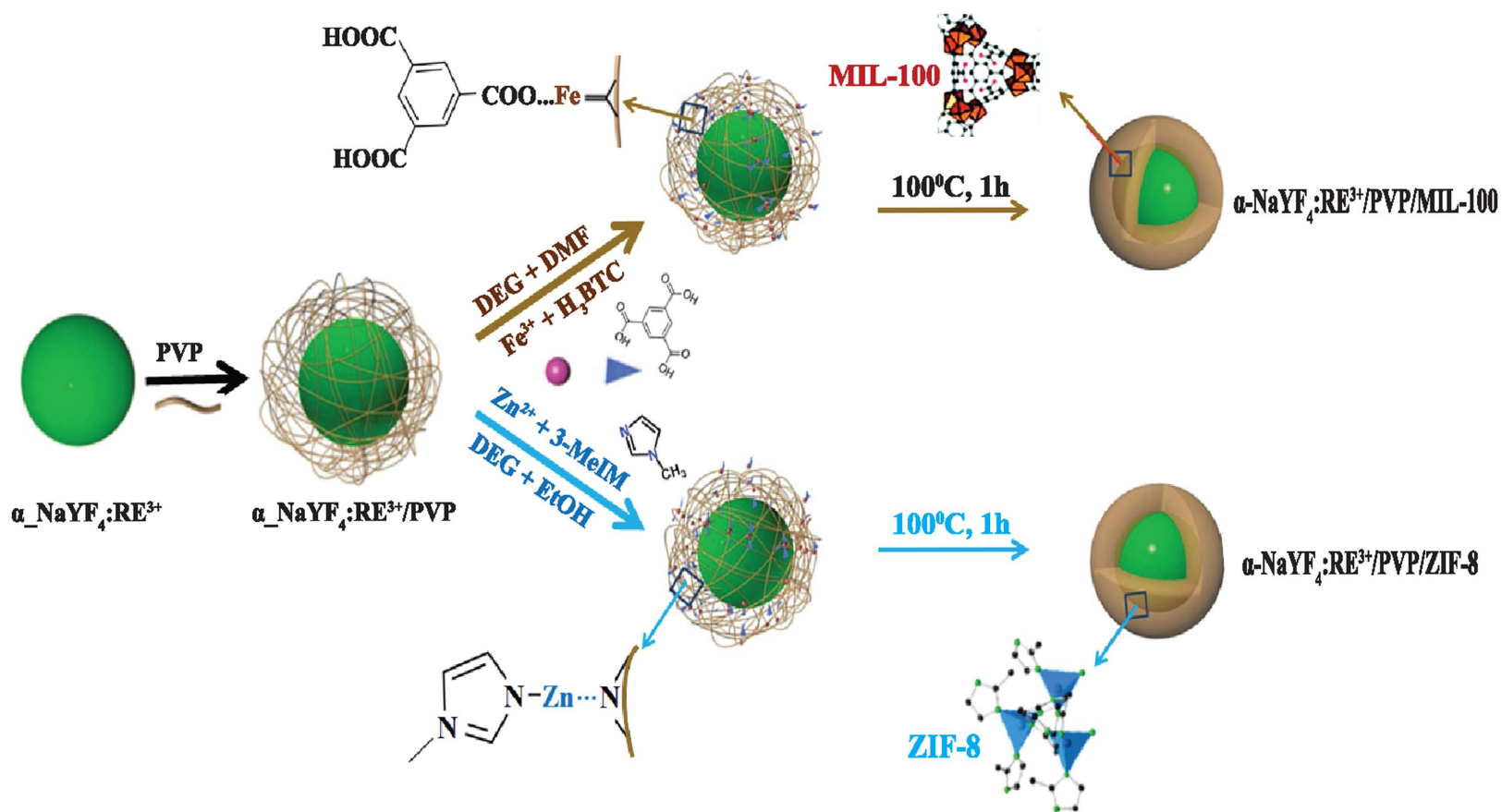




Synthesis, characterization and up-conversion luminescence properties of $\alpha\text{-NaYF}_4:\text{Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{MOFs}$ multilayer nanocrystals

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

4. Geosciences

- 4.1. Geography
- 4.2. Geology
- 4.3. Geophysics
- 4.4. Oceanography

5. Nanoscience and Nanotechnology

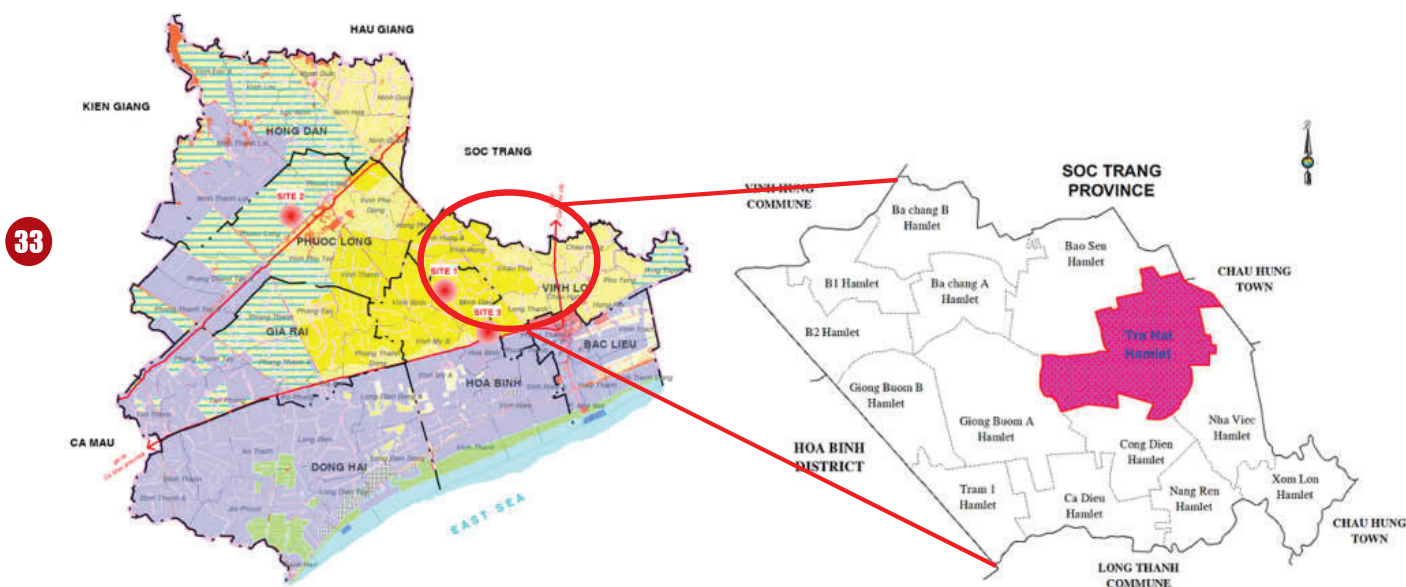
- 5.1. Nanophysics
- 5.2. Nanochemistry
- 5.3. Nanobiotechnology
- 5.4. Nanomedicine
- 5.5. Nanoengineering

6. Environmental Sciences

- 6.1. Ecology
- 6.2. Climatology

CONTENTS

SEPTEMBER 2017 • VOL.59 NUMBER 3



PHYSICAL SCIENCES

- 3** Optimization of congo red removal by adsorption onto $\text{NiFe}_2\text{O}_4/\text{GO}$ nanocomposites.

Van Thuan Tran, Van Thinh Pham, Thi Phuong Quynh Bui, Duy Trinh Nguyen, Long Giang Bach

- 7** Studies on thermo-oxidative degradation of synthetic rubbers 1-4 and 1-2 polybutadienes.

Quoc Hung Nguyen, Van Hai Chu, Ngoc Cuong Hoang, Van Huong Pham

- 14** Comparison of the adsorption of Zn(II) on α - and γ - MnO_2 nanostructures.

Van Phuc Dinh, Ngoc Chung Le, Ngoc Tuan Nguyen, Thien Hoang Ho, Van Dong Nguyen

- 20** The development of the GC-MS analytical method used for the determination of the isotope ratio of linalool in yuzu essential oil from different geographic origins.

Thi Thao Nguyen, Masayoshi Sawamura

- 25** Immobilization of ZnO nanoparticles on fluorinated perlite granules for the photocatalytic degradation of methylene blue.

Nguyet Anh Pham, Thi Huynh Nhu Nguyen, Tuan Ngoc Tran, Quy Tu Nguyen, Tien Khoa Le

LIFE SCIENCES

- 32** Integrating indigenous knowledge for participatory land use planning (PLUP) in Tra Hat hamlet, a climate-smart village in the Mekong River Delta, Vietnam.

Thanh Vu Pham, Hieu Trung

Nguyen, Tuan Huy Vuong, Pham Dang Tri Van, Quang Tri Le

- 39** Genome-wide identification and annotation of the Nuclear factor YA gene family in cassava (*Manihot esculenta* Crantz).

Duc Ha Chu, Thi Thuy Tam Do, Xuan Dac Le, Thi Ly Thu Pham

- 44** Molecular detection and identification of a phytoplasma associated with cinnamon (*Cinnamomum cassia* B.) witches' broom disease in Quang Ngai province, Vietnam.

Thi Nguyen Duong, Thanh Van Dao



CONTENTS

SEPTEMBER 2017 • VOL.59 NUMBER 3

48 Promising rice mutants with high yields, good grain quality, and disease resistance due to irradiation technology.

Duy Duong Tran, Manh Ha Nguyen, Duy Quy Tran, Thi Dung Quan, Hoang Dung Tran

53 Establishment of ultrasensitive PCR assay targeting cell-free EBV DNA for early detection of nasopharyngeal carcinoma.

Huu Tho Ho, Nguyen Quynh Anh Vu, Tram Anh Do, Dinh Ung Nguyen, Thi Thu Hang Dinh, Tien Sy Bui, Dao Chinh Hoang, Minh Ky Le, Truong Phong Vu, Thanh Tung Ngo, Kim Luu Nguyen, Truong Sinh Luu, Van Ba Nguyen, Anh Son Ho, Van Luong Hoang, Duc Thuan Nghiem

60 Neuroprotective effects of *Diospyros kaki* and *Gardenia jasminoides* against ischemic

neuronal injury in mice.

Thi Xoan Le, Thi Nguyet Hang Pham, Van Tai Nguyen, Viet Dung Le

GEOSCIENCES

65 An overview of petroleum potential and investment opportunities in Southeast Asia.

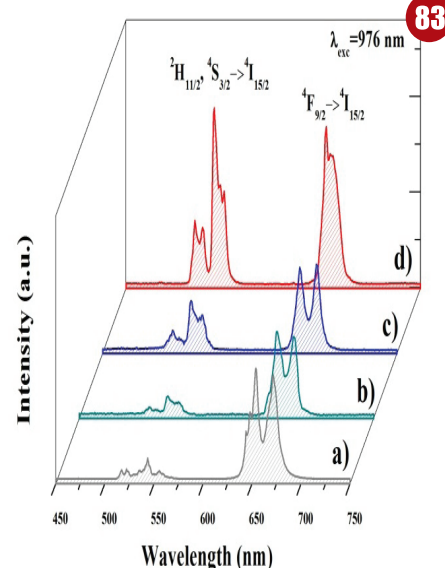
Minh Hoang Cu, Khac Hoan Phung, Hai An Le

71 Assessment of landfill leachate in Nam Son, Hanoi using 3D electrical imaging and self-potential data.

Van Giang Nguyen, Quang Dung Nguyen, Ba Duan Nguyen

NANOSCIENCE AND NANOTECHNOLOGY

79 Synthesis, characterization and up-conversion luminescence properties of α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$



PVP/MOFs multilayer nanocrystals.

Thi Kieu Giang Lam, Lukasz Marciniak, Quang Huy Tran, Manh Tien Dinh, Duc Chinh Vu, Vu Nguyen, Thu Huong Tran, Thi Khuyen Hoang, Thanh Huong Nguyen, Duc Roan Pham, Thanh Binh Nguyen, Quoc Minh Le

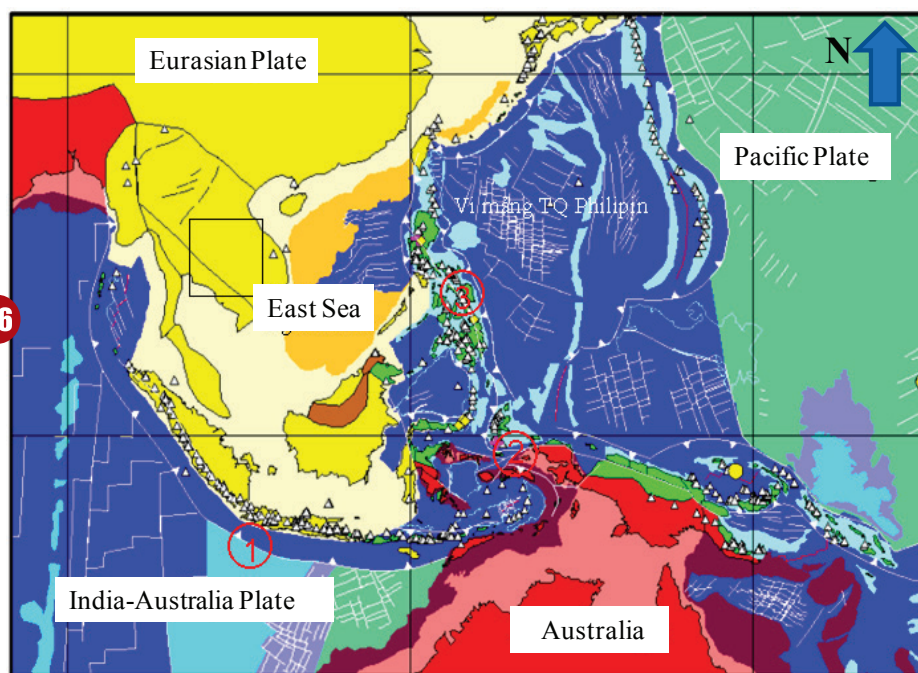
ENVIRONMENTAL SCIENCES

85 Effects of ENSO on the intraseasonal oscillations of sea surface temperature and wind speed along Vietnam's coastal areas.

Quoc Huy Le, Thuc Tran, Xuan Hien Nguyen, Van Uu Dinh

91 Development of a methodological framework for calculation of carbon footprint of rice production in Vietnam.

Minh Trang Dao, Thi Lan Huong Huynh



Optimization of congo red removal by adsorption onto $\text{NiFe}_2\text{O}_4/\text{GO}$ nanocomposite

Van Thuan Tran¹, Van Thinh Pham^{2,3}, Thi Phuong Quynh Bui⁴, Duy Trinh Nguyen¹, Long Giang Bach^{1*}

¹Nguyen Tat Thanh University, Ho Chi Minh city, Vietnam

²Dong Nai Technology University, Dong Nai province, Vietnam

³Graduate University of Science and Technology, Vietnam Academy of Science and Technology

⁴Ho Chi Minh city University of Food Industry, Vietnam

Received 14 June 2017; accepted 11 September 2017

Abstract:

The present study focused on the use of the magnetic nanocomposite $\text{NiFe}_2\text{O}_4/\text{GO}$ (GO - Graphene oxide) as an efficient adsorbent for the removal of congo red (CR). The $\text{NiFe}_2\text{O}_4/\text{GO}$ was synthesized from NiFe_2O_4 and GO via a facile route, and the structure of this nanocomposite was analyzed by X-ray powder diffraction (XRD), scanning electron microscope (SEM), and Vibrating sample magnetometer (VSM). By applying the response surface methodology, the profound relationship was found between variables (initial concentration, adsorbent dosage, and pH) and CR removal efficiency. Moreover, the model was optimized to give a favourable condition for the adsorption. Up to 94.7% of CR removal was obtained via a confirmation test, and this result indicated that the magnetic $\text{NiFe}_2\text{O}_4/\text{GO}$ was a promising material in terms of treatment for CR-contaminated wastewater.

Keywords: congo red, $\text{NiFe}_2\text{O}_4/\text{GO}$, optimization, response surface methodology.

Classification number: 2.2

Introduction

Nowadays, environmental pollution has increasingly become a universal issue as a consequence of the discharge of many hazardous chemicals [1]. Among the pollutants, organic dyes contribute to a large proportion because their contamination in wastewater often originates from fundamental and widespread industries such as textile [2]. Such compounds at high concentrations are considered as toxic sources due to toxicological and aesthetical reasons, and thus they are able to cause carcinogenic effects [3]. Meanwhile, these organic pollutants can accumulate a great amount in the environment over a period of time and result in persistent pollutions because of their low biodegradability [4]. CR is a crucial representative of secondary diazo anionic dyes, which leads to an allergic reaction and will be metabolized to benzidine, a human carcinogen [5]. Therefore, it is strongly expected to develop an eco-friendly method for treatment of CR-bearing wastewater.

