study aimed to demonstrate the significant impact of ground conditions, pitch characteristics, and opposition teams on batsman performance. By elucidating these differences and their implications, the study sought to provide insights into the most effective methods for predicting performance in diverse cricketing scenarios.

5. Conclusions

This study underscores the pivotal role played by contextual factors such as the playing field, pitch conditions, and the quality of opposition teams in accurately predicting cricket player performance. We conducted an evaluation of various machine learning algorithms, including decision tree, random forest, SVR, AdaBoost, XGBoost, and lightGBM. Notably, random forest emerged as the most promising model, achieving an impressive accuracy of 81.3%. Moreover, it effectively explained 91.2% of the variance in batting performance. This superiority over the decision tree model, which exhibited an accuracy rate of 73.5%, was further substantiated by its smaller RMSE and MAE values, indicating fewer prediction errors. The potential applications of sports analytics and data science extend beyond cricket, with these models offering valuable insights for other sports as well. The outcomes of this study lay a strong foundation for the development of more intricate prediction models and data-driven insights, benefiting cricket players, teams, and enthusiasts alike.

CRedit author statement

Rameshwari Lokhande: Conceptualisation, Methodology, Data collection and analysis, Interpretation of the result, Original draft preparation, Revision; Rawal Awale: Interpretation of the result, Revision; Rahul Ingle: Revision.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] R. Chaudhary, S. Bhardwaj, S. Lakra (2019), "A DEA model for selection of Indian cricket team players", *2019 Amity International Conference on Artificial Intelligence (AICAI)*, Dubai, United Arab Emirates, pp.224-227, DOI: 10.1109/AICAI.2019.8701424.
- [2] R.A. Stretch, R. Bartlett, K. Davids (2000), "A review of batting in men's cricket", *J. Sports Sci.*, **18**(12), pp.931-949, DOI: 10.1080/026404100446748.
- [3] S.E. Arefin, T.A. Heya, D.M. Zaber (2021), "Predictive analysis of Chikungunya", *arXiv*, DOI: 10.48550/arXiv.2101.03785
- [4] R.P. Bunker, F. Thabtah (2019), "A machine learning framework for sport result prediction", *Appl. Comput. Informatics*, **15**(1), pp.27-33, DOI: 10.1016/j.aci.2017.09.005.
- [5] H.H. Lemmer (2014), "Perspectives on the use of the combined bowling rate in cricket", *International Journal of Sports Science & Coaching*, **9**(3), pp.513-523, DOI: 10.1260/1747-9541.9.3.513.
- [6] A. Malhotra, S. Krishna (2018), "Release velocities and bowler performance in cricket", *J. Appl. Stat.*, **45**(9), pp.1616-1627, DOI: 10.1080/02664763.2017.1386772.
- [7] A. Malhotra, S. Krishna (2018), "Release velocities and bowler performance in cricket", *Journal of Applied Statistics*, **45**(9), pp.1616-1627, DOI: 10.1080/02664763.2017.1386772.
- [8] K.P. Jayalath (2017), "A machine learning approach to analyze ODI cricket predictors", *J. Sport. Anal.*, **4**(1), pp.73-84, DOI: 10.3233/JSA-17175.
- [9] T. Allen, O.F. Brault, D. James, et al. (2014), "Finite element model of a cricket ball impacting a bat", *Procedia Eng.*, **72**, pp.521-526, DOI: 10.1016/j.proeng.2014.06.090.
- [10] H. Mittal, D. Rikhari, J. Kumar, et al. (2012), "A study on machine learning approaches for player performance and match results prediction", *arXiv*, DOI: 10.48550/arXiv.2108.10125.
- [11] Z. Ahmed (2023), "5 types of pitches in cricket, kind of pitch can lead to thrilling performance by batsman or bowler", *TeamCric*, <https://teamcric.com/5-types-of-pitches-in-cricket>, accessed 1 May 2024.
- [12] A. Bandulasiri, T. Brown, I. Wickramasinghe (2016), "Factors affecting the result of matches in the one day format of cricket", *Oper. Res. Decis.*, **4**(4), pp.21-32, DOI: 10.5277/ord160402.
- [13] A.I. Anik, S.Yeaser, A.G.M.I. Hossain, et al. (2018), "Player's performance prediction in ODI cricket using machine learning algorithms", *4th International Conference on Electrical Engineering and Information & Communication Technology (iCEEICT)*, pp.500-505, DOI: 10.1109/CEEICT.2018.8628118.
- [14] G.D.I. Barr, B.S. Kantor (2004), "A criterion for comparing and selecting batsmen in limited overs cricket", *Journal of The Operational Research Society*, **55**(12), pp.1266-1274, DOI: 10.1057/palgrave.jors.2601800.
- [15] H. Lemmer (2022), "The combined bowling rate as a measure of bowling performance in cricket", *South African J. Res. Sport. Phys. Educ. Recreat.*, **24**(2), pp.37-44, DOI: 10.4314/sajrs.v24i2.25839.
- [16] D.G. Pahinkar, J. Srinivasan (2010), "Simulation of reverse swing of the cricket ball", *International Journal of Sport Science and Engineering*, **4**(1), pp.53-64.
- [17] M. Shanthi, L.S. Sarah (2008), "Bowler performance prediction for one-day international cricket using neural networks", *IIE Annu. Conf. Expo*, pp.1391-1395.
- [18] H. Saikia, D. Bhattacharjee, U.K. Radhakrishnan (2016), "A new model for player selection in cricket", *International Journal of Performance Analysis in Sport*, **16**(1), pp.373-388, DOI: 10.1080/24748668.2016.11868893.
- [19] G.D. Sharp, W.J. Brettigny, J.W. Gonsalves, et al. (2011), "Integer optimisation for the selection of a twenty 20 cricket team", *J. Oper. Res. Soc.*, **62**(9), pp.1688-1694, DOI: 10.1057/jors.2010.122.
- [20] M.W. Ahmad, J. Reynolds, Y. Rezgui (2018), "Predictive modelling for solar thermal energy systems: A comparison of support vector regression, random forest, extra trees and regression trees", *J. Clean. Prod.*, **203**, pp.810-821, DOI: 10.1016/j.jclepro.2018.08.207.
- [21] S.N. Omkar, R. Verma (2023), "Cricket team selection using genetic algorithm", *Int. Congr. Sport. Dyn.*, pp.1-3.
- [22] A.J. Lewis (2005), "Towards fairer measures of player performance in one-day cricket", *J. Oper. Res. Soc.*, **56**(7), pp.804-815, DOI: 10.1057/palgrave.jors.2601876.
- [23] H. Saikia, D. Bhattacharjee, U. Krishnan (2016), "A new model for player selection in cricket", *International Journal of Performance Analysis in Sport*, **16**(1), pp.373-388, DOI: 10.1080/24748668.2016.11868893.
- [24] M.G. Jhanwar, V. Pudi (2016), "Predicting the outcome of ODI cricket matches: A team composition based approach", *Conference: Machine Learning and Data Mining for Sports Analytics, ECML-PKDD'16*.
- [25] T.B. Swartz, P.S. Gill, D. Beaudoin, et al. (2006), "Optimal batting orders in one-day cricket", *Comput. Oper. Res.*, **33**(7), pp.1939-1950, DOI: 10.1016/j.cor.2004.09.031.
- [26] N. Marković, S. Milinković, K.S. Tikhonov, et al. (2015), "Analysing passenger train arrival delays with support vector regression", *Transportation Research Part C: Emerging Technologies*, **56**, pp.251-262, DOI: 10.1016/j.trc.2015.04.004.
- [27] P. Kecman, R.M. Goverde (2015), "Predictive modelling of running and dwell times in railway traffic", *Public Transport*, **7**, pp.295-319, DOI: 10.1007/s12469-015-0106-7.

- [28] D. Li, W. Daamen, R.M. Goverde (2016), "Estimation of train dwell time at short stops based on track occupation event data: A study at a Dutch railway station", *Journal of Advanced Transportation*, **50**(5), pp.877-896, DOI: 10.1002/atr.1380.
- [29] L. Oneto, E. Fumeo, G. Clerico, et al. (2016), "Advanced analytics for train delay prediction systems by including exogenous weather data", *2016 IEEE International Conference on Data Science and Advanced Analytics (DSAA)*, pp.458-467, DOI: 10.1109/DSAA.2016.57.
- [30] L. Oneto, E. Fumeo, G. Clerico, et al. (2018), "Train delay prediction systems: A big data analytics perspective", *Big Data Research*, **11**, pp.54-64, DOI: 10.1016/j.bdr.2017.05.002.
- [31] Y. Liu, T. Tang, J. Xun (2017), "Prediction algorithms for train arrival time in urban rail transit", *2017 IEEE 20th International Conference on Intelligent Transportation Systems (ITSC)*, pp.1-6, DOI: 10.1109/ITSC.2017.8317609.
- [32] A. Lulli, L. Oneto, R. Canepa, et al. (2018), "Large-scale railway networks train movements: A dynamic, interpretable, and robust hybrid data analytics system", *2018 IEEE 5th International Conference on Data Science and Advanced Analytics (DSAA)*, pp.371-380, DOI: 10.1109/DSAA.2018.00048.
- [33] C. Jiang, P. Huang, J. Lessan, et al. (2019), "Forecasting primary delay recovery of high speed railway using multiple linear regression, supporting vector machine, artificial neural network and random forest regression", *Canadian Journal of Civil Engineering*, **46**(5), DOI: 10.1139/cjce-2017-0642.
- [34] M. Arshad, M. Ahmed (2021), "Train delay estimation in Indian railways by including weather factors through machine learning techniques", *Recent Advances in Computer Science and Communications (Formerly: Recent Patents on Computer Science)*, **14**(4), pp.1300-1307, DOI: 10.2174/2666255813666190912095739.
- [35] Z.C. Li, C. Wen, R. Hu, et al. (2021), "Near-term train delay prediction in the Dutch railways network", *International Journal of Rail Transportation*, **9**(6), pp.520-539, DOI: 10.1080/23248378.2020.1843194.
- [36] R. Nair, T.L. Hoang, M. Laumanns, et al. (2019), "An ensemble prediction model for train delays", *Transportation Research Part C: Emerging Technologies*, **104**, pp.196-209, DOI: 10.1016/j.trc.2019.04.026.
- [37] W. Mou, Z. Cheng, C. Wen (2019), "Predictive model of train delays in a railway system", *Proc. of The 8th International Conference on Railway Operations Modelling Analysis (Rail-Norrköping)*, pp.913-929.
- [38] R. Shi, J. Wang, X. Xu, et al. (2020), "Arrival train delays prediction based on gradient boosting regression trees", *Proceedings of The 4th International Conference on Electrical and Information Technologies for Rail Transportation (EITRT) 2019: Rail Transportation Information Processing and Operational Management Technologies*, Springer, pp.307-315, DOI: 10.1109/ITSC45102.2020.9294365.
- [39] P. Huang, C. Wen, L. Fu, et al. (2020), "A deep learning approach for multi-attribute data: A study of train delay prediction in railway systems", *Information Sciences*, **516**, pp.234-253, DOI: 10.1016/j.ins.2019.12.053.
- [40] J. Ma, Z. Yu, Y. Qu, et al. (2020), "Application of the XGBoost machine learning method in PM_{2.5} prediction: A case study of Shanghai", *Aerosol Air Qual. Res.*, **20**(1), pp.128-138, DOI: 10.4209/aaqr.2019.08.0408.
- [41] M. Asgari, M. Farnaghi, Z. Ghaemi, et al. (2017), "Predictive mapping of urban air pollution using Apache Spark on a Hadoop cluster", *Proceedings of The 2017 International Conference on Cloud and Big Data Computing*, pp.89-93, DOI: 10.1145/3141128.314111.
- [42] M.Z. Joharestani, C. Cao, X. Ni, et al. (2019), "PM_{2.5} prediction based on random forest, XGBoost, and deep learning using multisource remote sensing data", *Atmosphere*, **10**(7), DOI: 10.3390/atmos10070373.
- [43] H. Karimian, Q. Li, C. Wu, et al. (2019), "Evaluation of different machine learning approaches to forecasting PM_{2.5} mass concentrations", *Aerosol and Air Quality Research*, **19**(6), DOI: 10.4209/aaqr.2018.12.0450.
- [44] J.K. Deters, R. Zalakeviciute, M.G. Zalez, et al. (2017), "Modeling PM_{2.5} urban pollution using machine learning and selected meteorological parameters", *Journal of Electrical and Computer Engineering*, DOI: 10.1155/2017/5106045.
- [45] I.M.Gad, H. Doreswamy, K.S. Harishkumar, et al. (2020), "Forecasting air pollution particulate matter (PM_{2.5}) using machine learning regression models", *Procedia Computer Science*, **171**, pp.2057-2066, DOI: 10.1016/j.procs.2020.04.221.
- [46] R.O. Sinnott, Z. Guan (2018), "Prediction of air pollution through machine learning approaches on the cloud", *IEEE/ACM 5th International Conference on Big Data Computing Applications and Technologies (BDCAT)*, pp.51-60, DOI: 10.1109/BDCAT.2018.00015.
- [47] M. Lee, L. Lin, C.Y. Chen, et al. (2020), "Forecasting air quality in Taiwan by using machine learning", *Scientific Reports*, **10**(1), DOI: 10.1038/s41598-020-61151-7.
- [48] A. Masood, K. Ahmad (2010), "A model for particulate matter (PM_{2.5}) prediction for Delhi based on machine learning approaches", *Procedia Computer Science*, **167**, pp.2101-2110, DOI: 10.1016/j.procs.2020.03.258.
- [49] X. Yu, Y. Wang, L. Wu, et al. (2020), "Comparison of support vector regression and extreme gradient boosting for decomposition-based data-driven 10-day streamflow forecasting", *J. Hydrol.*, **582**, DOI: 10.1016/j.jhydrol.2019.124293.
- [50] Y.G. Wang, J. Wu, Z.H. Hu, et al. (2023), "A new algorithm for support vector regression with automatic selection of hyper parameters", *Pattern Recognit.*, **133**, DOI: 10.1016/j.patcog.2022.108989.
- [51] A. Banga, R. Ahuja, S.C. Sharma (2021), "Performance analysis of regression algorithms and feature selection techniques to predict PM_{2.5} in smart cities", *Int. J. Syst. Assur. Eng. Manag.*, **14**, pp.732-745, DOI: 10.1007/s13198-020-01049-9.
- [52] J. Xiong, S.Q. Shi, T.Y. Zhang (2021), "Machine learning of phases and mechanical properties in complex concentrated alloys", *J. Mater. Sci. Technol.*, **87**, pp.133-142, DOI: 10.1016/j.jmst.2021.01.054.
- [53] W. Yuchi, E. Gombojav, B. Boldbaatar, et al. (2019), "Evaluation of random forest regression and multiple linear regression for predicting indoor fine particulate matter concentrations in a highly polluted city", *Environ. Pol.*, **245**, pp.746-753, DOI: 10.1016/j.envpol.2018.11.034.
- [54] H. Laifa, R. Khcherif, H.H.B. Ghezalaa (2021), "Train delay prediction in Tunisian railway through lightGBM model", *Procedia Comput. Sci.*, **192**, pp.981-990, DOI: 10.1016/j.procs.2021.08.101.
- [55] W. Dai, T.S. Brisimi, W.G. Adams, et al. (2015), "Prediction of hospitalization due to heart diseases by supervised learning methods", *Int. J. Med. Inform.*, **84**(3), pp.189-197, DOI: 10.1016/j.ijmedinf.2014.10.002.
- [56] M.Y. Shams, O.M. Elzeki, L.M.A. Magd, et al. (2021), "Hana: A healthy artificial nutrition analysis model during COVID-19 pandemic", *Comput. Biol. Med.*, **135**, DOI: 10.1016/j.combiomed.2021.104606.
- [57] A. Shehadeh, O. Alshboul, R.E.A.M. Look, et al. (2020), "Machine learning models for predicting the residual value of heavy construction equipment: An evaluation of modified decision tree, lightGBM, and XGBoost regression", *Autom. Constr.*, **129**, DOI:10.1016/j.autcon.2021.103827.
- [58] T. Thongthammachart, S. Araki, H.S. Madera, et al. (2022), "Incorporating light gradient boosting machine to land use regression model for estimating NO₂ and PM_{2.5} levels in Kansai region, Japan", *Environ. Model. Softw.*, **155**, DOI: 10.1016/j.envsoft.2022.105447.
- [59] D.N. Wang, L. Li, D. Zhao (2022), "Corporate finance risk prediction based on LightGBM", *Inf. Sci. (Ny)*, **602**, pp.259-268, DOI: 10.1016/j.ins.2022.04.058.

Researching open source WebGIS technology in building software to support optimal search and rescue equipment

Yen Phan Quoc*

Le Quy Don Technical University, 236 Hoang Quoc Viet Street, Co Nhue 1 Ward, Bac Tu Liem District, Hanoi, Vietnam

Received 2 March 2023; revised 11 April 2023; accepted 24 January 2024

Abstract:

Securing combat equipment for urgent special missions, such as rescue, fire protection, chemical prevention, and military operations, is crucial in technical logistics. Rapid preparation and mobilisation of forces in response to the reality of the tasks are essential. A software system that stores a complete database of equipment, human resources, and navigation maps is required. This article explores the application of Open Source WebGIS technologies, such as Geoserver, PostGIS, OpenLayer, GraphHopper, and Python, in experimentally building management software for searching equipment, units, and navigation. It supports decision-making by allowing users to select the optimal response plan based on digital maps. The software integrates spatial analysis and routing techniques to locate units with suitable equipment, either nearest or within a specified radius. Users can thereby determine the optimal route from the selected unit to the mobilisation site. Testing with fire prevention data in Hanoi city showed that the search results for the optimal route are precisely equivalent to those provided by current Google Maps. This research has initially mastered data and technology to proactively update and manage data in areas lacking industry-specific information that existing software does not cover.

Keywords: decision support, equipment, search and rescue, spatial analysis, WebGIS.

Classification numbers: 1.2, 1.3, 4.1

1. Introduction

The Department of Defense of Canada defines search and rescue (SAR) as the search for and provision of assistance to persons or vehicles in or at risk of impending distress [1]. The logistics component of disaster preparedness is complex, encompassing the management of entire supply networks. Logistics assurance is defined as the process of planning, implementing, and controlling the storage and movement of goods, materials, personnel, and facilities to most effectively and cost-efficiently support search and rescue operations [2]. Various vehicles, such as helicopters, cars transporting relief goods, ambulances evacuating casualties, and specialised vehicles for different fields (chemical equipment, fire trucks, excavators, bulldozers), are categorised as rescue equipment. These facilities need management through software that links equipment data to the spatial data of the unit, enabling commanders to swiftly draft rescue plans, choose optimal manoeuvring routes, designate rescue vehicle parking areas, and identify suitable medical facilities.

A geographic information system (GIS) is a computer-based system that supports the collection, storage, retrieval, mapping, analysis, and distribution of geospatial data for

decision support [3, 4]. Geospatial data represent natural earth objects like roads, fire stations, and parks. GIS applications visualise this data, enabling easy analysis of relationships and patterns [4]. Decisions on site selection, network routing, relief item location, and service coverage are based on this data [5]. The determination of optimal locations in GIS involves spatial analysis to compare attribute data across locations and identify the most suitable spatial position [4]. Recent advances have seen GIS-based systems widely applied across various fields, utilising spatial analysis techniques and road network analyses to optimise rescue mission responses. Notably, routing systems aid rescue crews in navigating efficiently from base stations to disaster sites, crucial in complex urban environments where factors like road length and speed impact route optimisation [6]. Currently, various algorithms, such as Dijkstra, A*, K-mean, Bellman-Ford, and Floyd-Warshall, are embedded in routing support tools like GraphHopper, Pgrouting, OSRM, Valhalla, and Openrouteservice to determine the optimal paths [7, 8].

Additionally, road network analysis to identify the optimal route is critical within a spatial decision support system, particularly for emergency response in rescue missions. A

*Email: phanquocyen@lqdtu.edu.vn

routing system that facilitates navigation between origin and destination points enables rescue teams to travel from base stations to disaster sites with maximum efficiency. In densely populated urban areas, complex road networks present specific challenges, where factors like road length and traffic speed significantly influence route optimisation [6]. Presently, several algorithms, including Dijkstra, A*, K-mean, Bellman-Ford, and Floyd-Warshall, have been integrated into routing support tools such as GraphHopper, Pgrouting, OSRM, Valhalla, and Openrouteservice, to compute the most efficient paths [7, 8].

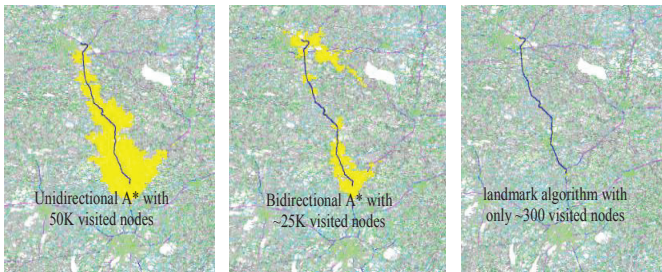


Fig. 1. GraphHopper's path finding algorithm improvement results [9].

GraphHopper stands out as an open-source, fast, and efficient routing library implementing multiple routing algorithms (unidirectional Dijkstra, one-to-many Dijkstra, and uni- and bidirectional A*, CH - Contraction Hierarchies) [9]. It offers options for routing, like the fastest or shortest route for pedestrians, motorbikes, and cars [10]. Particularly, GraphHopper boasts a faster and more accurate processing speed than other technologies. Its landmark algorithm, an enhancement using A* with a new heuristic based on landmarks and the triangle inequality for goal direction, exemplifies this [9]. Additionally, GraphHopper's ease of installation, configuration, and extension makes it highly convenient for developers [9, 11] (Fig. 1).

To build management software and ensure the equipment meets search and rescue work demands, various supporting technologies globally are considered. Since SDSS architecture is a distributed system [12], this study focuses on technologies for distributing and storing spatial data over the Internet. The open-source geospatial software family (FOSS/FOSS4G) has become foundational in the geospatial software ecosystem, providing core geospatial functionality and services, on par with commercial software [13]. FOSS4Gs are particularly vital for developing countries, allowing them to develop their technology rather than importing commercial software. This approach offers the closest path to digitalisation and is the right technology for creating a software system for unit management and equipment for SAR work in contexts of scarce development resources and system maintenance [14, 15].

Table 1. Summary of popular open source geospatial technologies [16-18].

Type	Software/libraries/programming language
Frontend libraries	OpenLayers , Leaflet, Cesium, Geomajas, Mapbender, GeoExt, GeoMoose, GeoNode, Vue , Angular, React...
WebGIS Server	GeoServer , MapServer, MapCache, Deegree, ncWMS, EOxServer, GeoNetwork, pycsw, PyWPS, MapProxy, QGIS Server, istSOS, 52 North SOS, 52 North WPS, Zoo Project, t-rex, Actinia...
Backend libraries	Libraries: GRASS GIS, GeoDjango , GDAL/OGR, GeoTools, GEOS, libLAS, JTS, PROJ4, CyberGIS Toolkit; Tools: GMT, Orfeo ToolBox, Mapnik, MapSlicer, R; Programming Language: PHP, Java, Python , NodeJS, GraphHopper ...
Database management system	PostGIS , Spatialite, Rasdaman, pgRouting, MySQL Spatial, MongoDB, CouchDB, Rasdaman

Table 1 highlights various technology options within the open-source ecosystem for building a WebGIS system. These technologies are noted for their modernity, convenience, and flexibility, which facilitate development and adaptation [19]. This study aims to employ these technologies to develop a WebGIS software system for managing and searching unit data and spatial equipment data for SAR operations. The software not only provides functions for decentralized administration of users and management of equipment and common units but also incorporates advanced spatial analysis functions. These include searching for equipment and unit types by radius based on their location on the map and finding the shortest path from any unit to the incident location. The system is developed using the MVC model of the GeoDjango Framework, which is easy to inherit, adjust, and extend to other applications using a single database platform [20].

2. Methods

2.1. Back-end technology solutions

Programming languages and libraries: The backend utilises the Python programming language, a high-level programming language. To process geospatial data effectively, we integrated additional software packages into the Django Framework, including: 1. Django psycopg2 - is a PostgreSQL and PostGIS adapter for Python; 2. Geo Django - is a module for Django that turns it into a Web Framework for geospatial data. GeoDjango has the function of creating WebGIS applications. Its features include Extensions to Django's ORMs for querying and manipulating spatial data; High-level Python interfaces for GIS geometry operations and data manipulation in various formats; 3. Django REST-Framework - is a powerful and flexible toolkit for building Web APIs based on REST architecture. Django REST Framework provides robust model serialization, displaying data using standard function-based views or with views for more complex functions; 4. Django REST-framework-GIS - provides Django REST Framework geo plugins such as

GeometryField fields and GeoJSON serializers; 5. Django GeoJSON - is a toolkit for manipulating GeoJSON with Django for GeoDjango objects, query sets, and lists, etc.; base view to serve GeoJSON map layers from models; GeoJSON models and form fields to avoid spatial databases at the server; 6. Django Redis Cache - is a server cache software for the Django Web Framework, featuring a key-value data structure [20-23].

Django differentiates between an "application" and a "project". An application is a system designed for a specific function, while a project hosts multiple applications and their settings to form an entire website. Projects can host multiple applications, and applications can be migrated to multiple projects without issues. Table 2 presents the basic commands for creating software system projects and applications within Django.

Table 2. Basic commands to build a Django application [20].

No	Commands	Command Description
1	django-admin startproject CSDL_SDI	Create a project
2	python manage.py startapp GOIGIAOTHONG	Create a Goigiahong application in a project
3	python manage.py runserver	Start the test server
4	python manage.py runserver 0.0.0.0:8000	Start the test server to access the Internet

Map Server: The system's GIS server utilises GeoServer, and the web server employs Tomcat. GeoServer is a mature open-source map service publishing platform capable of easily publishing WCS, WFS, WMS, and other map services. Data imported into the PostgreSQL database through PostGIS is published, and the map service can be invoked in the frontend framework Vue using OpenLayers with the space name and namespace URL, allowing visual display in the frontend [24].

2.2. Front-end development environment

The client-side technology comprises two primary libraries: OpenLayer and Vue.js. Vue is a framework designed for building user interfaces, enabling and encouraging step-by-step application development. To integrate Vue.js, the user must embed the library using the following statement [25]:

```
<script src="https://cdn.jsdelivr.net/npm/vue/dist/vue.js"> </script>
```

OpenLayers is an open-source client-side library for creating web-based spatial data object interactions, displaying maps in WMS/WFS format standards in most current Web browsers without requiring server-side software or installation. To use OpenLayers, execute the following import statement: *import {Map, View} from 'ol'* [26].

2.3. The solution to finding the unit location

The search for suitable units or equipment is carried out in six steps (Fig. 2). Steps 1 and 2 are built on the frontend using OpenLayers and Vue libraries; step 3 is constructed on the backend using Python to query data from the PostgreSQL database management system, with results returned in the form of GeoJSON files; step 4 involves checking the returned results-if a suitable unit is found, proceed to steps 5 and 6. If not, the process concludes; step 5 filters the space in the returned dataset by calculating the distance between the points in the returned data to the incident point. If determining the nearest point, the point with the smallest distance is selected. If within a certain radius, points whose calculated distance is less than or equal to that radius are chosen; step 6 displays the results on the map.

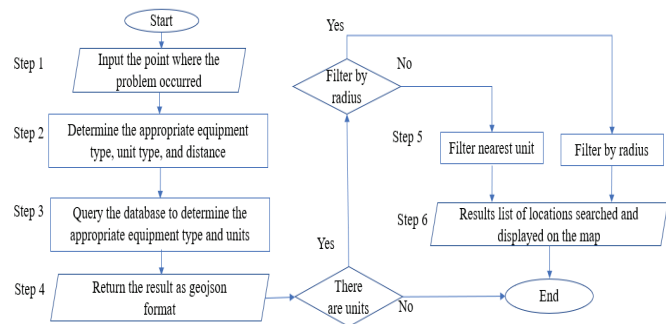


Fig. 2. Algorithm diagram for searching unit location with suitable equipment.

2.4. The solution to finding the optimal route

We consider the road system (edge-E) and the intersections of the roads (vertex-V) as a directed, non-negatively weighted graph $G(E, V)$. The problem is to find the path from vertex x to vertex y that is the shortest or has the smallest weight. E.W. Dijkstra's algorithm is as follows [27]:

$dist[s] \leftarrow 0$ (distance to source vertex is zero)

for all $v \in V - \{s\}$

do $dist[v] \leftarrow \infty$ (set all other distances to infinity)

$S \leftarrow \emptyset$ (S , the set of visited vertices is initially empty)

$Q \leftarrow V$ (Q , the queue initially contains all vertices)

while $Q \neq \emptyset$ (while the queue is not empty)

do $u \leftarrow \min_distance(Q, dist)$ (select the element of Q with the min. distance)

$S \leftarrow S \cup \{u\}$ (add u to the list of visited vertices)

for all $v \in neighbors[u]$

do if $dist[v] > dist[u] + w(u, v)$ (if new shortest path found)

then $d[v] \leftarrow d[u] + w(u, v)$ (set new value of shortest path)

return $dist$

Dijkstra's algorithm has a running time of $O(|V|^2 + |E|)$. For sparse graphs, which have few edges relative to the number of nodes, the algorithm can be implemented more efficiently using an adjacency list combined with a binary heap or priority queue, achieving a running time of $O((|E| + |V|) \log |V|)$. This algorithm has been enhanced by scientists to increase processing speed through the use of appropriate heuristic evaluations, leading to optimal performance. This enhanced version is known as the A* algorithm and has been integrated into the GraphHopper library [28, 29].

To determine the optimal route in GIS, the process begins with the user entering the starting and destination points using OpenLayers and Vue libraries on the frontend. In this step, users click on the map background to select these points. The second step involves determining the type of transport-car, motorbike, or walking-based on the purpose of the journey. The third step, determining the optimal route, is conducted using the GraphHopper route search server. This step involves adjusting weights, determining priority criteria values, and sorting nodes by importance before establishing the optimal path. The formula for calculating the weight of line segments (edges) is as follows [29]:

$$\text{edge_weight} = \text{edge_distance} / (\text{speed} * \text{priority}) + \text{edge_distance} * \text{distance_influence}$$

where: edge_weight is the weight of the line segment; edge_distance is the length of the line segment; speed is the travel speed on that segment; priority is the priority of the segment, default is 1; distance_influence is the effect of distance, default is 0 [29].

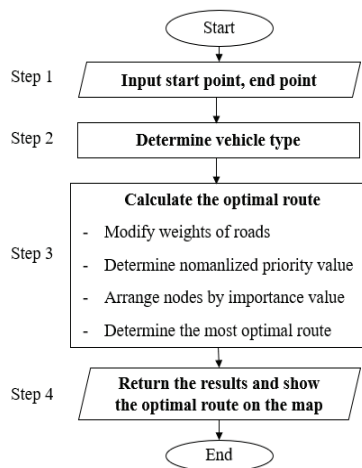


Fig. 3. Steps to find optimal path and A* algorithm.

The results are then processed using the improved algorithms on the GraphHopper server, typically the A* algorithm (Fig. 3). The final step involves returning the

result to the client as GeoJSON and displaying the route on a map using OpenLayers and Vue libraries.

For GraphHopper, data processing and preparation play a particularly important role in the speed of returning results of the program. Initially, GraphHopper reads the input data, builds a graph structure for the data, and stores important feature information in a directory (graph-cache). This includes a set of information about edges, nodes, spatial relationships, and related configurations. Each time there is a request, the system combines the use of original data with data in the graph-cache to process and return results, thus optimising time without having to perform repetitive tasks.

Step 1. Set the distance to infinity for all points except for the starting point, which is set at 0.

Step 2. Mark all points, including the start point, as non-visited nodes.

Step 3. Select the non-visited node with the smallest current distance as the current node "C"

Step 4. For each neighbour "N" of "C": add the current distance of "C" with the weight of the edge connecting "C" to "N" and the weight to the destination point (heuristic). If it is smaller than the current distance of "N," set it as the new current distance of "N"

Step 5. Mark the current node "C" as visited.

Step 6. Repeat steps 3 to 5 until one of the neighbours "N" is the destination point.

3. Results and discussion

3.1. Data and experimental area

Basemap data: The base map is sourced from OpenStreetMap, open data released under the Open Data Commons Open Database License (ODbL) by the OpenStreetMap Foundation (OSMF).

Traffic class thematic data: Use GIS processing software to extract the Hanoi area to obtain the file HaNoi.osm.pbf. To prepare data, create a configuration file config2.yml with data input as Hanoi's pbf file, options for car (weighting: fastest), bike (weighting: fastest), and foot (weighting: shortest). Starting the GraphHopper server for the first time, the system builds a graph structure for the data and saves important feature information in the graph-cache (Fig. 4B). The structure of the data tables is stored to suit the optimal improvement of the applied algorithm (Figs. 4A, 4C).

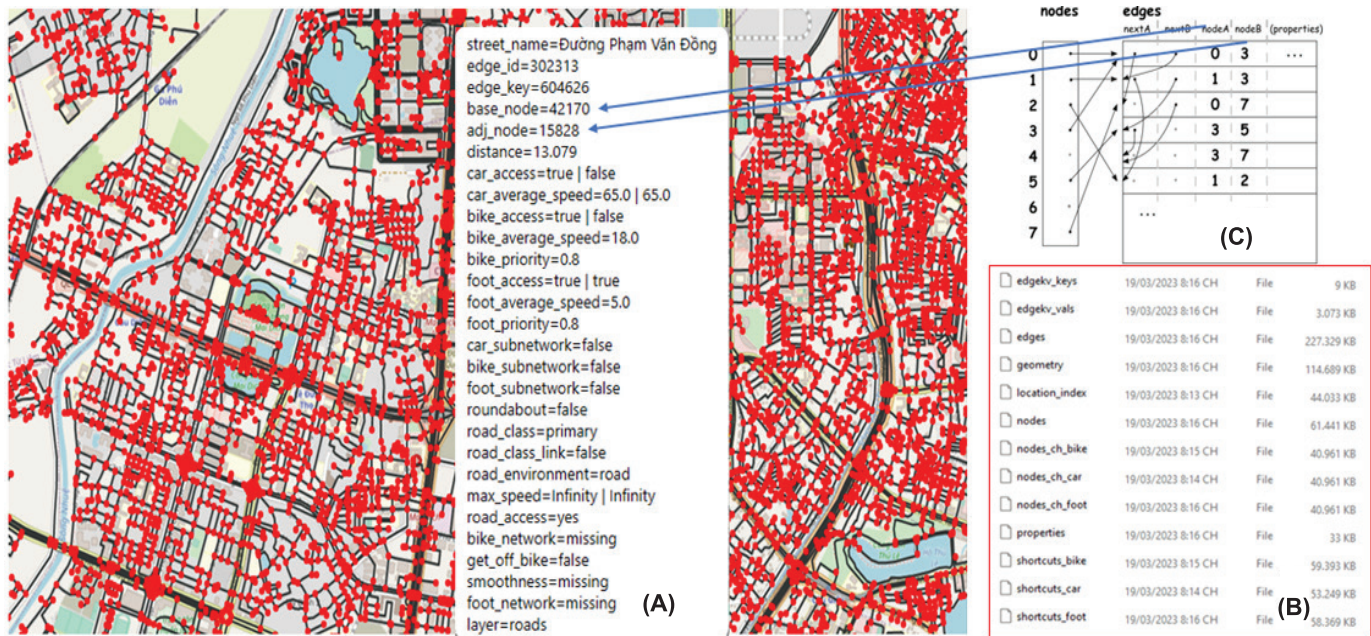


Fig. 4. Traffic thematic data prepared in Graphhopper.

Data of units and equipment: The structure of information fields and database tables are fully and wholly surveyed and designed at the centers, fire prevention, and fighting departments of the districts of Hanoi. Inside, some equipment related to the chemical defence of the Army.

3.2. Results and discussion

Architectural and functional model of the system: The system is designed with three layers: data tier, logic tier, and application tier (Fig. 5). The data layer includes spatial data (base map, unit map layer, traffic map layer) and attribute data (user data, equipment data). The logical layer comprises the Web application and the map server (Geoserver). The web application server hosts the web page, responsible for receiving HTTP requests from the client, parsing the request, and processing and responding to the HTTP client. The map server has map services according to OGC standards (WMS, WFS, WCS, etc.), provides map distribution services according to Raster and Vector standards, and performs querying, analysis, and filtering of spatial data, in addition to allowing services to add, edit, and delete spatial data. The application layer is a web browser, with accounts

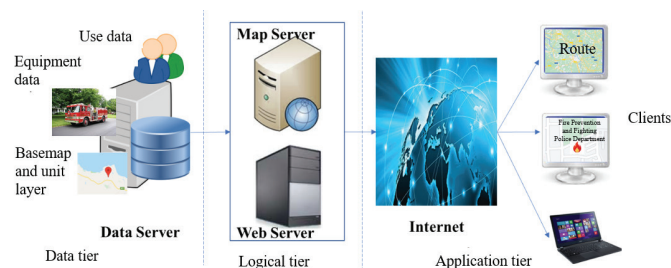


Fig. 5. Deployment architecture of the system.

belonging to the system administration groups, equipment data management, and user accounts to look up, statistics equipment information, search for optimal routes, etc.

Functional diagram: The system has many functions, generally divided into four primary groups: account administration function group and user account group. This group has the function of creating/deleting/editing/authorizing accounts for a group or a single user, etc.; unit information management group, users can add/edit/delete/search/display units on the map/view, search for equipment from the map, etc.; a functional group of equipment management, including add/edit/delete/quick search/exact search/multi-criteria search/search on the map/export the list of equipment to the report form, etc.; and a group of spatial analysis functions, including network analysis to find the optimal path and buffer zone analysis to find the type of unit or type of equipment within a certain radius. Some typical functional models are shown in Fig. 6.

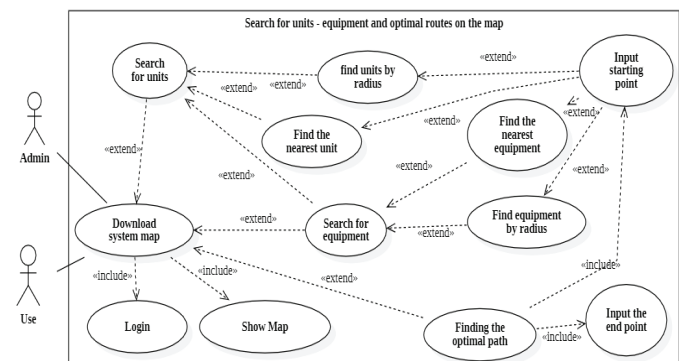


Fig. 6. Typical use case diagram of the system.

The main functional interface results of the system:

- The general layout interface of the system includes the following components: (1) Area describing information about the path, current user information, login/logout/change account information, and information about the number of device lists waiting for approval and viewing the user's operation history; (2) Working area with data tables or maps, including quick search with data, functions to add equipment data, accurately filter equipment by various criteria, output system charts list and output a report that prints the results after filtering; (3) The area where the equipment information list is displayed if the equipment menu is selected, and the selection operations to view, delete, and edit the equipment information. If you choose to enter the map menu, this area will display a map of units performing particular tasks. Users can perform operations to view unit information or analyse spatial problems to support decision support; (4) Menu area, to access specific functional groups such as Map function group, query execution, search for units and equipment on the map, optimal route search; The functional group views the equipment chart and validates the unit's equipment; Functional group of equipment management; Functional group to manage information describing the unit, including unit management, unit location management on the map; The system administration function group includes

managing user accounts, managing user groups and adding data fields to the equipment table. In addition, financial equipment will be displayed in the list of equipment. You can click on the equipment name for detailed information, pictures, and attached equipment (Fig. 7).

- The function of spatial analysis is crucial in searching for units with equipment suitable for the task: it enables the search for units or equipment by radius at any location on the map, typically starting from where the disaster occurs. The search area is marked with a red teardrop, the centre of the circle on the map, and units within the circle radius are marked with a blue teardrop shape. This functionality is vital in mobilising the nearest response force within a certain radius, offering a rapid means to locate the appropriate equipment or units for the mission in the area to be mobilised, thereby assisting the commander in making prompt decisions (Fig. 8A). Currently, typical equipment management software does not include this function. If using standard spatial analysis, such as searching for firefighting units on map.google.com, the process involves mobilising all units that match keywords on the map (Fig. 8B).

- By applying the software as depicted in Fig. 8A, in the search area, there are four units, with only two matching units that can be filtered with the blue water drop icon. Next, the user will perform the second search phase to determine

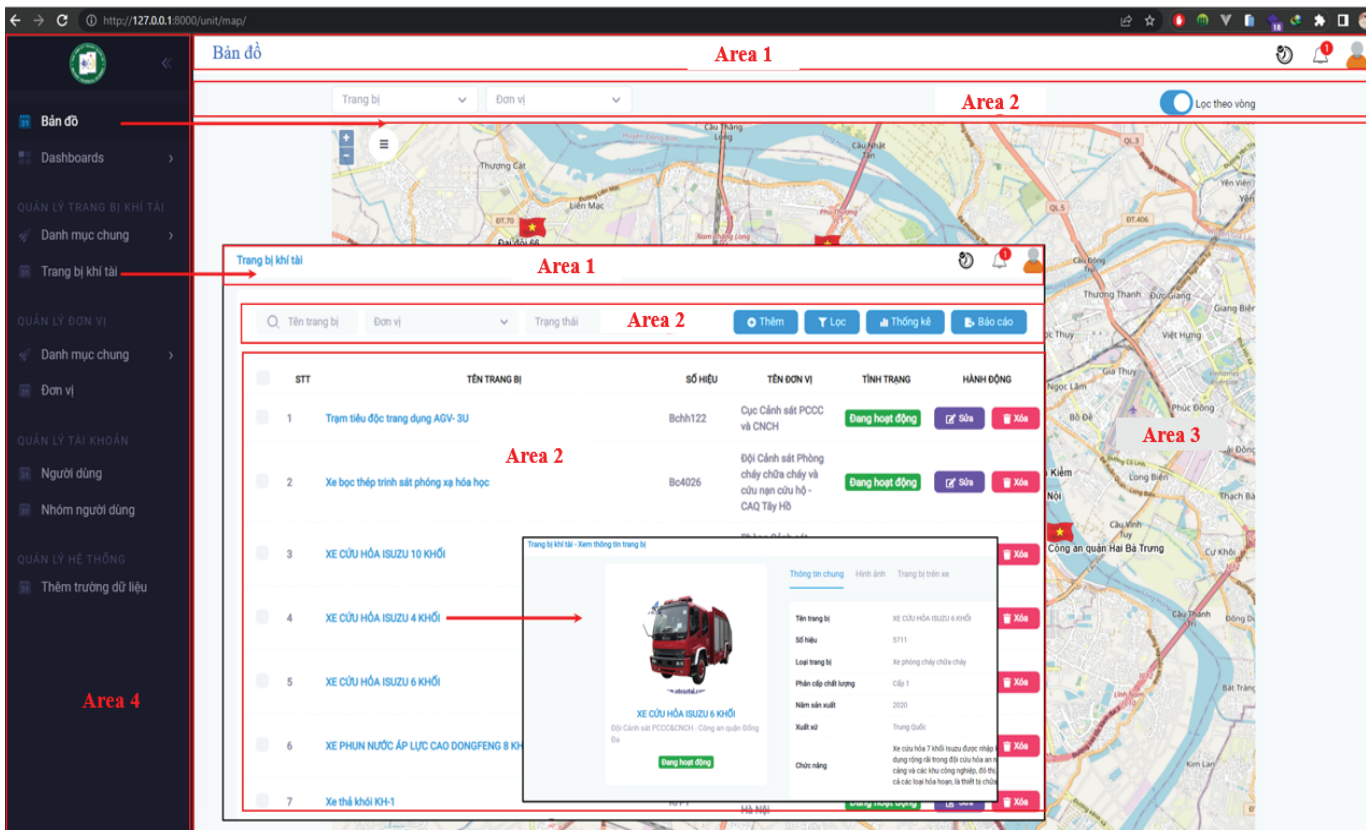


Fig. 7. The general interface of the software system.

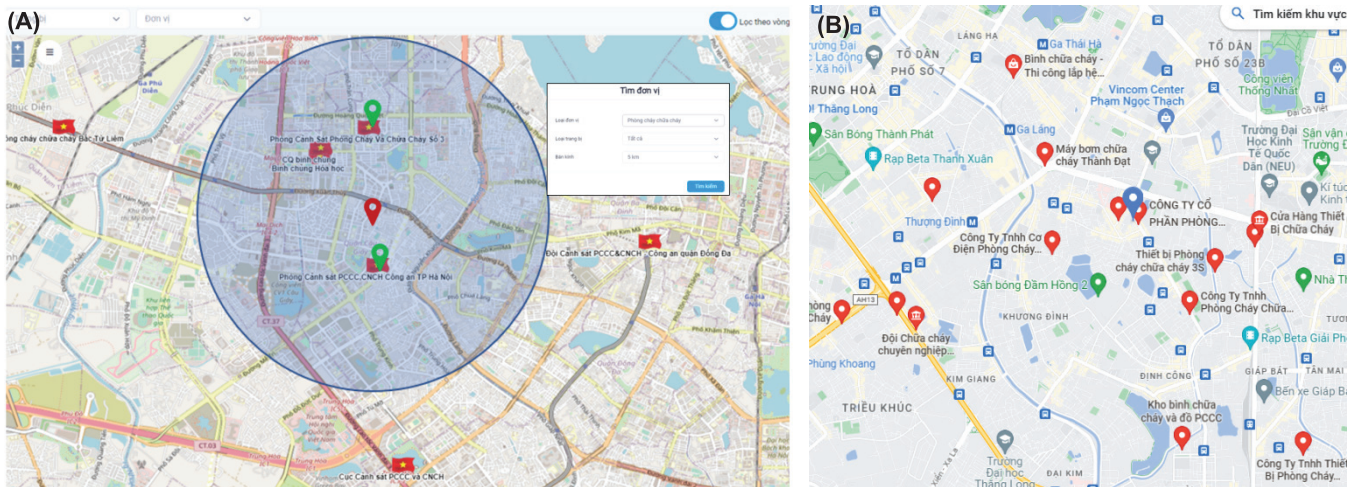


Fig. 8. Equipment type or unit type spatial query function by radius.

if the shortest path from the unit's location to the incident area is feasible. The results were tested with two modes of travel: by car and on foot. These results are compared and evaluated against the optimal route-finding results of Google Maps:

Option 1, from Fire Prevention and Fighting Police Team No. 3 to the rescue point (Fig. 9): Travelling by car,

the software calculated a distance of 1.09 km, while Google Maps calculated 1.2 km. Generally, the overall routes are the same; the primary difference lies in the starting point location (circled area), which stems from the different granularities in the route datasets. Both algorithms selected the closest route from the starting position on the map. For walking, the shorter route is preferred: 943 m for the software compared to 1.1 km for Google Maps.

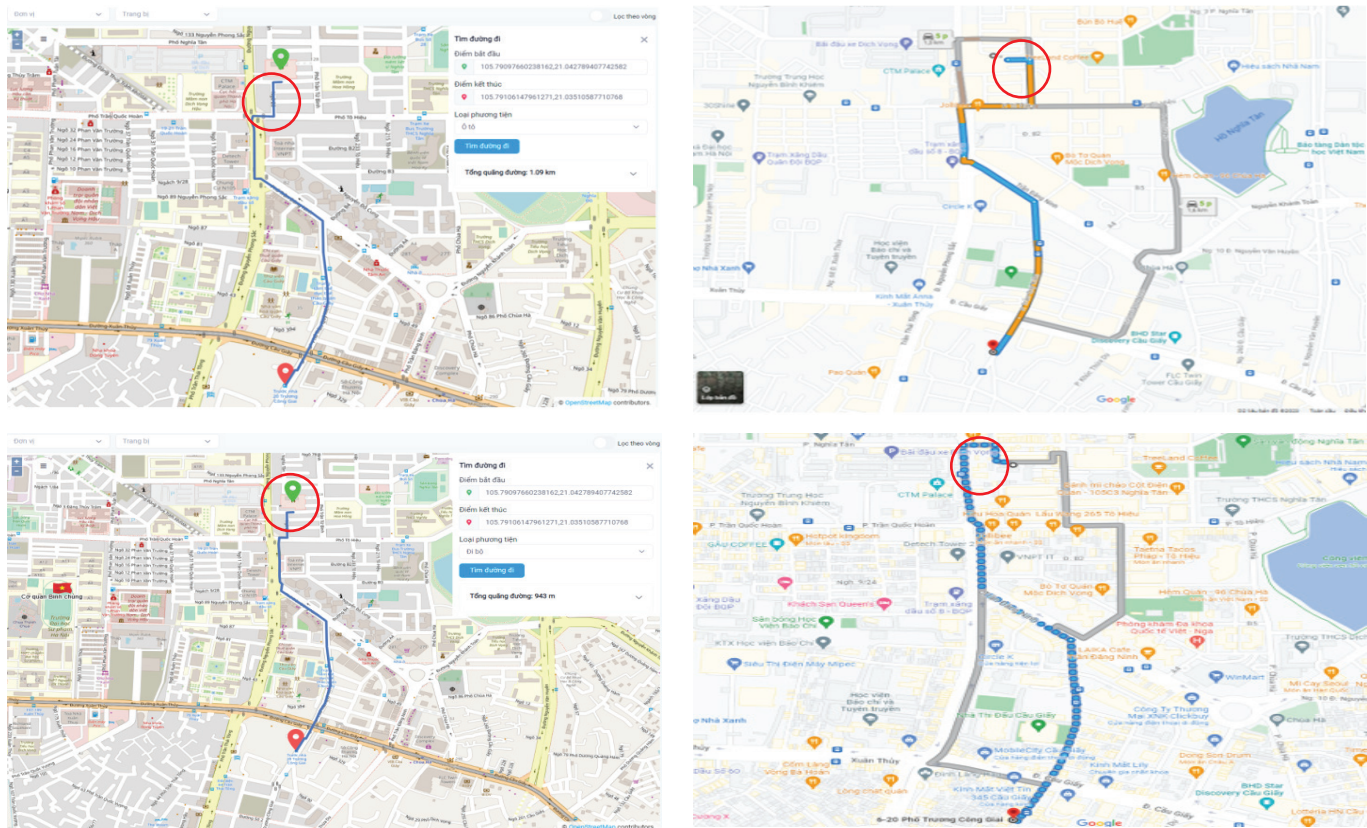


Fig. 9. Optimal path search results of option 1 (left side of software, right of Google Maps).

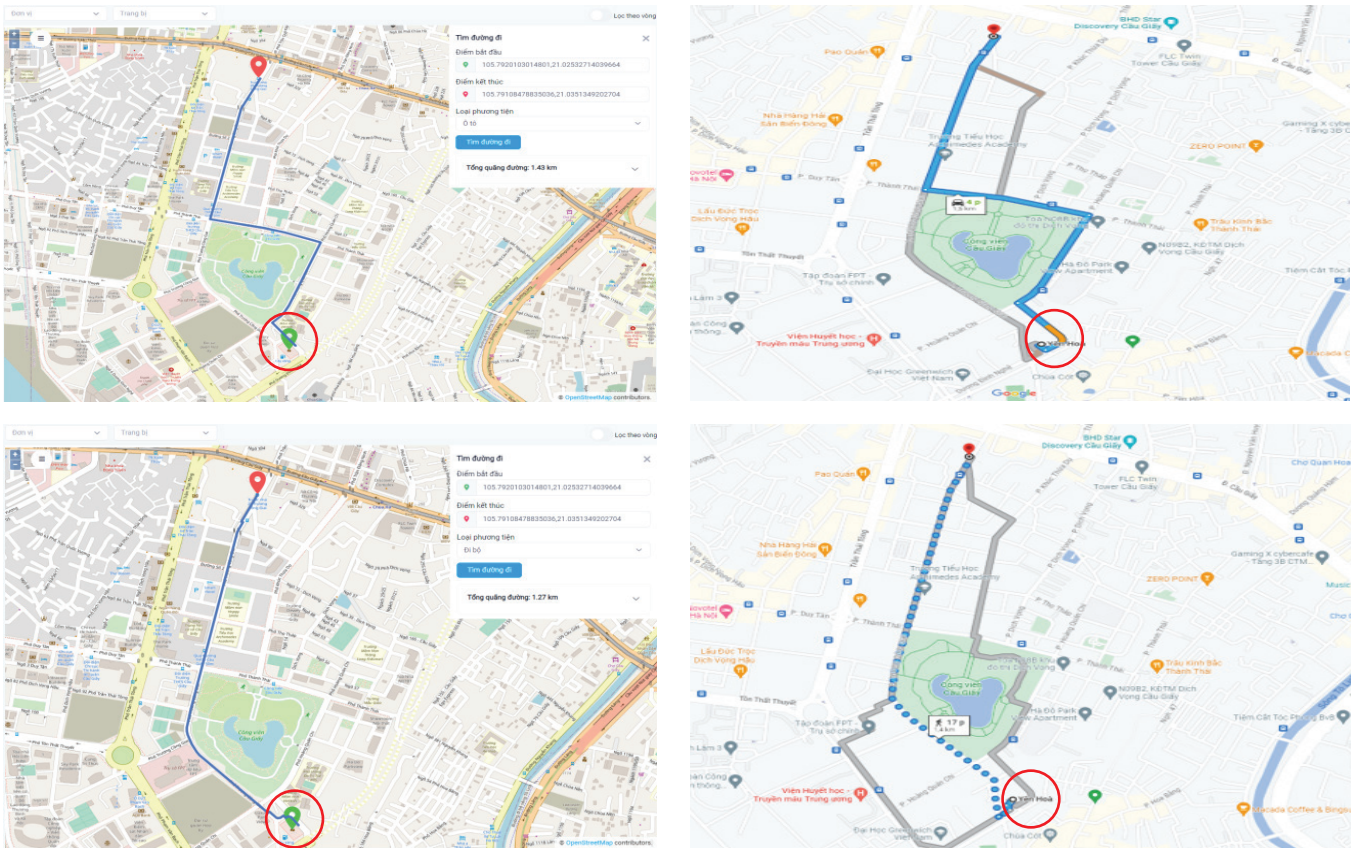


Fig. 10. Optimal path search results of option 2 (left side of software, right of Google Maps).

Option 2, from Hanoi City Fire and Rescue Police Department to the rescue point (Fig. 10): The shortest road length when travelling by car is 1.43 km for the software and 1.4 km for Google Maps. The optimal walking length is 1.27 km for the software and 1.4 km for Google Maps. This discrepancy, similar to the previous case, arises due to the differences in the route data near the starting points and the algorithms' nearest point capture mechanism. The optimal solution requires that the spatial data for the area be complete, adhering to the correct geographic database standards. This involves using spatial analysis methods to determine the connection point of the starting and destination areas with the connecting route to it, necessitating sufficient data collection between the area boundary layer and the traffic layer to ensure the system operates accurately.

Based on these test results, the software can select the most suitable option, achieving accuracy equivalent to the widely-used Google Maps navigation system. This is a crucial step in mastering the technology for building data management and decision support software for specialised fields involving sensitive and highly confidential data. The system operates efficiently and synchronously. In addition to the described functions, the system also includes a group of general administrative functions (monitoring user

activity history, equipment statistics by chart, approving new or edited equipment by the administrator), user account modifications, and system login and logout functions; a functional group for user administration and a functional group for device-unit administration.

4. Conclusions

Rescue missions or operations are often urgent and require swift action to be effective. Thus, tools for managing information about human and material resources to support prompt and optimal planning are increasingly vital. The development of open-source geospatial technology (including maps, GIS, WebGIS, and Industry 4.0 technology) provides a favourable condition for developing a spatial decision support system. This study has investigated, designed, and programmed a software system for managing the spatial data of units and technical equipment to ensure specific combat tasks such as rescue and firefighting, chemical assurance, and engineer assurance are met. The paper focuses on spatial analysis to locate units with suitable firefighting equipment and the optimal manoeuvring route in the Hanoi area. The results have been evaluated to possess the same accuracy as Google Maps, marking an essential first step as a foundation to tackle more

complex spatial decision-making and management issues, such as spatial analysis using AHP techniques to find the optimal path based on multi-criteria, overlap analysis, etc. This will further develop systems for drafting spatial plans based on shared maps, approving plans, monitoring plan implementation, and gradually mastering specific systems.

COMPETING INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] M. Wysokiński, R. Marcjan, J. Dajda (2014), "Decision support software for search & rescue operations", *Procedia Computer Science*, **35**, pp.776-785, DOI: 10.7494/csci.2015.16.3.281.
- [2] J. Malczewski, P. Jankowski (2020), "Emerging trends and research frontiers in spatial multi-criteria analysis", *International Journal of Geographical Information Science*, **34**(7), pp.1257-1282, DOI: 10.1080/13658816.2020.1712403.
- [3] R. Tierno, B. Puig, B. Vera, et al. (2013), "The retail site location decision process using GIS and the analytical hierarchy process", *Applied Geography*, **40**, pp.191-198, DOI:10.1016/j.apgeog.2013.03.005.
- [4] A. Mardoukhi, N. Kordzadeh (2016), "Site location determination using geographic information systems: The Process and a case study, *The Proceedings of The Southern Association for Information Systems Conference*, USA, 5pp.
- [5] P.B. Keenan, P. Jankowski (2019), "Spatial decision support systems: Three decades on", *Decision Support Systems*, **116**, pp.64-76, DOI: 10.1016/j.dss.2018.10.010.
- [6] B.A. Widyanoro, P.B. Santosa (2021), "Network analysis to determine the optimal route for firefighters in Makassar city", *IOP Conference Series: Earth and Environmental Science*, **936**(1).
- [7] O. Khaing, H. Wai, E. Myat (2018), "Using Dijkstra's algorithm for public transportation system in Yangon based on GIS", *Int. J. Sci. Eng. Appl.*, **7**(11), pp.442-447, DOI: 10.7753/IJSEA0711.1008.
- [8] D. Das, A.K. Ojha, H. Kramsapi, et al. (2019), "Road network analysis of Guwahati city using GIS", *SN Applied Sciences*, **1**(8), pp.1-11, DOI: 10.1007/s42452-019-0907-4.
- [9] N. Nolde (2020), "Open source routing engines and algorithms - An overview", <https://gis-ops.com/open-source-routing-engines-and-algorithms-an-overview/>, accessed 5 May 2023.
- [10] N. Krismer, D. Silbernagl, M. Malfertheiner, et al. (2016), "Elevation enabled bicycle router supporting user-profiles", *28th GI-Workshop on Foundations of Databases*, **2016**, pp.74-79.
- [11] K. Samah, S. Ibrahim, N. Ghazali, et al. (2020), "Mapping a hospital using OpenStreetMap and GraphHopper: A navigation system", *Bulletin of Electrical Engineering and Informatics*, **9**(2), pp.661-668, DOI: 10.11591/eei.v9i2.2082.
- [12] R. Sugumaran, J. Degroote (2010), *Spatial Decision Support Systems: Principles And Practices*, CRC Press, 174pp.
- [13] R. Can, S. Kocaman, A.O. Ok (2021), "A WEBGIS framework for semi-automated geodatabase updating assisted by deep learning", *The International Archives of Photogrammetry, Remote Sensing and Spatial Information Sciences*, **43**, pp.13-19, DOI: 10.5194/isprs-archives-XLIII-B5-2021-13-2021.
- [14] R.M. Sanchez (2012) "Free and open source software for Geospatial Applications (FOSS4G): A mature alternative in the Geospatial technologies arena", *Transactions in GIS*, **16**(2), pp.81-88, DOI: 10.1111/j.1467-9671.2012.01314.x.
- [15] M. Molina, S. Bayarri (2011). "A multinational SDI-based system to facilitate disaster risk management in the Andean community", *Computers & Geosciences*, **37**(9), pp.1501-1510, DOI: 10.1016/j.cageo.2011.01.015.
- [16] OpenSource for GeoSpatial Fosters (2019), *Welcome to OSGeoLive 13.0-OSGeoLive 13.0 Documentation*, <https://live.osgeo.org/en/index.html>, accessed 19 October 2019.
- [17] S. Coetzee, I. Ivánová, H. Mitasova, et al. (2020), "Open geospatial software and data: A review of the current state and a perspective into the future", *ISPRS International Journal of Geo-Information*, **9**(2), DOI: 10.3390/ijgi9020090.
- [18] M. Li, D. Peak, W. Zeng, et al. (2017), *An Open Source Webgis Framework For Collaborative Research In West Africa*, Spatial Knowledge Initiative Conference, Banff, AB, Canada, pp.1-9.
- [19] M.A. Brovelli, M. Minghini, R.M. Sanchez, et al. (2017), "Free and open source software for geospatial applications (FOSS4G) to support future Earth", *International Journal of Digital Earth*, **10**(4), pp.386-404, DOI: 10.1080/17538947.2016.1196505.
- [20] Django (2023a), *Django Documentation Contents*, <https://docs.djangoproject.com/en/4.1/contents/>, accessed 5 May 2023.
- [21] Django (2023b), *GeoDjango*, <https://docs.djangoproject.com/en/4.1/ref/contrib/gis/>, accessed 5 May 2023.
- [22] Python Package Index (2023a), *Geographic addons for Django Rest Framework*, <https://pypi.org/project/djangorestframework-gis/>, accessed 5 May 2023.
- [23] Python Package Index (2023b), *Django-GeoJson 4.1.0*, <https://pypi.org/project/django-geojson/>, accessed 5 May 2023.
- [24] L. Cui, W. Li, X. Wang, et al. (2022), "Design and implementation of open-source WebGIS system for orchard land management", *Advances in Computer, Signals and Systems*, **6**(3), pp.26-34, DOI: 10.23977/acss.2022.060304.
- [25] Vue.js (2023), *What is Vue.js?*, <https://vi.vuejs.org/v2/guide/>, accessed 5 May 2023.
- [26] OpenLayers (2023), *Tutorials*, <https://openlayers.org/doc/tutorials/>, accessed 5 May 2023.
- [27] E.W. Dijkstra (1959), "A note on two problems in connection with graphs", *Numerische Mathematik*, **1**, pp.269-271.
- [28] P.E. Hart (1968), "A formal basis for the heuristic determination of minimum cost paths", *IEEE Transactions on Systems Science and Cybernetics SSC4*, **2**, pp.100-107.
- [29] GraphHopper (2023), *GraphHopper Directions API*, <https://docs.graphhopper.com/#section/Custom-Model>, accessed 5 May 2023.

Hydration number and allied parameters of aqueous solutions of ammonium bromide through ultrasonic study

J.H. Rakini Chandrasekaran¹, S. Nithiyanantham^{2*}

¹Centre for Research-Department of Physics, Arignar Anna Government Arts College, Musiri - 621211, Tiruchirappalli, India

²PG and Research Department of Physics, Thiru. Vi. Kalyansundaram Govt Arts & Science College, Thiruvavur, Tamil Nadu, India

Received 19 April 2023; revised 4 July 2023; accepted 27 November 2023

Abstract:

Ammonium bromide, a water-soluble wood preservative used in pharmaceutical preparations, is the focus of this economic study aimed at understanding its physical properties in aqueous solutions. A straightforward and reliable method for examining interactions between molecules in liquids and solutions is ultrasonic research. When ultrasonic waves pass through liquids, they disturb the equilibrium between the molecules of the solute and solvent. Ion-ion and ion-solvent interactions are just two aspects of solvation chemistry that ultrasonic velocity measurement can elucidate. This study investigates the hydration number of aqueous solutions of ammonium bromide at temperatures ranging from 35 to 55°C, assessing both density and ultrasonic velocity across various concentrations. From the adiabatic compressibility, the hydration number and related parameters are derived, all of which are sensitive to changes in concentration and temperature. Thus, we explore how these parameters vary with changes in temperature and concentration, providing insight into the interactions of ions and molecules in the solutions, as well as their molecular structure.

Keywords: allied parameters, density, hydration number, molecular interactions, ultrasonic velocity.

Classification numbers: 1.3, 2.1, 2.2

1. Introduction

Ammonium bromide, a water-soluble wood preservative used in pharmaceutical preparations, is the focus of this economic study aimed at understanding its physical properties in aqueous solutions. We investigate whether Ammonium bromide has a structure-making or -breaking property with water by examining its hydration number and allied parameters such as apparent molal compressibility, apparent molal volume, and molar solvated volume.

When an electrolyte dissolves in water, both volume and compressibility are altered due to solute-solvent interaction. The interaction of ions with water molecules is quantified by the hydration number, representing the number of water molecules surrounding the solute molecules during interaction. Previous research has explored the hydration number, assuming the incompressibility of ions and water molecules in the primary hydration sheaths directly bonded to the ions [1, 2]. The methodology for calculating the hydration number has been documented [3, 4], along with techniques for determining allied parameters such as apparent molal compressibility, apparent molal volume, and molar solvated volume using the electrolyte solution's hydration number [5-7]. For each of these parameters, three formulas were derived using adiabatic compressibility and the number of moles of solute and solvent, corroborated by traditional equations [8].

In this article, we determine the hydration number for aqueous solutions of Ammonium bromide within the temperature range

of 35 to 55°C. From these values, we estimate apparent molal compressibility, apparent molal volume, and molar solvated volume, examining their variations.

2. Experimental study

AnalaR grade salts and double-distilled water were used to prepare aqueous solutions of ammonium bromide at various concentrations. The ultrasonic velocity of the solutions was measured using a Mittal type ultrasonic interferometer equipped with a 2 MHz crystal and an accuracy of 0.1 m/s. Density was determined using a 10 ml specific gravity container and a single pan digital balance. Temperature control between 35 and 55°C was achieved using a thermostat accurate to 0.1°C [8].

3. Computation

The compressibility method, noted for its accuracy and the insightful data it provides, was employed to estimate the hydration number [9, 10]. Previous studies have established the methodology for calculating the hydration number [11-13], presuming that the free solvent molecules primarily contribute to the solution's compressibility. As every solvent possesses a unique structure, its molecules are bound more or less tightly to the solute during hydration, significantly altering the solvent's surrounding structure. Ultrasonic analysis presents a direct but precise method for determining adiabatic compressibility, which is calculated from the ultrasonic velocity and density measurements. From the

*Corresponding author: Email: s_nithu59@rediffmail.com

adiabatic compressibility values, the hydration number is derived [14, 15].

Using the standard relation, the adiabatic compressibility may be calculated by:

$$\beta = \frac{1}{u^2 \rho} \text{ m}^2/\text{N} \quad (1)$$

where β is the adiabatic compressibility of a material, a measure of how much the material can be compressed adiabatically (without heat exchange) under the influence of pressure with the units are typically Pa^{-1} (inverse of pressure); u^2 is the speed of sound in the material with the units are typically meters per second (m/s); ρ (rho) is the density of the medium, usually in kilograms per cubic meter (kg/m^3); m^2/N : this part represents area per number of particles or units.

The molar hydration number n_h is calculated using Barnartt's relation [16]:

$$n_h = \frac{1}{n_2} \left[n_1 - \frac{\beta}{\beta_0} N \right] \quad (2)$$

where N is the number of solvent molecules in 1000 cc of solvent, n_1 is the number of solvent moles present in 1000 cc of solution of molar concentration n_2 , and β_0 is the solvent's compressibility.

The molal hydration number n_h' is computed through Passynski's equation [17]:

$$n_h' = \frac{N'}{n_2'} \left[1 - \frac{\beta}{\beta_0} \right] \quad (3)$$

where n_2' is the molal concentration of the solution and N' is the number of solvent molecules in 1000 g of the solvent.

Apparent molal volume ϕ_v of the mixture is calculated using the relations:

$$\phi_v = \frac{(V - n_1 V_m)}{n_2} \quad (4)$$

$$\phi_v = V_1 \left[\frac{(N \Delta \beta)}{n_2 \beta_0} \right] - n_h V_m \text{ (molar scale)} \quad (5)$$

$$\phi_v = \frac{\beta_0 n_h' \left(\frac{V'}{N'} - V_m \right)}{\Delta \beta} \text{ (molar scale)} \quad (6)$$

$$\phi_v = \frac{1000}{n_2 \rho_0} (\rho_0 - \rho) + \frac{M_2}{\rho_0} \text{ (Traditional formula)} \quad (7)$$

where n_1 is the number of moles of the solvent and n_2 is the number of moles of the solute present in the solution, and $\Delta \beta = \beta - \beta_0$. V is the total volume of the solution, V_m is the molar volume of the solvent, V_1 is a reference volume per mole, n_h is the number of moles of component "h", V' is a volume associated with a specific condition, ρ_0 is the reference density and M_2 is the molar mass or mass of component 2.

The volume of the solution measured in the molal scale is given by:

$$V' = (N' M_1 + n_2' M_2) / \rho$$

where M_1 is the molar mass or mass of component 1, M_2 is the molar mass or mass of component 2, n_h and n_h' are found using Eqs. (2) and (3), respectively.

Apparent molal compressibility ϕ_k of the solution is calculated using the relations:

$$\phi_k = \left(\frac{\beta V - n_1 \beta_0 V_m}{n_2} \right) \quad (8)$$

$$\phi_k = -V_m \beta_0 n_h \text{ (molar scale)} \quad (9)$$

$$\phi_k = -n_h' V_m \beta_0 + \phi_v \beta \text{ (molar scale)} \quad (10)$$

$$\phi_k = \frac{1000}{n_2 \rho_0} (\rho_0 \beta - \beta_0 \rho) + \frac{M_2}{\rho_0} \beta_0 \text{ (Traditional formula)} \quad (11)$$

where ϕ_v is calculated using Eq. (7).

Molar solvated volume ϕ_s of the solution is computed from the following equations:

$$\phi_s = \left(\frac{n_h' V_m \beta_0}{\beta} \right) - \left(\frac{n_h V_m \Delta \beta}{\beta} \right) \quad (12)$$

$$\phi_s = \phi_v + n_h V_m \text{ (molar scale)} \quad (13)$$

$$\phi_s = \frac{V n_h'}{n_1} \text{ (molar scale)} \quad (14)$$

$$\phi_s = \frac{V \Delta \beta}{n_2 \beta_0} \text{ (Traditional formula)} \quad (15)$$

where $\Delta \beta = \beta - \beta_0$.

Variation of three allied parameters ϕ_v , ϕ_k and ϕ_s parameters with concentration: The equations for the variation of the three allied parameters ϕ_v , ϕ_k , and ϕ_s with the square root of molar and molal concentration ($\sqrt{n_2}$ & $\sqrt{n_2'}$) are

$$\phi_v = \phi_v^0 + n_{hv} \sqrt{n_2} \quad (16)$$

$$\phi_v = \phi_v^{0'} + n_{hv}' \sqrt{n_2'} \quad (17)$$

$$\phi_k = \phi_k^0 + n_{hk} \sqrt{n_2} \quad (18)$$

$$\phi_k = \phi_k^{0'} + n_{hk}' \sqrt{n_2'} \quad (19)$$

$$\phi_s = \phi_s^0 + n_{hs} \sqrt{n_2} \quad (20)$$

$$\phi_s = \phi_s^{0'} + n_{hs}' \sqrt{n_2'} \quad (21)$$

where ϕ_v^0 , ϕ_k^0 , ϕ_s^0 and $\phi_v^{0'}$, $\phi_k^{0'}$, $\phi_s^{0'}$ are the intercepts in molar and molal scale of the linear equations and n_{hv} , n_{hk} , n_{hs} and n_{hv}' ,

n'_{hk} , n'_{hs} are the slopes in molar and molal scale of the linear equations.

4. Results and discussion

Using adiabatic compressibility, the molar and molal hydration numbers of aqueous solutions of ammonium bromide across all concentrations and at various temperatures have been calculated and are presented in Table 1 and Fig. 1.

Table 1. Adiabatic compressibility, molar and molal hydration numbers (n_h and n'_h) of ammonium bromide solutions.

Temp °C	Molality (n_2) M	Density Kg/m ³	Velocity m/s	$\beta(^{\circ}\text{E}10)$ m ² /N	n_2	n_1	n_h	n'_h
35°C	0.5	1.015	1531.5	4.1991	0.49110	54.518	3.7	5.0
	1.0	1.046	1533.5	4.0666	0.96462	53.543	2.5	4.1
	1.5	1.070	1535.2	3.9670	1.42308	52.66	2.1	3.7
	2.0	1.091	1537.5	3.8769	1.86089	51.645	1.7	3.4
	2.5	1.114	1539.9	3.7859	2.28220	50.671	1.5	3.2
	3.0	1.134	1541.7	3.7104	2.67951	49.576	1.2	2.9
	3.1	1.139	1542.9	3.6886	2.75658	49.357	1.2	2.9
40°C	0.5	1.014	1539.9	4.1605	0.48891	54.275	3.4	4.8
	1.0	1.042	1541.7	4.0373	0.95943	53.255	2.2	3.9
	1.5	1.066	1543.1	3.9398	1.41236	52.263	1.8	3.5
	2.0	1.088	1545.3	3.8487	1.84283	51.144	1.4	3.2
	2.5	1.111	1547.4	3.7599	2.25951	50.167	1.2	3.0
	3.0	1.131	1549.5	3.6835	2.65354	49.096	0.9	2.8
	3.1	1.135	1550.9	3.6622	2.72971	48.876	0.9	2.8
45°C	0.5	1.014	1544.7	4.1319	0.48824	54.201	3.2	4.7
	1.0	1.041	1546.5	4.0155	0.95661	53.098	2.0	3.8
	1.5	1.065	1548.7	3.9149	1.40687	52.060	1.6	3.4
	2.0	1.087	1550.7	3.8254	1.83446	50.912	1.2	3.1
	2.5	1.109	1552.3	3.7411	2.25168	49.993	1.0	3.0
	3.0	1.130	1554.4	3.6643	2.64218	48.886	0.8	2.8
	3.1	1.134	1555.1	3.6469	2.7202	48.706	0.8	2.7
50°C	0.5	1.015	1551.9	4.0909	0.48657	54.016	3.0	4.6
	1.0	1.040	1553.5	3.9832	0.95260	52.875	1.8	3.7
	1.5	1.064	1555.7	3.8853	1.40098	51.842	1.4	3.4
	2.0	1.085	1557.3	3.8006	1.82426	50.629	1.0	3.0
	2.5	1.108	1559.3	3.7139	2.23220	49.560	0.8	2.8
	3.0	1.127	1561.7	3.6368	2.63035	48.667	0.7	2.7
	3.1	1.132	1562.9	3.6168	2.70712	48.472	0.7	2.7
55°C	0.5	1.014	1554.4	4.0839	0.48538	53.883	2.8	4.4
	1.0	1.039	1556.3	3.9759	0.94951	52.703	1.6	3.6
	1.5	1.062	1558.4	3.8783	1.39627	51.668	1.3	3.3
	2.0	1.083	1560.7	3.7894	1.81841	50.467	0.9	3.0
	2.5	1.106	1562.7	3.704	2.22336	49.364	0.7	2.8
	3.0	1.126	1564.6	3.6289	2.60925	48.277	0.5	2.6
	3.1	1.129	1565.1	3.6156	2.68500	48.076	0.5	2.6

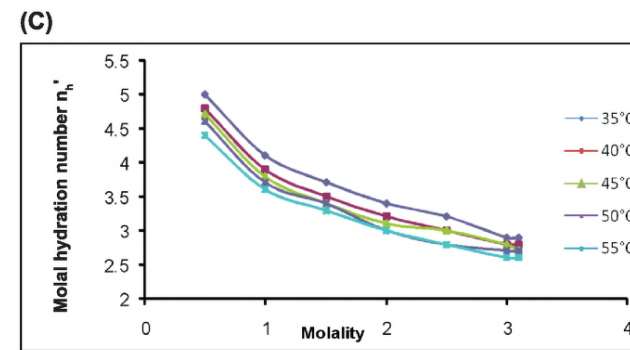
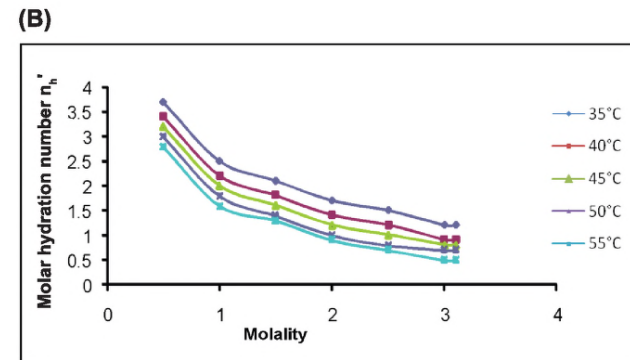
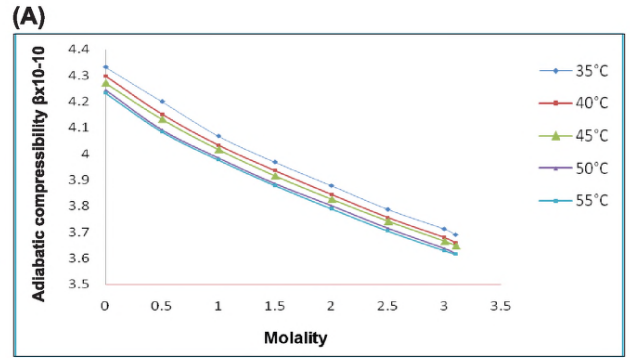


Fig. 1. (A) Adiabatic compressibility, (B) molar hydration number, and (C) molal hydration number - water + ammonium bromide.

These variations indicate that ion-solvent interactions exist within the system. The molar and molal hydration numbers decrease with increases in concentration and temperature in aqueous solutions of ammonium bromide. For instance, the molar hydration number declines from 4 to 1 and the molal hydration number from 5 to 3 at 35°C, as detailed in Table 1. A similar pattern of reduction is observed at other temperatures. Temperature influences a slight decrease in both molar and molal hydration numbers.

The reduction in molar hydration numbers with increasing molality is attributed to ion-ion association, consequently decreasing ion-solvent interaction [18]. The number of solvent molecules surrounding an ion also diminishes due to the presence of ion pairs in highly concentrated solutions. Therefore, hydration by water molecules is reduced at higher concentrations, which is reflected by the declining molar

Table 2. Allied parameters of ammonium bromide solutions.

Apparent molal volume ϕ_v of ammonium bromide salt in water.