Over the past years, some feasible approaches for the removal of dyes have been reported such as various physical, chemical, and biological methods, including adsorption, biosorption coagulation/flocculation, catalytic reduction, electrolytic reduction membrane filtration, and

liquid-liquid extraction [6]. Although the aforementioned methods generally remain their own limitations towards either cost-effectiveness or performance, adsorption is a highly effective separation technique because its advantage is superior to that of other methods in terms of initial cost, simplicity, good operation, and cyclability [7]. There are various kinds of common adsorbents which can be highly compatible with adsorption systems. However, adsorbents need to be reusable towards “green chemistry” trend and can be easily separated from the aqueous solution, so we have developed a class of nickel-based spinel ferrite magnetic material NiFe_2O_4 [8]. To enhance the surface properties by means of adding various functional groups, graphene oxide can be loaded to modify a rigid structure of NiFe_2O_4 and form a nanocomposite $\text{NiFe}_2\text{O}_4/\text{GO}$ [9]. These types of materials can, therefore, be easy to perform magnetic separation and recycling as they hold unique properties of facile synthesis, magnetic recoverability and stability.

The present work aims to show the influential factors including initial CR concentration, the dosage of $\text{NiFe}_2\text{O}_4/\text{GO}$, and pH of the solution on the removal of CR by adsorption onto $\text{NiFe}_2\text{O}_4/\text{GO}$ using the response surface methodology (RSM). The second-order RSM-based polynomial regression model was used

*Corresponding author: Email: blgiang@ntt.edu.vn

to evaluate the mathematical relationship of factors and find the optimum region for removal of dye ions.

Materials and methods

Chemicals and instruments

All chemicals for this study were commercially purchased from Merck. The XRD was implemented on D8 Advance Bruker powder diffractometer with a Cu-K α excitation source. The SEM was recorded with the Japanese instrument S4800 and used an accelerating voltage source of 10 kV with a magnification of 7000. The magnetic properties were determined by VSM.

Production of NiFe₂O₄/GO

NiFe₂O₄ and GO were performed by a two-step procedure as early reported [8, 10]. In the typical experiment, a beaker containing 1.0 g of NiFe₂O₄ 50 ml in 50 ml ethanol and another beaker containing 5 ml of GO colloidal suspension in 45 ml of water were put in an ultrasonic bath for one hour. After heating to 60°C, the mixtures were transferred into a 500 ml beaker and then stirred to vaporize. The solid was dried at 90°C and used for the present study.

Adsorption batch

The NiFe₂O₄/GO (0.16-1.84 g/l) was poured into an Erlenmeyer flask containing 50 ml of CR solution (66.4-133.6 mg/l). After the absorption equilibrium in three hours, the solid was removed from the mixture. The residual concentrations were confirmed by AAS. Twenty experiments were performed by using central composite design (CCD) (Table 1) at five levels including the low (encoded -1), high (encoded +1), and rotatable (encoded ± 1.68).

between graphene oxide and active open-metal sites of the NiFe₂O₄ were formed [8-11]. Moreover, other shape diffraction peaks at 26.4°, 30.4°, 35.8°, 43.4°, 63.0° revealed the crystalline nature which fitted well with spinel structure of ferrites reported by a previous study [9].

Figure 1B showed that the saturation magnetization value was 2.38 emu/g, which was considered to be significantly lower than that of the previous report [8]. Magnetization decrease could be

Table 1. Independent variables matrix and their encoded levels.

No	Independent factors	Code	Levels				
			-1.68	-1	0	+1	+1.68
1	CR concentration (mg/l)	x_1	66.4	80	100	120	133.6
2	NiFe ₂ O ₄ /GO dosage (g/l)	x_2	0.16	0.5	1.0	1.5	1.84
3	pH (-)	x_3	3.6	5	7	9	10.4

Results and discussions

Textural characterization of NiFe₂O₄/GO

It can be seen from Fig. 1A that the typical peak of GO at a low angle of $2\theta = 10.8^\circ$ was absent from the given diagram [11]. This phenomenon can be explained as follows: the graphene oxide was remarkably reduced through the oxidative reaction, where new bonds

attributable to the presence of non-magnetic graphene loaded on the surface of ferrite NiFe₂O₄ in the preparation of NiFe₂O₄/GO [1]. The SEM image at various scales of 500 nm in Fig. 1C revealed the morphological property from NiFe₂O₄/GO. Obviously, the NiFe₂O₄/GO possesses a type of porous and defective surface. The average diameter of nanoparticles was calculated to be from 40 to 50 nm.

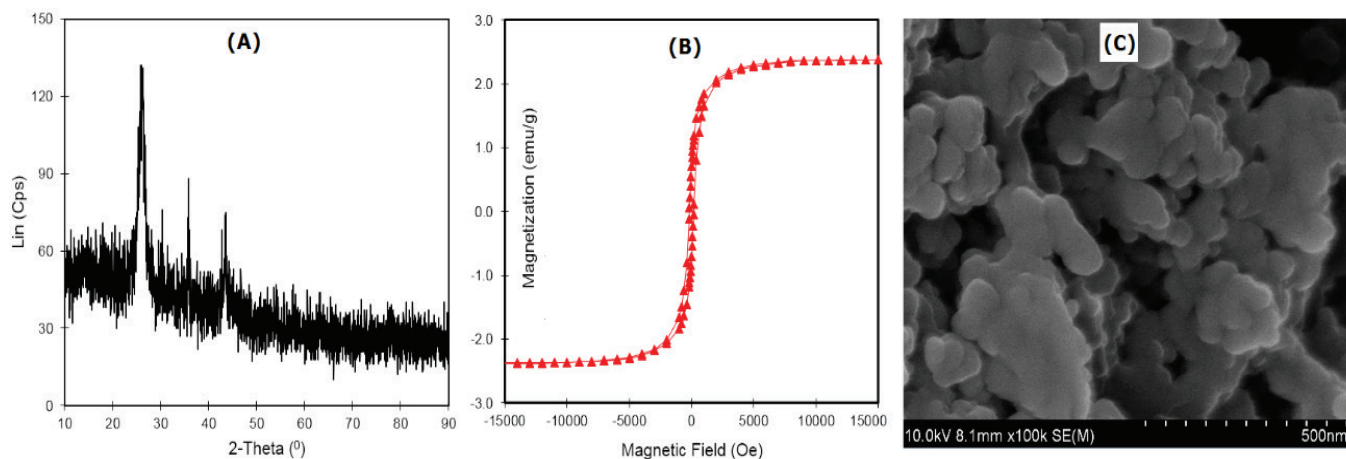


Fig. 1. XRD spectra (A), VSM (B), and SEM image (C) of the NiFe₂O₄/GO.

Table 2. The matrix of observed and predicted values.

No	Variables			Response	
	x_1 (C_i , ppm)	x_2 (dosage, g/l)	x_3 (pH)	Actual (%)	Predicted (%)
1	80	0.5	5	43.9	45.6
2	120	0.5	5	30.3	29.0
3	80	1.5	5	91.9	93.8
4	120	1.5	5	80.8	84.0
5	80	0.5	9	56.8	56.0
6	120	0.5	9	42.4	42.9
7	80	1.5	9	60.4	70.1
8	120	1.5	9	62.9	63.7
9	66.4	1	7	80.2	77.5
10	133.6	1	7	59.0	58.2
11	100	0.16	7	18.5	19.5
12	100	1.84	7	90.8	77.5
13	100	1	3.6	74.0	71.9
14	100	1	10.4	70.0	63.6
15	100	1	7	71.0	73.8
16	100	1	7	75.8	73.8
17	100	1	7	73.7	73.8
18	100	1	7	76.6	73.8
19	100	1	7	73.1	73.8
20	100	1	7	72.1	73.8

Table 3. ANOVA for response surface quadratic model.

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob. > F	Comment
Model	6393.26	9	710.36	77.88	< 0.0001 ^s	SD = 3.02
x_1	44840	1	448.40	49.16	< 0.0001 ^s	Mean = 64.82
x_2	4057.32	1	4057.32	444.81	< 0.0001 ^s	CV(%) = 4.66
x_3	82.35	1	82.35	9.03	0.0132 ^s	Press = 563
$x_1 x_2$	22.44	1	22.44	2.46	0.1478 ⁿ	$R^2 = 0.9859$
$x_1 x_3$	5.78	1	5.78	0.63	0.4445 ⁿ	$R^2_{(adj.)} = 0.9733$
$x_2 x_3$	584.82	1	584.82	64.11	< 0.0001 ^s	AP = 34.767
x_1^2	63.48	1	63.48	6.96	0.0248 ^s	
x_2^2	1151.82	1	1151.82	126.27	< 0.0001 ^s	
x_3^2	65.64	1	65.64	7.20	0.0230 ^s	
Residuals	91.22	10	9.12			
Lack of Fit	68.19	5	13.64	2.96	0.1293 ⁿ	
Pure Error	23.03	5	4.61			

^ssignificant at $p < 0.05$; ⁿnot significant at $p > 0.05$.

ANOVA: Analysis of variance.

Assessment of experimental results with DX10

To investigate a wide range of parameters (Table 2), the table for response and predicted values was

applied by the Design-Expert 10 (DX10). This table gives information about actual experiments obtained by independent runs and the predicted values (via DX10) built from these true runs. In this study, there were 20

actual runs including 14 fluctuation experiments of three variables and six replications at the central point. A proposed model was considered to be highly fitted with actual data if its parameters from ANOVA table satisfied a series of mentioned conditions: (1) Correlation coefficient (R^2) approaches 1.0; (2) Values of P or P-values are lower than 0.05 at 95% confidence level; (3) Adequate precision (AP) ratio is greater than 4.0; (4) Lack-of-fit value is higher than 0.05. It is inevitable that all the above standards met these requirements (Table 3). Moreover, Fig. 2A and Fig. 2B showed that the predicted values versus actual values distributed on the straight line and the residuals versus runs plot demonstrated that the obtained results of total 20 runs were experimentally random. Therefore, the proposed model was statistically significant.

Effect of independent variables on the removal of CR

Figure 2C shows “desirability” of the model, in which the experiments could be possible to obtain the highest results (probability = 100%) if they were conducted by the optimization condition. The region of maximum “desirability” was obviously spreading, thus it allowed obtaining good CR removal efficiencies. To describe the optimal regions that were plotted by altering two variables and holding another at zero level, the response surfaces were drawn and shown in Fig. 3.

To begin with, three-dimensional response surfaces were firstly plotted in Fig. 3A. The CR removal efficiency would rise by increasing the amount of $\text{NiFe}_2\text{O}_4/\text{GO}$. The main cause for this phenomenon was that when adding the $\text{NiFe}_2\text{O}_4/\text{GO}$ into the solution, the number of active adsorption sites rose to create more functional groups. Consequently, an optimum zone was positioned at the higher side of dosage. Meanwhile, the initial concentration of CR anions had a negligible impact on the CR removal efficiency.

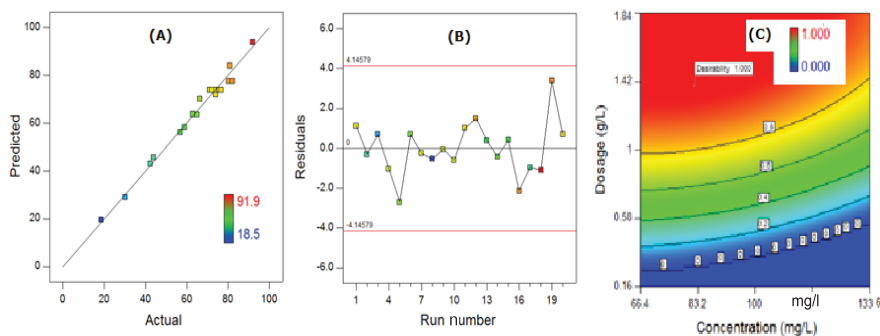


Fig. 2. Actual plot versus predicted plot (A), residuals versus runs (B), and “desirability” (C).

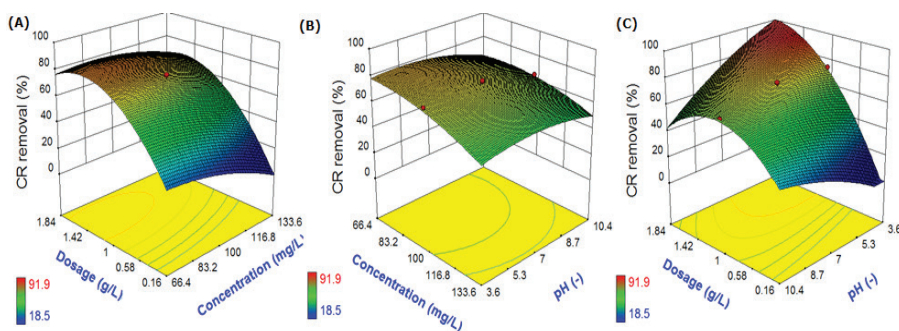


Fig. 3. Surface response plot (A-C) for the removal of CR by $\text{NiFe}_2\text{O}_4/\text{GO}$.

Table 4. Model confirmation.

C_i (mg/l)	Dosage (g/l)	pH (-)	Desirability	Ni^{2+} removal (%)	
				Predict	Test
82.2	1.4	4.0	1.00	94.3	94.7

Figure 3B indicated a significant effect of initial concentration and pH on CR removal efficiency. In contrast, there was a strong interaction between dosage and pH against the percentage of CR removal. The CR was markedly removed from the aqueous solution at a high level of dosage and low level of pH (Fig. 3C). When pH in solution decreased, the material surface would be charged positively. Thus, new bonds between positive-charged material and CR anions were favourably formed to enhance the adsorption.

The predicted optimal-condition-based model experiment was further conducted to verify the suitability of the

proposed model: $C_i = 82.2$ mg/l, dosage = 1.4, and pH = 4.0 with the highest desirability of 1.0 (Table 4). Thereby, the test for the percentage of CR removal was obtained at 94.3%, which was nearly closed to the predicted value of 94.7%. This result demonstrated the high compatibility of the proposed models with the experimental data.

Conclusions

The porous magnetic nanocomposite $\text{NiFe}_2\text{O}_4/\text{GO}$ was successfully synthesized and characterized by several techniques. The results indicated that the $\text{NiFe}_2\text{O}_4/\text{GO}$ had a highly crystalline nature with defective structure. By evaluating parameters from ANOVA, the proposed

model was proved to be statistically significant. Moreover, the DX10 obtained the optimal condition for the removal of CR from solution at $C_i = 82.2$ mg/l, dosage = 1.4, and pH = 4.0. With a high result of removal efficiency (94.7%), the $\text{NiFe}_2\text{O}_4/\text{GO}$ was an efficient adsorbent to remove the CR from contaminated groundwater.

REFERENCES

- [1] W. Yin, H. Cao (2017), “Solvothermal synthesis of magnetic $\text{CoFe}_2\text{O}_4/\text{rGO}$ nanocomposites for highly efficient dye removal in wastewater”, *RSC Adv.*, **7**, pp.4062-4069.
- [2] S. Han, K. Liu, Y. Zhu (2017), “Superior Adsorption and Regenerable Dye Adsorbent Based on Flower-Like Molybdenum Disulfide Nanostructure”, *Sci. Rep.*, **7**, pp.43-59.
- [3] Ghanizadeh (2011), “Adsorption kinetics and isotherm of methylene blue and its removal from aqueous solution using bone charcoal”, *React. Kinet. Mech. Catal.*, **102**(1), pp.127-142.
- [4] L. Ai, Z. Chen (2011), “Removal of methylene blue from aqueous solution by a solvothermal-synthesized graphene/magnetite composite”, *J. Hazard. Mater.*, **192**(3), pp.1515-1524.
- [5] V.K. Gupta, S. Suhas (2009), “Application of low-cost adsorbents for dye removal - A review”, *J. Environ. Manage.*, **90**(8), pp.2313-2342.
- [6] Q. Zhao, H. Zhao, T. Jiang (2017), “Efficient Removal of Pb(II) from Aqueous Solution by $\text{CoFe}_2\text{O}_4/\text{Graphene Oxide}$ Nanocomposite: Kinetic, Isotherm and Thermodynamic”, *J. Nanosci. Nanotechnol.*, **17**(6), pp.28-31.
- [7] A.A. Inyinbor, G.A. Olatunji (2016), “Kinetics, Isotherms and thermodynamic modeling of liquid phase adsorption of Rhodamine B dye onto *Raphia hookerie* fruit epicarp”, *Water Resour. Ind.*, **15**, pp.14-27.
- [8] S. Yáñez Vilar, M. Sánchez-Andújar, C. Gómez-Aguirre, J. Mira, M.A. Señarís Rodríguez, S. Castro García (2009), “A simple solvothermal synthesis of MFe_2O_4 (M = Mn, Co and Ni) nanoparticles”, *J. Solid State Chem.*, **182**(10), pp.2685-2690.
- [9] K. Hareesh, S.D. Dhole (2016), “PSS wrapped $\text{NiFe}_2\text{O}_4/\text{rGO}$ tertiary nanocomposite for the super-capacitor applications”, *Electrochim. Acta.*, **201**, pp.106-116.
- [10] C. Tan, X. Huang, H. Zhang (2013), “Synthesis and applications of graphene-based noble metal nanostructures”, *Materialstoday*, **16**(1-2), pp.29-36.
- [11] F. Hongbin, L. Yueming, L. Jinghong (2012), “Strong reduced graphene oxide-polymer composites: hydrogels and wires”, *RSC Adv.*, **2**, pp.6988-6993.