System	Temp. molality	ϕ_v (*10 ⁶) m ³ /mole				
		35°C	40°C	45°C	50°C	55°C
Water + ammonium bromide	0.5	24.574	27.414	27.967	31.105	31.223
	1.0	30.838	33.302	35.257	37.713	38.673
	1.5	32.143	35.381	37.399	39.090	39.854
	2.0	34.461	38.155	40.067	42.145	42.677
	2.5	35.840	38.982	40.073	43.172	43.967
	3.0	37.925	40.529	41.776	42.830	45.083
	3.1	38.305	40.862	41.781	42.931	45.178

Apparent molal compressibility ϕ_k of ammonium bromide salt in water.

System	Temp. molality	ϕ_k (*10 ¹⁴) m ⁵ /N/mole				
		35°C	40°C	45°C	50°C	55°C
Water + ammonium bromide	0.5	-2.875	-2.640	-2.472	-2.317	-2.142
	1.0	-1.942	-1.739	-1.522	-1.368	-1.229
	1.5	-1.645	-1.377	-1.208	-1.104	-1.006
	2.0	-1.352	-1.061	-0.904	-0.755	-0.708
	2.5	-1.166	-0.919	-0.816	-0.604	-0.541
	3.0	-0.934	-0.740	-0.632	-0.577	-0.402
	3.1	-0.908	-0.722	-0.626	-0.573	-0.387

Molar solvated volume ϕ_s of ammonium bromide salt in water.

System	Temp. Molality	ϕ_s (*10 ⁶) m ³ /mole				
		35°C	40°C	45°C	50°C	55°C
Water + ammonium bromide	0.5	90.950	88.832	88.842	85.686	81.825
	1.0	75.665	73.750	70.887	69.940	67.719
	1.5	70.112	67.415	65.689	65.107	63.630
	2.0	65.685	62.836	61.231	59.929	59.396
	2.5	62.748	60.356	59.174	57.405	56.738
	3.0	59.490	57.748	56.572	56.428	54.580
	3.1	59.268	57.663	56.441	56.422	54.310

hydration number values [19]. Regarding temperature, a slight decrease in molar hydration number may be due to the disruption of the hydration sphere around ions.

The computed values of the above parameters for ammonium bromide solutions are provided in Table 2 and Fig. 2. Apparent molal volume is observed to increase with ammonium bromide at all temperatures in the aqueous solutions. The rate of change of ϕ_v with concentration is more pronounced at lower concentrations than at higher concentrations. Concerning temperature, the values of

ϕ_v increase in the case of ammonium bromide solutions. Apparent molal compressibility values are negative, and this parameter increases with both concentration and temperature. Molar solvated volume decreases with increases in concentration and temperature, mirroring the trends seen with the hydration number. It is evident that the hydration number significantly influences interactions and, consequently, the value of ϕ_s [20]. In ammonium bromide solutions, the hydration number has a more substantial impact on the molar solvated volume than the apparent molal volume. Thus, molality affects the three allied parameters-apparent molal volume, apparent molal compressibility, and molar solvated volume-predominantly at lower concentrations, while temperature has a minimal effect on these values.

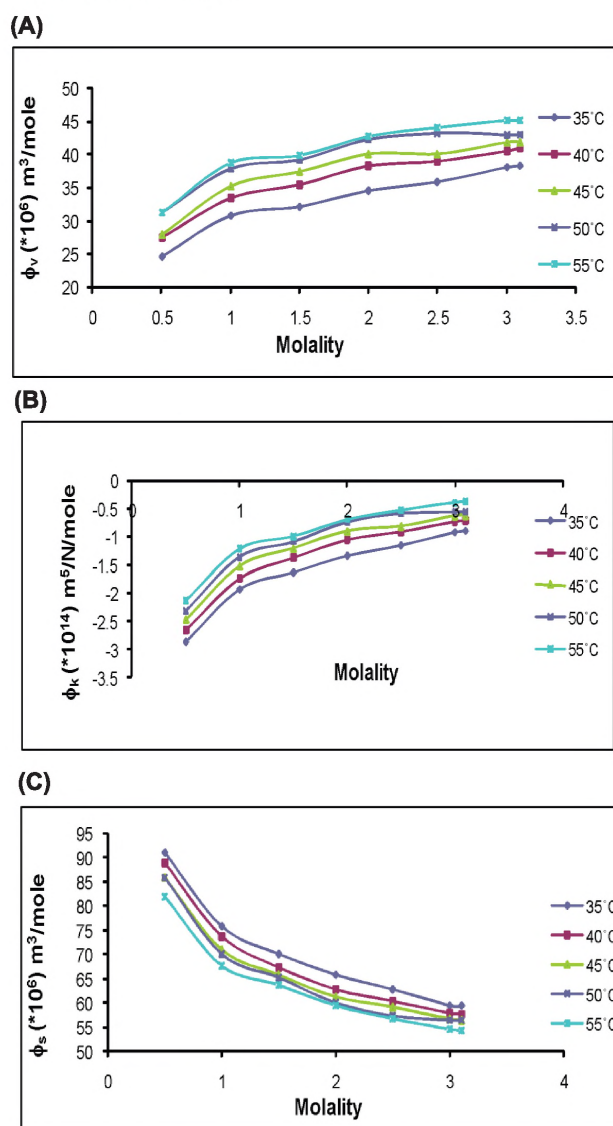


Fig. 2. (A) Apparent molal volume, (B) apparent molal compressibility, and (C) molar solvated volume of water + ammonium bromide.

Table 3. Variation of allied parameters due to hydration with molality at 35°C for water + ammonium bromide.

	Apparent molal volume ϕ_v ($\ast 10^6$) m ³ /mole				Apparent molal compressibility ϕ_k ($\ast 10^{14}$) m ⁵ /N/mole				Molar solvated volume ϕ_s ($\ast 10^6$) m ³ /mole			
Molality	Equations											
	4	5	6	7	8	9	10	11	12	13	14	15
0.5	24.585	23.902	24.78	24.574	-2.874	-2.904	-2.907	-2.875	91.707	90.950	91.713	90.950
1.0	30.833	30.362	31.210	30.838	-1.942	-1.962	-1.980	-1.942	76.579	75.665	76.574	75.665
1.5	32.143	32.058	32.211	32.143	-1.645	-1.648	-1.651	-1.645	70.262	70.112	70.262	70.113
2.0	34.465	34.879	34.538	34.461	-1.352	-1.334	-1.358	-1.352	65.902	65.685	65.834	65.685
2.5	35.836	35.566	36.071	35.840	-1.166	-1.177	-1.182	-1.166	63.136	62.748	63.152	62.748
3.0	37.928	37.745	37.291	37.925	-0.934	-0.942	-0.895	-0.934	58.393	59.490	58.496	59.490
3.1	38.307	37.523	37.974	38.305	-0.908	-0.942	-0.888	-0.908	58.567	59.268	58.756	59.269

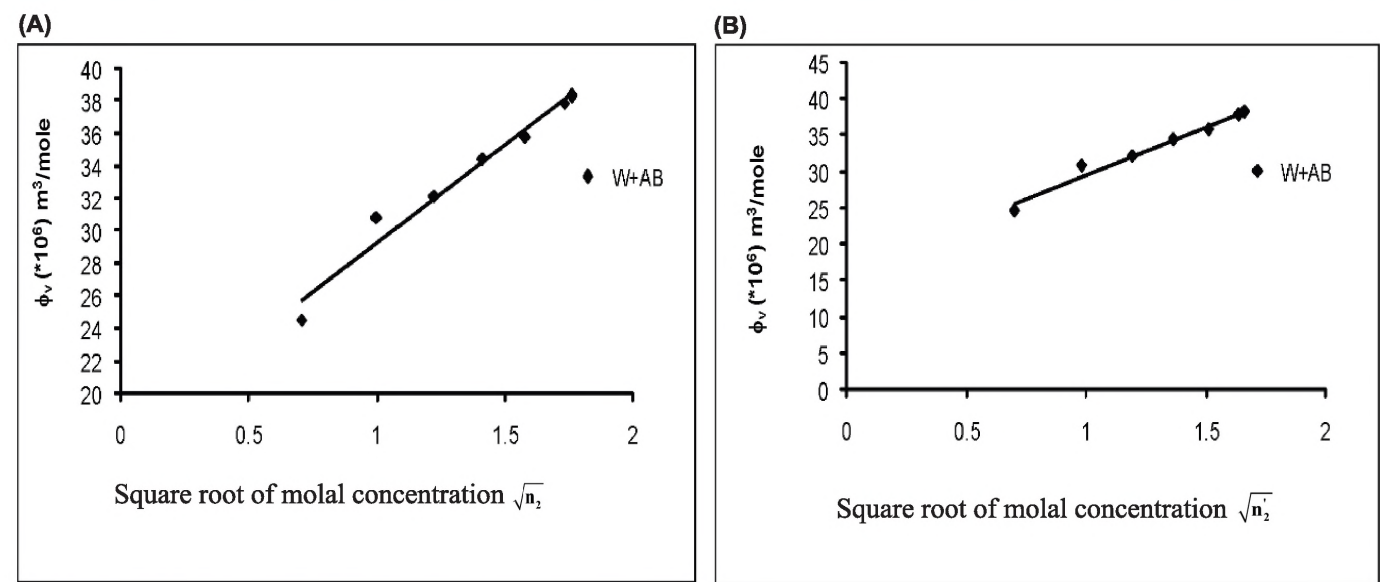


Fig. 3. (A) $\sqrt{n_2}$ and (B) $\sqrt{n_2}$ vs. ϕ_v in water at 35°C.

Validity of three equations for ϕ_v , ϕ_k and ϕ_s from hydration studies: From the hydration studies, the three parameters ϕ_v , ϕ_k and ϕ_s are calculated using three sets of equations and their values are compared with those obtained by traditional equations. This comparison, for values at 35°C, is presented in Table 3. It is evident from the table that the values calculated from the three equations are in good agreement with those obtained from the traditional equations. This concordance suggests that the assumptions made by Passynski, Barnartt, and the authors are valid, thereby affirming the correctness of the methodology adopted for deriving these equations.

Variation of three allied parameters ϕ_v , ϕ_k and ϕ_s parameters with concentration: The variation of the three allied parameters ϕ_v , ϕ_k and ϕ_s with the square root of molar and molal concentration ($\sqrt{n_2}$ & $\sqrt{n_2}$) are found to be linear. The variation of ϕ_v , ϕ_k and ϕ_s and molar solvated volume with concentration are studied and the corresponding plots of ϕ_v vs. $\sqrt{n_2}$ and $\sqrt{n_2}$, ϕ_k vs. $\sqrt{n_2}$ and $\sqrt{n_2}$, ϕ_s vs. $\sqrt{n_2}$ and $\sqrt{n_2}$ are constructed at 35°C and presented in Figs. 3 to 5. The values of slope, intercept, and correlation coefficient at 35°C in molar and molal scale for the three allied parameters are given in Table 4. The correlation coefficient is found to be 0.99 suggesting that the linear equations provide the best fit, thus indicating their appropriateness for modelling these relationships.

Table 4. Values of slope, intercept and correlation co-efficient in molar and molal scale for the three allied parameters.

Values of n_{hv} , ϕ_v^0 , n'_{hv} , $\phi_v^{0'}$ and correlation co-efficient r at 35°C.

System	Molar scale			Molal scale		
	Slope n_{hv} (*10 ⁶ m ³ /M ²)	Intercept ϕ_v^0 (*10 ⁶ m ³ /mole)	Correlation co-efficient r	Slope n'_{hv} (*10 ⁶ m ³ /M ²)	Intercept $\phi_v^{0'}$ (*10 ⁶ m ³ /mole)	Correlation co-efficient r
Water + ammonium bromide	13.255	016.308	0.987	12.0242	017.260	0.985

Values of n_{hk} , ϕ_k^0 , n'_{hk} , $\phi_k^{0'}$ and correlation co-efficient r at 35°C.

System	Molar scale			Molal scale		
	Slope n_{hk} (*10 ¹⁴ m ⁵ /N/M ²)	Intercept ϕ_k^0 (*10 ¹⁴ m ⁵ /N/mole)	Correlation co-efficient r	Slope n'_{hk} (*10 ¹⁴ m ⁵ /N/M ²)	Intercept $\phi_k^{0'}$ (*10 ¹⁴ m ⁵ /N/mole)	Correlation co-efficient r
Water + ammonium bromide	01.915	-04.022	0.983	01.734	-03.880	0.980

Values of n_{hs} , ϕ_s^0 , n'_{hs} , $\phi_s^{0'}$ and correlation co-efficient r at 35°C.

System	Molar scale			Molal scale		
	Slope n_{hs} (*10 ³ m ³ /M ²)	Intercept ϕ_s^0 (*10 ⁶ m ³ /mole)	Correlation co-efficient r	Slope n'_{hs} (*10 ³ m ³ /M ²)	Intercept $\phi_s^{0'}$ (*10 ⁶ m ³ /mole)	Correlation co-efficient r
Water + ammonium bromide	-030.962	109.151	-0.979	-028.009	106.823	-0.975

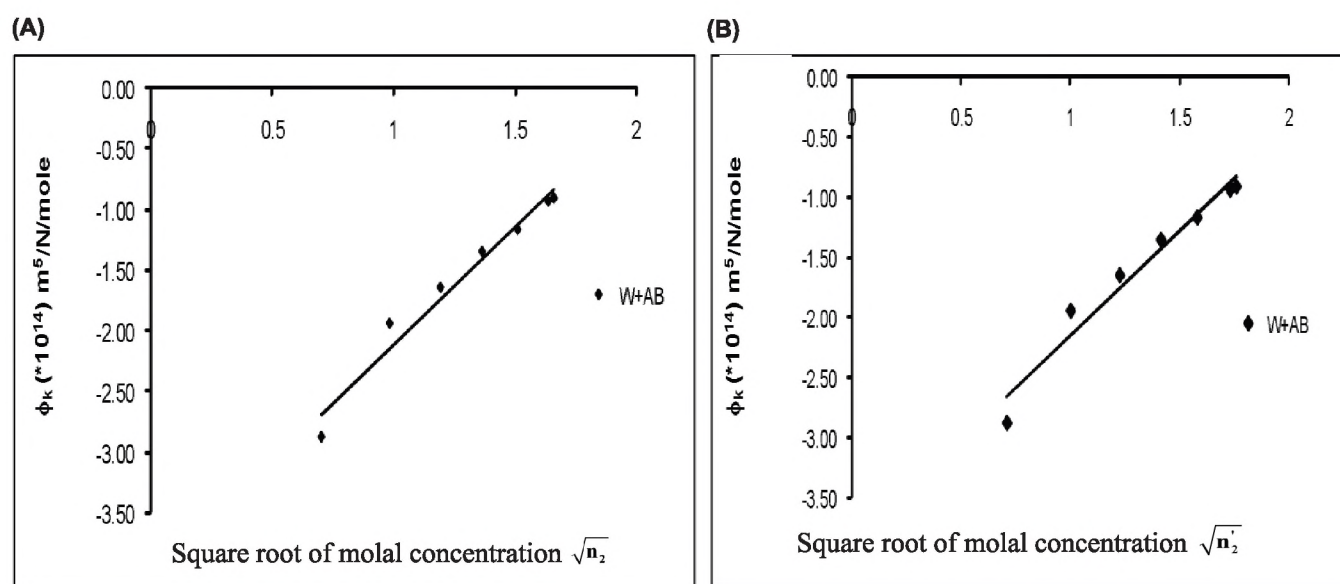


Fig. 4. (A) $\sqrt{n_2}$, (B) $\sqrt{n_2}$ vs. ϕ_k in water at 35°C.

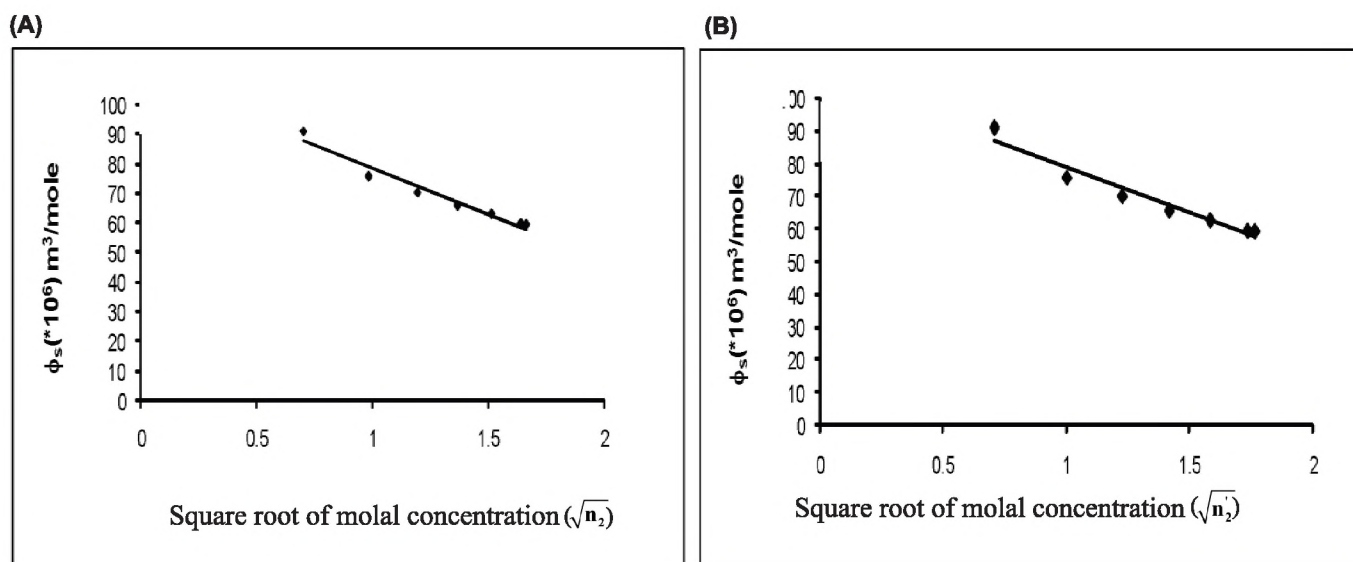


Fig. 5. (A) $\sqrt{n_2}$, (B) $\sqrt{n_2}$ vs. ϕ_s in water at 35°C.

5. Conclusions

The hydration number and some related allied parameters for ammonium bromide at various temperatures are reported. Variations in adiabatic compressibility and related parameters against concentration and temperature reveal the existence of molecular association among solute and solvent molecules. The results of the hydration number and their allied parameters indicate the molecular interactions taking place in the solution, and the trends of these parameters are in good agreement with the structure-promoting behaviour of the solutes.

CRediT author statement

J.H. Rakini Chandrasekaran: Conceptualisation, Data curation, Investigation; S. Nithiyanantham: Methodology, Formal analysis, Original draft preparation, Visualisation.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] D. Feakins, F.M. Bates, W.E. Waggoner, et al. (1993), "Relative viscosities and quasi-thermodynamics of solutions of tert-butyl alcohol in the methanol-water system: A different view of the alkyl-water interaction", *J. Chem. Soc. Faraday Trans*, **89**, pp.3381-3388, DOI: 10.1039/FT993890 3381.
- [2] L. Palaniappan, S. Nithiyanantham (2020), "Acoustical and physico-chemical study of binary azeotropes (aniline)", *Journal of Molecular Liquids*, **312**, DOI: 10.1016/j.molliq.2020.113423.

- [3] S. Yella, M.S. Dougherty, N.K. Gupta (2006), "Artificial intelligence techniques for the automatic interpretation of data from non-destructive testing", *Insight-Non-Destructive Testing and Condition Monitoring*, **48(1)**, pp.10-20, DOI: 10.1784/insi.2006.48.1.10.

- [4] A.P. Mishra, S.K. Gautam (2001), "Viscometric and volumetric studies of some transition metal chlorides in glycine water solution", *Indian J. Chem.*, **40A**, pp.100-104.

- [5] S. Kalyanasundaram, S. Saraswathi (1993), "Determination of apparent molal volume through ultrasonic velocity measurements", *Indian Journal of Pure & Applied Physics*, **31(12)**, pp.936-941.

- [6] A. Pal, R. Gaba (2008), "Densities, excess molar volumes, speeds of sound and isothermal compressibilities for {2-(2-hexyloxyethoxy) ethanol n-alkanol} systems at temperatures between (288.15 and 308.15)", *K. J. Chem. Thermodyn.*, **40**, pp.750-758, DOI: 10.1016/j.jct.2008.01.015.

- [7] J.H.R. Chandrasekaran, S. Nithiyanantham (2022), "Study of water and water-ethanol solutions of potassium salts through ultrasonic and spectroscopic approach", *Chem. Africa*, **5(5)**, pp.1397-1415, DOI: 10.1007/s42250-022-00412-6.

- [8] S. Nithiyanantham, L. Palaniappan (2012), "Thermodynamic studies of lactose with amylase in aqueous media at 298.15 K", *J. Comput. Theor. Nanosci.*, **9(12)**, pp.2193-2197, DOI: 10.1166/jctn.2012.2638.

- [9] X.L. Wu, X.Y. Sang, Z.M. Li, et al. (2020), "Study on physicochemical properties and basicity of carbanion-functionalised ionic liquids", *J. Mol. Liq.*, **312**, DOI: 10.1016/j.molliq.2020.113405.

- [10] A. Kaur, N. Kaur, G.R. Chaudhary (2020), “Volumetric and acoustic approach for investigating molecular interactions of choline acetate ionic liquid in a, &-alkanediols at different temperatures”, *J. Mol. Liq.*, **312**, DOI: 10.1016/j.molliq.2020.113330.
- [11] P. Verdía, M. Hernaiz, E.J. González, et al. (2014), “Effect of the number, position and length of alkyl chains on the physical properties of polysubstituted pyridinium ionic liquids”, *J. Chem. Thermodyn.*, **69**, pp.19-26, DOI: 10.1016/j.jct.2013.09.028.
- [12] B. Zhang, Q. Daniel, L. Fan, et al. (2018), “Identifying MnVII-oxo species during electrochemical water oxidation by manganese oxide”, *iScience*, **4**, pp.144-152, DOI: 10.1016/j.isci.2018.05.018.
- [13] K. Rajagopal, S. Jayabalakrishnan (2009), “Volumetric and viscometric studies of 4-aminobutyric acid in aqueous solutions of salbutamol sulphate at 308.15, 313.15 and 318.15 K”, *Chin. J. Chem. Eng.*, **17(5)**, pp.796-804, DOI: 10.1016/S1004-9541(08)60279-7.
- [14] F. Yang, Q. Ma, X. Wang, et al. (2017), “Influence of aprotic cosolvents on the thermophysical properties of imidazolium-based ionic liquid”, *J. Chem. Eng. Data*, **62(5)**, pp.1628-1638, DOI: 10.1021/acs.jced.7b00002.
- [15] S. Sarmad, M.T.Z. Moattar, D. Nikjoo, et al. (2020), “How different electrolytes can influence the aqueous solution behavior of 1-ethyl-3-methylimidazolium chloride: A volumetric, viscometric, and infrared spectroscopy approach”, *Front. Chem.*, **8**, DOI: 10.3389/fchem.2020.593786.
- [16] M.T.Z. Moattar, S. Sarmad (2012), “Apparent molar volumes, apparent isentropic compressibilities, and viscosity B-coefficients of 1-ethyl-3-methylimidazolium bromide in aqueous di-potassium hydrogen phosphate and potassium di-hydrogen phosphate solutions at T=(298.15, 303.15, 308.15, 313.15, and 318.15) K”, *J. Chem. Thermodyn.*, **54**, pp.192-203, DOI: 10.1016/j.jct.2012.03.032.
- [17] R. Sadeghi, A. Mahdavi (2012), “(Vapour liquid) equilibria, volumetric and compressibility behaviour of binary and ternary aqueous solutions of 1-hexyl-3-methylimidazolium chloride, methyl potassium malonate, and ethyl potassium malonate”, *J. Chem. Thermodyn.*, **47**, pp.347-357, DOI: 10.1016/j.jct.2011.11.014.
- [18] H. Kumar, G. Singh, R. Kataria, et al. (2020), “Volumetric, acoustic and infrared spectroscopic study of amino acids in aqueous solutions of pyrrolidinium based ionic liquid, 1-butyl-1-methyl pyrrolidinium bromide”, *J. Mol. Liq.*, **303**, DOI: 10.1016/j.molliq.2020.112592.
- [19] S. Nithiyanantham (2020), “Ultrasonic velocity models in liquids (micro- and nanofluids): Theoretical validations”, *International Nano Letters*, **9**, pp.89-97, DOI: 10.1007/s40089-019-0269-3.
- [20] M.T.Z. Moattar, H. Shekaari, P. Jafari (2020), “Volumetric, acoustic and viscometric investigation of some choline amino acid ionic liquids in aqueous solutions of polypropylene glycol 400 and polyethylene glycol 400”, *J. Chem. Thermodyn.*, **142**, DOI: 10.1016/j.jct.2019.106019.

Analysis of a novel 3C-SiC thin layer on silicon diaphragms for enhanced stress amplification in MEMS piezoresistive pressure sensors

Nguyen Chi Cuong*, Trinh Xuan Thang, Lam Minh Thinh, Vuong Dinh Duy Phuc,
Truong Huu Ly, Ngo Vo Ke Thanh, Le Quoc Cuong

Research Laboratories of Saigon High-Tech-Park, Lot 13, N2 Street, Saigon High-Tech-Park, District 9, Ho Chi Minh City, Vietnam

Received 24 February 2023; revised 13 April 2023; accepted 7 July 2023

Abstract:

This study analyses the square diaphragm structure of a micro-electro-mechanical system (MEMS) pressure sensor using the finite element method (FEM). The research investigates an enhancement in stress distribution achieved by coating a silicon (Si) diaphragm with a thin layer of silicon carbide (3C-SiC). Applying a thin layer of 3C-SiC material onto a Si diaphragm enhances the stress distribution of MEMS pressure sensors. The design structure of the square diaphragm of a MEMS pressure sensor is simulated and analysed using 3D FEM in COMSOL multiphysics. Four piezo-resistors are configured in a Wheatstone bridge to translate resistance variations into an output voltage. Then, the stress distributions are calculated by solving the 3D structures of MEMS pressure sensors in the stationary solver of the solid mechanics module in COMSOL multiphysics. The findings indicate a significantly higher stress distribution in the 3C-SiC layer compared to a solely silicon diaphragm. Consequently, it is posited that the stress distribution in MEMS pressure sensors could be considerably augmented with increasing pressure, increasing diaphragm length, and decreasing diaphragm thickness. Also, the output voltage of more than 20 mV can be achievable with this design for using the thin layer of 3C-SiC material on a Si diaphragm in designing MEMS piezoresistive pressure sensors.

Keywords: micro-electro-mechanical system pressure sensors, silicon, silicon carbide, stress.

Classification numbers: 2.1, 2.3

1. Introduction

Pressure sensors are extensively utilised in various technical applications including medical [1], automotive [2], wearable devices [3], and aerospace sectors [4]. Micro-electro-mechanical systems (MEMS) pressure sensors offer numerous advantages, such as compact size, reduced power consumption, high sensitivity, and linearity [1]. Based on their working principles, pressure sensors can be categorised into piezoresistive [5-7], capacitive [8], optical fibre [9], resonant [10], and piezoelectric types [11]. For the enhancement of stress amplification, sensitivity, and linearity in MEMS pressure sensors, piezoresistivity [5, 6] is favoured. This involves converting the applied pressure signal into an electrical output signal, i.e., resistance, to measure the stress states in materials. An increase in material stress amplification leads to heightened sensitivity in MEMS pressure sensors.

Various electrical materials' piezoresistive properties are explored to measure stress states in materials [12]. Literature reviews suggest that conventional piezoresistive sensors, constructed using metal films (such as Au [13], Cu [14], Mn [15]), alloy films (like Bi-Sb [16]), and cermet films (such as Au-glass [17], RuO₂ [18]), exhibit limited

sensitivity, substantial temperature dependence, and high power consumption. Consequently, metal strain sensors have been substituted by semiconducting materials like silicon (Si) [19, 20], polysilicon (Poly-Si) [21], silicon carbide (3C-SiC) [22, 23], graphene [24], and diamond [25], enhancing the stress distribution and sensitivity of MEMS piezoresistive pressure sensors. Silicon carbide (3C-SiC) is particularly suited for MEMS piezoresistive pressure sensors in harsh environmental conditions due to its wide bandgap, corrosion resistance, excellent chemical inertness, and high Young's modulus. Recently, MEMS silicon carbide (SiC) pressure sensors have been operational under high pressure and high-temperature conditions, where conventional silicon sensors are ineffective [26-29]. The use of square diaphragms made of SiC materials, which show greater stress distribution, can lead to higher sensitivity in MEMS piezoresistive pressure sensors [30, 31]. However, the combination of a thin 3C-SiC layer on a Si diaphragm (3C-SiC/Si wafer) has not been previously explored for enhancing the stress distribution in MEMS piezoresistive pressure sensors.

This study investigates the application of a 3C-SiC layer on a Si diaphragm to achieve higher stress amplification

*Corresponding author: Email: cuong.nguyenchi@shiplabs.org

and robust performance in MEMS piezoresistive pressure sensors. A square-shaped diaphragm of a MEMS pressure sensor is modelled using the finite element method (FEM) in COMSOL multiphysics [32] to calculate its stress states. A thin layer of silicon carbide (3C-SiC) is applied atop a (100) plane Si wafer to augment the diaphragm's stress. Subsequently, four piezoresistors, configured in a Wheatstone bridge, are employed to translate resistance variations into the output voltage. The resulting output voltage from the 3C-SiC on the Si square diaphragm is then evaluated across a broad spectrum of pressure (p) and diaphragm dimensions.

2. Materials and methods

2.1. Stress-strain relations for single crystal Si and SiC materials

To examine the linear elastic behaviour of orthotropic and homogeneous materials, such as silicon (Si) and silicon carbide (SiC), it is essential to understand how an external force acting upon a solid body generates internal forces that cause deformation, stress, and strain. Fig. 1 illustrates the stress components within a small segment of a solid body.

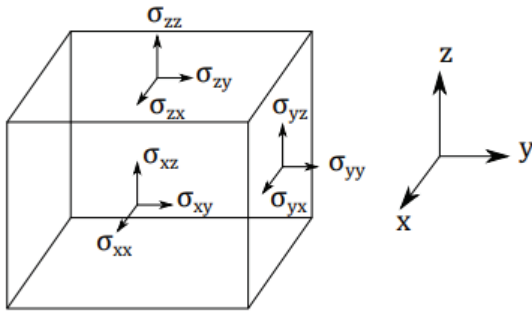


Fig. 1. Stress components in a small cubic element of a material. Variables σ_{xx} , σ_{yy} , σ_{zz} are the normal stress components in the x , y , and z direction, respectively. Variables σ_{xy} , σ_{xz} , σ_{yz} represent the shear stress components in the x , y , and z direction, respectively.

A linear relationship between stress and strain is established by Hooke's Law [33], as shown in Eq. (1):

$$\begin{bmatrix} \sigma_{xx} \\ \sigma_{yy} \\ \sigma_{zz} \\ \sigma_{yz} \\ \sigma_{xz} \\ \sigma_{xy} \end{bmatrix} = \hat{E} \begin{bmatrix} 1-\nu & \nu & \nu & 0 & 0 & 0 \\ \nu & 1-\nu & \nu & 0 & 0 & 0 \\ \nu & \nu & 1-\nu & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1-2\nu}{2} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1-2\nu}{2} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1-2\nu}{2} \end{bmatrix} \begin{bmatrix} \epsilon_{xx} \\ \epsilon_{yy} \\ \epsilon_{zz} \\ 2\epsilon_{yz} \\ 2\epsilon_{xz} \\ 2\epsilon_{xy} \end{bmatrix} - \frac{E\alpha\Delta T}{1-2\nu} \begin{bmatrix} 1 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{bmatrix} \quad (1)$$

where $\hat{E} = \frac{E}{(1+\nu)(1-2\nu)}$, E and ν are Young's modulus and Poisson's ratio of the material, respectively, α is the

coefficient of thermal expansion, and ΔT is the change in temperature of the solid, ϵ_{xx} , ϵ_{yy} , ϵ_{zz} are the normal strain components in the x , y , and z directions, respectively, and ϵ_{xy} , ϵ_{xz} , ϵ_{yz} are the shear strain components in x , y , and z directions, respectively.

Considering that a thin plate is only subjected to load forces in the x - y directions, the plane stress assumption ($\sigma_{yz} = \sigma_{xz} = \sigma_{zz} = 0$) is applied. Therefore, the stress components in Eq. (1) are simplified as shown in Eq. (2):

$$\begin{bmatrix} \sigma_{xx} \\ \sigma_{yy} \\ \sigma_{xy} \end{bmatrix} = \frac{E}{1-\nu^2} \begin{bmatrix} 1 & \nu & 0 \\ \nu & 1 & 0 \\ 0 & 0 & \frac{1-\nu}{2} \end{bmatrix} \begin{bmatrix} \epsilon_{xx} \\ \epsilon_{yy} \\ 2\epsilon_{xy} \end{bmatrix} - \frac{E\alpha\Delta T}{1-\nu} \begin{bmatrix} 1 \\ 1 \\ 0 \end{bmatrix} \quad (2)$$

Si and SiC are known to be anisotropic materials [34-36]. Thus, the relationship between stress and strain for these materials, considering their anisotropic coefficients, can be generally expressed as shown in Eq. (3):

$$\begin{bmatrix} \sigma_{xx} \\ \sigma_{yy} \\ \sigma_{zz} \\ \sigma_{yz} \\ \sigma_{xz} \\ \sigma_{xy} \end{bmatrix} = \begin{bmatrix} C_{11} & C_{12} & C_{12} & 0 & 0 & 0 \\ C_{12} & C_{11} & C_{12} & 0 & 0 & 0 \\ C_{12} & C_{12} & C_{11} & 0 & 0 & 0 \\ 0 & 0 & 0 & C_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & C_{44} & 0 \\ 0 & 0 & 0 & 0 & 0 & C_{44} \end{bmatrix} \begin{bmatrix} \epsilon_{xx} \\ \epsilon_{yy} \\ \epsilon_{zz} \\ \epsilon_{yz} \\ \epsilon_{xz} \\ \epsilon_{xy} \end{bmatrix} \quad (3)$$

where C_{11} , C_{12} , and C_{44} are components in a 6x6 matrix, often referred to as the coefficients of elasticity for anisotropic materials.

The specific stress-strain relationship for anisotropic Si in the $\langle 100 \rangle$ direction on a (100) plane Si wafer is expressed in Eq. (4) [33]:

$$\begin{bmatrix} \sigma_{xx} \\ \sigma_{yy} \\ \sigma_{zz} \\ \sigma_{yz} \\ \sigma_{xz} \\ \sigma_{xy} \end{bmatrix} = \begin{bmatrix} 165.7 & 63.9 & 63.9 & 0 & 0 & 0 \\ 63.9 & 165.7 & 63.9 & 0 & 0 & 0 \\ 63.9 & 63.9 & 165.7 & 0 & 0 & 0 \\ 0 & 0 & 0 & 79.6 & 0 & 0 \\ 0 & 0 & 0 & 0 & 79.6 & 0 \\ 0 & 0 & 0 & 0 & 0 & 79.6 \end{bmatrix} \begin{bmatrix} \epsilon_{xx} \\ \epsilon_{yy} \\ \epsilon_{zz} \\ \epsilon_{yz} \\ \epsilon_{xz} \\ \epsilon_{xy} \end{bmatrix} \quad [\text{GPa}] \quad (4)$$

Similarly, for anisotropic 3C-SiC in the $\langle 100 \rangle$ direction on a (100) plane Si wafer, the relationship is given in Eq. (5) [36, 37]:

$$\begin{bmatrix} \sigma_{xx} \\ \sigma_{yy} \\ \sigma_{zz} \\ \sigma_{yz} \\ \sigma_{xz} \\ \sigma_{xy} \end{bmatrix} = \begin{bmatrix} 395 & 132 & 132 & 0 & 0 & 0 \\ 132 & 395 & 132 & 0 & 0 & 0 \\ 132 & 132 & 395 & 0 & 0 & 0 \\ 0 & 0 & 0 & 236 & 0 & 0 \\ 0 & 0 & 0 & 0 & 236 & 0 \\ 0 & 0 & 0 & 0 & 0 & 236 \end{bmatrix} \begin{bmatrix} \epsilon_{xx} \\ \epsilon_{yy} \\ \epsilon_{zz} \\ \epsilon_{yz} \\ \epsilon_{xz} \\ \epsilon_{xy} \end{bmatrix} \quad [\text{GPa}] \quad (5)$$

Table 1 lists the Young's modulus (E), shear modulus (G), Poisson's ratio (ν), and mass density (ρ) of Si and 3C-SiC materials in the (100) plane Si wafer. These values are incorporated in the finite element method (FEM) in COMSOL multiphysics [32] for calculating the stress and strain of Si and 3C-SiC diaphragms in MEMS pressure sensors.

Table 1. The mechanical properties of silicon (Si) and silicon carbide (SiC) in the <100> orientation on the (100) plane of an Si wafer [35-37].

Materials	Orientation	Young's modulus (GPa)	Shear modulus (GPa)	Poisson's ratio	Mass density (kg/m ³)
Si	<100>	129.8	79.5	0.278	2330
3C-SiC	<100>	395	236	0.25	3210

For calculating stress and strain in homogeneous anisotropic materials like Si and 3C-SiC, solutions to the system equations of Eq. (1) are provided for 3D problems without body force, applied load, and boundary conditions. However, to simulate the complex structure of MEMS pressure sensors, FEM is required, incorporating specific boundary conditions such as applied pressure (p) and clamped boundaries. After solving, the stress and strain distributions in MEMS pressure sensors can be evaluated.

2.2. Piezoresistive effect for single crystal Si and SiC materials

Figure 2 demonstrates the alignment of all piezoresistors in the n-type SiC/p-type Si diaphragm. These are arranged in a

rotated square diaphragm at 45° for the <100> crystallographic orientation, which has the highest piezoresistive gauge factors for n-type SiC [36]. The change in resistivity of the piezoresistive sensing element is given by:

$$\frac{\Delta\rho}{\rho} = \frac{\Delta R}{R} = \pi_l \sigma_l + \pi_t \sigma_t = \pi_{11} \sigma_l + \pi_{12} \sigma_t \quad (6)$$

where ρ is the resistivity of the material, R is the resistance of the material, σ_l and σ_t are the longitudinal and transverse stresses in the piezoresistors, respectively, π_l and π_t are the longitudinal and transverse piezoresistive coefficients, respectively, and π_{11} and π_{12} are the fundamental piezoresistive coefficients of the single crystal 3C-SiC and Si materials [33, 34].

Table 2. The piezoresistive coefficients (π_{11} , π_{12} , π_{44}) in the <100> direction of (100) silicon (Si) [35].

Resistivity, ρ	π_{11} (10 ⁻¹¹ /Pa)	π_{12} (10 ⁻¹¹ /Pa)	π_{44} (10 ⁻¹¹ /Pa)
p-Si (7.8 Ω.cm)	6.6	-1.1	-138.1
n-Si (11.7 Ω.cm)	-102.2	53.4	-13.6

Table 3. The piezoresistive coefficients (π_{11} , π_{12} , π_{44}) in direction <100> of (100) silicon carbide (3C-SiC) [34, 35].

Resistivity, ρ	π_{11} (10 ⁻¹¹ /Pa)	π_{12} (10 ⁻¹¹ /Pa)	π_{44} (10 ⁻¹¹ /Pa)
p-3C-SiC (0.14 Ω.cm)	1.5	-1.4	18.1
n-3C-SiC (0.7 Ω.cm)	-9.6	5.8	1.6

For the design of the sensing element, the opposing signs of π_{11} and π_{12} , as presented in Tables 2 and 3, suggest that it is advantageous to maximise the longitudinal stress (σ_l) while minimising the transverse stress (σ_t). This approach facilitates a more significant change in the resistance (ΔR) of the piezoresistors. Consequently, the sensing elements are subjected to a simple uniaxial loading beam along the longitudinal axis. Under these conditions, only the longitudinal stress component σ_l is considered, and the transverse stress component σ_t is disregarded. Thus, Eq. (6) can be simplified to Eq. (7):

$$\frac{\Delta\rho}{\rho} = \frac{\Delta R}{R} = \pi_l \sigma_l = \pi_{11} \sigma_l \quad (7)$$

This simplification underscores that the resistance of the sensing elements will increase under compressive stress (indicated by a positive sign) and decrease under tensile stress (indicated by a negative sign). This relationship between stress and resistance change is crucial for the effective functioning of the sensing elements in piezoresistive applications.

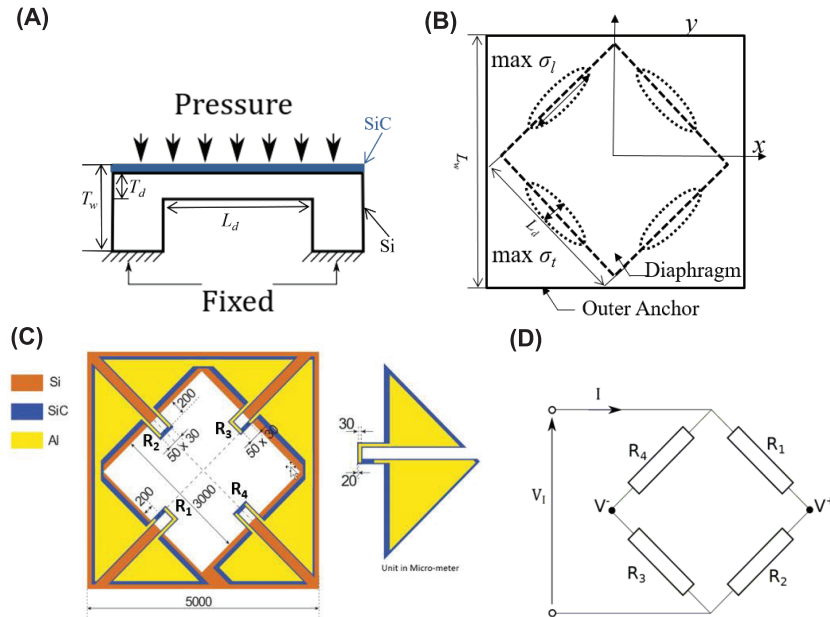


Fig. 2. (A) Schematic of the working principle of the device; (B) top-down view of maximum distributed stresses; (C) arrangement of the four piezoresistors; and (D) circuit diagram of resistors in full Wheatstone bridge forming the square diaphragm of the MEMS piezoresistive pressure sensors.

2.3. Sensor design and working principles

As depicted in Fig. 2A, a thin square diaphragm is supported by a thick Si substrate. A rotated square diaphragm of 45 degrees is proposed to maximise sensitivity, designed for the <100> direction on a (100) plane n-type 3C-SiC/p-type Si wafer. Additionally, a thin layer of 3C-SiC (approximately 500 nm) is applied on top of the Si diaphragm to enhance its stress distribution, as shown in Fig. 2A. Upon the application of pressure (p) to the diaphragm, stress concentrates at the corners of the thin SiC on Si diaphragm. This results in maximum distributed stresses (σ_l and σ_t) along the edges of the diaphragm and the substrate of the MEMS pressure sensor, as illustrated in Fig. 2B. Fig. 2C displays the placement of four piezoresistors (R_1, R_2, R_3, R_4) at these points of maximum stress on the diaphragm. When pressure is applied to the sensor diaphragm, the pairs of piezoresistors (R_1, R_3) and (R_2, R_4) undergo an equal and opposite change in resistance [35]. Subsequently, the piezoresistors R_1, R_2, R_3 , and R_4 are interconnected in a Wheatstone bridge formation, as shown in Fig. 2D, to transform the resistance changes into an output voltage. The output voltage (V_{out}) of the Wheatstone bridge, resulting from bridge unbalance, is given by Eq. (8):

$$V_{out} = V^+ - V^- = \frac{R_1 R_3 - R_2 R_4}{(R_1 + R_2)(R_3 + R_4)} V_I \quad (8)$$

where V_{out} is the output voltage and V_I is the source voltage.

When R_1 and R_3 experience stress, their relative change in resistance is given by:

$$\frac{\Delta R_1}{R_1} = \frac{\Delta R_3}{R_3} = \pi_l \sigma_{l_1} + \pi_t \sigma_{t_1} = 0 + \pi_l \sigma_{l_1} = \alpha_1 \quad (9)$$

where $\sigma_{l_1} (= \sigma_{xy})$ and $\sigma_{t_1} (\approx -\sigma_{xy})$ are the longitudinal and transverse stress components at the locations of R_1 or R_3 , respectively.

Similarly, the relative change in resistance for R_2 and R_4 is given by:

$$\frac{\Delta R_2}{R_2} = \frac{\Delta R_4}{R_4} = \pi_l \sigma_{l_2} + \pi_t \sigma_{t_2} = \pi_l \sigma_{l_2} + 0 = -\alpha_2 \quad (10)$$

where $\sigma_{l_2} (= \sigma_{xy})$ and $\sigma_{t_2} (\approx -\sigma_{xy})$ are the longitudinal and transverse stress components taken at the locations of R_2 or R_4 , respectively.

The relative change of voltage at the bridge output (or bridge sensitivity) can be rewritten as:

$$\frac{\Delta V}{V} = \frac{\alpha_1 + \alpha_2}{2 + \alpha_1 - \alpha_2} \quad (11)$$

Since the quantity $\alpha_1 - \alpha_2$ is sufficiently small, Eq. (11) can be simplified by

$$\frac{\Delta V}{V} \approx \frac{\alpha_1 + \alpha_2}{2} \quad (12)$$

2.4. Finite element simulation in COMSOL multiphysics

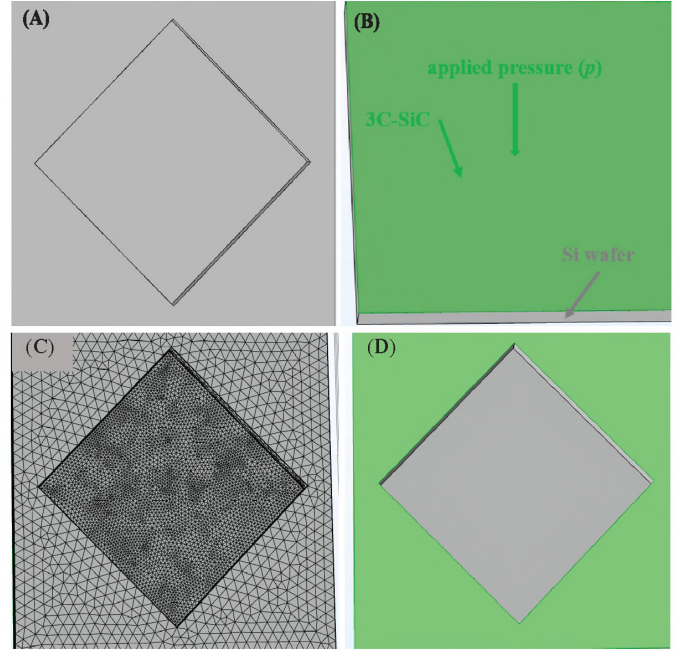


Fig. 3. (A) Proposed rotated square structures; (B) materials settings for silicon, silicon carbide (3C-SiC), and applied pressure; (C) mesh settings; and (D) clamping boundary conditions used for FEM in COMSOL multiphysics [32].

In Fig. 3, the finite element method (FEM) model in COMSOL multiphysics [32] is employed to calculate the stress and strain on the square diaphragm of MEMS pressure sensors. The simulated FEM model for the square structure involves four steps to determine the stress in the MEMS pressure sensor. Initially, a square structure of the MEMS pressure sensor is proposed, as shown in Fig. 3A. Subsequently, a thin layer of 3C-SiC material is applied to the top of a Si (100) wafer, as depicted in Fig. 3B. The anisotropic properties of Si and 3C-SiC materials, detailed in Table 1, along with the geometric conditions listed in Table 2, are incorporated. Additionally, pressure (p) is applied to create stress distribution on the thin diaphragm. In the mesh setting, a tetrahedral mesh density with 1,468,130 elements is employed throughout the sensor structure. An extremely high mesh density is specifically designated for the thin diaphragm, where stress concentration occurs, as illustrated in Fig. 3C. The fourth step involves setting a clamping boundary for the substrate, as shown in Fig. 3D. Ultimately, the stress distributions are obtained by solving the 3D structure of MEMS pressure sensors in the stationary solver of the solid mechanics module in COMSOL multiphysics [32].

3. Results and discussion

Figure 4 presents the stress distributions of square and circular diaphragm structures of MEMS pressure sensors, plotted using FEM in COMSOL multiphysics [32]. The results indicate that maximum stress concentrates along the corners of the thin diaphragm and the thick substrate. The stress distribution of the square diaphragm (see Fig. 4A) is higher than that of the circular diaphragm (see Fig. 4B) for MEMS pressure sensor diaphragms, as demonstrated in Table 4. Furthermore, the stress distributions of 3C-SiC on Si diaphragms are considerably higher than those of Si diaphragms alone, as indicated in Table

Table 5. Resultant von Mises stress (σ_{mises}) for a square diaphragm of a MEMS piezoresistive pressure sensor with Si only and Si with 3C-SiC.

Geometries	Materials	von Mises stress (σ_{mises})
Square Diaphragm	Si	$\sigma_{mises} \cong 38.279$ (MPa)
	Si with 3C-SiC	$\sigma_{mises} \cong 95.579$ (MPa)
Circular Diaphragm	Si	$\sigma_{mises} \cong 28.678$ (MPa)
	Si with 3C-SiC	$\sigma_{mises} \cong 81.560$ (MPa)

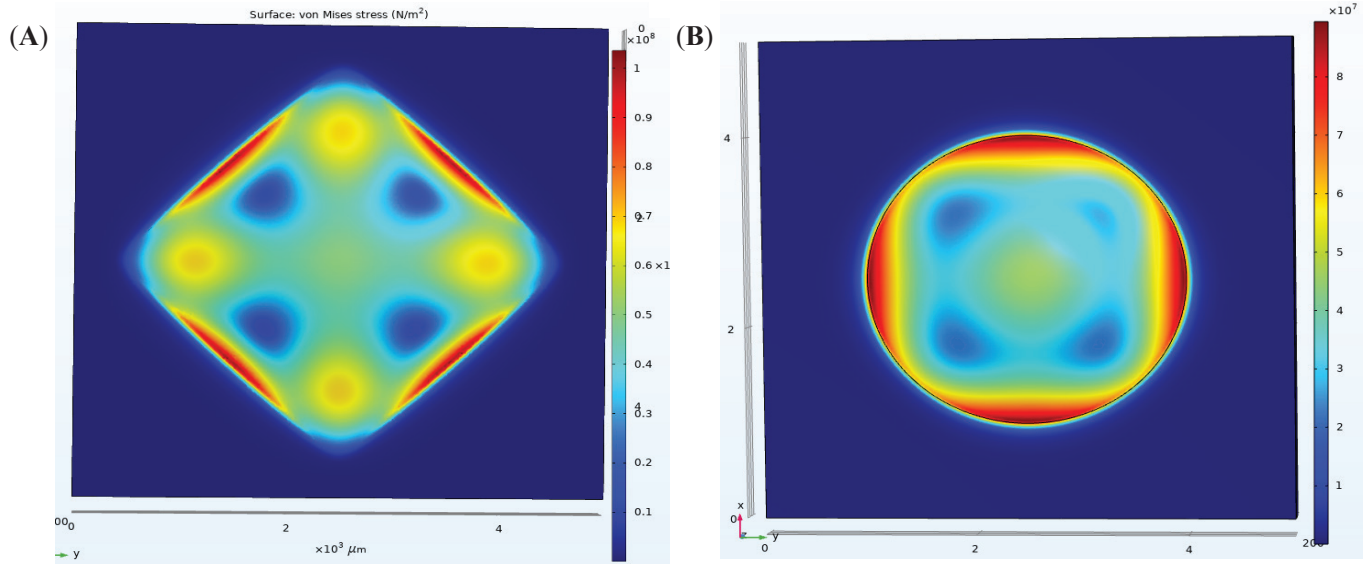


Fig. 4. The von Mises stress distribution (σ_{mises}) for (A) square and (B) circular diaphragms for MEMS pressure sensors.

Table 4. Basic geometric and operating conditions used in COMSOL multiphysics [32].

Parameters	Name	Value
Wafer length Si (100)	L_w	5000 μm
Wafer thickness Si (100)	T_w	380 μm
Diaphragm length Si (100)	L_d	3000 μm
Diaphragm thickness Si (100)	T_d	50 μm
3C-SiC layer thickness	T_{3C-SiC}	500 nm
Applied pressure	p	50 kPa
Young's modulus of (100) Si	E_{Si}	130 GPa
Poisson's ratio of (100) Si	ν_{Si}	0.28
Density of materials of (100) Si	ρ_{Si}	2330 kg/m ³
Young's modulus of 3C-SiC	E_{SiC}	394 GPa
Poisson's ratio of 3C-SiC	ν_{SiC}	0.25
Density of materials of 3C-SiC	ρ_{SiC}	3210 kg/m ³
Elastic limit of Si	$\sigma_{Si, limit}$	180 MPa
Strain limit of Si	$\epsilon_{Si, limit}$	1385 $\mu\epsilon$
Elastic limit of 3C-SiC	$\sigma_{SiC, limit}$	1245 MPa
Strain limit of 3C-SiC	$\epsilon_{SiC, limit}$	

5. Therefore, the principal stress distribution of the square structure of 3C-SiC on the Si diaphragm is investigated over a wide range of applied pressures (p) and diaphragm dimensions (i.e., length (L_d) and thickness (T_d)) to design for higher stress amplification in MEMS piezoresistive pressure sensors.

In Fig. 5, the principal stress (σ_{xy}) is plotted against the thickness (T_d) and length (L_d) of the diaphragm for varying applied pressures (p). Fig. 5A shows that the principal stress (σ_{xy}) increases significantly as the thickness (T_d) decreases. In Fig. 5B, the stress (σ_{xy}) increases as the length (L_d) increases and rises linearly with increasing pressure (p). Consequently, the stress distribution of MEMS pressure sensors can be significantly enhanced by increasing pressure, extending the length of the diaphragm, and reducing its thickness. Moreover, the elastic limit of 3C-SiC ($\sigma_{limit, SiC} = 1245$ MPa) is listed in Table 2. The observed stress (σ) levels remain within the elastic limit of 3C-SiC materials, ensuring the safe operation of MEMS pressure sensors. Thus, the stress distribution in MEMS pressure sensors can be considerably amplified across a

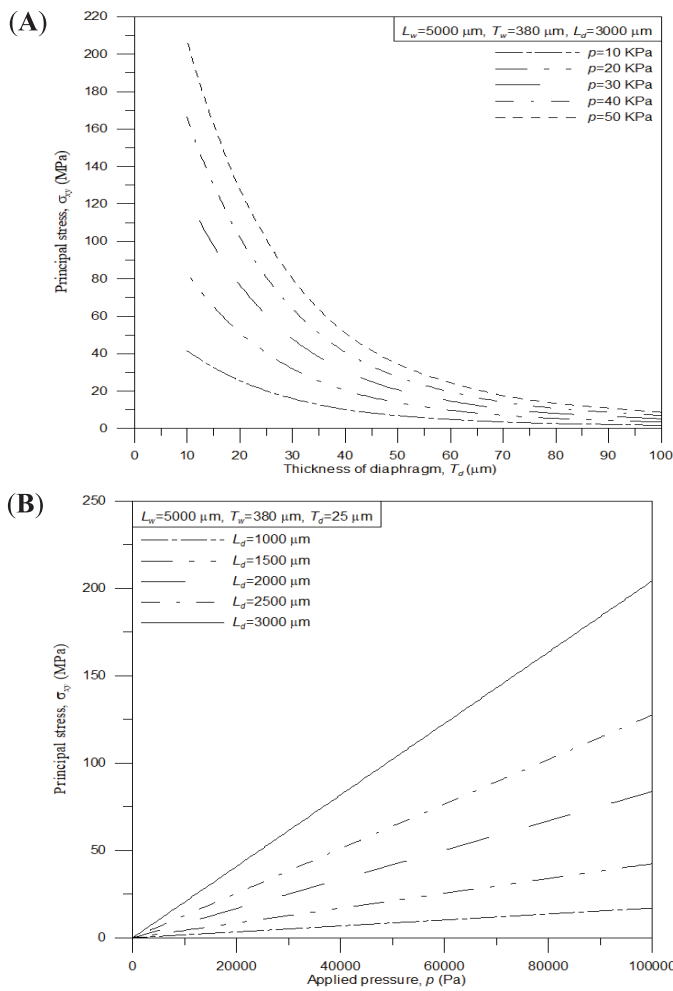


Fig. 5. Principle stress, σ_{xy} (MPa), versus (A) thickness of diaphragm (T_d) and (B) length of diaphragm (L_d) for different applied pressures (p) of MEMS pressure sensors.

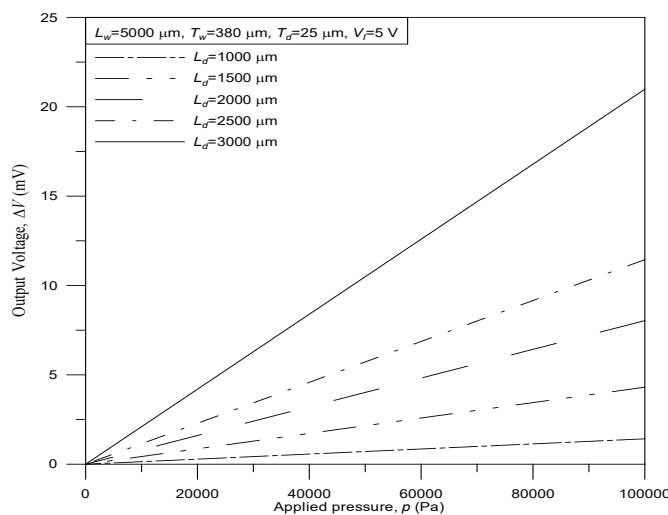


Fig. 6. Output voltage, ΔV (mV), versus applied pressure, p (Pa), for different diaphragm lengths (L_d) of MEMS pressure sensors.

wide range of diaphragm dimensions and pressure conditions.

In Fig. 6, the output voltage (ΔV) is calculated using Eq. (12) with varying applied pressure (p) for different lengths (L_d) of the diaphragm. The results indicate a linear increase in the output voltage (ΔV) as the pressure (p) rises. Additionally, there is an observed increase in the output voltage (ΔV) with an increase in the length (L_d) of the diaphragm. Consequently, a high output voltage (ΔV), exceeding 20 mV, can be achieved across a wide range of diaphragm lengths and thicknesses at an input voltage (V_i) of 5 V. Thus, the sensitivity of the MEMS piezoresistive pressure sensor, featuring a combination of a thin layer of 3C-SiC material on a Si diaphragm, can be significantly enhanced over a broad spectrum of diaphragm dimensions and pressure conditions.

4. Conclusions

This study has simulated and analysed the square diaphragm structure of a MEMS pressure sensor using 3D FEM in COMSOL multiphysics. A thin layer of 3C-SiC material, applied onto a Si diaphragm, enhances the stress distribution of MEMS pressure sensors. The stress distributions are calculated by solving the 3D structures of MEMS pressure sensors in the stationary solver of the solid mechanics module in COMSOL multiphysics. Notable results from this study include:

1. The stress distribution of the 3C-SiC material on a Si square diaphragm is considerably enhanced compared to that of a Si-only diaphragm for MEMS pressure sensors.
2. The stress distribution of the 3C-SiC material on a Si square diaphragm significantly increases under higher pressure, greater diaphragm length, and reduced diaphragm thickness. These stress values do not exceed the elastic limit of 3C-SiC materials. Hence, the stress distributions in MEMS pressure sensors with 3C-SiC on Si square diaphragms can be significantly amplified. An output voltage of more than 20 mV is achievable with this design. The results, demonstrating the utility of the thin layer of 3C-SiC material on a Si diaphragm, are pivotal for designing MEMS piezoresistive pressure sensors with enhanced stress amplification and robust performance, particularly in hazardous environments. Future work will involve the design and fabrication process of the 3C-SiC on Si square diaphragm for MEMS piezoresistive pressure sensors, employing MEMS technologies.

CRediT author statement

Nguyen Chi Cuong: Writing - Original draft preparation, Software; Trinh Xuan Thang: Conceptualisation, Methodology; Lam Minh Thinh, Vuong Dinh Duy Phuc: Investigation, Data curation; Truong Huu Ly: Software; Ngo Vo Ke Thanh, Le Quoc Cuong: Reviewing and Editing.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] P. Song, Z. Ma, J. Ma, et al. (2020), "Recent progress of miniature MEMS pressure sensors", *Micromachines*, **11**(1), DOI: 10.3390/mi11010056.
- [2] H. Soy, İ. Toy (2021), "Design and implementation of smart pressure sensor for automotive applications", *Measurement*, **176**, DOI: 10.1016/j.measurement.2021.109184.
- [3] Y. Kim, J.H. Oh (2020), "Recent progress in pressure sensors for wearable electronics: From design to applications", *Applied Sciences*, **10**(18), DOI: 10.3390/app10186403.
- [4] S.J. Prosser (1993), "Advances in sensors for aerospace applications", *Sensors and Actuators A: Physical*, **37-38**, pp.128-134, DOI: 10.1016/0924-6427(93)80024-B.
- [5] C.S. Smith (1954), "Piezoresistance effect in germanium and silicon", *Physical Review Journals Archive*, **94**(1), pp.42-49, DOI: 10.1103/PhysRev.94.42.
- [6] P. Song, C. Si, M. Zhang, et al. (2020), "A novel piezoresistive MEMS pressure sensors based on temporary bonding technology", *Sensors*, **20**(2), DOI: 10.3390/s20020337.
- [7] V. Belwanshi (2021), "Analytical modeling to estimate the sensitivity of MEMS technology-based piezoresistive pressure sensor", *Journal of Computational Electronics*, **20**, pp.668-680, DOI: 10.1007/s10825-020-01592-5.
- [8] P. Starr, K. Bartels, C.M. Agrawal, et al. (2016), "A thin-film pressure transducer for implantable and intravascular blood pressure sensing", *Sensors and Actuators A: Physical*, **248**, pp.38-45, DOI: 10.1016/j.sna.2016.06.035.
- [9] J.W. Arkwright, I.D. Underhill, S.A. Maunder, et al. (2014), "Fiber optic pressure sensing arrays for monitoring horizontal and vertical pressures generated by traveling water waves", *IEEE Sensors Journal*, **14**(8), pp.2739-2742, DOI: 10.1109/JSEN.2014.2311806.
- [10] B. Morten, G.D. Cicco, M. Prudenziati (1992), "Resonant pressure sensor based on piezoelectric properties of ferroelectric thick films", *Sensors and Actuators A: Physical*, **31**(1-3), pp.153-158, DOI: 10.1016/0924-6427(92)80096-L.
- [11] M. Akiyama, Y. Morofuji, T. Kamohara, et al. (2006), "Flexible piezoelectric pressure sensors using oriented aluminum nitride thin films prepared on polyethylene terephthalate films", *Journal of Applied Physics*, **100**(11), DOI: 10.1063/1.2401312.
- [12] J.C. Suhling, R.C. Jaeger (2001), "Silicon piezoresistive stress sensors and their application in electronic packaging", *IEEE Sensors Journal*, **1**(1), pp.14-30.
- [13] K. Rajanna, S. Mohan (1987), "Longitudinal and transverse strain sensitivity of gold film", *Journal of Materials Science Letters*, **6**, pp.1027-1029, DOI: 10.1007/BF01729121.
- [14] K. Rajanna, S. Mohan (1988), "Studies on meandering path thin film strain gage", *Sensors and Actuators*, **15**(3), pp.297-303, DOI: 10.1016/0250-6874(88)87018-5.
- [15] K. Rajanna, S. Mohan (1989), "Strain sensitive property of vacuum evaporated manganese films", *Thin Solid Films*, **172**(1), pp.45-50, DOI: 10.1016/0040-6090(89)90116-8.
- [16] S. Sanpath, V. Ramanaiah (1986), "Behavior of Bi-Sb alloy thin films as strain gages", *Thin Solid Films*, **137**(2), pp.199-205, DOI: 10.1016/0040-6090(86)90020-9.
- [17] G.R. Witt (1972), "Some effects of strain and temperature on the resistance of thin gold-glass cermet films", *Thin Solid Film*, **13**(1), pp.109-115, DOI: 10.1016/0040-6090(72)90163-0.
- [18] M. Hrovat, J. Holc, Z. Samardzija, et al. (2001), "The influence of firing temperature on gauge factors and the electrical and microstructural characteristics of thick-film resistor", *Journal of Materials Science Letters*, **20**, pp.701-705, DOI: 10.1023/A:1010954823442.
- [19] K.J. Suja, E.S. Raveendran, R. Komaragiri (2013), "Investigation on better sensitive silicon based MEMS pressure sensor for high pressure measurement", *International Journal of Computers and Applications*, **72**(8), pp.40-47.
- [20] V. Belwanshi, S. Philip, A. Topkar (2022a), "Performance Study of MEMS piezoresistive pressure sensors at elevated temperatures", *IEEE Sensors Journal*, **22**(10), pp.9313-9320, DOI: 10.1109/JSEN.2022.3164435.
- [21] S.S. Kumar, B.D. Pant (2015), "Polysilicon thin film piezoresistive pressure microsensor: Design, fabrication and characterization", *Microsystem Technology*, **21**, pp.1949-1958, DOI: 10.1007/s00542-014-2318-1.
- [22] P. Verma, D. Punetha, S.K. Pandey (2020), "Sensitivity optimization of MEMS based piezoresistive pressure sensor for harsh environment", *Silicon*, **12**, pp.2663-2671, DOI: 10.1007/s12633-019-00362-8.
- [23] S.K. Jindal, S.P. Magam, M. Shaklya (2018), "Analytical modeling and simulation of MEMS piezoresistive pressure sensors with a square silicon carbide diaphragm as the primary sensing element under different loading conditions", *Journal of Computational Electronics*, **17**, pp.1780-1789, DOI: 10.1007/s10825-018-1223-8.
- [24] M. Nag, J. Singh, A. Kumar, et al. (2019), "Sensitivity enhancement and temperature compatibility of graphene piezoresistive MEMS pressure sensor", *Microsystem Technologies*, **25**, pp.3977-3982, DOI: 10.1007/s00542-019-04392-5.
- [25] V. Belwanshi, A. Topkar (2022b), "Quantitative analysis of MEMS piezoresistive pressure sensors based on wide band gap materials", *IETE Journal of Research*, **68**(1), pp.667-677, DOI: 10.1080/03772063.2019.1620641.
- [26] S. Li, Z. Zhang, J. Tang, et al. (2013), "A novel signal conditioning circuit for piezoresistive pressure sensor", *Unifying Electrical Engineering and Electronics Engineering*, **238**, pp.1707-1713, DOI: 10.1007/978-1-4614-4981-2_187.
- [27] L. Jiang, R. Cheung (2009), "A review of silicon carbide development in MEMS applications", *Int. J. Computational Materials Science and Surface Engineering*, **2**(3-4), pp.297-299, DOI: 10.1504/IJCMSE.2009.027484.
- [28] V. Belwanshi, A. Topkar (2019), "Design optimization and simulation of a diamond based piezoresistive pressure sensor for high temperature application", *Materials Today Proceedings*, **18**, pp.1017-1024, DOI: 10.1016/j.matpr.2019.06.542.
- [29] V. Belwanshi, A. Topkar (2017), "Quantitative analysis of temperature effect on SOI piezoresistive pressure sensors", *Microsystem Technologies*, **23**, pp.2719-2725, DOI: 10.1007/s00542-016-3102-1.
- [30] R. Khakpour, S.R.M. Mansouri, A.R. Bahadorimehr (2010), "Analytical comparison for square, rectangular and circular diaphragms in MEMS applications", *International Conference on Electronic Devices, Systems and Applications*, DOI: 10.1109/ICEDSA.2010.5503057.
- [31] K.Y. Madhavi, K.A. Sumithradevi, M. Krishna, et al. (2011), "Analysis of square and circular diaphragms for a MEMS pressure sensor using a data mining tool", *International Conference on Electronic Devices, Systems and Applications*, DOI: 10.1109/CSNT.2011.63.
- [32] COMSOL (2022), *COMSOL Multiphysics® 6.0 Release Highlights*, "MEMS module updates", <https://www.comsol.com/>, accessed 20 February 2023.
- [33] M. Bao (2010), *Analysis and Design Principles of MEMS Devices*, Elsevier, 311pp.
- [34] M.A. Hopcroft, W.D. Nix, T.W. Kenny, et al. (2010), "What is the Young's modulus of Silicon?", *Journal of Microelectromechanical Systems*, **19**(2), pp.229-238, DOI: 10.1109/JMEMS.2009.2039697.
- [35] P. Djemia, Y. Roussigné, G.F. Dirras, et al. (2004), "Elastic properties of β -SiC films by Brillouin light scattering", *Journal of Applied Physics*, **95**(2), pp.2324-2330, DOI: 10.1063/1.1642281.
- [36] P.H. Phan, D.V. Dao, K. Nakamura, et al. (2015), "The piezoresistive effect of SiC for MEMS sensors at high temperatures: A review", *Journal of Microelectromechanical Systems*, **24**(6), pp.1663-1677, DOI: 10.1109/JMEMS.2015.2470132.
- [37] M. Olszacki (2009), *Mod'Elisation Et Optimisation De Capteurs De Pression Pi'Ezor'Esisifs*, Ph.D. Thesis, INSA de Toulouse.

Determination of phosphorus additives in instant noodle samples by Phosphorus-31 nuclear magnetic resonance (^{31}P -NMR)

Trung Quang Pham, Lan Anh Thi Nguyen, Nhung Thi Dao, Thao Thi Ta*

Faculty of Chemistry, University of Science, Vietnam National University - Hanoi,
334 Nguyen Trai Street, Thanh Xuan Trung Ward, Thanh Xuan District, Hanoi, Vietnam

Received 7 August 2023; revised 26 February 2024; accepted 13 May 2024

Abstract:

Instant noodles are widely consumed in Vietnam and are especially popular among young people. In the production of instant noodles, phosphate additives are commonly added to enhance the crispiness and chewiness of the product. In this study, three phosphate species, including sodium pyrophosphate (SPP), sodium tripolyphosphate (STPP), and sodium hexametaphosphate (SHMP), in instant noodle samples collected from the Vietnamese market were rapidly analysed using the ^{31}P -NMR method. The phosphate species in instant noodle samples were simply extracted using deionised water. For ^{31}P -NMR measurement, a D_2O /deionised water mixture (9:1) was added. Chlorpyrifos ethyl was used as an internal standard and the pH was adjusted to 9-10. The ^{31}P -NMR spectra were then obtained in 1D mode using a pulse program that involved a 90° hard pulse. The method detection limits for SPP, STPP, and SHMP determination were 0.60, 0.30, and 0.74 mg/g, respectively. The repeatability of SPP in instant noodles was found to be smaller than 5%. The linearity was tested with R^2 (R-squared) values greater than 0.99 for all three analytes. The analysis of instant noodle samples revealed the presence of only SPP, a common additive known for its significant impact on enhancing food quality. The SPP content was estimated to be around 25-54% of the total phosphate, determined by UV-Vis based on the heteropoly blue phosphomolybdate complex.

Keywords: instant noodle, phosphomolybdate, sodium pyrophosphate, total phosphate, ^{31}P -NMR.

Classification numbers: 2.2, 2.3

1. Introduction

Phosphates are crucial substances for life, playing vital roles in metabolising and absorbing nutrients from food [1]. Phosphorus (P) additives are incorporated into convenience and processed foods for various purposes, such as leavening, colour and moisture retention, anti-caking properties, and flavour enhancement [2]. In food technology, P additives (inorganic, organic, and natural P-containing) exist in different forms (single, di-, tri-phosphate, and polyphosphate), identified by E numbers [3, 4]. Previous research has suggested that SPP, STPP, SHMP, sodium acid pyrophosphate (SAPP), and their phosphate blends at various concentrations (0.3, 0.5, and 0.7%) significantly impact the quality and characteristics of food products, such as pH, water-holding capacity, and colour of ground meat patties [5]. Acceptable levels of phosphorus content vary depending on the type of food, ranging from 251 mg of phosphorus per person per day in infants to 1,625 mg per person per day for adults. High exposure levels range from 331 mg of phosphorus per person per day in infants to 2,728 mg per person per day for adults [1]. Various types of phosphorus additives are observed across different food categories, with lecithin (E 322), pyrophosphate (E 450), and triphosphate (E 451)

being the most common [3]. Therefore, it is essential to analyse phosphate species in food to ensure food safety.

In terms of conventional analytical methods for determining total phosphate in food samples, spectrophotometric techniques such as the molybdenum blue, vanadomolybdate complex, and malachite green methods are commonly employed [6]. These methods offer sufficient sensitivity and accuracy. However, for determining phosphorus species, chromatography is the preferred method in laboratories due to its selectivity and low detection limit [7]. Some studies have utilised high-performance liquid chromatography (HPLC) for the quantitative analysis of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) in litchi fruit pericarp tissue [8], as well as for analysing soluble reactive phosphate (SRP) in marine and freshwater samples [9].

Comparatively, quantitative nuclear magnetic resonance (NMR) spectroscopy offers several advantages over time-consuming chromatography when applied to food analysis. It presents a simple operation, allows for simultaneous procedures, and enables quick analysis without the need for extensive sample preparation, making it an inexpensive method [10]. Specifically, quantitative ^{31}P -NMR serves as a

*Corresponding author: Email: tathithao@hus.edu.vn

flexible analytical tool for phosphate determination across various food samples, including meat [11-14], milk [15], and beverages [16-18]. While sample preparation for ^{31}P -NMR analysis may require some time, the method offers a high method detection limit and good specificity comparable to other analytical methods. However, this high detection limit may potentially limit its effectiveness in detecting trace levels of polyphosphates in complex food matrices like certain types of food products.

In studies focusing on phosphorus additives, considerable attention is directed towards compounds such as $\text{Na}_5\text{P}_3\text{O}_{10}$, $\text{Na}_3\text{P}_3\text{O}_9$, $\text{K}_4\text{P}_2\text{O}_7$, $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$, and $\text{K}_4\text{P}_2\text{O}_7$, among others. Researchers highlight the utility of the proposed method for monitoring the hydrolysis of poly- and pyrophosphates, alongside the analysis of samples resulting from such hydrolysis or alkaline extraction. To ensure the completeness of the method, the use of internal reference standards such as hexamethylphosphoramide (HMPA) [14], methylphosphonic acid (MPA) [19], or triphenylphosphate with solvents like D_2O and modified $\text{CDCl}_3/\text{MeOH}/\text{D}_2\text{O}$ -EDTA- Cs^+ (1.0 M) (400:80:5 v/v) [13] is essential. The effectiveness of these techniques relies on the types and concentrations of phosphorus compounds present in food samples, ensuring good selectivity and high accuracy, particularly when applied to complex matrix samples.

This study aims to introduce a method for determining SPP, STPP, and SHMP in instant noodle samples using ^{31}P -NMR. The experimental conditions are optimised to enhance accuracy and reliability. An innovative approach is employed by utilising Chlorpyrifos ethyl, a readily available and cost-effective internal standard, for quantification. The validation and efficacy of the ^{31}P -NMR method in accurately assessing specific phosphate additives in instant noodle samples offer a novel and accessible approach for quality control in food analysis.

2. Materials and methods

2.1. Chemicals and reagents

The following primary standards were purchased from Sigma-Aldrich, with mass fraction purities indicated in parentheses: Sodium triphosphate pentabasic ($\text{Na}_5\text{P}_3\text{O}_{10}$, $\geq 98.0\%$), SPP tetrabasic ($\text{Na}_4\text{P}_2\text{O}_7$, $\geq 95.0\%$), and SHMP ($(\text{NaPO}_3)_6$, 96%). Working standard solutions were prepared by diluting the original stock standards, which were stored in Falcon tubes and kept in the freezer. These solutions were used within a month of preparation.

A stock standard solution of 1000 ppm phosphorus (P) was prepared from KH_2PO_4 stock standard solution obtained from Merck. A 0.08 M ammonium molybdate solution was prepared by dissolving 4.996 grams of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 50 ml of deionised water, followed by complete dissolution using an ultrasonic machine. Additionally, a mixture of ascorbic acid (0.375 M) and sulphuric acid (2.5 M) was prepared using

commercially available reagents. An internal standard solution of Chlorpyrifos ethyl was prepared from a phosphate-based plant protection drug containing 44.5% Chlorpyrifos ethyl. This was achieved by taking 1.0 ml of the pesticide and diluting it to 10 ml in a 10 ml volumetric flask using deionised water.

D_2O solution (100 atom %D), utilised in this research, was sourced as a pure chemical and was exclusively used in the laboratory of ARMAR Chemicals (Switzerland).

All reagents used were of analytical grade, and deionised water (DI) was utilised throughout. Solutions were prepared in volumetric flasks, transferred to glass jars, and stored at 25°C , shielded from sunlight. These solutions were used within 24 hours of reconstitution to maintain their integrity and accuracy.

2.2. Equipment and instruments

The UV-Vis spectrophotometer (UV-1650PC, Shimadzu, Japan) equipped with a deuterium lamp and tungsten filament, along with a plastic cuvette (1 cm thickness), was utilised for the spectrophotometric analysis of total phosphate.

For the analysis of phosphorus species, the NMR spectrometer Bruker Avance III equipped with a 5 mm PABBO BB/ 19F-1H/ D probe with Z gradient, featuring auto-matching and auto-tuning capabilities, operating at a frequency of 550 Hz in the mode of liquid sample, was used. Nitrogen (N_2) was used as the cooling liquid gas.

2.3. Samples and sample preparation

Sampling: Three instant noodle samples from different manufacturers were collected randomly from grocery stores and supermarkets in Vietnam, with expiry dates at least three months away. The samples were kept in the manufacturer's packaging and stored in a refrigerator until sample processing. All samples were used to determine total phosphate concentration by the UV-Vis method.

Sample preparation for phosphomolybdate method: Instant noodle samples were treated as described in AOAC 995.11 [20]. A mixture of 1.5 g of sample with 0.5 g of ZnO catalyst was heated in an oven at 550°C for 4 hours, cooled, then dissolved with 5 ml of HCl (3:1) and 5 ml of DI water, then boiled for 15 minutes. After filtering, the solution was neutralised with 0.1 M NaOH solution until a white precipitate ($\text{Zn}(\text{OH})_2$) appeared. Then a few drops of dilute acid (0.1 M HCl) were added to the precipitate to dissolve it. The sample was filtered and transferred to a 50 ml volumetric flask and then graduated up to the mark. Accurately pipette 5.0 ml of treated solution into a 50 ml volumetric flask containing 15 ml of DI. To this flask, 7 ml molybdate-ascorbic acid solutions (1.5 ml of 0.08 M ammonium molybdate, 3.5 ml of 2.5 M sulphuric acid, and 2 ml of 0.375 M ascorbic acid) were added. The blank samples were also prepared by a home-made instant noodle manufacturing process involving the combination of wheat flour and acetylated starch to create adhesion and enhance the chewiness of the noodle strands.