Studies on thermo-oxidative degradation of synthetic rubbers 1-4 and 1-2 polybutadienes

Quoc Hung Nguyen^{1*}, Van Hai Chu¹, Ngoc Cuong Hoang², Van Huong Pham³

¹Center of Analytical Services and Experimentation HCMC, Vietnam

²University of Science, Vietnam National University - Ho Chi Minh city, Vietnam

³University Bordeaux, IMS, UMR5255 CNRS, Bordeaux, France

Received 12 May 2017; accepted 6 July 2017

Abstract:

The production of natural latex is limited, and the related demand for terrestrial transportation has increased. The synthesis of rubber has become necessary and the resulting synthetics' properties must be known. Thermo-oxidative degradation of synthetic rubbers 1,4- and 1,2-polybutadienes caused by heating the compounds at 100°C in the air, was monitored. The volatile compounds released during the degradation were identified using coupled modern physical methods, including NMR, TGA, MS, FTIR, etc.. Thermal gravimetric analysis showed that oxidation occurred for both 1,4 and 1,2-PB. Below 400°C, major functional groups were formed, including peroxides, ketone, aldehyde, ester, etc.. Oxidation was found to start on the methylene (CH₂) carbon of 1,4-PB and on the methylene (CH₂) and methine (CH) carbons of 1,2-PB. The polycyclohexane-like structures were identified resulting from the oxidation of 1,2-PB. Above 400°C, the main resulting compounds were carbon dioxide, carbon monoxide, water, and some hydrocarbons and aromatics. Radical mechanisms for thermo-oxidative degradation of 1,4 and 1,2-PBs were proposed.

Keywords: FTIR, latex, mechanism, MS, NMR, polybutadiene, rubber, TGA, thermo-oxidative degradation.

Classification number: 2.2

Introduction

In contact with oxygen, polybutadienes can be oxidised even at low temperatures (0°C) [1]. Thermo-oxidative degradation of polymers can match the oxidation of the polybutadiene chain as well as the decomposition of oxidized products. Studies of 1,4-polybutadiene have shown that the major oxidised products have been hydroperoxide, cycloperoxide, alcohol, ether, carboxylic acid, ester, aldehyde, carbon dioxide, carbon monoxide, and water [1-5]. The mechanism for the formation of these products has been only proposed from observations of the FTIR spectrum. The polymer used for the studies has a high molecular weight which limited the resolution of analytical techniques. For 1,2-polybutadiene, the thermo-oxidative degradation seemed not to be mentioned in previous publications.

In this paper, we aim to use polybutadiene's low molecular weight. The thermo-oxidative degradation of the polybutadiene (PB) with various chemical structures was investigated. The oxidation process of product formation was monitored by heating the PBs in the air and identifying the corresponding residues using FTIR-ATR and NMR. The volatile compounds released during the degradation were investigated using TGA/FTIR and TGA/TD-GC/MS. Most probable degradation mechanisms were proposed.

Experimental

Materials and sample preparation

Polybutadienes (PBs) were provided by Polymer Laboratories Ltd. The PB samples were synthesised using sec-butyl lithium as an initiator. The structure of the PB samples was characterised using ¹³C-NMR spectrometry. Average molecular weight was determined by Size Exclusion Chromatography (SEC) combined with a laser scattering detector. The result was summarised in Table 1 [6].

*Corresponding author: Email: hungnq@case.vn

Table 1. Properties of PBs.

	% 1,2-vinyl ^b	% 1,4-cis ^b	% 1,4-trans ^b	Mn ^a
1,4-PB	8	33	59	7,600
1,2-PB	88	6	6	8,000

^aDetermined by SEC with laser scattering detector.

^bDetermined by ¹³C NMR.

Characterization methods

Thermal gravimetric analysis: PBs were studied using a TA Instrument Hi-Res 2,950 apparatus. Analyses were conducted on samples weighing about 10 mg, in a platinum pan, under air flowing at a flow-rate of 90 ml.min⁻¹, in an oven and 10 ml.min⁻¹ of balance. The heat cycle gradient was 10°C.min⁻¹ from 25 to 600°C in the air [6].

Fourier Transform Infrared Spectroscopy combined with Attenuated Total Reflection (FTIR-ATR): Infrared spectra were recorded at room temperature on a NICOLET Nexus Fourier Transform Infrared spectrometer. Recordings were obtained with a resolution of 4 cm⁻¹, and a spectral width between 400 and 4,000 cm⁻¹. ATR has a diamond crystal (128 scans) [6].

Nuclear Magnetic Resonance (NMR): The ¹H and ¹³C NMR spectra were recorded using a Bruker Avance 400 MHz spectrometer in CDCl₃, 30°C. ¹H and ¹³C NMR measurements were done at frequencies of 400.16 and 100.63 MHz, respectively. ¹H NMR spectra were acquired using 32 K data points, a spectral width of 4,789 Hz, an acquisition time of 3.42 s, a relaxation delay of 2 s, and a pulse width of 90° (10 μs), at 64 scans. ¹³C NMR spectra were acquired using 131 K data points, a spectral width of 25, at 126 Hz, an acquisition time of 2.61 s, a relaxation delay of 5 s, and a pulse width 90° (8.5 μs), at 16,384 scans, and the nuclear Overhauser effect (NOE) was suppressed by gating the decoupler sequence [6].

Thermal gravimetric Analysis combined with FTIR (TGA/FT-IR): TGA Diagrams were recorded on a TA Instrument 2,050 apparatus. Analyses were conducted on samples weighing about 10 mg, in a platinum pan, under air at a flow-rate of 90 ml.min⁻¹, in an oven, and with 10 ml.min⁻¹ on the balance. The heat cycle gradient was 10°C.min⁻¹ from 30 to 700°C in the air. The temperature of the transfer line was 250°C. FTIR spectra were recorded on a NICOLET Nexus Fourier Transform Infrared spectrometer. Recordings were obtained with a resolution of 4 cm⁻¹ and a spectral width between 400 and 4,000 cm⁻¹ (32 scans) [6].

TGA combined with thermal desorption (TD), combined with GCMS (TGA-TD/GC/MS): About 8-15 mg of samples were placed in a platinum pan of TGA. Analyses were performed under a stream of air at 45 ml.min⁻¹ in an oven, and with 10 ml.min⁻¹ on the balance. The heating rate of the cycles was 10°C.min⁻¹ ranging from 30 to 700°C. At the oven outlet, an adsorbent tube (Tenax) was placed to trap volatile organic compounds that were within the chosen temperature range. After that, an adsorbent tube was placed on a thermal desorber.

Desorption conditions on the TD system were T (desorption): 300°C, t (desorption): 20 min, T (trap): -10°C, P (He): 99 kPa, T (trap injector): 300°C, and T (column head): 140°C. Compounds were injected and separated in a gas chromatography column with an Agilent HP of 5MS, and to the dimensions of 30 m x 0.25 mm x 0.25 μm. The oven was conducted as follows: 35°C isotherm for 15 min, 35 min at 120°C temperature rise with a gradient of 2°C min⁻¹, 120 min at 300°C temperature rise with a gradient of 3°C min⁻¹, and 3 min isotherm at 300°C. The mass spectrometer (Agilent GC-MS 6890) was adjusted for an emission current of 35 μA, and an electron multiplier voltage between 1,423 and 1,628 V. MS quad temperature was 150°C and the transfer line was set at 250°C. The ion source was 230°C. The mass range was 15-600 u [6].

Results and discussions

Thermo-oxidative degradation of 1,2 and 1,4-PB by TGA-FTIR

TGA diagrams obtained for the 1,4-PB and 1,2-PB (Fig. 1) exhibited a similar degradation profile. A mass increase began near 130°C up to 328°C for the 1,4-PB, and 375°C for 1,2-PB, which can be attributed to the oxidation of the PBs [1]. From 400°C, the PBs were rapidly decomposed with a mass loss of about 77% between 400 and 475°C. The thermo-oxidative degradation completes at 600°C. The loss of mass rapidly occurred when the temperature increased.

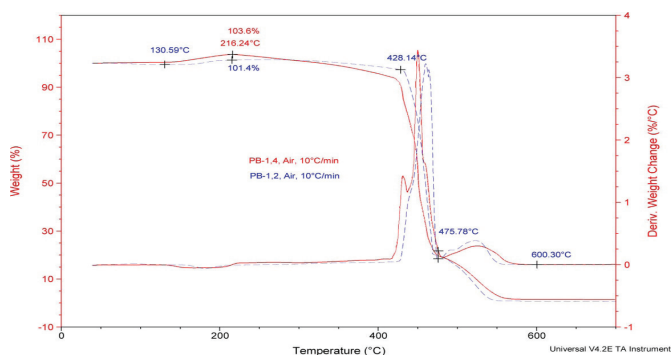


Fig. 1. TGA diagrams of 1,4-PB (red) and 1,2-PB (blue), heating 10°C.min⁻¹ in the air.

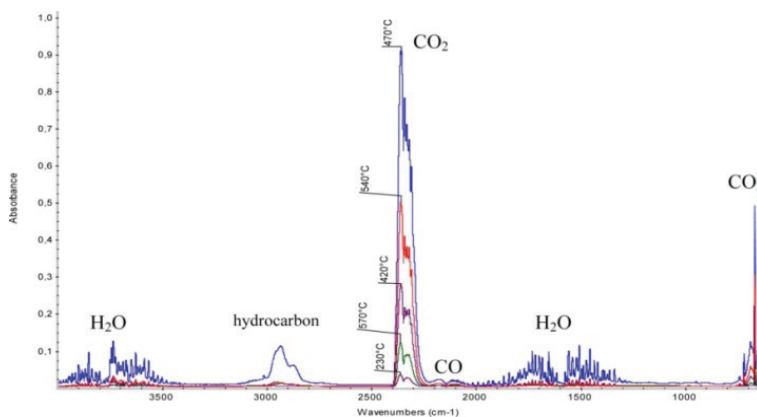


Fig. 2. FTIR spectra of volatile compounds of the 1,4-PB heated 10°C.min⁻¹, in the air.

FTIR spectra of the volatile compounds released from the TGA show that the decomposition begun at 130°C, at the same time of the oxidation. The main compounds were carbon dioxide (2,362, 2,330 cm⁻¹), carbon monoxide (2,172, 2,107 cm⁻¹), H₂O (3,739, 1,709, 680 cm⁻¹), and a low content of hydrocarbon (2,926, 2,867 cm⁻¹), as identified by FTIR.

Besides this, the FTIR spectra (Fig. 2) didn't show any volatile compounds at a temperature below 130°C. This confirmed that decomposition of PBs had not yet occurred. So, to exclusively observe the oxidation, PBs were isothermally heated at 100°C, in the air. Structural modifications during heating were monitored by FTIR, ¹H, ¹³C NMR.

Thermo-oxidative degradation of 1,2-PB and 1,4-PB at 100°C

The previous studies showed that oxidation of polybutadiene

forms a film which prevents oxygen diffusion inside materials and limits oxidation [7-8]. A thin layer of PBs was prepared on a glass surface in a solution of 5% of PBs in chloroform, and then heated at 100°C in an oven under air. Then, the samples were withdrawn at selected time intervals.

The FTIR spectra of 1,4-PB and 1,2-PB after heating at 100°C in the air (Fig. 3A, 3B) showed that the groups of 1,2-vinyl (909 cm⁻¹), 1,4-cis (729 cm⁻¹), and 1,4-trans (964 cm⁻¹) decreased. Besides this, new bands were also noted at 3,436 cm⁻¹ (ν_{OH}), 2,730 cm⁻¹ (ν_{CH=O}), 1,771 cm⁻¹ (γ-lactone), 1,717 cm⁻¹ (ν_{C=O}), 1,177, and 1,052 cm⁻¹ (ν_{C-O}). These bands can be assigned to hydroperoxide, alcohol, aldehyde, carboxylic acid, ester, ketone, and ether functions.

The films after heating were soaked in a concentrated solution of ammonia for 24 hours at an ambient temperature. The FTIR spectra of the heated 1,4 and 1,2-PB showed, after

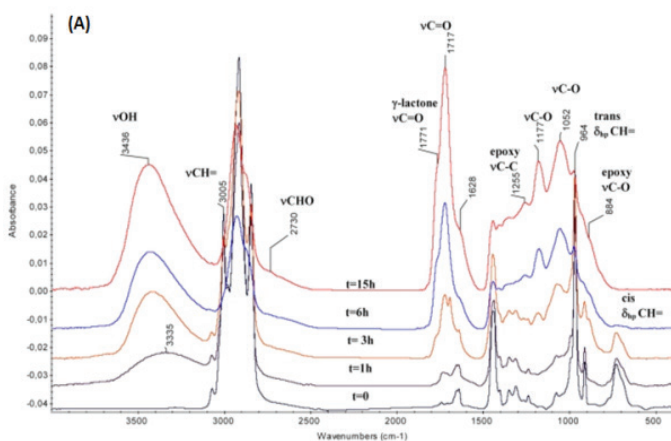


Fig. 3A. FTIR spectra (ATR-diamond) of 1,4-PB heated 100°C, in the air, $t = 1, 3, 6, 15$ h.

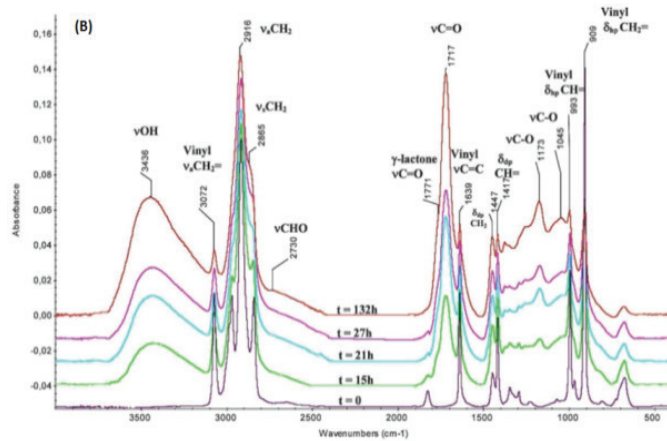


Fig. 3B. FTIR spectra (ATR-diamond) of 1,2-PB heated 100°C, in the air, $t = 15, 21, 27, 132$ h.

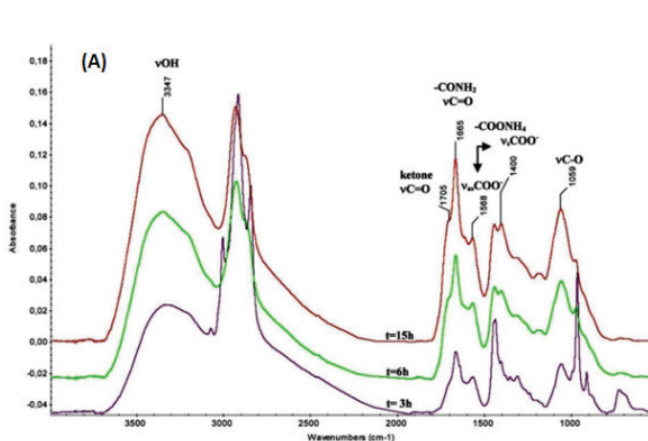


Fig. 4A. FTIR spectra (ATR-diamond) of 1,4-PB heated 100°C, in the air, $t = 3, 6, 15$ h, and then derivatized with ammonia, 24 h at ambient temperature.

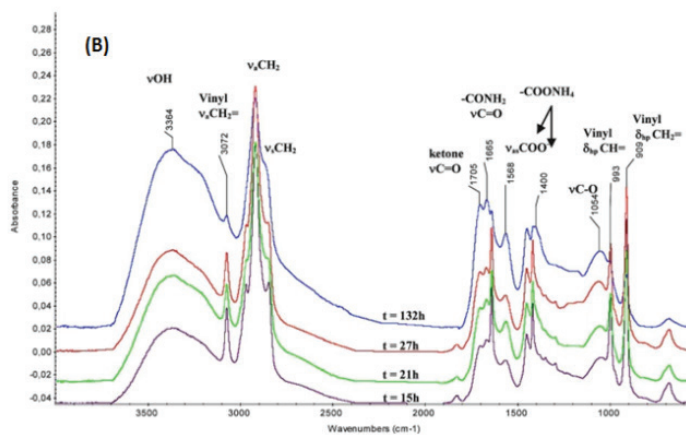


Fig. 4B. FTIR spectra (ATR-diamond) of 1,2-PB heated 100°C, in the air, $t = 15, 21, 27, 132$ h, and then derivatized with ammonia, 24 h at ambient temperature.

derivatization with ammonia (Fig. 4A, 4B), that the appearance of the primary amide group corresponding to bands at 1,665, 3,214, 3,347 cm^{-1} and the disappearance of the band at 1,717 cm^{-1} permit to confirm a formation of a carbonyl ester function during heating of the 1,4 and 1,2-PB in the air. Two bands at 1,568 and 1,400 cm^{-1} can be assigned to asymmetric and symmetric vibrations of ammonium carboxylate, which is due to the carboxylic acid function. The band at 1,705 cm^{-1} was not changed by the action of ammonia which confirms the presence of a saturated ketone.

The ^1H NMR spectrum of 1,4-PB, after 3 hours of heating (Fig. 5), showed that a doublet at 9.5 ppm with a coupling constant $J = 8$ Hz can be assigned to a proton of an unsaturated aldehyde function (compared to $\text{CH}_2=\text{CH}-\text{CHO}$ with $J = 8$ Hz [9]). The peak at 9.8 ppm can be attributed to protons from the saturated aldehyde function. The group of signals at 8.1 ppm can be assigned to the protons from hydroperoxide [10]. Peaks at 6.1 and 6.8 ppm correspond to the conjugated diene structure $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$. Peaks at 3.5 to 4.5 ppm can be attributed to protons in the carbon atoms connected to oxygen atoms with ether, alcohol, peroxide, and hydroperoxide [10]. Peaks found at around 2.7 ppm can be assigned to protons from epoxide. The ^{13}C NMR spectrum of 1,4-PB, heated for 3 hours (Fig. 6), showed that two peaks were at 201 and 194 ppm and can be respectively assigned to carbons of ketone and aldehyde functions. The intense signals at 58 ppm (CH), 32.1 ppm (CH_2), 29.0 ppm (CH_2), 23.8 ppm (CH_2) permit to be assigned to an epoxide ring structure (Fig. 7), which was formed as a reaction from the peroxide radical with a double bond of 1,4-cis or 1,4-trans [11]. Besides this, the ^1H and ^{13}C NMR (Fig. 5, 6) also showed that the dominant oxidative product was the epoxide structure. Then, the secondary oxidation of the oxidised structures had rapidly occurred.

For 1,2-PB, after 8 hours of heating, the sample could not be soluble in the solvent, causing difficulties of observation using the liquid NMR. The ^1H NMR spectrum of 1,2-PB, after heating at 100°C for 7 hours in the air (Fig. 8A), also permitted identification of new functional groups, such as epoxide rings, ether, hydroperoxide, and unsaturated structures (respectively at 2.62, 4.09, 8.04, 9.48 ppm) corresponding to oxidised products. This result was similar to what was found with the former IR spectra method (Fig. 3A, 3B). Besides this, an intense signal at 1.25 ppm can be assigned to a saturated structure. The ^{13}C NMR (Fig. 8B) showed that the signals at 38.7 and 40.9 ppm correspond to carbon methine ($-\text{CH}-$) and methylene ($-\text{CH}_2-$) of the 1,2-vinyl isomer, and had decreased after heating. The appearance of signals of CH_2 at 29.7 ppm and CH at 38.5 ppm permits the assignment of carbon methylene and methine of polycyclohexane like structures. The polycyclohexane structure is more stable than the 1,2-PB one. Consequently, the 1,4-PB (containing secondary allylic carbons) was more quickly

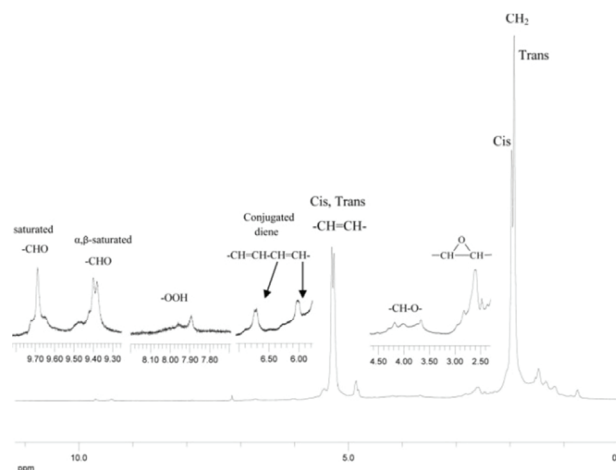


Fig. 5. ^1H NMR spectrum of 1,4-PB heated at 100°C, 3 h, in the air, 400.13 MHz, CDCl_3 , TMS, 30°C.

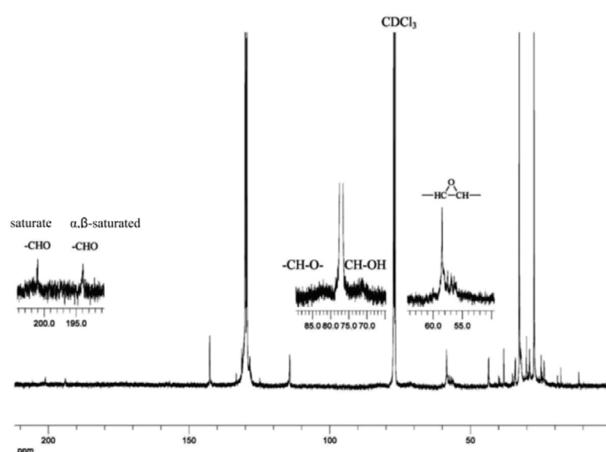


Fig. 6. ^{13}C NMR spectrum of 1,4-PB heated at 100°C, 3 h, in the air, 100.63 MHz, CDCl_3 , TMS, 30°C.

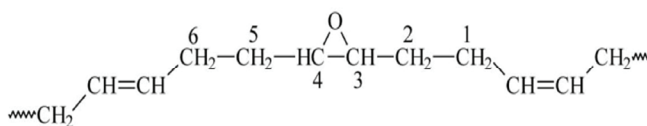


Fig. 7. Epoxide structure of the oxidized 1,4-PB.

C_1 : 23.8; $\text{C}_{2,5}$: 32.1; C_6 : 29.0; $\text{C}_{3,4}$: 58 ppm; $\text{H}_{3,4}$: 2.7 ppm.

oxidised than the 1,2-PB (containing tertiary allylic carbons) that had been also confirmed by the IR spectra (Fig. 3A, 3B).

VOCs releasing from the thermo-oxidation degradation of the PB in course of heating were trapped and identified by TGA-TD/GCMS system.

Observation of VOCs of the thermo-oxidative degradation of PB by TGA-TD/GCMS

In a temperature range from 130 to 400°C, for 1,4-PB (Fig. 9A), major products were detected, including alcohols (1-butene-2-ol, 1-pentene-3-ol, and 2-pentene-1-ol), which can

be formed from additional hydrogen on the alkoxide radicals [1, 2, 12]; aldehydes (2-butanal, 3-methyl pentanal, 4-hexene-1-al, 2-hexenal, butanedial, furfural, and benzaldehyde), which can be formed by β scission of alkoxide radicals and polycycloperoxide [1]; ketones (2-butanone, 1-pentene-3-one, 3-pentene-2-one, cyclopentanone, 2-cyclopentene-1-one,

and 2-cyclohexene-1-one), which can be formed from “cage” reactions between alkoxide radicals and hydroxide radicals; and carboxylic acid (acetic acid, etc.), which can be formed by oxidation of an aldehyde [12].

For 1,2-PB (Fig. 9B), VOCs are mainly as follows: 3-butene-

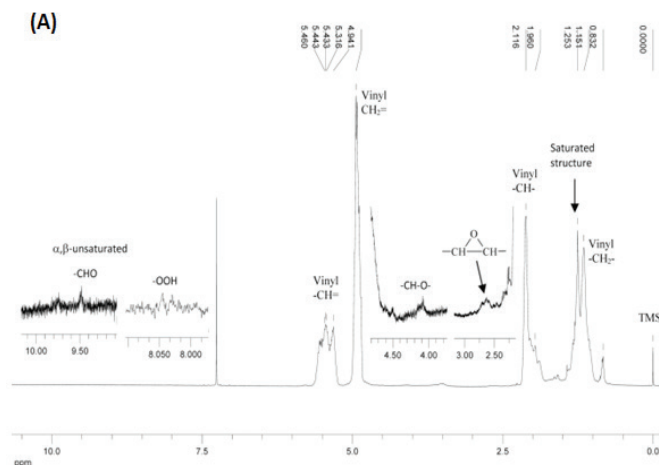


Fig. 8A. ^1H NMR spectrum of 1,2-PB heated at 100°C , 7 hours, in the air, 400.13 MHz, CDCl_3 , TMS, 30°C .

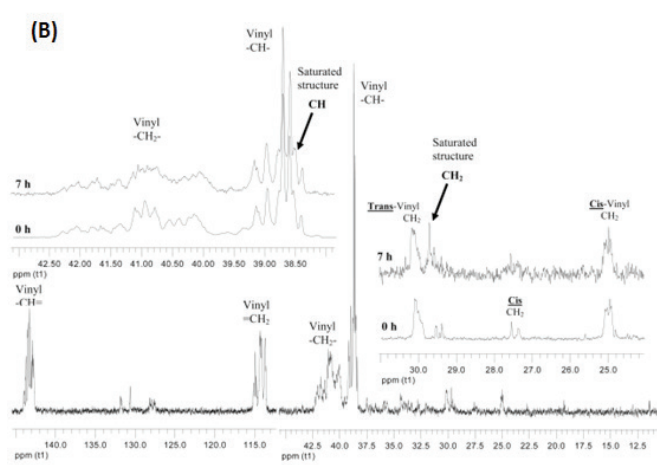


Fig. 8B. ^{13}C NMR spectrum of 1,2-PB heated at 100°C , 7 hours, in the air, 400.13 MHz, CDCl_3 , TMS, 30°C .

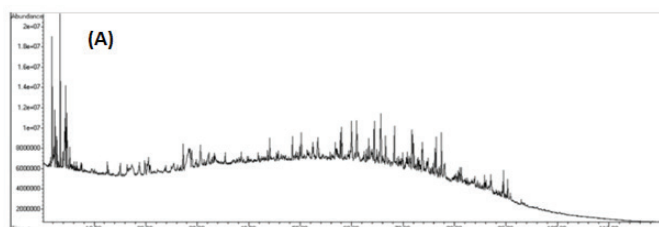


Fig. 9A. Chromatogram of released VOCs from 1,4-PB at $130\text{--}400^\circ\text{C}$, gradient $10^\circ\text{C}\cdot\text{min}^{-1}$, in the air.

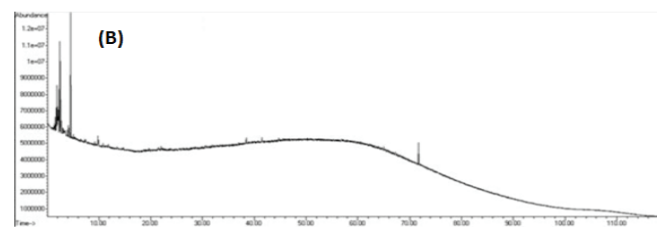


Fig. 9B. Chromatogram of released VOCs from 1,2-PB at $130\text{--}400^\circ\text{C}$, gradient $10^\circ\text{C}\cdot\text{min}^{-1}$, in the air.

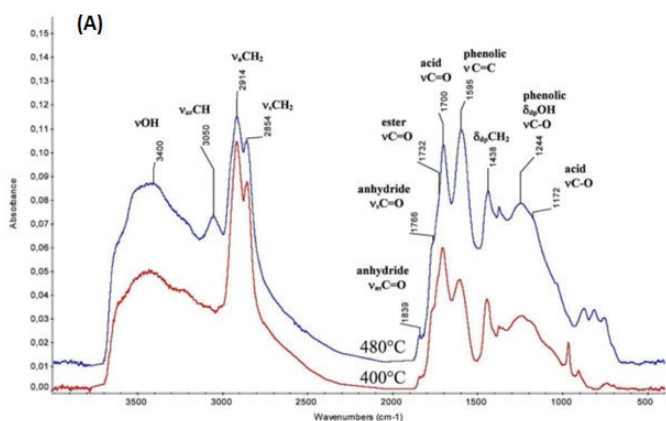


Fig. 10A. FTIR spectra (ATR-diamond) of residues at 400°C , 480°C of 1,4-PB heated $10^\circ\text{C}\cdot\text{min}^{-1}$, under the air.

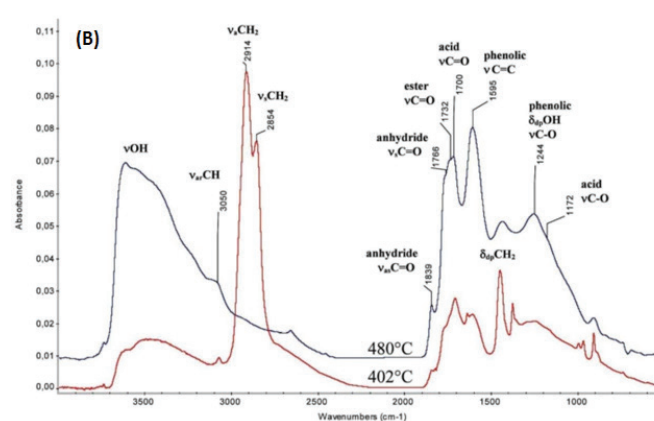
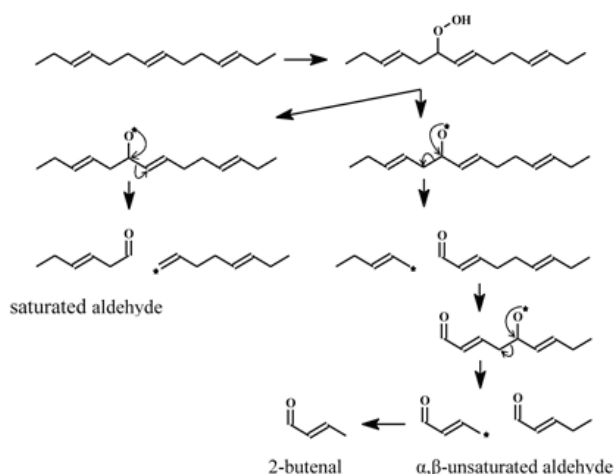


Fig. 10B. FTIR spectra (ATR-diamond) of residues at 402°C , 480°C of 1,2-PB heated $10^\circ\text{C}\cdot\text{min}^{-1}$, under the air.



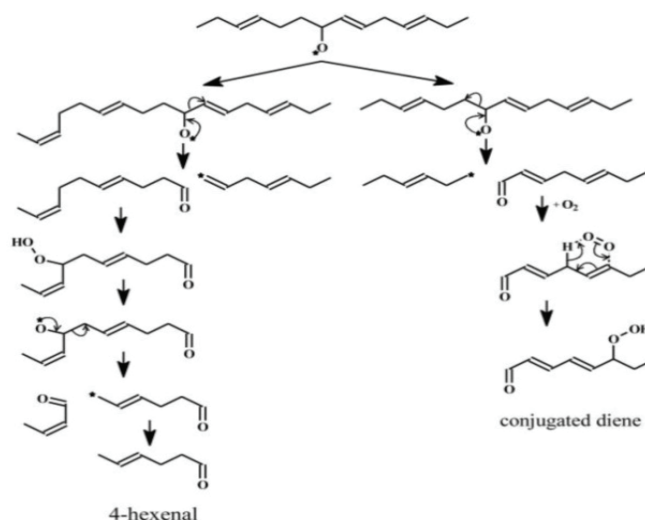
Scheme 1. Formation of saturated and α,β -unsaturated aldehydes from the oxidation of 1,4-PB.