The absorbance of the produced complex was measured at the maximum wavelength (820 nm) and the calibration curve method was studied and applied in this study. The instrument detection limit and quantification limit were 0.09 and 0.29 ppm, respectively. The method detection limit was 0.47 mg/g of total phosphorus species.

Sample preparation for ^{31}P -NMR method: Samples were prepared according to the E. Szlyk, et al. (2011) [13]'s process with slight modification. To the exact weight of ground sample (1.5 g for instant noodles), 10 ml of DI was added, then it was shaken for 60 minutes. Next, the solution was centrifuged at 4000 rpm for 1 hour. The clear solution was transferred into a 50 ml volumetric flask and filled to the mark. For quantitative determination of phosphorus content by ^{31}P -NMR, the standard and D_2O were mixed in a ratio of 9:1. An internal standard was added at 0.089%, and the pH was adjusted to about 9-10 with 0.1 M NaOH solution. Then 1 ml of these solutions was transferred into NMR tubes, and NMR spectra were recorded. To quantify the phosphate species by ^{31}P -NMR, the relationship between the area of the obtained signal and the concentration of the compounds was analysed.

The ^{31}P -NMR spectra were obtained in 1D mode. The pulse programme involved a 90° hard pulse. The relaxation delay was set at 2 seconds, five times the relaxation time of the ^{31}P nucleus, to ensure total spin relaxation, followed by an acquisition time of 0.402 seconds. The receiver gain was calculated by the command "rga" in Topspin 3.2 (Bruker Biospin). Other parameters were fixed: spectral width 81521.7 Hz (-400 to 400 ppm),

acquired size 32768. A line broadening function of 1.0 Hz was applied prior to Fourier transform. It resulted in a spectral size of 65536 points.

3. Results and discussion

3.1. Optimisation of experimental conditions

Optimisation of experimental conditions was studied by recording ^{31}P -NMR spectra of standards and the internal standard (Chlorpyrifos ethyl) in a mixture (Fig. 1). The signal was calibrated with an instrumental blank, which is a mixture of H_2O and D_2O in a 90:10 % v/v ratio.

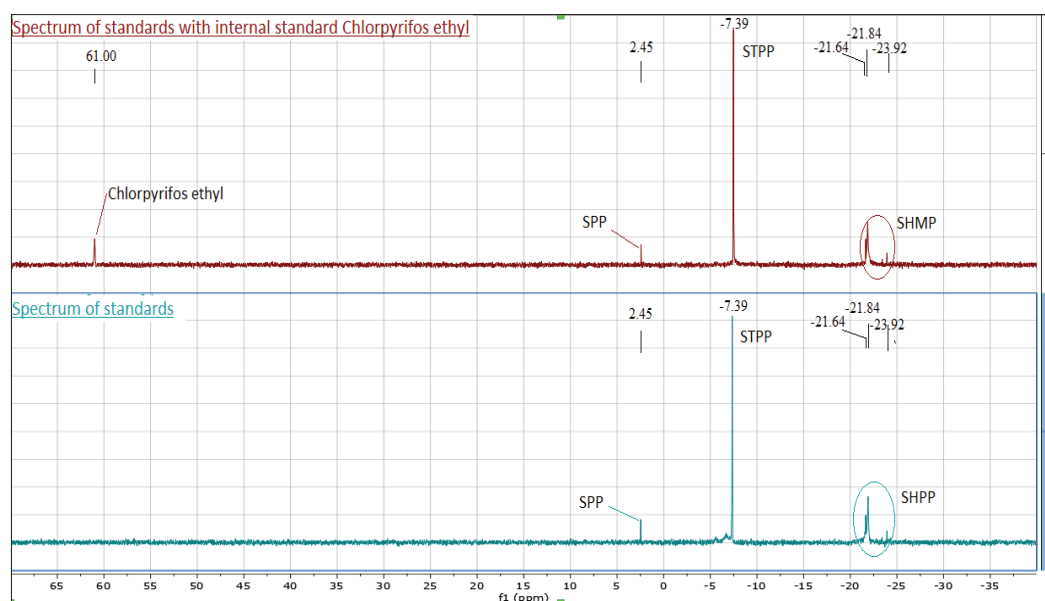


Fig. 1. ^{31}P -NMR spectra of standards and internal standard (chlorpyrifos ethyl) in a mixture of H_2O and D_2O (90:10 % v/v).

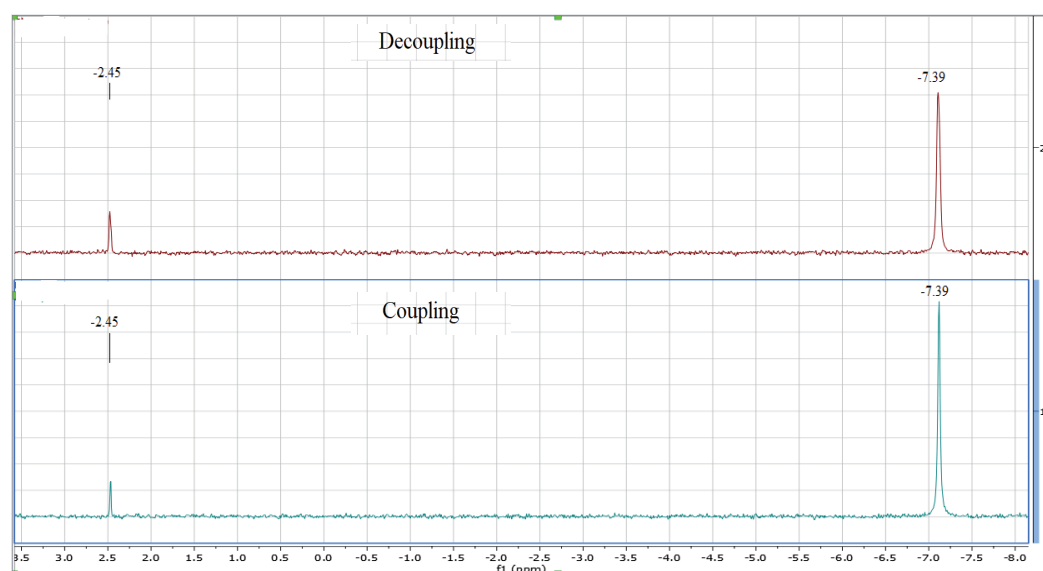


Fig. 2. Effect of scan mode on the ^{31}P -NMR signal.

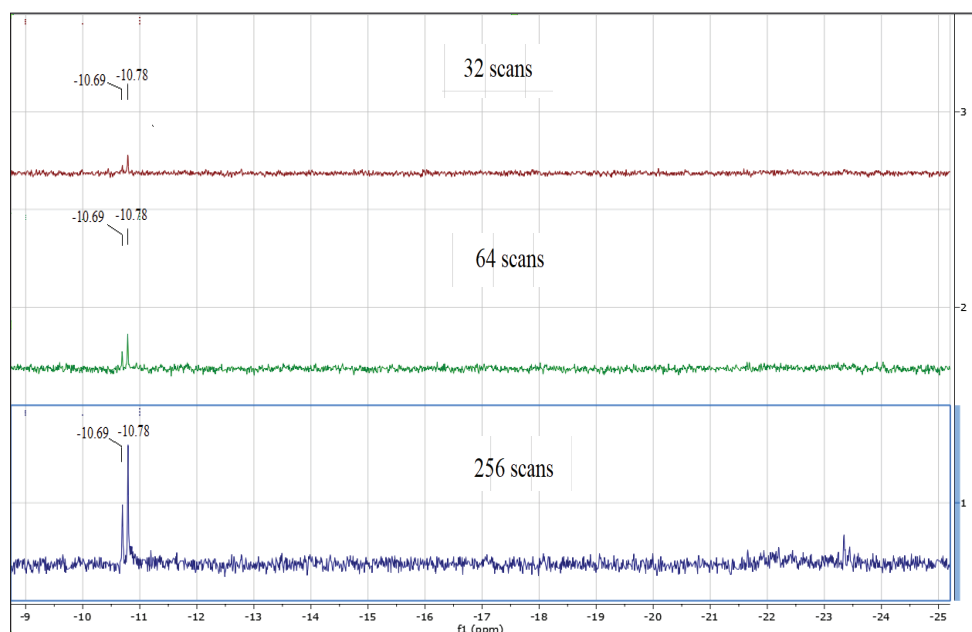


Fig. 3. Effect of the number of scans on the ^{31}P -NMR signal.

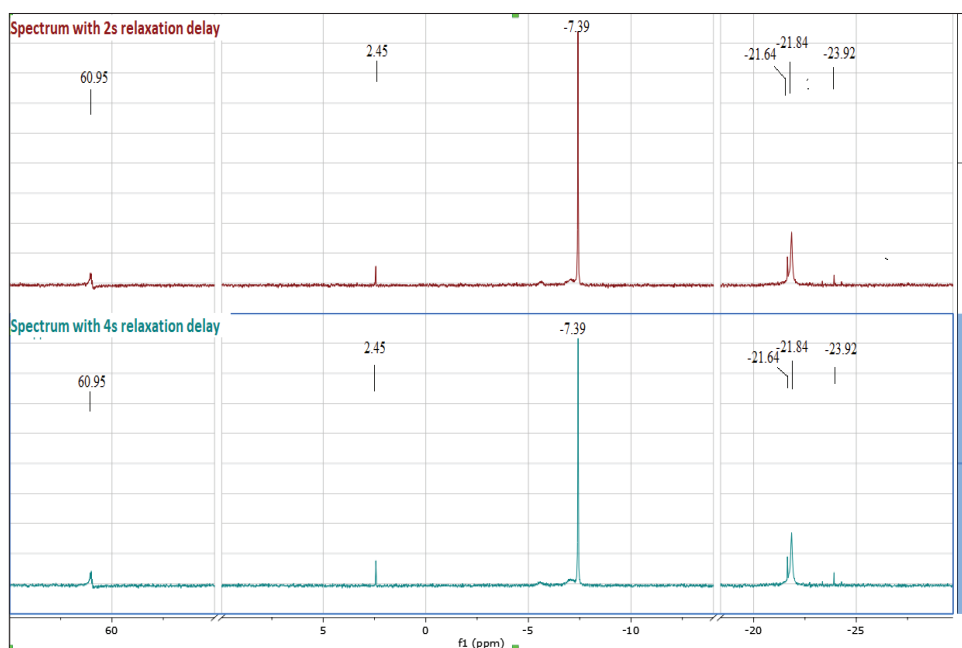


Fig. 4. ^{31}P -NMR spectra of standards and internal standard at different relaxation times.

Effect of measurement mode: Two measurement modes, coupling and decoupling, were investigated. The coupling mode scans the effects and interactions of atoms in the analyte molecule, while the decoupling mode ignores these interactions, resulting in a clearer measurement spectrum. However, the signal intensity in coupling mode is observed to be higher than in decoupling mode, despite a small difference in the area integral of the two modes (Fig. 2). Consequently, utilising decoupling mode may result in more challenging quantification. Therefore, experiments on measuring signals were conducted with pyrophosphate $\text{Na}_4\text{P}_2\text{O}_7$ 300 ppm with

64 scans and a relaxation delay time of 2 seconds at 26°C (Fig. 3).

Effect of the number of scans: As the number of scans increases, the spectral signal becomes clearer. However, excessive scans result in longer scanning times, which is not necessary.

In practice, 1024 scans were selected in this case as it provided a sufficiently clear signal for analysis.

Effect of relaxation time: Relaxation time theoretically affects signal clarity similar to the number of scans. However, experiments with different relaxation times showed very similar results, with insignificant differences (Fig. 4).

As the relaxation time increases, the measurement duration also becomes longer. Therefore, a relaxation time of 2 seconds was deemed reasonable and selected for future experiments.

Effect of pH: Variations in pH lead to changes in the measuring signal. At higher pH levels, the inorganic spectra shift towards the positive (left side) and the signal intensity increases, whereas the organic compound signals remain almost unchanged (Fig. 5).

The pH 10 sample controlled by the NaOH and HCl were considered as suitable experiment conditions.

Investigation of selection of internal standards: The ^{31}P -NMR spectrum of instant noodles shows a clear peak at 2.5 ppm and a smaller peak at approximately 5.5-6 ppm (Fig. 6). The peak at 2.5 ppm is identified as SPP, but the peak at 5.5-6 ppm does not match any of the three reference standards studied and thus remains unidentified [17-19]. Therefore, it is evident that the current method, with optimised conditions and Chlorpyrifos ethyl as the internal standard, can determine the presence of SPP in instant noodle samples.

It was found that the additive in instant noodles contains only SPP and no STPP or SHMP. Therefore, the experiments in this study focused on analysing SPP in instant noodles.

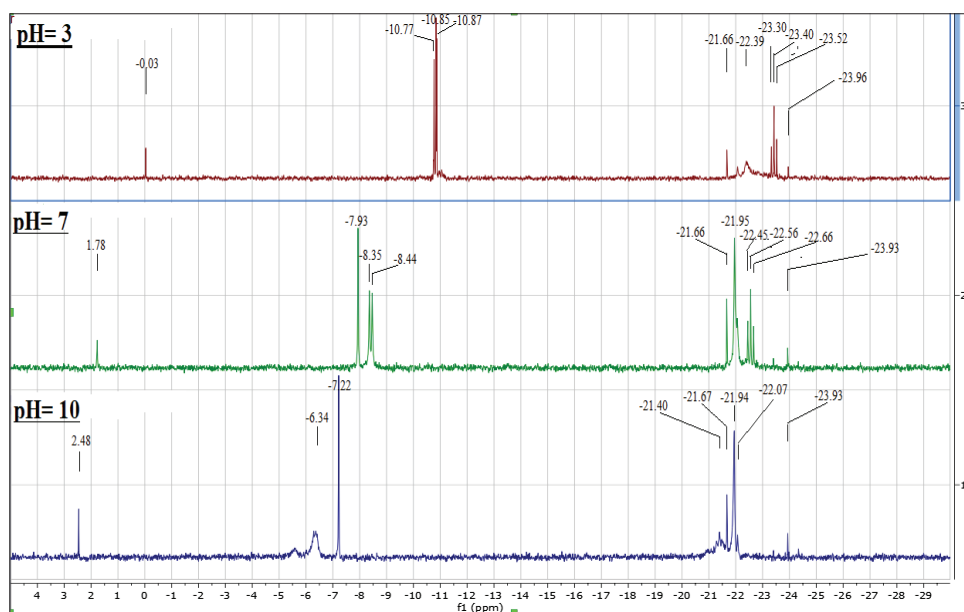


Fig. 5. Effect of pH on ^{31}P -NMR spectra.

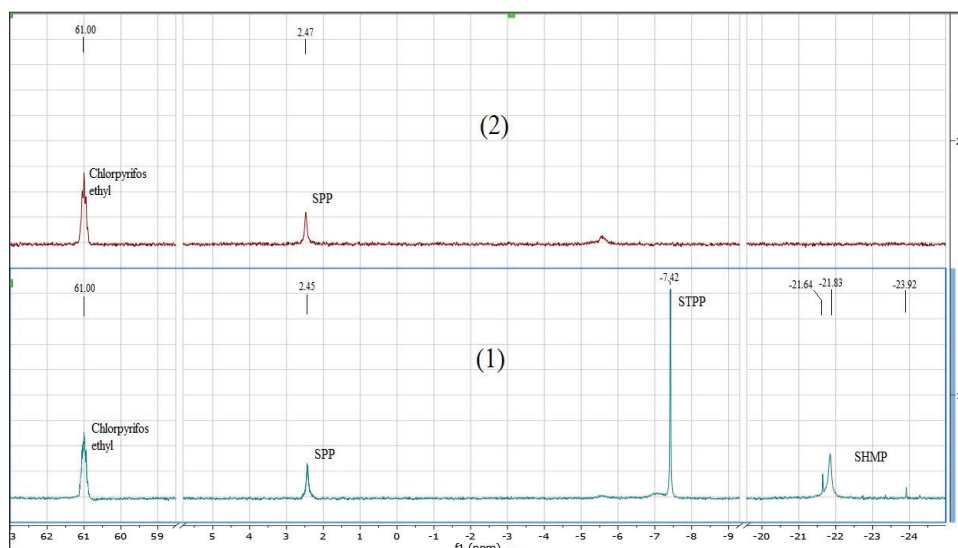


Fig. 6. ^{31}P -NMR spectra of (1) instant noodle with chlorpyrifos ethyl as the internal standard and (2) instant noodles added to standards sodium pyrophosphate, sodium triphosphate, and sodium hexametaphosphate with chlorpyrifos ethyl as the internal standard.

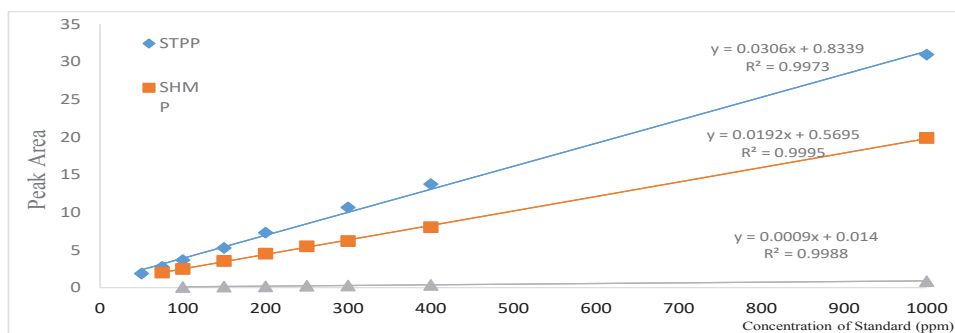


Fig. 7. Calibration curves of sodium pyrophosphate, sodium hexametaphosphate, and sodium triphosphate in ^{31}P -NMR.

3.2. Validations of the ^{31}P -NMR method

Calibration curves and linearity: The peak areas obtained from the analysis of 12 standard samples for the ^{31}P -NMR method were used to establish calibration curves and assess linearity. For the evaluation of linearity, correlation coefficients and working ranges were considered. The calibration curve for SPP utilised a concentration range of 100-1000 ppm. The STPP calibration curve was prepared within the range of 50-1000 ppm, while the SHMP calibration curve was established within the range of 75-1000 ppm. The obtained correlation coefficients for all three calibration curves were greater than 0.99, indicating excellent linearity of the regression models. Additionally, the relative deviation values of concentrations calculated from the calibration curves were all below 15%. This suggests that the calibration curves are reliable and accurately predict the concentrations of SPP, STPP, and SHMP within the specified concentration ranges (Fig. 7).

Detection and quantitative limits: For the method detection limit (MDL) and method quantitative limit (MQL), instant noodle samples of the same product (IN2) were processed and analysed. Six samples of IN2 noodles (containing only SPP) and spiked noodle samples (with low concentrations of STPP and SHMP added) were processed according to the same procedure and analysed under the same conditions to determine the MDL and MQL of SPP. The instrument detection limits (IDL)

indicate that the method is not highly sensitive and is not suitable for determining trace or hypertrace concentrations directly. However, for food samples, the detection limit and quantitative limit of the method are reasonable and acceptable (Table 1).

Table 1. Instrument detection limit, method detection limit, and method quantitative limit of sodium pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate determination by ^{31}P -NMR method.

Parameters	SPP	STPP	SHMP
IDL (mg/g)	0.6	0.3	0.74
MDL (mg/g) (For instant noodles samples)	0.31	0.25	0.32
MQL (mg/g) (For instant noodles samples)	1.00	0.83	1.10

Trueness: To determine the trueness of the method, the recovery of the method was assessed using spiked samples of instant noodles. The results in Table 2 show recoveries for SPP, STPP, and SHMP in instant noodle samples between 90 and 110%, indicating good trueness of the analytical procedure.

Table 2. Recovery of sodium pyrophosphate determined in instant noodle samples.

	Conc. added (ppm)	Conc. in sample (ppm)	Conc. found (ppm)	Spiked conc. found (ppm)	Recovery (%)
SPP	100	63.89	157.71	93.82	93.82
STPP	100	N.D.	104.36	104.36	104.36
SHMP	100	N.D.	95.05	95.05	95.05

Accuracy: Repeatability was evaluated by analysing the same sample 6 times, and reproducibility was validated by analysing the previously used sample 6 times the following day (Table 3). Relative standard deviations (RSD_r) for repeated analysis of phosphorus in instant noodle sample matrices within the same day were all lower than 3%, and RSD_R (%) values were smaller than 3%, indicating consistent results between the two sets of experiments.

Table 3. Repeatability and reproducibility.

Entry	Day 1	Day 2
	SPP (ppm)	SPP (ppm)
1	64.29	65.00
2	63.89	63.99
3	63.23	64.15
4	64.00	64.19
5	63.51	63.97
6	64.44	64.69
Mean	63.89	64.33
SD	0.459	0.418
RSD_r (%)	0.719	0.651

Precision: Relative standard deviations (RSD_r) for repeated analysis of phosphorus in instant noodle sample matrices within the same day were all lower than 3%, confirming the repeatability of the analytical procedure. Comparison between two sets of experiments on day 1 and day 2 was carried out to validate reproducibility across different days. The t_{cal} and s_{pooled} values for SPP concentration in instant noodles were 1.736 and 0.439, respectively. The t_{cal} value (1.736) was smaller than $t_{0.95,10}$ (2.228), and the RSD_R (%) value (0.745) was smaller than 3%, indicating consistent results between the two sets of experiments.

3.3. ^{31}P -NMR quantitative determination of phosphate compounds in instant noodle sample

^{31}P -NMR spectra of three analytical subjects (SPP, STPP, and SHMP) were detected separately from each other and from the internal standard spectrum (Chlorpyrifos ethyl) (Fig. 1). Quantitative determination of phosphate types in some instant noodle samples was performed using ^{31}P -NMR. The results are shown in Table 4.

Table 4. Concentration of phosphate in samples.

Sample	Conc. of SPP in analytical solution (ppm)	Conc. of SPP in instant noodles sample (mg/g)	Total phosphate (determined by UV-Vis) (mg/g)
IN1	120±1.3	1.2±0.045	4.3±0.082
IN2	128±1.63	2.6±0.054	4.7±0.12
IN3	93±0.41	0.93±0.02	3.6±0.08

The concentration of SPP in three instant noodle samples from three different brands ranged from approximately 0.93 to 2.6 mg/g. The percentage of SPP to total phosphate in the sample, as analysed by the UV-Vis method, ranged from approximately 28 to 54%, depending on the product of each producer. The percentage of SPP additives in instant noodles was relatively low, and other additives (STPP and SHMP) were not detected. An unknown spectrum appearing at approximately 5.5 ppm was recorded. This suggests that the phosphate in instant noodles may also include various other forms of phosphorus compounds, such as water-insoluble phosphorus (since the reporting solvent used was water) and other unreported phosphorus compounds. The amount of SPP additives used in instant noodle products varied among different companies. IN2 noodles were found to have the highest percentage of SPP additives and the largest amount of total phosphate among the three types of noodles. Meanwhile, the percentage of SPP in IN1 and IN3 was similar.

4. Conclusions

The ^{31}P -NMR method successfully detected the presence of SPP as the main phosphate additive in three instant noodle samples from different brands. The percentage of SPP to total phosphate in these samples, as analysed by the

UV-Vis method, ranged from approximately 28 to 54%, varying depending on the specific product of each producer. Interestingly, other additives such as STPP and SHMP were not detected in any of the samples. This suggests that the phosphate content in instant noodles may also consist of various other forms of phosphorus compounds, including water-insoluble phosphorus (as the reporting solvent used was water) and potentially other unreported phosphorus compounds. Moreover, it was observed that the amount of SPP additives used in instant noodle products varied among different companies. The proposed ^{31}P -NMR procedure proved to be highly useful for the quantitative analysis of phosphate types in food due to its excellent selectivity and straightforward implementation. This method offers valuable insights into the composition of phosphate additives in food products, aiding in quality control and regulatory compliance efforts within the food industry.

CRedit author statement

Trung Quang Pham: Conceptualisation, Methodology, Data collection, Writing - Original draft preparation; Lan Anh Thi Nguyen: Validation, Formal analysis; Nhung Thi Dao: Sampling and Sample preparation; Thao Thi Ta: Methodology, Investigation, Supervision, Writing - Reviewing and Editing.

ACKNOWLEDGEMENTS

This research was funded by the Vietnam National University - Hanoi, under project number QG.20.23.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] European Food Safety Authority, M. Younes, G. Aquilina, et al. (2019), "Re-evaluation of phosphoric acid-phosphates-di-, tri- and polyphosphates (E 338-341, E 343, E 450-452) as food additives and the safety of proposed extension of use", *EFSA Journal*, **17**(6), DOI: 10.2903/j.efsa.2019.5674.
- [2] R.A. Molins (1991), *Phosphates in Food*, CRC Press, 272pp.
- [3] M. Tuominen, H.J. Karp, S.T. Itkonen (2022), "Phosphorus-containing food additives in the food supply - An audit of products on supermarket shelves", *Journal of Renal Nutrition*, **32**(1), pp.30-38, DOI: 10.1053/j.jrn.2021.07.010.
- [4] A.S.R. Anjaneyulu, N. Sharma, N. Kondaiah (1989), "Evaluation of salt, polyphosphates and their blends at different levels on physicochemical properties of buffalo meat and patties", *Meat Science*, **25**(4), pp.293-306, DOI: 10.1016/0309-1740(89)90047-8.
- [5] W. Li, J.A. Bowers, J.A. Craig, et al. (1993), "Sodium tripolyphosphate stability and effect in ground turkey meat", *Journal of Food Science*, **58**(3), pp.501-504, DOI: 10.1111/j.1365-2621.1993.tb04310.x.
- [6] E.A. Tosi, A.F. Cazzoli, L.M. Tapiz (1998), "Phosphorus in oil. Production of molybdenum blue derivative at ambient temperature using noncarcinogenic reagents", *Journal of The American Oil Chemists' Society*, **75**(1), pp.41-44, DOI: 10.1007/s11746-998-0007-x.
- [7] G. Helmerich, P. Koehler (2003), "Comparison of methods for the quantitative determination of phospholipids in lecithins and flour improvers", *Journal of Agricultural Food Chemistry*, **51**(23), pp.6645-6651, DOI: 10.1021/jf0345088.
- [8] H. Liu, Y. Jiang, Y. Luo, et al. (2006), "A simple and rapid determination of ATP, ADP and AMP concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography", *Food Technology Biotechnology*, **44**(4), pp.531-534.
- [9] J.L. Haberer, J.A. Brandes (2003), "A high sensitivity, low volume HPLC method to determine soluble reactive phosphate in freshwater and saltwater", *Marine Chemistry*, **82**(3-4), pp.185-196, DOI: 10.1016/S0304-4203(03)00069-0.
- [10] V. Ramakrishnan, D.L. Luthria (2017), "Recent applications of NMR in food and dietary studies", *J. Sci. Food Agric.*, **97**(1), pp.33-42, DOI: 10.1002/jsfa.7917
- [11] A. Jastrzębska, E. Szlyk (2009), "Application of ^{31}P NMR for added polyphosphate determination in pork meat", *Chemical Papers*, **63**(4), pp.414-419, DOI: 10.2478/s11696-009-0042-6.
- [12] H.C. Bertram, H.J. Ersen (2004), "Applications of NMR in meat science", *Annual Reports on NMR Spectroscopy*, **53**, pp.157-202, DOI: 10.1016/S0066-4103(04)53003-X.
- [13] E. Szlyk, P. Hrynyszyn (2011), "Phosphate additives determination in meat products by ^{31}P -phosphorus nuclear magnetic resonance using new internal reference standard: Hexamethylphosphoramide", *Talanta*, **84**(1), pp.199-203, DOI: 10.1016/j.talanta.2010.12.046.
- [14] A. Chiba, M. Hamaguchi, M. Kosaka, et al. (1991), "Quality evaluation of fish meat by ^{31}P -phosphorus nuclear magnetic resonance", *Journal of Food Science*, **56**(3), pp.660-664, DOI: 10.1111/j.1365-2621.1991.tb05351.x.
- [15] J. Belloque, M.A.D.L. Fuente, A.M. Ramos (2000), "Qualitative and quantitative analysis of phosphorylated compounds in milk by means of ^{31}P -NMR", *Journal of Dairy Research*, **67**(4), pp.529-539, DOI: 10.1017/s0022029900004477.
- [16] S.M. Ackermann, K. Dolsophon, Y.B. Monakhova, et al. (2017), "Automated multicomponent analysis of soft drinks using 1D ^1H and 2D ^1H - ^1H J-resolved NMR spectroscopy", *Food Analytical Methods*, **10**, pp.827-836, DOI: 10.1007/s12161-016-0643-y.
- [17] M. Kõllo, M. Kudrjašova, M. Kulp, et al. (2013), "Methylphosphonic acid as a ^{31}P -NMR standard for the quantitative determination of phosphorus in carbonated beverages", *Analytical Methods*, **5**(16), pp.4005-4009, DOI: 10.1039/C3AY40743H.
- [18] L. Medhurst, F. Shahnaz, N. Ramnarine, et al. (2016), "Measurement of phosphates in soft drinks: A general chemistry experiment using NMR, in NMR spectroscopy in the undergraduate curriculum: First year and organic chemistry courses", *ACS Symposium Series*, **2**, pp.31-37, DOI: 10.1021/bk-2016-1221.ch003.
- [19] P. Hrynyszyn, A. Jastrzębska, E. Szlyk (2010), "Determination of phosphate compounds in meat products by ^{31}P -phosphorus nuclear magnetic resonance spectroscopy with methylenediphosphonic acid after alkaline extraction", *Analytica Chimica Acta*, **673**(1), pp.73-78, DOI: 10.1016/j.aca.2010.05.018.
- [20] AOAC International (1995), "AOAC official method 995.11 phosphorus (total) in foods: Colorimetric methods", <https://img.21food.cn/img/biaozhun/20100108/177/11285243.pdf>, accessed 5 June 2023.

Optimisation of calcinothemic reduction for metallic dysprosium preparation

Nguyen Trong Hung*, Nguyen Thanh Thuy, Le Ba Thuan

Institute for Technology of Radioactive and Rare Elements, 48 Lang Ha Street, Lang Ha Ward, Dong Da District, Hanoi, Vietnam

Received 21 February 2023; revised 23 May 2023; accepted 15 February 2024

Abstract:

This research focuses on optimising the process of preparing metallic dysprosium through calcinothemic reduction. The study employed response surface methodology (RSM) based on a central composite face-centred (CCF) design to model the interactive effects of three independent variables: reduction temperature, Ca/DyF₃ molar ratio, and reduction time, on the dependent response, which was the reduction yield. Initially, experiments were conducted to determine the experimental matrix or planning region. Subsequently, experimental studies were carried out within this matrix to develop a quadratic response surface methodology - central composite face (RSM-CCF) model for the calcinothemic reduction of DyF₃ using MODDE 5.0 software. The resulting model demonstrated good agreement between calculated and actual data, establishing a reliable framework for the calcinothemic reduction process. Further analysis involved assessing the contributions of the model's coefficients to the dependent response to optimise the process. The findings indicate that reduction temperature plays a significant role in governing the calcinothemic reduction. The optimal parameters for the calcinothemic reduction of DyF₃ were identified as a reduction temperature of 1450-1460°C, a reduction time of 50 minutes, and a Ca/DyF₃ molar ratio of 2.15. Under these conditions, the maximum reduction yield achieved was 85.7%.

Keywords: calcinothemic reduction, metallic dysprosium, modelling, optimising.

Classification numbers: 2.2, 2.3, 4.2

1. Introduction

Dysprosium (Dy) is a valuable heavy rare earth element. Along with iron and terbium, Dy is a component of the magnetic material Terfenol-D (TbxDy_{1-x}Fe₂; $x \approx 0.3$), which exhibits the highest room-temperature magnetostriction of any known material. Dy is also a critical component in the production of high-energy permanent magnets, such as Nd-Fe-B (Nd_{13.4-x}Dy_{0.2+x}Fe_{79.7}M_{0.4}B₆; $x = 0-0.3$; M is Co or Cu), which are used in modern electrical generators and motors for hybrid and electric vehicles, as well as wind turbine generators. Nearly 95% of the total demand for Dy is for its use in Nd-Fe-B permanent magnets. Additionally, Dy is required in hard disc drives, transducers, wide-band mechanical resonators, and high-precision liquid-fuel injectors. In the nuclear sector, Dy has a high thermal-neutron absorption cross-section, and consequently, dysprosium-oxide-nickel CERMET material is used as neutron-absorbing control rods in nuclear reactors. Dy is also utilised in dosimeters for measuring ionising radiation [1-3].

In metallurgy, molten salt electrolysis and metallothermic reduction are the two main techniques for producing rare earth metals. Light rare earth metals, such as lanthanum (La), cerium (Ce), praseodymium (Pr), and neodymium

(Nd), which have low melting points (920, 795, 935, and 1024°C, respectively), are commonly produced via molten salt electrolysis. In contrast, heavy rare earth metals such as Dy and Tb, with higher melting points (1407 and 1356°C, respectively), are typically produced using metallothermic reduction [4-7].

Both techniques employ anhydrous free-oxygen chloride or fluoride rare earth salts as feed materials. Although the salt electrolysis or metallothermic reduction temperature of anhydrous free-oxygen chloride salts is lower than that of fluoride salts, chloride salts have a highly hygroscopic nature, which is a major disadvantage. In contrast, anhydrous free-oxygen fluoride rare earth salts are preferred due to their superior stability. The metallothermic reduction of anhydrous free-oxygen fluoride salts using metallic calcium as the reductant is known as calcinothemic reduction [8].

The production of metallic rare earth by molten salt electrolysis or metallothermic reduction involves two key steps: (i) fabrication of anhydrous free-oxygen fluoride and (ii) molten salt electrolysis or calcinothemic reduction of the anhydrous fluoride to obtain metallic rare earth. In the case of Dy, metallic dysprosium is fabricated by the calcinothemic reduction of anhydrous free-oxygen fluoride Dy, which can be produced from, for example, the

*Corresponding author: Email: nthungvaec@gmail.com

fluorination of dysprosium oxide (Dy_2O_3) using ammonium bifluoride (NH_4HF_2) [9-11]. Previous studies have reported the fluorination of dysprosium oxide, terbium oxide (Tb_4O_7), and terbium chloride (TbCl_3) using ammonium bifluoride for the preparation of anhydrous free-oxygen fluoride dysprosium and terbium [12-14]. However, to the authors' knowledge, no previous study has investigated the calcinothemic reduction of DyF_3 for the preparation of metallic Dy.

This study aims to fill this research gap by investigating and optimising the calcinothemic reduction process of anhydrous free-oxygen DyF_3 to obtain metallic Dy. The relevant process parameters influencing the yield of calcinothemic reduction were identified using an Ishikawa diagram, which depicted various factors affecting the reduction yield of DyF_3 , including material factors (e.g., purity of DyF_3 , metallic Ca, powder form), equipment factors (e.g., tooling conditions, scale accuracy, temperature profile, calibration), and process parameters (e.g., reduction temperature, reduction time, Ca/ DyF_3 molar ratio, moisture, inert gas environment).

The primary process parameters identified for investigation were reduction temperature, reduction time, and Ca/ DyF_3 molar ratio. Subsequently, the quantitative relationship between these parameters and the reduction yield was evaluated. The RSM approach, based on CCF design, was employed to empirically model the interactions between the process parameters (independent variables) and the calcinothemic reduction yield (dependent response). This approach facilitated the optimisation of the process by identifying the optimal values for each variable and reducing the number of experiments required. Ultimately, the regression model developed through RSM enabled accurate prediction of the reduction process outcomes and optimisation accordingly.

2. Materials and methods

2.1. Preparation of dysprosium fluoride (DyF_3) material

Dysprosium oxide (Dy_2O_3) with a purity of 99.9% was obtained from dysprosium chloride (DyCl_3) solution, which resulted from the separation and purification of total rare earth oxides (TREO) from Yen Phu, Vietnam, using the solvent extraction method. The extraction was carried out using PC88A as the extractant at the Institute for Technology of Radioactive and Rare Elements (ITRRE) [15-18]. The procedure for Dy_2O_3 preparation is as follows: the DyCl_3 solution was first precipitated using a saturated oxalic acid solution; chloride (Cl^-) anions were then completely removed from the dysprosium oxalate [$\text{Dy}_2(\text{C}_2\text{O}_4)_3$] precipitate using dilute oxalic acid as a scrubbing solution. The resulting dysprosium oxalate was then annealed in air at 600°C for 2 hours, converting the oxalate salts into dysprosium oxide. The fluorinating reagent used was

ammonium bifluoride (NH_4HF_2), with a purity of $\geq 99\%$ (purchased from Shanghai Epoch Material Co., Ltd).

A mixture of NH_4HF_2 and Dy_2O_3 in a stoichiometric ratio of 3.5:1 was prepared using laboratory ceramic ball mill grinding equipment for approximately 0.5 hours. The fluorination of Dy_2O_3 by NH_4HF_2 is a solid-phase chemical reaction, requiring sufficient contact between the reactants to optimise performance. To achieve this, the mixture of Dy_2O_3 and NH_4HF_2 was ground into smaller particles, increasing the surface area and enhancing contact between the solid phases. This process improved the fluorination yield, leading to higher conversion rates and more efficient reactions. The reduction of particle size also promoted a uniform distribution of reactants, minimising local concentration gradients and enabling more homogeneous reactions. Therefore, reducing particle size plays a crucial role in solid-phase chemical reactions, significantly impacting their efficiency and effectiveness.

The fluorination was carried out in a tube furnace (Nabertherm) at $350\text{--}450^\circ\text{C}$ for 3 hours. Upon loading the mixture into the furnace, it was necessary to vent the steam produced during the reaction to maintain stable furnace conditions. A water aspirator was attached to the outlet of the furnace, while two compressed air cylinders with pressure-reducing valves were connected to the furnace inlet. These measures ensured a continuous flow of compressed air, removing the steam produced during the reaction and maintaining appropriate temperature and pressure conditions. This approach facilitated a smooth reaction process and the production of high-quality fluorinated products. The flow of compressed air also enhanced safety and efficiency, minimising the risk of overheating or other hazards that could compromise the process. As a result, the conversion efficiency of the oxide to fluoride was estimated to be over 99%.

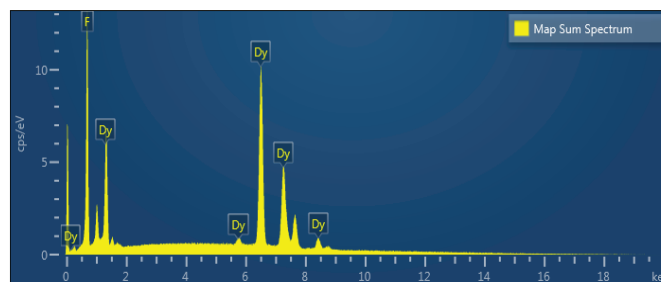


Fig. 1. The EDS pattern of DyF_3 [12].

Analysis of the data presented in Fig. 1 reveals no detectable peaks corresponding to oxygen in the EDS pattern. Furthermore, the calculated compositions of the F and Dy elements, based on the [DyF_3] formula, closely match those determined from the EDS results. These findings confirm the successful conversion of Dy_2O_3 into

the desired $[\text{DyF}_3]$ product. The absence of oxygen peaks further indicates that the reaction proceeded to completion, leaving no residual oxygen in the final product. Overall, the results provide strong evidence that the fluorination process effectively produced high-quality $[\text{DyF}_3]$ with accurate and consistent elemental compositions in line with the intended formula. The anhydrous DyF_3 is used as the raw material for the calcinothemic reduction to produce metallic dysprosium (Dy).

2.2. Chemicals and equipment

Metallic calcium clinkers (99% pure, 1 mm in size), stored under inert gas, were used as a reductant (purchased from Shanghai Epoch Material Co., Ltd). A 15 kW induction furnace, with a maximum operating temperature of 1800°C (Zhengzhou, China), was utilised for the reduction process (Fig. 2). The furnace consisted of a sealed silica chamber, approximately 180 mm in length, housing a graphite susceptor with a diameter of 80 mm. The susceptor featured a central hole in its lid. The reaction occurred inside a tantalum crucible placed within the susceptor for indirect heating.

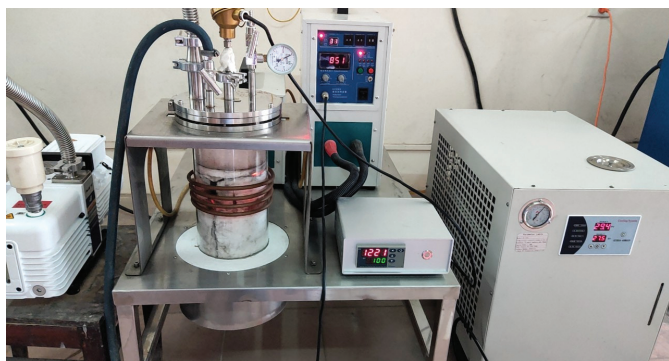


Fig. 2. The vacuum inductive furnace.

Temperature measurements were conducted using a two-colour pyrometer, directed at the central hole of the graphite susceptor lid. While no black body correction was applied, the ratio of the distance between the susceptor lid and the charged surface to the diameter of the hole was approximately 4-5. Although this configuration did not create a perfect black body, the recorded temperatures were reasonably close to the actual values, ensuring practical significance. The furnace employed a proportional-integral-derivative (PID) control mechanism, receiving temperature data from the pyrometer. This setup enabled precise and controlled heating with an accuracy of $\pm 2^\circ\text{C}$, even in an induction furnace. Additionally, the pyrometer allowed observation of changes in the charge throughout the process, despite the sealed system.

Prior to heating, the furnace chamber was purged with argon at a flow rate of approximately 2 l/min for a sufficient

period. This slight flow of argon, maintained via a bubbler, continued throughout the entire reduction process.

2.3. Experiments

(i) Sample preparation: 50 g of dry DyF_3 and a specified weight of metallic calcium were placed in a tantalum crucible with a volume of 100 ml. The mixture was stirred for 2 hours in a laboratory box filled with argon gas, after which the crucible was placed in the vacuum induction furnace.

(ii) Calcinothemic reduction of DyF_3 : The process was conducted at temperatures ranging from 1200 to 1500°C , with a Ca/DyF_3 molar ratio of 1.65-2.25, for 0.25-1 hour. Upon completion, metallic Dy in the reduction samples was dissolved in diluted hydrochloric acid (HCl), and Dy content in the solution was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Horiba, Japan). The reduction yield was calculated based on the Dy content in the original DyF_3 and in the solid after reduction, as follows (Eq. 1):

$$\text{Reduction yield (\%)} = \frac{\text{Initial Dy} - \text{Dy in solid}}{\text{Initial Dy}} \times 100 \quad (1)$$

A satisfactory mass balance was achieved for each reduction experiment, with five replications performed for each experiment.

2.4. Modeling

The method of modelling and statistical design of experiments, using a central composite face-centred (CCF) design in response surface methodology (RSM), was employed [15]. The dependent response in this study was the reduction yield (Y, %), while the independent variables, or factors, were the reduction temperature (X_1 , $^\circ\text{C}$), Ca/DyF_3 molar ratio (X_2 , mol/mol), and reduction time (X_3 , h). The levels of these variables are presented in both coded and actual values in Table 1.

Table 1. Independent variables and their corresponding levels.

Independent variable	Symbol coded	Coded variable levels		
		-1	0	1
Reduction temperature ($^\circ\text{C}$)	X_1	1400	1450	1500
Ca/DyF_3 molar ratio (mol/mol)	X_2	1.95	2.10	2.25
Reduction time (h)	X_3	0.50	0.75	1.00

To determine the optimal combination of these factors for achieving the highest reduction yield, 15 experimental runs were required. This was calculated as $2k+2k+n_0$, where k is the number of factors, in this case three, and n_0 is the number of replications at the centre points, set to one for this study. Therefore, 15 runs were necessary to obtain sufficient data for analysis and to identify the most effective combination of reduction temperature, Ca/DyF_3 molar ratio,

and reduction time for maximising the reduction yield. By carefully controlling these variables, it is possible to optimise the reduction process and maximise the production of high-quality reduced products.

The experimental results were entered into MODDE 5.0 software, which was used to fit the model via multiple linear regression. This process employed an RSM-CCF regression model, mathematically represented as follows Eq. 2:

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i,j=1(i \neq j)}^k b_{ij} X_i X_j \quad (2)$$

where Y is the dependent response; b_0 is the constant coefficient; b_i , b_{ii} and b_{ij} are the linear, quadratic, and interaction coefficients, respectively; X_i and X_j are the coded values of the independent variables; $X_i X_j$ and X_i^2 represent the interaction and quadratic terms, respectively.

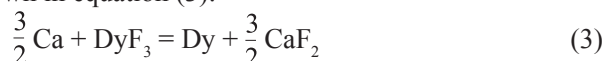
By applying this model to the experimental data, a deeper understanding of the relationships between the independent variables (factors) and the dependent response (reduction yield) was obtained. This information can be used to optimise the reduction process and improve the quality of the reduced products. The use of advanced statistical modelling techniques, such as multiple linear regression and RSM-CCF, is essential for achieving accurate and reliable results in experimental research, significantly enhancing the understanding of complex processes and systems.

3. Results and discussion

3.1. Determining the experimental planning region

A central composite face-centred (CCF) design, within the framework of response surface methodology (RSM), is capable of modelling the linear, quadratic, and interaction effects of independent variables on the dependent response. Thus, incorporating a model based on the CCF design provides a comprehensive understanding of the overall reduction process, leading to an accurate description of the system under investigation. Prior to implementing the CCF design in RSM for modelling the calciothermic reduction process, experimental studies were conducted to examine the effects of the independent variables on the dependent response. These studies are essential in determining the experimental planning region, which is necessary for obtaining accurate and reliable results.

By conducting these experimental studies and defining the planning region, a CCF design can be effectively used to model the calciothermic reduction process. This facilitates the optimisation of process parameters, thereby improving the quality of the final product and enhancing efficiency and productivity. The calciothermic reduction reaction of DyF_3 is shown in equation (3):



From the literature, the key parameters influencing the calciothermic reduction are reduction temperature, Ca/DyF_3 molar ratio, and reaction time. Studies investigating the effects of these parameters on the reduction yield were undertaken.

The investigation into the effect of reduction temperature on the reduction yield was conducted at a Ca/DyF_3 molar ratio of 2.1 and a reduction time of 0.5 hours. The results indicated that at temperatures below 1300°C , the reduction yield was very low ($<10\%$). At 1300°C , the yield increased to 35%, while in the temperature range of 1400 to 1500°C , the reduction yield reached approximately 80%.

The study on the effect of Ca/DyF_3 molar ratio on the reduction yield was conducted at a fixed reduction temperature of 1450°C and a reduction time of 0.5 hours. The reduction yields at Ca/DyF_3 molar ratios of 1.65, 1.84, 1.95, 2.1, and 2.25 were 54.3 ± 0.8 , 62.1 ± 1.0 , 80.8 ± 1.2 , 84.6 ± 1.0 , and $85.4 \pm 0.6\%$, respectively.

The investigation into the effect of reduction time on the reduction yield was carried out at a fixed Ca/DyF_3 molar ratio of 1.95 and a reduction temperature of 1400°C . The reduction yields for time intervals of 0.25, 0.5, 0.75, and 1 hour were 71.9 ± 1.4 , 73.3 ± 1.6 , 75.4 ± 1.0 , and $75.2 \pm 1.2\%$, respectively.

The effects of reduction temperature, Ca/DyF_3 molar ratio, and reduction time on the reduction yield of DyF_3 by metallic calcium are illustrated in Fig. 3. Based on these results, the planned experimental region was determined as follows: reduction temperatures of 1400 – 1500°C , Ca/DyF_3 molar ratios of 1.95–2.25, and reduction times of 0.5–1 hour. The subsequent experimental studies to model the calciothermic reduction of DyF_3 were implemented within this defined region.

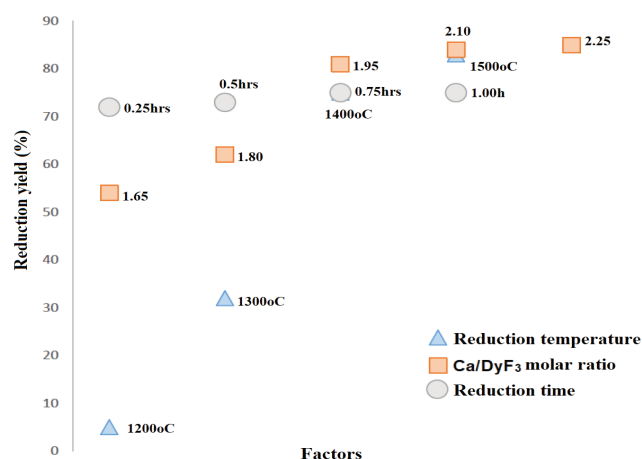


Fig. 3. The effects of factors on the reduction yield of DyF_3 by metallic calcium.

3.2. Modelling the calcinothemic reduction of DyF_3

A response surface methodology (RSM) with a CCF design was employed to evaluate the impact of independent variables on the dependent response, specifically the reduction yield. The study examined the effects of three variables: reduction temperature (ranging from 1400 to 1500°C), Ca/DyF₃ molar ratio (ranging from 1.95 to 2.25), and reduction time (ranging from 0.5 to 1 hour).

To accurately represent the relationship between these variables and the reduction yield, a quadratic model was selected, denoted by the variable Y. The MODDE 5.0 software was used to guide the adoption of this robust model, facilitating the analysis of the experimental data. Through this approach, optimal values for each independent variable were determined, enabling maximisation of the reduction yield and improving process efficiency.

In the RSM-CCF model, the independent variables were assigned codes for ease of analysis. Reduction temperature, Ca/DyF₃ molar ratio, and reduction time were designated as X_1 , X_2 , and X_3 , respectively. A coding scheme was applied, where high, centre, and low levels were represented by 1, 0, and -1, respectively, as shown in Table 2. This coding approach was crucial for conducting accurate experimental studies and modelling the effects of independent variables on the dependent response. By employing codes and levels,

Table 2. Central composite rotatable design arrangement and results.

Run	Independent variables			Responses				
	Coded levels		Real values	Reduction yield				
	X_1	X_2	X_3	Reduction temperature, in °C	Ca/DyF ₃ molar ratio, in mol/mol	Reduction time, in h	Experimental* (Actual), in %	Calculated (Predicted), in %
1	-1	-1	-1	1400	1.95	0.50	71.4	71.6
2	1	-1	-1	1500	1.95	0.50	75.3	75.8
3	-1	1	-1	1400	2.25	0.50	78.1	78.1
4	1	1	-1	1500	2.25	0.50	82.7	82.7
5	-1	-1	1	1400	1.95	1.00	74.8	74.8
6	1	-1	1	1500	1.95	1.00	78.9	79.0
7	-1	1	1	1400	2.25	1.00	80.2	79.7
8	1	1	1	1500	2.25	1.00	84.5	84.4
9	-1	0	0	1400	2.10	0.75	78.4	78.7
10	1	0	0	1500	2.10	0.75	83.6	83.1
11	0	-1	0	1450	1.95	0.75	80.3	79.4
12	0	1	0	1450	2.25	0.75	84.7	85.3
13	0	0	-1	1450	2.10	0.50	83.2	82.5
14	0	0	1	1450	2.10	1.00	84.5	84.9
15	0	0	0	1450	2.10	0.75	83.8	84.3

*Experimental mean of five replications per batch.

a structured and systematic framework was established, enhancing the accuracy and reliability of the results obtained.

The outcomes of the 15 experimental runs were recorded in Table 2 and input into the MODDE 5.0 software for further analysis. Using multiple linear regression, the software fitted a model to the experimental data, providing a comprehensive understanding of the factors influencing the dependent response. Furthermore, this model can be utilised for predicting the outcomes of future experiments and optimising the reduction process.

To evaluate the adequacy of the quadratic model in the calcinothemic reduction of DyF₃, a significance test and analysis of variance (ANOVA) were conducted. Table 3 provides a summary of the estimated regression coefficients, along with their corresponding 95% confidence intervals. The accuracy and variability of the model were assessed using the coefficient of determination (R^2), which was calculated to be 0.95. This indicates that the model explains 95% of the total variation in the response, leaving only 5% unexplained. Thus, the model exhibits a high level of confidence in predicting the response.

The ANOVA results, including the p-value, sum of squares, mean square, model significance (F-value), and degrees of freedom (Table 4), reveal that the p-value for the regression model was below 0.05, indicating that the model terms are statistically significant at the 95% confidence level. This demonstrates the overall goodness of fit of the model. Additionally, the lack-of-fit p-value suggests that there is no significant lack of fit at the 0.05% level, further validating the model's statistical soundness. The adjusted coefficient of determination (adjusted R^2) was calculated to be 0.94, reflecting a strong agreement between the predicted model and the experimental data.

Table 3. Estimated regression coefficients for sequential model.

Source	Coefficient	Standard error	p-value
Model	84.3378	0.418872	5.73439e-011
X_1	2.21	0.246443	0.000287564
X_2	2.95001	0.246443	7.17519e-005
X_3	1.22	0.246443	0.00428328
X_1^2	-3.47222	0.485992	0.000834294
X_2^2	-1.97223	0.485992	0.00974683
X_3^2	-0.622238	0.485992	0.056602
X_1X_2	0.112495	0.275532	0.099961
X_1X_3	-0.0124991	0.275532	0.065574
X_2X_3	-0.387508	0.275532	0.018609

Therefore, the results of the significance test, ANOVA, and evaluation metrics indicate that the quadratic model employed for the calciothermic reduction of DyF_3 is accurate and reliable. It effectively explains the variation in the response and demonstrates good agreement with the experimental data. The final predicted formula for the calciothermic reduction of DyF_3 to obtain metallic Dy, incorporating coded coefficients, is presented in Eq. (4):

$$Y = 84.3 + 2.2X_1 + 3.0X_2 + 1.2X_3 - 3.5X_1^2 - 2.0X_2^2 - 0.6X_3^2 + 0.1X_1X_2 - 0.4X_2X_3 \quad (4)$$

Table 4. The results of analysis of variance (ANOVA) of model.

Source	Degree of freedom	Sum of squares	Mean square	F-value	p-value	RSD
Regression	9	233.784	25.976	42.7699	0.000	5.09666
Residual	5	3.03671	0.607341			0.779321
Lack of fit	--	--	--	--	--	--
$R^2 = 0.95$; $R^2 \text{ adj.} = 0.94$						

In Fig. 4, a plot comparing the calculated reduction yield of DyF_3 by metallic calcium versus the experimental results is presented. The close distribution of the experimental data points around a straight line indicates a strong agreement between the predicted and actual results. This confirms that the RSM-CCF model for the calciothermic reduction is consistent with the experimental data.

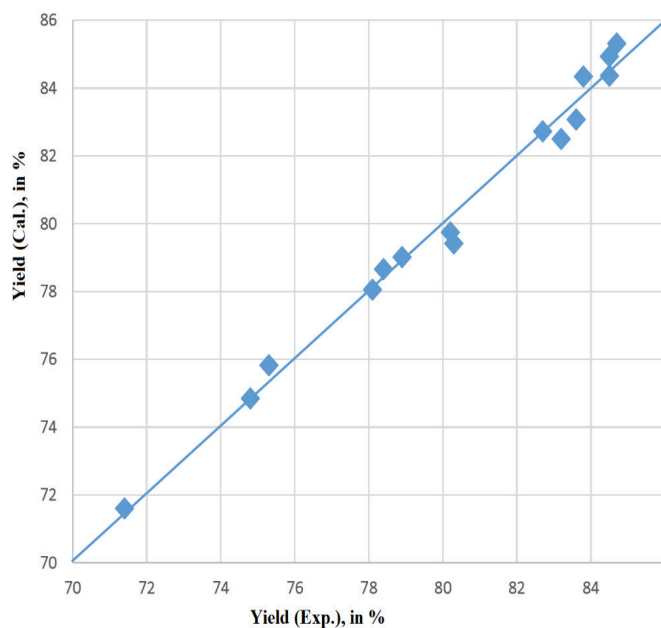


Fig. 4. Linear correlation between calculated and experimental values for the calciothermic reduction of DyF_3 .

3.3. Optimising the calciothermic reduction of DyF_3

3.3.1. Interaction among factors

From Eq. (4), several key insights can be drawn:

i. The contributions from the linear coefficients (b_i of X_i) and quadratic coefficients (b_i^2 of X_i^2) on Y are far more significant than those from the interaction coefficients (b_{ij} of X_iX_j). Therefore, the variables have largely independent effects on the response.

ii. The contributions from the coefficients b_1 and b_1^2 at the low level ($X_1 = -1$) and high level ($X_1 = 1$) on Y were calculated as $b_0 - 5.7$ and $b_0 - 1.3$, respectively. This indicates a significant decrease in the reduction yield at $X_1 = -1$. The highest reduction yield is achieved at temperatures between 1450 and 1460°C, with a calculated value of $b_0 = 84.3$ at $X_1 = 0$.

iii. The contributions from the coefficients b_2 and b_2^2 at the low level ($X_2 = -1$) and high level ($X_2 = 1$) on Y were calculated as $b_0 - 4.7$ and $b_0 + 0.8$, respectively. This suggests a significant decrease in yield at $X_2 = -1$, while the increase at $X_2 = 1$ is minimal. The highest yield is reached with Ca/DyF_3 molar ratios between 2.1 and 2.25.

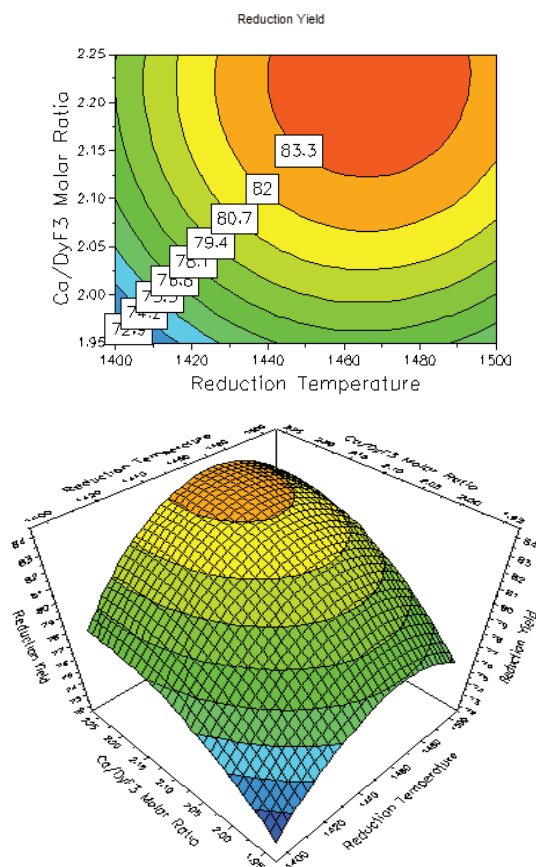


Fig. 5. Contours and response surface of the reduction yield of DyF_3 by metallic calcium vs reduction temperature and Ca/DyF_3 molar ratio at a low level of the reduction time.

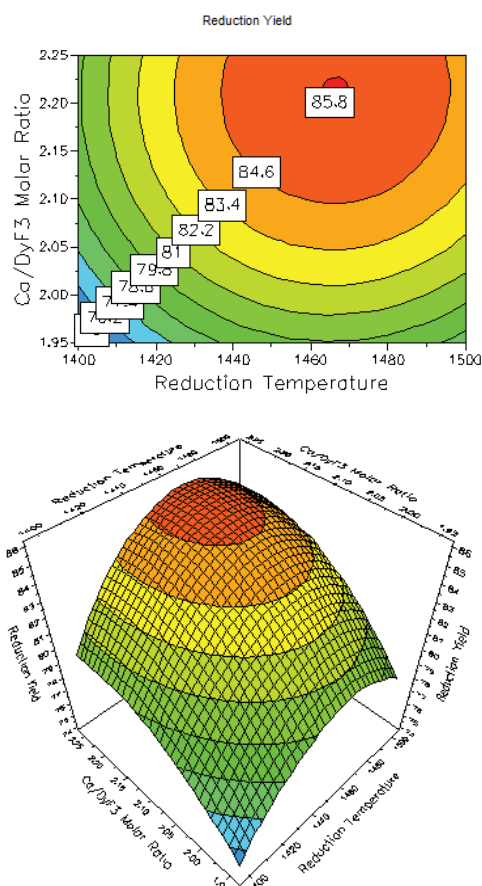


Fig. 6. Contours and response surface of the reduction yield of DyF_3 by metallic calcium vs reduction temperature and Ca/DyF_3 molar ratio at centre level of the reduction time.

iv. The contributions from the coefficients b_3 and b_3^2 at the low level ($X_3=-1$) and high level ($X_3=1$) on Y were calculated as $b_0-1.6$ and $b_0+0.4$, respectively, indicating that reduction time has an insignificant effect on the yield.

The comprehensive analysis of these independent variables' contributions to the response shows that reduction temperature (X_1) has the most significant effect on the reduction yield (Y). Therefore, optimal conditions for the calciothermic reduction of DyF_3 are primarily determined by careful control of the reduction temperature. Although the Ca/DyF_3 molar ratio (X_2) and reduction time (X_3) also influence the process, their effects are comparatively weaker. To achieve the best overall performance, a balanced optimisation of all independent variables should be pursued, taking their relative importance and interdependence into account.

3.3.2. Effect of factors on reduction yield

The influence of experimental factors on the reduction yield of DyF_3 using metallic calcium is shown in Figs. 5-7. The reduction temperature positively affects the yield within the range of 1400 to 1450°C. However, at temperatures

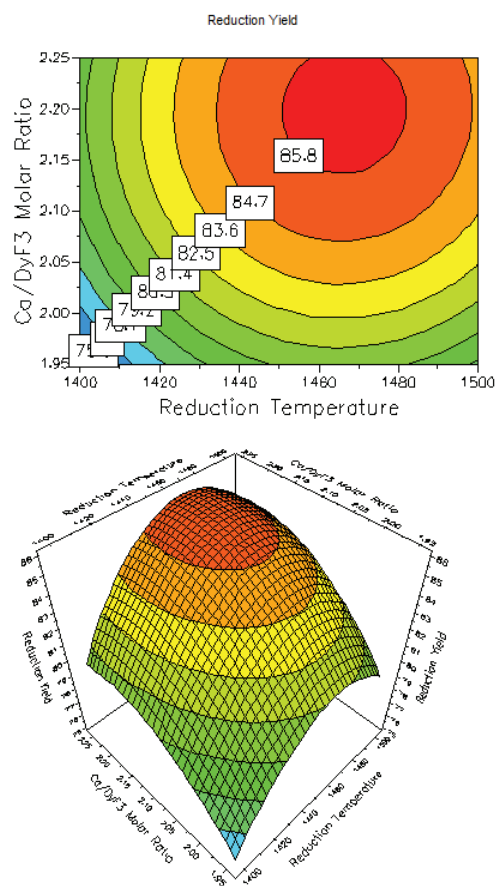


Fig. 7. Contours and response surface of the reduction yield of DyF_3 by metallic calcium vs. reduction temperature and Ca/DyF_3 molar ratio at a high level of the reduction time.

above 1450°C, the increase in yield is minimal, irrespective of the Ca/DyF_3 molar ratio or reduction time. A similar trend was observed for the effects of the Ca/DyF_3 molar ratio and reduction time on the yield, though the Ca/DyF_3 molar ratio had a stronger influence than reduction time.

Figure 8 provides a comprehensive visual overview of the effects of factors on the reduction yield. The linear positive effects of reduction temperature (X_1), time (X_3), and Ca/DyF_3 molar ratio (X_2) were quantified as 4.42 ± 1.27 , 2.44 ± 1.27 , and 5.90 ± 1.27 , respectively. Conversely, the quadratic negative effects of reduction temperature (X_1^2) and Ca/molar ratio (X_2^2) were -6.94 ± 2.50 and -3.94 ± 2.50 , respectively. The remaining factors, including the quadratic influence of reduction time (X_3^2), the interactive influences of the reduction temperature and time (X_1X_3), the influence of the reduction temperature and the Ca/DyF_3 molar ratio (X_1X_2), and the influence of the Ca/DyF_3 molar ratio and reduction time (X_2X_3) on the DyF_3 calciothermic reduction yield, were quantified as -0.02 ± 1.42 , 0.22 ± 1.42 , and -0.78 ± 1.42 , respectively. These influences exhibited larger uncertainties and were therefore deemed insignificant.

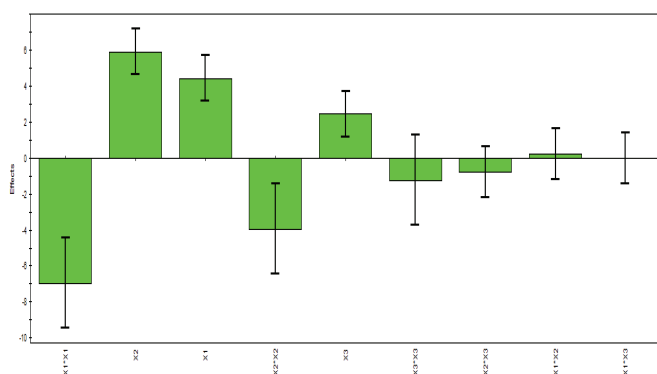


Fig. 8. The effects of terms on the reduction yield of DyF_3 by metallic calcium.

From these results, it can be concluded that reduction temperature exerts the strongest influence on the reduction yield, with values ranging from -6.94 to +4.42. The Ca/DyF_3 molar ratio has a weaker influence, ranging from -3.94 to +5.90, while reduction time has the weakest effect, at only +2.44. Combining the analysis of the contributions of the b_{ij} coefficients with the analysis of the influences of the X_i and X_{ij} factors, it can be concluded that the calcinothemic reduction of to produce metallic Dy is predominantly controlled by reduction temperature.

3.3.3. Optimising the calcinothemic reduction of DyF_3

To determine the most favourable conditions for achieving the highest yield in the calcinothemic reduction of DyF_3 , the RSM-CCF model was employed to explore desirable parameters within the design space. Using the RSM-CCF model in conjunction with MODDE 5.0 software, the optimal conditions for the calcinothemic reduction process were identified. These optimal parameters consist of a reduction temperature of 1466°C , a reduction time of 0.9 hours, and a Ca/DyF_3 molar ratio of 2.2. Under these conditions, the maximum reduction yield of using metallic calcium achieved was 86.2% (Table 5).

Table 5. Results of process optimisation and optimum levels of variables.

Reduction temperature ($^\circ\text{C}$)	Ca/DyF_3 molar ratio	Reduction time (hour)	Reduction yield	$\log(\text{D})$
1466,39	2,2034	0,9399	86,1725	-10
1466,25	2,2029	0,9381	86,1727	-10
1466,26	2,2011	0,9397	86,1725	-10
1480	2,25	1	85,662	-1,2129
1460	2,16	0,75	85,5144	-0,8992
1480	2,25	1	85,662	-1,2129
1470	2,25	0,75	85,6892	-1,2853
1450	2,16	1	85,6452	-1,1708

However, optimising the calcinothemic reduction process requires consideration of several key factors that can significantly influence the outcome. Striving for

the highest yield alone is insufficient, as other critical aspects such as energy consumption, chemical usage, and waste management must also be accounted for. Thus, the predictive model should not focus solely on maximising yield, but rather consider trade-offs associated with these factors. To achieve optimal overall performance, the model should incorporate variables that are adaptable to cost, yield, profitability, and other significant parameters. This approach not only predicts outcomes but also guides the process towards the best possible results while minimising adverse environmental and resource impacts.

Table 6. The calcinothemic reduction yield of DyF_3 calculated from Eq. (4).

No	X_1 ($^\circ\text{C}$)	X_2	X_3 (min)	Y (%)
1	1450	2.15	40	84.7
2	1460	2.10	40	84.2
3	1460	2.15	40	85.0
4	1470	2.10	40	84.2
5	1470	2.15	40	85.0
6	1450	2.10	50	84.7
7	1450	2.15	50	85.4
8	1460	2.10	50	85.0
9	1460	2.15	50	85.7
10	1470	2.10	50	85.0
11	1470	2.15	50	85.7

Table 6 presents the calcinothemic reduction yield of DyF_3 , calculated using Eq. (4). To optimise the performance of the calcinothemic reduction process, factors such as energy consumption, chemical usage, waste treatment, and the reduction yield must be carefully considered. Based on the results, it is recommended to set the reduction temperature between 1450 and 1460°C , with a reduction time of 50 minutes, and maintain a Ca/DyF_3 molar ratio of 2.15. This optimisation strategy, derived from the experimental data of samples 7 and 9 in Table 6, is expected to result in an efficient reduction process with minimal energy and chemical consumption, effective waste treatment, and a high reduction yield.

By carefully considering these factors, an optimised calcinothemic reduction process can be developed that meets both industrial and environmental standards. Following the identification of the optimised conditions for the calcinothemic reduction of DyF_3 , additional experiments were conducted to validate the model's accuracy in producing metallic Dy. The optimised parameters were applied in further experiments, and the resulting reduction yield was compared to the model's predicted yield. The outcomes were highly satisfactory, confirming the model's ability to accurately predict the reduction yield of DyF_3 for metallic Dy production. This successful validation represents a significant step towards the practical implementation of

the calciothermic reduction process on a larger scale, facilitating the preparation of metallic Dy with high yields while minimising energy and chemical usage.

4. Conclusions

The calciothermic reduction of DyF_3 to produce metallic Dy was examined using a response surface methodology (RSM) based on a central composite face (CCF) design. The developed RSM-CCF model demonstrated a strong correlation with the experimental results, underscoring its effectiveness for metallic Dy preparation. Analysis revealed that the reduction temperature had the most significant impact on the reduction yield, highlighting its critical role in the optimisation process.

Considering factors such as energy consumption, chemical usage, waste management, and reduction yield, the parameters for the calciothermic reduction were optimised. The optimal conditions identified were a reduction temperature range of 1450 to 1460°C, a reduction time of 50 minutes, and a Ca/ DyF_3 molar ratio of 2.15. Under these conditions, a maximum reduction yield of 85.7% was achieved.

The successful outcomes of the testing further validate the proposed model's effectiveness. This model offers promising potential for advancing the development of comprehensive processing technologies for the production of metallic Nd, Dy, Tb, and Y from Yen Phu xenotime concentrate.

CRedit author statement

Nguyen Trong Hung: Methodology, Conceptualisation, Idea, Writing - Reviewing and Editing; Nguyen Thanh Thuy: Investigation, Visualisation; Le Ba Thuan: Supervision, Methodology.

ACKNOWLEDGEMENTS

This research was financially supported by the Vietnam Ministry of Science and Technology under the projects 2022-2023 (code DTCB.11/19/VCNXH).

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] C.K. Gupta, N. Krishnamurthy (2005), *Extractive Metallurgy of Rare Earths*, CRC Press, 484pp.

[2] P. Zapp, J. Marx, A. Schreiber, et al. (2018), "Comparison of dysprosium production from different resources by life cycle assessment", *Resources, Conservation & Recycling*, **130**, pp.248-259, DOI: 10.1016/j.resconrec.2017.12.006.

[3] S. Hoenderdaal, L.T. Espinoza, F.M. Weidemann, et al. (2013), "Can a dysprosium shortage threaten green energy technologies?", *Energy*, **49**, pp.344-355, DOI: 10.1016/j.energy.2012.10.043.

[4] V.A. Ivanov, A.S. Dedyukhin, I.B. Polovov, et al. (2015), "Fabrication of rare-earth metals by metallothermic reduction: Thermodynamic modeling and practical realization", *AIP Conference Proceedings* 2015, DOI: 10.1063/1.5055106.

[5] C.K. Gupta, N. Krishnamurthy (2013), "Oxide reduction processes in the preparation of rare-earth metals", *Minerals & Metallurgical Processing*, **30**, pp.38-44, DOI: 10.1007/BF03402339.

[6] J.L. Moriarty (1968), "The industrial preparation of the rare earth metals by metallothermic reduction", *The Journal of The Minerals, Metals & Materials Society*, **20**, pp.41-45, DOI: 10.1007/BF03378760.

[7] B.J. Beaudry, K.A. Gschneidner (1978), "Chapter 2: Preparation and basic properties of the rare earth metals", *Handbook on the Physics and Chemistry of Rare Earths*, **1**, pp.173-232, DOI: 10.1016/S0168-1273(78)01006-5.

[8] H. Liu, Y. Zhang, Y. Luan (2020), "Research progress in preparation and purification of rare earth metals", *Metals*, **10**(10), DOI: 10.3390/met10101376.

[9] A. Mukherjee, A. Awasthi, N. Krishnamurthy (2016), "Studies on calcium reduction of yttrium fluoride", *Mineral Processing and Extractive Metallurgy*, **125**(1), pp.26-31, DOI: 10.1179/1743285515Y.0000000017.

[10] F.E. Block (1964), *Preparation of Yttrium and Rare Earth Metals by Metallothermic Reduction*, Thesis of Oregon State University, 118pp.

[11] T.M. Riedemann (2011), *High Purity Rare Earth Metals Preparation*, MPC Rare Earth Materials Section, <https://slideplayer.com/slide/2859004/>, accessed 7 January 2023.

[12] T.H. Nguyen, B.T. Le, T.T. Nguyen (2021), "Study on the fluorination of dysprosium oxide by ammonium bifluoride for the preparation of dysprosium fluoride", *Vietnam Journal of Science and Technology - MOST*, **63**(8), pp.9-13, DOI: 10.31276/VJST.63(8).09-13 (in Vietnamese).

[13] N.T. Hung, N.T. Thuy, L.B. Thuan, et al. (2021), "Anhydrous oxygen-free rare earth material preparation and characterization", *Materials Today Chemistry*, **22**, DOI: 10.1016/j.mtchem.2021.100608.

[14] T.H. Nguyen, T.T. Nguyen, B.T. Le, et al. (2022), "The wet preparation and dehydration of terbium fluoride salt for terbium metal processing", *Vietnam Journal of Science and Technology - MOST*, **64**(8), pp.47-52, DOI: 10.31276/VJST.64(8).47-52 (in Vietnamese).

[15] N.T. Hung, L.B. Thuan, T.C. Thanh, et al. (2020a), "Optimization of sulfuric acid leaching of a Vietnamese rare earth concentrate", *Hydrometallurgy*, **191**, DOI: 10.1016/j.hydromet.2019.105195.

[16] N.T. Hung, L.B. Thuan, T.C. Thanh, et al. (2020b), "Separation of thorium and uranium from xenotime leach solutions by solvent extraction using primary and tertiary amines", *Hydrometallurgy*, **198**, DOI: 10.1016/j.hydromet.2020.105506.

[17] N. Aoyagi, T.T. Nguyen, Y. Kumagai, et al. (2020), "Spectroscopic studies of mössbauer, infrared, and laser-induced luminescence for classifying rare-earth minerals enriched in iron rich deposits", *ACS Omega*, **5**(13), pp.7096-7105, DOI: 10.1021/acsomega.9b03247.

[18] N.T. Hung, L.B. Thuan, T.C. Thanh, et al. (2022), "Selective recovery of thorium and uranium from leach solutions of rare earth concentrates in continuous solvent extraction mode with primary amine N1923", *Hydrometallurgy*, **213**, DOI: 10.1016/j.hydromet.2022.105933.

Preliminary study on simultaneous adsorption of nitrate and phosphate using CaFe_2O_4 -modified biochar originated from lychee peels

Lan Huong Nguyen*, Tuan Hiep Tran

Ho Chi Minh City University of Industry and Trade (HUIT), 140 Le Trong Tan Street, Tay Thanh Ward, Tan Phu District, Ho Chi Minh City, Vietnam

Received 20 October 2023; revised 20 November 2023; accepted 22 January 2024

Abstract:

In this study, lychee peels derived-biochar modified with CaFe_2O_4 NPs (MLPB) were successfully fabricated to adsorb simultaneously both nitrate (NO_3^-) and phosphate (PO_4^{3-}) from aqueous solution. The preliminary investigation on the effect of operational parameters, including pyrolysis temperature and modification ratio on adsorption of nitrate (NO_3^-) and phosphate (PO_4^{3-}) in single-component and bi-component systems was performed. The characteristics of biochar (PLPB) and magnetic modified biochar (MLPB) were evaluated through the specific surface area, SEM, EDS and mapping, FTIR, and XRD. The primary results showed the adsorption of NO_3^- and PO_4^{3-} onto PLPB reached a peak at the pyrolysis temperature of 450°C with a NO_3^- and PO_4^{3-} adsorption capacity of 12.8 and 10.9 mg/g, respectively, in the single-component system while the adsorption capacity of NO_3^- and PO_4^{3-} in the bi-component system was 12.5 and 10.6 mg/g, respectively. The removal efficiency of NO_3^- and PO_4^{3-} maximised at a modification ratio of 5%. At this modification ratio, the adsorption capacity of NO_3^- and PO_4^{3-} in the single-component reached, respectively, 24.0 and 21.5 mg/g while the adsorption of NO_3^- and PO_4^{3-} was 24.0 and 22.6 mg/g in the bi-component system, respectively. The preliminary study on analysing characteristics of the adsorbent confirmed that the removal of NO_3^- and PO_4^{3-} was through ligand exchange and surface complexation mechanisms. The findings indicated that the MLPB was a feasible and fully promising adsorbent in the simultaneous removal of NO_3^- and PO_4^{3-} from the aqueous solution.

Keywords: calcium spinel ferrites, lychee peel biochar, magnetic modified biochar, nitrate, phosphate.

Classification numbers: 2.2, 5.3

1. Introduction

In recent years, with the massive growth of industry and agriculture, contamination of receiving water bodies by the excessive discharge of nitrate (NO_3^-) and phosphate (PO_4^{3-}) has become a vital issue worldwide. NO_3^- and PO_4^{3-} primarily originate from fertiliser residues in crop growth [1] and effluents from industries such as seafood processing and fertiliser production. Water bodies receiving excessive amounts of both NO_3^- and PO_4^{3-} simultaneously trigger eutrophication with algal blooms, leading to the depletion of dissolved oxygen in water, impairment of the self-cleaning capacity of receiving sources, and the death of aquatic organisms [1, 2]. Additionally, nitrate-contaminated drinking water can cause serious diseases such as methemoglobinemia, cancer, and blue baby syndrome [1, 3]. Therefore, it is essential to simultaneously remove both NO_3^- and PO_4^{3-} from wastewater before discharging into water bodies.

Techniques for removing NO_3^- and PO_4^{3-} include biological processes using microalgae *Chlorella vulgaris* [4] and pilot-scale SBR for nitrate removal [5], ion exchange [6, 7], chemical precipitation [8, 9], and adsorption [10, 11]. However, biological processes and chemical precipitation are not suitable for removing NO_3^- and PO_4^{3-} at very low concentrations and require a long biomass residence time [1]. In comparison, adsorption is considered a simple, low-cost, and highly efficient technique for removing NO_3^- and PO_4^{3-} at trace concentrations [1-3, 11].

Biochar is a product of the pyrolysis process of carbon-based materials, which has garnered more attention in the treatment of various pollutants, such as nitrate and phosphate. Especially, biochar derived from agro-industrial by-products has encouraged product development towards circular economic practices. Additionally, the utilisation of solid waste to fabricate biochar helps effectively manage solid waste and generate useful materials for pollutant removal from wastewater. To date, a large amount of agro-

*Corresponding author: Email: huongnl@huit.edu.vn

industrial by-products has been used to produce biochar for treating both NO_3^- and PO_4^{3-} from wastewater, such as biochar from sugarcane bagasse for NO_3^- adsorption [2]; MgO-modified magnetic biochar derived from anaerobic digestion residue for PO_4^{3-} removal [12]; Gigantochloa bamboo-derived biochar [13]; Copper modified activated bamboo charcoal for adsorption of trace metals and ruthenium dye wastes [14, 15], and activated carbon originated from musk-melon peel composited with ZnFe_2O_4 for simultaneous adsorption of both NO_3^- and PO_4^{3-} from wastewater [16]. Lychee peels are grown popularly in the Northern of Vietnam with an average yield of 40,000 tons/year [17]. The amount of lychee peel discharged makes up about 15% of the fresh weight of lychee. Besides, lychee peel has main components of cellulose, lignin which are suitable as potential carbon biomass for producing biochar [18].

However, the main drawback of applying agro-industrial by-products-derived biochars for pollutant removal is their low adsorption capacity towards pollutants due to their low specific surface area and inherent negatively charged surface. Thus, the biochar needs to be modified by chemical-physical methods to enhance pollutant adsorption capacity and efficiency. Among modification methods, loading spinel ferrite nanoparticles (MFe_2O_4) has proven to be highly efficient and overcome some limitations of biochars by increasing specific surface area (S_{BET}), well-developing a porous structure, and decreasing nanoparticle agglomeration. Specifically, L. Ma, et al. (2018) [8] reported magnesium ferrite (MgFe_2O_4)/biochar magnetic composites (MFB-MCs) exhibited superior PO_4^{3-} adsorption capacity over biochar due to enlarged porosity along with increased available binding sites; adsorption of Cr(VI) onto MnFe_2O_4 reached a high value of 89.18 mg/g [19]; simultaneous removal of NO_3^- and PO_4^{3-} from wastewater by biochar/ ZnFe_2O_4 composite achieved excellent adsorption capacities of 75.58 and 91.80 mg/g, respectively.

In the present work, lychee peels-originated biochar modified by spinel ferrite nanoparticles (CaFe_2O_4) was primarily studied for the simultaneous adsorption of both NO_3^- and PO_4^{3-} from wastewater in batch-mode tests. The primary study aims to investigate the effect of operational parameters, consisting of pyrolysis temperature, modification ratios between biochar and CaFe_2O_4 NPs on the simultaneous adsorption capacity and efficiency of NO_3^- and PO_4^{3-} from wastewater. Physical-chemical characteristics of biochar and modified biochar before and after loading both NO_3^- and PO_4^{3-} were determined using various analytical techniques such as nitrogen adsorption-desorption, scanning electron microscope (SEM), Fourier

transform infrared (FTIR), XRD, and EDS and mapping. Besides, the plausible mechanisms of NO_3^- and PO_4^{3-} adsorption onto modified biochar were briefly discussed.

2. Materials and methods

2.1. Chemicals and materials

The chemicals used for experiments, including KH_2PO_4 , NaNO_3 , KOH, CaCl_2 , FeCl_3 , glycerol, HCl and citric acid, were purchased from Kyamata (Japan). All chemicals were of analytical grade. Lychee peels were obtained from Bac Giang province, Vietnam.

The simulated wastewater containing NO_3^- and PO_4^{3-} was prepared by dissolving precisely 4.387 g KH_2PO_4 and 6.071 g NaNO_3 in 1000 ml double distilled water (DDW) separately to reach a stock solution of 1000 mg/l. The experimental solution was prepared by diluting the stock solution to the desired concentrations.

2.2. Preparation of biochars

2.2.1. Fabrication of biochar (PLPB)

After collection, the lychee peels (LPs) were repeatedly washed using distilled water to remove all impurities attached to the LP's surface. Then, the LPs were exposed under sunlight. The dried LPs were then blended using blender and sieved to achieve a diameter of 60 μm . The blended LPs were impregnated in KOH 0.1 M by a homogeniser agitator to enrich oxygen-containing functional groups. The KOH-soaked LP was then filtered and repeatedly cleaned by DDW until pH reached 7.0. The LP was continuously dried at 105°C for many hours to obtain the dried LP powder. The dried LP powder was continuously pyrolysed in temperature ranges from 300-550°C (interval=50°C) with a heating rate of 10°C for 2 h. Finally, the obtained black solid was denoted as lychee peel-derived biochar (PLPB). The biochars obtained by pyrolysing at various temperatures were used for the next experiments to determine the optimal pyrolysis temperature for the fabrication of biochar, which was then used to modify with CaFe_2O_4 NPs in the following steps. The scheme describing the fabrication procedure of biochar from lychee peels was demonstrated in Fig. 1A.

2.2.2. Fabrication of CaFe_2O_4 NPs

The spinel ferrite CaFe_2O_4 nanoparticles were formed using the sol-gel method as described in our previous study [3]. Briefly, the procedure for the fabrication of CaFe_2O_4 NPs can be described as follows: firstly, a 500 ml beaker containing 100 ml DDW, and 93 g of citric acid was homogenised and placed on a magnetic agitator at 120 rpm, 60°C for 15 min. Next, a certain amount of CaCl_2 and

FeCl₃ at a molar ratio of 0.0015:0.001 in ethylene glycol (C₂H₆O₂) was added to the above beaker. The mixture was then agitated at 120 rpm at 80°C to achieve a completely dissolved solution. The mixture was then dried continuously at 105°C for 12 h to form a gel form. The gel was then calcined at 300°C for 3 h and further calcined at 1000°C for the next 2 h. Finally, the obtained black solid was CaFe₂O₄ NPs.

2.2.3. The modification procedure of biochar with CaFe₂O₄ NPs

The biochar was modified with CaFe₂O₄ NPs using the wet impregnation method. In the first step of the procedure, a certain amount of CaFe₂O₄ NPs at ratios of 1, 3, 5, 7, and 9% (w/w) was added into a certain volume of methanol in a 500 ml beaker. The beaker was then sonicated at 80°C for 30 min. Next, a certain amount of biochar (PLPB) was also added to the above beakers. The beakers were then stirred at 120 rpm for 2 h to ensure all CaFe₂O₄ NPs were loaded onto the biochar's surface. The mixture was then centrifuged to collect the solid part. The obtained solid was cleaned with DDW and dried at 105°C for 2 h. The resulting product was magnetic modified biochar and labelled as MLPB-1; MLPB-3; MLPB-5; MLPB-7 and MLPB-9. Together with biochar (PLPB), the biochars at various modification ratios were used for simultaneous adsorption experiments of both NO₃⁻ and PO₄³⁻ to choose the optimal modification ratio.

2.3. Adsorption experiment design

All adsorption tests were conducted in batch-mode using Erlenmeyer flasks containing 100 ml NO₃⁻ and PO₄³⁻ (50 mg/l for each ion), 0.4 g of adsorbent with agitation at 150 rpm at room temperature (25±2°C) for 3 h of reaction time. All tests were performed in triplicate. The experimental data were expressed as the average value ± standard deviation. The adsorption capacity and performance of NO₃⁻ and PO₄³⁻ onto biochars were calculated using the following equations:

$$q = \frac{(C_o - C_e)V}{M} \quad (1)$$

$$\%H = \frac{(C_o - C_e)}{C_o} \times 100\% \quad (2)$$

where q (mg/g) and $\%H$ are the adsorption capacity and efficiency of nitrate and phosphate onto biochars, respectively; C_o and C_e are concentrations of NO₃⁻ and PO₄³⁻ (mg/l) at initial and equilibrium, respectively; V is the solution volume (l); and M is the mass of applied biochar (g).

2.4. Analysis method

The physical-chemical characteristics of adsorbents before and after the adsorption process were determined. Specifically, the nitrogen adsorption-desorption isotherm was applied to measure the textural properties of materials at 77 K using Quantachrome Instruments version NOVA4000e. A D8 Phaser X-ray diffractometer was used to analyse the crystalline phase structure of adsorbents with a scanning angle of 10-80°. The surface morphology was observed by a scanning electron microscope (SEM). The elements' distribution on the adsorbents was evaluated via energy-dispersive X-ray spectroscopy (EDS) and mapping using S-4800, Hitachi, Japan.

The residual NO₃⁻ and PO₄³⁻ concentrations in the solution were measured according to the standard method [20] by spectrophotometric method using Spectrophotometer photoLab®7600 UV-VIS (WTW, Germany). The NO₃⁻ was analysed according to 4500-NO₃⁻ - nitrogen at $\lambda=415$ nm with sulfosalicylic acid. The PO₄³⁻ was measured according to 4500 P-B&E using ammonium molybdate at $\lambda=880$ nm. Measurement of NO₃⁻ was based determination of the UV-Vis absorption spectrum of the yellow compound formed by the reaction of 10 g/l sulfosalicylic acid (formed by adding sodium salicylate and sulfuric acid to the test sample) with nitrate and subsequent treatment with alkali. Afterwards, measure the absorbance of the solution at 415 nm. Meanwhile, the phosphate was determined by Ascorbic Acid Method, 4500 P-B&E, Standard Methods for the Examination of Water and Wastewater 23rd edition. In this procedure, ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid that is reduced to intensely coloured molybdenum blue by ascorbic acid. Afterwards, measure the absorbance of the solution at 880 nm. The pH meter (HANNA, pH HI 2002-02, Romani, American) was used to determine solution pH values.

3. Results and discussion

3.1. Effect of pyrolysis temperature on the adsorption of NO₃⁻ and PO₄³⁻

The tests to evaluate the effect of pyrolysis temperature on the adsorption of NO₃⁻ and PO₄³⁻ in the single-system and bi-component system were conducted with fixing initial concentrations of NO₃⁻ and PO₄³⁻ at 50 mg/l, adsorbent dosage of 4.0 g/l with varying pyrolysis temperatures in the range from 300-550°C. The NO₃⁻ and PO₄³⁻ adsorption capacities on the biochar at different pyrolysis temperatures in both single- and bi-component systems are illustrated in Fig. 1.

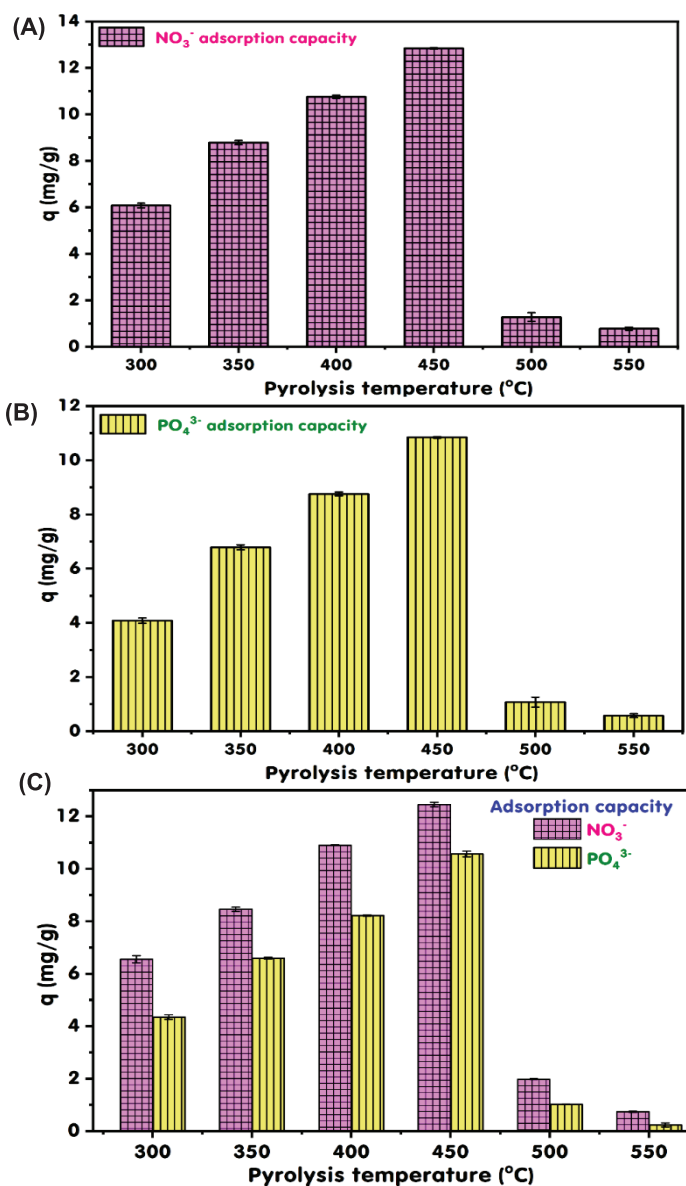


Fig. 1. Effect of pyrolysis temperature on NO_3^- and PO_4^{3-} by (A, B) single-component and (C) bi-component systems. Experimental conditions: NO_3^- and PO_4^{3-} (50 mg/l for each ion), 0.4 g of adsorbent, pH of 7.0 with varying of pyrolysis temperatures from 300-500°C.

The data in Fig. 1 demonstrate that the adsorption of NO_3^- and PO_4^{3-} strongly depended on the pyrolysis temperature [21, 22] in both single- and bi-component systems, and there was no significant difference in NO_3^- and PO_4^{3-} adsorption capacity in the single-component system and bi-component system. The NO_3^- and PO_4^{3-} adsorption capacities increased when the pyrolysis temperature rose. The NO_3^- and PO_4^{3-} adsorption capacity increased, respectively, from 6.08 to 12.84 mg/g and from 4.08 to 10.84 mg/g in the single-component system (Figs. 1A and 1B) and from 6.56 to 12.45 mg/g and from 4.36 to 10.57 mg/g in the bi-component

system when the pyrolysis temperature was raised from 300-450°C. The NO_3^- and PO_4^{3-} adsorption maximised at a pyrolysis temperature of 450°C with an adsorption capacity of 12.84 and 10.84 mg/g, respectively, in the single-component system and 12.45 and 10.57 mg/g, respectively, in the bi-component system. However, a further increase in pyrolysis temperature from 500-550°C led to a strong drop in NO_3^- and PO_4^{3-} adsorption capacity on the biochar. The results were because a very low or very high temperature is not favourable for the production of biochar because samples seem to have turned into ash. A very low temperature causes incomplete pyrolysis of carbon constituent in the material while a very high temperature leads to ashing of the carbon composition in the material, thus the adsorption capacity was decreased [23]. The highest adsorption capacity of NO_3^- and PO_4^{3-} at a pyrolysis temperature of 450°C was due to a well-development in porous property and S_{BET} of biochar at a suitable pyrolysis temperature [21, 24]. Therefore, the optimal pyrolysis temperature for the fabrication of biochar was chosen as 450°C in this work. Fig. 2 demonstrated biochar product images were produced at various pyrolysis temperatures.



Fig. 2. Images of the biochar products obtained at different pyrolysis temperatures.

3.2. Effect of modification ratio on NO_3^- and PO_4^{3-} adsorption in the single-component and bi-component systems

In this test, the lychee peels-derived biochar pyrolysed at 450°C was used to modify with CaFe_2O_4 NPs at various ratios of 0, 1, 3, 5, 7, and 9% (w/w) to promote NO_3^- and PO_4^{3-} adsorption capacity in both single-component and bi-component systems. The tests were performed by fixing initial concentrations of NO_3^- and PO_4^{3-} at 50 mg/l, biochar dosage of 4.0 g/l, and varying modification ratios of 0, 1, 3, 5, 7, and 9% (w/w) between biochar (PLPB) and CaFe_2O_4 NPs.

It can be seen from the data in Fig. 3 that the removal of both NO_3^- and PO_4^{3-} had a negligible difference in the single-component and bi-component systems, proving that there was no competition of binding sites for adsorption of these ions. When the amount of CaFe_2O_4 NPs loaded on the biochar increased from 1 to 5%, the NO_3^- and PO_4^{3-} adsorption capacity and performance onto modified biochar in both systems increased. Specifically, the adsorption capacity of NO_3^- and PO_4^{3-} increased from 13.79 to 24.05 mg/g and from 12.39 to 21.52 mg/g, respectively, in the single-component system. The NO_3^- and PO_4^{3-} adsorption

capacity in the bi-component system increased from 15.46 to 24.05 mg/g and from 14.60 to 22.57 mg/g, respectively, when the modification ratio increased from 1-5%. The adsorption capacity and performance of both NO_3^- and PO_4^{3-} reached a peak at a modification ratio of 5% in both single- and bi-component systems. At a modification ratio of 5%, the NO_3^- adsorption capacity was 24.05 mg/g with a NO_3^- removal efficiency of 95.36%, and the PO_4^{3-} adsorption capacity reached 21.52 mg/g with a removal efficiency of 87.09% in the single system. Similarly, the NO_3^- and PO_4^{3-} adsorption capacities in the bi-component system reached 24.05 mg/g and 22.57 mg/g, respectively. The NO_3^- and PO_4^{3-} adsorption capacity in both single- and bi-component systems onto biochar was the lowest. The NO_3^- and PO_4^{3-} adsorption capacities onto modified biochar at a ratio of 5% were higher than that of biochar (PLPB) by many times.

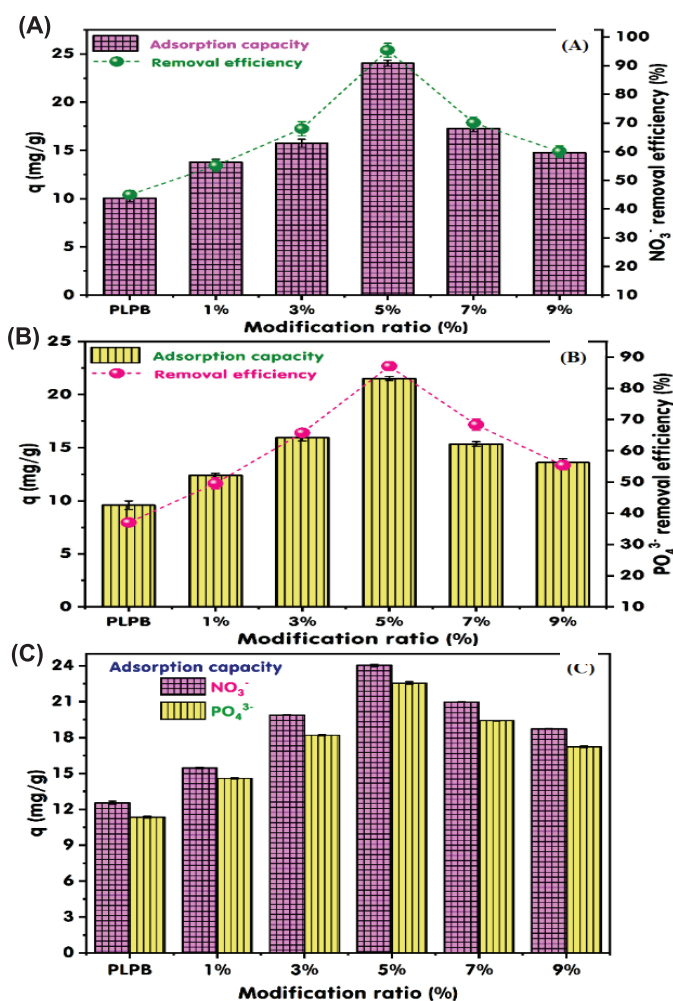


Fig. 3. NO_3^- and PO_4^{3-} adsorption capacity and efficiency in the (A, B) single-component and (C) bi-component systems. Experimental conditions: NO_3^- and PO_4^{3-} (50 mg/l for each ion), 0.4 g of adsorbent, pH of 7.0 with varying of modification ratios between biochar and CaFe_2O_4 from 1-9%.

However, a further growth in the loading ratio of nanoparticles onto biochar led to a moderate drop in NO_3^- and PO_4^{3-} adsorption capacity and efficiency in both single- and bi-component systems. The removal of NO_3^- was 17.28 and 14.77 mg/g, which decreased by 6.77 and 9.30 mg/g, while removal of PO_4^{3-} reached 15.34 and 13.59 mg/g, which dropped by 6.18 and 7.43 mg/g when the modification ratio increased from 5 to 7 and 9% in the single-component system. Meanwhile, the simultaneous adsorption of both NO_3^- and PO_4^{3-} in the bi-component system saw a similar trend. To be specific, the adsorption of NO_3^- dropped by 3.07 and 5.33 mg/g, and PO_4^{3-} adsorption fell by 3.14 and 5.33 mg/g when the modification ratio increased from 5 to 7 and 9%.

The results showed that there was an optimal modification ratio in which the NO_3^- and PO_4^{3-} adsorption capacity and efficiency in both single- and bi-component systems were the highest. A very low or very high loading ratio was not beneficial for the adsorption of NO_3^- and PO_4^{3-} onto biochar. A very low loading ratio of nanoparticles led to a lack of binding sites on the biochar's surface, while a very high loading ratio caused overlapping pores on the biochar's surface, thus the adsorption capacity was dropped. The similar results were also obtained by previous scholars. For instance, B. Wu, et al. (2017) [25] used magnetic $\text{La}(\text{OH})_3/\text{Fe}_3\text{O}_4$ nanocomposites with varied La-to-Fe mass ratios of 1:1, 2:1, 4:1, and 5:1 for adsorption of PO_4^{3-} and the result showed that La-to-Fe mass ratio of 4:1 had the highest removal efficiency of PO_4^{3-} . A low or high ratio of La was not favourable for adsorption of PO_4^{3-} . H.H.P. Quang, et al. (2022) [26] studied adsorption of NO_3^- onto drinking water sludge (DWS) coated by ZrO_2 nanoparticles ($\text{DWS}@ZrO_2$) and results indicated that either low (0.05 M) or high ZrO_2 concentrations (0.3 and 0.5 M) had a negative effect on the NO_3^- adsorption capacity. The NO_3^- adsorption capacity maximised at coating ZrO_2 concentration of 0.1 M. This result was because the limited active sites on the surface of adsorbents at low concentration of ZrO_2 caused a lack of adsorption sites for ion NO_3^- , while a high ZrO_2 concentration led to negligible active adsorption sites on the surface of adsorbents for nitrate adsorption, resulting in a drop in nitrate adsorption capacity. From the above discussions, a loading ratio of 5% CaFe_2O_4 NPs was selected to modify lychee peels-derived biochar for the adsorption promotion of both NO_3^- and PO_4^{3-} in single- and bi-component systems in this work.

3.3. Adsorbent characteristics and adsorption mechanism briefly discussion

In this present work, the chemical-physical properties of biochar (PLPB) and modified biochar at the optimal ratio of 5% (MLPB-5) were assessed to briefly determine NO_3^- and PO_4^{3-} adsorption mechanisms in the bi-component system.

Table 1. Nitrogen adsorption-desorption isotherm data of PLPB, MLPB-5 before adsorption, PLPB; MLPB-5 after adsorption in bi-component system.

	Unit	Before adsorption		After adsorption	
		PLPB	MLPB-5	PLPB	MLPB-5
S_{BET}	m^2/g	1.554	2.618	0.616	1.183
V_{pore}	cm^3/g	0.006	0.007	0.004	0.005
D_{pore}	nm	4.117	4.153	3.535	4.111

As can be seen from the data in Table 1, the biochar was modified by CaFe_2O_4 NPs, witnessing a growth in specific surface area by about 1.7 times. Moreover, the loading of CaFe_2O_4 NPs also led to a well-developed porous structure of the modified biochar. This result was due to the occurrence of both Ca and Fe on the modified biochar's surface. Similar results were also indicated in previous works. For example, the loading of MgFe_2O_4 onto biochar derived from *Undaria pinnatifida* roots for the adsorption of PO_4^{3-} caused an increase in the S_{BET} of the modified biochar (MFB-MCs) by 6.12 times [27]. The S_{BET} of ZrO_2 -modified biochar also increased by 2.81 times compared with biochar [26]. After the adsorption of both NO_3^- and PO_4^{3-} in the bi-component system, the S_{BET} of NO_3^- and PO_4^{3-} -laden PLPB and NO_3^- and PO_4^{3-} -laden MLPB-5 decreased due to filling porous holes on the biochar's surface by several NO_3^- and PO_4^{3-} ions. Similar results were also reported by previous research [1, 25, 26].

However, the S_{BET} data of biochars in Table 1 also indicated that both PLPB and MLPB-5 belong to non-porous material with a low S_{BET} . Thus, the NO_3^- and PO_4^{3-} adsorption by pore-filling mechanism was negligible in this study.

The surface morphology of PLPB and MLPB-5 before and after simultaneous adsorption of NO_3^- and PO_4^{3-} in the bi-component system are presented in Fig. 4.

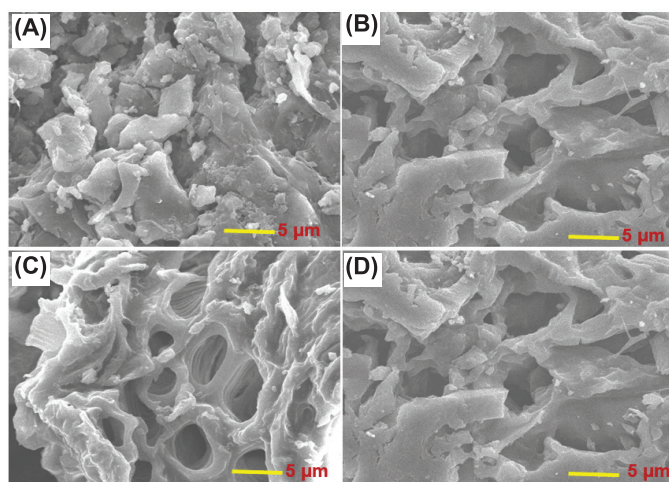


Fig. 4. SEM images of (A) PLPB and (C) MLPB-5 before adsorption; (B) PLPB and (D) MLPB-5 after adsorption in a bi-component system.

From Fig. 4A, it is evident that the PLPB before adsorption had a heterogeneous surface with a rough structure. Several tiny fine pores were observed in the SEM image of MLPB-5 before adsorption, confirming that there was an amount of CaFe_2O_4 NPs stacked onto the porous surface of biochar in the form of irregular flakes (Fig. 4C). Fig. 4C also showed MLPB-5 had a porous structure with several scroll layers on the surface. After adsorption, the porous structure on both PLPB and MLPB-5 was broken and blocked with NO_3^- and PO_4^{3-} ions. The result suggested that the Ca^{2+} and Fe^{2+} on the biochar's surface reacted with nitrate and phosphate through chemical or physical adsorption [2].

Based on EDS analysis results (Fig. 5), the biochar contained C, O, Mg, S, K and Ca in its constituent (Fig. 5A). After being loaded by CaFe_2O_4 NPs, there was an occurrence of Ca and Fe with a high amount in the modified biochar composition, proving that the CaFe_2O_4 NPs were successfully loaded on the biochar's surface (Fig. 5C).

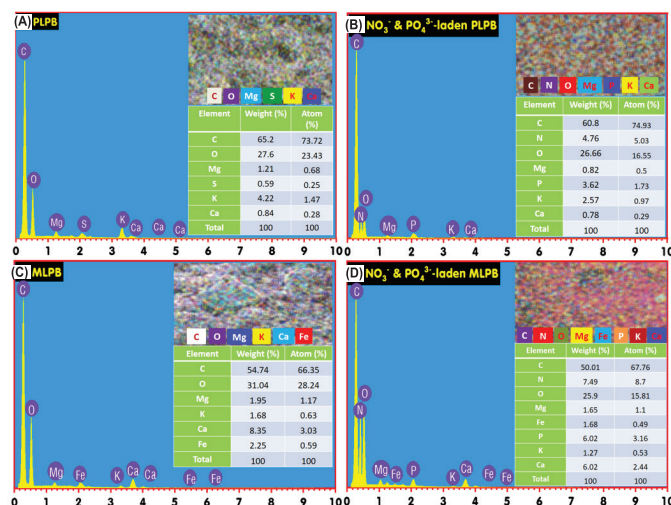


Fig. 5. EDS spectrums and elements mapping of (A) PLPB and (C) MLPB-5 before adsorption; and (B) PLPB and (D) MLPB-5 after adsorption in a bi-component system.

Especially, after adsorption, both N and P were present in the biochars' constituent, confirming that both NO_3^- and PO_4^{3-} ions were successfully adsorbed by PLPB and MLPB-5. With the same initial concentration of pollutants, the adsorbed amount of N and P on the MLPB-5 was higher than that of the PLPB by about 2 times, indicating that the NO_3^- and PO_4^{3-} adsorption capacity on the modified biochar was higher than that of biochar.

The XRD patterns of biochar and modified biochar before and after NO_3^- and PO_4^{3-} adsorption in the bi-component system are demonstrated in Fig. 6A. The successful loading of both Ca and Fe on MLPB-5's surface was also confirmed by the XRD pattern (Fig. 6A). As indicated in Fig. 6B, the biochar (PLPB) before and after adsorption exhibited an amorphous structure. This was also an inherent property of biochar

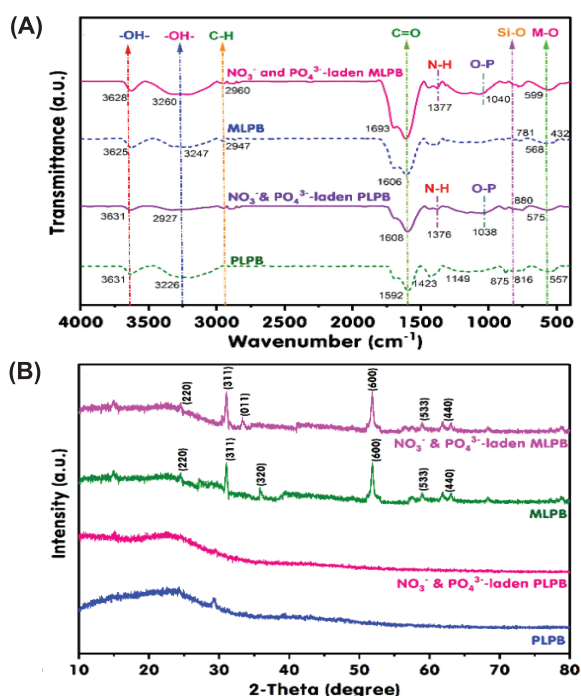


Fig. 6. FTIR of PLPB and MLPB-5 (A) XRD of PLPB and (B) MLPB-5 in a bi-component system.

produced from carbon-based materials. On the other hand, the CaFe_2O_4 -modified biochar expressed a nanocrystalline structure both before and after adsorption with the appearance of typical characteristic peaks of CaFe_2O_4 . All these typical peaks were well agreed with the standard CaFe_2O_4 spectra (JCPDS Card No. 74-2136) [3]. The 2θ values at 26.2° , 33.6° , 39.5° , 52.7° , 61.2° , and 67.7° corresponded with the typical crystal planes of calcium ferrite with good purity of CaFe_2O_4 at (220), (311), (320), (600), (533), and (440) planes, confirming that CaFe_2O_4 nanoparticles were successfully deposited on the PLPB's surface. In combination with data in Fig. 5 which demonstrated the distribution of Fe, Ca, O, N and P in the modified biochar constituent, leading to the formation of Fe-O-P, Ca-O-P, Fe-O-N and Ca-O-N complexes on the biochar's surface by covalent binding of nitrate and phosphate ions with -OH functional groups, confirming the surface complexation mechanism contributed to the removal of both NO_3^- and PO_4^{3-} by MPLP. The surface complexation mechanism was also confirmed by FTIR data (Fig. 6A) by the formation of complexes of P-O-M and N-H groups. Similar results were achieved by previous studies [26, 28].

The FTIR analysis revealed dense peaks density at 3228 and 3226 cm^{-1} in biochars before adsorption, indicating the presence of -OH stretching and vibration of -OH groups [2, 26]. However, the density of -OH groups decreased in NO_3^- and PO_4^{3-} -laden biochars, suggesting that the -OH groups participated in the adsorption process. Moreover, peaks fluctuating around 2947 and 2940 cm^{-1} were attributed to

C-H groups in biochar. The peaks density around 1592 and 1608 cm^{-1} corresponded to C=O groups. Notably, the occurrence of new peaks at 1376 and 1377 cm^{-1} after adsorption may indicate band vibration of O-N, while peaks at 1038 and 1040 cm^{-1} were associated with N-H groups. This result confirmed chemical/physical interactions between the biochars' surface and NO_3^- and PO_4^{3-} during the adsorption process, demonstrating successful adsorption of both ions onto the biochars. Additionally, peaks vibrating around 781 cm^{-1} to 880 cm^{-1} were attributed to Si-O bonds. The bands at 432 , 575 , and 599 cm^{-1} denoted the stretching vibration of M-O, P-O-M, and N-O-M ($\text{M}=\text{Fe}, \text{Ca}$), respectively. Analysis of the FTIR data indicated a ligand exchange reaction occurred between -OH groups on the surface-modified biochar and NO_3^- and PO_4^{3-} anions during the adsorption process. Thus, ligand exchange was identified as the main adsorption mechanism of NO_3^- and PO_4^{3-} anions onto modified biochar in the bi-component system.

In summary, based on the above-discussed physical-chemical characteristics of biochars, it can be concluded that the removal of both NO_3^- and PO_4^{3-} in the bi-component system occurred via surface complexation and ligand exchange mechanisms.

4. Conclusions

This study successfully developed a low-cost adsorbent from waste lychee peels and modified it with CaFe_2O_4 NPs for the removal of both NO_3^- and PO_4^{3-} from wastewater. The optimal pyrolysis temperature for fabricating biochar was determined to be 450°C . Furthermore, the results indicated that a modification ratio of 5% CaFe_2O_4 NPs with biochar provided the highest NO_3^- and PO_4^{3-} adsorption capacity and efficiency in both single- and bi-component systems, with negligible differences observed in the adsorption performance between the single-component and bi-component systems. The NO_3^- and PO_4^{3-} adsorption capacities onto MLPB were consistently higher than those onto PLPB, approximately double. Analysis of the physical-chemical properties data of biochars before and after adsorption primarily determined that the NO_3^- and PO_4^{3-} adsorption mechanisms were through surface complexation and ligand exchange. These findings suggest that CaFe_2O_4 -modified biochar holds significant promise for the simultaneous removal of both NO_3^- and PO_4^{3-} from wastewater. Moreover, the spent adsorbent containing NO_3^- and PO_4^{3-} can be utilised as a slow fertiliser for plant growth.

CRedit author statement

Lan Huong Nguyen: Supervision, Original draft preparation, Writing - Reviewing and Editing, Conceptualisation, Methodology, Software; Tuan Hiep Tran: Visualisation, Investigation, Data curation.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] W. Yang, X. Shi, J. Wang, et al. (2019), "Fabrication of a novel bifunctional nanocomposite with improved selectivity for simultaneous nitrate and phosphate removal from water", *ACS Appl. Mater. Interfaces*, **11**(38), pp.35277-35285, DOI: 10.1021/acsami.9b08826.
- [2] L.D.H. Hafshejani, A. Hooshmand, A.A. Naseri, et al. (2016), "Removal of nitrate from aqueous solution by modified sugarcane bagasse biochar", *Ecol. Eng.*, **95**, pp.101-111, DOI: 10.1016/j.ecoleng.2016.06.035.
- [3] M.T. Le, X.H. Nguyen, T.P. Nguyen, et al. (2023), "Lychee peels-derived biochar-supported CaFe_2O_4 magnetic nanocomposite as an excellent adsorbent for effective removal of nitrate and phosphate from wastewater", *J. Environ. Chem. Eng.*, **11**(5), DOI: 10.1016/j.jece.2023.110991.
- [4] M.B. Sabeti, M.A. Hejazi, A. Karimi (2019), "Enhanced removal of nitrate and phosphate from wastewater by *Chlorella vulgaris*: Multi-objective optimization and CFD simulation", *Chinese J. Chem. Eng.*, **27**(3), pp.639-648, DOI: 10.1016/j.cjche.2018.05.010.
- [5] D. Scaglione, G. Tornotti, A. Teli, et al. (2013), "Nitrification denitrification via nitrite in a pilot-scale SBR treating the liquid fraction of co-digested piggy/poultry manure and agro-wastes", *Chem. Eng. J.*, **228**, pp.935-943, DOI: 10.1016/j.cej.2013.05.075.
- [6] S. Guida, G. Rubertelli, B. Jefferson, et al. (2021), "Demonstration of ion exchange technology for phosphorus removal and recovery from municipal wastewater", *Chem. Eng. J.*, **420**, DOI: 10.1016/j.cej.2021.129913.
- [7] T.E. Bektaş, B.K. Ugürluoğlu, B. Tan (2021), "Phosphate removal by ion exchange in batch mode", *Water Pract. Technol.*, **16**(4), pp.1343-1354, DOI: 10.2166/wpt.2021.072.
- [8] L. Ma, S. Yuan, F. Ji, et al. (2018), "Ammonia and phosphorous precipitation through struvite crystallization from swine wastewater with high suspended solid", *Desalin. Water Treat.*, **116**, pp.258-266, DOI: 10.5004/dwt.2018.22589.
- [9] M.M. Maroneze, L.Q. Zepka, J.G. Vieira, et al. (2014), "Phosphorus removal technology: Management of the element in industrial waste", *Rev. Ambient. Água*, **9**(3), pp.445-458, DOI: 10.4136/1980-993X (in Portuguese).
- [10] H.A.T. Banu, P. Karthikeyan, S. Meenakshi (2021), "Removal of nitrate and phosphate ions from aqueous solution using zirconium encapsulated chitosan quaternized beads: Preparation, characterization and mechanistic performance", **3**, *Results in Surfaces and Interfaces*, DOI: 10.1016/j.rsufri.2021.100010.
- [11] A. Gizaw, F. Zewge, A. Kumar, et al. (2021), "A comprehensive review on nitrate and phosphate removal and recovery from aqueous solutions by adsorption", *Aqua Water Infrastructure, Ecosyst. Soc.*, **70**(7), pp.921-947, DOI: 10.2166/aqua.2021.146.
- [12] J. Liu, J. Jiang, A. Aihemaiti, et al. (2019), "Removal of phosphate from aqueous solution using MgO-modified magnetic biochar derived from anaerobic digestion residue", *J. Environ. Manage.*, **250**, DOI: 10.1016/j.jenvman.2019.109438.
- [13] N. Suhaimi, M.R.R. Kooh, C.M. Lim, et al. (2022), "The use of *Gigantochloa* bamboo-derived biochar for the removal of methylene blue from aqueous solution", *Adsorpt. Sci. Technol.*, **2022**, pp.22-24, DOI: 10.1155/2022/8245797.
- [14] A. Masanizan, C.M. Lim, M.R.R. Kooh, et al. (2021), "The removal of ruthenium-based complexes N_3 dye from DSSC wastewater using copper impregnated KOH-activated bamboo charcoal", *Water, Air, Soil Pollut.*, **232**, DOI: 10.1007/s11270-021-05333-7.
- [15] R. Thotagamage, M.R.R. Kooh, A.H. Mahadi, et al. (2021), "Copper modified activated bamboo charcoal to enhance adsorption of heavy metals from industrial wastewater", *Environ. Nanotechnology, Monit. Manag.*, **16**, DOI: 10.1016/j.enmm.2021.100562.
- [16] P. Karthikeyan, P. Sirajudheen, M.R. Nikitha, et al. (2020), "Removal of phosphate and nitrate via a zinc ferrite@activated carbon hybrid composite under batch experiments: Study of isotherm and kinetic equilibriums", *Environ. Nanotechnology, Monit. Manag.*, **14**, DOI: 10.1016/j.enmm.2020.100378.
- [17] M.K.V. Carr, C.M. Menzel (2014), "The water relations and irrigation requirements of lychee (*Litchi chinensis* Sonn.): A review", *Exp. Agric.*, **50**(4), pp.481-497, DOI: 10.1017/S0014479713000653.
- [18] J. Wu, J. Yang, P. Feng, et al. (2020), "High-efficiency removal of dyes from wastewater by fully recycling litchi peel biochar", *Chemosphere*, **246**, DOI: 10.1016/j.chemosphere.2019.125734.
- [19] N. Sezgin, A. Yalçın, Y. Köseoğlu (2016), "MnFe₂O₄ nano spinels as potential sorbent for adsorption of chromium from industrial wastewater", *Desalin. Water Treat.*, **57**(35), pp.16495-16506, DOI: 10.1080/19443994.2015.1088808.
- [20] L. Bridgewater, American Public Health Association, American Water Works Association, et al. (2012), *Standard Methods for The Examination of Water and Wastewater*, American Public Health Association, 724pp.
- [21] Y. Li, S. Ma, S. Xu, et al. (2020), "Novel magnetic biochar as an activator for peroxymonosulfate to degrade bisphenol A: Emphasizing the synergistic effect between graphitized structure and CoFe₂O₄", *Chem. Eng. J.*, **387**, DOI: 10.1016/j.cej.2020.124094.
- [22] T.H. Nguyen, X.H. Nguyen, T.G. Do, et al. (2023), "Development of biochar supported NiFe₂O₄ composite for peroxydisulfate (PDS) activation to effectively remove moxifloxacin from wastewater", *Chem. Eng. J. Adv.*, **16**, DOI: 10.1016/j.cej.2023.100550.
- [23] J. He, Y. Xiao, J. Tang, et al. (2019), "Persulfate activation with sawdust biochar in aqueous solution by enhanced electron donor-transfer effect", *Sci. Total Environ.*, **690**, pp.768-777, DOI: 10.1016/j.scitotenv.2019.07.043.
- [24] C. Zhao, B. Shao, M. Yan, et al. (2021), "Activation of peroxymonosulfate by biochar-based catalysts and applications in the degradation of organic contaminants: A review", *Chem. Eng. J.*, **416**, DOI: 10.1016/j.cej.2021.128829.
- [25] B. Wu, L. Fang, J.D. Fortner, et al. (2017), "Highly efficient and selective phosphate removal from wastewater by magnetically recoverable La(OH)₃/Fe₃O₄ nanocomposites", *Water Res.*, **126**, pp.179-188, DOI: 10.1016/j.watres.2017.09.034.
- [26] H.H.P. Quang, K.T. Phan, N.T. Dinh, et al. (2022), "Using ZrO₂ coated sludge from drinking water treatment plant as a novel adsorbent for nitrate removal from contaminated water", *Environ. Res.*, **212**, DOI: 10.1016/j.envres.2022.113410.
- [27] K.W. Jung, S. Lee, Y.J. Lee (2017), "Synthesis of novel magnesium ferrite (MgFe₂O₄)/biochar magnetic composites and its adsorption behavior for phosphate in aqueous solutions", *Bioresour. Technol.*, **245**, pp.751-759, DOI: 10.1016/j.biortech.2017.09.035.
- [28] M. Ahmad, M. Ahmad, A.R.A. Usman, et al. (2018), "Biochar composites with nano zerovalent iron and eggshell powder for nitrate removal from aqueous solution with coexisting chloride ions", *Environ. Sci. Pollut. Res.*, **25**(26), pp.25757-25771, DOI: 10.1007/s11356-017-0125-9.

Analysis of welding hazards from an occupational safety perspective

S. Senthil Murugan^{1*}, P. Sathiya²

¹Department of Mechanical Engineering, Rajalakshmi Engineering College, Chennai - 602 105, Tamilnadu, India

²Department of Production Engineering, National Institute of Technology, Tiruchirappalli - 620 015, Tamilnadu, India

Received 11 February 2023; revised 10 May 2023; accepted 7 August 2023

Abstract:

Hazards generated during welding work are risky to human health. Knowledge of occupational health and safety (OHS) while working with different welding processes is essential. Through theoretical and empirical methods, the effects of welding hazards faced by welders in industries and the safety precautions for different welding processes are explained in this article. The welding goggle with filter shade number 10 is recommended for eye protection, particularly in industry settings for heavy-duty welding work. Most research has proven that welding hazards can lead to diseases such as musculoskeletal disorders (MSD), cancer, auditory nerve failure, pulmonary oedema, lung damage, pulmonary disorders, dermatitis, renal dysfunction, and pneumoconiosis. Among the steps of the hazard control hierarchy, the “elimination” step offers an effective alternative to prevent major accidents. Controlling fumes is crucial and is discussed through ventilation and other fume extraction methods. Forced circulation ventilation (FCV) provides better performance in fume control than natural circulation ventilation (NCV) if the height from the welding room roof is less than 5 m. Air velocity should be maintained at or below 1 m/s. Adhering to international standards like OSHA, ISO, HSE Act, and ASME is beneficial to avoid or reduce hazards.

Keywords: chemical, hazards, health, occupational, safety, welding.

Classification number: 2.3

1. Introduction

Welding is a mechanical process of joining the faying surfaces of similar or dissimilar metals using heat or pressure where temporary fastening is not advisable. Metal joining is required in various sectors such as defence, nuclear power plants, aerospace, and automobiles. Welding work can be detrimental and poses risks if the right precautionary steps and safety measures are not followed by the welders [1]. Welding hazards can affect welders, compromising their physical conditions and health. The acute effects of the welding process include death, physical injuries, or mental distress [2].

New environmental and health policies focus on reducing industrial pollution and improving the safety conditions of workers. Ignorance of safety precautions can be hazardous for both the welding machines and the welders. It is crucial to follow the OSHA's guidelines since the welding process inherently has hazards during joining, cutting, gouging, chipping, and finishing. For welding in a hazardous area, a “hot work permit” is necessary, and a material safety data sheet (MSDS) should be consulted before starting.

The primary threats from welding that harm welders include heat, electricity, smoke, fumes, fire and gases [3],

dust, and UV & IR rays. If the welding operations are not optimally executed, the resulting issues can range from musculoskeletal injuries due to repetitive stresses, nose, eye, and throat irritations, burns, auditory damage [4], suffocation, exposure to contaminants, and physical discomforts like sprains. Welding fumes can affect nearby individuals and negatively impact an industry's productivity if safety measures are not adequate. Prioritising the safety and health of workers is essential for a successful business. If the arc welding process utilises a flux with higher current and larger electrodes, it produces more fumes and smoke than other welding methods [5]. Welding fumes originate from sources like consumable electrodes, shielding gases, paint on base metal, molten puddles, etc. Fusion welding processes, such as arc welding, pose more risks than the solid-state welding techniques where joining occurs below the melting temperature of base metals without significant heat. However, arc welding can generate temperatures of around 5000°C [6].

When addressing safety measures concerning welding, using safety data sheets is vital, helping identify health hazards, exposure limits, and preventative measures. Generally, welders should be well-trained in handling welding equipment, understanding Personal Protective

*Corresponding author: Email: gctsegan@gmail.com

Equipment (PPE), recognising welding hazards, and ways to mitigate them. Techniques like shielded metal arc welding, oxyacetylene welding, and gas metal arc welding are widely used and produce harmful visible, infrared & ultraviolet radiation. A notable hazard in welding is oxygen displacement in confined spaces. “Safety in welding” covers protection from electric shock, burns, UV and IR rays, chemicals, and airborne debris [7]. Other concerns include electromagnetic radiation, X-ray radiation [8], and intense heat present in high-energy welding methods like plasma, laser, and electron beam welding.

Though the literature on welding safety is limited, this article addresses that gap. A distinctive feature of this article is its comprehensive coverage of safety issues and precautions necessary for welding in industries. It aims to elucidate the various types of welding hazards, their causes, impacts on human health, and further discusses on hazard control and safety measures in different welding processes for the benefit of welders and surrounding workers.

2. Methods

This article is based on theoretical and empirical studies. The observation data was gathered by the authors during visits to welding industries, and the outcomes of discussions on welding safety with experts were used to prepare this manuscript.

3. Results and discussion

3.1. Welding hazards and their effects on health

In the welding industry, accidents are typically categorised into four: 1. Near miss incidents, 2. trivial, 3. minor, and 4. serious/major accidents. A near miss incident (a close call) describes a situation nearly leading to injury, such as a welder escaping hazards. ‘Trivial’ pertains to minimal damage like electrode damage or machine fault. ‘Minor’ accidents entail both injury and property damage, like electrocution or touching hot surfaces. Serious accidents lead to significant property damage and severe injuries, causing health complications, eye damage, and losses. These incidents can impact the owner, workers, and even have environmental implications.

In gas welding, damage to a gas cylinder’s valve can trigger accidents due to its inherent force. Common causes of accidents in welding include: 1. Hot metals and fire, 2. Fingers caught during resistance and friction welding, 3. Debris entering eyes after welding, 4. Tipping gas cylinders, and 5. Electric shocks from welding equipment. These accidents and their health implications, as detailed in Table 1, originate from three main factors: environmental, human, and mechanical. The human aspect is particularly significant in steering industrial accidents. The leading cause of these accidents is neglecting safety rules. Additionally,

poor communication and unfamiliarity with the welding machines and processes are also culprits.

Table 1. Risk factors in welding and health effects observed.

Factor	Causes of accidents/Risk/Hazard	Health effects and control measures
<i>Environmental conditions</i>	Inadequate lighting in the work area, rainy season, or presence of dust Electrocution, improper ventilation, poor housekeeping, discomfort due to room Temperature, heat, and radiation	Possibility for serious accidents and fatalities, lung damage, overstrain, suffocation, arc eye Can be controlled by good housekeeping and limits on welding time
<i>Human</i>	Working with welding machines without proper PPEs, lack of knowledge on the usage of welding machines and their parameters, fatigue due to overtime work Psychological factors of welders regarding working with hot surfaces, smoke-filled environment, fear of electrocution, poor housekeeping, poor ergonomics	Fatality due to improper usage of welding machines, minor and serious injuries, neck pain, burns, eye and nose irritation, overstress, and dehydration Health and mental fitness, use of PPEs, knowledge of proper machine usage to control accidents
<i>Mechanical</i>	Poorly maintained welding equipment, overspeed of solid-state welding machines Material breakage, overheating, unfamiliarity with machines and operation	Fatality, serious and minor injuries, electrical shock. Burns due to hot surfaces of machines and materials Proper machine maintenance, welding machines with safety guards can control risks

Welding hazards can be physical or chemical, both affecting welder health. Chemical agents include gases, fumes, filler materials, the chemical compositions of metals, and fire-related hazards. Respiratory dangers are divided into particles and vapours, with elements like iron oxide, lead, and zinc representing the former, and gases like argon and carbon monoxide representing the latter. Physical risks encompass noise, radiation, and electrocution. Harmful fumes from welding have been linked to cancer [9], and most welding operations produce noise above 90 dBA [10]. A study by O. Aminian, et al. (2021) [11] found that approximately 38% of workers are exposed to noise levels above 85 dBA, which, over time, can be damaging.

Welding emits fumes, forming solid particles around 1 µm in size, as a result of oxidation and condensation. These can settle in the lungs and lead to health issues. Some research suggests a relationship between fume production and welding process parameters in arc welding, noting increased emissions at higher parameters.

Hazards may arise from the chemical compositions of materials being welded, the specific welding processes, consumables used, and exposure length. Welding of galvanized iron, for instance, poses a lung hazard due to long-term exposure to iron fumes. Ergonomic considerations also play a role. Additional risk factors include lack of awareness, cigarette smoking, and welder psychology. Even minor burns in welding are significant, but if such injuries don’t lead to lost work time, they may not be reported. Hazards specific to different welding methods are outlined in Table 2.

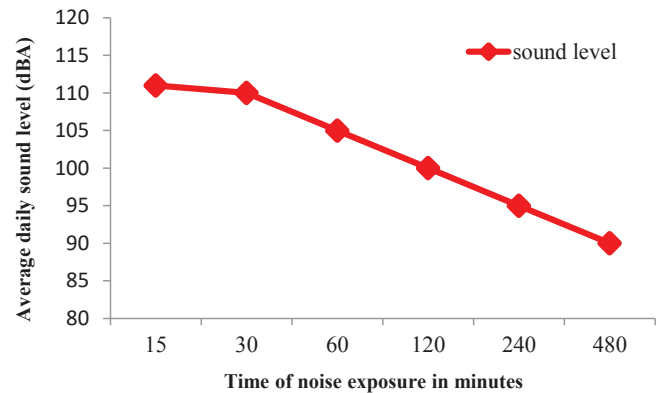
Table 2. Hazards of welding processes.

Hazards	Welding processes					
	PAW/Arc welding	Oxy-fuel	SMAW/TIG/MIG	SAW	FW	FCAW
Noise	y	n	n	n	y	n
Heat, fire & burns	y	y	y	y	n	y
UV radiation	y	n	y	#	n	y
Electric shock	y	n	y	y	y	y
Ergonomics	y	y	y	y	y	y
Toxic fumes and gases	y	y	y	#	n	y
Bright light	y	y	y	#	n	y
Explosive gases	n	y	n	n	n	n

Note: y - hazard present, n - no hazard, # - hazard present if SAW flux is absent, PAW - plasma arc welding, SMAW - shielded metal arc welding, TIG - tungsten inert gas welding, MIG - metal inert gas welding, SAW - submerged arc welding, FW - friction welding, FCAW - flux-cored arc welding.

Heat stress is a primary hazard in the industry, influenced by proximity to heat, room temperature, and clothing. Other health issues for welders include: 1. Vibration from welding tasks, 2. Physical strain due to prolonged postures, 3. Unsafe handling of equipment, and 4. UV and IR radiation exposure. Burns, a consequence of intense welding heat, can be detrimental. Proper protective clothing, such as leather attire, can mitigate these risks. Radiation impacts welders' eyes and skin but can be managed using appropriate protective equipment.

Electricity can pose hazards such as electrocution and electrical shock. Damaged electrical appliances or equipment, such as electrodes or frayed wires, may be primary culprits. Fumes, produced during the arc welding of metals by an electrode and the introduction of filler metals, can lead to cancer and respiratory disorders. Fumes from cadmium (Cd) and manganese (Mn) are particularly hazardous. These risks can be reduced with appropriate respiratory and ventilation systems. Managing mechanical hazards is demanding. This includes situations like handling sheets or metals intended for welding and managing welding equipment. Sheet metals might have sharp edges that could injure the welder's hand, and metal rods or plates could pose ergonomic risks to welding staff due to their weight. Such hazards are typically mitigated through proper training in material handling and the appropriate use of PPEs, such as masks, gloves, and safety shoes. It is widely recognised that MSDs can be triggered by the use of vibrating tools, such as chipping and grinding tools, and pneumatic equipment in welding. Fig. 1 depicts the hazard curve established by the relationship between exposure time and the sound level that might harm the hearing of personnel [12]. If a welder is exposed to a sound level of 110 dBA for 15 minutes, it could affect his auditory nerves. Similarly, exposure to a 90 dBA sound for 8 hours (480 minutes) could lead to auditory disorders.

**Fig. 1. Relationship between sound level and exposure time.**

The literature identifies 5 hazards involved in nearly all welding processes, as illustrated in Fig. 2. The nature of welding tasks is influenced by ergonomic factors. Welding methods that neglect ergonomic considerations can lead to musculoskeletal injuries (MSIs) and MSD. Such injuries often result from strains and back pain experienced by welders working in static postures. The weight of the PPE a welder wears can also contribute to neck and hand discomfort. Repetitive motions during welding heighten the risk of injuries. It is crucial to assess ergonomic factors in the industry to manage and mitigate health risks. Table 3 itemises various chemical agents produced during fusion welding processes. Key gaseous hazards in welding environments include NO_x , O_3 , CO_2 and CO [13]. These harmful gases can lead to symptoms such as nausea, headaches, dizziness, throat irritation, shivering, sweating, and dryness - often referred to as metal fume fever. Physical agents can also impact welder health, as detailed in Table 4. For instance, prolonged exposure to heat, a physical agent, can result in heat-related ailments like heat cramps, heat rash, heat exhaustion, heat oedema, and heat syncope [14]. Noise, considered non-beneficial in the welding setting, also poses a health risk due to its detrimental effects.

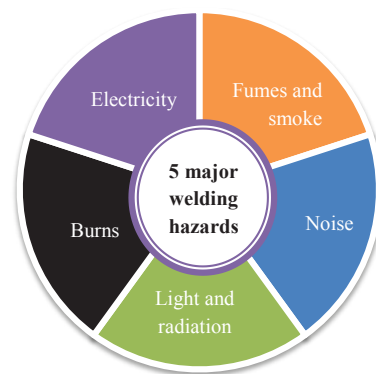
**Fig. 2. Five major hazards in welding processes.**

Table 3. Health effects identified due to chemical agents in the welding process.

Agents	Type	Source	Health effects
Cadmium oxide	Fumes	Cadmium-related materials, electroplating	Respiratory problems, muscle aches, carcinogenic effects, headache, pulmonary oedema, fever, lung damage, death
CO	Gas	CO ₂ welding, coating	Nausea, dizziness, headache, cardiovascular symptoms
Phosphine, Phosgene	Vapour	Metal-coating, incomplete combustion	Eye irritation, kidney damage
ZnO ₂	Fumes	Painted and galvanized metals	Metal fume fever, zinc poisoning, death
O ₃	Gas	Welding arc	Lung issues, pulmonary disorders
Nitrogen oxide	Gas	Welding arc	Chronic bronchitis, emphysema, pulmonary fibrosis, pneumonitis
Ni	Fumes	Nickel-containing metals	Lung cancer, respiratory tract irritation, dermatitis, renal dysfunction
Mn	Fumes	Welding, high tensile steel	Metal fume fever, kidney damage, insomnia, nervous system disorders
Magnesium oxides	Fumes	Magnesium alloys, base metals	Eyes and nose irritation, cough, fever, chest pain, fire or burn
Iron oxide	Fumes	All ferrous metals, base metals	Siderosis, lung irritation, nose irritation, pneumoconiosis
Compressed gases	Gas	Gas welding	Asphyxiation and respiratory problems, fainting
Beryllium	Fumes	Beryllium alloys	Lung inflammation
Lead	Fumes	Lead alloys, coatings, paints	Damage to the liver, heart, and kidney

Table 4. Health hazards and control measures owing to physical agents in welding process.

Agent	Causes	Symptoms/hazards	Control measures
Hot surfaces and the environment	Air movement and humidity in the welding area, hot surfaces of welds	Excessive sweating, weakness, burns, fainting, confusion, body heat, reduced urine output, etc.	Proper ventilation, and proper PPE needed for insulation from heat
Noise	Gouging, arc cutting and welding	Hearing loss, auditory nerve failure, poor sleep quality, psychological torture, echo	Sound barriers around the welding area, noise absorbers, earplugs/ear-muffs, helmet usage
Radiation	Welding light (UV, IR, visible light), skin contact	Eye irritation, skin inflammation, melancholy, mental stress, headache, itchy skin, ultraviolet keratitis, eye pain caused by exposure to ultraviolet rays	Goggles, shield/ helmet, band-pass filter

Workplace exposure limits (WELs) are established to mitigate the overexposure of welders to welding hazards. Eight-hour and fifteen-minute time periods represent the exposure limits for the long-term exposure limit (LTEL) and short-term exposure limit (STEL), respectively. The exposure limits for 8 hours of various hazardous substances released during the welding process, based on the UK system, are illustrated in Fig. 3. It is advised to keep exposure below WEL. For example, the WEL values for iron oxide fume

hazard stand at 5 mg/m³ for LTEL and 10 mg/m³ for STEL. These values vary depending on the specific hazard. The limit for nickel and manganese should ideally not exceed 0.5 mg/m³, and it is emphatically suggested that the limits for cobalt and cadmium be 0.1 mg/m³.

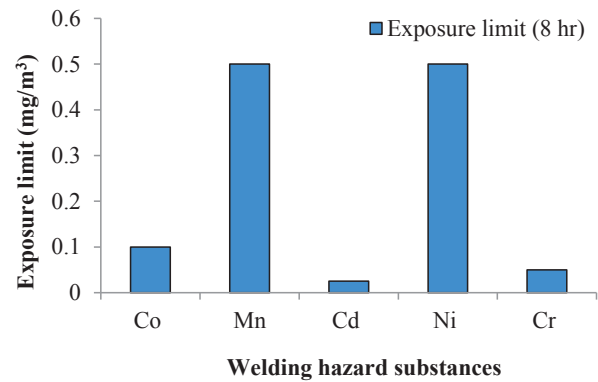


Fig. 3. Exposure limits of hazardous welding substances for 8 hours.

Gas cylinders are utilised as they are the source of heat production for welding operations. LPG, hydrogen, acetylene, propane, and butane gases are typically employed in welding. These cylinders can pose hazards, as their gas pressure increases when exposed to elevated temperatures. The risks associated with these gases include explosion, fire, propulsion of the cylinder, and more. They are characterised by their toxicity, flammability, and difficulty in manual handling.

Oxygen, being odourless, can easily ignite and explode. Acetylene, with a maximum flame temperature of 1755°C with air and 3200°C with oxygen, is used as fuel gas in gas welding for joining metals and emits a garlic-like odour. Misunderstandings related to gas cylinders can lead to accidents; this can be mitigated by recognising that acetylene (maroon-coloured cylinder) has left-hand threads, while oxygen (black-coloured cylinder) has right-hand threads. It is crucial first to close the acetylene valve and then the oxygen to prevent backfires in the event of an accident. Acetylene, being lighter than air, accumulates in roof spaces. The gas is stored in a cylinder at 15 bar pressure and a temperature of 15°C. It is explosive and highly reactive. Conditions are such that the time interval from initiation to explosion could span several hours.

Explosions can be triggered by overheating, dropping, and mechanical shocks as they induce decomposition within the acetylene gas cylinder. When reacting with silver and copper-containing materials, it forms explosive compounds called acetylides. The recommended safety precautions include wearing an oxy-acetylene safe outfit, ensuring cylinder hoses are not entangled, refraining from using acetylene gas at pressures above 100 kPa, keeping a fire extinguisher on hand during welding, and opening the

acetylene gas cylinder valve only one and a half turns while keeping a wrench on it. It is advised to avoid piecing hoses together and having any damage on the hoses connecting the cylinder and welding torch in gas welding.

Storing gas cylinders separately in a yard is essential. Ideally, a firewall of a minimum of 2 m should separate different categories of gas cylinders. If a firewall is impractical, the distance between LPG cylinders and other gas cylinders should be at least 3 m. The acetylene cylinder, when in an upright position, should be double-chained at 1/3 and 2/3 of its height for safety. Such measures help avoid potential explosions. For instance, if a cylinder's height is 1.25 m in an upright position, one chain should be positioned at 0.83 m and another at 0.4 m from its base. Every 1/3 m of its total height, the cylinder must be chained for optimal safety.

Cylinders should not come into contact with heating sources or electrical systems and should be stored in well-ventilated areas. In the work environment, gas cylinders must be positioned at least 6 m away from combustible/flammable paints. Oxygen and acetylene cylinders should ideally be stored separately. If any partition exists between acetylene and oxygen storage areas, this barrier should be 0.45 m taller than the tallest cylinder and extend 0.45 m from the outermost cylinder. It is crucial not to use oxygen near any flammable materials due to its combustion-enhancing properties. When transporting the acetylene cylinder, ensure it is either in a vertical position or tilted no more than 60°, using a trolley.

Rolling cylinders from one location to another is not advised. When utilising inert argon gas in a confined space, it tends to settle at the bottom of the welding area since it is denser than air, successively reducing the oxygen content. When ceasing the gas welding process for an extended period, it is necessary to close the cylinder valves and then discharge the gas pressures from regulators and hoses through the welding torch. However, gases can still pollute the welding area. For instance, helium fills spaces from top to bottom as it is lighter than air.

During arc welding, there is a potential risk of electric shock or electrocution. Human skin, when wet, exhibits reduced resistance to current compared to dry skin [15]. An electric shock from sources such as 240 volts (domestic appliances) or 415 volts (industrial machinery) can result in fatal accidents or severe injuries, often causing the victim to be thrown a considerable distance due to convulsion. Electric shocks can also arise from welding apparatuses [16]. A current of 50 milliamperes (mA) can be lethal; at this magnitude, respiration becomes difficult and can cease. The voltage in the electrode holder can surge to 80 V quickly when an arc is not established. In such scenarios, if an operator contacts a conductive part of the electrode

holder, they may experience an electric shock leading to significant injury. Devices known as automatic electric shock prevention devices can mitigate this hazard as they promptly reduce the voltage. Moreover, proper grounding and insulation can further minimise risks. Electric shocks can sometimes be felt due to the deterioration of a flexible cable or electrode holder. Other times, shocks result from improper wiring of an arc welding machine. Factors such as wet clothing or welding environments during rainy seasons, poorly insulated connections, ungrounded plugs, and worn-out cables typically induce electric shocks. Table 5 discloses the electrical hazards related to high and low voltage in arc welding and their ramifications on humans.

Table 5. Arc welding electric shock hazard and health issues at high and low voltage.

Fault/hazard	Health issue
Lack of welding machine earthing	Shock, severe burns, fatal
Poor insulation and damaged cable	Fire, property damage, shock, fatal,
Oversize fuse	Shock, burn
Inadequate connection	Fire, overheating

3.2. Safety issues and measures in different welding processes

Fumes, electric shock, heat, brightness, radiation, ergonomics, and noise constitute the primary hazards in the majority of welding processes. Welding is intrinsically a high-temperature operation. Welding safety encompasses the comprehensive protection of workers from hazards such as fumes, hot metal, splatters, heat, and radiation. Therefore, it is imperative to secure a “hot work permit” when operating under these conditions. Beyond complying with Environment, Health, and Safety (EHS) standards, this permit is a legal requisite according to both Indian and international statutes. The enactment of ISO standards necessitates this. The engineer or supervisor overseeing the welding or other high-temperature works applies for this permit. The plant manager, in collaboration with the safety professional, evaluates the work site and approves the hot work, provided all criteria on the permit checklist are met. This encompasses considerations such as PPE, protocols for hot work, welder training, and more.

A suitable working environment is pivotal in mitigating hazards during welding and reducing exposure to health-damaging substances. Welders must have ample space to manoeuvre and operate effectively. Appropriate ventilation and roof height are compulsory in welding areas to dissipate fumes and smoke, ensuring workers remain undisturbed. In terms of safety, it's crucial to have fire extinguishers, hydrant systems, fire monitoring systems, and machine guarding systems proximate to welding machines to shield operators from potential harm. Welders should receive specialised training on the correct use of PPEs and

adherence to safety protocols. Welding machines should be halted when substituting materials and subjected to a preliminary inspection before initiating the welding process. Welders must also be mindful of psychological factors, such as stress, complacency, and job interest, to preclude human errors.

Anticipated hazards for gas welding include hot surfaces, fumes, toxic gases, and potential cylinder explosions. In contrast, arc welding may introduce risks of electrocution, spatters, hot surfaces, fumes, smoke, and toxic gas emissions when welding materials that contain harmful substances [17]. In heavy industrial settings, adherence to the safety checklist is paramount to prevent mishaps during welding activities. The electrical supply to the welding machine needs rigorous examination to ensure its hazard-free. Any paint, coatings, or plating on the base metal must be removed prior to welding to prevent metal fume generation. Welding tasks should only be undertaken by competent individuals under stringent supervision. From an electrical safety perspective, the open-circuit voltage of the power unit must not be excessive, limited to a maximum of 80 V AC, or 48 V AC in damp areas [18].

Gas cylinder valves, hoses, their positioning, and the isolation of cylinders are essential to circumvent heat source interaction before and post gas welding. Welding zones should maintain good ventilation and be devoid of flammable materials. The functionality of all welding machines and related equipment should undergo regular assessment. The state of PPEs, firefighting tools, and welding machine insulation against both natural and artificial hazards must be routinely inspected. The “job card” for welding tasks requires meticulous scrutiny by the sanctioned authority, ensuring the qualification of welders, the equipment in use, requisite PPEs, their accessibility, welding duration, and the designated work space. Conclusively, it is imperative to confirm the deactivation of welding machines post-operation. Generally, the employment of gas cylinders in gas welding is indispensable. However, mishandling or improper storage can precipitate accidents. Table 6 enumerates the precautions to be heeded during arc and gas welding.

The safe storage and handling of gas cylinders is imperative. Herein, we propose several recommendations for cylinder safety to mitigate hazards. Cylinders should be stored in a well-ventilated area, and the use of uninsulated cylinder hoses across trafficked areas should be avoided. A mixture of water and soap or detergent is preferable for detecting leakage, as opposed to using a flame [19]. When stored, the valves of the cylinders should be oriented uppermost. Secure cylinders with a chain to prevent unnecessary movement during storage, and ensure cylinder valves are closed after use. It is prudent to avoid storing cylinders near sources of heat or electricity. During welding

Table 6. Safety measures in arc and gas welding.

Arc welding process	Gas welding process
Bright light, IR, and UV rays are produced by arc welding. Eye and face protection equipment is essential for all involved.	Special attention must be paid to welding materials, especially toxic ones.
Appropriate PPEs must be worn. Ensure welding machines are switched off when not in operation.	Hoses connected to gas cylinders must adhere to safety standards.
Earplugs can mitigate the disturbance caused by the arc sound.	Appropriate PPEs are essential during the joining process.
Ensure welding materials are clean and free from rust. Maintain a tidy work area.	Protect workers from skin contact with hot surfaces, fluxes, and similar hazards.
Guarantee proper insulation of the welding torch, cables, and machine.	Handle acetylene with care.
To avoid electrical shocks, stand on wooden or rubber mats while welding.	Before welding, clean weld surfaces with acetone and remove any coatings.
Avoid wearing wet clothes or gloves. Implement measures to prevent welders from inhaling harmful fumes.	Ensure gas cylinders are securely stored and handled safely during transportation.

operations, welders should manage any flashback into the hoses. The use of oil, water, and grease on cylinder appliances should be avoided, and trolleys are recommended for cylinder transport between workplaces. Prevent cylinder rust and the introduction of dust, grit, and sand into the hose or valve. If gas welding is utilised within a boiler, cylinders should be stored externally due to the confined nature of the working space. While forced fans can be utilised for ventilation during welding [20], the use of oxygen is not recommended as it exacerbates potential fire hazards. Flashback arrestors can halt the backflow of gas when the flame begins burning within the hose. Welding on painted surfaces poses risks of nausea and inhalation of chemical contaminants, necessitating appropriate PP). During gas welding, maintaining a gas torch welding gap of 5-10 mm and a standoff distance of a minimum of 160 mm is advised.

Understanding welding protection utilities and their functions is essential. Fume extraction systems [12] remove fumes from the welding space, ensuring the welder’s health is not compromised. Appropriate selection of breathing or extraction systems is crucial for a safe welding environment. Welding curtains or fireproof canvases protect personnel working in proximity to the welding area. As arc welding poses multiple injury risks, the proper use of PPE, including boots, fire retardants, goggles, masks, helmets, protective clothing, and leather aprons, is crucial. For fresh air supply, managing oxygen content and supplying fresh air to welders during operations is essential. Safety boots with steel toes protect from potential injuries and electric shocks due to their shock-resistant soles [21]. In respirators with blowers,

embedded air purification systems introduce fresh air to the user.

During resistance welding, resistance spot welding, commonly used in automobile manufacturing, poses risks such as finger entrapment between electrodes and welding spatter. Additional hazards include electric shocks, hot metal exposure, and electromagnetic fields. Safety precautions, such as protective screens and the use of PPE, are recommended. Sensor-embedded equipment in resistance spot welding machines detects obstructions and halts operations, reducing accidents [22]. Minimising the

gap between electrodes can prevent finger entry. For safety, resistance welding machines can be leg-operated to reduce hand injuries.

Guarding, particularly fixed or interlocking types, is a vital safety feature. Robotic spot welding requires safety interlock systems due to the rapid movement of robot arms. Hazards associated with spot welding, depicted in Fig. 4D, include MSDs in the neck, wrist, shoulder, and back. Technological advancements like the “Car-O-Liner CTR9”, illustrated in Fig. 4E, reduce these ergonomic issues. In Figs. 4D and 4E, welders are shown performing spot welding tasks on automobile bodies. Thanks to innovations such as the Advanced Resistance Spot Welder, the incidence of MSDs in welders is reduced, as evident in Fig. 4E.

While considering safety in laser and explosive welding, laser welding (LW) should be conducted within an enclosure in line with safety guidelines. It has been determined that the primary hazards associated with LW include fumes, glare, radiation, and direct exposure to the laser beam. The laser beam may harm the operator’s skin and eyes. To mitigate beam-related hazards, it is prudent to minimise direct interaction between the operator and LW. Implementing enclosures around the machine’s working area, safety curtains, and interlocking guards is strongly advised. However, LW presents a unique hazard distinct from other welding techniques: the risk associated with high laser voltage. Therefore, technicians handling LW machine maintenance should be well-versed in procedures and thoroughly familiar with the equipment. Existing literature notes that CO₂ does not transmit through glass materials. Consequently, doors of CO₂ laser-embedded welding machines should be constructed of such materials to shield operators from beam-related hazards. Both operators and repair personnel should undergo training about the operations, safety hazards, and preventive measures associated with the LW machine.

Explosive welding is a solid-state welding method that does not melt the material; joining occurs due to the high-impact energy provided by chemical explosives. Here, metals like sheets and plates are bonded using energy from chemical explosives. Careful attention must be given to factors such as the distance of the process, the specific chemical utilised, its handling, the target plate angle, applied pressure, and detonation speed.

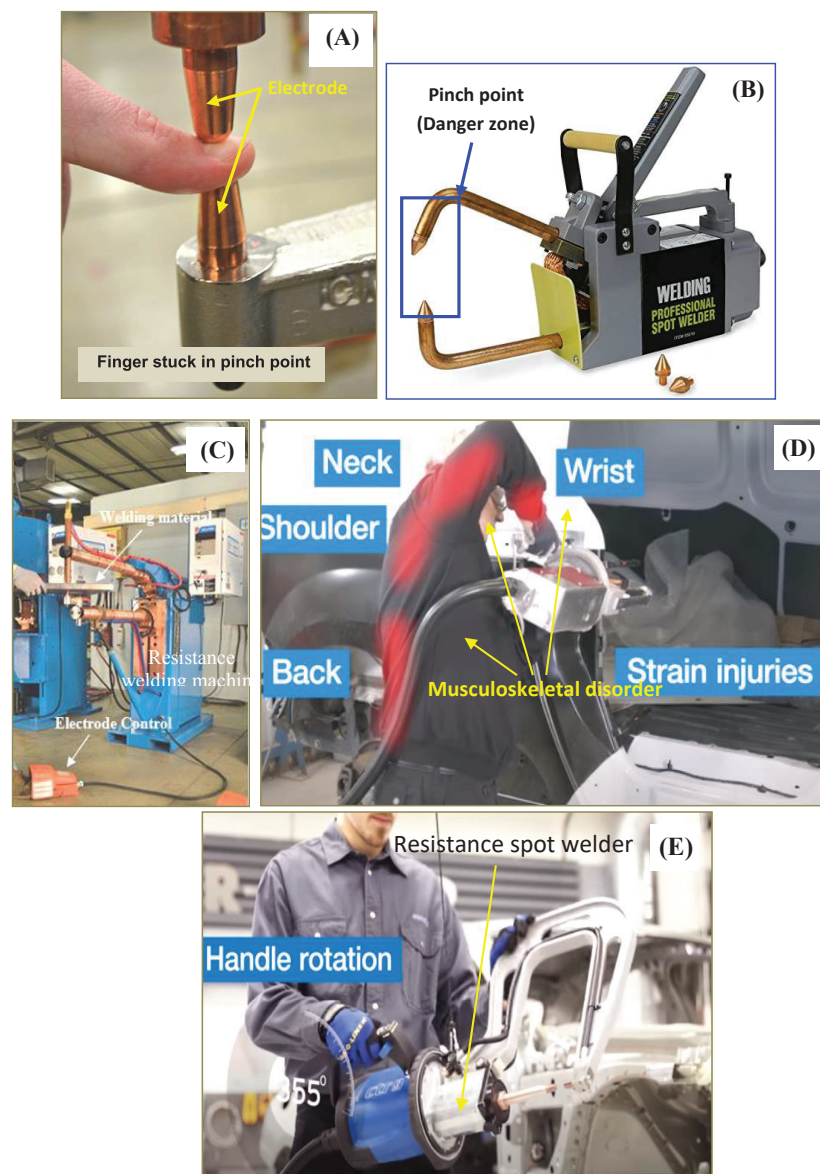


Fig. 4. (A) Finger caught between electrodes in resistance welding, (B) portable resistance spot welding machine with pinch point and danger zone, (C) welding of material with leg operation, (D) resistance welding hazards, and (E) advanced resistance spot welder (Car-O-Liner CTR9) with zero hazards.

The hazards inherent in the rotary friction welding (RFW) process include noise [23] from the motor and chuck rotation, pinch points, elevated forging and friction pressures, and the movement of the hydraulic actuator during welding. There is also the risk of an operator's hand getting trapped between the axially moving system and the chuck in RFW [24]. The welding region, or "danger zone" in friction welding, is a high-risk area where weld specimens are placed. Although FW is considered environmentally friendly [25], it is advisable to employ machine guards and noise-controlling enclosures around the FW machine to mitigate noise levels [26]. When operating the FW machine, safety protocols must be adhered to. Operators should avoid loose clothing, unrestrained hair, wrist bracelets, and necklaces, as these items can pose risks. A transparent sliding door guard or a sliding grilled gate is recommended for the machine's front side to facilitate the inspection of weld specimens during loading and unloading. A fixed guard is preferable for the machine's rear [27]. Implementing interlocking mechanisms or automation is advised to shield welders from potential hazards. Additionally, a sensing system that detects human movement and promptly halts machine operation can be instrumental in preventing accidents.