2-ol, 3-butene-2-one, 2-butanone, acetic acid, 2-butenal, 2-methyl 2-butenal, butanedial, 3-methyl 2,5-furandione, benzaldehyde, phenol, acetophenone, 3-methyl phenol, 4-methyl benzaldehyde, and phthalic anhydride.

The analytical results showed that the thermo-oxidative degradation of the 1-4 and 1-2 PB have differently occurred in the region below 400°C.

In a range from 400 to 600°C, volatile compounds were trapped and identified by TGA-FTIR and TGA-TD/GCMS. The main compounds were CO_2 , CO , H_2O , and some hydrocarbons and the same for both 1,4 and 1,2-PB, which is comparable to the former observed in an inert atmosphere [6-13]. At high temperatures, under N_2 , PBs can rapidly decompose [6-13]. In this study, the decomposition of PBs had also occurred, but there was not a participation by oxygen. The hydrocarbon residues showed that the competition between two reactions seemed to belong to the rate of oxygen diffusion on the surface of the sample and the oxygen concentration in the oven of TGA.

The residues from the thermo-oxidative degradation of PBs were obtained by performing a TGA analysis in an air flow at $10^\circ\text{C}.\text{min}^{-1}$, and stopped at 400°C and 480°C. The FTIR spectra of the 1,4 and 1,2-PB residues (Fig. 10A, 10B) showed the appearance of bands corresponding to the phenolic structures (ν_{OH} between 3,000 and 3,700 cm^{-1} , $\nu_{\text{C-H aromatic}}$ at 3,050 cm^{-1} , $\nu_{\text{C=C aromatic}}$ at 1,595 cm^{-1} , and $\nu_{\text{C=O}}$ at 1,244 cm^{-1}), carbonyl anhydride (1,839, 1,766 cm^{-1}), ester (1,732 cm^{-1}), and carboxylic acid (1,700 cm^{-1}). This result showed that above 400°C, thermo-oxidative degradation has rapidly occurred. The oxidative products have been already formed and continuously oxidised. Consequently, oxidised products could not be detected by GCMS.



Scheme 2. Formation of saturated, α,β -unsaturated aldehydes and conjugated diene structure from the oxidation of 1,4-PB.

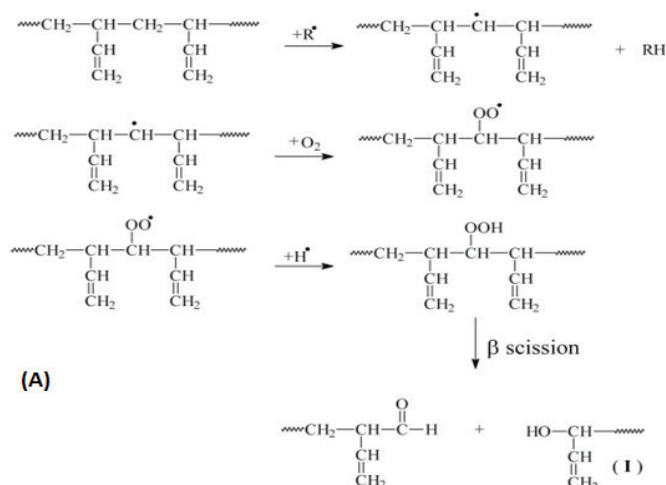
Proposed reaction mechanisms

Based on these analytical results, some reaction mechanisms could be proposed as follows:

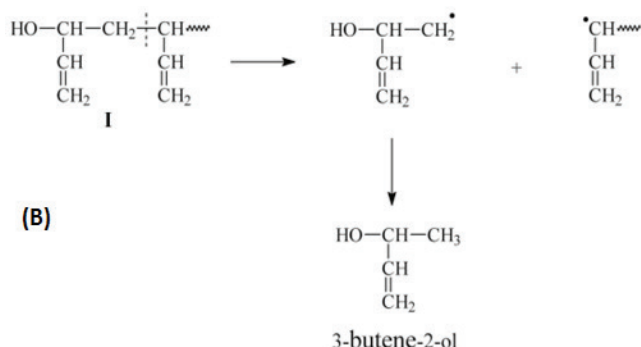
Oxidation on 1,4-PB: The hydroperoxide could be easily formed on allylic carbon (Scheme 1). The alkoxide radical was formed by the decomposition of this hydroperoxide. Saturated and unsaturated aldehyde products can be explained by β -Scission of the alkoxide (Scheme 1, 2). The direct reaction of an oxygen molecule with an allylic carbon and an adjacent double bond led to form the conjugated diene structures (Scheme 2).

Oxidation on 1,2-PB: Two products 3-butene-2-ol and 3-butene-2-one formed from the thermo-oxidative degradation of 1,2-PB, allowing the consideration of two targets of oxidation on 1,2-vinyl, which are methylene (CH_2) and methine (CH) carbons. The radical attack of a hydrogen of the methylene group (CH_2) led to the formation of a radical, which was added to an oxygen molecular and a proton, forming a hydroperoxide. Then, the hydroperoxide compound is decomposed to aldehyde and alcohol by β -scission (Scheme 3A). The formation of the 3-butene-2-ol compound can be explained by rupture of the C-C bond of the alcohol I, followed by addition of a proton as shown in the Scheme 3B.

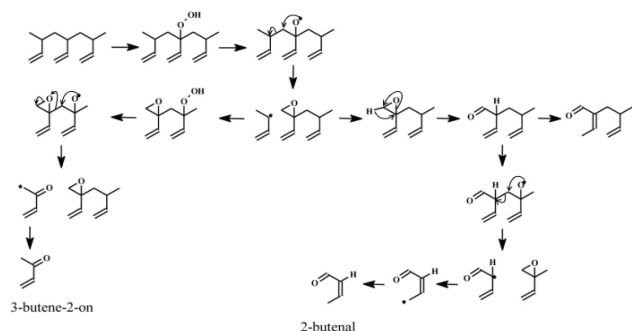
A radical will attack to the hydrogen on a tertiary CH group of 1,2-vinyl, forming a molecular radical. The addition of an oxygen molecule and a proton leads to the formation of a hydroperoxide, the decomposition of which forms alkoxide and hydroxide radicals. The decomposition of the epoxide ring formed from the alkoxide radical gives 3-butene-2-one and 2-butenal compounds (Scheme 4).



Scheme 3A. Oxidation of the CH₂ carbon of 1,2-vinyl.

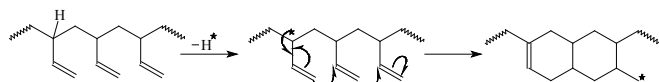


Scheme 3B. Formation of 3-butene-2-ol.



Scheme 4. Formation of 3-butene-2-one and 2-butenal.

The tertiary allylic carbon on the 1,2-PB was dominant for the formation of a tertiary stable radical. The allylic resonance between the radical and allylic double bond formed a new radical which reacted with the adjacent 1,2-vinyl groups. Consequently, the polycyclohexane-like structure had been formed (Scheme 5).



Scheme 5. Formation of polycyclohexane like structure from 1,2-PB.

Conclusions

The thermo-oxidative degradation of polybutadienes with various structures was studied and their different degradation mechanisms were discussed. Below 400°C, the oxidation of 1,4 and 1,2-PB released products with the same functions, such as ketone, alcohol, aldehyde, ester, carboxylic acid, and anhydride by different radical mechanisms. For 1,4-PB, oxidation starts on the methylene (CH₂) carbon of the 1,4 group. The dominant oxidised structure was the epoxide ring. For 1,2-PB, targets of oxidation were methylene (CH₂) and methine (CH) carbons of 1,2-vinyl. The polycyclohexane-like structure was also identified in the oxidation process of the 1,2-PB. Above 400°C, the oxidation of PB rapidly occurred. This stage corresponds to the oxidation of oxidised structures. The major products detected were carbon dioxide, carbon monoxide, water, and some hydrocarbons and aromatics for both 1,4 and 1,2-PB. Oxidation and thermal degradation took place at the same time. Their competition may belong to the rate of oxygen diffusion and oxygen concentration. This result will help to adapt the production of various synthetic rubbers and latex for suitable uses.

ACKNOWLEDGEMENTS

The financial aid from CNRS France is highly appreciated.

REFERENCES

- [1] I.C. McNeill, W.T.K. Stevenson (1985), "The structure and stability of oxidised polybutadiene", *Polymer Degradation and Stability*, **11**(2), pp.123-143.
- [2] V.V. Pchelintsev, Y.T. Denisov (1985), "Mechanisms of the oxidative degradation of diene rubbers", *Review Polymer Science U.S.S.R.*, **27**(6), pp.1253-1270.
- [3] M. Coquillat, J. Verdu, X. Colin, L. Audouin, R. Nevière (2007a), "Thermal oxidation of polybutadiene. Part 2: Mechanistic and kinetic schemes for additive-free non-crosslinked polybutadiene", *Polymer Degradation and Stability*, **92**(7), pp.1334-1342.
- [4] M. Coquillat, J. Verdu, X. Colin, L. Audouin, R. Nevière (2007b), "Thermal oxidation of polybutadiene. Part 3: Molar mass change of additive-free non-crosslinked polybutadiene", *Polymer Degradation and Stability*, **92**(7), pp.1343-1349.
- [5] M.L. Kaplan, P.G. Kelleher (1970), "Photo-oxidation of polymers without light: Oxidation of polybutadiene and an ABS polyblend with singlet oxygen", *Journal of Polymer Science, Part A: Polymer Chemistry*, **8**(11), pp.3163-3175.
- [6] C. Sanglar, N.Q. Hung, M.F. Grenier-Loustalot (2010), "Studies on thermal degradation of 1-4 and 1-2 polybutadienes in inert atmosphere", *Polymer Degradation and Stability*, **95**(9), pp.1870-1876.
- [7] C.F. Cullis, H.S. Laver (1978), "The thermal degradation and oxidation of polybutadiene", *European Polymer Journal*, **14**(8), pp.571-573.
- [8] M. Coquillat, J. Verdu, X. Colin, L. Audouin, R. Nevière (2007c), "Thermal oxidation of polybutadiene. Part 1: Effect of temperature, oxygen pressure and sample thickness on thermal oxidation of hydroxyl-terminated polybutadiene", *Polymer Degradation and Stability*, **92**(7), pp.1326-1333.
- [9] E. Pretsh, T. Clerc, J. Seibl, W. Simon (1989), *Tables of Spectral Data for structure determination of organic compounds*, Springer Verlag, Berlin, Heidelberg.
- [10] M.A. Golub (1980), "Photosensitized oxidation of unsaturated polymers", *Pure and Applied Chemistry*, **52**(2), pp.305-323.
- [11] M. Guyader, I. Audouin, X. Colin, J. Verdu, S. Chevalier (2006), "Epoxides in the thermal oxidation of polybutadiene", *Polymer Degradation and Stability*, **91**(11), pp.2813-2815.
- [12] M. Piton, A. Rivaton (1996), "Photooxidation of polybutadiene at long wavelengths ($\lambda > 300$ nm)", *Polymer Degradation and Stability*, **53**(3), pp.343-359.
- [13] N.Q. Hung, C. Sanglar, M.F. Grenier-Loustalot, P.V. Huong, H.N. Cuong (2011), "New structures from the thermal rearrangement of polybutadiene revealed by 2D HSQC NMR", *Polymer Degradation and Stability*, **96**(7), pp.1255-1276.

Comparison of the adsorption of Zn(II) on α - and γ -MnO₂ nanostructures

Van Phuc Dinh^{1*}, Ngoc Chung Le², Ngoc Tuan Nguyen³, Thien Hoang Ho¹, Van Dong Nguyen⁴

¹Dong Nai University

²Dalat University

³Vietnam Atomic Energy Institute

⁴University of Science, Vietnam National University, Ho Chi Minh city

Received 11 April 2017; accepted 28 August 2017

Abstract:

In this report, the adsorption of Zn(II) ion on γ - and α -MnO₂ nanostructures was compared. The results showed that the maximum adsorption was obtained at pH = 4.0 for both materials and after 60 minutes for γ -MnO₂ and 80 minutes for α -MnO₂. Adsorption isotherm models demonstrated that the Langmuir was the best model to describe the adsorption of Zinc(II) on γ - and α -MnO₂ because of the highest correlation coefficient (R^2), the smallest root mean square error (RMSE), and the nonlinear chi-squared test (χ^2) values. The maximum adsorption capacity of γ -MnO₂ calculated from Langmuir model was 55.23 mg/g, which was roughly double α -MnO₂. The lower 1/n value from Freundlich model for α -MnO₂ revealed that it was not as favourable as γ -MnO₂. The heat of the adsorption as well as the mean free energy estimated from Temkin and Dubinin - Radushkevich models to be less than 8 kJ/mol indicated that the adsorption on both materials followed a physical process. Kinetic studies showed that pseudo-second-order model was accurate to describe both materials in three stages.

Keywords: adsorption, kinetics, Zinc, α -MnO₂, γ -MnO₂.

Classification number: 2.2

Introduction

Zinc is an essential trace element that can be found in cells throughout the human body as well as animals and plants. However, Zinc can cause depression, lethargy, neurological signs, and excessive thirst [1]. Zinc is widely used in many important industrial applications such as mining, coal and waste combustion, and steel processing [2]. Various treatment techniques have been applied to remove Zinc(II) ions from contaminated water such as chemical precipitation, flotation, adsorption, ion exchange, and electrochemical deposition [3]. Adsorption technology is considered as one of the most efficient and promising methods for the treatment of trace amount of heavy metal ions in

a large volume of water because of its enrichment efficiency and the ease of phase separation [4-9].

Manganese dioxide is a low-cost and environmentally friendly material. Along with many types of crystalline structures such as α -, β -, and γ -, etc., manganese dioxide has been extensively studied due to its excellent chemical characteristics. Therefore, this material is applied in different areas such as batteries, molecular sieves, catalysts, and adsorbents [10-12]. However, a systematic comparison of the adsorption of Zn(II) from the aqueous solution onto α - and γ -MnO₂ nanomaterials has not been reported.

Our goal is to compare the adsorption

capacity of Zinc(II) from aqueous solution by using α - and γ -MnO₂ nanomaterials as adsorbents. Four non-linear adsorption isotherm models, namely Langmuir, Freundlich, Temkin, and Dubinin - Radushkevich and three kinetic models, namely pseudo-first-order, pseudo-second-order, and intra-diffusion were used to assess the uptake capacity and to predict the adsorption mechanism.

Material and methods

Chemicals

Potassium permanganate (KMnO₄), ethyl alcohol (C₂H₅OH), HNO₃, and NaOH with analytical grade were purchased from Merck. Zn(II) ion was used as the adsorbate. 1000 mg/l of zinc standard stock solution was prepared by dissolving Zn(NO₃)₂ respectively in double-distilled water.

Analytical methods

Atomic Absorption Spectrometry (flame technique) was used to determine the concentration of zinc in aqueous solution by using an atomic absorption spectrophotometer AA - 7000 (Shimadzu, Japan).

The pH measurements were done with a pH-meter (MARTINI Instruments Mi-150 Romania) which was standardized by using HANNA instruments with three buffer solutions with the pH values of 4.01±0.01, 7.01±0.01, and 10.01±0.01.

Temperature-controlled shaker (Model IKA R5) was used for the equilibrium studies.

*Corresponding author: Email: dinhvanphuc82@gmail.com

Preparation α -MnO₂ and γ -MnO₂

The γ -MnO₂ was successfully synthesized by L. Ngoc Chung and D. Van Phuc [11] from ethanol and potassium permanganate; whereas, α -MnO₂ was formed by heating γ -MnO₂ at 600°C [12]. The synthesized γ -MnO₂ and α -MnO₂ characterized by X-ray Diffractometer D5000 with X-ray radiation: CuK α , λ = 1.54056Å, Ultra High Resolution Scanning Electron Microscopy S - 4800, Transmission Electron Microscope with physical absorption system Micrometrics Gemini VII, and BET-BJH analysis were used as adsorbents to adsorb Zinc(II) ions from aqueous solution.