3.3. Hazard control

The hierarchy of hazard control is presented in Fig. 5. It encompasses personal protection, as well as administrative and engineering controls. In this context, "PPE" is deemed the least effective for welding hazard control because its efficacy depends on various factors, such as the availability of PPE, individual users, the working environment, and the duration of welding, among others. PPE is utilised either singularly or in combination in accordance with OSHA standards [28]. However, the effectiveness of PPE in countering health hazards remains minimal. PPE includes earplugs, gloves, goggles, safety glasses, welding helmets, protective shields, aprons, respirators, and more. Welding fume hazards can be mitigated using PPE-respiratory devices. Auto-darkening filters (ADF) in welding helmets have been employed to minimise injuries such as arc-eye burns caused by UV rays during welding [29, 30]. Safety gloves safeguard the welders' hands, while asbestos or leather aprons shield their torso and thighs. Protection for the head during overhead welding is essential, and a leather skull cap, helmet, or peaked cap (kepi) proves valuable for this purpose. The welder's body can be further protected using leather jackets and leggings.

Training for welders falls under administrative controls. Three types of training can be provided: basic, technical, and specialised. The nature and depth of training are

contingent on the welders' tasks and their experience level. "Administrative control" involves modifying work procedures by implementing new safety policies within the welding sector. On the other hand, "engineering controls" aim to separate individuals from hazards by enclosing the welding area, utilising methods such as guarding systems and adequate ventilation. Another method of control is "substitution", which entails replacing the hazard. If certain hazards are particularly severe, they might need to be entirely removed or "eliminated", a strategy which is highly effective within the control hierarchy.

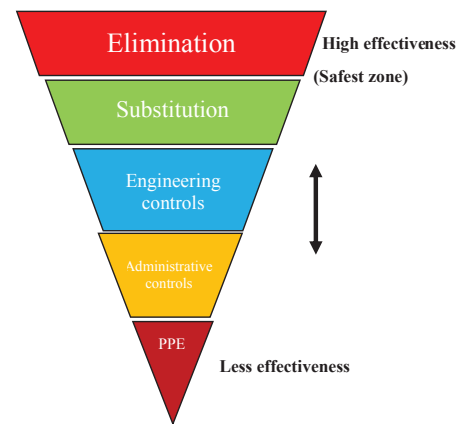


Fig. 5. Hierarchy of hazard control.

In welding, both major and minor accidents necessitate immediate first-aid intervention. Electric shocks, burns, and eye injuries are predominantly linked with welding hazards [31]. Keeping a first-aid kit in the vicinity is advantageous. To circumvent electric shock risks, one should disconnect the power source, avoid touching any surfaces with bare hands, and don appropriate non-conductive clothing and gloves. If an individual is injured, measures should be taken to ensure their airway remains open, and trained personnel should administer first-aid. In the event of burns, the affected area should be cooled with water for a minimum of 10 minutes and then dressed. Depending on the burn's severity - such as internal damage or deep burns categorised as 1st, 2nd, or 3rd-degree - further medical attention might be necessary. If foreign substances like dust or fumes contact the eyes, immediate flushing with a suitable solution is imperative. Following this, if any debris remains, a professional examination is recommended. Goggles should be worn during activities like gouging, etching, and chipping of weldments. For brief welding tasks, a face shield can be utilised, whereas extended welding sessions might necessitate a helmet with a hood. Prolonged exposure to UV rays mandates consultation with

an eye specialist. When analysing welding fume samples, occupational chemical agent guidelines offer insights into sampling procedures and calculation methods. Additionally, guidelines on thermal stress in workplaces are invaluable for assessing hazards related to high-temperature conditions. It is prudent to adhere to approved regulatory requirements and welding manuals, which outline fundamental criteria for hazard assessment, welder qualifications, and control measures [32]. If risk levels escalate in the vicinity of the welding area, they should be reduced and maintained below stipulated thresholds. Given that injured workers require immediate first-aid, specially trained medical staff or ambulance personnel should be available around the clock within industrial settings to assist casualties.

3.4. Eye protection and fumes control

The welding process, particularly arc welding, produces intense light which can adversely affect the welders' eyes and potentially lead to blindness. Thus, provisions must be made for eye protection to shield against this light. The standard OSHA's 29 CFR 1910.252 section (b) (2) mandates eye protection during welding [33]. Wearing a welding helmet (ANSI Z49.1) equipped with the appropriate shade number for the filter ensures the protection of both face and eyes. The correct lens shade numbers [34] for different welding processes should be adhered to as delineated in Table 7. Welding with intense glare requires a higher shade number. For instance, oxy-fuel gas welding utilises lenses between shade numbers 4 to 8. The shade numbers for flux-cored arc welding, plasma arc welding, and gas metal arc welding range from 8 to 14. Similarly, for the safety of welders, the recommended lens shade numbers for gas tungsten arc welding and shielded metal arc welding are between 9 to 14 and 10 to 14, respectively.

Table 7. Recommended lens shade number for the welding process.

Metal welding processes	Range of lens shade number
Oxy-fuel gas welding	04-08
Gas metal arc welding	08-12
Flux-cored arc welding	08-14
Plasma arc welding	08-14
Shielded metal arc welding	10-14
Gas tungsten arc welding	09-14

The construction of ADF and its efficacy against UV light are pivotal. It comprises liquid crystal (LC) placed between each polarising filter to enhance its effectiveness.

Likewise, a band-pass filter (BPF) is employed to shield against UV and IR rays, permitting only visible light to pass through. The arrangement within the face shield, designed for the welding helmet, includes a protective glass made of polycarbonate positioned closest to our eyes to defend against chipping particles. This is followed by the filter shade glass which screens out the IR and UV rays emitted by the arc welding light. Finally, another protective glass is installed to guard against spatter during welding. Fig. 6 depicts the appropriate shades in relation to the amperage used for welding operations to protect eyes from potential hazards. The choice of filter glasses is contingent on the current utilised for welding. For instance, for a current of 100 A, a shade number below 10 is suitable. As amperage increases, so does the required shade number, with numbers 12 and above recommended for currents exceeding 300 A. Typically, shade number 10 is adequate for most welding processes.

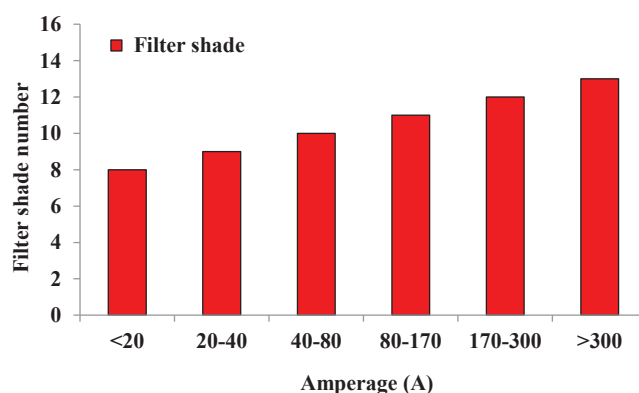


Fig. 6. Relationship between amperage in welding and filter shade number.

Adequate ventilation is imperative in the welding area to mitigate welding smoke and fumes. Welding fume exposure control is typically achieved using smoke extraction and ventilation systems. However, an overly congested area can compromise the efficiency of these systems. Two prevalent types of ventilation are utilised in industries: dilution ventilation and local exhaust ventilation. The former involves wall or roof exhaust fans and functions by diluting contaminants to levels below exposure limits (e.g., threshold limit values and permissible exposure limits) by introducing large volumes of air. However, this method can occasionally prove insufficient as it might allow contaminants to enter the welder's breathing space. Conversely, local exhaust ventilation, designed specifically to accompany welding machines, efficiently extracts gases and fumes produced during welding. It comprises a

hood, duct, fan, and air cleaner, crafted to prevent fumes from entering the welder's breathing zone. The efficacy of the ventilation system is determined by the distance of the welding booth's hood from the welding source and the exhaust-air velocity (recommended at 0.5 m/s) [35]. Due to this reduced air velocity, fumes, gases, and smoke generated during the welding process remain away from the welder's breathing zone. Welding within confined spaces is inadvisable unless adequate ventilation is ensured.

Figures 7 and 8 illustrate ventilation and fume extraction techniques designed to minimise hazardous concentrations, and a welder's face shielded with PPE during welding. Fig. 7A showcases the extraction of fumes and particles through the ventilation duct. M.H. Lee, et al. (2007) [36] introduced a new model, as seen in Fig. 7B, tailored for fume control. The ventilation system atop a booth, depicted in Fig. 7C, is devised to regulate particle concentrations around the welder's face [36]. Fig. 7D [37] displays a movable ventilation duct, adept at capturing fumes wherever welding occurs and facilitating easy discharge without environmental impact [38]. In this fume control setup, the maintained air velocity is approximately one m/s. The Health and Safety Executive (HSE) strongly advocates the Control of Substances Hazardous to Health (COSHH) regulations, specifically concerning welding fumes,

to uphold public health and safety. Typically, the HSE recommends the employment of local exhaust ventilation and respiratory protective equipment in environments with welding fumes. This is applicable to welding processes such as gas, TIG, MIG, arc, and resistance spots.

The welding fumes, linked to conditions such as asthma, cancer, and pneumonia, have not been entirely eliminated during fusion welding processes but can be safely managed with appropriate welding fume control techniques. Some of the fume extraction techniques endorsed by HSE to expel the fumes are depicted in Figs. 8A-8C [39]. In one technique (Fig. 8A), the fumes produced during welding are removed through slots or holes in the welding chamber. The fume-capturing hoods can be automated and mobile, while battery-powered PPE hoods can prevent welders from inhaling fumes (Fig. 8B). The fume extraction via slots or holes, positioned near the welding torch (Fig. 8C), ensures immediate removal of fumes before reaching the welders' respiratory zone. These methods effectively regulate the volume of fumes accumulated in the welding booth. By utilising these ventilation and fume extraction approaches, the build-up of fumes within the welding area can be controlled.

The extent of dust and fumes exposure to welders is contingent upon the welding technique, ventilation efficacy, practices, and locale [40, 41]. When ventilation is not viable, respiratory protection systems must be employed. Respiratory conditions are prevalent amongst welders [42], including respiratory tract cancer [43], especially for gas and arc welders. Thus, it is imperative to curtail exposure to welding fumes. According to NIOSH (National Institute for Occupational Safety & Health), an approved respirator is essential, and each welder should be adept at its efficient usage. Every respirator is characterised by an assigned protection factor (APF), indicating its protective capacity. The maximal permissible concentration of a substance during welding can be determined by multiplying the APF value of the respirator with the exposure threshold for that substance. Atmosphere-supplying respirators operate independently of ambient conditions, whereas air-purifying respirators cleanse the inhaled air, filtering out particulates. Welding respirators should incorporate particle filtration since fumes predominantly contain particles.

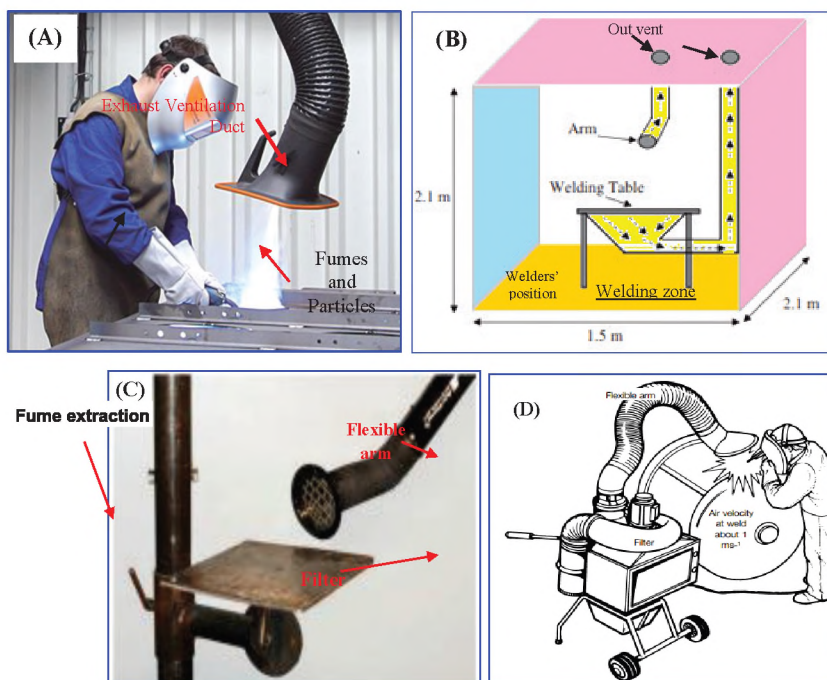


Fig. 7. Ventilation setup: (A) Modified welding room with duct; (B) Welding table with modified ventilation setup; (C) Exhaust pickup arrangement; and (D) Mobile fume control setup.

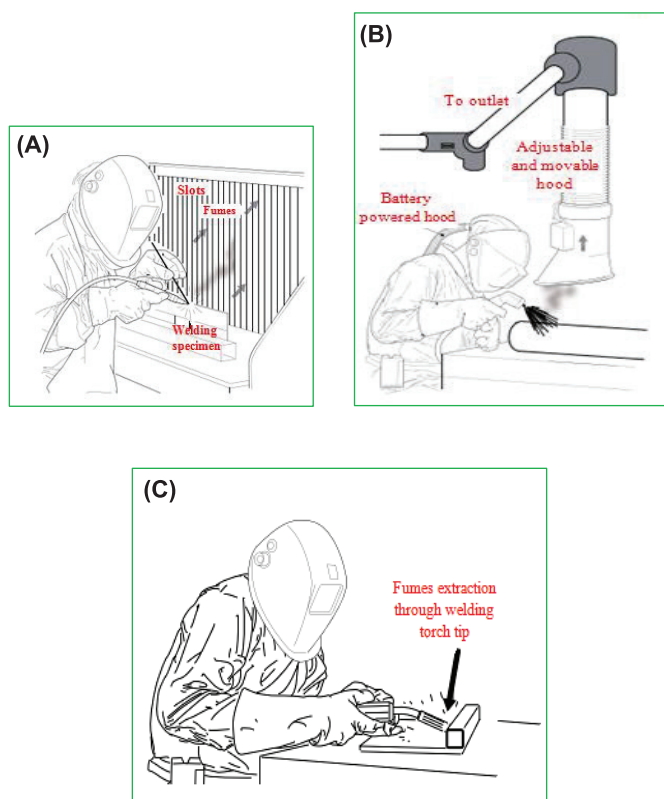


Fig. 8. Welding fume control [39].

4. Conclusions

Given the paucity of research articles in the realm of welding safety, this article elucidates the causes and effects of welding hazards across diverse processes on welders' health, alongside safety precautions and hazard control strategies. The principal contributors to welders' health deterioration encompass inhaling welding fumes, perilous practices during welding, flawed specimens and equipment, UV and IR emissions from welding lights, and heat radiation. A Hot Work Permit (HWP) is imperative for high-risk welding tasks, and risk assessments or safety audits should be systematically conducted within industries to safeguard both workers and assets. Welding hazards can lead to ailments such as nausea, headaches, respiratory conditions, and vertigo. Symptoms of metal fume fever include throat discomfort, chills, perspiration, and dryness. A 3-m distance should be maintained between oxygen, LPG, and acetylene cylinders, and gas containers should be situated at least 6 m away from hazardous zones. In many welding processes, filter shade numbers between 8-10 are utilised for ocular protection. As the working amperage escalates, a higher shade number for the welder's face shield is recommended. The design of fume extraction systems and ventilation efficiency is pivotal in determining fume

control. For welding in confined spaces, a forced circulation fan is recommended for fume dispersal, whereas natural circulation is favoured in spaces with a 5.5 m ceiling height. Fume accumulation can be efficaciously managed through slots and holes in welding tables or at the torch's tip. In the hierarchy of hazard control, elimination and substitution remain paramount for ensuring a secure environment. An ambient light intensity of approximately 200 lux is advised for the welding workspace.

CRediT author statement

S. Senthil Murugan: Investigation, Methodology, Data collection, Writing - Original draft preparation; P. Sathiya: Revision, Conceptualisation, Supervision.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] T.S. Hong, M. Ghobakhloo (2014), "6.12 - Safety and security conditions in welding processes", *Comprehensive Materials Processing*, Elsevier, pp.213-225, DOI: 10.1016/B978-0-08-096532-1.00608-7.
- [2] P.J. Hewitt (1996), "Occupational health in metal arc welding", *Indoor and Built Environment*, **5**(5), pp.253-262, DOI: 10.1159/000463728.
- [3] I. Pires, L. Quintino, T. Rosado (2010), "Reduction of fume and gas emissions using innovative gas metal arc welding variants", *International Journal of Advanced Manufacturing Technology*, **50**, pp.557-567, DOI: 10.1007/s00170-010-2551-4.
- [4] D. Mckeown (2009), "Welding safety hazards and precautions", *Health & Safety International*, <https://www.healthandsafetyinternational.com/article/1842126/welding-safety-hazards-precautions>, accessed 1 October 2009.
- [5] Employment and Social Development Canada (2019), *Welding and Allied Processes - A Guide to Health Hazards and Hazard Control Measures*, 18pp.
- [6] J. Petkovsek (2014), "Be prepared: Five potential welding safety hazards to avoid", *Occup. Health Saf.*, **83**(4), pp.34-39.
- [7] Unitor (2017), *Maritime Welding Handbook*, 561pp.
- [8] S. Ozcelik, K. Moore (2003), *Modeling, Sensing and Control of Gas Metal Arc Welding*, Elsevier, 372pp.
- [9] J. Ninness (2018), "Welding fume cancer risk warning", *Australasian Mine Safety Journal*, <https://www.amsj.com.au/welding-fume-cancer-risk/>, accessed 9 January 2023.
- [10] N. Ahmed (2005), *New Developments in Advanced Welding*, Woodhead Publishing, 308pp.
- [11] O. Aminian, M. Saraie, M. Ahadi, et al. (2021), "Association of the working environment noise with occupational stress in industrial workers", *J. Public Health (Berl.)*, **31**, pp.979-984, DOI: 10.1007/s10389-021-01605-y.

- [12] L. Jeffus (2011), *Welding and Metal Fabrication*, Cengage Learning, 800pp.
- [13] Y. Mehrifar, S.K. Zeverdegani, M. Faraji, et al. (2018), "Risk assessment of welders exposure to the released contaminated gases in different types of welding process in a steel industry", *Health Scope*, **8(1)**, DOI: 10.5812/jhealthscope.58267.
- [14] M.T. Khan, E.A. Karpinski (2018), *Thermal Stress in The Workplace*, Evaluation Directorate Employment and Social Development Canada, 32pp.
- [15] C.T. Mgonja (2017), "The effects of arc welding hazards to welders and people surrounding the welding area", *International Journal of Mechanical Engineering and Technology*, **8(3)**, pp.433-441.
- [16] R. Timings (2008), *Fabrication and Welding Engineering*, Elsevier, 565pp.
- [17] R. Singh (2006), *Introduction to Basic Manufacturing Processes and Workshop Technology*, New Age International Publisher, 506pp.
- [18] K. Weman (2003), *Welding Process Handbook*, Woodhead Publishing, 193pp.
- [19] Health and Safety Executive (1999), *Health and Safety in Engineering Workshops*, 118pp.
- [20] Bureau of Indian Standards (1975), *Handbook for Gas Welders*, 134pp.
- [21] Centre for Advanced Maintenance Technology, Indian Railways (2015), *Handbook on Welding Techniques*, 83pp.
- [22] Health and Safety Executive (2021a), "Controlling the risks from welding", <https://www.hse.gov.uk/welding/welding-controls.htm>, accessed 2 January 2023.
- [23] K. Maheswaran, N.G. Serrao, S.S. Murugan, et al. (2020), "Acoustic level measurement by noise mapping method and mitigation using acoustic plaster in power press industry", *International Journal of Advanced Science and Technology*, **29(7)**, pp.11200-11207.
- [24] S.S. Murugan, N.A. Haq, P. Sathiya (2020a), "Effect of welding parameters on the microstructure and mechanical properties of the friction-welded dissimilar joints of AA6063 alloy and faying surface-tapered AISI304L alloy", *Welding in The World*, **64(10)**, pp.483-499, DOI: 10.1007/s40194-020-00846-x.
- [25] S.S. Murugan, N.A. Haq, P. Sathiya (2020b), "Eco-friendly frictional joining of AA6063 and AISI304L dissimilar metals and characterisation of bimetal joints", *Journal of New Materials for Electrochemical Systems*, **23(2)**, pp.101-111, DOI: 10.14447/jnmes.v23i2.a07.
- [26] P. Alam, K. Ahmad, A.H. Khan, et al. (2021), "2D and 3D mapping of traffic induced noise near major roads passing through densely populated residential area of South Delhi, India", *PLOS ONE*, **16(3)**, DOI: 10.1371/journal.pone.0248939.
- [27] Manufacturing Technology Inc. (2021), <https://www.mtiwelding.com/#>, accessed 12 December 2021.
- [28] D. Thornton (2014), "Improving welder safety", *Welding Journal*, **93(3)**, pp.36-38.
- [29] Saif Corporation (2015), *Welding Health and Safety*, https://ehs.oregonstate.edu/sites/ehs.oregonstate.edu/files/pdf/occsafety/hot_work/saif_welding_health_safety.pdf, 21pp, accessed 9 January 2023.
- [30] K. Martinelli (2018), "Welding hazards in the workplace: Safety tips & precautions", *High Speed Training*, <https://www.highspeedtraining.co.uk/hub/welding-hazards-in-the-workplace/>, accessed 21 December 2022.
- [31] J. Blunt, N.C. Balchin (2002), *Health and Safety in Welding and Allied Processes*, Woodhead Publishing, 272pp.
- [32] The Minister of Justice (2018), *Canadian Occupational Health and Safety Regulations*, <https://lois-laws.justice.gc.ca/eng/regulations/SOR-86-304/20181123/P1TT3xt3.html>, accessed 25 December 2018.
- [33] J. Laws (2017), "Welding safety best practices", *Occupational Health And Safety*, **86(4)**, pp.22-23.
- [34] J.E. Brumbaugh, R. Miller (2007), *Audel Welding Pocket Reference*, Wiley, 448pp.
- [35] The Federal Register Washington D.C. (1998), *ACGIH: Industrial Ventilation Manual*, American Conference of Governmental Industrial Hygienists, <https://law.resource.org/pub/us/cfr/ibr/001/acgih.manual.1998.pdf>, accessed 14 January 2023.
- [36] M.H. Lee, W.J. McClellan, J. Candela, et al. (2007), "Reduction of nanoparticle exposure to welding aerosols by modification of the ventilation system in a workplace", *J. Nanopart Res.*, **9**, pp.127-136, DOI: 10.1007/s11051-006-9181-7.
- [37] International Finance Corporation, World Bank Group (2007), *Environmental, Health, and Safety Guidelines*, <https://www.ifc.org/content/dam/ifc/doc/2000/2007-general-ehs-guidelines-en.pdf>, 99pp, accessed 9 January 2023.
- [38] H. Tukur, Q. Zhoude (2013), "A review of safety procedures and guidelines in welding and cutting", *International Journal of Science and Research*, **2(3)**, pp.119-123.
- [39] Health and Safety Executive (2021b), *Welding Fume Control*, <https://www.hse.gov.uk/pubns/guidance/wl3.pdf>, accessed 25 December 2021.
- [40] Hobart Institute of Welding Technology (2009), *Safety & Health of Welders*, <https://www.welding.org/wp-content/uploads/2014/08/363.pdf>, 28pp, accessed 9 January 2023.
- [41] K. Singh, A. Anand (2013), "Safety considerations in a welding process: A review", *International Journal of Innovative Research in Science, Engineering and Technology*, **2(2)**, <https://www.semanticscholar.org/paper/SAFETY-CONSIDERATIONS-IN-A-WELDING-PROCESS%3A-A-Singh-Anand/550e80554bc8e25b618b92b9017a2c84e682f526>, accessed 25 December 2021.
- [42] M.B. Wanjari, P. Wankhede (2020), "Occupational hazards associated with welding work that influence health status of welders", *International Journal of Current Research and Review*, **12(23)**, pp.51-55, DOI: 10.31782/IJCRR.2020.122303.
- [43] J.L. Orr (1993), "Effects of welding on health VIII", *Safety and Health Committee, American Welding Society*, 64pp.

Investigation for subchronic oral toxicity and evaluation of analgesic activities of *Stephania tetrandra* S. Moore liquid extract in experimental animals

Thanh Mai Phuong¹, Trang Tran Thi Thu², Khanh Do Thi Hong³, Ngoc Tran Thi Hong³, Dat Tran Tien³,
Trang Nguyen Thi Mai³, Minh Phan Hong^{3*}

¹Hanoi Medical University, 1 Ton That Tung Street, Trung Tu Ward, Dong Da District, Hanoi, Vietnam

²Hanoi University of Pharmacy, 13-15 Le Thanh Tong Street, Phan Chu Trinh Ward, Hoan Kiem District, Hanoi, Vietnam

³University of Medicine and Pharmacy, Vietnam National University - Hanoi, 144 Xuan Thuy Street, Dich Vong Hau Ward, Cau Giay District, Hanoi, Vietnam

Received 13 July 2023; revised 11 September 2023; accepted 5 February 2024

Abstract:

This research evaluates subchronic toxicities and analgesic effects of *Stephania tetrandra* S. Moore (*S. tetrandra*) liquid extract through oral administration in experimental animals. Over 4 weeks of subchronic toxicity research, *S. tetrandra* was administered to Wistar rats at a dose of 1.6 g/kg body weight (BW) (the dose is equivalent to the expected human dose) and 4.8 g/kg BW (three times the human expected dose), based on World Health Organization (WHO) and Organisation for Economic Co-operation and Development (OECD) recommendations. The analgesic effect was assessed using three models: hot plate, tail-flick, and acetic acid-induced writhing in Swiss mice. Regarding the subchronic toxicity test, after oral administration of *S. tetrandra* liquid extract, haematological parameters, hepato-renal functions, and microscopic images of the liver and kidney were unchanged compared with the control group. As a result, *S. tetrandra* liquid extract at both doses of 3.2 g/kg/day and 9.6 g/kg/day tended to reduce pain in the models. The low dose significantly showed peripheral analgesic effects, while the high dose demonstrated better analgesic activity at both central and peripheral sites. In conclusion, *S. tetrandra* liquid extract did not produce subchronic toxicities in Wistar rats, and has shown experimental analgesic effects.

Keywords: analgesic activity, experimental animals, *Stephania tetrandra* S. Moore (*S. tetrandra*), subchronic toxicity.

Classification number: 3.3

1. Introduction

Nature has been a source of medicinal agents since ancient times, and medicinal plants form a wide variety of traditional medicines used in various countries worldwide [1]. The exclusive use of herbal drugs for managing various ailments continues due to easy access, better compatibility, and economic reasons. According to the World Health Organization (WHO), up to 80% of the population in developing countries uses traditional medicine for primary health care. However, the lack of evidence-based approaches and toxicological profiling of herbal preparations remains the biggest concern regarding the use of medicinal plants. Thus, evaluating their toxicity plays a vital role in recognising these effects, in support of their characterisation, evaluation for human risk, and thus proposing measures to mitigate the risk, particularly in early clinical trials [2].

Toxicity refers to unwanted effects on biological systems. The toxicity of a substance can be impacted

by many factors, such as the route of exposure (skin absorption, ingestion, inhalation, or injection), the exposure time (acute or chronic administration), the physical form of the toxin (solid, liquid, or gas), the organ system involved (cardiovascular, nephro-, haemo-, nervous-, or haematopoietic-system), and genetic structure [3]. Subchronic systemic toxicity is defined as adverse effects occurring after repeated or continuous administration of a test sample for up to 12 weeks or not exceeding 10% of the animal's lifespan [4, 5]. In Vietnam, *S. tetrandra* has been recently exploited and used as medicine. Historically, *S. tetrandra* has been used since ancient times and in folklore to treat joint pain, muscle fatigue, etc., but no report available on the safety of the product and little scientific evidence is available on the topical analgesic activity. Therefore, our intention was to investigate subchronic toxicities and antinociceptive activities of *S. tetrandra* liquid extract in animals in this study.

*Corresponding author: Email: phanhongminh.hmu@gmail.com

2. Materials and methods

2.1. Preparation of *S. tetrandra* liquid extract

S. tetrandra were harvested in Ba Vi, Hanoi, Vietnam. The samples were assessed by the Department of Medicinal Materials at the University of Medicine and Pharmacy, Vietnam National University - Hanoi. The tubers were washed and dried at 60°C. A total of 800 g of the dried tubers were ultrasonically extracted with 96% ethanol for 45 minutes and then evaporated under reduced pressure to obtain the extract (97 g) with an extraction yield of 12.1%. The extracts were stored in a refrigerator at 2-8°C. The extract was completely dissolved in distilled water before administration.

2.2. Preparation of machinery and chemicals

- + Codeine phosphate was provided by the Central Institute of Drug Testing.
- + Acetic acid solution was supplied by Guangdong Guanghua Sci-Tech Co., Ltd, China.
- + Aspirin tablets (100 mg) were obtained from Traphaco Joint Stock Company, Vietnam.
- + Distilled water.
- + Sodium chloride 0.9% solution was acquired from Braun, Vietnam.
- + 70% alcohol was sourced from HD Pharma, Vietnam.
- Machines and tools for research:
 - + Precisa LX2200c electronic scale (Switzerland).
 - + Hot plate machine model - DS37 (Ugo-Basile, IDEA).
 - + Dynamic Plantar pain response meter Aesthesiometer 37450 (Ugo Basile, Italy).
 - + Special needles for administering medicine to mice.
 - + Various tools and materials used in the research (cotton, trays, gauze, scissors, pints, etc.).

2.3. Experimental animals

This study utilised *Wistar* rats weighing 150-200 g obtained from the Vietnam Military Medical University and *Swiss* mice weighing 20-22 g sourced from the National Institute of Hygiene and Epidemiology, Vietnam. The animals were divided into cages (each containing 10 mice or rats) and housed in a room maintained at a temperature of 22±2°C and 18-20% humidity. They were subjected to a 12-hour light/dark cycle and had free access to food and water ad libitum. The animals were acclimated to the housing conditions for at least one week prior to the commencement of the study.

2.4. Subchronic toxicity study

A subchronic toxicity study was conducted according to WHO and OECD guidelines [6, 7]. The study spanned a continuous four weeks. *Wistar* rats were divided into three groups of ten animals each:

- Group 1 (control group) received distilled water.
- Group 2 was orally administered *S. tetrandra* liquid extract at a dose of 1.6 g/kg/day (equivalent to the human recommended dose, conversion ratio 6);
- Group 3 was orally administered *S. tetrandra* liquid extract at a dose of 4.8 g/kg/day (3 times higher than that of Group 2).

Animals received oral administration of distilled water and *S. tetrandra* liquid extract at a volume of 10 ml/kg body weight daily for four consecutive weeks and were observed daily to detect clinical signs and determine time points for laboratory tests. The liquid extract was dissolved in distilled water (the solvent for *S. tetrandra* liquid extract) daily before administration to the rats.

The parameters monitored during the study included general conditions, mortality, and clinical signs such as:

- Body weight changes.
- Haematopoietic function: red blood cells (RBC), haemoglobin (HGB), haematocrit, total white blood cells (WBC), WBC differentials, and platelet count (PLT).
- Serum biochemistry tests: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol, and creatinine levels.

These parameters were checked before treatment, two weeks, and four weeks after treatment. At the end of the experiment, all animals underwent a full gross necropsy. The livers and kidneys of 30% of the rats from each group were taken for histopathological examinations. The micro-histological examination was carried out at the Department of Pathology, 103 Military Hospital, Hanoi, Vietnam.

2.5. Analgesic activity

Hot plate test: The hot plate device used in this research was the Ugo Basile, Model DS 37, and was maintained at 54.5-56.5°C. Mice were randomly divided into four groups of ten each. The experiment involved placing mice on a hot plate within a beaker and observing their response to heat-induced pain. The baseline reaction time was considered the mean reaction time obtained before the oral administration of normal saline, codeine phosphate at a dose of 20 mg/kg/day, and *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day, defined as the normal reaction of the animal

to temperature. Thirty minutes after treatment, the reaction time (in seconds) was recorded when mice licked their fore or hind paws and then jumped, indicating their response to heat-induced pain. Animals showing a reaction time greater than 30 seconds were discarded. A cut-off time of typically 30 seconds was established to minimise the risk of tissue damage from prolonged exposure to hot surfaces [8, 9].

Tail-flick test: The tail-flick method was applied to evaluate the analgesic activity in mice. Reaction latencies were the index used in this study, measured by placing the tip (last 3 cm) of the tail on the radiant heat source. Removing the tail from the radiant heat source was considered the endpoint. A time limit of 15 seconds was used to avoid heat damage to the tail. Mice were divided into four groups (n=10) and administered with normal saline, codeine phosphate at a dose of 20 mg/kg/day, and *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day. The latency of the tail-flick reaction was recorded 30 minutes after dosing [10].

Acetic acid induced writhing in mice: Swiss mice were randomly divided into four groups (n=10). Group I was treated with distilled water (20 ml/kg), Group II with codeine phosphate at a dose of 20 mg/kg/day; Groups III and IV received *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day, respectively, for five consecutive days. On the last day, one hour after codeine phosphate and *S. tetrandra* liquid extract administration, groups II, III, and IV received 1% acetic acid at a dose of 10 ml/kg b.w, intraperitoneally, and writhing reflex was observed for 30 minutes. The mice exhibited writhing movements (stretching of hind limbs and bending of the trunk).

2.6. Statistical analysis

All data were expressed as mean $\pm$ standard deviation. The statistical analysis of the experimental data was carried out to identify differences between each treated group and the control group using Student's t-test and paired t-test. A difference was considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Acute and subchronic toxicity study

3.1.1. Acute toxicity

Swiss mice were administered *S. tetrandra* liquid extract in doses ranging from the highest dose that did not cause death (65.18 g/kg) to the lowest dose that resulted in 100% mortality (195.29 g/kg). The administrations were given three times within 24 hours, with each administration separated by 3 hours. As a result, Swiss mice exhibited mortality and some abnormal signs at a dose of 81.38 g/kg (Table 1).

Table 1. Results of acute toxicity studies according to a dose of *S. tetrandra* liquid extract.

Group	n	Dose (ml/kg)	Dose (g/kg)	Lethal dose	Abnormal sign
Group 1	10	15	48.83	0	No
Group 2	10	20	65.10	0	
Group 3	10	25	81.38	20	
Group 4	10	30	97.66	40	Convulsions, cyanosis
Group 5	10	35	113.95	40	
Group 6	10	40	130.22	60	
Group 7	10	45	146.50	70	
Group 8	10	50	162.79	80	
Group 9	10	55	179.18	90	
Group 10	10	60	195.29	100	

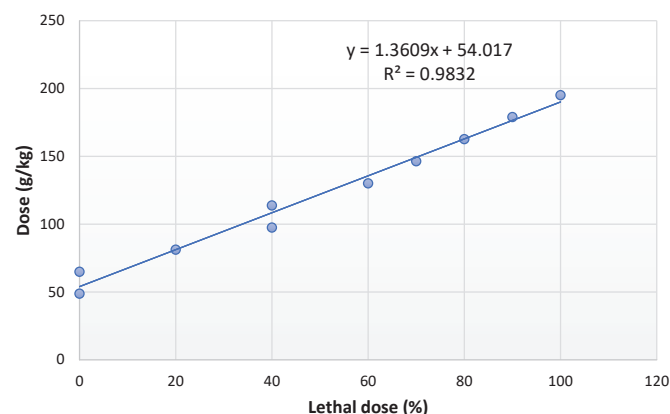


Fig. 1. Graph representing the dose vs. the lethal dose of *S. tetrandra* liquid extract.

From the graph above (Fig. 1), a 50% lethal dose of extract can be calculated as: $LD_{50} = 1.3609 \times 50 + 54.017 = 122.1$ g/kg. This calculation includes adults weighing 50 kg. The extrapolation coefficient for mice was 12 and for rats was 6.

3.1.2. Subchronic toxicity

General condition: Animals exhibited normal locomotor activities and good feeding habits. None of the animals in any of the treated groups showed any macroscopic or gross pathological changes compared with the control group.

Body weight changes: Fig. 2 shows that after two weeks and four weeks, the body weight in all groups increased slightly compared with the time point "Before treatment". There was no significant difference in body weight between the *S. tetrandra* liquid extract-treated groups and the control group ($p > 0.05$).

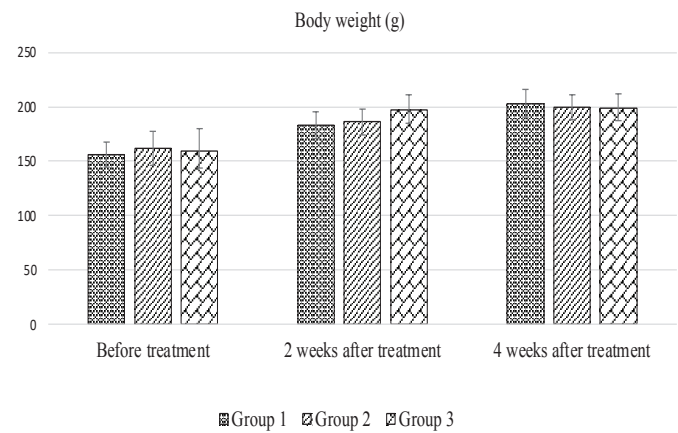


Fig. 2. The effect of *S. tetrandra* liquid extract on body weight.

Table 2. The effect of *S. tetrandra* liquid extract on hematopoietic function.

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
Red blood cells count (T/L)	Group 1	8.41±0.69	8.24±1.01	8.07±1.09
	Group 2	8.96±1.89	8.99±0.64	8.12±0.86
	Group 3	8.30±0.42	8.12±0.86	8.53±0.65
	p	>0.05	>0.05	>0.05
Haemoglobin level (g/dL)	Group 1	11.08±1.11	11.21±1.06	11.57±0.94
	Group 2	11.65±1.73	11.68±1.03	11.82±0.93
	Group 3	1093±1.27	11.01±0.91	11.61±1.15
	p	>0.05	>0.05	>0.05
Haematocrit (%)	Group 1	44.60±3.07	43.02±4.16	44.12±2.91
	Group 2	43.81±4.60	42.04±3.85	42.66±2.63
	Group 3	43.87±4.80	42.12±6.09	43.84±2.15
	p	>0.05	>0.05	>0.05
Platelet count (G/L)	Group 1	558.50±109.76	572.70±111.23	601.50±112.65
	Group 2	567.50±111.34	581.20±110.29	582.30±99.07
	Group 3	539.40±114.21	551.40±95.99	548.60±106.10
	p	>0.05	>0.05	>0.05
Total WBC count (G/L)	Group 1	6.68±1.07	7.20±1.76	7.09±1.19
	Group 2	6.85±1.70	7.19±2.03	6.99±1.73
	Group 3	6.34±0.93	673±1.53	6.85±1.56
	p	>0.05	>0.05	>0.05
Lymphocytes (%)	Group 1	79.38±5.32	75.00±5.27	78.33±5.35
	Group 2	75.92±6.54	78.48±3.77	76.32±5.78
	Group 3	75.82±4.38	77.94±9.09	76.28±5.36
	p	>0.05	>0.05	>0.05
Neutrophils (%)	Group 1	8.44±2.84	7.74±1.26	8.58±2.25
	Group 2	7.93±2.20	7.75±1.35	7.23±2.06
	Group 3	8.96±2.61	7.45±2.05	9.08±2.40
	p	>0.05	>0.05	>0.05

p: comparison with the control group and “Before treatment”.

The effect of S. tetrandra liquid extract on the haematological system: There were no significant differences in red blood cell count, haematocrit, haemoglobin level, platelet count, total WBC count, and WBC between the *S. tetrandra* liquid extract-treated groups and the control group (p>0.05) (Table 2).

Table 3. The effect of *S. tetrandra* liquid extract on liver function.

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
AST level (UI/L)	Group 1	84.98±8.15	79.82±13.76	79.98±20.88
	Group 2	85.12±9.06	80.62±14.77	86.14±22.52
	Group 3	87.34±9.17	87.85±14.06	89.00±21.27
	p	>0.05	>0.05	>0.05
ALT level (UI/L)	Group 1	36.08±4.11	38.00±6.06	33.57±3.94
	Group 2	32.25±6.73	33.68±8.03	34.82±4.93
	Group 3	35.13±5.27	34.01±5.81	34.69±7.85
	p	>0.05	>0.05	>0.05
Total bilirubin (mmol/l)	Group 1	13.35±0.46	13.55±0.75	13.39±0.42
	Group 2	13.52±0.38	13.53±0.29	13.57±0.35
	Group 3	13.31±0.53	13.40±0.30	13.52±0.28
	p	>0.05	>0.05	>0.05
Albumin concentration (g/dL)	Group 1	3.55±0.22	3.71±0.21	3.67±0.32
	Group 2	3.49±0.21	3.51±0.34	3.50±0.26
	Group 3	3.45±0.31	3.47±0.31	3.50±0.32
	p	>0.05	>0.05	>0.05
Total cholesterol concentration (mmol/l)	Group 1	1.66±0.29	1.62±0.30	1.65±0.27
	Group 2	1.65±0.29	1.63±0.24	1.59±0.23
	Group 3	1.59±0.31	1.55±0.23	1.72±0.29
	p	>0.05	>0.05	>0.05

p: compared with the control group and the time point “Before treatment”.

The effect of S. tetrandra liquid extract on liver function: There were no significant differences in aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels, total bilirubin, and albumin concentration between the *S. tetrandra* liquid extract-treated groups and the control group (p>0.05). The results are shown in Table 3.

The effect of S. tetrandra liquid extract on kidney function: Table 4 demonstrated that after two weeks and four weeks of treatment, *S. tetrandra* liquid extract caused no significant difference in serum creatinine levels between the control group and the two treated groups ($p>0.05$).

Table 4. The effect of *S. tetrandra* liquid extract on serum creatinine.

Group	Creatinine "Before treatment" (mg/dL)	Creatinine "After treatment" (mg/dL)	
		Two weeks	Four weeks
Group 1	0.78±0.05	0.81±0.06	0.79±0.03
Group 2	0.80±0.02	0.82±0.01	0.75±0.05
Group 3	0.79±0.04	0.83±0.07	0.78±0.07
p	>0.05	>0.05	>0.05

p: compared with the control group and the time point "Before treatment".

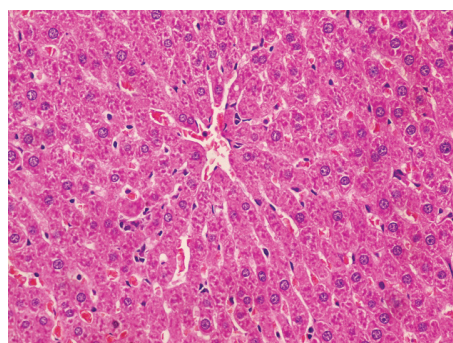
Histopathological examination: No gross lesions or changes in size were observed when all experimental rats underwent a full gross necropsy, which examined the hearts, livers, lungs, kidneys, and abdominal cavities.

There was no significant difference in the histopathological examinations of the livers and kidneys between the *S. tetrandra* liquid extract-treated mice and the control group after four weeks of treatment (Figs. 3 and 4).

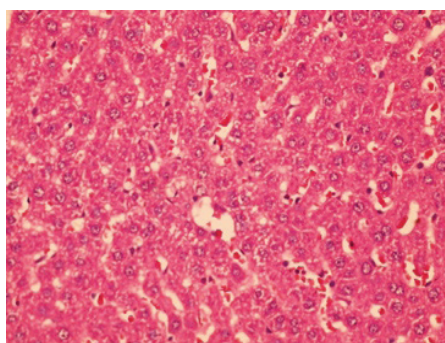
3.2. Analgesic activity

Hot plate test and tail flick test

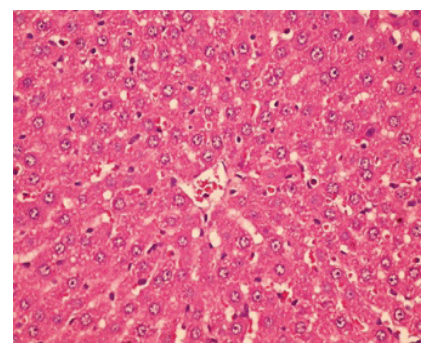
Time (seconds): Observing Fig. 5, the treatment with codeine phosphate showed a significant prolongation of reaction time compared with the control group in both the tail-flick assay and hot plate test. The results obtained with the administration of *S. tetrandra liquid extract* increased the latency to pain on the hot plate ($55.5\pm10^{\circ}\text{C}$) and in the tail-flick assay. The animals treated with *S. tetrandra* liquid extract at doses of 3.2 and 9.6 g/kg/day both showed increased resistance to the hot painful stimulation inflicted throughout this test. At the dose of 9.6 g/kg/day, the extract demonstrated significant results in antinociceptive activity, increasing the latency of tail movement in rats.



Group 1

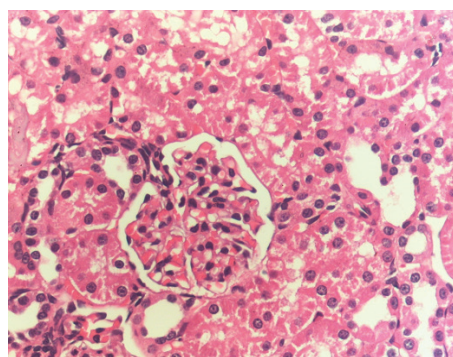


Group 2

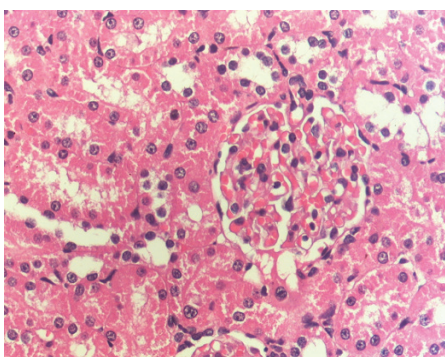


Group 3

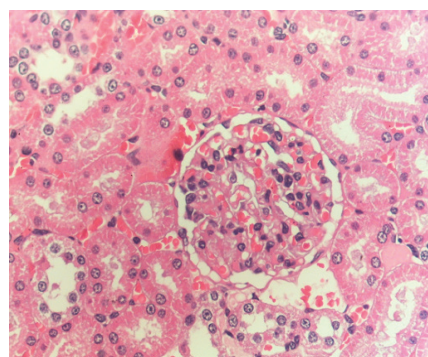
Fig. 3. Histopathological morphology of liver (HE × 400).



Group 1

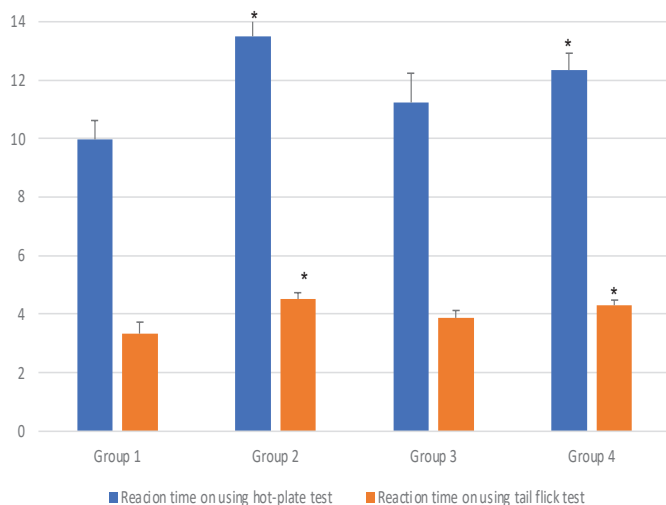


Group 2



Group 3

Fig. 4. Histopathological morphology of kidney (HE × 400).



* $p < 0.05$; compared with control (Student's t-test).

Fig. 5. The effects of *S. tetrandra* liquid extract on reaction time on using hot plate and tail flick test.

Table 5. Analgesic activity of *S. tetrandra* liquid extract by acetic acid-induced writhing method.

Group (n=10)	Number of writhing					
	0-5 min	>5-10 min	>10-15 min	>15-20 min	>20-25 min	>25-30 min
Group 1	10.70±2.09	17.20±3.80	15.60±4.61	13.80±3.80	10.90±3.21	6.90±1.84
Group 2	4.80±1.17***	14.20±1.05*	10.10±2.27*	9.20±2.06**	7.60±1.79*	4.50±1.13*
Group 3	7.60±2.22***	16.70±3.17	12.50±2.90*	9.70±2.27*	7.10±1.98*	5.50±1.18
Group 4	8.40±1.16***	15.90±3.16	11.80±2.07*	10.50±2.08*	6.80±2.22**	6.30±2.01*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with control (Student's t-test); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with group 2 (Student's t-test).

Statistical analysis indicated that codeine phosphate significantly reduced the number of writhes when compared to the control (Table 5) (* $p < 0.05$; $p < 0.01$). Both dose levels of the test extract statistically significantly reduced the number of abdominal constrictions at three time points: >10-15 minutes, >15-20 minutes, and >20-25 minutes.

4. Discussion

4.1. Subchronic toxicity

Toxicity is the degree to which a substance can harm humans or animals. It can affect anything from a single cell to an organ or even the entire organism [11]. To evaluate the safety of drugs or products for human use, toxicity testing is first performed on various experimental animal models to detect toxicity and, based on that, to provide guidance and safety treatment for humans. Subchronic toxicity

research provides knowledge on the effects of repeated oral exposure and offers evidence for longer-term studies [6, 12]. Subchronic studies assess the undesirable effects of continuous or repeated exposure to plant extracts or compounds over a portion of an animal's average lifespan, such as in rodents, specifically providing information on target organ toxicity [13].

The most basic indicator to reflect organ and system toxicity is the body weight change index [13]. For all experimental animals, general signs should be observed daily, and body weight should be measured periodically [12]. Based on the obtained results, it can be asserted that *S. tetrandra* did not interfere with the animals' normal metabolism because there were no significant differences compared to the control group.

The main function of the blood circulatory system is to deliver oxygen, nutrients, hormones, and drugs to cells, muscles, tissues, and organs. The haematopoietic system is one of the most sensitive targets of toxic compounds and is an essential parameter for the physiological and pathological status of humans and animals [6, 12]. Furthermore, such analysis is relevant to risk evaluation as changes in the haematological system have a higher predictive value for human toxicity when the data are translated from animal studies. After two weeks and four weeks of treatment, there was no significant difference in total red blood cells, haematocrit, haemoglobin level, platelet count, total WBC count, and WBC differentials between the *S. tetrandra* liquid extract-treated groups and the control group, so it can be concluded that the *S. tetrandra* liquid extract does not affect the haematological system.

Analysis of the kidney and liver is an important indicator in the assessment of the toxicity of drugs and plant extracts, as they are vital organs essential for the survival of living organisms. Biochemical indices were performed to assess plant product-induced changes in liver and renal function. Changes in serum transaminases are sensitive indicators to reflect the degree of hepatocellular injury. Transaminase would be released from hepatocellular injury when chronic liver cell damage occurs, increasing serum concentrations of these enzymes [11]. Creatinine levels can be used to describe the function of the kidneys [12]. There are no significant ALT and AST changes in both male and female rats at all doses, which indicates that *S. tetrandra* liquid extract had no deleterious effect on liver function. The blood biochemistry levels of the control and *S. tetrandra* liquid extract-treated rats at various doses presented no significant differences between the *S. tetrandra* liquid extract-treated groups and the control group ($p > 0.05$). This evidence shows that *S. tetrandra* liquid extract did not affect liver and kidney functions.

The liver and kidneys are important organs in the metabolism and elimination of drugs from the body. The histopathological examination revealed alterations in cell structure under the light microscope. Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of the *S. tetrandra* liquid extract. Our study showed no significant differences in histopathological examinations of the livers and kidneys between the *S. tetrandra* liquid extract-treated groups and the control group.

Overall, this study's findings indicated that no significant differences were observed in blood parameters, biochemistry parameters, and histopathological observations of liver and kidney tissues between the *S. tetrandra* liquid extract-treated groups and the control group.

4.2. Analgesic activity

In this study, the antinociceptive effect of *S. tetrandra* liquid extract was evaluated using classical *in vivo* models of nociception induced by thermal stimuli, as in the hot plate and tail-flick tests, as well as chemical stimuli, such as acetic acid-induced writhing in mice.

The hot plate test, similar to the tail-flick test, evaluates the effectiveness of pain relievers by indicators of response to heat-induced pain. The tail flick test is primarily a response

at the spinal level, while the hot plate test response occurs at the supraspinal level. These methods are mainly used to evaluate centrally-acting analgesics [14]. Experimental evidence obtained from the hot plate and tail flick tests in mice showed a tendency to prolong the reaction time to thermal stimuli with *S. tetrandra* liquid extract at both tested doses, with a significant difference observed at the higher dose level of 9.6 g/kg/day.

Acetic acid-induced abdominal writhing is a sensitive procedure for establishing the efficacy of peripherally-acting analgesics. The reaction is thought to involve local peritoneal cells and be mediated by prostaglandin pathways [14]. The data in Table 5 show that *S. tetrandra* liquid extract tended to reduce the number of writhing motions. Both dose levels significantly reduced the number of abdominal cramps at three time points: >10-15 minutes, >15-20 minutes, and >20-25 minutes.

Pain and inflammation often occur together, possibly due to tissue damage, chemical irritation, or autoimmune processes. Stimuli that induce the release of chemical mediators of pain and inflammation [14]. These processes can lead to central sensitization and hypersensitivity. Its sub-plantar injection of acetic acid is followed by the sequential liberation of several mediators of inflammation, such as histamine, 5-hydroxytryptamine, bradykinin, and finally prostaglandins [14]. These mediators stimulate sensory nerve endings and cause pain. Therefore, the above models can be used to evaluate the analgesic activity of *S. tetrandra*.

From the above results, *S. tetrandra* liquid extract expressed both central and peripheral analgesic activities. The peripheral analgesic activity was clearly demonstrated at the two tested doses, while a significant central analgesic effect was observed only at the higher dose. Thus, the dose of 9.6 g/kg/day showed stronger analgesic activity than the dose of 3.2 g/kg/day. This finding suggests that the lower dose should be chosen in cases of mild pain, while the higher dosage appears to be more appropriate for more severe pain requiring central analgesia.

5. Conclusions

For a continuous four weeks, *S. tetrandra* liquid extract at oral doses of 1.6 and 4.8 g/kg/day did not create any toxic signs or symptoms of subchronic toxicity in Wistar rats. *S. tetrandra* liquid extract at the dose of 9.6 g/kg exhibited

pain-inhibitory activities that were characterised by a significantly prolonged reaction time in hot-plate and tail-flick tests and induced a significant decrease in the number of writhes in acetic acid-induced writhing in mice.

CRediT author statement

Thanh Mai Phuong: Conceptualisation, Methodology, Supervision; Trang Tran Thi Thu: Data curation, Writing - Original draft preparation; Khanh Do Thi Hong: Investigation, Writing - Reviewing and Editing; Ngoc Tran Thi Hong: Investigation; Dat Tran Tien: Software, Validation; Trang Nguyen Thi Mai: Project administration; Minh Phan Hong: Formal analysis, Writing - Reviewing and Editing, Methodology, Visualisation.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] N. Guite (2010), "International protocol and indigenous knowledge on medicine and health care: An overview", *The Asian Man*, **4**, pp.1-12.
- [2] World Health Organization (2019), *WHO Global Report on Traditional and Complementary Medicine*, 226pp.
- [3] G.D. Venkatasubbu, S. Ramasamy, P.R. Gaddam, et al. (2015), "Acute and subchronic toxicity analysis of surface modified paclitaxel attached hydroxyapatite and titanium dioxide nanoparticles", *Int. J. Nanomedicine*, **10(1)**, pp.137-148, DOI: 10.2147/IJN.S79991.
- [4] W.H. Jong, J. Carraway, R. Geertsma (2012), "In vivo and in vitro testing for the biological safety evaluation of biomaterials and medical devices", *Biocompatibility and Performance of Medical Devices*, pp.120-158, DOI: 10.1533/9780857096456.2.120.
- [5] S.A. Saganuwan (2017), "Toxicity studies of drugs and chemicals in animals: An overview", *Bulgarian Journal of Veterinary Medicine*, **20(4)**, pp.291-318, DOI:10.15547/bjvm.983.
- [6] Organization for Economic Cooperation and Development iLibrary (2008), *OECD Guidelines for the Testing of Chemicals, Section 4*, DOI: 10.1787/20745788.
- [7] World Health Organization (2007), *WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues*, 118pp.
- [8] H.G. Vogel (2008), *Drug Discovery and Evaluation: Pharmacological Assays*, 3rd Edition, Springer, 2071pp, DOI: 10.1007/978-3-540-70995-4.
- [9] D. Mishra, G. Ghosh, S.K. Paidesetty, et al. (2011), "An experimental study of analgesic activity of selective COX-2 inhibitor with conventional NSAIDs", *Asian Journal of Pharmaceutical and Clinical Research*, **4(1)**, pp.78-81.
- [10] G. Mulder, K.P. Corning (2004), "Rodent analgesiometry: The hot plate, tail flick and Von Frey hairs", *Contemporary Topics in Laboratory Animal Science/American Association for Laboratory Animal Science*, **43(3)**, pp.54-55.
- [11] J.T.L. Jr, F. Wilcoxon (1949), "A simplified method of evaluating dose-effect experiments", *J. Pharmacol Exp. Ther.*, **96(2)**, pp.99-113.
- [12] Regional Office for the Western Pacific of the World Health Organization (1993), *Working Group on The Safety and Efficacy of Herbal Medicine, Philippines, 5-9 October 1992*, Report series RS/92/GE/15(PHL), 56pp.
- [13] M.Y. Lee, C.S. Seo, S.W. Cha, et al. (2014), "Safety assessment of So-cheong-ryong-tang: Subchronic toxicity study in crl:cd Sprague dawley rats", *Mol. Med. Rep.*, **9(6)**, pp.2273-2282, DOI: 10.3892/mmr.2014.2114.
- [14] M.F. Yam, Y.C. Loh, C.W. Oo, et al. (2020), "Overview of neurological mechanism of pain profile used for animal "pain-like" behavioral study with proposed analgesic pathways", *International Journal of Molecular Sciences*, **21(12)**, DOI: 10.3390/ijms21124355.

Studies on the growth and essential oil accumulation of *Elsholtzia ciliata* (Thunb.) Hyland and the effect of gibberellic acid treatment on growth and essential oil accumulation

Luong Thi Le Tho¹, Luu Tang Phuc Khang^{1*}, Nguyen Xuan Hieu¹, Dinh Thi Bich Thuy¹,
Vo Ngoc Khoi Nguyen¹, Chau Minh Hai Dang¹, Tran Thi Phuong Dung²

¹Department of Biology, Ho Chi Minh City University of Education, 280 An Duong Vuong Street, Ward 4, District 5, Ho Chi Minh City, Vietnam

²Department of Primary Education, Ho Chi Minh City University of Education, 280 An Duong Vuong Street, Ward 4, District 5, Ho Chi Minh City, Vietnam

Received 22 February 2023; revised 6 September 2023; accepted 5 February 2024

Abstract:

Vietnamese balm (*Elsholtzia ciliata* (Thunb.) Hyland) (*E. ciliata*) is known as an aromatic herb and a valuable remedy in oriental medicine thanks to the essential oil contained in the leaves. In plant life, plant growth regulators play an important role in the growth and accumulation of essential oils. This article investigates the effect of gibberellic acid (GA₃) on the growth and essential oil accumulation of *E. ciliata* at the 4-week-old plant stage based on a randomised complete block design with 3 replications. The results showed that the effect of GA₃ (0, 5, 10, 15, 20, and 25 ppm) on growth parameters of *E. ciliata*. GA₃ increases plant height, leaf length, leaf width, whole plant fresh weight, dry weight, nitrate content, and essential oil percentage significantly ($p \leq 0.05$). With an increasing amount of GA₃, all of these parameters increased significantly compared to the control. The treatment with 10 ppm GA₃ resulted in the highest plant height, leaf length, leaf width, and production of fresh weight, dry weights, nitrate content, and more than the control. The highest essential oil yield (1.41%) was obtained by applying 10 ppm GA₃. The results of this study showed that the quantitative and qualitative yield of Vietnamese balm could be increased by treatment with an appropriate amount of gibberellic acid.

Keywords: *Elsholtzia ciliata* (Thunb.) Hyland, essential oil accumulation, gibberellic acid, growth.

Classification numbers: 3.3, 3.4

1. Introduction

Vietnam is a country located in an area with a tropical monsoon climate and many geographical regions favourable for plant growth. The diversity of Vietnamese flora is very high, with about 13,000 vascular plants [1]. The Family Lamiaceae (Labiatae), also known as the mint family, is represented by 236 genera and about 7,200 species distributed almost all around the world [2]. Numerous species of the family Lamiaceae, which are rich in volatile oils, are of great importance as medicinal plants and spices.

As people become more and more aware of the effectiveness and benefits of plant aromatics, medicinal plants, and their metabolites in protecting health and beauty, the demand for products of plant origin is constantly increasing. Essential oils (EOs) - secondary metabolites from plants - are widely used in the chemical industry, perfumery, flavouring, and cosmetic industries. Many of their therapeutic properties have been demonstrated in recent years, such as antioxidant, antimicrobial, anti-inflammatory, antiviral, antispasmodic, antiproliferative, and neuroprotective, among others [3]. Nowadays, EOs are

likely to be used as safer alternatives to synthetic antibiotics, antifungals, anti-mosquitos, insecticides, and materials used for beauty treatments. With such outstanding values, many plant species containing essential oils, including species belonging to the Lamiaceae family, have become popular crops, and many essential oils are commodities with high economic value.

E. ciliata, a flowering plant belonging to the family Lamiaceae, is a wild plant native to Asia. In traditional medicine, *E. ciliata* has been used for the treatment of headache, fever, diarrhoea, oedema, blood clotting, gastralgia, dysphonia, nephritis, and throat infections [4, 5]. According to scientific literature, *E. ciliata* is a valuable bioactive source of natural antioxidants [6]. *E. ciliata* also possesses anti-inflammatory, antiviral, antibacterial, antioxidant, anticancer, and vasorelaxant effects [6-9]. The chemical composition of *E. ciliata* leaf essential oil is characterised by high amounts of geranial (19.5-26.5%), neral (15.2-20.5%), limonene (10.9-14.2%), and (Z)-p-farnesene (10.8-11.7%) as major constituents [10]. J.H. Jeong, et al. (2005) [11] reported that EOs extracted from

*Corresponding author: Email: ltpkcmue@gmail.com

E. ciliata inhibit lipid peroxidation. In addition, Y.J. Kim, et al. (2006) [12] showed that a methanol extract of *E. ciliata* showed potent inhibitory activity against pancreatic lipase in a screen of medicinal plants for anti-obesity agents.

Plant growth regulators have immense commercial importance in intensive agriculture and are valuable in the agribusiness of high-value crops for organic cultivation [13]. Efforts to improve EO production by applying plant growth regulators have been suggested [14]. Plant growth regulators have been found to enhance herb and oil yield in some species. Gibberellins (especially gibberellic acid GA_3) are the most critical and valuable compounds for enhancing the productivity of commercial crops. Gibberellins are associated with various growth and development processes such as seed germination, stem and hypocotyl elongation, leaf expansion, floral organ development, flowering time reduction, flower number and size increase, and induction of some hydrolytic enzymes in the aleurone of cereal [15, 16]. In its role in essential oil accumulation, GA_3 has been studied previously; B.R.R. Rao, et al. (1997) [17] sprayed geranium with GA_3 promoted oil yield and prolonged flowering. Reports are available indicating a direct correlation between trichome number and oil production [18]. In parallel, GA_3 also increases leaf area leading to an increase in trichome parameters. The increase in oil accumulation per bed was because of an increase in trichome parameters (density, trichome diameter, total number of trichomes per leaf), leaf area, and oil biogenetic capacity per plant.

There have been much research works on *E. ciliata*. However, these studies focused mainly on extracting and analysing the chemical composition or biological activities of secondary compounds obtained from *E. ciliata* [19, 20]. On the contrary, the number of studies on the effects of phytohormones on the growth and accumulation of essential oil of *E. ciliata* is still limited. Based on the reference information, this study was carried out to evaluate the effects of GA_3 on plant height, leaf length, leaf width, whole plant fresh biomass, dry biomass, nitrate content, and essential oil percentage of *E. ciliata*.

2. Materials and methods

2.1. Plant materials

The seeds of Vietnamese balm (*E. ciliata*) were obtained from Huong Nong Seeds Co., Ltd (Vietnam). *E. ciliata* at the 4-week-old seedling stage.

2.2. Growth conditions

Prior to seed sowing, a homogeneous mixture of soil and organic manure in the ratio of 4:1 was filled in the nursery

garden [21]. A uniform basal dose of nitrogen, phosphorus, and potassium was given at a rate of 15, 25, and 25 kg ha⁻¹, respectively. The soil was maintained at proper moisture to ensure better growth of the plants. Relative humidity was set to 60-80% [22]. The seeds were sown directly at a depth of 2 cm in a nursery garden located in Can Giuoc district, Long An province.

2.3. Experimental design

2.3.1. Plant growth assessment experiment

The growth of *E. ciliata* grown from seeds was assessed for 8 weeks (from the time the plant has 2 true leaves to the flowering stage). A randomised complete block design (RCBD) with three replications was applied for the experimental layout. After each week, the plant's growth was recorded for the parameters of plant height, leaf length, leaf width, total plant fresh weight, total plant dry weight, nitrate content, photosynthesis intensity, and respiration rate.

2.3.2. Gibberellic acid experiments

Nursery-raised healthy, vigorously growing 4-week-old seedlings (based on the result of the plant growth assessment experiment) were sprayed once on the foliage of the plants with six levels of GA_3 solution (adjusted to pH 7.0 with NaOH) (0, 5, 10, 15, 20, 25 ppm) (1.5 l) with the help of an atomiser [23]. The spraying volume for each treatment was maintained at 0.5 l. After a lapse of 2 hours following the spraying, regular watering procedures were resumed. Treatments were triplicated in a completely randomised block design three times in factorial experiments. The performance of *E. ciliata* was assessed for the above-mentioned parameters at week 6 (W6) and week 8 (W8) after spraying.

2.3.3. Growth analysis

Growth parameters were measured as follows:

1. Plant height (PH, cm): The plant height was measured from the area where the stem contacts the growing medium to its apex with a ruler.
2. Leaf length (LL, cm): The length of the 4th leaf was measured at its longest size using a technical Palmer ruler (Mitutoyo digital micrometre, Japan).
3. Leaf width (LW, cm): The width of the 4th leaf was measured at its widest size using a technical Palmer ruler (Mitutoyo digital micrometre, Japan).
4. Fresh biomass (FB, g): Randomly chosen plants were taken from each experimental unit. After cleaning them

from dust and separating them from the root system, the total plant fresh weight was recorded with a sensitive scale.

5. Dry biomass (DB, g): After weighting, the plant was wrapped in a clean paper bag, labelled, and oven-dried at 65°C for 48 h to determine the dry weight following the methodology established by M. Corell, et al. (2012) [24].

6. Nitrate content (NC, mg.kg⁻¹/FW): Nitrate content in plants was determined by colour reaction with phenol sulphonic acid solution. The absorbance of the standards and samples was measured with a Shimadzu (Model: 2401) UV-Vis spectrophotometer after setting the blank at 100% transmittance at the wavelength of 410 nm [25].

7. Photosynthesis intensity (PI, $\mu\text{mol O}_2/\text{dm}^2/\text{h}$) and respiration rate (RR, $\mu\text{mol O}_2/\text{dm}^2/\text{h}$): *E. ciliata* leaves were cut with specialised cutting tools ($\leq 10 \text{ cm}^2$), then placed in the measuring chamber LD₂ (Hansatech, the UK) for 10 minutes. Gas exchange was measured with the instrument's oxygen electrode and was calculated as an increase (when leaves were illuminated with a white LED at 150 $\mu\text{mol}/\text{m}^2/\text{s}$) or decrease (when leaves were placed in the dark) of the amount of oxygen in the measuring chamber. The chamber temperature was 27 $\pm$ 0.2°C under light-intensity conditions. The photosynthetic intensity and respiratory intensity were calculated at 4 to 7 minutes based on a graph showing the change in oxygen content displayed on the computer [26].

2.3.4. Essential oil contents

Essential oil contents (EO, %) of the air-dried and ground plants (150 g each) were determined by hydrodistillation for 3 h using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and stored at 4°C in the dark until further analysis [27].

2.3.5. Morphology of trichomes

Leaf samples were transversely cut into thin slices and directly observed for the appearance of hairs under an optical microscope after each week of the growth cycle.

2.4. Data analysis

The data of growth of *E. ciliata* at each week (growth experiment); weeks 6 and 8 (GA₃ treatment experiment); and the data of essential oil contents of the GA₃ treatment experiment at week 8 were tested. One-way ANOVA with a significance level of 5% ($p < 0.05$) was used to compare the mean values at each time point. These analyses were performed using SPSS statistical software to test the significant difference at probability $p < 0.05$. All data were presented as mean $\pm$ standard errors (SE).

3. Results and discussion

3.1. Growth of *E. ciliata* in nursery garden for 8 weeks

E. ciliata in the nursery garden grew slowly from week 1 to week 4. By week 5, the plants exhibited faster growth, as evidenced by the increase in growth parameters such as plant height (9.10 to 15.58 cm), leaf length (3.34 to 6.94 cm), and leaf width (1.91 to 3.71 cm) (Table 1). The leaves were dark green and deeply lobed, with each axillary bud having 2-4 branches. Stems and roots were sturdy. During weeks 6 and 7, plants continued to grow, showing increased tillering, and flowered at the end of weeks 7 and 8 (Fig. 1). This is consistent with the role of leaves as the organs carrying out the function of photosynthesis and synthesising metabolic compounds for the plant. The rapid growth of leaves before flowering is to create conditions for the plant to accumulate enough energy and achieve physiological maturity, preparing for the flowering stage [28].

Table 1. Growth analysis of *E. ciliata* in the nursery garden.

Parameters	Week							
	1	2	3	4	5	6	7	8
PH cm	1.75 $\pm$ 0.14 ^a	2.81 $\pm$ 0.34 ^b	5.40 $\pm$ 0.36 ^c	9.10 $\pm$ 0.44 ^d	15.58 $\pm$ 1.96 ^e	26.33 $\pm$ 1.78 ^f	28.23 $\pm$ 0.70 ^g	28.34 $\pm$ 0.90 ^g
LL cm	1.02 $\pm$ 0.07 ^a	1.18 $\pm$ 0.10 ^a	2.80 $\pm$ 0.03 ^b	3.34 $\pm$ 0.35 ^b	6.94 $\pm$ 0.56 ^d	7.02 $\pm$ 0.11 ^d	7.13 $\pm$ 0.28 ^d	7.17 $\pm$ 0.09 ^d
LW cm	0.72 $\pm$ 0.10 ^a	0.77 $\pm$ 0.11 ^a	1.78 $\pm$ 0.03 ^b	1.91 $\pm$ 0.36 ^b	3.71 $\pm$ 0.57 ^c	3.99 $\pm$ 0.11 ^d	4.07 $\pm$ 0.28 ^d	4.22 $\pm$ 0.09 ^d
FB g	1.06 $\pm$ 0.06 ^a	1.16 $\pm$ 0.06 ^a	3.45 $\pm$ 1.11 ^b	9.81 $\pm$ 0.41 ^c	25.18 $\pm$ 0.43 ^d	31.95 $\pm$ 0.49 ^e	32.27 $\pm$ 0.63 ^e	32.36 $\pm$ 0.45 ^e
DB g	0.05 $\pm$ 0.01 ^a	0.06 $\pm$ 0.01 ^a	0.13 $\pm$ 0.03 ^b	0.62 $\pm$ 0.08 ^c	1.31 $\pm$ 0.02 ^d	2.79 $\pm$ 0.05 ^e	2.82 $\pm$ 0.02 ^e	2.83 $\pm$ 0.11 ^e
NC mg.kg ⁻¹ /FW	4.75 $\pm$ 0.14 ^a	4.94 $\pm$ 0.32 ^a	12.81 $\pm$ 0.59 ^b	28.61 $\pm$ 0.46 ^c	55.94 $\pm$ 0.45 ^d	58.81 $\pm$ 0.58 ^e	59.10 $\pm$ 0.98 ^e	59.52 $\pm$ 0.65 ^e

Values are mean $\pm$ SE, the superscript letters in each row indicate significant differences between weeks ($p < 0.05$).



Fig. 1. Growth of *E. ciliata* in the nursery garden over 8 weeks. Scale bar: 5 cm.

The flowering phase commenced at the end of week 7. At that time, plant growth slowed down, reaching its maximum in plant height (28.34 cm), leaf length (7.17 cm), and leaf width (4.22 cm) due to the vegetative meristem transforming into the floral meristem. Therefore, it was no longer able to lengthen the internodes and create new leaves (Table 1), aiding the plant's transition from the vegetative stage to the flowering stage [28].

During the developmental stages, plants absorb and accumulate nutrients. Nitrate accumulation depends on the plant's requirements during each growth stage. During the vegetative stage, the plant requires a large amount of nitrogen for the development of stems and leaves. As the plant transitions to the reproductive stage, nutrients are prioritised for flowering and fruiting, thereby slowing down or halting growth to concentrate energy on flowering [29]. This was consistent with the increase in FB (3.45 to 31.95 g); DB (0.13 to 2.79 g); nitrate content (12.81 to 58.81 mg.kg⁻¹/FW) from weeks 3 to 6. From weeks 6 to 8, the increase in FB (31.95-32.36 g), DB (2.79 to 2.83 g), and nitrate content (58.81 to 59.52 mg.kg⁻¹/FW) was slow and showed no significant difference ($p>0.05$) (Table 1).

The photosynthetic intensity gradually increased from week 1 to week 6 (4.95 to 18.06 $\mu\text{mol O}_2/\text{dm}^2/\text{h}$), peaked at week 6, and then gradually decreased at weeks 7 and 8 (18.06 to 17.93 $\mu\text{mol O}_2/\text{dm}^2/\text{h}$) (Table 2). Strong photosynthesis during the nutrient stage helps plants to grow quickly, with the energy generated in photosynthesis preferentially used for stem elongation, leaf formation, and branching [30]. Photosynthetic intensity started to decrease slightly from week 7 to week 8 but there is no statistical difference. On the other hand, respiratory intensity increased from week 7 to week 8 (5.77 to 9.84 $\mu\text{mol O}_2/\text{dm}^2/\text{h}$) to prepare for the flowering stage. In the flowering stage, *E. ciliata* favourably uses a lot of energy from cellular respiration to support flower morphogenesis energy [31].

Table 2. Photosynthetic intensity and respiration rate of *E. ciliata* in the nursery garden.

Parameters	Week							
	1	2	3	4	5	6	7	8
PI $\mu\text{mol O}_2/\text{dm}^2/\text{h}$	4.95±0.09 ^a	5.37±0.17 ^a	6.39±0.10 ^b	9.16±0.19 ^c	17.65±0.48 ^d	18.06±0.22 ^d	17.98±0.22 ^d	17.93±0.07 ^d
RR	22.17±0.50 ^a	20.34±1.04 ^d	20.03±0.75 ^d	11.19±0.66 ^c	5.54±0.41 ^a	5.77±0.26 ^a	5.77±0.19 ^a	9.84±0.35 ^b

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

3.2. Morphological changes and accumulation of essential oil in *E. ciliata* trichomes for 8 weeks

On the cross-section of the leaves, trichomes accumulating essential oil in *E. ciliata* have a 1-cell head and a 2-cell head. At weeks 1 and 2, a new ciliary system formed into undivided cells and there was almost no oil accumulation (Fig. 2A). At week 3, trichomes divided into a 2-cell epithelium (Fig. 2B) and began to accumulate essential oil (Fig. 2D) or did not divide but still accumulated essential oil (Figs. 2C, 2D). By week 4, the accumulation of

essential oil in the hair secretion system was enhanced. This transformation was maintained and did not differ from week 4 to week 8 (Fig. 2).

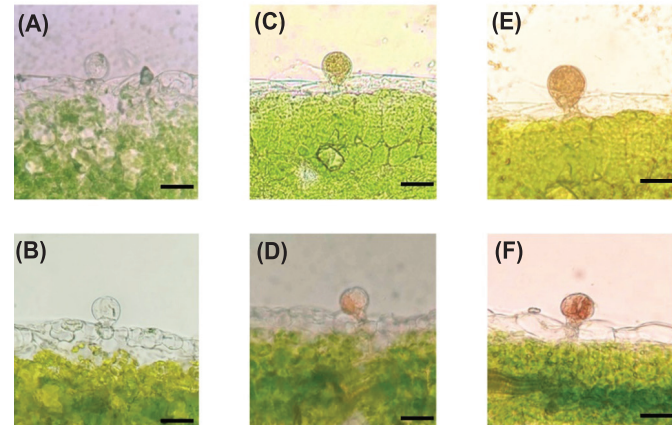


Fig. 2. Feathers in *E. ciliata* 1 cell head has not accumulated essential oil at week 1, 2 (A); the head of 2 cells has not yet accumulated essential oil at week 3 (B); 1 cell head accumulates essential oil at week 3 (C); the head of 2 cells accumulates essential oils at weeks 4-8 (E); the head of 2 cells accumulates essential oil at weeks 4-8 (F). Scale bar: 10 μm .

3.3. Effect of GA₃ on the growth and accumulation of essential oil in *E. ciliata*

3.3.1. Plant height

The results in Tables 3 and 4 indicated that spraying GA₃ on *E. ciliata* caused a significant increase in plant height. Increasing GA₃ concentrations from 5 to 10 ppm significantly increased plant height (33.68-45.58 cm at W6; 55.94-77.01 cm at W8). The height of *E. ciliata* was highest (with significant difference) when sprayed with 10 ppm GA₃, reaching 45.58 cm at W6, and 77.01 cm at W8, compared with the control which gave 26.42 cm at W6; and 28.55 at W8.

Table 3. Effect of GA₃ on growth of *E. ciliata* after 2 weeks of treatment.

GA ₃ concentrations (ppm)	Parameters					
	PH (g)	LL (cm)	LW (cm)	FB (g)	DB (g)	NC (mg.kg ⁻¹ /FW)
0	26.42±0.84 ^a	7.01±0.10 ^a	3.93±0.18 ^a	29.98±0.98 ^a	2.81±0.04 ^a	58.15±0.43 ^a
5	33.68±0.57 ^c	7.37±0.12 ^b	4.35±0.15 ^b	34.66±1.80 ^c	4.09±0.30 ^c	69.39±0.77 ^c
10	45.58±0.64 ^d	8.73±0.34 ^d	5.50±0.43 ^d	44.02±1.20 ^d	6.39±1.18 ^c	83.39±1.27 ^c
15	44.24±0.48 ^c	8.64±0.46 ^d	5.41±0.34 ^d	43.09±1.56 ^c	6.28±0.32 ^{bc}	83.01±1.44 ^c
20	37.90±0.49 ^d	8.64±0.46 ^d	4.72±0.28 ^c	39.81±1.72 ^d	5.73±0.36 ^d	76.41±1.48 ^d
25	29.38±0.57 ^b	7.29±0.13 ^{ab}	4.27±0.10 ^b	31.89±1.54 ^b	3.63±0.07 ^b	65.86±0.91 ^b

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

Table 4. Effect of GA₃ on growth of *E. ciliata* after 4 weeks of treatment.

GA ₃ concentrations (ppm)	Parameters					
	PH (g)	LL (cm)	LW (cm)	FB (g)	DB (g)	NC (mg.kg ⁻¹ /FW)
0	28.55±0.42 ^a	7.17±0.12 ^a	4.30±0.09 ^a	34.47±1.35 ^a	2.96±0.12 ^a	61.25±0.78 ^a
5	55.94±0.48 ^c	8.52±0.41 ^b	5.31±0.34 ^b	49.62±0.67 ^c	7.49±0.22 ^c	95.03±0.67 ^c
10	77.01±0.49 ^f	9.50±0.51 ^c	6.03±0.29 ^d	65.44±1.63 ^f	11.10±0.30 ^f	83.39±1.27 ^e
15	75.05±0.78 ^e	8.81±0.35 ^c	5.79±0.31 ^{cd}	62.76±2.49 ^e	9.74±0.37 ^e	128.71±1.03 ^e
20	66.89±0.80 ^d	8.64±0.46 ^c	5.51±0.33 ^{bc}	58.56±1.58 ^d	8.38±0.37 ^d	109.61±0.58 ^d
25	44.45±0.56 ^b	7.52±0.15 ^a	4.46±0.15 ^a	37.15±0.46 ^b	6.13±0.07 ^b	71.09±0.92 ^b

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p < 0.05$).

3.3.2. Leaf length and leaf width

The data in Tables 3 and 4 indicated that spraying GA₃ on *E. ciliata* caused a considerable increase in the leaf length and leaf width, which reached 7.29-8.73 cm (LL at W6), 4.27-5.50 cm (LW at W6). The highest length and width of the leaves (9.50 cm (LL) and 6.03 cm (LW)) were observed when the plant was sprayed with 10 ppm GA₃ at W8, which differs significantly from all other treatments studied.

3.3.3. Fresh and dry biomass

The results in Tables 3 and 4 showed that the total plant fresh biomass (FB) and dry biomass (DB) were significantly highest (44.02 and 6.39 g (W6); 65.44 and 11.10 g (W8), respectively) when the plants were sprayed with GA₃ at 10 ppm, showing an increase of 47.32%.

3.3.4. Nitrate content

The outcomes in Tables 3 and 4 demonstrate that spraying *E. ciliata* with GA₃ from 5-25 ppm caused significant increase in nitrate content, which ranged from 65.86 to 83.39 mg.kg⁻¹/FW at W6, and from 71.09 to 128.71 mg.kg⁻¹/FW at W8. The nitrate content had the significantly highest value of 83.39 mg.kg⁻¹/FW at W6 with 10 ppm and 128.71 mg.kg⁻¹/FW at W8 when plants were treated with 15 ppm GA₃.

3.3.5. Photosynthesis intensity and respiration rate

After 4 weeks of GA₃ treatment, the PIs of all treated plants increased (PI: 19.24-32.49 μmol O₂/dm²/h at W8) when compared with control (PI: 7.04 μmol O₂/dm²/h at W8). Plants treated with 10 and 15 ppm GA₃ had increased PIs compared to those treated with 5; 20; and 25 ppm GA₃ and the control. The PI was highest in the 10-ppm GA₃ treatment (29.53 μmol O₂/dm²/h at W6; 32.49 μmol O₂/dm²/h at W8) (Table 5, Fig. 3). In contrast, the RIs of the GA₃-treated plants decreased (from 6.94 to 2.90 μmol O₂/dm²/h at W6; 7.04 to 1.52 μmol O₂/dm²/h at W8) and were different from that of the control (11.16 μmol O₂/dm²/h at W6; 9.84 μmol O₂/dm²/h at W8) (Table 5).



Fig. 3. Effect of GA₃ treatment on the morphology of *E. ciliata* in the nursery garden at week 6: control (A), GA₃ 5 ppm (B), GA₃ 10 ppm (C), GA₃ 15 ppm (D), GA₃ 20 ppm (E), GA₃ 25 ppm (F). Scale bar: 5 cm.

Table 5. Effect of GA₃ on photosynthetic intensity and respiration rate of *E. ciliata* in the nursery garden.

Parameters	Gibberellic acid concentrations (ppm)					
	0	5	10	15	20	25
After 2 weeks						
PI	9.15±0.51 ^a	19.96±0.43 ^b	29.53±0.48 ^d	27.66±0.93 ^c	20.17±0.72 ^b	19.57±0.15 ^b
RR	11.16±0.63 ^a	4.59±0.02 ^b	2.90±0.06 ^c	3.08±0.04 ^d	5.45±0.35 ^e	6.94±0.11 ^d
After 4 weeks						
PI	7.04±0.31 ^a	22.09±0.17 ^c	32.49±0.27 ^e	30.01±0.17 ^d	19.36±0.14 ^b	19.24±0.15 ^b
RR	9.84±0.35 ^c	3.03±0.07 ^c	1.52±0.05 ^a	2.03±0.07 ^b	6.79±0.04 ^d	7.04±0.16 ^d

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p < 0.05$).

Gibberellins (especially GA₃) are the most important group of compounds among the known plant growth regulators for enhancing the productivity of commercial crops. However, the high cost and tedious production technology restrict its field application to high-value crops only [32]. As shown in Tables 3 and 4, spraying *E. ciliata* with 10 ppm GA₃ caused a considerable increase in PH, LL, LW, FB, DB, and NC when compared with the control. The increase in plant height under treatments with gibberellic acid in *Mentha* plants (0.001-0.1 mM GA₃) was also previously reported [33]. The physiological role of gibberellic acid in plants, which involves stimulating and activating the processes of cell division and expansion, can be linked to these results. Additionally, by promoting the synthesis of DNA, RNA, and proteins, gibberellic acid activates many enzymes that are crucial for the growth and development of plants and increases the activity of several biological and physiological processes of the cell [34]. Additionally, gibberellins increase the permeability of cell walls and raise

the water content of cells [35]. It is possible to interpret that the gibberellic acid's ability to enhance auxin levels leads to an increase in cell division and cell elongation, resulting in increased plant height [36, 37]. Alternatively, this could be explained by GA₃'s capacity to stimulate the synthesis of mRNA for hydrolytic enzymes and by the fact that larger cells eventually result in longer internodes [38]. Bioactive GA promotes growth by opposing the functions of DELLA growth-repressing proteins (DELLAs), members of the GRAS family of transcriptional regulators [39]. The GA pathway is a major regulator of this feed forward loop interconnecting plant growth to nitrate availability. This leads to GA₃ supplementation increasing nitrate content as previously reported by L. Camut, et al. (2021) [40].

3.3.6. Essential oil content

The results obtained in Table 5 reveal that the oil content of *E. ciliata* was positively affected by spraying with 5 to 25 ppm GA₃, reaching values of 1.09 to 1.41%. The oil percentage was highest (1.41%) for plants treated with 10 ppm GA₃. However, between treatments supplemented with GA₃ at concentrations of 10, 15, 20 ppm, there was no statistical difference in essential oil yields (Table 6, Fig. 4). For comparison, the oil content of the control was 0.93%.

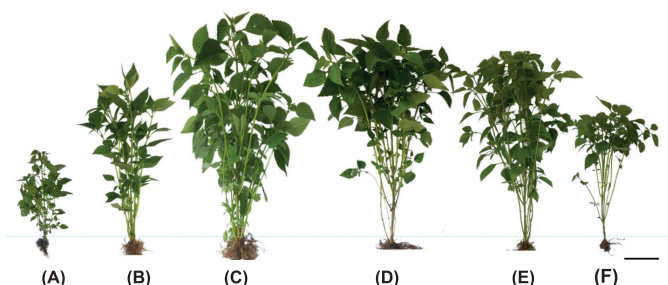


Fig. 4. Effect of GA₃ treatment on the morphology of *E. ciliata* in the nursery garden at week 8: control (A), GA₃ 5 ppm (B), GA₃ 10 ppm (C), GA₃ 15 ppm (D), GA₃ 20 ppm (E), GA₃ 25 ppm (F). Scale bar: 5 cm.

Table 6. Effect of GA₃ on essential oil content of *E. ciliata* in the nursery garden.

Parameter		Gibberellic acid concentrations (ppm)					
		0	5	10	15	20	25
EO content	%	0.93±0.04 ^b	1.11±0.02 ^c	1.41±0.02 ^c	1.38±0.03 ^{bc}	1.29±0.04 ^d	1.09±0.06 ^c

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks (p<0.05).

Plant growth regulators have been reported to influence the yield and quality of essential oil in many crops [41]. Growth regulators such as GA₃ can influence essential oil production through effects on plant growth, essential oil biosynthesis, and the number of oil storage structures. On the other hand,

the increase in oil content may also be due to their effects on enzymatic pathways of terpenoid biosynthesis [42-44]. GA₃ affects the key enzyme activity of terpenoid biosynthetic pathways (DXS and HMGR) based on decreasing DXS activity in plants. GA₃ treatment caused a decrease in the 1-deoxy-D-xylulose5-phosphate synthase (DXS) activity in male and female *Cannabis sativa* plants [45]. Many reports support a regulatory role of DXS for the production of MEP-derived isoprenoids in plants [46]. J.M. Estevez, et al. (2001) [47] reported that transgene-mediated up or down regulation of DXS levels in *Arabidopsis* was correlated with concomitant changes in the levels of MEP-derived isoprenoid end products. Additionally, D.J. Wilk, et al. (1999) [48] showed that gibberellin works to protect the destruction of chlorophyll by inhibiting the effect of ethylene on chlorophyllase transcript accumulation. Similarly, GA₃ also increased oil yield and quality in *Ocimum sanctum* and *Ocimum basilicum* [49]. The result was in conformity with the finding of E.S.M. Rashad, et al. (2009) [50] on *Calendula officinalis* L., A. Dadkhah, et al. (2016) [51] on *Satureja hortensis*, and M.N. Hasan, et al. (2020) [52] on *Rosmarinus officinalis* L.

4. Conclusions

The growth and development of *E. ciliata* (Thunb.) Hyl. can be divided into 3 stages: the slow growth phase (from sowing time to week 4), the fast growth phase (week 4 to week 7), and the flowering phase (stops growing and flowering from week 7 to week 8). The formation of the trichomes of *E. ciliata* starts from the young leaves (1 to 2 weeks old). There are 2 types of hairs, 1-cell hairs and 2-cell hairs, which begin to accumulate essential oil from week 3.

In addition, the results clearly showed that the exogenous application of GA₃ considerably affected the plant growth attributes, oil productivity and plant biomass. Foliar treatment with GA₃ (10 ppm) could be used to promote the growth and accumulation of EOs in Vietnamese balm (*E. ciliata* (Thunb.) Hyl.).

CRedit author statement

Luong Thi Le Tho: Conceptualisation, Writing - Original draft preparation, Literature review; Luu Tang Phuc Khang: Experimental design, Data collection and analysis, Revision; Nguyen Xuan Hieu: Experimental implementation, Data collection; Dinh Thi Bich Thuy: Writing - Original draft preparation, Literature review; Vo Ngoc Khoi Nguyen: Experimental implementation, Data collection; Chau Minh Hai Dang: Experimental implementation, Data collection; Tran Thi Phuong Dung: Conceptualisation, Writing - Original draft preparation, Literature review.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] N.T. Nguyen (2008), *Flora and Species Diversity*, Vietnam National University Publishing House, 146pp (in Vietnamese).
- [2] R.M. Harley, S. Atkins, A.L. Budantsev, et al. (2004), "Labiateae", *The Families and Genera of Vascular Plants*, pp.167-282, DOI: 10.1007/978-3-642-18617-2_11.
- [3] M.D.J.R. Alanis, J.B. Gonzalez, C.T. Alvarez, et al. (2019), "Chemical composition and biological activities of oregano essential oil and its fractions obtained by vacuum distillation", *Molecules*, **24**(10), pp.1904-1914, DOI: 10.3390/molecules24101904.
- [4] P.M. Zhao, C.X. Liu, D. Lai, et al. (2016), "Analysis of the essential oil of *Elsholtzia ciliata* aerial parts and its insecticidal activities against *Liposcelis bostrychophila*", *Helvetica. Chimica. Acta.*, **99**, pp.90-94, DOI: 10.1002/hlca.201500232.
- [5] X. Wang, L. Gong, H. Jiang (2017), "Study on the differences between volatile constituents of the different parts from *Elsholtzia ciliata* by SHS-GC-MS", *American Journal of Analytical Chemistry*, **8**(10), pp.625-635, DOI: 10.4236/ajac.2017.810045.
- [6] M.S. Choi, B. Choi, S.H. Kim, et al. (2015), "Essential oils from the medicinal herbs upregulate dopamine transporter in rat pheochromocytoma cells", *Journal of Medicinal Food*, **18**(10), pp.1-9, DOI: 10.1089/jmf.2015.3475.
- [7] Z. Guo, Z. Liu, X. Wang, et al. (2012), "Elsholtzia: Phytochemistry and biological activities", *Chemistry Central Journal*, **6**, DOI: 10.1186/1752-153X-6-147.
- [8] T.P.K. Luu, H.T. Nguyen, V.L. Cao, et al. (2023), "Chemical composition of *Elsholtzia ciliata* (Thunb.) Hyland essential oil in Vietnam with multiple biological utilities: A survey on antioxidant, antimicrobial, anticancer activities", *Academia Journal of Biology*, **45**(3), pp.99-110, DOI: 10.15625/2615-9023/18230.
- [9] L. Pudziulyte, V. Jakstas, L. Ivanauskas, et al. (2018), "Different extraction methods for phenolic and volatile compounds recovery from *Elsholtzia ciliata* fresh and dried herbal materials", *Industrial Crops and Products*, **120**, pp.286-294, DOI: 10.1016/j.indcrop.2018.04.069.
- [10] X.D. Nguyen, V.H. Le, H.H. Le, et al. (1996), "Composition of the essential oils from the aerial parts of *Elsholtzia ciliata* (Thunb.) Hyland from Vietnam", *Journal of Essential Oil Research*, **8**(1), pp.107-109.
- [11] J.H. Jeong, H.O. Sohn, H.J. Shin, et al. (2005), "Biological activity of flavor components extracted from *Elsholtzia ciliata* and *Elsholtzia splendens*", *Journal of The Korean Society of Tobacco Science*, **27**, pp.19-30.
- [12] Y.J. Kim, B.H. Kim, S.Y. Lee, et al. (2006), "Screening of medicinal plants for development of functional food ingredients with antiobesity", *Journal of The Korean Society for Applied Biological Chemistry*, **49**, pp.221-226.
- [13] W. Rademacher (2015), "Plant growth regulators: Backgrounds and uses in plant production", *Journal of Plant Growth Regulation*, **34**, pp.845-872, DOI: 10.1007/s00344-015-9541-6.
- [14] M. Moghaddam, L. Mehdizadeh (2017), "Chemistry of essential oils and factors influencing their constituents", *Soft Chemistry and Food Fermentation*, pp.379-419, DOI: 10.1016/B978-0-12-811412-4.00013-8.
- [15] S.M. Swain, D.P. Singh (2005), "Tall tales from sly dwarves: Novel functions of gibberellins in plant development", *Trends in Plant Science*, **10**(3), pp.123-129, DOI: 10.1016/j.tplants.2005.01.007.
- [16] A.N.M. Khassawneh, N.S. Karam, R.A. Shibli (2006), "Growth and following of black iris (*Iris nigricans* Dinsm) following treatment with plant growth regulators", *Scientia Horticulturae*, **107**(2), pp.187-193, DOI: 10.1016/j.scienta.2005.10.003.
- [17] B.R.R. Rao, A.K. Bhattacharya, A.K. Singh, et al. (1997), "Growth, biomass production and essential oil profile of rose-scented geranium (*Pelargonium* species) as influenced by plant growth regulators", *Pafai J.*, **19**, pp.11-14.
- [18] H.K. Lichtenthaler, M. Rohmer, J. Schwender (2006), "Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants", *Physiologia Plantarum*, **101**(3), pp.643-652, DOI: 10.1111/j.1399-3054.1997.tb01049.x.
- [19] D.T. Nguyen, H. Tran, S. Schwaiger, et al. (2021), "Effect of non-volatile constituents of *Elsholtzia ciliata* (Thunb.) hyl. from southern Vietnam on reactive oxygen species and nitric oxide release in macrophages", *Chemistry & Biodiversity*, **18**(1), DOI: 10.1002/cbdv.202000577.
- [20] T.B. Hoang, N. Nguyen (2022), "The antimicrobial activity and chemical composition of *Elsholtzia blanda* (Benth.) Benth. essential oils in Lam Dong province, Vietnam", *Can Tho University Journal of Science*, **14**(3), pp.72-77, DOI: 10.22144/ctu.jen.2022.044.
- [21] M.M.A. Khan, N. Hashmi, Moinuddin, et al. (2014) "Changes in growth, yield, photosynthetic characteristics, enzyme activities and essential oil production of fennel (*Foeniculum vulgare* Mill.) under growth regulator treatments", *Journal of Essential Oil Research*, **26**(2), pp.105-113, DOI: 10.1080/10412905.2013.860408.
- [22] N. Lu, E.L. Bernardo, C. Tippayadarapanich, et al. (2017), "Growth and accumulation of secondary metabolites in perilla as affected by photosynthetic photon flux density and electrical conductivity of the nutrient solution", *Frontiers in Plant Science*, **8**, DOI: 10.3389/fpls.2017.00708.
- [23] W.A.A.A. Rawi, M.E.A.A. Hadethi, A.A.A. Kareem (2016), "Effect of foliar application of gibberellic acid and seaweed extract spray on growth and leaf mineral content on peach trees", *Iraqi Journal of Agricultural Science*, **47**(7), pp.98-105.
- [24] M. Corell, M.C. Garcia, J.I. Contreras, et al. (2012), "Effect of water stress on *Salvia officinalis* L. bioproductivity and its bioelement concentrations", *Communications in Soil Science and Plant Analysis*, **43**(1-2), pp.419-425, DOI: 10.1080/00103624.2012.641811.
- [25] S. Ramalingam, A. Subramania (2021), "Effective removal of nitrates from the drinking water by chemical and electrochemical methods", *Engineered Science*, **15**, pp.80-88, DOI: 10.30919/es8d456.

- [26] P.T. Doan, A.T. Le, N.H. Phan (2020), "Effect of thidiazuron and monochromatic light on shoot development *in vitro* culture of *Polygonum cuspidatum* Sieb et Zucc.", *Science & Technology Development Journal - Natural Sciences*, **4(2)**, pp.468-477 (in Vietnamese).
- [27] T.Q.A. Tran, T.T.H. Vo (2022), "Study on the chemical composition and antibacterial properties of Vietnamese balm essential oil (*Elsholtzia ciliata*)", *Journal of Agricultural Science and Technology of the University of Agriculture and Forestry*, **6(1)**, pp.2881-2890 (in Vietnamese).
- [28] T.V. Bui (2000), *Plant Physiology. Part II: Development*, Vietnam National University Publishing House, 333pp (in Vietnamese).
- [29] A.J. Bloom, F.S. Chapin, H.A. Mooney (1985), "Resource limitation in plants-an economic analogy", *Annual Review of Ecology and Systematics*, **16(1)**, pp.363-392, DOI: 10.1146/annurev.es.16.110185.002051.
- [30] B. Marambe, L. Amarasinghe, K. Silva, et al. (2004), "Distribution, biology and management of *Mimosa pigra*", *Sri Lanka Research and Management of Mimosa pigra Canberra, Australia: CSIRO Entomology*, pp.85-90.
- [31] L. Taiz, E. Zeiger (2002), "Photosynthesis: Physiological and ecological considerations", *Plant Physiology*, **9**, pp.172-174.
- [32] S.K. Bose, R.K. Yadav, S. Mishra, et al. (2013), "Effect of gibberellic acid and calliterpenone on plant growth attributes, trichomes, essential oil biosynthesis and pathway gene expression in differential manner in *Mentha arvensis* L.", *Plant Physiology and Biochemistry*, **66**, pp.150-158, DOI: 10.1016/j.plaphy.2013.02.011.
- [33] F. Haider, G.D. Bagchi, A.K. Singh (2009), "Effect of calliterpenone on growth, herb yield and oil quality of *Mentha arvensis*", *International Journal of Integrative Biology*, **7**, pp.53-57.
- [34] I.M.A. Alsudays, H.E.S.A. El Sayed (2020), "Influence of ascorbic acid, gibberellic acid and *Moringa oleifera* extract for alleviating salinity stress by enhancing antioxidant enzymatic activity and some physiological studies on two tomato cultivars", *Journal of Applied Life Sciences International*, **23(8)**, pp.39-62, DOI: 10.9734/jalsi/2020/v23i830180.
- [35] W. Hopkins, N. Huner (2009), *Introduction to Plant Physiology*, John Wiley & Sons, 503pp.
- [36] S. Kuraishi, R.M. Muir (1964), "The mechanism of gibberellic action in dwarf pea", *Plant and Cell Physiology*, **5(3)**, pp.259-271, DOI: 10.1093/oxfordjournals.pcp.a079041.
- [37] L. Taiz, E. Zeiger (2010), *Plant Physiology, 5th Edition*, Sinauer Associates Inc., Publishers Sunderland, Massachusetts USA, 782pp.
- [38] N.T. Baliah, P.C. Sheeba, S. Mallika (2018), "Encouraging effect of gibberellic acid on the growth and biochemical characters of green gram (*Vigna radiata* L.)", *Journal of Global Biosciences*, **7**, pp.5522-5529.
- [39] J.M. Daviere, P. Achard (2013), "Gibberellin signaling in plants", *Development*, **140(6)**, pp.1147-1151, DOI: 10.1242/dev.087650.
- [40] L. Camut, B. Gallova, L. Jilli, et al. (2021), "Nitrate signaling promotes plant growth by upregulating gibberellin biosynthesis and destabilization of DELLA proteins", *Current Biology*, **31(22)**, pp.4971-4982, DOI: 10.1016/j.cub.2021.09.024.
- [41] N.S. Sangwan, A.H.A. Farooqi, F. Shabih, et al. (2001), "Regulation of essential oil production in plants", *Plant Growth Regulation*, **34(1)**, pp.3-21, DOI: 10.1023/A:1013386921596.
- [42] W.N. Ali, A.O.A. Atrakchii (2022), "Effect of gibberellic, salicylic acids, and NPK fertilizers on growth and chemical constituents of Rosemary plants (*Rosmarinus officinalis* L.)", *Journal of Pharmaceutical Negative Results*, **13(3)**, pp.1842-1850, DOI:10.47750/pnr.2022.13.S03.281.
- [43] S. Sharafzadeh, M. Zare (2011), "Influence of growth regulators on growth and secondary metabolites of some medicinal plants from Lamiaceae family", *Advances in Environmental Biology*, **5(8)**, pp.2296-2302.
- [44] C.L. Prins, I.J. Vieira, S.P. Freitas (2010), "Growth regulators and essential oil production", *Brazilian Journal of Plant Physiology*, **22(2)**, pp.91-102, DOI: 10.1590/S1677-04202010000200003.
- [45] H. Mansouri, Z. Asrar, M. Mehrabani (2009), "Effect of gibberellic acid on primary terpenoids and tetrahydrocannabinol in *Cannabis sativa* at flowerin stage", *Journal of Integrative Plant Biology*, **51**, pp.553-561, DOI: 10.1111/j.1744-7909.2009.00833.x.
- [46] M.R. Concepcion, A. Boronat (2002), "Elucidation of the methylerythritol phosphate pathway for isoprenoids biosynthesis in bacteria and plastids: A metabolic milestone achieved through genomics", *Plant Physiology*, **130(3)**, pp.1079-1089, DOI: 10.1104/pp.007138.
- [47] J.M. Estevez, A. Cantero, A. Reindl, et al. (2001), "1-deoxy-D-Xylulose 5- phosphate synthase, limiting enzyme for plastidic isoprenoid biosynthesis in plants", *Journal of Biological Chemistry*, **276**, pp.22901-22909, DOI: 10.1074/jbc.M100854200.
- [48] D.J. Wilk, D. Holland, E.E. Goldschmidt, et al. (1999), "Chlorophyll breakdown by chlorophyllase: Isolation and functional expression of the chlase1 gene from ethylene-treated citrus fruit and its regulation during development", *The Plant Journal*, **20(6)**, pp.653-661, DOI: 10.1046/J.1365-313X.1999.00637.X.
- [49] M.N.A. Eid, S.S. Ahmed (1976), "Preliminary studies on the effect of GA₃ and CCC on growth and essential oil content of *Ocimum basilicum* L.", *Egyptian Journal of Horticulture*, **3(1)**, pp.83-87.
- [50] E.S.M. Rashad, M.E. Wahed, A.A. Amin (2009), "Effect of sitosterol and gibberellic acid on leaf angle, growth, flowering and biochemical constituents of marigold (*Calendula officinalis* L.)", *Medicinal Aromatic Plant Science Biotechnology*, **3(1)**, pp.21-27.
- [51] A. Dadkhah, S. Ghorbanian, G. Rassam (2016), "Foliar application effect of gibberellic acid on growth, volatile oil and nutrients content of *Satureja hortensis* L.", *Zeitschrift für Arznei- & Gewürzpflanzen*, **21(2)**, pp.76-79.
- [52] M.N. Hasan, S.M. Sultan (2020), "Effect of Fe and GA₃ on growth and oil characteristics of Rosemary plant (*Rosmarinus officinalis* L.)", *Plant Cell Biotechnology and Molecular Biology*, **21(40)**, pp.55-63.

Evaluating antioxidant and anti-inflammatory activities of *Dracaena cambodiana* Pierre ex Gagne extracts on RAW 264.7 and SW 1353 cell lines

Huynh Cong Duy¹, Nguyen Trung Quynh Nhu¹, Mai Kieu Tien¹, Nguyen Phuc Thinh¹, Nguyen Thi Le Thuy¹,
Le Thi Truc Linh², Tran Thi Van Anh³, Pham Thi Kim Tram^{1*}

¹Division of Medical Biotechnology, Biotechnology Center of Ho Chi Minh City, Highway 1, Quarter 2, Trung My Tay Ward, District 12, Ho Chi Minh City, Vietnam

²Faculty of Biotechnology, Ho Chi Minh City Open University, 35-37 Ho Hao Hon Street, Co Giang Ward, District 1, Ho Chi Minh City, Vietnam

³Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, 41 Dinh Tien Hoang Street, Ben Nghe Ward, District 1, Ho Chi Minh City, Vietnam

Received 26 October 2023; revised 27 November 2023; accepted 18 January 2024

Abstract:

Dracaena cambodiana Pierre ex Gagnep (Huyết Giác in Vietnamese) is widely utilised in traditional remedies to alleviate joint pain. Indeed, *D. cambodiana* contains chemical components with biological properties such as cytotoxicity, antimicrobial, and antioxidant activities. This research aims to determine the antioxidant and anti-inflammatory properties of *D. cambodiana* extract in RAW 264.7 and SW 1353 cell lines. The *D. cambodiana* was extracted with two solvents: dichloromethane (HGA) and methanol (HGB) and subsequently assessed for the total polyphenols and flavonoids content before evaluating its antioxidant and anti-inflammatory properties in RAW 264.7 and SW 1353 cell lines. The extract with HGB exhibited 1.73 times higher total polyphenol content than HGA. The antioxidant capacity (EC_{50}) of HGA was lower than that of HGB. HGA displayed significant cytotoxicity towards the cells, while HGB did not induce cell death. HGB effectively inhibited nitric oxide production, LPS-induced *iNOS*, and IL-1 β -stimulated *MMP13* expression. These findings demonstrate that HGB derived from *D. cambodiana* Pierre ex Gagnep possesses antioxidant and anti-inflammatory properties, offering potential applications in the treatment of inflammation and osteoarthritis in the future.

Keywords: anti-inflammatory, antioxidant, *Dracaena cambodiana*, osteoarthritis, RAW 264.7, SW 1353.

Classification numbers: 3.3, 3.5, 3.6

1. Introduction

Inflammation is defined as a biological response of the immune system to harmful stimuli such as irritants, pathogens, or damaged cells. Its progression involves the participation of immune cells, blood vessels, and molecular mediators. The primary purpose of the inflammatory response is to eliminate the original cause of cell injury, remove necrotic cells and damaged tissues, and initiate tissue repair.

Arthritis, particularly osteoarthritis, is characterised by the destruction of articular cartilage and local inflammation. It is one of the diseases that significantly impact our health and quality of life, especially in the elderly population. Statistics reveal that 61.1% of individuals over 60 years old are affected, whereas the range is 8-30% among those aged 40-59 [1]. However, there is currently no definitive treatment for these diseases, only several intervention methods to mitigate disease progression, including the application of traditional remedies. Given Vietnam's rich and diverse reservoir of medicinal herbs with potential therapeutic properties, we were determined to conduct

research to identify their chemical composition and mechanism of action. Identifying the chemical components and biological activities of these herbs holds practical value for human health.

D. cambodiana Pierre ex Gagnep belongs to the genus *Dracaena*, which comprises more than 50 species, primarily distributed in tropical and subtropical Asia and Africa. In Vietnam, there are approximately 12 species [2]. According to Oriental medicine, *D. cambodiana* is documented to have effects on sprains, joint pain, and anticoagulation [2, 3]. Currently, several compounds found in *D. cambodiana* extract have exhibited antifungal activity against *Aspergillus niger* and cytotoxic activity against HepG2 at an IC_{50} dose of 2 μ g/ml [4]. Of particular interest is the ethyl acetate fraction of *D. cambodiana* extract, which displays the highest antioxidant activity [5]. Three new flavonoid derivatives have demonstrated cytotoxicity against the K562 cell line and resistance to methicillin-resistant *Staphylococcus aureus* (MRSA) [6]. Moreover, the 96% ethanol extract of the *D. cambodiana* plant did not exhibit acute oral toxicity [7]. The identification of the chemical composition, antibacterial, antifungal, and cytotoxic activities of *D.*

*Corresponding author: Email: phamthikimtram@gmail.com

cambodiana extract underscores its potential for treating various diseases. In this study, we further investigate the *in vitro* antioxidative, anti-inflammatory activity, and the inhibition of joint degeneration of the *D. cambodiana* plant extract.

2. Materials and methods

Extract and cell lines: The extracts of *D. cambodiana* Pierre ex Gagnep were provided by the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City. The redwood part of the tree was collectively extracted using HGA and HGB. The crude extract of each sample was weighed and then dissolved in 100% dimethyl sulfoxide (DMSO) (final concentration was 10 mg/ml) and stored at -20°C for further experiments. RAW 264.7 mouse macrophage cell line (ATCC TIB-71) and human SW 1353 chondrocytes cell line (ATCC HTB-94).

Equipment: Biological safety cabinet class II (ESCO), CO₂ incubator (Panasonic Healthcare), inverted microscope (Carl Zeiss), 4-decimal balance (Mettler Toledo), microplate reader VersaMax (Molecular Devices), cooling micro-centrifuge (Eppendorf), Realtime Lightcycler 480 (Roche), PCR cabinet (Biosan), vortexer (Cleaver Scientific).

Chemicals and reagents: Folin-Ciocalteu (Merck), gallic acid (Sigma), AlCl₃ (Merck), quercetin (Sigma), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck), 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) (Biobasic), Dulbecco's Modified Eagle Medium (DMEM) high glucose (GE), heat-inactivated fetal bovine serum (FBS) (Sigma), Lipopolysaccharide (LPS) (Sigma), DMSO (Merck), Trizol (Thermo), RevertAid First Strand cDNA Synthesis Kit (Thermo), Maxima SYBR Green qPCR Master Mix (Thermo), and other chemicals.

Quantification of total polyphenols in the extract: The total polyphenolic content of the extract was analysed using the Folin-Ciocalteu method as follows: 10 µl of the extract was mixed with 50 µl of Folin-Ciocalteu reagent. Then, 40 µl Na₂CO₃ (10% w/v) was added, and the reaction mixture was incubated in the dark at room temperature for 2 hours on a shaker. The absorbance of the sample was measured at 760 nm using a spectrophotometer. The total polyphenolic content was determined based on the gallic acid standard curve.

Quantification of total flavonoid in the extract: Total flavonoid content was determined using the AlCl₃ colorimetric method as follows: 50 µl of the extract was mixed in a reaction mixture consisting of 25 µl AlCl₃ 2%, 25 µl H₂O, 25 µl HCl 1M, and 25 µl CH₃COONH₄ 1M. The reaction mixture was incubated in the dark at room temperature on a shaker for 15 min. Absorbance of the sample was measured at 425 nm using a spectrophotometer.

The total flavonoid content was determined based on the quercetin standard curve.

Antioxidative activity evaluation by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay: 100 µl of 0.3 mM DPPH radical solution was added to 100 µl of the extract. The reaction mixture was incubated for 30 min. The absorbance was then measured at 517 nm using a spectrophotometer. The radical scavenging activity of the extract was calculated using the following equation:

$$\% SC = \left(1 - \frac{As - Ab}{Ac}\right) \times 100\%$$

where A_c is the absorbance at 517 nm of DPPH, A_b is the absorbance at 517 nm of the negative control sample, and A_s is the absorbance at 517 nm that contains the test sample.

Evaluation of cytotoxicity effect of the extract on RAW 264.7 cells by MTT assay: RAW 264.7 cells were inoculated at a density of 2×10⁴ cells/well in DMEM high glucose medium supplemented with 10% FBS, incubated for 24 hours at 37°C, 5% CO₂ on a 96-well plate. Then, cells were treated with extracts at concentrations of 10, 20, and 50 µg/ml (the extract was diluted in DMEM high glucose medium supplemented with 10% FBS). The treated cell plate was incubated for 24 hours at 37°C and 5% CO₂. Then, 10 µl of MTT solution (5 mg/ml) was added to all wells, followed by incubation for 3 hours. After treatment with MTT, the old medium was removed, and 100 µl of DMSO was added to each well. The plate was incubated in the dark at room temperature for 10 min to dissolve the formazan crystals. Finally, the percentage of surviving cells was determined by comparing the absorbance at 540 nm of the test well (with extract treatment) with the absorbance value of the negative control (not treated with extract).

Evaluation of the ability to inhibit nitric oxide secretion induced by Lipopolysaccharide (LPS) on RAW 264.7 model: RAW 264.7 cells were prepared similarly to the MTT assay. Then, cells were treated with extracts at concentrations of 10 and 20 µg/ml. Next, LPS (diluted in PBS at pH 7.4) was added to all the wells at a final concentration of 1 µg/ml after 1 hour of treatment with the extract. A negative control (cells not treated with extract and LPS), a positive control (treated with dexamethasone and LPS), and a control treated with LPS only (no extract treatment) were also included. After 24 hours, cultures were collected to measure nitric oxide (NO) secretion using the Griess reagent. Briefly, 100 µl of cell culture fluid was mixed with the corresponding volume of the Griess reagent. The reaction mixture was incubated for 10 min at room temperature, and the absorbance was then measured at 540 nm. The experiment was repeated three times. The procedure was adapted from the study of G. Yang, et al. (2012) [8].

Evaluation of the expression of pro-inflammatory genes *iNOS* induced by LPS on RAW 264.7 model: Cells were grown on 6-well plates at a density of 8×10^5 cells/well. After overnight proliferation, cells were treated with the extract at different concentrations and incubated at 37°C , 5% CO_2 . 1 hour after treatment with the extract, LPS was added to the wells at a final concentration of 1 $\mu\text{g/ml}$ to induce inflammation. A negative control (cells not treated with extract and LPS), a positive control (treated with dexamethasone and LPS), and a control treated with LPS only (no extract treatment) were also included. Total RNA from cells was isolated after 24 hours of treatment to evaluate the expression of inflammatory genes. Total RNA from cells was obtained using the Trizol kit according to the manufacturer's instructions. NanoDrop was used to quantify RNA concentration and purity by spectrophotometry. RNA after extraction was synthesised into cDNA using the RevertAid First Strand cDNA synthesis kit according to the instructions. The expression of *iNOS* genes was evaluated by real-time PCR reaction with SYBR Green fluorescence, and the internal control was the GAPDH gene. The specific primer sequences and thermal cycles of the real-time PCR reaction were referenced from S. Bose, et al. (2013) [9].

Evaluation of cytotoxicity effect of the extract on SW 1353 cells by MTT assay: Experiments were arranged similarly to the toxicity test of the extract on RAW 264.7 cells. SW 1353 cells were seeded at a density of 5×10^3 cells/well in DMEM high glucose medium supplemented with 10% FBS, incubated for 24 hours at 37°C , and 5% CO_2 on a 96-well plate. Then, cells were treated with extracts at concentrations of 50, 20, and 10 $\mu\text{g/ml}$ (extracts were diluted in DMEM medium supplemented with 10% FBS). The toxicity of the extract on SW 1353 cells was tested using the same MTT assay as previously described.

Evaluation of *MMP13* gene expression in SW 1353 cells stimulated by *IL-1 β* : SW 1353 cells were cultured on a 6-well plate at a density of 2×10^5 cells/well in DMEM high glucose medium supplemented with 10% FBS, incubated for 24 hours at 37°C and 5% CO_2 . Then, cells were treated with HGB extracts. After 1 hour of treatment, *IL-1 β* was added to each well at a final concentration of 10 ng/ml, excluding the negative control wells (treated with medium only). A negative control (cells not treated with extract and *IL-1 β*), a positive control (treated with dexamethasone and *IL-1 β*), and a control treated with *IL-1 β* only (no extract treatment) were also included. Cells were then incubated for 24 hours. Total RNA extraction was performed as previously described. A real-time PCR reaction was performed to check the expression of the *MMP13* gene with GAPDH as the internal control. The specific primer sequences and thermal cycling of real-time PCR with SYBR were referenced from J.E. Huh, et al. (2014) [10].

Statistical analysis of target gene expression: The quantification cycle value (the Q_q) generated by real-time PCR constituted the initial quantitative data for the relative expression study [11]:

$$R = 2^{-\Delta\Delta C_q}, \Delta\Delta C_q = (C_{q,\text{target}} - C_{q,\text{GAPDH}})_{\text{treat}} - (C_{q,\text{target}} - C_{q,\text{GAPDH}})_{\text{untreat}}$$

where R is the relative expression of target gene.

3. Results and discussion

Quantification of total polyphenols and flavonoids: The analysis of total polyphenolic content in the extract using HGB revealed a concentration of 234.47 ± 5.55 GAE/g DW, which was 1.73 times higher than that obtained using HGA (135.92 ± 0.32 GAE/g DW). However, the quantification of total flavonoids in the two extracts indicated that HGA (31.11 ± 0.86 mg QE/g DW) had a higher flavonoid content than HGB (9.28 ± 0.36 mg QE/g DW). This preliminary assessment of the chemical composition suggests that the extract from *D. cambodiana* Pierre ex Gagnep contains numerous compounds with potential applications as a medicinal herb.

Antioxidative activity evaluation: The *in vitro* antioxidative effect of HGA and HGB extracts was assessed by their ability to neutralise DPPH free radicals, using the EC_{50} value as a measure. The EC_{50} value (EC_{50} - half maximal effective concentration) represents the highest concentration of the extract that inhibits 50% of DPPH free radicals. The extract of *D. cambodiana* Pierre ex Gagnep in HGB displayed an EC_{50} value of 159.1 $\mu\text{g/ml}$, which was lower than that of HGA (242.2 $\mu\text{g/ml}$). However, the EC_{50} values of both solvents were lower when compared to vitamin C ($\text{EC}_{50} = 8.196$ $\mu\text{g/ml}$). According to Y. Luo, et al. (2010) [5], the EC_{50} values of *D. cambodiana* extractions using petroleum ether, ethyl acetate, n-butanol, and water were 5.007, 1.613, 2.441, and 4.874 mg/ml, respectively. This result suggests that the HGA and HGB extracts in this study possess higher antioxidative effects. Moreover, HGB shows more potential and can be considered for further research.

Effect of the extract on the viability of RAW 264.7: The cytotoxicity of HGA and HGB extracts on RAW 264.7 cells was evaluated using the MTT assay. The results (Fig. 1) indicated that the extract in HGA exhibited cytotoxicity at the investigated concentrations (ranging from 10 to 50 $\mu\text{g/ml}$). In contrast, the extract of *D. cambodiana* in HGB showed lower cytotoxicity at the investigated concentrations, with cells remaining viable at concentrations from 10 to 20 $\mu\text{g/ml}$. Previously, a research by W.L. Mei, et al. (2013) [12] showed that a newly isolated flavonoid compound (*Cambodianin G*) could be toxic to K562 and SGC-7901 cell lines, with IC_{50} values of 9.5 and 16.2 g/ml, respectively.

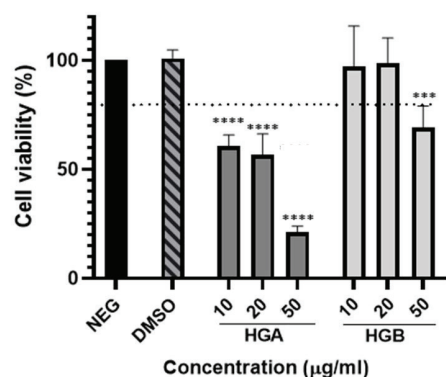


Fig. 1. Effects of dichloromethane and methanol extracts on the cell viability of RAW 264.7. NEG: untreated control; DMSO at 0.5%. Data are expressed as means $\pm$ standard deviation of the three independent experiments (*** p <0.001; **** p <0.0001) vs. untreated control.

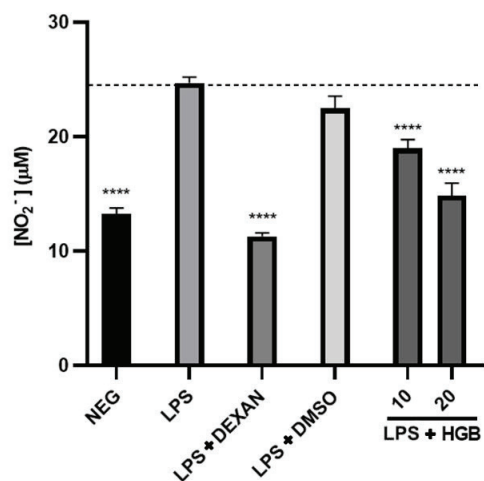


Fig. 2. Inhibition of nitric oxide by methanol in lipopolysaccharide-stimulated RAW 264.7. NEG: untreated control. DEXAN: dexamethasone 15 μ M. Data are expressed as means $\pm$ standard deviation of the three independent experiments (**** p <0.0001) vs. LPS-induced cell.

Attenuation of LPS-induced nitric oxide by the methanol extract in RAW 264.7: The anti-inflammatory activity of the extract was assessed by its ability to inhibit NO secretion in inflammatory RAW 264.7 cells induced by LPS at a concentration of 1 μ g/ml. To evaluate the cytotoxicity of HGA and HGB extracts on RAW 264.7 cell line, two concentrations of 10 and 20 μ g/ml of the extract with HGB were prepared, followed by an assessment of their ability to inhibit NO secretion. The positive control was dexamethasone at 15 μ M. The results are shown in Fig. 2. The NO concentration in inflammatory cells induced by LPS significantly increased (1.8 times higher than in untreated cells). RAW 264.7 cells treated with dexamethasone showed a significant inhibitory effect on NO secretion

compared to untreated cells. DMSO at a concentration of 0.2% had no significant effect on NO secretion. The HGB extract at all three concentrations also exhibited marked inhibition of NO secretion, especially at concentrations of 20 μ g/ml (more than a 40% reduction compared to the LPS control). Therefore, the anti-inflammatory potential of the HGB extract can be observed through its ability to inhibit NO secretion. Previous studies have reported that certain types of inflammatory tissue injury are mediated by reactive oxygen metabolites, and administering specific antioxidants has shown an effective reduction of tissue inflammation and injury [13, 14]. Furthermore, polyphenols are known to possess anti-inflammatory properties through their ability to modulate MAPK, Akt, and NF- κ B signalling pathways, suppress several cytokines related to inflammation, as well as hinder the activity of COX and iNOS [15]. Considering the above, we believe that as the HGB extract carries a high total polyphenol content and has significant antioxidant capacity, it is capable of reducing the inflammatory response. This led us to conduct further experiments to demonstrate the HGB extract's ability to attenuate inducible nitric oxide synthase (iNOS) expression.

Reduction of LPS-stimulated iNOS expression by HGB in RAW 264.7: The extract of *D. cambodiana* Pierre ex Gagnep with HGB at a concentration of 20 μ g/ml exhibited the ability to inhibit NO secretion in LPS-stimulated RAW 264.7 cells without inducing cell death. NO is produced from the amino acid L-arginine by the enzymatic action of iNOS, which is induced by inflammatory stimuli such as bacterial lipopolysaccharide. Therefore, the HGB extract was further evaluated for its ability to inhibit the expression

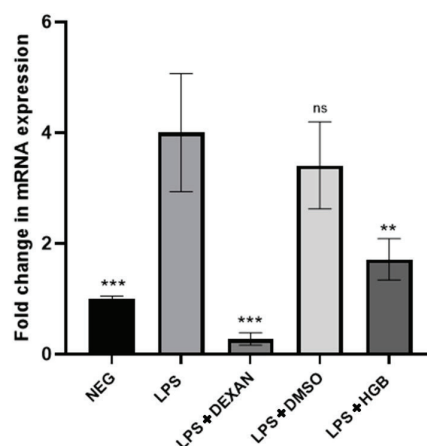


Fig. 3. Effects of methanol extract on the expression of iNOS in lipopolysaccharide-stimulated RAW 264.7 cells. Cells were stimulated with 1 μ g/ml LPS after 1-hour treatment with HGB extract. GAPDH was used as an internal control for the RT-qPCR. NEG: untreated control; DEXAN: dexamethasone 15 μ M; DMSO 0.2%. Data are represented as mean $\pm$ SD; number of RT-qPCR experiments (n)=3. ** p <0.01; *** p <0.001 vs. control treated with lipopolysaccharide.

of mRNAs responsible for encoding proinflammatory cytokines such as *iNOS*. The results indicated that HGB extract significantly inhibits the expression of inflammatory genes (Fig. 3). Compared with the LPS-stimulated cells, the inhibitory activity on the expression of the *iNOS* gene was 1.8 times higher. However, the effect of reducing the expression of the HGB extract at 20 $\mu\text{g/ml}$ was lower than that of dexamethasone at 15 μM , suggesting that the methanolic extract of *D. cambodiana* Pierre ex Gagnep

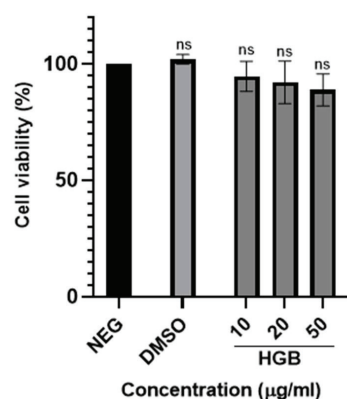


Fig. 4. Effects of methanol extract on cell viability of SW 1353 cell line. NEG: untreated control, DMSO at 0.5%. Data are expressed as mean $\pm$ SD of the three independent experiments (ns: no significant vs. untreated control).

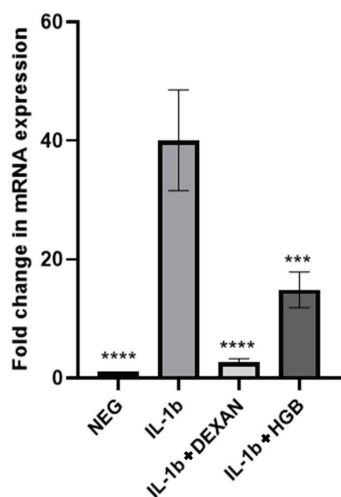


Fig. 5. Effects of methanol extract on the expression of *MMP13* in IL-1 β -stimulated SW 1353 chondrocytes. Cells were stimulated with 10 ng/ml IL-1 β after 1-hour treatment with HGB extract. GAPDH was used as internal controls for the RT-qPCR. NEG: untreated control; DEXAN: dexamethasone 15 μM ; Data are represented as mean $\pm$ SD; number of RT-qPCR experiments (n)=3. *** p <0.001; **** p <0.0001 vs. control treated with IL-1 β .

was effective but had a relatively lower potency. Further isolation of compounds in the solvent may help identify those with higher activity.

Effect of the HGB extract on the viability of SW 1353 cells: The cytotoxicity on SW 1353 cells of the methanol extract was evaluated by MTT assay (Fig. 4). The results showed that HGB extract did not induce cytotoxicity in SW 1353 cells at all three concentrations investigated. However, the HGB extract at a concentration of 50 $\mu\text{g/ml}$ was toxic to RAW 264.7 cells (60% live cells compared with control). We aim to select a concentration that is effective on both cell lines while still conserving the amount of extract used. Therefore, the HGB extract at a concentration of 20 $\mu\text{g/ml}$ was used to evaluate the ability to inhibit the expression of genes associated with osteoarthritis.

Inhibitory effects of HGB extract on *MMP13* expression in IL-1 β -stimulated SW 1353 cell line: Osteoarthritis has been described as an inflammatory process of the joints. Previous research has indicated that low-grade systemic and local inflammation is involved in the progression of osteoarthritis. Several inflammatory mediators, such as interleukin (IL)-1 β , nitric oxide (NO), prostaglandin E2 (PGE2), and fragments of cartilage proteins, including matrix metalloproteinases (MMPs), have been reported to be elevated in patients with osteoarthritis [16]. IL-1 β is an important proinflammatory cytokine during the inflammatory response, which has been reported to serve critical roles in joint inflammation and cartilage destruction processes. IL-1 β elevates NO production and upregulates the expression of MMPs, including *MMP13*, which is associated with extracellular matrix (ECM) degradation in cartilage. Based on the significant role of inflammation in osteoarthritis, anti-inflammatory analysis may have significant value in the treatment of osteoarthritis. In this study, the expression levels of the *MMP13* gene were evaluated in the SW 1353 chondrocytes stimulated by IL-1 β . The results are shown in Fig. 5, HGB at 20 $\mu\text{g/ml}$ inhibited the overexpression of the *MMP13* gene (reduced by more than 60% compared to IL-stimulated cells). Dexamethasone - a commercial drug - significantly reduced *MMP13* gene expression at 15 μM (93.22% reduction compared to IL-1 β treated cells). In a rabbit osteoarthritis model, W.P. Chen, et al. (2011) [17] proved that chlorogenic acid (CGA), a polyphenol isolated from natural plants, had the ability to reduce *MMP13* expression at both mRNA and protein levels. Other polyphenols were also reported to be capable of downregulating *MMP13*, such as curcumin in human and bovine chondrocytes [18], or epigallocatechin-3-O-gallate (EGCG) in human OA chondrocytes [19]. Through antioxidative action, these polyphenols downregulated transcription factors including NF- κ B, AP1, Sp1, β -catenin, ERK1/2, p38, and Hsp27, all of which were in control of MMP expression [20]. These findings suggest that as HGB

possesses a high total polyphenolic content, it holds the potential to reduce *MMP13* production, which, as a result, attenuates osteoarthritis.

4. Conclusions

D. cambodiana Pierre ex Gagnep extracted in HGA and HGB was reported to contain polyphenolic and flavonoid components. Both extracts exhibited antioxidant activity *in vitro*. Besides, methanol extracts from *D. cambodiana* also showed anti-inflammatory activity and inhibited joint degeneration without causing cell cytotoxicity at 20 µg/ml. These results suggest that *D. cambodiana* Pierre ex Gagnep, as a medicinal herb, is a potential candidate in the treatment of inflammation and osteoarthritis. Further research is needed to identify the biologically active compounds to provide more scientific evidence about the species and promote the potential usage of this medicinal plant.

CRedit author statement

Huynh Cong Duy: Writing - Original draft preparation, Methodology, Data analysis; Nguyen Trung Quynh Nhu: Formal analysis, Writing; Mai Kieu Tien, Nguyen Phuc Thinh: Methodology; Nguyen Thi Le Thuy: Support in the evaluation of expression of pro-inflammatory genes; Le Thi Truc Linh: Methodology, Writing - Reviewing and Editing; Tran Thi Van Anh: Preparing the extracts; Pham Thi Kim Tram: Project administration, Supervision, Investigation, Formal analysis, Writing - Reviewing and Editing.

ACKNOWLEDGEMENTS

The results of this article are part of a project carried out at the Biotechnology Center of Ho Chi Minh City, sponsored by the People's Committee of Ho Chi Minh City, Vietnam.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] L.T.H. Pham, T.Q. Lai, L.D. Mai, et al. (2014), "Prevalence of radiographic osteoarthritis of the knee and its relationship to self-reported pain", *PLOS ONE*, **9**(4), DOI: 10.1371/journal.pone.0094563.
- [2] J.L. Zhao, L. Zhang, S. Dayanandan, et al. (2013), "Tertiary origin and Pleistocene diversification of dragon blood tree (*Dracaena cambodiana* - Asparagaceae) populations in the Asian tropical forests", *PLOS ONE*, **8**(4), DOI: 10.1371/journal.pone.0060102.
- [3] T.L. Do (2004), *Vietnamese Medicinal Plants and Herbs*, Medical Publishing House, Hanoi, 1294pp (in Vietnamese).
- [4] H.D. Nguyen, V.K. Phan, V.M. Chau, et al. (2008), "Antifungal constituents from the stems of *Dracaena cambodiana*", *Vietnam Journal of Chemistry*, **46**(4), pp.503-508.
- [5] Y. Luo, H. Wang, X. Xu, et al. (2010), "Antioxidant phenolic compounds of *Dracaena cambodiana*", *Molecules*, **15**(12), pp.8904-8914, DOI: 10.3390/molecules15128904.

- [6] Y. Luo, H. Wang, Y.X. Zhao, et al. (2011), "Cytotoxic and antibacterial flavonoids from dragon's blood of *Dracaena cambodiana*", *Planta Medica*, **77**(18), pp.2053-2056, DOI: 10.1055/s-0031-1280086.
- [7] L.T.T. Nguyen, T.H.D. Doan, T.N.Y. Ngo, et al. (2018), "Investigation of acute toxicity and anticoagulant effects of hemoglobin (*Dracaena cambodiana* Pierre ex Gagnep, *Dracaenaceae*)", *Journal of Pharmacy*, **58**(6), pp.50-53 (in Vietnamese).
- [8] G. Yang, K. Lee, M. Lee, et al. (2012), "Inhibition of lipopolysaccharide-induced nitric oxide and prostaglandin E₂ production by chloroform fraction of *Cudrania tricuspidata* in RAW 264.7 macrophages", *BMC Complement. Altern. Med.*, **12**, DOI: 10.1186/1472-6882-12-250.
- [9] S. Bose, H. Kim (2013), "Evaluation of *in vitro* anti-inflammatory activities and protective effect of fermented preparations of Rhizoma Atractylodis Macrocephalae on intestinal barrier function against lipopolysaccharide insult", *Evidence-Based Complementary and Alternative Medicine*, **2013**, DOI: 10.1155/2013/363076.
- [10] J.E. Huh, P.S. Koh, B.K. Seo, et al. (2014), "Mangiferin reduces the inhibition of chondrogenic differentiation by IL-1β in mesenchymal stem cells from subchondral bone and targets multiple aspects of the SMAD and SOX9 pathways", *International Journal of Molecular Sciences*, **15**(9), pp.16025-16042, DOI: 10.3390/ijms150916025.
- [11] K.J. Livak, T.D. Schmittgen (2001), "Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔC_T} method", *Methods*, **25**(4), pp.402-408, DOI: 10.1006/meth.2001.1262.
- [12] W.L. Mei, Y. Luo, H. Wang, et al. (2013), "Two new flavonoids from dragon's blood of *Dracaena cambodiana*", *Bulletin of the Korean Chemical Society*, **34**(6), pp.1791-1794, DOI: 10.5012/bkcs.2013.34.6.1791.
- [13] U. Skalic, J.B. Allen, P.D. Smith, et al. (1991), "Inhibitors of reactive oxygen intermediates suppress bacterial cell wall-induced arthritis", *J. Immunol.*, **147**(8), pp.2559-2564.
- [14] D.A. Clark, D.M. Fomabaio, H. McNeill, et al. (1988), "Contribution of oxygen-derived free radicals to experimental necrotizing enterocolitis", *Am. J. Pathol.*, **130**(3), pp.537-542.
- [15] B. Veres (2012), "Anti-inflammatory role of natural polyphenols and their degradation products", *Severe Sepsis and Septic Shock - Understanding a Serious Killer*, pp.379-410, DOI: 10.5772/27889.
- [16] C.C. Liu, Y. Zhang, B.L. Dai, et al. (2017), "Chlorogenic acid prevents inflammatory responses in IL-1β-stimulated human SW-1353 chondrocytes, a model for osteoarthritis", *Mol. Med. Rep.*, **16**(2), pp.1369-1375, DOI: 10.3892/mmr.2017.6698.
- [17] W.P. Chen, J.L. Tang, J.P. Bao, et al. (2011), "Anti-arthritis effects of chlorogenic acid in interleukin-1β-induced rabbit chondrocytes and a rabbit osteoarthritis model", *Int. Immunopharmacol.*, **11**(1), pp.23-28, DOI: 10.1016/j.intimp.2010.09.021.
- [18] A. Liacini, J. Sylvester, W.Q. Li, et al. (2002), "Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes", *Matrix Biol.*, **21**(3), pp.251-262, DOI: 10.1016/s0945-053x(02)00007-0.
- [19] Z. Rasheed, A.N. Anbazhagan, N. Akhtar, et al. (2009), "Green tea polyphenol epigallocatechin-3-gallate inhibits advanced glycation end product-induced expression of tumor necrosis factor-alpha and matrix metalloproteinase-13 in human chondrocytes", *Arthritis Res. Ther.*, **11**(3), DOI: 10.1186/ar2700.
- [20] T. Suzuki, T. Ohishi, H. Tanabe, et al. (2023), "Anti-inflammatory effects of dietary polyphenols through inhibitory activity against metalloproteinases", *Molecules*, **28**(14), DOI: 10.3390/molecules28145426.

Impact of pasteurisation and storage conditions on the physicochemical properties of acerola jelly

Binh Hoang Quang^{1*}, Uyen Tran Phuong Ho², Diep Ngoc Thi Duong^{2*}

¹Research Institute for Biotechnology and Environment, Nong Lam University, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

²Faculty of Chemical Engineering and Food Technology, Nong Lam University, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

Received 13 May 2023; revised 26 July 2023; accepted 11 September 2023

Abstract:

Acerola, a fruit rich in bioactive compounds such as polyphenols and vitamin C, is highly susceptible to degradation under temperature influences. This study aimed to evaluate the impact of pasteurisation and storage conditions on the stability of these compounds in acerola jelly. Analytical findings revealed that thermal treatment at 80°C for 5 minutes effectively eradicated total aerobic bacteria and mould yeast content. Furthermore, this treatment preserved the vitamin C and phenolic content, as well as the sensory quality of the jelly, better than other samples. The study also discovered that during storage, the degradation of vitamin C and polyphenols in acerola jelly adhered to the first-order model and the Arrhenius model. In contrast, attributes such as total soluble solid (TSS), pH, and titratable acidity (TA) remained constant during pasteurisation and storage. Kinetic parameters, including D-value, k-value, z-value, and activation energy (E_a), indicated high stability of the product when stored at a low temperature of 4-6°C. These findings suggest that acerola jelly has considerable potential for commercial production.

Keywords: acerola jelly, pasteurisation, polyphenol, storage, vitamin C.

Classification numbers: 3.4, 3.5

1. Introduction

Acerola, renowned for its richness in bioactive compounds, possesses a vitamin C content notably higher (over ten times) than many other fruits, such as oranges, tangerines, and mangos. Besides vitamin C, acerola also encompasses an array of phenolic compounds, which include simple phenols, phenolic acids (derivatives of hydroxybenzoic and hydroxycinnamic acids), flavonoids, coumarins, stilbenes, and lignans [1]. Acerola extracts have been reported to exhibit robust antioxidant, anti-inflammatory, anti-hyperglycaemic, anti-tumour, anti-toxic, and hepatoprotective properties [2]. However, acerola fruits present several challenges, such as a succulent, thin peel, a soft texture, and a propensity to ripen rapidly, complicating distribution and storage. Consequently, various acerola-based food products, like powder, jam, and juice, have been the focus of several studies [3-5].

Acerola jelly, derived from acerola juice with added flavouring and stabilising agents, not only offers a pleasing taste but is also rich in vitamin C and polyphenols. Previous studies have indicated that the biological compounds in acerola products undergo changes during production, storage, and transportation. Such changes are predominantly due to undesirable reactions like oxidation and non-enzymatic

browning, which occur continuously during storage and lead to the deterioration of the physicochemical properties of fruit products. These degradations are notably pronounced at elevated temperatures. Given these detrimental effects on nutritional composition and sensory quality, understanding the deterioration causes and identifying optimal storage conditions for food products is essential. However, reports on the stability of acerola jelly during processing, storage, and under varying temperature conditions are scarce. Hence, this study aims to evaluate the physicochemical property changes in acerola jelly under different thermal treatment and storage conditions.

2. Materials and methods

2.1. Materials

The chosen acerola fruits belonged to the *Malpighia glabra* variety, commonly known in Vietnam as Sô ri chua or sour acerola. These fruits were sourced from a farm in Go Cong, Tien Giang province, Vietnam. Selection criteria included fruits that were ripe, red-orange in colour, and free from physical or microbiological damage (e.g., dark brown, bruised, surface mouldy, fermentable, or strange smells). Post-selection, the fruits were washed, drained, and stored at -18°C until use.

*Corresponding authors: Email: qbinh93@gmail.com; duongngocdiep@hcmuaf.edu.vn

The stabilising agent used was Aquagel WD-7183, procured from Marcel Carrageenan (Philippines). This additive, prevalent in fruit jelly product processing, consists of carrageenan, sodium dihydro citrate, konjac powder, and potassium chloride, as per supplier information.

2.2. Chemicals

Chemicals utilised in this study included ascorbic acid, gallic acid (Sigma Aldrich, Singapore), Folin-Ciocalteu (Merck, Germany), 99% methanol (Chemsol, Vietnam), hydrochloric acid (HCl), sodium chloride (NaCl), potassium iodide (KI), iodine (I₂), starch (Xilong, China), plate count agar (PCA), and potato dextrose agar (Himedia, India).

2.3. Experiment design

Effect of pasteurisation conditions: Thawed acerola fruits were processed using a kitchen juicer (HR 1863, Philips, China). The extracted juice was then blended with other ingredients, following the proportions outlined in Table 1. These proportions were based on preliminary survey results. The mixture underwent heating at 80°C for 5 minutes to ensure complete dissolution of all ingredients. Subsequently, the hot sample solution was poured into aluminium luminate bags (6×11 cm), each containing 30 g of the mixture. The sealed bags were pasteurised using a retort (ALP Model MCY-40L, Japan) at various time intervals (5 and 10 minutes) and temperatures (80, 85, and 90°C). Post-pasteurisation, the samples were cooled to room temperature in a water bath and then refrigerated for further analysis.

Table 1. Formulation of acerola jelly.

Material	Content (g)
Acerola juice	20.00
Sugar	19.57
Citric acid	0.23
Stabiliser agent (Aquagel)	1.50
Water	58.70
Total	100.00

Effect of storage conditions: The samples were prepared identically to those in the initial experiment. They were then stored at three different temperatures: 8-10°C in a refrigerator (Zanotti S.p.A., Italy), 29-31°C (room temperature), and 40°C in an incubator (IN 110, Memmert, Germany). Every five days over a period of 30 days, the samples were assessed for their physicochemical properties.

2.4. Analytical method

Determination of total soluble solids, pH and titratable acidity: The TSS content was measured using a refractometer (Atago, 0-32°Bx, Japan). Total acidity (TA) was determined

by titration with 0.1N NaOH using 1% phenolphthalein as an indicator [6]. The pH was recorded using a pH meter (Hanna 211, USA). Prior to these assessments, samples were macerated with distilled water.

Determination of total phenolic content: Following the extraction method described by G. Xu, et al. (2008) [7], the total phenolic content (TPC) was determined in accordance with Y.Y. Lim, et al. (2007) [8]. In this process, 0.3 ml of the diluted sample was mixed with 1.5 ml of 10% Folin-ciocalteu reagent. The mixture was allowed to stand for five minutes before the addition of 1.2 ml of 7.5% Na₂CO₃. After thorough mixing, the mixture was left for 30 minutes at room temperature (29-31°C). The analysis of optical density was then performed at 765 nm using a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan), under dark conditions. TPC was calculated using Eq. (1) and expressed as mg gallic acid equivalents per 100 g dry matter (mg GAE/100 g dm):

$$TPC = \frac{(y - b) \times V \times DF \times 100}{a \times m \times (100\% - \text{moisture content}\%) \times 1000} \quad (1)$$

where y is the OD value of the analysed sample; a and b are the coefficients of the gallic acid standard curve equation (10-70 g/ml); V is the extract volume (ml); DF is the dilution; m is the mass of the sample (g).

Determination of vitamin C content: The vitamin C content was determined using the method of K. Pathy (2018) [9]. The sample, post-crushing, was weighed (0.1 g) into a 100 ml volumetric flask, to which 2 drops of 2% HCl and distilled water up to the mark were added. After thorough mixing, the sample was filtered to obtain the extract. For analysis, 10 ml of this extract was taken, mixed with 2 drops of a 0.5% starch-saturated solution, and titrated with 0.01 N iodine solution until a persistent blue coloration was observed for 30 seconds. Vitamin C content is calculated using Eq. (2) and expressed as mg/100 g of dry matter (mg/100 g dm):

$$X \text{ (mg/100 g dm)} = \frac{V \times V_1 \times 0.88 \times 100}{V_2 \times m} \quad (2)$$

where V is the volume of 0.01 N I₂ solution used for titration (ml); V₁ is the volume of test sample solution (ml); V₂ is the volume of sample solution taken for determination (ml); and 0.88 is the mass of vitamin C (g) corresponding to 1 ml of 0.01 N I₂ solution.

Determination of microbial count: Initially, one gram of the sample was blended with 9 ml of 0.85% sterile saline. Subsequently, the sample underwent further dilution to achieve the appropriate density. Next, 1 ml of the diluted sample was evenly spread over the surface of a pre-prepared medium plate, where potato dextrose agar was used for measuring total yeast and mould, and plate count agar

(PCA) for total aerobic bacteria. After allowing 15 minutes for stabilisation at room temperature, the Petri dishes were inverted and incubated for 72 hours at 30°C in an incubator (IN110, Memmert, Germany). The microbial density was expressed in log CFU/g. This method aligns with the approach outlined by V. Santhirasegaram, et al. (2013) [10].

Sensory evaluation: The sensory evaluation involved a panel assessing the organoleptic qualities of the samples, including texture, taste, colour, and overall preference. A 7-point scale was used, with 1 indicating least favourite and 7 most favourite. The sensory panel consisted of 20 individuals, aged between 18-25 years. Between sample tastings, panellists cleansed their palates with odourless water. Each 20-g sample was presented in a white porcelain dish, labelled with a random 3-digit code. The evaluation method was adapted from P.H.D. Sousa, et al. (2010) [6], with minor modifications.

Degradation kinetic: The degradation kinetics of vitamin C and polyphenols in acerola agar during storage follow a first-order model, as demonstrated by Eq. (3):

$$\ln C = \ln C_0 - kt \quad (3)$$

where C is the content of vitamin C or polyphenols after storage time t, C_0 is the content of vitamin C or polyphenols before storage (i.e., sample after thermal treatment), and k is the reaction rate constant (day^{-1}) calculated from the base coefficient of the method C/C_0 process according to storage time t (days). The half-life ($t_{1/2}$), representing the time for a 50% reduction in content, is determined by Eq. (4):

$$t_{1/2} = \ln(2)/k \quad (4)$$

The time required for a 90% reduction in content (D) is calculated using Eq. (5) and Eq. (6):

$$D = \ln(10)/k \quad (5)$$

$$D = D_0 10^{-T/z} \quad (6)$$

where T is the storage temperature (°C); z is the temperature required to reduce one log of price value D (°C); D_0 is the value of D when at $T=0^\circ\text{C}$.

The relationship between loss of vitamin C, polyphenols, and temperature during storage follows the Arrhenius model, as indicated in Eq. (7):

$$k = Ae^{-E_a/RT} \quad (7)$$

where A is constant; E_a (kJ/mol) is the activation energy (minimum energy required for a chemical reaction to occur) and calculated based on the base coefficient obtained from the straight line of the Eq. (7); R is the universal gas constant (8.314 J/mol K); T is the absolute storage temperature (°K).

The kinetic models (Eqs. (3)-(7)) are based on the report by M.A.V. Boekel (2008) [11].

The predicted shelf life for vitamin C and polyphenols is determined using Eq. (8), as referenced in M.M.A. Zubaidy, et al. (2007) [12]:

$$E_x = e^{-\left[\frac{S}{T} + I - \ln\left(-\ln\left(\frac{p\%}{100}\right)\right)\right]} \quad (8)$$

where E_x is the shelf life of the product (days); S and I are the original coefficients and constants obtained from the Arrhenius equation; T is the absolute storage temperature (°K); p% is the residual percentage of vitamin C and polyphenols after storage.

2.5. Statistical analysis

The study employed one-way analysis of variance (ANOVA) and the least significant difference (LSD) test for statistical analysis, using JMP software (version 13.0). Excel 2019 was utilised for data calculation, graph plotting, and verification of statistical results.

3. Results and discussion

3.1. Effect of pasteurisation conditions

Microbial count: Thermal treatment in fruit and vegetable processing is crucial for controlling microbiological safety. However, heat treatment can also negatively affect the chemical composition, particularly the content of healthy components such as vitamins. Despite this, pasteurisation is still one of the most effective methods of product preservation, ensuring long shelf life and safety [13]. It is also an easy method to apply with low investment costs. In this experiment, the effects of different pasteurisation regimes on the microbial count and physicochemical properties of acerola jelly were evaluated, as shown in Tables 2 and 3.

Table 2. Effect of pasteurisation regimes on the microbial count in acerola jelly.

Time (min)	Temperature (°C)	Aerobic count (CFU/g)	Yeast and mould (CFU/g)
Before pasteurisation		2×10^1	-
5	80	-	-
	85	-	-
	90	-	-
10	80	-	-
	85	-	-
	90	-	-

-: not detected.

Table 3. Effect of pasteurisation regimes on physicochemical properties of acerola jelly.

Time (minute)	Temperature (°C)	TSS (%)	TA (%)	pH	TPC (mg/100 g dm)	Vitamin C (mg/100 g dm)
Before pasteurisation		22,83±0,76	0.35 ± 0,05	0.41±0,07	853.12±7,65	947.56±10,22
5	80	22,70±0,10	0.28±0,01	4.22±0,03	427,38±20,13	394,13±0,77
	85	22,97±0,32	0.24±0,01	4.34±0,05	412,5±11,65	369,7±6,06
	90	22,93±0,06	0.24±0,01	4.36±0,06	379,39±29,37	276,31±2,20
10	80	22,87±0,12	0.26±0,01	4.29±0,06	407,29±7,18	353,03±4,30
	85	22,93±0,21	0.29±0,01	4.35±0,06	384,23±25,77	290,76±4,46
	90	22,90±0,10	0.24±0,01	4.35±0,04	353,35±28,48	251,99±2,49

The results were expressed as mean ± standard deviation. In the same column, values denoted with different superscript letters had statistically different significance ($p < 0.05$).

The microbial analysis showed that no aerobic bacteria or mould yeast were detected in any pasteurisation regime. The acerola jelly, with a pH ranging from 4.2 to 4.4, exhibited acidic characteristics that reduced the heat resistance of microorganisms, facilitating their destruction after heat treatment [5]. Additionally, high temperatures can rupture cellular membranes and denature nucleic acids and proteins, effectively eliminating spoilage microorganisms [10]. Previous research has indicated that aerobic bacteria, yeast, and mould were not detected in mango juice treated at 90°C for 15 seconds, in blended orange and carrot juice at 98°C for 21 seconds, and in pineapple juice at 80°C for 15 minutes [10, 14, 15].

Physicochemical properties: Generally, all heated jelly samples maintained TSS values of approximately 23%, titratable acidity (TA) from 0.24% to 0.29%, and pH levels between 4.2 and 4.4 (Table 3). However, the content of biological compounds, such as vitamin C and polyphenols, varied significantly among the samples ($p < 0.05$). The concentration of these compounds decreased with increasing temperatures and extended treatment times. Notably, the vitamin C content was observed to be 394.13 mg/100 g dm at 80°C, 369.97 mg/100 g dm at 85°C, and 276.31 mg/100 g dm at 90°C, all for a heating duration of 5 minutes. This trend is consistent with previous studies on acerola-based products [3-5, 13, 16].

The increase in temperature from 85 to 95°C led to an escalation in the degradation of vitamin C in acerola juice, from 46.75 to 50.65% [5]. Similarly, acerola powder dried at 130°C showed a recovery yield of vitamin C and polyphenols of 43.52 and 49.54%, respectively, which were higher than the yields at 170°C (31.30 and 30.66%,

respectively) [3]. Another study on acerola jam indicated that reducing the heating time enhanced the retention of vitamin C, increasing the percentage remaining from 71.99 to 93.68% [4]. Research on other fruits has also noted that heat-sensitive compounds, such as vitamin C and polyphenols, degrade rapidly at high temperatures over time [13, 16].

Sensory evaluation: Regarding colour, acerola contains compounds like cyanidin 3-O-rhamnoside and pelargonidin 3-O-rhamnoside (anthocyanins), and neochrome, lutein, and zeaxanthin (carotenoids), which contribute to the ripe fruit's hue [2]. Although these pigments are susceptible to degradation during thermal processing, the colour of acerola jelly varied only slightly across different pasteurisation regimes. All samples presented an attractive orange-yellow colour, with the highest score (5.70/7 points) observed for the sample treated at 85°C for 10 minutes, and the lowest (4.80/7 points) for the one treated at 85°C for 5 minutes (Table 4). Additionally, pasteurisation conditions appeared to have minimal impact on consumer preferences for texture, with sensory scores ranging from 4.85 to 5.55 out of 7 points.

Table 4. Effect of pasteurisation temperature and time on sensory quality of acerola jelly.

Time (minute)	Temperature (°C)	Colour	Texture	Flavour	Overall liking
5	80	5.45 ^{ab} ±0.94	5.25 ^a ±0.90	5.60 ^a ±1.10	5.15 ^{bc} ±0.88
	85	4.80 ^b ±1.24	4.85 ^{ab} ±1.63	5.10 ^{ab} ±1.25	5.25 ^{bc} ±1.02
	90	5.15 ^{ab} ±1.27	5.35 ^a ±1.14	4.70 ^b ±0.98	4.90 ^a ±0.97
10	80	5.45 ^{ab} ±0.89	5.45 ^a ±1.23	5.10 ^{ab} ±1.29	5.15 ^{ac} ±1.35
	85	5.70 ^a ±1.03	5.40 ^a ±1.43	5.50 ^a ±0.95	5.95 ^a ±0.76
	90	5.40 ^{ab} ±1.14	5.55 ^a ±0.89	5.35 ^{ab} ±1.14	5.55 ^{ab} ±0.94

The results were expressed as mean ± standard deviation. In the same column, values denoted with different superscript letters had statistically different significance ($p < 0.05$).

Most panellists noted that the acerola jelly had a balanced sweet and sour taste, consistent across the samples. This taste profile is primarily influenced by the acid and sugar content (dissolved solids), with little variation in TA, TSS, and pH between the jelly samples, as shown in Table 3. Furthermore, the aroma of acerola was perceptible in the product, although differences in odour intensity were not pronounced between the samples. Volatile compounds such as esters, terpenoids, aldehydes, ketones, alcohols, and acids have been identified as key contributors to acerola's characteristic aroma [17]. The jelly's structure might have aided in retaining these aromatics during processing.

Given the analysed results, the pasteurisation condition of 80°C for 5 minutes was chosen as optimal. This regime ensured that the content of spoilage microorganisms was within safe limits, while maintaining good organoleptic quality and a higher content of vitamin C and polyphenols compared to other samples.

3.2. Effect of storage conditions

Kinetic modelling of vitamin C and polyphenol degradation: To ascertain the shelf life of food products, the conventional approach involves monitoring changes in product quality during storage. Nevertheless, heat-treated products often have extended life cycles, rendering the determination of their shelf life time-consuming and thus challenging for commercialisation. To address this issue, the accelerated method has been employed. The first-order model and the Arrhenius model are extensively used to assess the impact of temperature on the kinetics of chemical and biological reaction mechanisms in food. These models are instrumental in predicting optimal preservation conditions and minimising quality losses during food storage [18].

During storage, the degradation of vitamin C and polyphenols in acerola jelly was modelled using the first-order and Arrhenius models (Fig. 1). The negative sign preceding the slope of the first-order equation indicates an inverse relationship between time and the concentration of biological compounds. An increase in temperature results in a heightened degradation rate of antioxidant compounds. For example, as the temperature rose from 8 to 40°C, the rate constant (k) for vitamin C increased from 0.0141 to 0.0446 day⁻¹, leading to a significant reduction in the half-life ($t_{1/2}$) from 49.16 to 15.51 days. This pattern was also observed in previous studies. For instance, in kiwifruit puree, an increase in storage temperature from 5 to 45°C resulted in the k value for polyphenols rising from 1.59 to 6.54×10⁻³ hour⁻¹, while $t_{1/2}$ decreased from 18.19 to 4.42 days [19]. Similarly, with the storage temperature of lingonberry jam rising from 4 to 25°C, the k value increased from 0.87 to 1.42 day⁻¹, but $t_{1/2}$ decreased from 256 to 157 days [20].