Adsorption study

0.1 g of adsorbents was placed into 50 ml of Zn(II) ion solution in a 100 ml conical flask. The effect of pH (2-6), contact time (20-240 min), and initial concentration of Zn(II) ions were examined. The obtained mixture was centrifuged at 5500 rpm within 10 minutes, then was purified by PTFE Syring Filters with 0.22 μ m of pore size to get the filtrate. The concentrations of Zn(II) ions in the filtrate before and after the adsorption were determined by F-ASS.

The adsorption capacity was calculated by using the mass balance equation for the adsorbent [12].

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

The removal efficiency (%) was calculated using the following formula:

$$\% \text{ Removal} = \frac{(C_o - C_e) \cdot 100\%}{C_o} \quad (2)$$

where: q is the adsorption capacity (mg/g) at equilibrium, C_o and C_e are the initial and the equilibrium concentrations (mg/l), respectively. V is the volume (l) of the solution, and m is the mass (g) of the adsorbent used.

Some adsorption isotherm formula and kinetic models which were applied to predict both the adsorption capacities of materials and the adsorption mechanisms were given in Table 1 and Table 2 [13].

Table 1. The non-linear, error functions, and meaning of some models.

Isotherm	Non-linear form	Meaning	List of error functions
Langmuir	$q_e = \frac{q_m \cdot K_L \cdot C_e}{1 + K_L \cdot C_e}$	Assuming the adsorption occurred on monolayer on the material surface. Also, estimating the maximum adsorption capacity on the material surface.	$R^2 = 1 - \frac{\sum_{n=1}^n (q_{e,meas} - q_{e,calc})^2}{\sum_{n=1}^n (q_{e,meas} - \overline{q_{e,calc}})^2}$
Freundlich	$q_e = K_F \cdot C_e^{1/n}$	Assuming the adsorption occurred on multilayers on the material surface.	$RMSE = \sqrt{\frac{1}{n-1} \sum_{n=1}^n (q_{e,meas} - q_{e,calc})^2}$
Temkin	$q_e = \frac{RT}{b_T} \ln(K_T C_e)$	Evaluating the adsorption potentials of the adsorbent for adsorbates as well as the heat of the adsorption process.	$\chi^2 = \sum_{n=1}^n \frac{(q_{e,meas} - q_{e,calc})^2}{q_{e,calc}}$
Dubinin - Radushkevich	$q_e = q_m \cdot e^{(-\beta \cdot \epsilon^2)}$	Evaluating the value of mean sorption energy which gives information about chemical and physical sorption	The small values of RMSE and χ^2 indicate firstly a better fitting model, and secondly the correspondence of the model with the experimental data.

Where: q_e: the adsorption capacity at equilibrium (mg/g); q_m: the maximum adsorption capacity (mg/g); C_e: the equilibrium concentration (mg/l); K_L: Langmuir constant; K_F: Freundlich constant; n: adsorption intensity; R: the universal gas constant (8.314.10⁻³ kJ/K.mol); T: the temperature (K); b_T: Temkin isotherm constant related to the adsorption heat (kJ/mol); K_T: the equilibrium binding constant (l/mol); β : Dubinin-Radushkevich isotherm constant (mol²/kJ²); ϵ : Dubinin-Radushkevich isotherm constant; E: mean free energy (kJ/mol); R²: correlation coefficient; RMSE: Root mean square error; χ^2 : Non-linear chi-squared test.

Table 2. Models and kinetic parameters.

Kinetic models		Kinetic parameters	
		γ -MnO ₂	α -MnO ₂
Pseudo-first-order model	$\log(q_e - q_t) = \log q_e - \frac{k_1 \cdot t}{2,303}$	q _{e(exp)} (mg/g)	25.5 mg/g
		K ₁ (min)	7.60.10 ⁻³
		R ²	0.5594
		q _{e(cal)} (mg/g)	1.88
Pseudo-second-order model	$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right) \cdot t$	K ₂ (g/mg.min)	5.86.10 ⁻³
		R ²	1.0000
		q _{e(cal)} (mg/g)	25.91
Intra-particle diffusion model	$q_t = k_d t^{1/2} + C$	k ₁	4.209
		k ₂	0.3078
		k ₃	0.0026

Where: q_e: the amount of solute adsorbed at equilibrium per unit weight of adsorbent (mg/g); q: the amount of solute adsorbed at any time (mg/g); k₁, k₂, k₃: the adsorption constant; t, t^{1/2}: adsorption time.

Results and discussions

Characterization of γ - and α - MnO_2 nanomaterials

Figure 1 shows the X-ray diffraction patterns of two samples at room temperature and at 600°C. The results indicated that γ - MnO_2 was formed at room temperature with some specific peaks at $2\theta = 22.2^\circ$, 37.8° , 42.5° , 56.3° , and 65.7° corresponded with orthorhombic γ - MnO_2 (JCPDS card No. 82-2169); whereas, α - MnO_2 was formed by heating γ - MnO_2 at 600°C with specific peaks at $2\theta = 28.58^\circ$, 37.48° , 49.78° , 59.98° , and 68.98° (JCPDS card No. 01-072-1982). Surface properties, which affect the adsorption capacity of both materials, were determined by Scanning Electron Microscope (SEM) (Fig. 2) and TEM (Fig. 3). The comparison between SEM and TEM images of γ - MnO_2 and α - MnO_2 provided that γ - MnO_2 nanomaterial had a porous surface including many nanospheres while α - MnO_2 consisted of a lot of nanorods which were bigger than nanospheres. Moreover, the surface area of γ - MnO_2 was $65.00 \text{ m}^2/\text{g}$, which was approximately 6.5 times higher than that of α - MnO_2 (about $9.37 \text{ m}^2/\text{g}$) (Table 3). It can be predicted that adsorption properties of γ - MnO_2 were more favourable than that of α - MnO_2 .

Investigation of factors affecting the adsorption

The pH and adsorption contact time are important factors affecting the adsorption of Zinc(II) ions on α - and γ - MnO_2 nanomaterials. As shown in Fig. 4A, at low pH values, the uptake of Zn(II) onto these materials was lower because the H^+ ions effectively compete with the Zn^{2+} [14]. At high pH values, the adsorption of Zinc(II) ion also decreased due to the formation of different types of Zinc(II) such as $\text{Zn}(\text{OH})^+$, $\text{Zn}(\text{OH})_2$ or ZnO_2^{2-} [15]. Although the charging behaviour of MnO_2 could induce

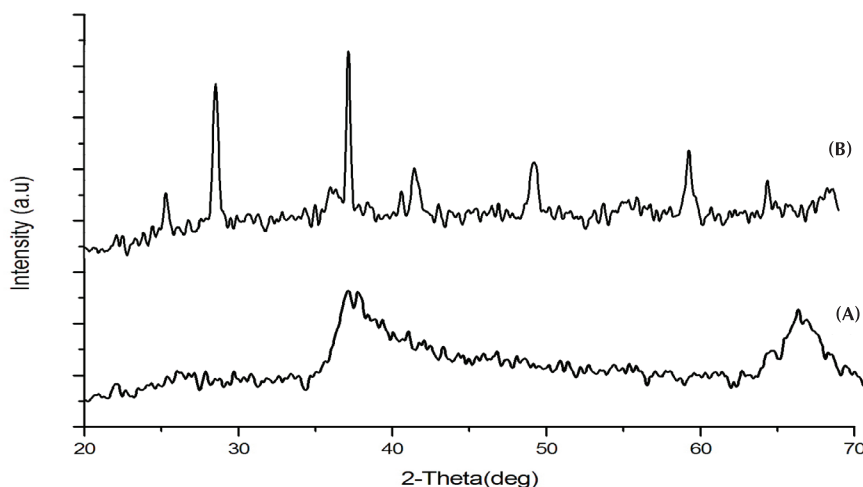


Fig. 1. XRD patterns of γ - MnO_2 (A) and α - MnO_2 (B).

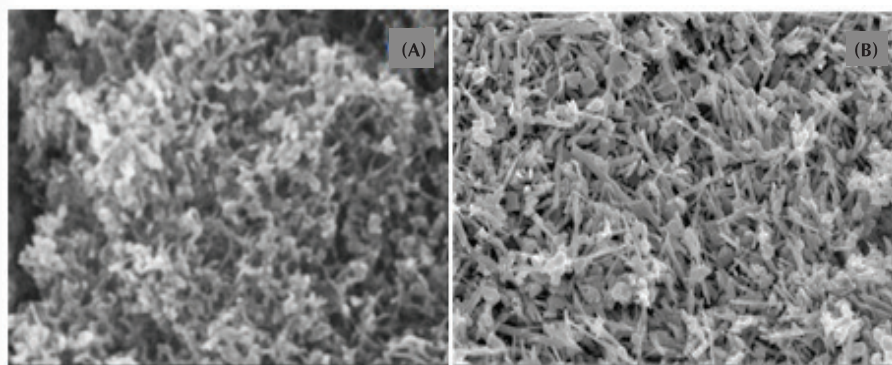


Fig. 2. SEM images of γ - MnO_2 (A) and α - MnO_2 (B).

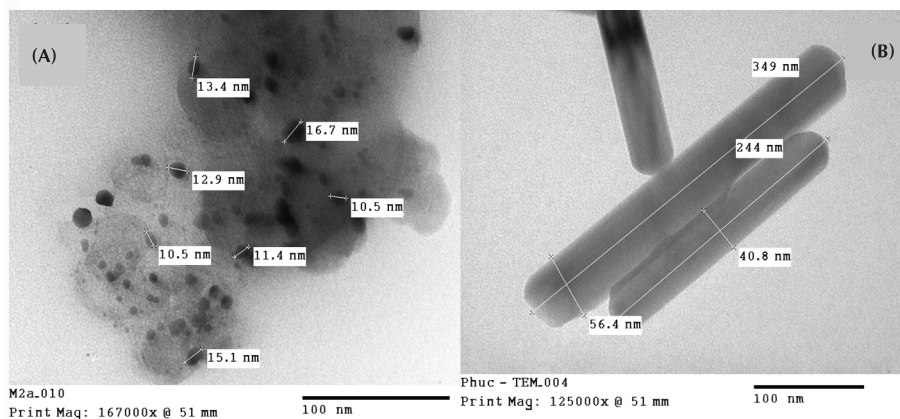


Fig. 3. TEM images of γ - MnO_2 (A) and α - MnO_2 (B).

Table 3. BET and BJH analysis results of γ - MnO_2 and α - MnO_2 .

Materials	BET surface area	BJH adsorption pore size	BJH desorption pore size
γ - MnO_2	$65.00 \text{ m}^2/\text{g}$	$417.83 \text{ \AA}$	$340.23 \text{ \AA}$
α - MnO_2	$9.37 \text{ m}^2/\text{g}$	$162.95 \text{ \AA}^0$	$734.37 \text{ \AA}^0$

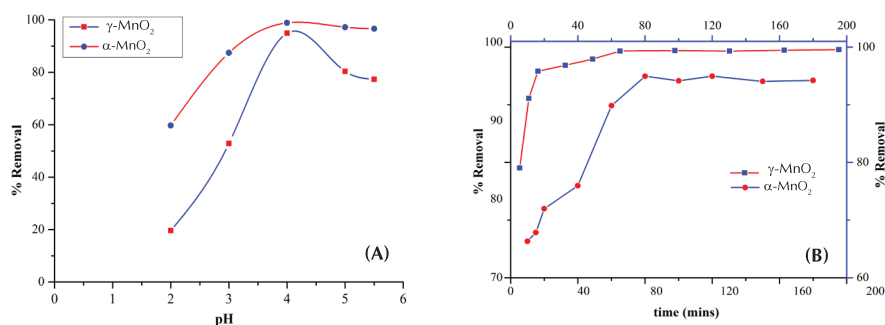


Fig. 4. The influence of pH (A) and adsorption contact time (B) to the removal of Zinc(II) by α - and γ -MnO₂ (240 rpm of shaking speed and 50 ppm of initial concentration).

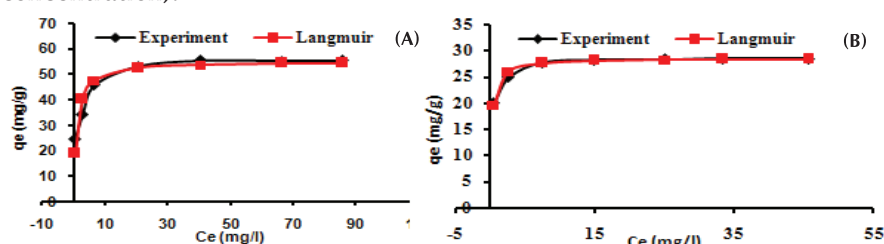


Fig. 5. Plots of non-linear isotherm Langmuir models of γ -MnO₂ (A) and α -MnO₂ (B).

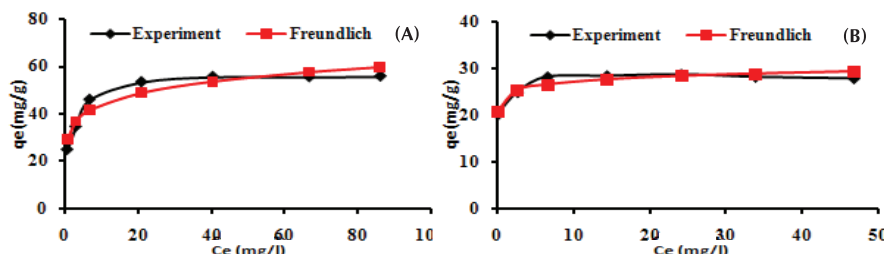


Fig. 6. Plots of non-linear isotherm Freundlich models of γ -MnO₂ (A) and α -MnO₂ (B).

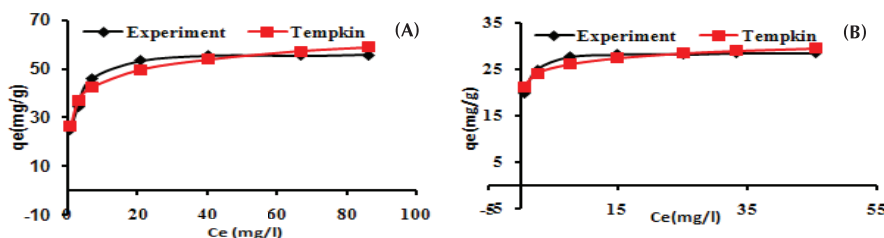


Fig. 7. Plots of non-linear isotherm Temkin models of γ -MnO₂ (A) and α -MnO₂ (B).

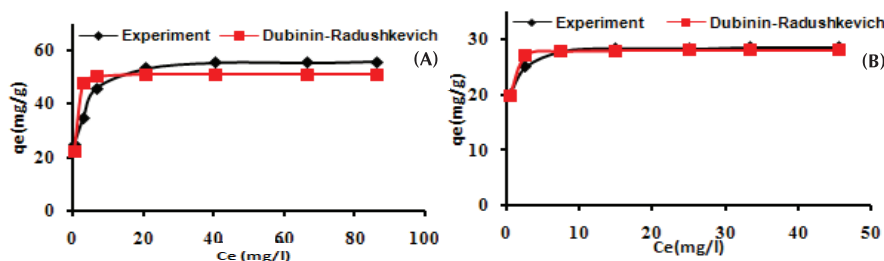


Fig. 8. Plots of non-linear isotherm Dubinin - Radushkevich models of γ -MnO₂ (A) and α -MnO₂ (B).

adsorption, this effect was not mentioned in the present study. Therefore, a range of pH values was chosen from 2.0 to 5.5. As a result, the maximum adsorption capacity obtained at pH=4.0 for both α - and γ -MnO₂ nano-adsorbents was approximately 94.96% removal for α -MnO₂ and nearly 98.90% removal for γ -MnO₂.

Figure 4B shows that the adsorption increases with the increase in the contact time and reaches equilibrium after 80 minutes for α -MnO₂ and 60 minutes for γ -MnO₂. However, the adsorption capacity of γ -MnO₂ was better than that of α -MnO₂ at any time.

Adsorption models studies

Isotherm models:

In order to predict adsorption mechanisms and assess the adsorption capacities of α - and γ -MnO₂ materials, four models namely Langmuir, Freundlich, Temkin, and Dubinin - Raduskevich were chosen and fitted with the experimental data.