The adverse impact of temperature on the stability of vitamin C and polyphenols in acerola jelly was further corroborated through the D value, which significantly

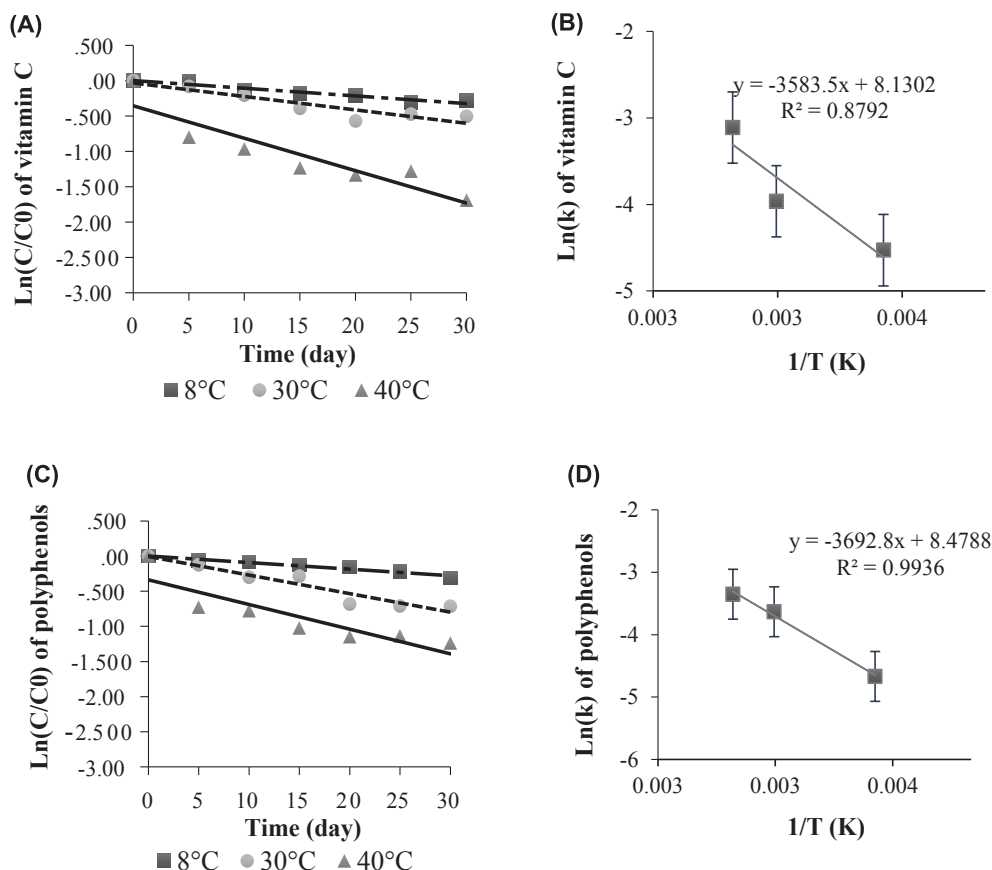


Fig. 1. (A) First order model plot and (B) Arrhenius model plot for degradation of vitamin C; (C) First order model plot and (D) Arrhenius model plot for degradation of TPC in acerola jelly under various storage temperatures.

Table 5. Degradation parameters of vitamin C and polyphenols of acerola jelly under various storage temperatures.

Temperature (°C)	k (day ⁻¹)	t _{1/2} (day)	*R ²	D (day)	E _a KJ/mol	**R ²	Z (°C)	***R ²
<i>Vitamin C</i>								
8-10	0.0108	64.18	0.95	213.20				
29-31	0.019	36.48	0.84	121.19	29.79	0.88	87.72	0.89
40	0.0446	15.54	0.79	51.63				
<i>TPC</i>								
4-6	0.0094	73.74	0.96	244.96				
29-31	0.0264	26.26	0.91	87.22	30.70	0.99	54.64	0.99
40	0.035	19.80	0.79	65.79				

The coefficient obtains from*: first order model, **: arrhenius model, ***: ball model.

diminished at higher temperatures. Specifically, the D value for polyphenols was 244.96 days at 8-10°C, 87.22 days at room temperature, and 65.79 days at 40°C. The temperature rise likely accelerated undesirable reactions such as oxidation and non-enzymatic browning, contributing to the degradation of vitamin C and polyphenols [20, 21].

As presented in Table 5, the activation energy (E_a) of vitamin C (27.69 kJ/mol) was lower than that of polyphenols (30.70 kJ/mol), while the z value of vitamin C (87.72°C) was greater than that of polyphenols (54.64°C), indicating that polyphenols in acerola jelly were more temperature-sensitive. The lower z and higher E_a values suggest a more pronounced temperature dependence of the reactions [11]. In other research, the activation energy of vitamin C in orange and pear juice was recorded as 44.81 and 55.44 kJ/mol, respectively [22]. Meanwhile, the E_a value for polyphenols was reported as 3.5 kJ/mol in cherry jam [21], and 28.15 kJ/mol in kiwi puree [19].

Shelf-life prediction of vitamin C and polyphenol: In this study, the recovery yield of vitamin C in acerola jelly was found to be 75.05, 60.51, and 18.41% after 30 days of storage at 8-10°C, room temperature, and 40°C, respectively. Similarly, the remaining percentages of TPC under the same storage conditions were 73.05, 48.95, and 29.04%, respectively. These parameters are likely to continue degrading with prolonged storage time. The predictive equations for the degradation of vitamin C and polyphenols are presented in Table 6.

Table 6. Change in total soluble solid, pH, and titratable acidity of acerola jelly over time at various storage temperatures.

Temperature (°C)	Storage time (day)						
	0	5	10	15	20	25	30
<i>TSS (%)</i>							
8-10	23.57±0.51	23.57±0.64	23.2±0.40	23.17±0.25	23.53±0.70	23.53±0.12	23.4±0.53
29-31	23.57±0.14	23.13±0.15	23.4±0.20	23.27±0.12	23.13±0.31	23.87±0.12	23.47±0.7
40	23.57±0.05	23.2±0.00	23.8±0.53	23.87±0.58	23.93±0.46	23.53±0.31	23.67±0.12
<i>pH</i>							
8-10	4.07±0.51	4.08±0.13	3.91±0.03	3.92±0.05	3.92±0.05	3.97±0.03	3.97±0.04
29-31	4.07±0.14	3.93±0.04	4.04±0.17	4.08±0.29	4.08±0.29	3.95±0.09	3.97±0.03
40	4.07±0.05	4.04±0.29	3.88±0.05	4.01±0.17	4.01±0.17	3.96±0.03	4.02±0.12
<i>TA (%)</i>							
8-10	0.28±0.51	0.36±0.06	0.29±0.01	0.28±0.02	0.28±0.01	0.28±0.02	0.29±0.00
29-31	0.28±0.14	0.35±0.00	0.29±0.01	0.28±0.02	0.30±0.02	0.3±0.02	0.28±0.01
40	0.28±0.05	0.35±0.00	0.30±0.02	0.30±0.01	0.32±0.00	0.3±0.01	0.31±0.01

The results were expressed as mean ± standard deviation.

The decline of these compounds during storage has been noted in various acerola products. For instance, acerola jam, after a month, retained 55.98% of its polyphenol content and 23.98% of its vitamin C content [4]. A study examining the impact of storage temperature on fresh acerola fruits found that after three days of storage at room temperature, the content of vitamin C and polyphenols was reduced to 40 and 70% respectively, while at colder temperatures, these figures were 15 and 30% respectively [3]. These findings indicate that lower temperatures are more effective in preserving antioxidant compounds in acerola products and suggest that vitamin C is less affected than polyphenols during storage.

Research by N.J. Wurlitzer, et al. (2019) [23] observed no significant changes in the content of vitamin C and polyphenols in blended acerola juice over 180 days of storage at 5°C. Conversely, another study on acerola juice reported that vitamin C was lost not only during pasteurisation but also throughout the storage period [5]. After six weeks of storage at room temperature, the loss rate of vitamin C in acerola juice was 37.4%. This study also suggested that the rate of vitamin C degradation decreases over time, possibly due to the gradual reduction in the amount of dissolved oxygen inside the packaging and the intrinsic properties of the product, leading to fewer oxidation reactions [24].

Table 7. Microbial count in acerola jelly before and after storage.

Sample	Temperature (°C)	Aerobic count (CFU/g)	Yeast and mould (CFU/g)
Before	8-10	-	-
	29-31	-	-
After 30 days	8-10	-	-
	29-31	-	-

-: not detected.

Table 8. Change in total soluble solid, pH, and titratable acidity of acerola jelly over time at various storage temperatures.

Temperature (°C)	Storage time (day)						
	0	5	10	15	20	25	30
TSS (%)							
8-10	23.57±0.51	23.57±0.64	23.2±0.40	23.17±0.25	23.53±0.70	23.53±0.12	23.4±0.53
29-31	23.57±0.14	23.13±0.15	23.4±0.20	23.27±0.12	23.13±0.31	23.87±0.12	23.47±0.7
40	23.57±0.05	23.2±0.00	23.8±0.53	23.87±0.58	23.93±0.46	23.53±0.31	23.67±0.12
pH							
8-10	4.07±0.51	4.08±0.13	3.91±0.03	3.92±0.05	3.92±0.05	3.97±0.03	3.97±0.04
29-31	4.07±0.14	3.93±0.04	4.04±0.17	4.08±0.29	4.08±0.29	3.95±0.09	3.97±0.03
40	4.07±0.05	4.04±0.29	3.88±0.05	4.01±0.17	4.01±0.17	3.96±0.03	4.02±0.12
TA (%)							
8-10	0.28±0.51	0.36±0.06	0.29±0.01	0.28±0.02	0.28±0.01	0.28±0.02	0.29±0.00
29-31	0.28±0.14	0.35±0.00	0.29±0.01	0.28±0.02	0.30±0.02	0.3±0.02	0.28±0.01
40	0.28±0.05	0.35±0.00	0.30±0.02	0.30±0.01	0.32±0.00	0.3±0.01	0.31±0.01

The results were expressed as mean ± standard deviation.

Microbial count, total soluble solid, titratable acidity, and pH: The results displayed in Table 7 indicate that after 30 days of storage at 8-10°C and 29-31°C, the acerola jelly samples did not exhibit growth of aerobic bacteria and mould yeast.

Over the course of 30 days of storage, this study observed fluctuations in the physicochemical properties of acerola jelly at various temperatures (Table 8). Generally, the acerola jelly maintained a TSS range of 23-24%, a pH of 3.9-4.1, and a TA of 0.28-0.35%. Similarly, in guava juice, TSS, pH, and TA remained relatively unchanged over 240 days of storage at 10°C [24], with TSS varying between 23% and 24%, pH between 3.70 and 3.8, and TA between 0.22 and 0.25%. Another study on orange, pear, and grape juices [22] suggested that pH and TSS experienced minor modifications when stored at 4, 25, and 37°C. Research by

P.H.D. Sousa, et al. (2010) [6] noted that after 180 days of storage at 4°C, grapefruit juice retained a pH of 3.0 and a TSS of 10%, consistent with its initial values. Additionally, after 60 days of storage at 26°C, pasteurised pineapple juice exhibited a TSS range of 15.5 to 16.0% and a pH of approximately 4.0, while TA decreased slightly [15].

4. Conclusions

The various pasteurisation and storage conditions did not significantly alter the TSS, titratable acidity, or pH of acerola jelly. However, temperature and time were identified as key factors influencing the degradation of vitamin C and polyphenols. The longer the heat treatment duration and the higher the processing temperature, the greater the loss of these compounds. The optimal pasteurisation conditions for acerola products were determined to be 80°C for 5 minutes; under these conditions, the product met microbiological safety standards and maintained high sensory quality, along with substantial vitamin C and polyphenol content. To preserve these qualities, it is recommended that the product be stored at a low temperature of 4-6°C. At this temperature, the predicted time for the vitamin C and polyphenol content of the product to decrease by 50% is estimated to be 64.17 days and 73.74 days, respectively.

CRediT author statement

Binh Hoang Quang: Methodology, Formal analysis, Writing, Editing; Uyen Tran Phuong Ho: Material synthesis, Data analysis, Editing; Diep Ngoc Thi Duong: Methodology, Formal analysis, Writing, Editing.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] L. Delva, R.G. Schneider (2013), "Acerola (*Malpighia emarginata* DC): Production, postharvest handling, nutrition, and biological activity", *Food Reviews International*, **29**(2), pp.107-126, DOI: 10.1080/87559129.2012.714433.
- [2] T. Belwal, H.P. Devkota, H.A. Hassan, et al. (2018), "Phytopharmacology of Acerola (*Malpighia* spp.) and its potential as functional food", *Trends in Food Science & Technology*, **74**, pp.99-106, DOI: 10.1016/j.tifs.2018.01.014.
- [3] T.T. Le, H.Q. Luong, D.C. Angeli, et al. (2018a), "Changes of total polyphenolics and vitamin C in acerola during storage and spray drying process", *The Journal of Agriculture and Development*, **17**(3), pp.69-76, DOI: 10.52997/jad.10.03.2018.

- [4] T.T. Le, T.T.D. Le (2018b), "Processing acerola jam and changes in concentrations of vitamin C and polyphenolic compounds during processing and storage", *The Journal of Agriculture and Development*, **17(2)**, pp.87-92 (in Vietnamese).
- [5] M.H. Le, T.M.D. Nguyen, N.T. Vo, et al. (2009), "Studying on the mixed fruit juice processing", *CTU Journal of Science*, **11B**, pp.235-244 (in Vietnamese).
- [6] P.H.D. Sousa, G.A. Maia, H.M.D. Azeredo, et al. (2010), "Storage stability of a tropical fruit (cashew apple, acerola, papaya, guava, and passion fruit) mixed nectar added caffeine", *International Journal of Food Science & Technology*, **45(10)**, pp.2162-2166. DOI: 10.1111/j.1365-2621.2010.02383.x.
- [7] G. Xu, D. Liu, J. Chen, et al. (2008), "Juice components and antioxidant capacity of citrus varieties cultivated in China", *Food Chemistry*, **106(2)**, pp.545-551.
- [8] Y.Y. Lim, T.T. Lim, J.J. Tee (2007), "Antioxidant properties of several tropical fruits: A comparative study", *Food Chemistry*, **103(3)**, pp.1003-1008.
- [9] K. Pathy (2018), "Process for preparation of vitamin C and method for determination of vitamin C in tablets", *Sur. Cas. Stud. Op. Acc.*, **1(3)**, pp.52-65, DOI: 10.32474/SCSOAJ.2018.01.000114.
- [10] V. Santhirasegaram, Z. Razali, C. Somasundram (2013), "Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice", *Ultrasonics Sonochemistry*, **20(5)**, pp.1276-1282, DOI: 10.1016/j.ultsonch.2013.02.005.
- [11] M.A.V. Boekel (2008), "Kinetic modeling of food quality: A critical review", *Comprehensive Reviews in Food Science and Food Safety*, **7(1)**, pp.144-158, DOI: 10.1111/j.1541-4337.2007.00036.x.
- [12] M.M.A. Zubaidy, R.A. Khalil (2007), "Kinetic and prediction studies of ascorbic acid degradation in normal and concentrate local lemon juice during storage", *Food Chemistry*, **101(1)**, pp.254-259, DOI: 10.1016/j.foodchem.2006.01.024.
- [13] M. Mieszczakowska-Frąc, K. Celejewska, W. Płocharski (2021), "Impact of innovative technologies on the content of vitamin C and its bioavailability from processed fruit and vegetable products", *Antioxidants*, **10(1)**, DOI: 10.3390/antiox10010054.
- [14] A. Rivas, D. Rodrigo, A. Martínez, et al. (2006), "Effect of PEF and heat pasteurisation on the physical-chemical characteristics of blended orange and carrot juice", *Lebensmittel-Wissenschaft & Technologie*, **39(10)**, pp.1163-1170, DOI: 10.1016/j.lwt.2005.07.002.
- [15] C. Lagnika, Y.C. Adjovi, L. Lagnika, et al. (2017), "Effect of combining ultrasound and mild heat treatment on physicochemical, nutritional quality and microbiological properties of pineapple juice", *Food and Nutrition Sciences*, **8(2)**, pp.227-241, DOI: 10.4236/fns.2017.82015.
- [16] Y. Gao, W. Xia, P. Shao, et al. (2022), "Impact of thermal processing on dietary flavonoids", *Current Opinion in Food Science*, **48**, DOI: 10.1016/j.cofs.2022.100915.
- [17] J.A. Pino, R. Marbot (2001), "Volatile flavor constituents of acerola (*Malpighia emarginata* DC.) fruit", *Journal of Agricultural and Food Chemistry*, **49(12)**, pp.5880-5882, DOI: 10.1021/jf010270g.
- [18] M. Peleg, M.D. Normand, M.G. Corradini (2012), "The Arrhenius equation revisited", *Critical Reviews in Food Science and Nutrition*, **52(9)**, pp.830-851, DOI: 10.1080/10408398.2012.667460.
- [19] A.N. Kim, H.J. Kim, J. Chun, et al. (2018), "Degradation kinetics of phenolic content and antioxidant activity of hardy kiwifruit (*Actinidia arguta*) puree at different storage temperatures", *Lebensmittel-Wissenschaft & Technologie*, **89**, pp.535-541, DOI: 10.1016/j.lwt.2017.11.036.
- [20] T. Scrob, S.M. Varodi, G.A. Vintilă, et al. (2002), "Estimation of degradation kinetics of bioactive compounds in several lingonberry jams as affected by different sweeteners and storage conditions", *Food Chemistry X*, **16**, DOI: 10.1016/j.fochx.2022.100471.
- [21] T.M. Rababah, M.A. U'Datt, M.A. Mahasneh, et al. (2014), "Effect of jam processing and storage on phytochemicals and physicochemical properties of cherry at different temperatures", *Journal of Food Processing and Preservation*, **38(1)**, pp.247-254, DOI: 10.1111/j.1745-4549.2012.00770.x.
- [22] N. Touati, F.J. Barba, H. Louaileche, et al. (2016), "Effect of storage time and temperature on the quality of fruit nectars: Determination of nutritional loss indicators", *Journal of Food Quality*, **39(3)**, pp.209-217, DOI: 10.1111/jfq.12189.
- [23] N.J. Wurlitzer, A.P. Dionísio, J.R. Lima, et al. (2019), "Tropical fruit juice: Effect of thermal treatment and storage time on sensory and functional properties", *Journal of Food Science and Technology*, **56(5)**, pp.184-193, DOI: 10.1007/s13197-019-03987-0.
- [24] I. VanBree, J.M. Baetens, S. Samapundo, et al. (2012), "Modelling the degradation kinetics of vitamin C in fruit juice in relation to the initial headspace oxygen concentration", *Food Chemistry*, **134(1)**, pp.207-214, DOI: 10.1016/j.foodchem.2012.02.096.

Assessing the effect of the Trier social stress test (TSST) on psychological and physiological measurements of Vietnamese students

Quang Lam Nguyen^{1,2,†}, Ngan Huu Phuc Nguyen³, Anh Phuc Hoang Le^{1,2,†}, Bao Hoai Pham^{1,2}, Vy Kim Huynh^{2,4}, Thien Hoang Minh Cao^{1,2}, Dong An Bui⁵, Khon Huynh^{1,2*}, Huong Thi Thanh Ha^{1,2*}

¹School of Biomedical Engineering, International University, Vietnam National University - Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

²Vietnam National University - Ho Chi Minh City, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

³Department of Psychology, Fulbright University Vietnam, 105 Ton Dat Tien Street, Tan Phu Ward, District 7, Ho Chi Minh City, Vietnam

⁴Faculty of Biology and Biotechnology, University of Science, Vietnam National University - Ho Chi Minh City, 227 Nguyen Van Cu Street, Ward 4, District 5, Ho Chi Minh City, Vietnam

⁵Impact Technical Resources, 3 Nguyen Luong Bang Street, Tan Phu Ward, District 7, Ho Chi Minh City, Vietnam

Received 17 February 2023; revised 23 May 2023; accepted 2 August 2023

Abstract:

The Trier social stress test (TSST) is considered as the most appropriate standardized protocol for studying stress hormone reactivity. The TSST is distinct from other stress induction protocols in laboratory settings due to its unique ability to create a natural context that includes elements of social and psychological acute stress. However, there has been no investigation of the efficacy of the TSST among the Vietnamese population. Therefore, the purpose of this study is to examine the impact of the TSST on Vietnamese university students. A group of 10 healthy male and female volunteers underwent the TSST protocol, and self-reported values (the positive and negative affect schedule and the visual analogue scale) as well as physiological stress response (heart rate) were measured. The results suggest that the TSST mildly induces acute psychological stress among Vietnamese participants. To enhance the potency of the TSST in future studies, a larger sample size and more consistent adherence to the TSST protocol are crucial.

Keywords: electrocardiogram, questionnaires, stress, Trier social stress test, university students.

Classification number: 3.6

1. Introduction

Stress, as defined by the World Health Organization, is the body's response to stimuli that require attention or action, resulting in physical, emotional, or psychological pressure. Stressors are increasingly prevalent in modern society, encompassing factors from family to work stress [1, 2]. As stress affects individuals of all ages, stress-related disorders such as depression, anxiety, physiological changes (cardiovascular, hormonal), and cognitive impairments have become more common [3-7]. Consequently, there is a pressing need for a deeper understanding of stress mechanisms to develop effective treatments. Laboratory-based protocols that can induce the human stress response are essential [8].

To establish a stress-inducing protocol in a laboratory setting, it is crucial to differentiate between different types of stress. Chronic stress arises from long-term stressors that individuals frequently encounter over an extended period, such as unemployment, financial strain, or caregiving responsibilities [9-11]. Acute stress, on the other hand, results from sudden, traumatic, or surprising events, like having an argument, presenting to a large audience, or facing imminent work deadlines. While chronic stress is a more severe and

pervasive form of stress associated with disease and mortality [12], acute stress is particularly relevant for understanding how individuals respond to threatening situations. Exploring acute stress responses is vital for informing adaptive stress management strategies that promote long-term well-being. Achieving this requires a laboratory protocol capable of inducing significant changes in endocrine and cardiovascular activities.

Regarding acute psychological stressors, a meta-analysis of 208 studies conducted by S.S. Dickerson, et al. (2004) [13] identified the TSST as one of the few stress protocols consistently eliciting hypothalamic-pituitary-adrenocortical (HPA) axis responses. Social-evaluative threat, where individuals face potential negative judgment from others, and uncontrollability are key psychological components that generate the largest HPA axis response. These components are prominently present in the TSST's procedural stages. Social-evaluative threat is incorporated by requiring participants to prepare a presentation in front of expert judges while being video recorded. Elements of uncontrollability are integrated throughout the procedure: participants are abruptly informed of a short preparation time and are not informed about the mental arithmetic task [14].

*Corresponding authors: Email: hckhon@hcmu.edu.vn; htthuong@hcmu.edu.vn

† These authors have contributed equally to this manuscript and share first authorship

Up until 2007, the TSST had been employed in over 4000 studies, consistently producing results that established its position as the gold standard for inducing laboratory-based acute psychological stress [15]. The TSST's applicability in non-western populations, such as Chinese [16-22], Japanese [23], and African [24], further enhances its desirability. However, due to its wide usage across cultures, variations in how researchers implement the TSST protocol have been observed. A recent systematic review by N.N. Linares, et al. (2020) [25] identified several discrepancies in testing procedures, particularly in exclusion criteria, selected time intervals for physiological data collection, and the number of judges. These variations may complicate research comparison and replication. To address these issues, the authors provided comprehensive guidance for the systematic implementation and detailed reporting of the TSST methodology, which this study aims to follow.

The effects of the TSST are typically analyzed using multiple methods at different levels of analysis: physiological (electrocardiogram), self-reports, and cortisol. Electrocardiogram (ECG) is a valuable tool for assessing stress levels during specific stages [26]. During the stress experience, the release of adrenaline triggers the body's fight-or-flight response, resulting in physiological changes, including an increased heart rate compared to a non-stress state. Therefore, heart rate (beats per minute) is a reliable metric for evaluating current stress levels [27]. Recent studies have also utilized heart rate variability (HRV) as an indicator of stress [28]. Additionally, common data used to assess the effectiveness of the TSST in inducing stress include heart rate, standard deviation of HRV (RMSSD), root mean square of successive differences (RMSSD), and the percentage of N-N intervals larger than 50 ms (pNN50) [25, 29, 30]. Previous studies have shown that RMSSD and pNN50 decrease in the presence of stressors [28, 31]. Skewness and kurtosis are also employed to understand the distribution of HRV values [32, 33]. Alongside physiological measures, a minimum of two self-report stress questionnaires are typically recommended to assess participants' subjective stress levels before and after the TSST. The positive and negative affect schedule (PANAS), the visual analogue scale (VAS), and the state-trait anxiety inventory (STAI) are commonly selected scales due to their sensitivity to change and validity across languages [25].

Despite the TSST's essential role in studying the neurobiological response to acute stress, no previous studies have investigated its effects on Vietnamese or Southeast

Asian samples. K. Lewin's field theory, proposed in 1997, asserts that behavior is a product of the individual and the environment [34]. This notion is supported by numerous studies demonstrating significant differences in mental health outcomes across ethnicities, highlighting the role of culture in perceiving stress and anxiety [35]. Therefore, this preliminary study aims to lay the foundation for future investigations by evaluating the effects of the translated TSST protocol on a small sample of Vietnamese students. Ten healthy Vietnamese university students were exposed to the TSST protocol, during which self-reported measures (PANAS and VAS) and electrocardiogram activity were recorded. This study hypothesizes that post-TSST, participants would exhibit lower positive affectivity, higher negative affectivity, and higher perceived psychological stress. Additionally, a significant increase in heart rate was predicted, while the remaining electrocardiogram values (standard deviation of HRV, root mean square of successive differences between normal heartbeats (RMSSD), and pNN50) during the resting phase with speech and arithmetic tasks were expected to significantly decrease. Furthermore, the study employed kurtosis and skewness of HRV to understand the distribution of HRV values.

2. Materials and methods

2.1. Participants

Ten participants (six men and four women), aged 19 to 22, all of whom were current university students, took part in this study. Exclusion criteria for the participants included regular consumption of energy drinks or beverages containing stimulant compounds (less than two cups per week), diagnosis of mental illness by professionals, use of psychotherapeutic therapies, and exhibiting signs of anxiety or obsession with their surroundings.

This study received approval from the Institutional Review Board (IRB) of the School of Biomedical Engineering, International University, Vietnam National University, Ho Chi Minh City. Prior to the study, all participants were provided with the informed consent form to read and sign if they agreed to participate.

2.2. The Trier social stress test protocol

The TSST was utilised to induce psychosocial stress [14]. This study followed the recommendations of a systematic review conducted by N.N. Linares, et al. (2020) [25], which synthesized data on the procedures from 35 articles.

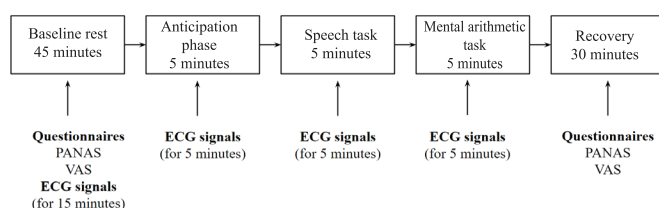


Fig. 1. The Trier social stress test laboratory protocol (total of 90 minutes).

In our study, all participants underwent a 45-minute resting stage, which involved completing questionnaires (PANAS and VAS) and a 15-minute acquisition of EEG signals. Subsequently, the participants had 5 minutes to prepare for a 5-minute speech task. This was followed by a 5-minute mental arithmetic task, concluding the TSST. Finally, there was another 30-minute resting stage during which the participants completed the two questionnaires once again (Fig. 1).

2.2.1. Baseline rest (resting stage) (45 minutes)

The participants were instructed to wait comfortably for 30 minutes. After the 30-minute waiting period, the participants completed questionnaires (Supplementary Tables 1 and 2) to assess their baseline stress level and subjective well-being, including stress symptoms, positive and negative affect (using the PANAS), and current stress level (using the VAS). Physiological measures (subjects' ECG signals) were collected for 15 minutes following the initial 30-minute waiting period.

2.2.2. The Trier social stress test protocol

Anticipation phase (5 minutes): The researcher informed the participant that they were applying for their ideal job and would need to give a 5-minute speech describing why they would be the ideal candidate for the position in front of a judge. The researcher explained that the presentation would be audio and video-recorded for later analysis. The participants were provided with a sheet of paper and a pen and given five minutes to prepare for the presentation. The researcher then started a digital timer to count down from five minutes and left the room. Subjects' ECG signals were collected for five minutes during this stage.

Speech task (5 minutes): Immediately after the preparation stage, the researcher returned to the testing room and instructed the participant to deliver their speech. The researcher set a timer for five minutes. If the participant paused for more than 20 seconds during the speech or finished before five minutes, the judge would use a standardized prompt to ask the participant to continue. If necessary, the judge would ask prepared questions to ensure the participant spoke for the entire allotted time.

Mental arithmetic task (5 minutes): At the conclusion of the speech task, the judge introduced a new task to

the participant. The participant was asked to subtract the number 13 from 1022 sequentially, aloud, as quickly and accurately as possible. The judge informed the participants that their progress would be closely monitored, and any mistakes would result in starting over from the beginning. Pauses lasting longer than 15 seconds were also counted as miscalculations. The task began immediately after the researcher set a timer to count down from five minutes. Subjects' ECG signals were collected for five minutes during this stage.

2.2.3. Recovery (30 minutes)

After the mental arithmetic task, the researcher returned the participants to the original waiting room and instructed them to wait comfortably for 30 minutes. After the 30-minute period, the participants completed the questionnaires again, and post-stress physiological measures were taken.

During the debrief, the researcher informed the participants that their performance was not recorded and that no analysis of their speech or math performance would be conducted. Additionally, the tasks presented to them were intentionally challenging and did not reflect their true abilities.

2.3. Psychological measures

Affect was measured using the PANAS, which is a self-reported questionnaire consisting of 20 multiple-choice questions, with 10 questions measuring positive affect and 10 measuring negative affect [36]. Each question presented a word describing a feeling or emotion experienced at the present moment, and participants rated their response on a scale from one (very slightly or not at all) to five (extremely). The positive and negative affect subscales each comprised 10 words, with a possible score range of 10 to 50. Higher scores indicated a higher level of positive or negative affectivity.

Perceived stress was assessed using the VAS, which has been reliably used to measure psychological distress in Vietnam [37-39]. Participants rated their current stress level on a scale from 1 (not at all) to 10 (extremely).

2.4. Physiological measures: ECG

To measure participants' ECG activities, ECG sensors developed by the ITR company (Fig. 2), and approved by the FDA, were used. The device was placed in the middle of the participants' chest, and the sensors were in direct contact with their skin to record their heart activities. Participants were instructed to remain seated and avoid sudden movements during the TSST procedure to minimize noise caused by muscular movement.

The collected data was then processed using a bandpass filter (range from 0.5 to 30 Hz), and a fifth-order Butterworth filter was applied to remove any remaining noise, particularly

electromyography noise [40, 41] (Fig. 3). The data processing was conducted in Python, utilizing the WFDB processing tools (<https://wfdb.readthedocs.io/en/latest/index.html>) for signal analysis (Fig. 4). Five values were selected for further analysis, calculated using Python: average heart rate, standard deviation, kurtosis and skewness of HRV, root mean square of successive differences (RMSSD) between normal heartbeats, and PNN50 (the ratio of the number of standard heartbeat intervals larger than 50 ms to the total of the N-N intervals). These values indicate the participants' physiological responses to each task condition [42, 43]. Additionally, the average heart rate was calculated by summing all heart rate values between 30 and 200 bpm and dividing them by the total number of suitable heart rate values.



Fig. 2. The ITR electroencephalogram sensor.

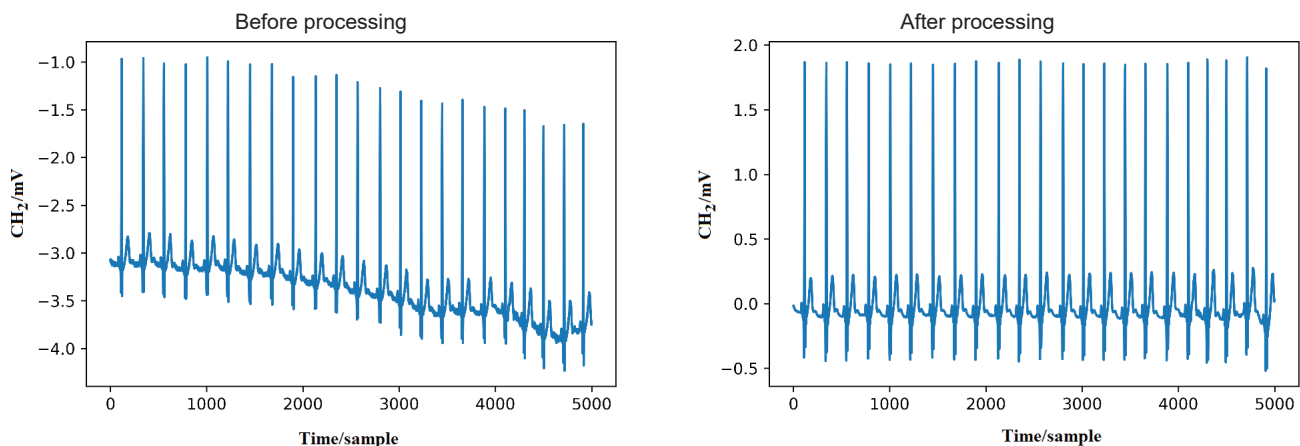


Fig. 3. Acquired data before and after signal processing.

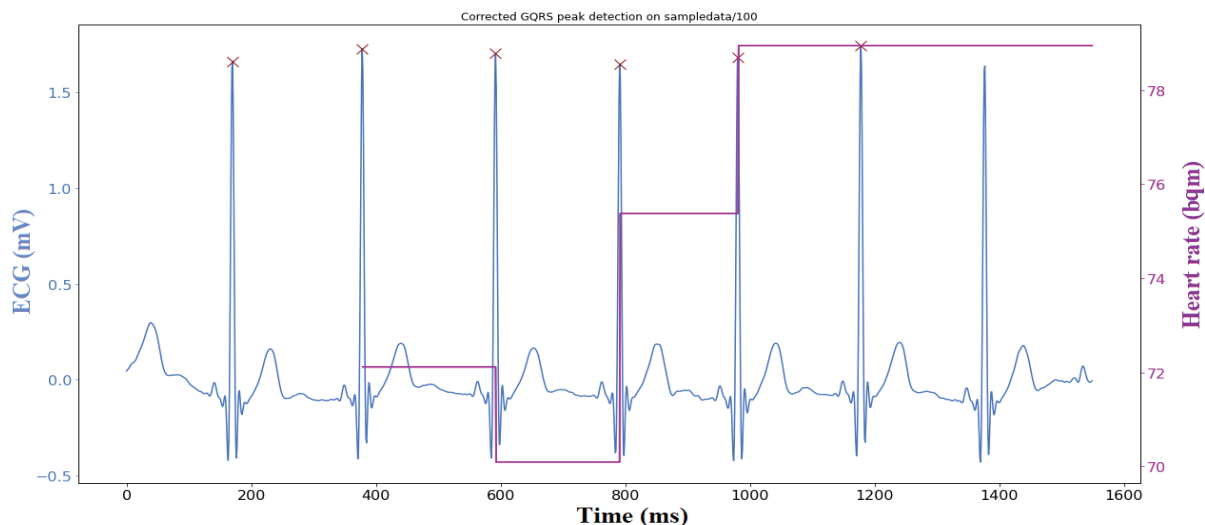


Fig. 4. Peak finding using WFDB functions.

2.5. Statistical analysis

Paired samples t-tests were conducted to compare affective states and perceived psychological stress before and after the administration of the TSST. Additionally, repeated-measures one-way ANOVA was used to compare the ECG signal results across the four stages of the TSST. All statistical analyses were performed using GraphPad Prism. Unless otherwise stated, all values and graphs presented in this article are reported as data $\pm$ standard error (SE). In addition, the asterisk symbol (*) in the graph indicates that there are significant differences between the two comparison groups. The statistical power and effect size of the testing population were calculated using G*Power 3.1.9.7 [44].

3. Results

3.1. Psychological measures

3.1.1. Positive affective state

We hypothesised that the positive affective score, as measured by the PANAS, would significantly decrease after the induction of stress. The average positive affect score prior to the TSST was

28.9±8.621. This score slightly decreased to 26.9±8.212 after the administration of the TSST. However, there was no significant difference in the positive affect scores before and after stress induction ($t(9)=0.956$, $p=0.364$) (Fig. 5). The statistical power of the conducted t-test was 0.668 with an effect size of 0.849.

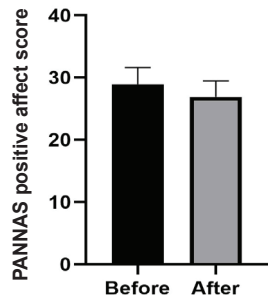


Fig. 5. Positive affect score before and after Trier social stress test.

3.1.2. Negative affective state

We also tested the hypothesis that participants would experience higher negative affectivity after undergoing the TSST. The average negative affective score during the baseline rest was 18.8±8.230. After the participants underwent the TSST, the average score increased to 25.8±11.094, indicating an increase in negative emotions. Our analysis revealed a significant effect of the TSST on negative affect ($t(9)=-2.881$, $p=0.020$) (Fig. 6). The statistical power of the conducted t-test was 0.266 with an effect size of 0.472.

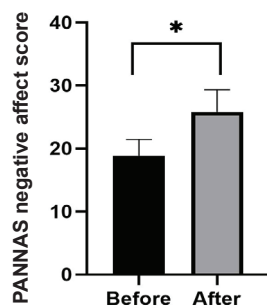


Fig. 6. Negative affect score before and after Trier social stress test.

3.1.3. Perceived stress

Our prediction was that participants would experience increased perceived stress after completing the TSST procedure. Comparing the VAS scores before and after the TSST, we observed a 42% increase in the self-reported stress score (mean of 4.8±1.751 and 6.8±1.687, respectively).

The paired sample t-test indicated a significant effect of the TSST on participants' perceived stress ($t(9)=-3.078$, $p=0.013$) (Fig. 7). The statistical power of the conducted t-test was 0.29 with an effect size of 0.497.

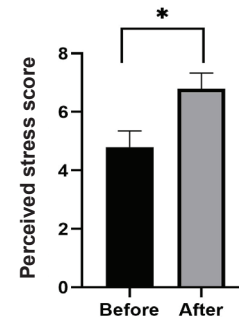


Fig. 7. Perceived stress score before and after Trier social stress test.

3.2. Physiological measures

3.2.1. Heart rate

To assess the influence of different phases of the TSST on participants' heart rate, a one-way repeated measures ANOVA was conducted. We expected significant differences between the baseline rest and the speech and mental arithmetic task phases (Fig. 8). Comparing the four phases of the TSST, significant differences were observed in the participants' average heart rates after applying the repeated measures ANOVA one-way statistical analysis between the rest and anticipation phase (means of 80.4±2.95 and 86.8±3.04, $p=0.043$), the rest and speech phase (means of 80.4±2.95 and 90.8±2.77, $p=0.012$), and the rest and arithmetic phase (means of 80.4±2.95 and 88.0±3.02, $p=0.010$). No significant differences were observed among the remaining phases (anticipation and speech task, anticipation and arithmetic task, speech and arithmetic task). In summary, the TSST significantly affected participants' heart rate, with an increase observed during the speech task and arithmetic task stages.

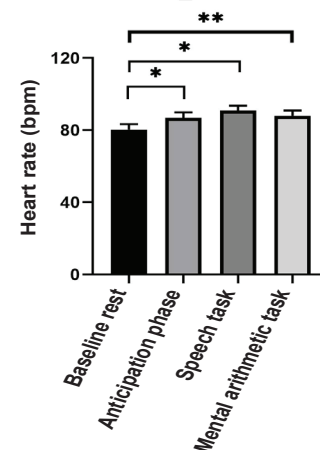


Fig. 8. Subject's heart rate during the four sessions of Trier social stress test.

3.2.2. Heart rate variability (HRV) measurement

Standard deviation of R-R intervals: Assessing the standard deviation of the R-R Interval, a significant difference was only found between the Anticipation and Speech phases (means of 0.0507 ± 0.0052 and 0.062 ± 0.0051 , respectively, $p=0.016$). No significant differences were observed in the remaining comparisons between any two phases (Fig. 9).

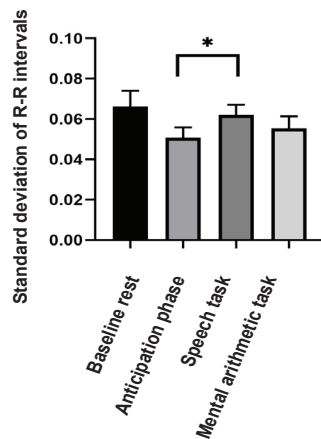


Fig. 9. Subjects' standard deviation of R-R intervals between four sessions of Trier social stress test.

Kurtosis and skewness of R-R intervals: Analysing the participants' kurtosis of R-R intervals, no significant differences were observed. For the skewness of R-R intervals, significant differences were found between the rest and anticipation phases (means of -0.181 ± 0.153 and 0.267 ± 0.13 , respectively, $p=0.015$), the rest and speech task phase (means of -0.181 ± 0.153 and 0.337 ± 0.165 , respectively, $p=0.005$), and the rest and arithmetic phase (means of -0.181 ± 0.153 and 0.536 ± 0.156 , respectively, $p<0.0001$). No differences were observed in the remaining comparison groups. In summary, no

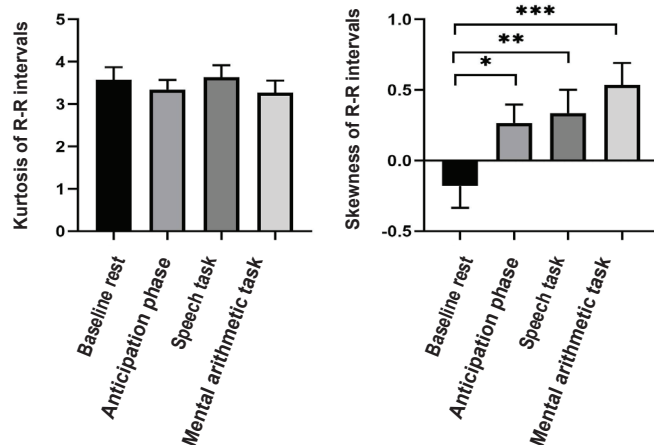


Fig. 10. Subjects' kurtosis and skewness of R-R intervals among the four sessions of Trier social stress test.

conclusive findings can be drawn from the results of kurtosis. However, the significant increase in skewness between each phase of the TSST indicates that the R-R intervals tend to lean towards smaller values, indicating lower variance among R-R intervals and suggesting increased stress in later stages (Fig. 10).

Root mean square of successive difference (RMSSD) and pNN50: The results of the root mean square of successive differences and pNN50 indicate a decrease in their mean values between the rest session and the anticipation and speech sessions (Fig. 11 and Supplementary Table 3). However, no significant differences were found among the four stages of TSST in these two results. Therefore, no conclusive indications can be interpreted from the RMSSD and pNN50 values (Fig. 11).

The statistical power of all heart rate measures ranged from 0.139 to 0.479, with effect sizes ranging from 0.3 to 0.676.

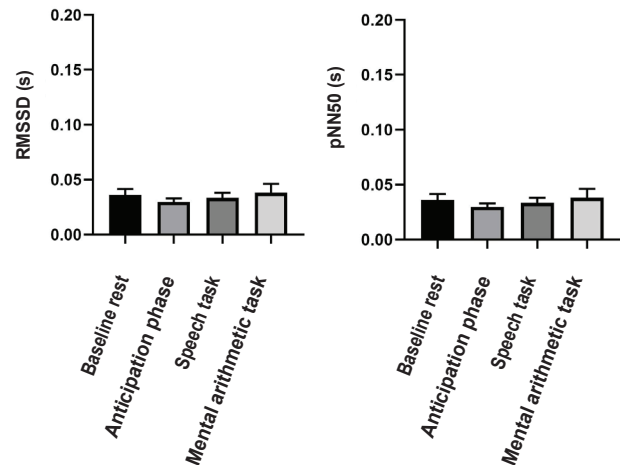


Fig. 11. Subjects' root mean square of successive difference (RMSSD) and pNN50 among the four sessions of Trier social stress test.

4. Discussion

In our experiment investigating the effect of the TSST on stress responses in the Vietnamese population using questionnaires and electrocardiogram (ECG) measurements, we observed several notable results indicating the stress-inducing effects of the TSST. The analysis of the questionnaires revealed a significant increase in participants' negative affective state after the TSST, supporting our hypothesis that negative emotions would intensify during the speech and arithmetic task stages, leading to a more stressful state. However, no significant differences were found in the positive affective state, indicating that positive emotions remained unaffected by the TSST. Additionally, the VAS questionnaire confirmed a significant increase in participants' perceived stress levels after the TSST. Analysing the ECG

measurements, we observed a significant increase in heart rate between the resting stage and the speech and arithmetic stages. The increase in skewness of HRV further indicated that participants' heart rates beat at a faster rate than usual after these two final stages, suggesting a heightened state of stress.

However, some of the remaining ECG results contradicted our hypotheses. There were no differences in the standard deviation and kurtosis of HRV, root mean square of successive differences, and pNN50 values between the resting stage and the speech and arithmetic tasks. These mixed results deviated from previous studies that demonstrated the effectiveness of the TSST in inducing stress in other Asian populations [16-23]. We will discuss potential explanations and recommendations for these contradictions in the following section.

5. Conclusions

The results of our project indicate that the TSST had a stimulating effect on the stress levels of participants, as evidenced by the increased heart rate and alternations in psychological measurements (increased negative affective state and VAS scores). However, non-significant findings in measures such as HRV (kurtosis, RMSSD, and pNN50) may be attributed to confounding variables, including familiarity with the test procedure and ethnic characteristics. Future experiments using the TSST to induce stress should consider several modifications. Firstly, an increased number of interviewers, preferably more than two as in previous TSST studies, should be employed. Secondly, interviewers' rigor during the speech and arithmetic tasks should be consistent to minimise participant bias. Furthermore, future extensions of the study should incorporate a control group and a larger number of participants to enhance the study's validity.

6. Limitations and recommendations

Some of the results that contradict our hypotheses can be attributed to several reasons, which also serve as limitations of our preliminary study. *Firstly*, in the speech task and the arithmetic task, the presence of only one interviewer may have been insufficient to induce a significant level of stress or intensity in the participants. Additionally, the current level of arithmetic difficulty might not have been challenging enough to elicit stress in Vietnamese students. This could be attributed to the exceptional mathematical skills of the Vietnamese population, as evidenced by their performance in the PISA examination compared to other nations (India, Ethiopia, Peru) [45]. *Secondly*, variations in the interviewers' strictness could have led to different emotional responses from participants, resulting in varying stress levels. Furthermore, the lack of uniformity in the questions asked during the speech task may

have led to some participants perceiving the interview as easier than others. *Thirdly*, the small number of participants in our study may have yielded undesirable results and affected the statistical analysis. Particularly for this preliminary study, with only ten participants, the statistical analysis may not provide conclusive findings. The statistical power of the conducted analyses was all below 0.8, which is the ideal threshold for statistical tests [46]. However, the effect size was notable, ranging from 0.472 to 0.849 for psychological measures, indicating strong effects of the TSST intervention on these measures. Conversely, the effect size ranged from 0.3 to 0.67 for physiological measures, indicating a moderate independence between the TSST intervention and these measures [47]. *Fourthly*, some participants may have previously been in similar situations, particularly in the speech task, as reported after the study. This familiarity with job interviews could have reduced the perceived stress of the interview task. Consequently, participants may have experienced a milder response to stress during the TSST. *Fifthly*, the absence of a control group (groups that do not undergo the TSST stage) limits our ability to confirm further details about the effects of the TSST. *Lastly*, the small sample size (N=10) may have reduced the study's ability to replicate the TSST, as the original study by C. Kirschbaum, et al. (1993) [14] found that a sample size of at least 20 participants was necessary to detect significant cortisol responses to the TSST.

SUPPLEMENTARY

Table 1. Positive and negative affective schedule questionnaire's English version.

Positive and negative affective schedule (PANAS-SF)					
Indicate the extent you feel this way right now:					
	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
Interested	☐	☐	☐	☐	☐
Distressed	☐	☐	☐	☐	☐
Excited	☐	☐	☐	☐	☐
Upset	☐	☐	☐	☐	☐
Strong	☐	☐	☐	☐	☐
Guilty	☐	☐	☐	☐	☐
Scared	☐	☐	☐	☐	☐
Hostile	☐	☐	☐	☐	☐
Enthusiastic	☐	☐	☐	☐	☐
Proud	☐	☐	☐	☐	☐
Irritable	☐	☐	☐	☐	☐
Alert	☐	☐	☐	☐	☐
Ashamed	☐	☐	☐	☐	☐
Inspired	☐	☐	☐	☐	☐
Nervous	☐	☐	☐	☐	☐
Determined	☐	☐	☐	☐	☐
Attentive	☐	☐	☐	☐	☐
Jittery	☐	☐	☐	☐	☐
Active	☐	☐	☐	☐	☐
Afraid	☐	☐	☐	☐	☐

Table 2. Visual analogue scale questionnaire (English version).

0-1 Numeric visual analogue scale - VAS

Choose a number from 0 to 10 that best describes your stress level:

No stress	1	2	3	4	5	6	7	8	9	10	Extremely stressed
○	○	○	○	○	○	○	○	○	○	○	

Table 3. Mean values of different heart signal information in four sessions of the Trier social stress test.

	Baseline rest (mean ± SE)	Anticipation phase (mean ± SE)	Speech task (mean ± SE)	Mental arithmetic task (mean ± SE)
Average heart rate	80.4±2.95	86.8±3.04	90.8±2.77	88.0±3.02
R-R standard deviation (s)	0.066±0.0078	0.05±0.0052	0.062±0.0051	0.055±0.006
R-R kurtosis	3.58±0.296	3.34±0.229	3.63±0.285	3.27±0.291
R-R skewness	-0.18±0.153	0.26±0.130	0.33±0.165	0.536±0.156
RMSSD (s)	0.036±0.0053	0.029±0.0032	0.033±0.0046	0.038±0.008
pNN50 (%)	16.1±4.55	9.85±3.01	10.05±2.36	13.0±3.26

CRedit author statement

Lam Quang Nguyen: Conceptualisation, Software, Validation, Formal analysis, Writing - Original draft preparation; Huu Phuc Ngan Nguyen: Conceptualisation, Methodology, Formal analysis, Writing - Original draft preparation; Phuc Hoang Anh Le: Conceptualisation, Methodology, Investigation, Writing - Reviewing and Editing; Hoai Bao Pham, Kim Vy Huynh, Hoang Minh Thien Cao: Investigation, Writing - Reviewing and Editing; An Dong Bui: Software, Resources; Khon Huynh, Thi Thanh Huong Ha: Conceptualisation, Project administration, Writing - Reviewing and Editing.

ACKNOWLEDGEMENTS

This work was supported by the Vietnam National University - Ho Chi Minh City under the Project C Grant (#C2022-28-01). We would like to express our sincere gratitude to Mr. Nguyen Hoang Phuc and Ms. Nguyen Thi Tra My for their support during the experiment and to all the subjects who participated in this experiment.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] N.P. Podsakoff, J.A. LePine, M.A. LePine (2007), "Differential challenge stressor-hindrance stressor relationships with job attitudes, turnover intentions, turnover, and withdrawal behavior: A meta-analysis", *Journal of Applied Psychology*, **92**(2), pp.438-454, DOI: 10.1037/0021-9010.92.2.438.
- [2] D.S. Carlson, P.L. Perrewé (1999), "The role of social support in the stressor-strain relationship: An examination of work-family conflict", *Journal of Management*, **25**(4), pp.513-540, DOI: 10.1177/014920639902500403.
- [3] R. Beiter, R. Nash, M. McCrady, et al. (2015), "The prevalence and correlates of depression, anxiety, and stress in a sample of college students", *Journal of Affective Disorders*, **173**, pp.90-96, DOI: 10.1016/j.jad.2014.10.054.
- [4] P.F. Lovibond (1998), "Long-term stability of depression, anxiety, and stress syndromes", *Journal of Abnormal Psychology*, **107**(3), pp.520-526, DOI: 10.1037//0021-843x.107.3.520.
- [5] J.R. Turner (1994), "Cardiovascular reactivity and stress: Patterns of physiological response", *The Springer Series in Behavioral Psychophysiology and Medicine (SSBP)*, DOI: 10.1007/978-1-4757-9579-0.
- [6] A.J. Crum, M. Akinola, A. Martin, et al. (2017), "The role of stress mindset in shaping cognitive, emotional, and physiological responses to challenging and threatening stress", *Anxiety, Stress & Coping*, **30**(4), pp.379-395, DOI: 10.1080/10615806.2016.1275585.
- [7] A. Saleh, G.G. Potter, D.R. McQuoid, et al. (2017) "Effects of early life stress on depression, cognitive performance and brain morphology", *Psychological Medicine*, **47**(1), pp.171-181, DOI: 10.1017/S0033291716002403.
- [8] D.B. O'Connor, J.F. Thayer, K. Vedhara (2021), "Stress and health: A review of psychobiological processes", *Annual Review of Psychology*, **72**, pp.663-688, DOI: 10.1146/annurev-psych-062520-122331.
- [9] L. Dettenborn, A. Tietze, F. Bruckner, et al. (2010), "Higher cortisol content in hair among long-term unemployed individuals compared to controls", *Psychoneuroendocrinology*, **35**(9), pp.1404-1409, DOI: 10.1016/j.psyneuen.2010.04.006.
- [10] S. Gallagher, R.C. Sumner, O.T. Muldoon, et al. (2016), "Unemployment is associated with lower cortisol awakening and blunted dehydroepiandrosterone responses", *Psychoneuroendocrinology*, **69**, pp.41-49, DOI: 10.1016/j.psyneuen.2016.03.011.
- [11] Y. Kim, R. Schulz (2008), "Family caregivers' strains: Comparative analysis of cancer caregiving with dementia, diabetes, and frail elderly caregiving", *Journal of Aging and Health*, **20**(5), pp.483-503, DOI: 10.1177/0898264308317533.
- [12] E.S. Epel, A.D. Crosswell, S.E. Mayer, et al. (2018), "More than a feeling: A unified view of stress measurement for population science", *Frontiers in Neuroendocrinology*, **49**, pp.146-169, DOI: 10.1016/j.yfme.2018.03.001.
- [13] S.S. Dickerson, M.E. Kemeny (2004), "Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research", *Psychological Bulletin*, **130**(3), pp.355-391, DOI: 10.1037/0033-2909.130.3.355.
- [14] C. Kirschbaum, K.M. Pirke, D.H. Hellhammer (1993), "The 'Trier social stress test' - A tool for investigating psychobiological stress responses in a laboratory setting", *Neuropsychobiology*, **28**(1-2), pp.76-81, DOI: 10.1159/000119004.
- [15] B.M. Kudielka, D.H. Hellhammer, C. Kirschbaum (2007), "Ten years of research with the Trier social stress test - revisited", *Social Neuroscience: Integrating Biological and Psychological Explanations of Social Behavior*, The Guilford Press, pp.56-83.
- [16] J. Fu, T. Weng, F. Tao (2012), "Cortisol response to the Trier social stress test among Chinese adolescents", *Journal of Pediatric Endocrinology & Metabolism*, **25**(11-12), pp.1213-1216, DOI: 10.1515/jpem-2012-0234.

- [17] Y. Juan, H.O.U. Yan, Y. Yu, et al. (2011), "Impact of Trier social stress test (TSST) on salivary cortisol secretion", *Acta Psychologica Sinica*, **43**(04), pp.403-409.
- [18] Y. Sun, F. Li, T. He, et al. (2023), "Physiological and affective responses to green space virtual reality among pregnant women", *Environmental Research*, **216**, Part 1, DOI: 10.1016/j.envres.2022.114499.
- [19] Y. Wei, W. Tianfang, H. Wu, et al. (2018), "Biological mechanisms underlying the liver's regulation of emotions in women: A study using the Trier social stress test", *Journal of Traditional Chinese Medical Sciences*, **5**(2), pp.110-118, DOI: 10.1016/j.jtcms.2018.03.003.
- [20] L. Ying, Q. Yan, X. Shen, et al. (2022), "Social value orientation moderated the effect of acute stress on individuals' prosocial behaviors", *Frontiers in Psychology*, **13**, DOI: 10.3389/fpsyg.2022.803184.
- [21] C.L.J. Lai, D.Y.H. Lee, M.O.Y. Leung (2021), "Childhood adversities and salivary cortisol responses to the Trier social stress test: A systematic review of studies using the children trauma questionnaire (CTQ)", *International Journal of Environmental Research and Public Health*, **18**(1), DOI: 10.3390/ijerph18010029.
- [22] Q. Liu, W. Zhang (2020), "Sex differences in stress reactivity to the Trier social stress test in virtual reality", *Psychology Research and Behavior Management*, **2020**(13), pp.859-869, DOI: 10.2147/PRBM.S268039.
- [23] Y. Yamanaka, H. Motoshima, K. Uchida (2018), "Hypothalamic-pituitary-adrenal axis differentially responses to morning and evening psychological stress in healthy subjects", *Neuropsychopharmacology Reports*, **39**(1), pp.41-47, DOI: 10.1002/npr2.12042.
- [24] C. Plooy, K. Thomas, M. Henry, et al. (2014), "The fear-factor stress test: An ethical, non-invasive laboratory method that produces consistent and sustained cortisol responding in men and women", *Metabolic Brain Disease*, **29**, pp.385-394, DOI: 10.1007/s11011-014-9484-9.
- [25] N.N. Linares, V. Charron, A.J. Ouimet, et al. (2020), "A systematic review of the Trier social stress test methodology: Issues in promoting study comparison and replicable research", *Neurobiology of Stress*, **13**, DOI: 10.1016/j.nynstr.2020.100235.
- [26] S. Pourmohammadi, A. Maleki (2020), "Stress detection using ECG and EMG signals: A comprehensive study", *Computer Methods and Programs in Biomedicine*, **193**, DOI: 10.1016/j.cmpb.2020.105482.
- [27] S. Sriramprakash, V.D. Prasanna, O.R. Murthy (2017), "Stress detection in working people", *Procedia Computer Science*, **115**, pp.359-366, DOI: 10.1016/j.procs.2017.09.090.
- [28] H.G. Kim, E.J. Cheon, D.S. Bai, et al. (2018), "Stress and HRV: A meta-analysis and review of the literature", *Psychiatry Investigation*, **15**(3), pp.235-245, DOI: 10.30773/pi.2017.08.17.
- [29] J.A. Seddon, V.J. Rodriguez, Y. Provencher, et al. (2020), "Meta-analysis of the effectiveness of the Trier social stress test in eliciting physiological stress responses in children and adolescents", *Psychoneuroendocrinology*, **116**, DOI: 10.1016/j.psyneuen.2020.104582.
- [30] J. Hellhammer, M. Schubert (2012), "The physiological response to Trier social stress test relates to subjective measures of stress during but not before or after the test", *Psychoneuroendocrinology*, **37**(1), pp.119-124, DOI: 10.1016/j.psyneuen.2011.05.012.
- [31] E. Filaire, H. Portier, A. Massart, et al. (2010), "Effect of lecturing to 200 students on HRV and alpha-amylase activity", *European Journal of Applied Physiology*, **108**(5), pp.1035-1043, DOI: 10.1007/s00421-009-1310-4.
- [32] A.L. Rivera, B. Estanol, H.S. Madrid, et al. (2016), "Heart rate and systolic blood pressure variability in the time domain in patients with recent and long-standing diabetes mellitus", *PLOS ONE*, **11**(2), DOI: 10.1371/journal.pone.0148378.
- [33] A.V. Machado, M.G. Pereira, G.G. Souza, et al. (2021), "Association between distinct coping styles and HRV changes to an acute psychosocial stress task", *Scientific Reports*, **11**(1), DOI: 10.1038/s41598-021-03386-6.
- [34] K. Lewin (1997), *Resolving Social Conflicts and Field Theory in Social Science*, American Psychological Association, 422pp, DOI: 10.1037/10269-000.
- [35] L. Jobson, S. Haque, S.Z. Abdullah, et al. (2022), "Examining cultural differences in the associations between appraisals and emotion regulation and posttraumatic stress disorder in Malaysian and Australian trauma survivors", *International Journal of Environmental Research and Public Health*, **19**(3), DOI: 10.3390/ijerph19031163.
- [36] D. Watson, L.A. Clark, A. Tellegen (1988), "Development and validation of brief measures of positive and negative affect: The PANAS scales", *Journal of Personality and Social Psychology*, **54**(6), pp.1063-1070, DOI: 10.1037//0022-3514.54.6.1063.
- [37] J.C. Beckham, A.L. Crawford, M.E. Feldman, et al. (1997), "Chronic posttraumatic stress disorder and chronic pain in Vietnam combat veterans", *Journal of Psychosomatic Research*, **43**(4), pp.379-389, DOI: 10.1016/s0022-3999(97)00129-3.
- [38] G.B. Langley, H. Sheppard (1985), "The visual analogue scale: Its use in pain measurement", *Rheumatology International*, **5**(4), pp.145-148, DOI: 10.1007/BF00541514.
- [39] F.X. Lesage, S. Berjot, F. Deschamps (2012), "Clinical stress assessment using a visual analogue scale", *Occupational Medicine*, **62**(8), pp.600-605, DOI: 10.1093/occmed/kqs140.
- [40] P. Zhou, B. Lock, T.A. Kuiken (2007), "Real time ECG artifact removal for myoelectric prosthesis control", *Physiol. Meas.*, **28**(4), pp.397-413, DOI: 10.1088/0967-3334/28/4/006.
- [41] H.L. Butler, R. Newell, C.L.H. Kozey, et al. (2009), "The interpretation of abdominal wall muscle recruitment strategies change when the electrocardiogram (ECG) is removed from the electromyogram (EMG)", *Journal of Electromyography and Kinesiology*, **19**(2), pp.e102-e113, DOI: 10.1016/j.jelekin.2007.10.004.
- [42] B.M. Kudiella, A. Buske-Kirschbaum, D.H. Hellhammer, et al. (2004), "Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: The impact of age and gender", *International Journal of Behavioral Medicine*, **11**(2), pp.116-121, DOI: 10.1207/s15327558ijbm1102_8.
- [43] A. Arza, J.M. Garzón, A. Hemando, et al. (2015), "Towards an objective measurement of emotional stress: Preliminary analysis based on HRV", *37th Annual International Conference of The IEEE Engineering in Medicine and Biology Society (EMBC)*, **2015**, pp.3331-3334, DOI: 10.1109/EMBC.2015.7319105.
- [44] F. Faul, E. Erdfelder, A.G. Lang, et al. (2007), "G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences", *Behavior Research Methods*, **39**(2), pp.175-191, DOI: 10.3758/bf03193146.
- [45] P. Glewwe, Z. James, J. Lee, et al. (2021), *What Explains Vietnam's Exceptional Performance in Education Relative to Other Countries? Analysis of The Young Lives Data from Ethiopia, Peru, India and Vietnam*, RISE Working paper 21/078, 75pp.
- [46] C.C. Serdar, M. Cihan, D. Yücel, et al. (2021), "Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies", *Biochemia Medica*, **31**(1), pp.27-53, DOI: 10.11613/BM.2021.010502.
- [47] T. Schäfer, M.A. Schwarz (2019), "The meaningfulness of effect sizes in psychological research: Differences between sub-disciplines and the impact of potential biases", *Frontiers in Psychology*, **10**, DOI: 10.3389/fpsyg.2019.00813.

Geotechnical properties of cement-stabilised fill material: Developing a linear regression model for predicting unconfined compression strength and undrained shear stress

Muntasir Ahmed, Mostakim Ahmed, Lubaba Afzal*

Civil Engineering Department, Bangladesh University of Engineering and Technology, Dhaka-1000, Bangladesh

Received 7 November 2023; revised 18 December 2023; accepted 15 February 2024

Abstract:

The use of cement stabilisation is considered a practical and cost-effective method in the construction sector for enhancing the geotechnical properties of fill materials. This research has the potential to introduce significant innovations in soil stability and strength, which are crucial for the design and construction of various geotechnical structures, such as foundations, embankments, and retaining walls. This study observed changes in unconfined compressive strength (UCS) and undrained shear stress (USS) by varying the percentage of cement content and curing period. Comprehensive geotechnical characterisation was conducted, including specific gravity determination, sieve analysis, maximum and minimum density tests, standard and modified Proctor compaction tests, and unconfined compression testing. Results were analysed and visualised through graphical representations. Cement content (2-7%) and curing time (7-28 days) led to substantial increases in soil UCS and USS, ranging from 18 to 43% for UCS and 13 to 39% for USS. This research employs a linear regression model for predicting these results, which outperforms existing models. This model contributes to the body of geotechnical engineering knowledge and provides direction for further study and real-world applications in soil stabilisation. The research culminates in a compelling demonstration of the transformative impact of controlled increases in cement content and curing time on the geotechnical properties of silty-sand.

Keywords: cement stabilisation for construction, compaction test, linear regression model, unconfined compressive strength, undrained shear stress.

Classification numbers: 4.2, 4.3

1. Introduction

Unconfined compressive strength and undrained shear stress are two important properties for cement stabilisation that indicate the material's ability to resist deformation and failure under load. Cement-stabilised soil serves as a building material that possesses sufficient strength and durability while having low embodied energy [1]. The use of cement-stabilised soil in construction can address some of the challenges associated with earth construction, such as shrinkage cracking, low strength, and lack of durability [2]. Determining the engineering properties of soil for dynamic stability analysis remains a significant challenge [3]. The unconfined compressive strength test reveals that as the blending ratio increases, the axial strain reduces and brittle fracture becomes more evident. An empirical relationship accounting for the combined effects of cement mixing proportion and curing time is developed to determine UCS and USS. Several methods, including the addition of adhesive materials like cement and lime, are employed to strengthen loose soils for construction; each of these additives has its own economic, environmental, and practical considerations [4]. Saturated sands, often found in coastal areas, experience volumetric instability and significant deformation under seismic pressure, a phenomenon known as liquefaction [5]. For problematic sands, soil improvement is achieved using cement [6]. It is essential to determine the particle size distribution of sand for a

wide range of engineering applications. Significant properties such as setting time, hydration heat curves, water demand, adsorption properties, flowability performance, and compressive strength may vary among cement types with different particle size distributions, even if they have the same Blaine fineness [7-10]. Recent advancements have been made in understanding the influence of grain size on erosion potential. However, relatively few studies have quantified the effects of various geotechnical properties, despite their potential to significantly affect the mechanisms governing the erosion of coarse-grained soil particles. Silty sand has lower shear strength than pure sand because the fine silt particles reduce interparticle friction. Cementitious products are formed as a result of hydration interactions between cement and water during the curing process. These hydration by-products, such as calcium hydroxide (CH) and calcium silicate hydrates (C-S-H), help the cement-mixed sand build interparticle connections and gain strength [11].

The objective of this research is to elucidate the correlation between cement content and curing time on the geotechnical properties of stabilised soil, supported by results from various tests, including specific gravity, sieve analysis, maximum and minimum density, compaction, and unconfined compression strength tests. Existing research indicates that a large amount of test data is needed as predictor variables for a regression model to achieve desirable

*Corresponding author: Email: lubaba.afzal@gmail.com

target variables. This article presents a prediction model that achieves high accuracy despite the limited availability of test data, providing more reliable predictions for undrained shear strength than unconfined compressive strength. This model could be useful for geotechnical engineers who need to estimate the strength and stability of sand-cement mixtures based on various inputs.

2. Materials and methods

2.1. Sample collection

Laboratory experiments were conducted to examine key stabilising elements. The water-cement ratio, mixing duration, and curing conditions were prioritised as they affect the performance of stabilised sand. Particle size distribution, specific gravity, maximum density, and minimum density were tested using standard procedures.

2.2. Physical properties tests

Specific gravity test: A representative sample was prepared, dried in an oven, submerged in water, and weighed. The specific gravity of the sample was determined according to ASTM D854.

Sieve analysis: The ASTM C136 test method encompasses sample preparation, sieve selection, sieving procedure, weighing, and particle size distribution analysis. The particle size distribution of aggregates was analysed to calculate the coefficient of uniformity (C_u) and coefficient of curvature (C_c).

Maximum and minimum density test: The accepted test procedure for measuring the maximum and minimum densities of soil samples is ASTM D4254. These densities are used to evaluate the stability and engineering behaviour of soils.

Compaction test: The soil sample was compacted in a cylindrical mould using a freely falling hammer from a specific height to determine the optimal moisture content and maximum dry density. Fifteen samples were prepared, comprising five separate 2.5 kg samples, each receiving five different additions of water, and were subjected to three distinct experiments or different compaction energies (Table 1).

Table 1. Procedure of compaction tests.

Test type	Mold size	Weight of Hammer (lb)	Number of layers	Height of Hammer drop (in)	Number of blows per layer	Compactive energy per layer (ft-lb/cft)
15 Blow proctor	4.6" height x 4.0" diameter	5.5	3	12	15	7400
Standard proctor	4.6" height x 4.0" diameter	5.5	3	12	25	12375
Modified proctor	4.6" height x 4.0" diameter	10	5	18	25	56300

Specimen for unconfined compression test: Air-dried silty sand with 2, 5, and 7% cement content was compacted at optimum moisture using the standard proctor method. The specimens were then extruded and trimmed to a diameter of 1.5" and a height of

3". A total of 12 different specimens were water-cured for 7, 14, and 28 days, and unconfined compression tests were performed to compare and observe variations in the results for each case (i.e., cement content and curing period).

Procedure of unconfined compression test: This test was conducted according to ASTM D2166. After being removed from the moist curing environment, stabilised samples were subjected to a uniform and shock-free force in a compression testing apparatus, deforming at a rate of approximately 0.05 inches (1 mm) per minute until failure. The entire load and associated deformation were recorded. Untreated samples were later evaluated in compression after the preparation phase (Fig. 1).



Fig. 1. Unconfined compression test (UCS).

2.3. Determining the undrained shear stress

If the unconfined compression test results are known, the undrained shear stress can be easily calculated using the formula:

$$S_u = Q_u/2$$

where S_u is the undrained strength; Q_u denotes unconfined compressive strength of soil. The angle of internal friction is obtained as $\phi=0$ for the test. These specific conditions lead some engineers to refer to this test as an “unconsolidated undrained” test or UU for short [12].

2.4. Regression analysis

A multivariate regression model was developed to predict Q_u and S_u based on the percentage of cement content and curing period. Due to the limited dataset, synthetic data were generated to enhance model accuracy and minimise error. The model aligns with J.J. Binder’s (1985) [13] multivariate regression approach and exhibits characteristics of a first-order unbiased mean squared error estimator [14]. To evaluate model performance, the mean actual error and coefficient of determination were calculated.

3. Results and discussion

3.1. Grain size distribution

The D_{60} , D_{30} , and D_{10} values, derived from the soil gradation curve, correspond to the particle diameters at which 60, 30, and 10% of the soil's total mass, respectively, is composed of finer particles. The results obtained from sieve analysis are displayed in Table 2.

Table 2. Values from sieve analysis.

Parameter	Value
D_{60} (mm)	0.28
D_{30} (mm)	0.15
D_{10} (mm)	0.09
Coefficient of uniformity, C_u	3.11
Coefficient of curvature, C_c	0.89

As the coefficient of uniformity $C_u=3.11<6$ and $C_c=0.89<1$, along with a silt content of 7% (which is between 5 and 12%), the soil is classified as SP-SM, meaning poorly graded sand with silt. According to the correlations found, the gradation of the silty sand impacts the strength outcomes of cement-stabilised sand.

3.2. Compaction test

Both the standard proctor test and modified proctor tests were performed on the soil sample. The results from each test and a comparison between the values are discussed in the following sub-sections.

3.3 Standard proctor test

The standard proctor test produced a value for the optimal moisture content (OMC), which is the moisture content at which the material reaches its maximum dry density under the given compaction effort. The results of the standard proctor test are displayed in Table 3.

Table 3. Results from both compaction methods.

Parameters	Standard proctor test	Modified proctor test
Optimum moisture content, OMC (%)	14.3	13.7
Maximum dry density (kN/m^3)	15.9	16.7

3.4. Modified proctor test

In the modified proctor test, the optimal moisture content (OMC) denotes the moisture content at which the soil sample achieves maximum compaction due to the greater compaction energy applied. At this moisture level, soil particles are properly lubricated, enabling effective compaction and maximum density. The OMC for the modified proctor test is typically lower than that for the standard proctor test because the greater compaction energy in the modified proctor test breaks down more soil particles, creating additional water voids that need to be filled to achieve maximum compaction (Fig. 2). The

modified proctor method uses a heavier hammer and a greater drop distance compared to the standard proctor method. This increased compaction energy results in a denser soil structure with a higher unit weight [15]. The optimum moisture content and maximum dry density resulting from the modified proctor test is shown in Table 3.

Compaction curve for both methods

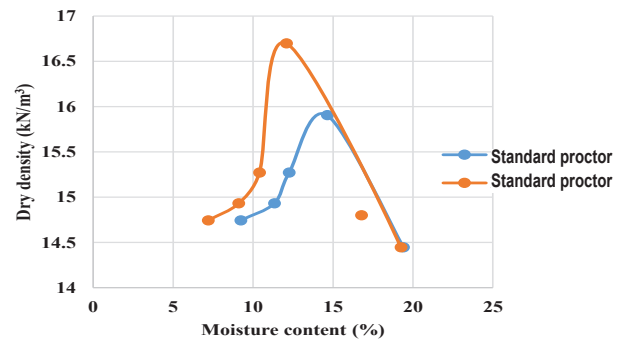


Fig. 2. Dry density in both standard and modified proctor tests.

Unconfined compressive stress vs. axial strain in %

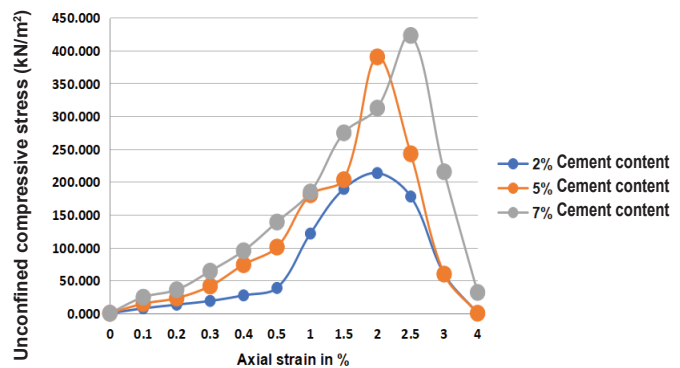


Fig. 3. Unconfined compressive stress (kN/m^2) vs. axial strain (%) for 7 curing days with different cement contents.

Unconfined compressive stress vs. axial strain in %

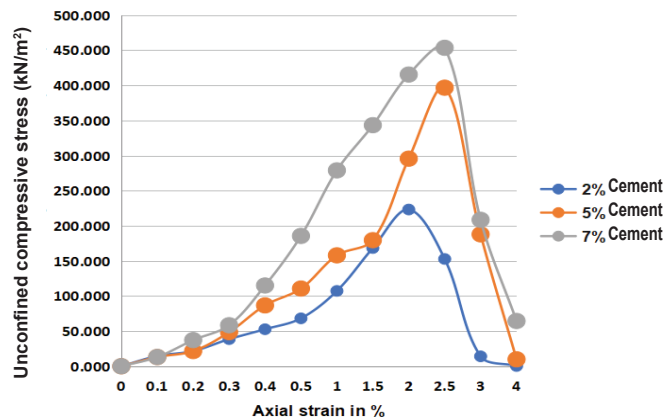


Fig. 4. Unconfined compressive stress (kN/m^2) vs. axial strain in % for 14 curing days with different cement contents.

Unconfined compressive stress vs. axial strain in %

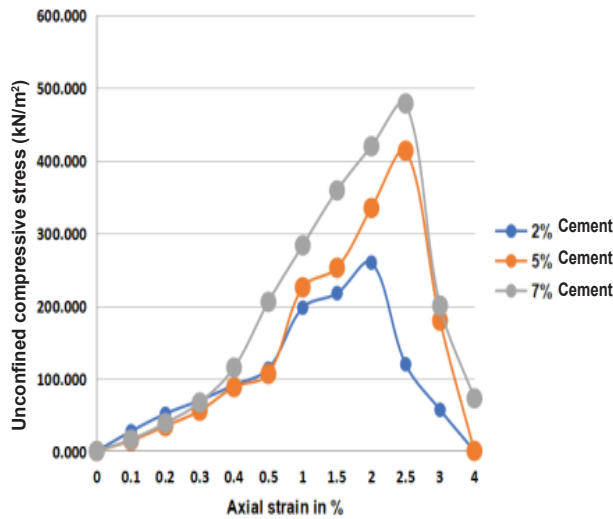


Fig. 5. Unconfined compressive stress (kN/m^2) vs. axial strain in % for 28 curing days with different cement contents.

 Q_u (kN/m^2) vs. curing period (in days)

with 2%, 5%, 7% cement

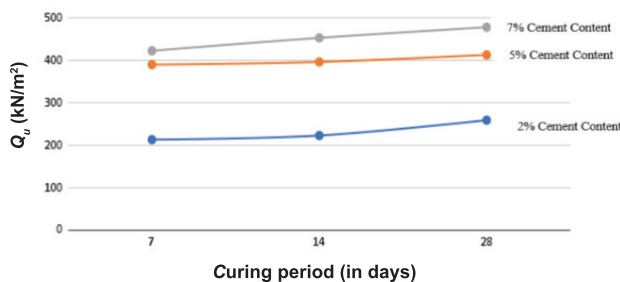


Fig. 6. Unconfined compressive stress Q_u (kN/m^2) vs. curing period.

 S_u (kN/m^2) vs. curing period (in days)

with 2%, 5%, 7% cement

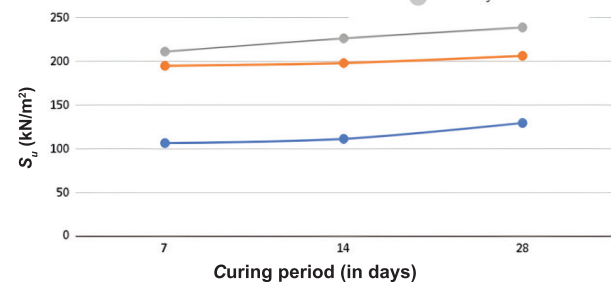


Fig. 7. Undrained shear strength S_u (kN/m^2) vs. curing period (days).

 S_u (kN/m^2) vs. cement (%)

Line for 7 days, 14 days & 28 days

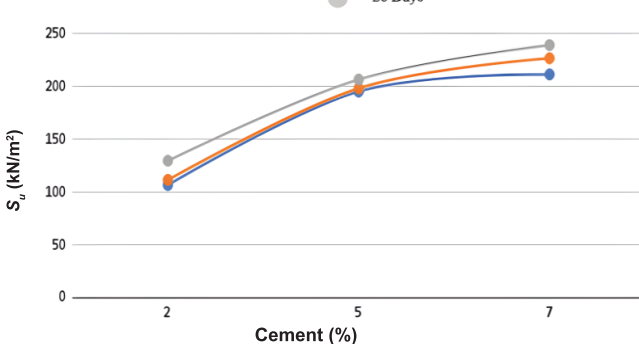


Fig. 8. Undrained shear strength S_u (kN/m^2) vs. cement content (%).

 Q_u (kN/m^2) vs. cement (%)

Line for 7 days, 14 days & 28 days

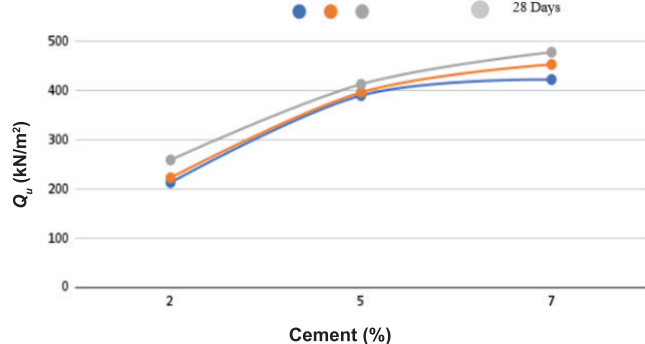


Fig. 9. Unconfined compressive stress Q_u (kN/m^2) vs. cement content (%).

The three graphs (Figs. 3-5) presented depict the relationship between unconfined compressive strength and axial strain (%) for different curing days and cement contents. The graphs indicate that as the cement content increases, the peak unconfined compressive strength also rises, resulting in enhanced load-bearing capacity. This trend is consistent across curing periods of 7, 14, and 28 days [16].

The unconfined compressive strength test shows that as the cement blending ratio increases, the axial strain decreases and brittle fracture becomes more pronounced. An empirical relationship accounting for the combined effects of cement mixing proportion and curing time has been developed to determine Q_u and S_u .

Figures 6 and 7 show that both Q_u and S_u increase with the curing period, indicating that the material becomes stronger and more resistant to deformation over time. This is due to the ongoing cement hydration reaction, which produces more gels that fill the pores and bond the soil particles.

Figures 8 and 9 show that both Q_u and S_u increase with cement content, suggesting that the material becomes stronger

and more resistant to failure with higher cement content. This improvement is attributed to the additional cementitious material, which enhances the cohesion and friction within the soil matrix.

3.5. Tabular summary of values from unconfined compression test

Table 4 illustrates a noteworthy trend: for a consistent curing period, an increase in cement content from 2 to 5% results in a substantial rise in unconfined compressive strength. Interestingly, the increase in undrained shear stress due to a higher cement percentage is less pronounced compared to the rise in unconfined compressive strength. Moreover, transitioning from 5 to 7% cement content results in a slower rate of increase in both Q_u and S_u compared to the previous change from 2 to 5%. This suggests a nuanced relationship between cement content and curing period, reflecting complex changes in these mechanical properties.

Table 4. Results from unconfined compression test.

Cement content	2%			5%			7%		
Curing days	7	14	28	7	14	28	7	14	28
Q_u (kN/m ²)	213.42	223.17	259.38	390.30	396.62	413.25	422.95	453.43	478.48
S_u (kN/m ²)	106.71	111.58	129.69	195.15	198.31	206.62	211.47	226.71	239.24

3.6. Result of regression analysis

The regression analysis conducted in this research was evaluated using statistical metrics for the correlation coefficient (R) (Figs. 10, 11). Table 5 contains the mean absolute error (MAE), mean squared error (MSE), and R^2 value of Q_u and S_u , respectively.

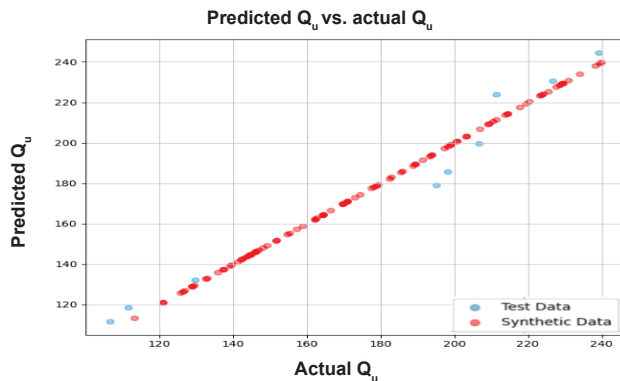


Fig. 10. Prediction analysis of unconfined compression test.

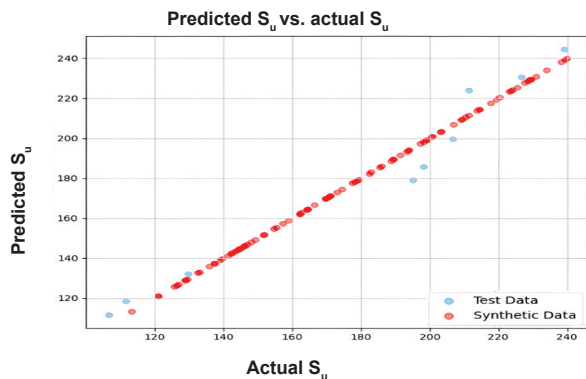


Fig. 11. Prediction analysis of undrained shear stress.

Table 5. Result of regression analysis.

	Mean absolute error	Mean squared error	R^2
Q_u	15.91	329.97	0.96
S_u	7.95	82.51	0.96

Mean absolute error: The MAE provides a quantitative measure of the average difference between the model's predictions and the actual values. An MAE of 15.91 kN/m² for Q_u indicates that, on average, the model's predictions for Q_u deviate from the actual values by approximately 15.91 kN/m². Similarly, an MAE of 7.95 kN/m² for S_u suggests that the average prediction error for S_u is around 7.95 kN/m². The lower value of MAE for S_u implies that the model's predictions for S_u are closer to the actual values compared to that of Q_u .

Mean squared error: The MSE measures the average squared difference between the model's predicted values and the actual values. A significantly higher MSE for Q_u (329.97) compared to that of S_u (82.51) suggests greater variability in the predictions or potential outliers affecting the model's performance for Q_u . This is because MSE is sensitive to outliers and large errors, magnifying their impact on the overall error measure. Conversely, the lower MSE for S_u implies that the model's predictions for S_u are more consistent and less prone to outliers.

Coefficient of determination: The R^2 statistic quantifies the proportion of the variance in the dependent variable that is explained by the independent variables in a regression model. An R^2 value close to 1 indicates a perfect fit, meaning that the regression predictions perfectly align with the observed data.

The high R^2 values of 0.96 for both Q_u and S_u indicate that the model explains approximately 96% of the variability in the respective target variables. This suggests a strong correlation between the selected features (cement content and curing days) and the target variables (Q_u and S_u).

4. Conclusions

A synergistic effect was observed with increases in cement content typically resulting in a 30-40% enhancement in strength and load-bearing capacity. This suggests that the addition of cement improves the strength and load-bearing capacity of the stabilised sand. As a binding agent, cement particles create a matrix that is more cohesive and improves particle interlocking, leading to increased compressive stress. The sand-cement mixture exhibited a significantly higher undrained shear strength with both longer curing times and higher cement content. On average, increasing the curing period led to a 20-30% increase in undrained shear strength. This enhancement is attributed to the cement's adhesive properties, which improve frictional resistance and inter-particle bonding. These findings indicate that stabilised sand is better able to withstand shear stresses, making it more suitable for engineering applications where stability and resistance to deformation are critical.

The results of the compaction tests showed that the modified proctor test yielded a higher maximum dry density and a lower optimum moisture content compared to the standard proctor test for the same soil sample. This indicates that the modified proctor test is a more effective method for compacting soil and improving its strength and stability. Increasing cement content improves the engineering properties of high-plasticity soils, such as reducing their plasticity and shrink/swell potential, and increasing their bearing capacity. Cement stabilisation also enhances soil strength and durability by forming hydration products that bind the soil particles together.

Through controlled increases in both cement content and curing time, silty-sand exhibited significantly enhanced geotechnical properties, including dry density, undrained shear strength, and unconfined compressive strength. These findings have significant implications for geotechnical engineers and professionals involved in construction and soil stabilisation projects.

The prediction model developed in this research demonstrates superior performance compared to existing models. The model exhibits strong predictive capabilities, as evidenced by high R^2 values for both output parameters, indicating a good fit between the model and the observed data. The prediction error for S_u is consistently lower than that for Q_u as measured by both MAE and MSE. This suggests that the model's predictive accuracy is higher for S_u compared to Q_u . Despite the relatively low prediction errors, it is important to consider the absolute magnitude of these errors in the context of the intended application. If precision is critical and the acceptable error threshold is very low, even these small errors may have significant consequences. This research underscores the pivotal role of optimised cement content and curing time in enhancing the geotechnical properties of silty-sand, paving the way for more resilient and sustainable construction and soil stabilisation projects.

5. Future works

If the higher MSE for Q_u is attributed to outliers or specific segments of the data where the model's performance is suboptimal, further investigation and potential refinement of the model may be necessary to address these areas of deficiency. Overall, the model demonstrates notable robustness and can be considered reliable for predicting Q_u and S_u based on the provided inputs. However, it is noteworthy that the model's predictions for S_u are slightly more reliable compared to those for Q_u .

CRedit author statement

Muntasir Ahmed: Conceptualisation, Methodology, Laboratory testing, Data collection, Data analysis, Visualisation, Revision, Original draft preparation, Reviewing and Editing; Mostakim Ahmed: Conceptualisation, Methodology, Laboratory testing, Data collection, Data analysis, Visualisation, Revision, Original draft preparation, Reviewing and Editing; Lubaba Afzal: Conceptualisation, Methodology, Laboratory testing, Data collection, Data analysis, Visualisation, Revision, Original draft preparation, Reviewing and Editing.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Dr. Abu Siddique and the Geotechnical Engineering Laboratory of Bangladesh University of Engineering and Technology.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] K.K.G.K.D. Kariyawasam, C. Jayasinghe (2016), "Cement stabilized rammed earth as a sustainable construction material", *Construction and Building Materials*, **105**, pp.519-527, DOI: 10.1016/j.conbuildmat.2015.12.189.
- [2] R. Bahar, M. Benazzoug, S. Kenai, (2004), "Performance of compacted cement-stabilised soil", *Cement and Concrete Composites*, **26**(7), pp.811-820, DOI: 10.1016/j.cemconcomp.2004.01.003.
- [3] W.F. Marcuson, P.F. Hadala, R.H. Ledbetter (1996), "Seismic rehabilitation of earth dams", *Journal of Geotechnical Engineering*, **122**(1), DOI: 10.1061/(ASCE)0733-9410(1996)122:1(7).
- [4] S. Talamkhani (2023), "Machine learning-based prediction of unconfined compressive strength of sands treated by microbially-induced calcite precipitation (MICP): A gradient boosting approach and correlation analysis", *Advances in Civil Engineering*, DOI: 10.1155/2023/3692090.
- [5] H.M. Abasi, B. Kordtabar, A. Kordnaei (2018), "Liquefaction prediction using CPT data by triangular chart identification", *International Journal of Geotechnical Engineering*, **12**(4), pp.377-382, DOI: 10.1080/19386362.2017.1282399.
- [6] H. MolaAbasi (2023), "Novel estimation of time-dependent unconfined compressive strength of sand treated with cement and zeolite", *Geotechnical and Geological Engineering*, **41**(5), pp.3217-3224, DOI: 10.1007/s10706-023-02428-2.
- [7] J. Assaad, S. Asseily, J. Harb (2010), "Effect of grinding aids on the clinker factor and energy consumption of Portland cement", *ACI Mater. J.*, 30pp.
- [8] M. Katsioti, P.E. Tsakiridis, P. Giannatos, et al. (2009), "Characterization of various cement grinding aids and their impact on grindability and cement performance", *Construction and Building Materials*, **23**(5), pp.1954-1959, DOI: 10.1016/j.conbuildmat.2008.09.003.
- [9] Y.M. Zhang, T.J.N. Munn (1995), "Effects of particle size distribution, surface area and chemical composition on Portland cement strength", *Powder Technology*, **83**(3), pp.245-252, DOI: 10.1016/0032-5910(94)02964-P.
- [10] D.P. Bentz, E.J. Garboczi, C.J. Haecker, et al. (1999), "Effects of cement particle size distribution on performance properties of Portland cement-based materials", *Cement and Concrete Research*, **29**(10), pp.1663-1671, DOI: 10.1016/S0008-8846(99)00163-5.
- [11] M. Ghanbari, M. Bayat (2022), "Effectiveness of reusing steel slag powder and polypropylene fiber on the enhanced mechanical characteristics of cement-stabilized sand", *Civil Engineering Infrastructures Journal*, **55**(2), pp.241-257, DOI: 10.22059/CEIJ.2021.319310.1742.
- [12] O. Gunaydin, A. Gokoglu, M. Fener (2010), "Prediction of artificial soil's unconfined compression strength test using statistical analyses and artificial neural networks", *Advances in Engineering Software*, **41**(9), pp.1115-1123, DOI: 10.1016/j.advengsoft.2010.06.008.
- [13] J.J. Binder (1985), "On the use of the multivariate regression model in event studies", *Journal of Accounting Research*, **23**(1), pp.370-383, DOI: 10.2307/2490925.
- [14] S. Chen, P. Lahiri (2008), "On mean squared prediction error estimation in small area estimation problems", *Proceedings of The Survey Research Methods Section*, **37**(11), pp.1792-1798.
- [15] J. Connelly, W. Jensen, P. Harmon (2008), *Proctor Compaction Testing*, <https://core.ac.uk/download/pdf/77939216.pdf>, accessed 10 October 2023.
- [16] W.A. Jabban, J. Laue, S. Knutsson, et al. (2019), "A comparative evaluation of cement and by-product petrit T in soil stabilization", *Applied Sciences*, **9**(23), DOI: 10.3390/app9235238.

The role of groundwater recharge in groundwater exploitation of the Red river delta plain

Viet Hung Le^{1,2}, Quy Nhan Pham^{2*}, Thi Linh Phung², Van Canh Doan³, Quoc Cuong Tran³, Tran Trung Dang⁴

¹Graduate University of Science and Technology, 18 Hoang Quoc Viet Street, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam

²Hanoi University of Natural Resources and Environment (HUNRE), 41A Phu Dien Street, Phu Dien Ward, Bac Tu Liem District, Hanoi, Vietnam

³Institute of Geological Sciences (IGS), 84 Chua Lang Street, Lang Thuong Ward, Dong Da District, Hanoi, Vietnam

⁴National Centre for Water Resources Planning and Investigation (NAWAPI), 93/95 Vu Xuan Thieu Street, Sai Dong Ward, Long Bien District, Hanoi, Vietnam

Received 14 November 2023; revised 28 December 2023; accepted 15 February 2024

Abstract:

The Red river delta plain (RRDP), the second largest delta in Vietnam, is situated in the northern region of the country and spans an area of 21,260 km² and supports a population of over 22.9 million inhabitants. Groundwater extraction primarily occurs from quaternary sedimentary aquifers, with a total discharge of approximately 1.5 million m³/day. However, certain localities, such as Hanoi and Nam Dinh, have exhibited signs of over-exploitation, leading to associated issues such as depletion, land subsidence, saltwater intrusion, and water pollution. Groundwater recharge in the study area primarily originates from various sources including rainfall, irrigation, wastewater, and the river system. The objective of this study is to evaluate the contribution of groundwater recharge to groundwater exploitation reserves within the Red river delta. To achieve this, a three-dimensional (3D) model employing the MODFLOW code was developed and refined through the comparison of modelled and actual groundwater levels within the surveillance network. The refined model's budget analysis indicated that the recharge of quaternary aquifers by means of rainfall, irrigation, and effluent occurs year-round, with the peak recharge happening during the monsoon season (approximately 68% of rainfall) and the nadir during the arid season (approximately 10% of rainfall). Throughout the monsoon period, the river system predominantly replenishes the quaternary aquifers, contributing approximately 9.51-17.36% to the overall water balance of the aquifers. The influx from fractured aquifers at the fringe of the plain to the quaternary aquifers remains consistently minimal year-round.

Keywords: groundwater modelling, groundwater recharges, quaternary aquifer, Red river delta.

Classification numbers: 4.2, 5.3

1. Introduction

Groundwater recharge is the process by which water infiltrates the ground, adding to the groundwater reservoir [1]. There are three main types of groundwater recharge: i) Direct recharge, where excess soil moisture percolates vertically down to the water table. ii) Indirect recharge, occurring through infiltration and percolation via riverbeds or lakes. iii) Localised recharge, happening at concentrated points such as joints, depressions, sinkholes, or rivulets [2, 3]. Groundwater recharge plays a critical role in the water balance of a basin and is key to the sustainable use of water resources. However, measuring groundwater recharge directly is challenging [4]. B.R. Scanlon, et al. (2002) [5] suggested using multiple methods to estimate groundwater recharge and then comparing the results of each. Understanding the contribution of groundwater recharge to the regional groundwater flow system is crucial for management and economic reasons, as indicated by J.G. Arnold, et al. (2000) [6], R. Hirata, et al. (2012) [7], and C.R. Hearne, et al. (2010) [8]. Groundwater recharge typically feeds local flow systems as base flow [9], while some may exit the basin into deeper aquifers, termed groundwater outflow to the regional system.

The Red river delta plain, covering over 21,260 km² and home to 22.9 million people in Vietnam, heavily relies on groundwater resources, with a daily extraction rate exceeding 3 million m³/d. However, signs of over-exploitation, such as depletion, land subsidence, saline water intrusion, and water contamination, have been observed in certain areas like Hanoi and Nam Dinh [10]. While various studies have focused on groundwater recharge in the RRDP, most have concentrated on specific points or local areas, and a comprehensive regional study has yet to be conducted. Earlier research by P.Q. Nhan (2000) [11] used modelling to estimate groundwater recharge, allocating 87% to the rainy season and 56.28% to the dry season. The limitations of data and lack of validation from alternative methods, however, cast uncertainties on these findings. T.L. Tran (2011) [12] applied isotope hydrology to assess groundwater recharge in Dan Phuong, Hanoi, identifying a linkage between the Holocene and Pleistocene aquifers with a 19.4% contribution from leaky sources. The study noted that during the rainy season, the Holocene aquifer was recharged by the river, whereas in the dry season, it discharged into the river. P.Q. Nhan, et al. (2019) [10] used isotope hydrology to assess the interaction between the Red river and groundwater, showing

*Corresponding author: Email: pqnhan@hunre.edu.vn

that Red River water contributed 50, 52, and 57% to well fields in Ha Dinh, Mai Dich, and Phap Van, respectively. D. Postma, et al. (2017) [13] dated groundwater and determined recharge from the Red river to aquifers using Tritium/Helium dating in the Nam Du area. F. Larsen, et al. (2008) [14] employed isotopes and modelling to assess recharge from the Red river and rainwater to quaternary aquifers in Dan Phuong. V.C. Doan (2015) [15] utilised GIS, MODFLOW, and water table fluctuation methods to evaluate groundwater resources in the RRDP. D.H. Trieu (2022) [16] employed modelling and water table fluctuation methods to categorise Red river boundary conditions and bedrock boundaries in the Southwest of Hanoi, with groundwater recharge and discharge varying from $-191 \text{ m}^3/\text{day}/\text{km}$ to $227 \text{ m}^3/\text{day}/\text{km}$. Recently, P.Q. Nhan, et al. (2022) [17] utilised GIS and remote sensing to analyse factors and to zone direct groundwater recharge in the RRDP. They quantified the recharge using ^3H dating, yet its role in aquifer systems remains unclear. Therefore, this study aims to comprehensively assess the contribution of direct groundwater recharge, stemming from sources such as rainwater, irrigation, and wastewater, to the aquifer system.

2. Study area

2.1. Location and natural conditions

The study area, delineated in Fig. 1, spans an extensive area exceeding $21,260 \text{ km}^2$ within the bounds of the RRDP. Positioned within a tropical humid monsoon climate, the territory experiences a pronounced dry season from November to April, accounting for 15% of annual precipitation, contrasted by a significant rainy season from May to October, delivering 85% of annual rainfall. The area is characterised by persistently high relative humidity levels, averaging 84.5% annually.

Annual climatic variations across the delta include precipitation ranging from 1,200 to 2,700 mm and evaporation from 828.2 to 1,057.1 mm. Temperature profiles show a marked seasonal variance, peaking at 35 to 39°C , with an annual mean between 23 and 23.5°C . Diurnal temperature fluctuations are substantial, with a range of 8 to 15°C in summer and 4 to 13°C in winter. The RRDP is defined by a complex hydrological network, with the Red river system and the Thai Binh river system as prominent features, where drainage densities vary from 0.4 to $0.7 \text{ km}/\text{km}^2$.

The terrain within the RRDP is varied, encompassing coastal areas below 1 m , terraced riverine zones from 7 to 15 m , hilly regions at 50 to 100 m on the plain, and mountainous regions ascending to 900 m on the western and north-western edges. The landscape is sculpted by fault systems, particularly the Northwest-Southeast, Northeast-Southwest, and sub-meridian faults, which dictate the tectonic activity and subdivide the plain. The eastern and south-eastern coastline stretches approximately 200 km from Quang Ninh to Thanh Hoa, affected by saline intrusion from waves, tides, and river dynamics, influencing the coastal ecosystem, agriculture, and shallow aquifers.

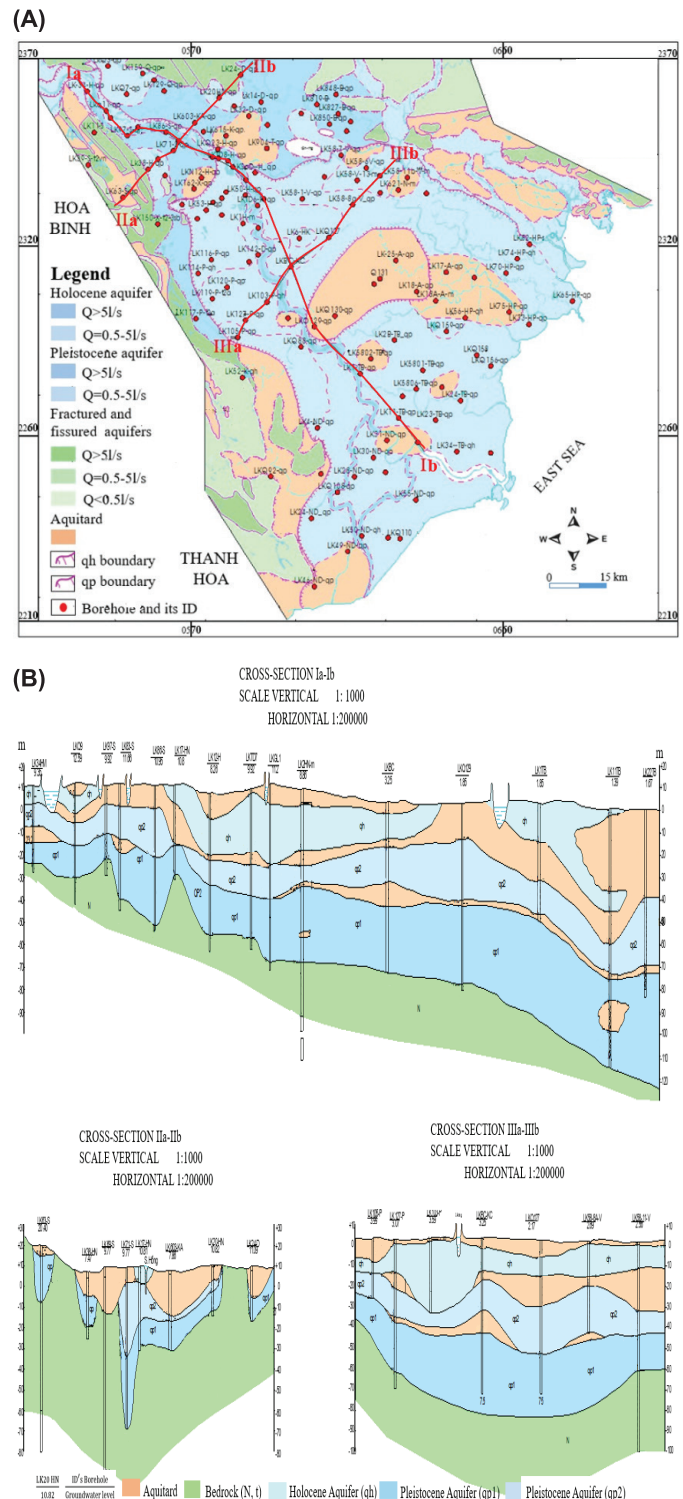


Fig. 1. Hydrogeological map of the Red river delta plain (A) and cross-sections of hydrogeology in the Red river delta plain (B). The fraction $\frac{\text{LK105}-P}{3.99}$ indicates the borehole over the static water level. The numerical values accompanying the borehole diagram denote the depths of the layers, screens, and depth of borehole. The colours and symbols representing the aquifers and aquitards on the map are given in the figure (modified from [18]).

2.2. Hydro-geology settings

The RRDP segment under study lies in the northwest of the Red River sedimentary basin, distinguished by sediment deposits from the Paleogene, Neogene, and Quaternary periods. Flanked by mountain ranges bearing Palaeozoic and Mesozoic strata, the sedimentary deposits of the RRDP have formed across five fining-upward cycles, outlined by T.L. Tran (2011) [12]. These cycles, ranging from the lower to upper Pleistocene and lower to upper Holocene, comprise varied sedimentary makeups from coarse-grained alluvial and fluvial to fine-grained deltaic-lacustrine and swamp deposits. The Pleistocene aquifers, recharged by the adjacent mountains, exhibit an average annual replenishment of 100 to 400 mm [11]. The hydraulic gradients typically span 0.05 to 0.15, and the groundwater flow velocity within Holocene aquifers is several tens of meters annually [12].

3. Materials and methods

3.1. Construction of the groundwater flow model and evaluation of the role of groundwater recharge in groundwater exploitation

The differential equation of motion of groundwater under heterogeneous and anisotropic environment is as follows:

$$\frac{\partial}{\partial x} \left(K_{xx} \frac{\partial h}{\partial x} \right) + \frac{\partial}{\partial y} \left(K_{yy} \frac{\partial h}{\partial y} \right) + \frac{\partial}{\partial z} \left(K_{zz} \frac{\partial h}{\partial z} \right) - W = S_s \frac{\partial h}{\partial t} \quad (1)$$

where K_{xx} , K_{yy} , and K_{zz} are the aquifer hydraulic conductivities in the x , y , and z directions, respectively; S_s specific storage of the aquifer; h hydraulic head in the aquifer; t is time; and W is the source and/or sink of the aquifers. Equation (1), in conjunction with the specified boundary and initial conditions for the aquifers, constitutes a mathematical model for the flow of groundwater. To address the aforementioned equation, it is imperative to identify the function $h(x, y, z, t)$ that complies with Eq. (1) and the prescribed boundary conditions. The temporal evolution of h will describe the characteristics of the groundwater flow, enabling the calculation of both the flow rate and the flow direction.

To evaluate the role of direct groundwater recharge to groundwater exploitation within the RRDP, we rely on recent research data as the basic inputs for building a groundwater flow model. These datasets include:

- Updated regional data derived from the hydrogeological map with scale of 1/200,000 (Fig. 1) compiled by V.T. Tam, et al. (2018) [19].
- A comprehensive survey of the current state of groundwater exploitation, conducted in support of the Hong-Thai Binh river basins planning, as presented by the Department of Water Resources Management (2020, 2022) [19, 20].
- Delineation and quantification of direct groundwater recharge across the delta, analysed by P.Q. Nhan, et al. (2022) [17].

- Assessment of groundwater recharge from the bedrock and the hydraulic interaction between the Red river and groundwater in Hanoi and its vicinity, as researched by D.H. Trieu (2022) [16].

- A comprehensive assessment of the impact of urbanisation on groundwater resources in Hanoi, executed with precision by V.T. Tam, et al. (2018) [18].

These datasets serve as the cornerstone for the groundwater flow model within the RRDP.

The groundwater flow model was constructed utilising GMS software by Aquaveo (2018) [21], with calibration performed via data from the national monitoring network and our recent investigations. Post-calibration, the RRDP model's results were analysed using the BUDGET package. These budget assessments and predictive scenarios highlighted the contribution of direct groundwater recharge to groundwater exploitation in the RRDP.

3.2. Input data and conceptual model for the groundwater system

- *Digital Elevation Model (DEM)*: This model is created from contour lines and elevation data on a 1:50,000-scale topographic map, covering 47 map sections with 500x500 m dimensions. The process within ArcGIS involves two steps:

1. Generation of a triangular irregular network (TIN) using contour lines, elevation points, and local key elevations. TIN modelling preserves elevation point shapes and sharp peaks.

2. Interpolation of the DEM map from the TIN using the natural neighbour method, ensuring accurate representation by referencing surrounding points and avoiding elevation anomalies.

- *Model grid development*: The modelling area, which has been expanded to 14,860 km², intentionally extends beyond the eastern boundary of the Pleistocene aquifer. Investigations have indicated that the Pleistocene aquifer in the eastern sector of the Northern region projects into the sea and is not confined by the current coastline, thus justifying the model's spatial extension. The research area has been methodically divided into a grid format, consisting of 162 rows and 223 columns, each cell measuring 1000x1000 m. Notably, within the Hanoi vicinity, grid resolution has been refined to 500x500 m in response to the dense clustering of extraction wells.

- *Stratigraphic layer classification*: The stratigraphic layout was interpolated from an extensive dataset of 728 well logs, as detailed by P.Q. Nhan, et al. (2022) [17]. This information has been stratified into four key layers for hydrogeological assessment, including the surface aquitard layer (Aquitard 1), the Holocene aquifer (qh), the interjacent Holocene-Pleistocene aquitard (Aquitard 2), and the Pleistocene aquifer (qp).

- *Hydrogeological parameters*: The model incorporates essential hydrogeological parameters hydraulic conductivity (K) and storativity (S), are integrated into the model in accordance

with previously established research findings. These parameters have been rigorously delineated and extensively documented in recent research conducted by P.Q. Nhan, et al. (2022) [17]. For the Holocene aquifer, hydraulic conductivity values range from a minimum of 0.02 m/day to a maximum of 158 m/day, with an average of 26.1 m/day. In the case of the Pleistocene aquifer, these values range from a minimum of 0.5 m/day to a maximum of 184 m/day, with an average of 34.75 m/day (Fig. 2).

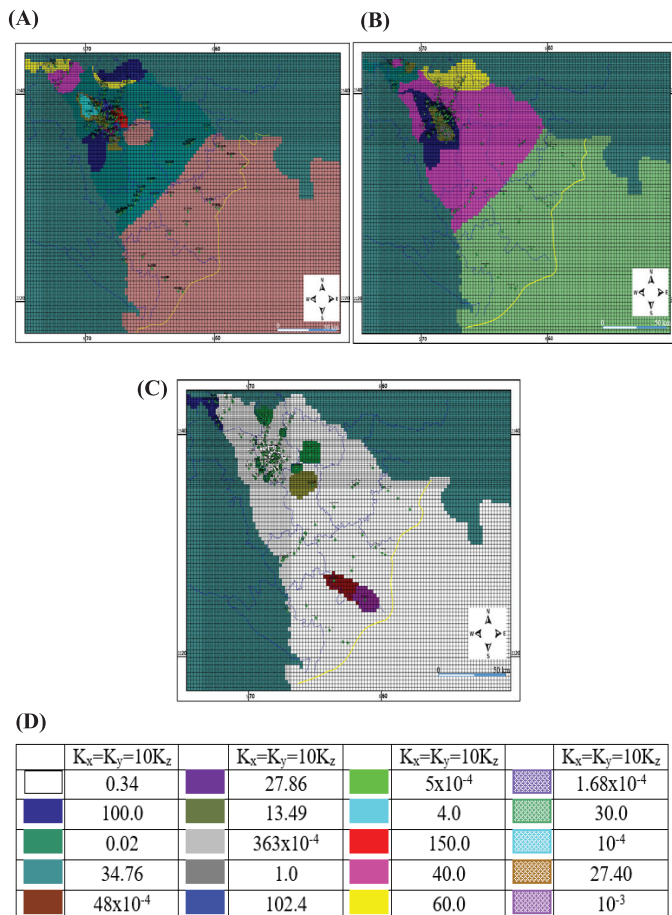


Fig. 2. Hydrogeological parameters. K_x , K_y , and K_z denote the hydraulic conductivity in the x, y, and z dimensions, respectively, for both the Holocene (A) and Pleistocene (B) aquifers. Additionally, specific storativity (S_s), specific yield (S_y), effective porosity (Eff Por), and total porosity (Tot Por) are defined for the Pleistocene aquifer (C). Notably, the storativity coefficient for the Holocene aquifer is explicitly set at 0.01. The hydraulic conductivities K_x , K_y and its colour of Holocene and Pleistocene aquifers (D).

- **Groundwater recharge:** The data on groundwater recharge that was integrated into the model stems from the recent research conducted by P.Q. Nhan, et al. (2022) [17] (Fig. 3). This encompasses a comprehensive methodology that utilises remote sensing for interpreting conditions conducive to groundwater recharge, coupled with an overlay analysis conducted via GIS techniques. To refine this approach, it is crucial to accurately determine the relative significance of various contributing factors

through the implementation of the analytical hierarchical process (AHP). Additionally, the application of the direct measurement method employing the radioactive isotope ^3H has proven to be exceedingly effective for verification purposes.

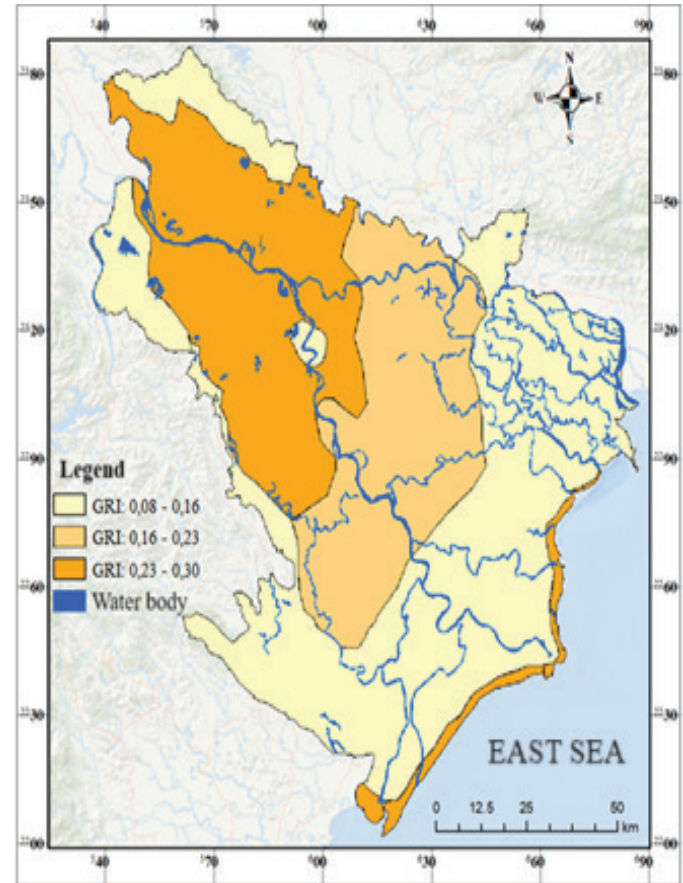


Fig. 3. Zonation of direct groundwater recharge within the Red river delta plain. The map is characterised by the GRI index, delineated into three ranges: 0.08-0.16, 0.16-0.23, and 0.23-0.30. These intervals correspond to direct groundwater recharge rates of 188, 372, and 429 mm/year, respectively [17].

- **Boundary conditions:** The boundary conditions for the model, as delineated in Section 3.1, are based on up-to-date scholarly findings. For the simulation of the Red-Thai Binh river systems, the general head boundary (GHB) method is adopted, utilising river bed conductance figures from P.Q. Nhan (2000) [11] and D.H. Trieu (2022) [16]. The evaporation boundary conditions are defined by averaging meteorological data collected over thirty years. Inputs concerning groundwater recharge from bedrock formations are derived from the findings of D.H. Trieu (2022) [16]. The seaward boundaries follow the hypotheses of V.H. Hoan, et al. (2022) [22], which postulate the extension of freshwater into the ocean as influenced by topographic elevation and geological bed characteristics. This assumption establishes constant head (CH) conditions along the model's boundaries, with head values informed by offshore freshwater equipotential levels (Fig. 4).

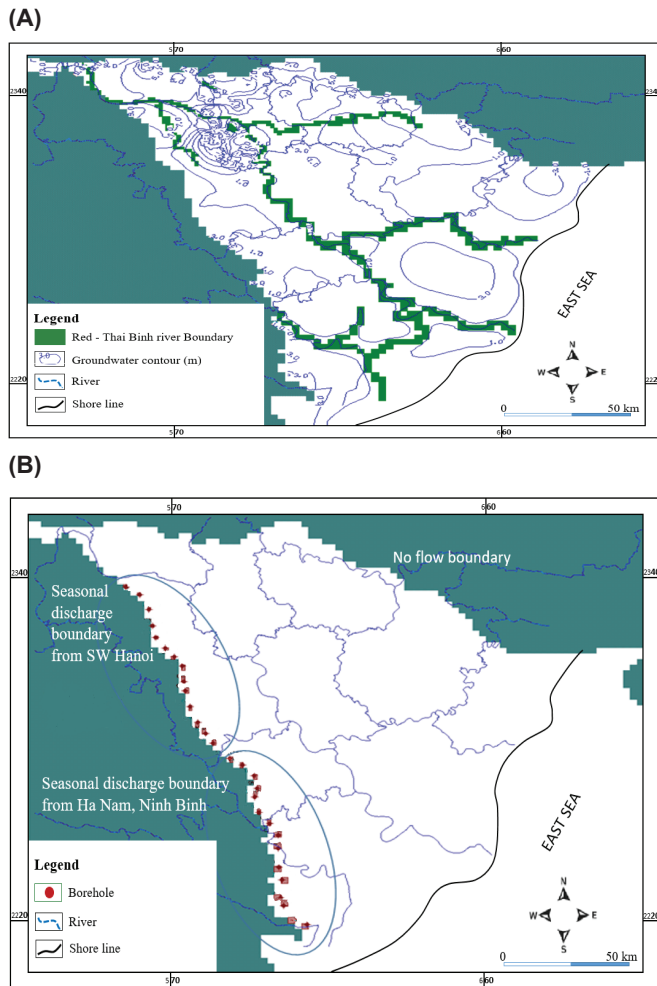


Fig. 4. Boundary conditions of the model. (A) The simulation of the Red - Thai Binh river systems incorporating the general head boundary, with equipotential head denoted by blue lines; (B) A no-flow boundary established in the Northeast, while a seasonal discharge boundary (time independent discharge) is implemented southwest of the plain.

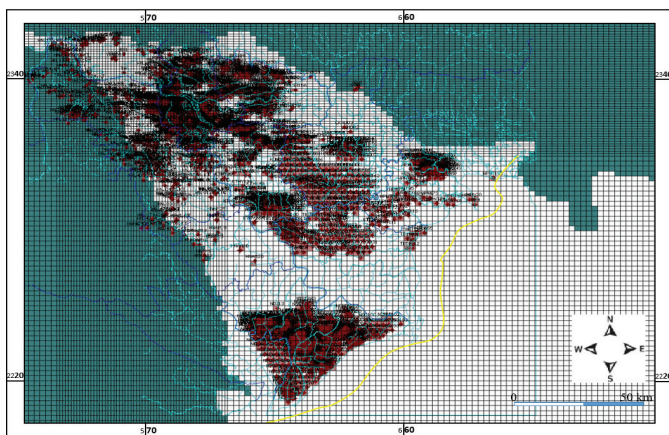


Fig. 5. The spatial arrangement of groundwater extraction sites within the Red river delta plain. The presence of extraction wells are denoted by red dots.

Table 1. Groundwater extraction across the entire Red river delta plain [18, 20].

No.	Province/city	Extraction (m ³ /day)		
		Industrial wells	Domestic wells	Total
1	Hanoi	670931	124180	795111
2	Vinh Phuc	25900	52361	78261
3	Hung Yen	114490	7800	122290
4	Bac Ninh	42000	55118	97118
5	Hai Duong	18200	64259	82459
6	Ha Nam	5508	24492	30000
7	Hai Phong	-	-	34000
8	Thai Binh	9546	39454	49000
9	Nam Dinh	-	-	120000

The current status of groundwater extraction in the RRDP over the period from 2005 to 2018 is illustrated in Fig. 5. The details of the cumulative groundwater extraction throughout the entire RRDP are provided in Table 1.

Thus, the total amount of groundwater exploited throughout the delta is approximately 1,500,000 m³/day.

4. Results and discussion

4.1. Model calibration

Within the designated study area, a three-dimensional model based on GMS software has been developed and subsequently calibrated against observed groundwater levels within the monitoring network. Calibration, particularly aimed at resolving any model instability, utilised data from both local and national monitoring wells spanning from January 1996 to January 2018. The national monitoring network utilises a borehole system within the aquifers as a benchmark for water level comparison in the model. The calibration data set encompasses 128 observation boreholes, with 88 situated in the Pleistocene aquifer and 40 in the Holocene aquifer. The monitoring data are systematically gathered and maintained within a database by the National Centre for Water Resources Planning and Investigation [23]. A comparative evaluation of water levels over time, recorded at the monitoring boreholes within both the Holocene and Pleistocene (Figs. 6-8) aquifers, is detailed below. This comparison clearly delineates the variations between the observed and modelled groundwater levels.

The comparison between observed and modelled groundwater levels at the monitoring boreholes throughout the RRDP is illustrated in Fig. 9.

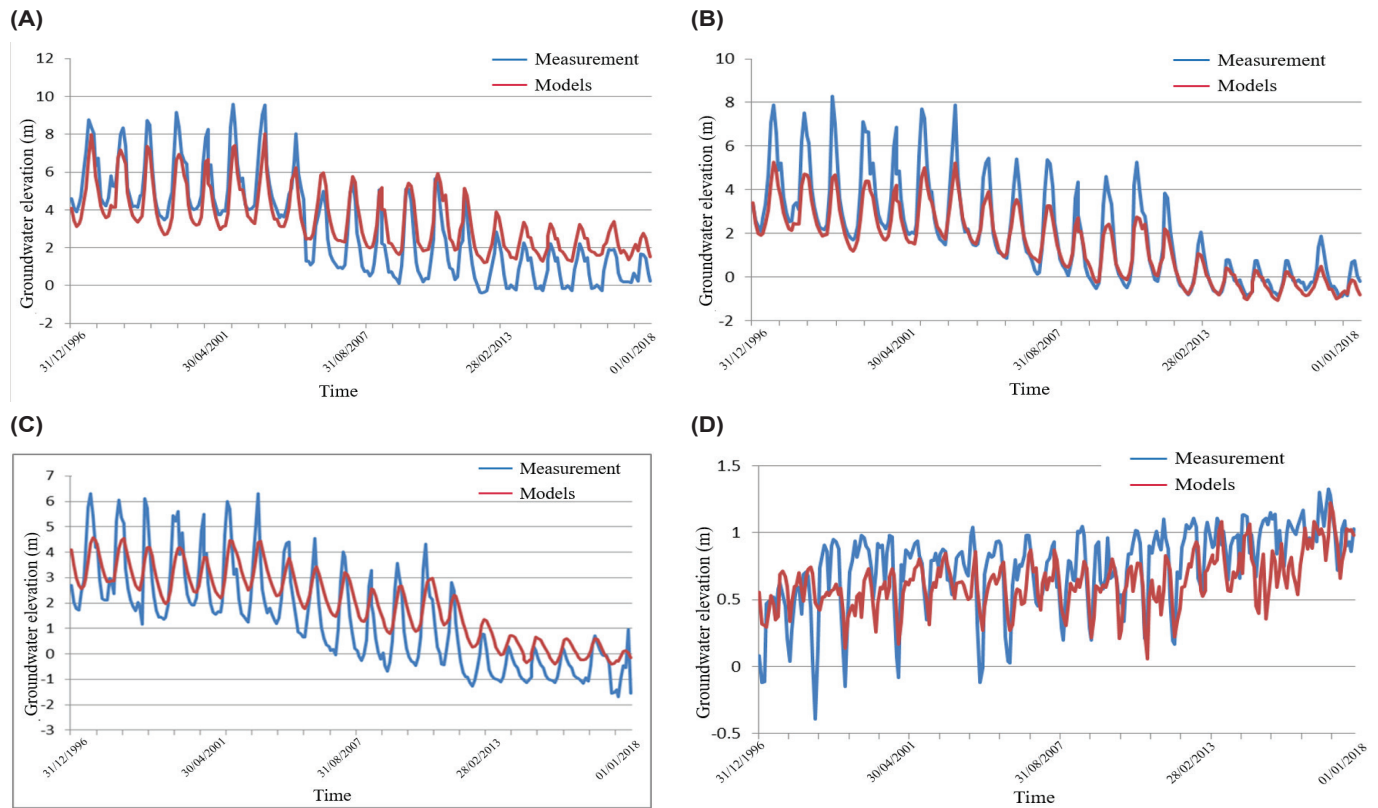


Fig. 6. Discrepancy analysis between modelled and observed groundwater levels at Holocene aquifer monitoring boreholes: (A) Q.33, (B) Q.76, (C) Q.77, and (D) Q.108.

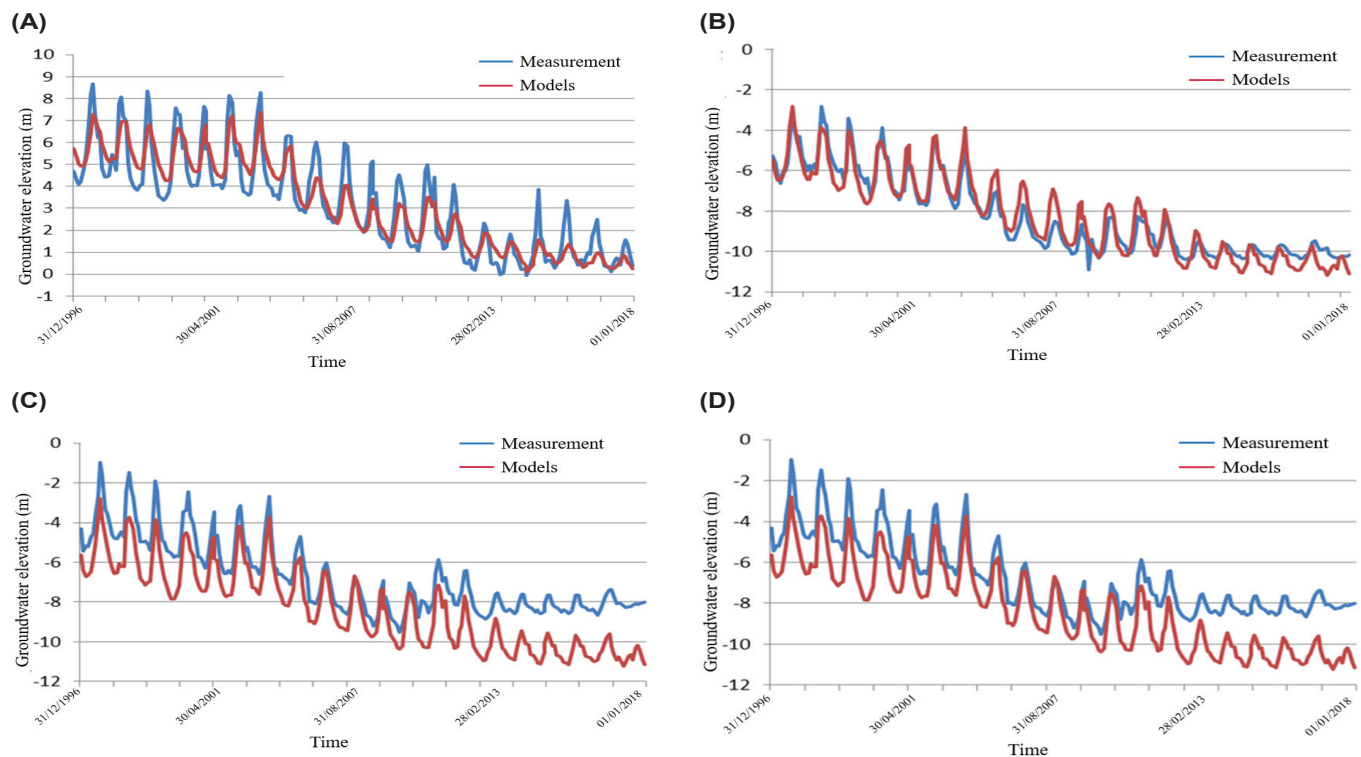


Fig. 7. Discrepancy analysis between modelled and observed groundwater levels at Pleistocene aquifer monitoring boreholes: (A) Q.23, (B) Q.32, (C) Q.35, and (D) Q.77.

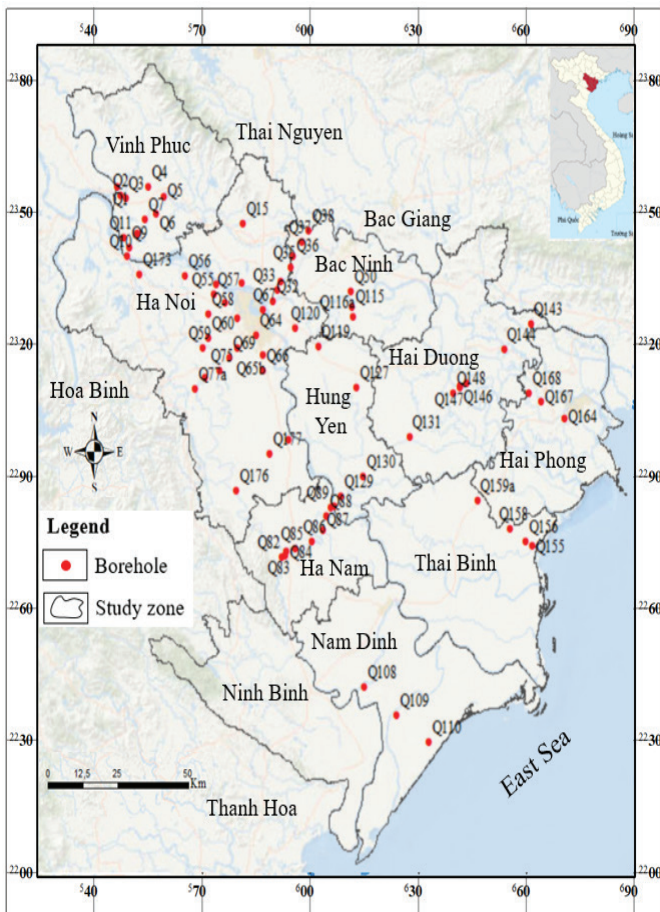


Fig. 8. Location of monitoring boreholes used for comparison of modelled and observed groundwater levels.

The error evaluation for the Holocene aquifer has identified a maximum discrepancy of 1.4 m at monitoring borehole Q.64, and conversely, a negligible error at monitoring borehole Q.85. The mean error across the dataset is calculated to be 0.197 m, with a mean absolute error of 0.861 m, and a normalised root mean square (NRMS) error stands at 5.9%. For the Pleistocene aquifer, error margins at monitoring sites peak at 1.96 m at borehole P.73a, and at borehole Q.164a, the error is again recorded at 0.0 m. The resultant average error is 0.067 m, with a mean absolute error of 0.906 m, and an NRMS error computed to be 2.621%.

The majority of data points are captured within the 95% confidence interval, suggesting a high level of precision. Despite some data points showing significant absolute errors, the relative errors are maintained within acceptable bounds, affirming adherence to prescribed error margins. It is observed that these points are notably situated near to extraction well fields, where modelled data and actual observations often show considerable variances. Thus, the scrutiny of model errors validates that the parameters align with the defined standards of reliability. When contrasted with preceding models for the RRDP [11], the current model exhibits enhanced reliability.

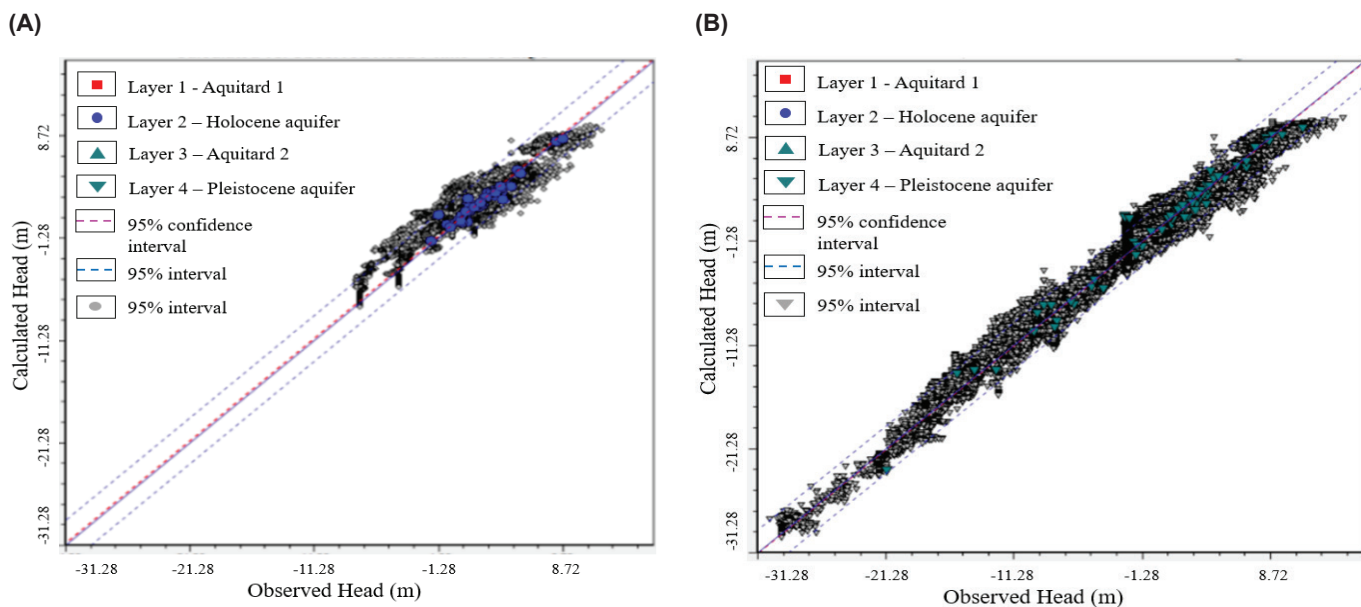


Fig. 9. Comparison of observed and modelled groundwater levels at monitoring boreholes in Holocene aquifer qh (A) and Pleistocene aquifer qp (B) throughout the entire the Red river delta plain. The red dashed line represents the zero-error value, while the green dashed line delineates the 95% confidence interval around its values.

4.2. Evaluating the role of groundwater recharge on groundwater exploitation in the Red river delta plain

Post-calibration, the BUDGET package was utilised to meticulously calculate the water balance for both the Holocene and Pleistocene aquifer systems across the RRDP. Through detailed examination of the water balance components, our objective is to thoroughly evaluate the impact of groundwater recharge on regional groundwater exploitation. The water budget figures, analysed via the ZONE BUDGET, are detailed for the qh and qp aquifer systems for the months of March, June, September, and December (Table 2, Fig. 10). It is important to note that the period from October to March is characterised as the dry season, whilst April to September marks the rainy season.

Table 2. Temporal distribution of water balance components (%) in quaternary aquifers within the Red river delta plain, indicating inflow and outflow with positive and negative signs, respectively.

	Storage	Sea	Exploitation	Direct recharge	Evaporation	River	Leakage	Fractured aquifers in the plain's edge
March	13.94	-2.47	-29.86	39.47	-21.32	-0.48	0.00	0.71
June	-29.32	-1.52	-18.38	67.63	-28.34	9.51	0.00	0.43
September	-53.56	-1.25	-13.91	66.67	-15.63	17.36	0.00	0.32
December	59.74	-1.86	-22.67	9.75	-33.24	-12.25	0.00	0.53

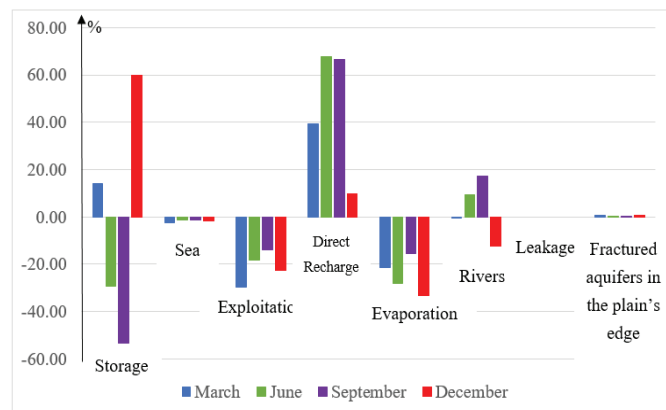


Fig. 10. Temporal representation of water balance components in quaternary aquifers within the Red river delta plain, illustrating inflow and outflow with positive and negative signs.

Figure 10 illustrates the constituents contributing to the groundwater balance within the quaternary aquifer system. Positive values signify inflows contributing to the aquifer system, whereas negative values denote outflows. This analysis specifically addresses the impact of groundwater recharge sources on the quaternary aquifer system within the RRDP. Based on the aforementioned results, several key observations can be stated as follows:

- Throughout the year, the direct groundwater recharge into the quaternary aquifer system shows considerable seasonal variation. The highest recharge rates, constituting 67.63% (equivalent to 5,607,868 m³/day), occur during the rainy season, which significantly decreases to about 9.75% (equivalent to 664,326 m³/day) in the dry season. A contrast between direct groundwater recharge and total groundwater extraction within the study area indicates a stark imbalance during the dry season, where extraction exceeds recharge. This imbalance suggests a potential for depletion of groundwater storage, which may lead to a drop in groundwater levels, especially in zones remote from river courses. The presence of depression cones throughout the study area corroborates this situation.

- The combined effects of aquifer system storage changes and ground evaporation persist year-round, collectively accounting for 13.94 to 59.74% of the total, which translates to a volume ranging from 705,693 to 4,085,660 m³/day.

- Recharge from river systems into the quaternary aquifer is most pronounced during the rainy season, ranging from 9.51 to 17.36% of the total recharge, equating to a volume between 788,378 and 1,928,548 m³/day.

- Recharge from bedrock boundaries at the delta's margins into the Quaternary aquifer system and the groundwater outflow to the sea from this system are consistent throughout the year, albeit in relatively minor volumes.

5. Conclusions

- Evaluating groundwater recharge sources is imperative for managing sustainable groundwater exploitation effectively. The recent quantitative research on groundwater recharge sources in the RRDP regions provides essential data, enabling precise impact assessment on a delta-wide scale through groundwater modelling.

- The Quaternary aquifer system's recharge in the RRDP primarily comes from direct groundwater recharge, such as precipitation, irrigation return flow, and wastewater. Additionally, water from fractured aquifers at the edge of the plain and river inflow into the aquifers also contribute significantly.

- There is temporal and spatial variability in the groundwater recharge sources for the quaternary sedimentary aquifer system within the RRDP. Direct recharge fluctuates by 4,943,542 m³/day across March, June, September, and December. Recharge from the fractured aquifers at the plain's edge alters by 35,886 m³/day, and the variance in river-sourced recharge is about 1,140,169 m³/day during these periods.

- Groundwater extraction in the RRDP is relatively constant throughout the year [19]. In the dry season (October to March), extraction primarily depends on aquifer storage, direct groundwater recharge, and fractured aquifers at the plain's edge. During the rainy season (April to September), extraction is influenced by direct recharge, followed by river water, with additional contributions from fractured aquifers at the plain's edge.

CRediT author statement

Viet Hung Le: Methodology, Data analysis, Validation, Writing; Quy Nhan Pham, Thi Linh Phung, Van Canh Doan: Methodology, Data analysis, Writing; Quoc Cuong Tran: Methodology, Validation, Reviewing, Editing; Tran Trung Dang: Methodology, Validation.

ACKNOWLEDGEMENTS

This study is part of the research activities conducted with the OKP project, hosted by Delft University of Technology (TU Delft), the Netherlands, and Hanoi University of Natural Resources and Environment (HUNRE). The authors express appreciation to Mr. Peter Nelemans from Delft University of Technology for his insightful discussions on this study.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] J.J.D. Vries, I. Simmers (2022), "Groundwater recharge: An overview of processes and challenges", *Hydrogeology Journal*, **10**, pp.5-17, DOI: 10.1007/s10040-001-0171-7.
- [2] A. Hartmann, T. Gleeson, R. Rosolem, et al. (2015), "A large-scale simulation model to assess karstic groundwater recharge over Europe and the Mediterranean", *Geoscientific Model Development*, **8**(6), pp.1729-1746, DOI: 10.5194/gmd-8-1729-2015.
- [3] A. Hartmann, T. Gleeson, Y. Wada, et al. (2017), "Enhanced groundwater recharge rates and altered recharge sensitivity to climate variability through subsurface heterogeneity", *Proceedings of The National Academy of Sciences of The United States of America*, **114**(11), pp.2842-2847, DOI: 10.1073/pnas.1614941114.
- [4] D.N. Lerner, A.S. Issar, I. Simmers (1992), "Groundwater recharge: A guide to understanding and estimating natural recharge (Volume 8, International Contributions to Hydrogeology)", *Journal of Environmental Quality*, **21**(3), pp.307-514, DOI: 10.2134/jeq1992.00472425002100030036x.
- [5] B.R. Scanlon, R.W. Healy, P.G. Cook, et al. (2002), "Choosing appropriate techniques for quantifying groundwater recharge", *Hydrogeology Journal*, **10**, pp.18-39, DOI 10.1007/s10040-0010176-2.
- [6] J.G. Arnold, R.S. Muttiah, R. Srinivasan, et al. (2000), "Regional estimation of base flow and groundwater recharge in the Upper Mississippi river basin", *J. Hydrol.*, **227**(1-4), pp.21-40, DOI: 10.1016/S0022-1694(99)00139-0.
- [7] R. Hirata, B.P. Conicelli (2012), "Groundwater resources in Brazil: A review of possible impacts caused by climate change", *An Acad. Bras. Cienc.*, **84**(2), pp.297-312, DOI: 10.1590/S0001-37652012005000037.
- [8] C.R. Hearne, G.D. Peterson, E.M. Bennett (2010), "Ecosystem service bundles for analyzing tradeoffs in diverse landscapes", *Proceedings of The National Academy of Sciences of The United States of America*, **107**(11), pp.5242-5247, DOI: 10.1073/pnas.090728410.
- [9] C.W Fetter (1994), *Applied Hydrogeology*, Prentice-Hall, EUA, 598pp.
- [10] P.Q. Nhan, T.T. Dang, T.L. Tran (2019), *Sustainable Groundwater Development in Hanoi City*, Science and Technics Publishing House, ISBN 978-604-67-1284-8, 219pp (in Vietnamese).
- [11] P.Q. Nhan (2000), *Groundwater Reserves in Red River Delta Plain and Its Sustainable Development*, PhD Thesis, Hanoi University of Mining and Geology (in Vietnamese).
- [12] T.L. Tran (2011), *Estimation of Groundwater Recharge and Hydraulic Interaction Between Quaternary Aquifer in Thach That - Dan Phuong, Hanoi by Using Isotopes*, MSc Thesis, Hanoi University of Mining and Geology (in Vietnamese).
- [13] D. Postma, T.H.M. Nguyen, M.L. Vi, et al. (2017), "Fate of arsenic during Red river water infiltration into aquifers beneath Hanoi, Vietnam", *Environmental Science and Technology*, **51**(2), pp.838-845, DOI: 10.1021/acs.est.6b05065.
- [14] F. Larsen, N.Q. Pham N.D. Dang, et al. (2008), "Controlling geological and hydrogeological processes in an arsenic contaminated aquifer on the Red River flood plain, Vietnam", *Appl. Geochem.*, **23**(11), pp.3099-3115, DOI: 10.1016/j.apgeochem.2008.06.014.
- [15] V.C. Doan (2015), *Research and Propose Criteria and Zoning for Sustainable Exploitation and Protection of Groundwater Resources in The Northern Deltas and Southern Deltas*, Summary report on state-level research, Code KC.08.06/11-15, Hanoi, p.281 (in Vietnamese).
- [16] D.H. Trieu (2022), *Determining The Role of The Red River and Bedrock in Groundwater Recharge in Quaternary Sediments in The Southwest, Hanoi*, PhD Thesis, Hanoi University of Mining and Geology (in Vietnamese).
- [17] P.Q. Nhan, L.V. Hung, T.T. Le, et al. (2022), "Zoning groundwater potential recharge using remote sensing and GIS technique in the Red river delta plain", *IOP Conf. Ser.: Earth Environ. Sci.*, **964**, DOI: 10.1088/1755-1315/964/1/012025.
- [18] Department of Water Resources Management, Vietnam (2020), *Report on Water Resources Planning for Red - Thai Binh Basin in The Period 2022 -2030 with Vision to 2050*, Archives of Ministry of Natural Resources and Environment (in Vietnamese).
- [19] V.T. Tam, T.T.V. Nga (2018), "Assessment of urbanization impact on groundwater resources in Hanoi, Vietnam", *Journal of Environmental Management*, **227**, pp.107-116, DOI: 10.1016/j.jenvman.2018.08.087.
- [20] Department of Water Resources Management, Vietnam (2022), *Synthetic Report on National Master Plan for Water Resources in the Period 2022-2030 with a Vision to 2050*, Approved Version, Archives of Department of Water Resources Management (in Vietnamese).
- [21] Aquaveo (2018), *GMS - Groundwater Modeling System v10.8*, <https://www.aquaveo.com/software/gms-groundwater-modeling-system-introduction>, accessed 1 October 2023.
- [22] V.H. Hoan, F. Larsen, Q.N. Pham, et al. (2022), "Recharge mechanism and salinization processes in coastal aquifers in Nam Dinh province, Vietnam", *Vietnam Journal of Earth Sciences*, **44**(2), pp.213-238 (in Vietnamese).
- [23] National Center for Water Resources Planning and Investigation (2022), *Data Base for National Water Resources Monitoring System. Hanoi, Vietnam*, <http://dwrm.gov.vn/index.php?language=vi&nv=laws&op=Ban-tin-thong-bao-du-bao-va-canh-bao-tai-nguyen-nuoc-duoi-dat>, accessed 1 October 2023 (in Vietnamese).

General Instructions on using this template and submitting a manuscript to VJSTE: Thank you for preparing a manuscript for submission to VJSTE. Using this template, or following the guidelines below, will help us in processing your article.

The structure of different types of articles may vary according to the type of study, and the content of the article.

The following are the detailed main components of the original research and review articles submitted to the journal.

From 2023 onwards, you can submit your article at: <https://mc04.manuscriptcentral.com/mostvjste>

Original research

The original research should have three following parts:

Preliminary section: It includes title, list of all contributing authors with their institutional affiliation, and abstract (160-250 words) with at least 3 keywords.

Title: How to format a science article (replace with your real title)

Author(s): List the author name(s) separated by commas. Use superscript numbers to link affiliations, and symbols *†‡ for author notes. For example, Y. Obsie^{1*}, S.A. Adem^{1,2}

Corresponding author(s): Include the email addresses or telephone numbers of the corresponding author(s). Please use the asterisk () symbol for the corresponding author information. This information is at the bottom of the manuscript's first page.

Author's affiliation addresses:

¹Affiliations should be preceded by superscript numbers corresponding to the author list

²For any institutions, please provide the institution name, street, district, city, country

Received ; revised ; accepted

Abstract:

Start your abstract here...

Keywords: keyword 1, keyword 2, keyword 3...

Classification number(s): 1.1, 3.4, 5.3, etc.

Abstract: The abstract should range between 160 and 250 words and be structured in a single paragraph following keywords in a separate line. The abstract should represent the main part of the research manuscript. It should reflect a brief and comprehensive background following the aim of the study (main questions to be addressed), methods used for investigating the problem, a summary of significant findings, main conclusions, and implications of the results.

Keywords: They should come immediately after the abstract and contain 3-7 specific and relevant keywords. They should reflect the research study and the relevant field of research.

Classification numbers: Depend on the Journal's Classification Scheme.

Author(s) are encouraged to write a "Highlights" that summarises the most exciting, interesting points from your manuscript.

Manuscript section: It is the main body of the scientific manuscript including: 1) Introduction; 2) Material(s) and method(s); 3) Results and discussion (separation of these two sections is acceptable); 4) Conclusions; 5) Recommendations/Suggestions/ Implications/Study limitations...(if any). These sections may slightly vary depending on the type of research approach and design used in the research. Each section should be numbered.

All of the Figures, Tables, and Equations should be cited in numerical order.

Figures

Fig. #: (Begin each figure caption with a label, "Fig. 1." for example, as a new paragraph).

Fig. 1. The figure caption should begin with an overall descriptive statement of the figure followed by additional text. They should be immediately after each figure. Figure parts are indicated with capital letters (A). If you prefer, you can place both the actual figures and captions logically through the text near where they are cited rather than at the end of the file (but not both). If a paragraph in the main text begins with the name of a figure, write out "Figure" in full (e.g., <para> "Figure 1 shows...").

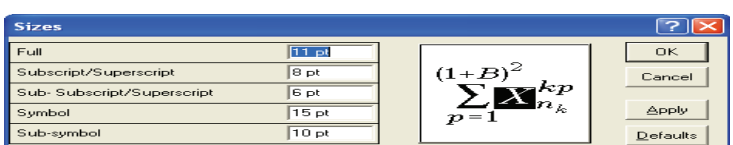


Fig. 1. The figure caption.

Tables

Table #: (Begin each table caption with a label "Table 1.", etc. as a new paragraph).

Table 1. Start this caption with a short description of your table. Format tables

using the Word Table commands and structures. Do not create tables using spaces or tabs characters.

Table 1. Caption.

a	b	c	d	e
f				
g				
h				

Equations

$$\int_a^b x dx \tag{1}$$

Equations can be included. We do not recommend using the native Word 2007, 2008, 2010 or 2011 equation editor. This can in some cases produce less reliable MathML, the online markup language we use, which may result in display errors. Instead, use the legacy equation editor in Word (Insert menu; select insert object; select word equation) or use MathType (recommended). If you enter equations in simple LaTeX, check that they will convert accurately (Word 2007 and higher can convert simple LaTeX equations).

Closing section: It includes appendices/supplementary materials/data availability statement (if applicable), CRediT author statement (for multiple authors), acknowledgements (if applicable), competing interests, ethics (if any) and a complete list of references corresponding to all the citations given in the text of the manuscript. The appendices/supplementary materials may include data files and other materials relevant to the research that should be submitted in separate files if required.

APPENDICES (if any)

The appendices should ideally be under 1000 words (approximately 2 A4 pages) for printing version. With longer appendices, they are only accepted for online publication.

CRediT author statement (for multiple authors)

CRediT offers authors the opportunity to share an accurate and detailed description of their diverse contributions to the published work.

ACKNOWLEDGEMENTS (if applicable)

This section is for the researchers who were funded for the research projects, and it should enclose all sources of financial support.

COMPETING INTERESTS

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

REFERENCES

For in-text citation: References should be cited in the bracket with a number [1]. Multiple reference citations are separated by commas [2, 3] or if a series, dashes [4-6]. When citing sources with the author(s) and the year, they should be shortened to the first author's name, followed by et al. (with more than one author) and the year.

For reference list: With less than or equal to three authors, list all the author(s). With more than three authors, the first three should be listed and followed by et al.

References are listed in numerical order, and in the same order in which they are cited in text. The reference list should include all and only those references you have cited in the text. The reference list appears at the end of the article.

The reference consists of: Initial(s) and first/last name of the author(s), author' names separated by commas (Year of publication), "Titles of journal articles or chapter of book", Title of Journal, Book or Other Publication, Volume(Number) (if any), page numbers (if any), DOI (if any), URL and accessed date (if any) (in its original language if not English).

To cite a website and online/electronic resources, please include the accessed date (day month year).

Example of a reference list:

[1] T. Binzoni, T.S. Leung, D. Boggett, et al. (2003), "Non-invasive laser Doppler perfusion measurements of large tissue volumes and human skeletal muscle blood RMS velocity", *Phys. Med. Biol.*, **48**(1), pp.2527-2549, DOI: 10.1088/0031-9155-48-15-318.
[2] H.W. Bischoff, H.C. Bold (1963), *Some Soil Algae from Enchanted Rock and Related Algal Species*, University of Texas Publications, 95pp.
[3] R. Busi (2016), "Weedy rice in the Philippines and Vietnam", <https://www.aciar.gov.au/publication/technical-publications/weedy-rice-philippines-and-vietnam-final-report>, accessed 15 July 2018.

Review articles

They should be different from the original research as they would contain different sections on the literature review. Authors may structure this part as per nature, background, and available literature on the issue argued in the manuscript.

For more detailed instructions, please visit our website: vietnamscience.vn or vietnamscience.vjst.vn

This manuscript template has been used from 2021 onwards.

EDITORIAL BOARD

Editorial Board

Chairman

Van Minh Chau (*Vietnam Academy of Science and Technology, Vietnam*)

Standing Members

Bao Chau Ngo (*The University of Chicago, USA*)

Gia Khanh Pham (*Vietnam Society of Organ Transplantation, Vietnam*)

Manh Huong Phan (*University of South Florida, USA*)

Sang-Wook Cheong (*Rutgers University, USA*)

Thuc Tran (*Vietnam Panel on Climate Change, Vietnam*)

Members

Chart Chiemchaisri (*Faculty of Engineering, Kasetsart University, Bangkok, Thailand*)

Cheorl-Ho Kim (*College of Science, Sungkyunkwan University, South Korea*)

Chi Buu Bui (*Vietnam Academy of Agricultural Sciences, Vietnam*)

Clark Gedney (*Bio Media Center for Instructional Design, Biological Sciences, Purdue University, USA*)

Dang Xuan Tran (*Hiroshima University, Japan*)

Dindo Campilan (*International Center for Tropical Agriculture (CIAT) - Asia Regional Office, Philippines*)

Dinh Duc Nguyen (*University of Engineering and Technology (UET) - Vietnam National University, Hanoi, Vietnam*)

Edgar Andreas Bohner (*VTT Technical Research Centre of Finland, Finland*)

Franz Nestmann (*Institute for Water and River Basin Management, Karlsruhe Institute of Technology, Germany*)

Gopalakrishnakone P (*National University Health System, Singapore*)

Hung Viet Pham (*University of Science, Vietnam National University, Hanoi, Vietnam*)

John Nieber (*University of Minnesota, USA*)

Phuc Phung-Van (*Ho Chi Minh City University of Technology, Vietnam National University, Ho Chi Minh City, Vietnam*)

Pierre Darriulat (*European Organization for Nuclear Research; Vietnam Academy of Science and Technology, Vietnam*)

Son Phan Lam Tran (*Institute of Genomics for Crop Abiotic Stress Tolerance, USA*)

Stefan Norra (*Institute of Applied Geosciences, Karlsruhe Institute of Technology, Germany*)

Thanh Hai Tran (*Hanoi University of Mining and Geology, Vietnam*)

Tjandra Setiadi (*Bandung Institute of Technology, Indonesia*)

Tomoaki Itayama (*Nagasaki University, Japan*)

Tri Nang Tran (*University of Minnesota, USA*)

Truong Duc Pham (*University of Birmingham, UK*)

Tu Bao Ho (*John Von Neumann Institute, Vietnam National University Ho Chi Minh City, Vietnam*)

Van Suong Hoa (*Concordia University, Canada*)

Van Thang Nguyen (*Vietnam Institute of Meteorology, Hydrology and Climate change, Ministry of Natural Resources and Environment, Vietnam*)

Van Toi Vo (*Tufts University, Massachusetts, USA; Vietnam National University Ho Chi Minh City, Vietnam*)

Editor-in-Chief

Nguyen Thi Huong Giang (*Ministry of Science and Technology, Vietnam*)

Deputy Editor-in-Chief

Pham Thi Minh Nguyet (*Ministry of Science and Technology, Vietnam*)

Associate Editors

Bach Tran (*Hanoi Medical University, Vietnam*)

Duc Thanh Dang (*University of South Florida, USA*)

Nguyen Hai Tran (*Duy Tan University, Vietnam*)

Thi-Hiep Nguyen (*International University, Vietnam National University Ho Chi Minh City, Vietnam*)

Thi Thuy Linh Tran (*Duy Tan University, Vietnam*)

Tien Dung Le (*Ho Chi Minh City, Vietnam*)

Van Tuan Tran (*Vietnam National University, Hanoi, Vietnam*)

Xuan Thanh Bui (*Ho Chi Minh City University of Technology, Vietnam*)

Managing Editors

Phi Cong Thuong (*Ministry of Science and Technology, Vietnam*)

Vu Van Hung (*Ministry of Science and Technology, Vietnam*)

Ninh Van Dien (*Ministry of Science and Technology, Vietnam*)

Le Thi Bac (*Ministry of Science and Technology, Vietnam*)

Tang Xuan Binh (*Ministry of Science and Technology, Vietnam*)

Le Thi Tuyet Hanh (*Ministry of Science and Technology, Vietnam*)

Truong Thi Thao Duong (*Ministry of Science and Technology, Vietnam*)

EDITORIAL OFFICE

113 Tran Duy Hung Str., Cau Giay Dist., Hanoi, Vietnam

Tel: (84.24) 39436793; Fax: (84.24) 39436794

Email: vjste@most.gov.vn

Website: vietnamscience.vn or vietnamscience.vjst.vn

PUBLICATION LICENCE

No. 459/GP-BTTTT 20th July 2021

No. 50/GP-BTTTT 1st February 2023