On the one hand, Langmuir model assumes the uptake of Zinc(II) onto both materials to be monolayer adsorption. Plots of Langmuir models in Fig. 5 and non-linear isotherm Langmuir models parameters given in Table 4 provided that the experimental data of the adsorption of Zinc(II) ions on α -MnO₂ fitted to the Langmuir model better than that of γ -MnO₂, which corresponded with higher R² value and smaller RMSE and χ^2 values. In contrast, the maximum capacity of α -MnO₂ (28.50 mg/g) was two times less than that of γ -MnO₂ (55.23 mg/g). It was completely concordant with the porous structure of γ -MnO₂ with many adsorption sites.

On the other hand, Freundlich model assumes the adsorption of Zinc(II) ions as the multilayer adsorption and the interaction between adsorbate and adsorbent. As shown in Fig. 6 and

Table 4. Isotherm equilibrium parameters.

Isotherm	Nonlinear forms	Isotherm Parameters		
			$\gamma\text{-MnO}_2$	$\alpha\text{-MnO}_2$
Langmuir	$q_e = \frac{q_m \cdot K_L \cdot C_e}{1 + K_L \cdot C_e}$	K_L	0.0379	1.805
		q_m (mg/g)	55.23	28.76
		RMSE	0.619	0.1899
		R^2	0.9928	0.9877
		χ^2	0.0561	0.0078
Freundlich	$q_e = K_F \cdot C_e^{1/n}$	n	3.17	18.79
		K_F	10.19	23.44
		RMSE	1.036	0.687
		R^2	0.9798	0.8395
		χ^2	0.2031	0.1089
Temkin	$q_e = \frac{RT}{b_T} \ln(K_T C_e)$	K_T (l/mg)	0.4156	$7.34 \cdot 10^6$
		b_T (kJ/mol)	0.21	1.69
		RMSE	0.6380	0.6544
		R^2	0.9923	0.8542
		χ^2	0.0693	0.0981
Dubinin - Radushkevich	$q_e = q_m \cdot e^{(-\beta \cdot \epsilon^2)}$	q_m (mg/g)	44.16	28.17
		β	57.13	0.2859
		E (kJ/mol)	0.094	1.32
		RMSE	2.262	0.2972
		R^2	0.9037	0.9699
		χ^2	0.8348	0.0192

Table 4, the experimental data of the uptake onto $\alpha\text{-MnO}_2$ did not fit well to Freundlich model as $\gamma\text{-MnO}_2$ did. In addition, Zinc(II) ions could interact with $\gamma\text{-MnO}_2$ stronger than $\alpha\text{-MnO}_2$ because of the smaller n value of $\gamma\text{-MnO}_2$. Nevertheless, the interactions between Zinc(II) and both materials were favourable since the 1/n values of

$\alpha\text{-MnO}_2$ (0.0505) and $\gamma\text{-MnO}_2$ (0.1425) were less than 1.

Temkin and Dubinin-Radushkevich models were used to estimate the heat of the adsorption and the mean free energy of the uptake of Zinc(II) ions onto both materials. Fig. 7, Fig. 8 and Table 4 showed that the experimental data fitted

to Temkin model better than Dubinin-Radushkevich model for $\gamma\text{-MnO}_2$; whereas, $\alpha\text{-MnO}_2$ followed Dubinin - Radushkevich model. Energy values calculated from both models to be less than 8 kJ/mol provided that there was a weak interaction between the absorbent and adsorbate [16] and the adsorption of Zinc(II) ions onto $\alpha\text{-MnO}_2$ and $\gamma\text{-MnO}_2$ was essentially a physical process [8].

Kinetic models:

The uptake rate of Zn^{2+} ions onto $\alpha\text{-MnO}_2$ and $\gamma\text{-MnO}_2$ surface was described by three kinetic models, namely pseudo-first-order, pseudo-second-order, and intra-particle diffusion model. The calculated results showed that although the adsorption process partially followed both pseudo-first-order and pseudo-second-order equations for different time, the adsorption of Zinc(II) ions onto both materials was controlled by the pseudo-second-order model because of its higher R^2 values (Fig. 9 and Table 2). In addition, intra-particle diffusion model developed by Weber and Morris [17] was applied to identify the diffusion mechanism involved in the adsorption process. Fig. 10 showed that there were three stages in the uptake of Zn^{2+} ions onto both $\alpha\text{-MnO}_2$ and $\gamma\text{-MnO}_2$ surfaces. In the first one, Zn^{2+} ions were transferred from the solution to the material's surfaces. A gradual adsorption stage, in which the intra-particle diffusion was the controlling factor, was occurred in the second part. However, the plot did not pass through the origin. It was thereby concluded that the sorption can be controlled by two or more diffusion mechanisms [18]. The last one constituted the final equilibrium stage where the intra-particle diffusion started to decelerate. This can be explained that firstly, Zn^{2+} ion concentration in the solution was extremely low; and secondly, the adsorbent equilibrium was obtained when the number of adsorption sites decreased [19].

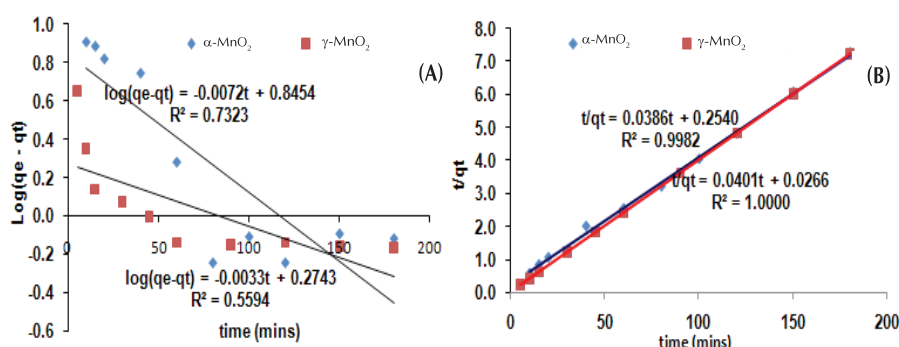


Fig. 9. Plots of pseudo-first-order (A), pseudo-second-order (B).

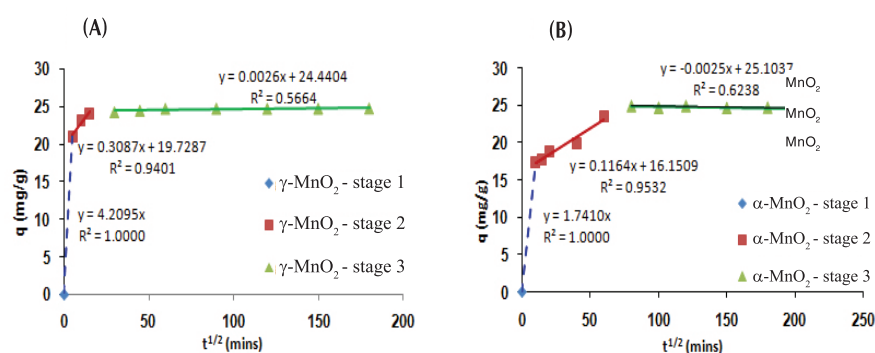


Fig. 10. Plots of intra-particle diffusion models of γ -MnO₂ (A) and α -MnO₂ (B).

Conclusions

To our knowledge, the comparison of the uptake of Zinc(II) ions onto α -MnO₂ and γ -MnO₂ nanomaterials in the optimal condition (4.0 of pH, 80 minutes of shaking time for α -MnO₂ and 60 minutes for γ -MnO₂, and 40-200 mg/l of initial concentration) is the first report. The results indicated that the maximum adsorption capacity calculated from Langmuir for γ -MnO₂ material was nearly two times higher than α -MnO₂. Energy values estimated from Temkin and Dubinin - Radushkevich models to be less than 8 kJ/mol informed that the uptake of Zinc(II) ions onto both materials was essentially a physical process. Kinetic studies showed that the adsorption data was well represented by pseudo-second-order models and the uptake of Zinc(II) ions onto both materials followed three stages.

REFERENCES

- [1] H. Ullah, S. Noreen, Fozia, A. Rehman, A. Waseem, S. Zubair, M. Adnan, I. Ahmad (2017), "Comparative study of heavy metals content in cosmetic products of different countries marketed in Khyber Pakhtunkhwa, Pakistan", *Arabian Journal of Chemistry*, **10**(1), pp.10-18.
- [2] C. Gakwisiri, N. Raut, A. Al-Saadi, S. Al-Aisri, A. Al-Ajmi (2012), "A Critical Review of Removal of Zinc from Wastewater", In: *Proceedings of the World Congress on Engineering*, London, U.K., p.4.
- [3] M.A. Barakat (2011), "New trends in removing heavy metals from industrial wastewater", *Arabian Journal of Chemistry*, **4**(4), pp.361-377.
- [4] K.S. Tushar, G. Dustin (2011), "Adsorption of zinc (Zn²⁺) from aqueous solution on natural Bentonite", *Desalination and Water Treatment*, **26**(2-3), pp.286-294.
- [5] M. Minceva, L. Markovska, V. Meshko (2007), "Removal of Zn²⁺, Cd²⁺ and Pb²⁺ from binary aqueous solution by natural zeolite and granulated activated carbon", *Macedonian Journal of Chemistry and Chemical Engineering*, **26**(2), pp.125-134.
- [6] K. Abidin, H. Ali (2005), "Oren adsorption of zinc from aqueous solutions to bentonite", *Journal of Hazardous Materials*, **125**(1-3), pp.183-189.
- [7] K. Rout, M. Mohapatra, B.K. Mohapatra, S.

Anand (2009), "Pb(II), Cd(II) and Zn(II) adsorption on low grade manganese ore", *International Journal of Engineering, Science and Technology*, **1**(1), pp.106-122.

[8] R.R. Bhatt, B.A. Shah (2015), "Sorption studies of heavy metal ions by salicylic acid-formaldehyde-catechol terpolymeric resin: Isotherm, kinetic and thermodynamics", *Arabian Journal of Chemistry*, **8**(3), pp.414-426.

[9] C. Necla, R.K. Ali, A. Salih, G.S. Eda, A. Ihsan (2011), "Adsorption of Zinc(II) on diatomite and manganese-oxide-modified diatomite: A kinetic and equilibrium study", *Journal of Hazardous Materials*, **193**, pp.27-36.

[10] J. Li, B. Xi, Y. Zhu, Q. Li, Y. Yan, Y. Qian (2011), "A precursor route to synthesize mesoporous γ -MnO₂ microcrystals and their applications in lithium battery and water treatment", *J. Alloy. Compd.*, **509**(39), pp.9542-9548.

[11] L. Ngoc Chung, D. Van Phuc (2015), "Sorption of lead(II), cobalt(II) and copper(II) ions from aqueous solutions by γ -MnO₂ nanostructure", *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, **6**(2), 025014.

[12] V.P. Dinh, N.C. Le, T.P.T. Nguyen, N.T. Nguyen (2016), "Synthesis of α -MnO₂ Nanomaterial from a Precursor γ -MnO₂: Characterization and Comparative Adsorption of Pb(II) and Fe(III)", *Journal of Chemistry*, **2016**(2016), 8285717.

[13] K.Y. Foo, B.H. Hameed (2010), "Insights into the modeling of adsorption isotherm systems", *Chemical Engineering Journal*, **156**(1), pp.2-10.

[14] C.P.J. Isaac, A. Sivakumar (2013), "Removal of lead and cadmium ions from water using Annona squamosa shell: kinetic and equilibrium studies", *Desalination and Water Treatment*, **51**(40-42), pp.7700-7709.

[15] A. Heidari, H. Younesi, Z. Mehraban, H. Heikkinen (2013), "Selective adsorption of Pb(II), Cd(II), and Ni(II) ions from aqueous solution using chitosan-MAA nanoparticles", *Int. J. Biol. Macromol.*, **61**, pp.251-263.

[16] J. Anwar, U. Shafique, Waheed-uz-Zaman, M. Salman, A. Dar, S. Anwar (2010), "Removal of Pb(II) and Cd(II) from water by adsorption on peels of banana", *Bioresource Technology*, **101**(6), pp.1752-1755.

[17] W.J. Weber, J.C. Morris (1963), "Kinetics of adsorption carbon from solutions", *Journal Sanitary Engineering Division: Proceedings of American Society of Civil Engineers*, **89**(2), pp.31-60.

[18] S. Vasiliu, I. Bunia, S. Racovita, V. Neagu (2011), "Adsorption of cefotaxime sodium salt on polymer coated ion exchange resin microparticles: Kinetics, equilibrium and thermodynamic studies", *Carbohydr. Polym.*, **85**(2), pp.376-387.

[19] F.C. Wu, R.L. Tseng, R.S. Juang (2000), "Comparative adsorption of metal and dye on flake- and bead-types of chitosans prepared from fishery wastes", *Journal of Hazardous Materials*, **73**(1), pp.63-75.

The development of the GC-MS analytical method used for the determination of the isotope ratio of linalool in yuzu essential oil from different geographic origins

Thi Thao Nguyen^{1*} and Masayoshi Sawamura²

¹School of Biotechnology and Food Technology, Hanoi University of Science and Technology

²Faculty of Agriculture, Kochi University

Received 6 April 2017; accepted 16 August 2017

Abstract:

A refined analytical method has been developed for the determination of the isotope ratio of oxygenated compounds in essential oils, using high-resolution gas chromatography-mass spectrometry (HRGC-MS), has been developed. Thirty-three samples of yuzu fruits from different production areas in Japan and South Korea were collected and prepared for cold-pressed peel oils. The oils were analysed by HRGC-MS for linalool concentration and isotope ratio based on the peak intensities of (M+2)⁺, (M+1)⁺ and M⁺ ions. A significantly lower isotope ratio m/z 156/154 and m/z 155/154 were observed for the yuzu essential oil from Goheung and Kyoto areas. Statistical analysis showed the isotope ratio of linalool to be useful in the discrimination of yuzu essential oils from different geographical origins.

Keywords: essential oil, GC-MS, geographical origin, isotope ratio, linalool, yuzu.

Classification number: 2.2

Introduction

Food authenticity is a term which simply refers to whether the food purchased by the consumer matches its description. There are a number of consumer-driven forces for reliable analytical methods to verify the provenance of the food we eat and there is growing enthusiasm amongst consumers for high-quality foods with clear regional identities. It is reasonable to suggest that there should be analytical methods in place that can verify the information provided on origin labels describing the origins of foods.

The stable isotope ratio of constituents is among the many criteria that have been used as discriminators of food authenticity [1, 2]. Naturally abundant isotope ratios of elements exist in a fixed ratio; however, many natural phenomena, classed as physicochemical effects, can also lead to isotope fractionation. The stable isotope ratios of water (oxygen and hydrogen), therefore, can yield unique geographic information [3], primarily because of the predictable spatial variation of precipitation stable isotope ratios across the Earth's surface [4, 5]. This spatial variation in precipitation composition is recorded in plant material since plants take up soil and water, which is derived generally from local precipitation and incorporates the hydrogen and oxygen atoms into the products needed for

photosynthesis [6]. This distinction of isotope content can be transferred to plants, creating an isotopic “fingerprint” for geographical characteristics of vegetation. The stable isotope content is determined by isotope ratio mass spectrometry (IRMS) and site-specific natural isotope fractionation determined by nuclear magnetic resonance (SNIF-NMR). These instruments, however, are expensive and rare in normal food and flavour analysis laboratories.

Gas chromatography-mass spectrometry (GC-MS) is an indispensable analytical instrument for analysing food and flavour compounds, especially for analysing volatile compounds. Sawamura, et al. have developed a new analytical method for the differentiation and characterization of citrus essential oils derived from different species and producing areas found on the basis of isotope ratio. The isotope ratio is determined based on the isotope peak and molecular peak of monoterpene hydrocarbons. A combination of the determination of the isotope ratio of multi-components and multivariate analysis results in good discrimination of citrus essential oils of different botanical [7, 8] and geographical origins [9].

In the essential oils of citrus species, oxygenated compounds account for a small but important fraction of keynote compounds. Among the oxygenated compounds, linalool is a main

*Corresponding author: Email: thao.nguyenthi@hust.edu.vn

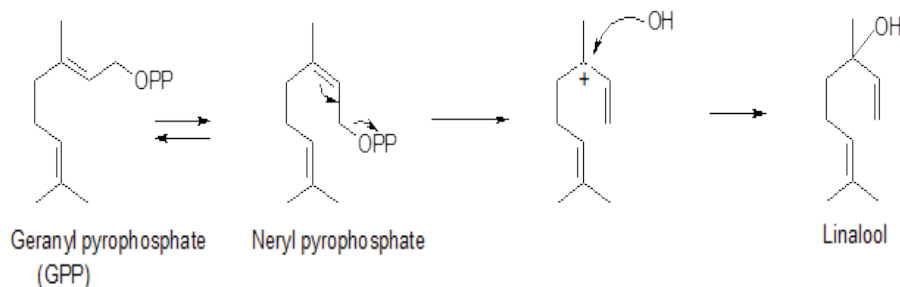


Fig. 1. The biosynthetic pathway of linalool from geranyl pyrophosphate (GPP).

oxygenated compound found in citrus essential oil [10]. Naturally, linalool is synthesised from the universal precursor geranyl pyrophosphate (GPP), catalysed by a membrane-bound enzyme, linalool synthase (Fig. 1) [11]. During this biosynthesis process, water is attached to the carbon frame and therefore, it is expected that it brings in an isotope ratio of hydrogen and oxygen, and thusly, the geographical information of isotope ratio. However, the determination of the isotope ratio of oxygenated compounds via current methods is challenging due to the weak signal strength of the isotope and molecular peaks. In this study, we designed a new approach to the determination of the isotope ratio of oxygen in order to find an additional analytical parameter to be used for the discrimination of essential oils and fruits from different origins. Yuzu (*Citrus junos* Tanaka), an important sour citrus fruit in Japan, and is especially found in Kochi prefecture, was investigated. Yuzu essential oils were derived from different producing areas in Japan and Korea, and were analysed for their isotope ratios through means of HRGC-MS.

Materials and methods

Materials

Authentic linalool was obtained from Tokyo Kasei Kogyo Co. Ltd. Standard solutions of linalool were prepared at different concentrations with purified acetone (purity $\geq 99.8\%$, Kanto Chemical Co., Inc.). 33 samples of yuzu were collected from Japan and South Korea (Fig. 2). The essential oil was prepared using the cold-pressing method to isolate citrus essential oils [12].



Fig. 2. Yuzu Sampling in Japan and Korea.

GC-MS

Analysis was carried out using a GC-6890N instrument (Agilent Technologies) coupled with a JMS-Q1000 GTA mass spectrometer (Jeol Datum) at an MS ionization energy of 70 eV; detector voltage, 1000 V; ionization current, 100 mA; and ion source temperature of 250°C. The GC column was a DB-Wax fused-silica capillary type (60 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness; J & W Scientific, Folsom, CA, USA). To determine the linalool peak relative percentage, scan mode was used. An oil sample of 1 μ l, which had been diluted with acetone (1:5), was automatically injected at a split ratio of 1:100. The column temperature was programmed from 70°C (2-min hold) to 100°C at a rate of 2°C/min and then heated to 230°C (held for 15 min) for sterilisation at the end of each run. The injector temperature was 250°C, and helium was used as the

carrier gas at a flow rate of 0.8 ml/min.

Determination of isotope ratio of linalool by GC-MS

Isotope ratio is defined as the ratio of the concentration of ions in an isotope and molecular peak, directly observed by the signal area of ions' peak in the mass spectrometry (MS). The selected ion monitoring mode (SIM) was employed to enable the sensitivity of MS analyses. Three ions of linalool were determined for ion concentrations: the molecular ion (M^+): m/z 154, the isotope ion ($M+1$) $^+$: m/z 155 and ($M+2$) $^+$: m/z 156. An optimised condition of MS was developed because the signal strength of an isotope peak m/z 156 is difficult to observe under normal analytical conditions. An oil sample of 1 μ l, which had been diluted with acetone (1:5), was automatically injected at a split ratio of 1:10. The ion source temperature was 150°C; ionisation current, 200 mA; the detector voltage, 1500 V; and the scanning rate of m/z 154 ion and m/z 155 ion was 50 cycles per second, while that of m/z 156 was 900 cycles per second.

The isotope ratio (Ir) was calculated using the following equation:

$$Ir (\%) = \frac{\text{Intensity of isotope peak}}{\text{Intensity of molecular peak}} \times 100$$

where: the isotope peaks were m/z 155 and m/z 156, respectively; and the molecular peak was m/z 154. Each value is the mean of replicate measurements of isotope ratio values.

Statistical analysis

All measurements were carried out in triplicate so that an average value and standard deviation of the isotope value could be calculated to evaluate the repeatability of the method. Analysis of variance (one-way ANOVA) was conducted to differentiate samples by means of the isotope ratio values. All statistical analyses were done using SPSS software for Windows (version 11.5, SPSS, Chicago, 2002).

Results and discussions

Accuracy of the isotope ratio by ordinary GC-MS

In principle, it is possible to obtain the isotope ratio from MS data [13]. The isotope peak contains the total isotopic abundance in the molecule. Authors have previously shown, in fact, a practical use for the isotope ratio of monoterpene hydrocarbon from mass spectrometry [7-9, 14]. However, the determination of isotope ratio in oxygenated compounds was difficult since the intensity of each molecular ion peak is not very strong after undergoing the fragmentation due to ionisation process of MS. In addition, the isotope peak ($M+1$) is approximately 10%, whereas the isotope peak ($M+2$) is approximately 1%, respectively, and of those observations, the molecular ion peak in the case of linalool in citrus essential oils is seen. Therefore, several experimental conditions have been developed to achieve a sensitivity of the analytical method for determining isotope ratio value. By choosing the softer ionisation energy (50 eV), suitable detector voltage, and scanning rate of the monitored ion, the desired ion peaks were enabled since optimisation of the analysis device and the analysis condition was attempted in the actual experiment. The mass peaks observed in the mass spectrum are m/z 154 (M)⁺, m/z 155 ($M+1$)⁺ and m/z 156 ($M+2$)⁺. When taking the ratio of those peak intensities, the isotope ratio m/z 155/154 accordingly gives the total ratios of $^{13}\text{C}/^{12}\text{C}$, $^2\text{H}/^1\text{H}$, and also $^{17}\text{O}/^{16}\text{O}$; and the isotope ratio m/z 156/154 gives information on $^{18}\text{O}/^{16}\text{O}$ and $^{13}\text{C}/^{12}\text{C}$.

The repeatability of the Ir values for linalool was examined using different linalool solutions. The concentrations of standard linalool solutions were 5 to 30 mg/g (w/w). Data is shown in Table 1, the isotope ratio m/z 155/154 varied from 11.50 to 11.60% whereas the isotope ratio value m/z 156/154 was 1.00%. These Ir values varied within a narrow range and in accordance with theoretical calculations from natural abundances

Table 1. The isotope ratio (%) of authentic linalool solutions at different concentrations.

Linalool concentration mg/g (w/w)	m/z 156/154		m/z 155/154	
	Mean ¹	SD	Mean ¹	SD
5	1.10	0.02	11.64	0.06
10	1.06	0.05	11.59	0.05
15	1.11	0.03	11.52	0.04
20	1.09	0.02	11.50	0.02
25	1.11	0.03	11.54	0.01
30	1.09	0.01	11.55	0.01

¹n=5.

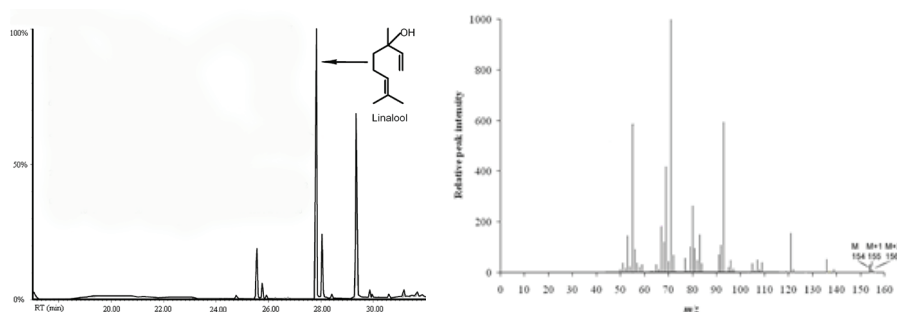


Fig. 3. GC chromatogram of yuzu essential oil by capillary GC-MS and MS spectrum of linalool.

of isotopes of carbon, hydrogen, and oxygen. The results obtained from the authentic linalool solution show that the reproducibility for determining the peaks of the molecular ion and its isotope using ordinary GC-MS is satisfactory and is applicable for practical use.

The repeatability, evaluated on relative chromatographic peak areas of the standard linalool solution using ten replicates that were analysed in the same day, was 3.1%. The reproducibility, calculated using five replicates of the same solution analysed in different days, was 8.8%. The limits of detection and the limits of quantitation were calculated from the concentration that would give up to three and ten times, respectively, and were also reported. The limit of detection (LOD) and limit of quantification (LOQ) were determined from a series of low-concentration measurements of the authentic linalool calibration solutions. The data

processing was proved under SIM mode to increase the specificity and sensitivity of the measurement. However, since the isotope peak of linalool was small compared to the molecular peak, we need to determine the isotope peaks m/z 155 (M)⁺ and m/z 156 (M)²⁺ so the detection limit was dependent on the appearance of those isotope peaks. The resulting LOD and LOQ were 10 and 35 mg/kg, respectively.

The isotope ratios of linalool in yuzu essential oils from different producing areas

A proper separation of linalool from other volatile compounds of the essential oil is a prerequisite to the determination of isotope ratio value. The linalool fraction was well separated from the other volatile compounds in the yuzu essential oil by employing appropriate column and column temperature program. The investigated compound

Table 2. Relative peak area percentage (%) and isotope ratio of linalool from yuzu essential oils.

No	Sample ¹	Relative peak area (%)	Isotope ratio (%)			
			<i>m/z</i> 155/154		<i>m/z</i> 156/154	
			Mean ²	SD	Mean ²	SD
1	EH1	3.04	14.35	0.49	1.02	0.01
2	EH2	2.19	12.91	0.06	0.91	0.00
3	EH3	1.97	12.38	0.09	0.88	0.00
4	OT1	2.95	14.46	0.63	1.03	0.02
5	OT2	2.31	13.24	0.09	0.95	0.01
6	OT3	2.24	13.28	0.13	0.94	0.02
7	WK1	2.90	14.78	0.17	1.04	0.02
8	WK2	2.36	13.54	0.03	0.96	0.01
9	WK3	2.61	13.83	0.13	0.98	0.01
10	TK1	2.71	14.07	0.60	1.00	0.02
11	TK2	2.59	13.86	0.14	0.98	0.01
12	TK3	2.58	13.84	0.09	0.98	0.01
13	KC1	2.98	13.05	0.69	0.88	0.18
14	KC2	2.14	12.59	0.35	0.90	0.01
15	KC3	1.72	11.77	0.19	0.83	0.00
16	KC4	1.25	10.84	0.06	0.91	0.17
17	KC5	1.28	10.89	0.12	0.92	0.16
18	KC6	1.27	11.45	0.05	1.10	0.01
19	KC7	1.31	11.10	0.66	0.94	0.03
20	KC8	1.28	10.67	0.05	0.97	0.05
21	KC9	1.24	10.71	0.09	0.97	0.04
22	KC10	1.28	10.76	0.20	0.92	0.05
23	KC11	1.31	10.76	0.10	0.97	0.03
24	KC12	1.28	10.69	0.09	0.96	0.02
25	KC13	1.18	10.78	0.06	1.05	0.07
26	KC14	1.32	11.13	0.73	0.96	0.01
27	KC15	1.35	10.69	0.17	0.94	0.03
28	KC16	1.40	10.64	0.02	0.96	0.04
29	KC17	1.38	11.08	0.03	1.00	0.03
30	KC18	1.46	11.04	0.61	0.94	0.00
31	KC19	1.46	10.93	0.04	0.99	0.01
32	KYO	2.55	11.61	0.02	0.93	0.04
33	GOH	3.58	11.53	0.04	0.91	0.06

¹Abbreviated name of samples: KC: Kochi; EH: Ehime; TK: Tokushima; OT: Oita; WK: Wakayama; KYO: Kyoto; GOH: Goheung (Korea).

²n = 3.

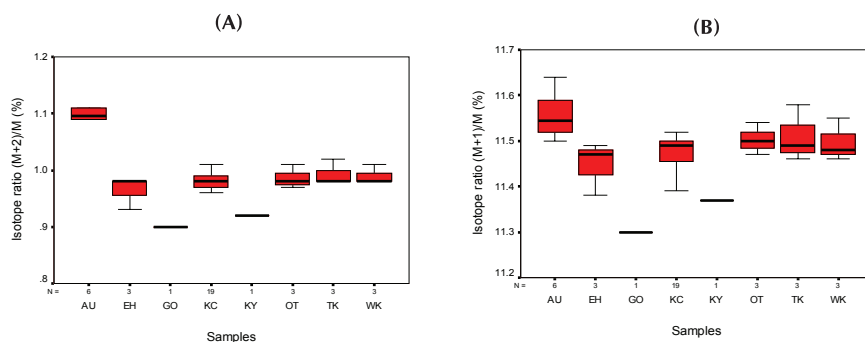


Fig. 4. Boxplot of isotope ratio (M+2)/M (A) and (M+1)/M (B) of linalool from authentic chemicals and yuzu essential oils from different producing areas. Samples from different areas are marked as Authentic chemicals, AU; Ehime, EH; Goheung, GO; Kochi, KC; Kyoto, KY; Oita, OT; Tokushima, TK; Wakayma, WK.

was best separated on DB-Wax column with high polarity. Fig. 3 shows the linalool peak in the GC chromatogram of yuzu essential oil under the actual experimental condition.

Relative peak area and isotope ratio of linalool from 33 yuzu essential oils are shown in Table 2. The isotope ratio *m/z* 156/154 of linalool in standard solution and in yuzu CPO from different producing areas is plotted in the boxplot (Fig. 4). It is obvious that the standard linalool has a significantly higher isotope ratio value (mean Ir=1.10% with SD=0.02%). The isotope ratio values of linalool from yuzu samples which were grown in Kochi (KC), Ehime (EH), Oita (OT), Tokushima (TK), and Wakayama (WK), were not different from one another. The average isotope ratio value ranged from 0.96 to 1.02% with a standard deviation less than 0.6%. The isotope ratio of linalool in GOH and KYO samples, on the other hand, significantly lower than the other yuzu samples. Ir values were 0.89 and 0.91% for GOH and KYO, respectively. These differences can be explained by the fact that authentic linalool from which the compound was isolated and/or synthesised was obtained from different sources.

Conclusions

In conclusion, to the best of our knowledge, the isotope ratio of oxygenated compounds in yuzu essential oils by HRGC-MS is reported here for the first time. The analytical method was developed and optimised for the observation of linalool isotope peaks and molecular peaks and showed high repeatability. The isotope ratio of linalool varied considerably among the samples from different regions, but could not readily be differentiated on the basis of isotope ratio value alone. The isotope ratio depends on the genealogy and geographical factors of the plant [6]. However, in this study, when all the

samples were obtained from the same botanical origin, the effect of genealogy may be eliminated. Thus the isotope ratio reflexes the effect of producing area. The significant lower isotope ratio of the samples from higher latitude is in agreement of lower isotope ratio in the region of higher latitude. This finding suggests a value for the application of GC-MS to authenticity control by means of component isotope ratio.

ACKNOWLEDGEMENTS

This work was financially supported by the Sasakawa Research Grant from The Japan Science Society. The authors are also indebted to Dr. Song Hee Sun, Gwangju Health College, Korea, for her kind cooperation with sample collection.

REFERENCES

- [1] A. Rossmann (2001), "Determination of stable isotope ratios in food analysis", *Food Reviews International*, **17**, pp.347-381.
- [2] Christopher N. Rhodes, Janice H. Lofthouse, Simon Hird, Paul Rose, Paul Reece, Julie Christy, Roy Macarthur, Paul A. Brereton (2010), "The use of stable carbon isotopes to authenticate claims that poultry have been corn-fed", *Food Chemistry*, **118**, pp.927-932.
- [3] S. Asche, A.L. Michaud, J.T. Brenna (2003), "Sourcing organic compounds based on natural isotopic variations measured by high precision isotope ratio mass spectrometry", *Current Organic Chemistry*, **7**, pp.1527-1543.
- [4] Y. Yuntseover, and J.R. Gat (1981), *Atmospheric water*, IAEA technical report series 210-stable isotope hydrology, pp.103-142.
- [5] H. Craig (1961), "Isotopic Variations in Meteoric Waters", *Science*, **133(3465)**, pp.1702-1703.
- [6] T.E. Dawson, S. Mambelli, A.H. Plamboeck, P.H. Templer, K.P. Tu (2002), "Stable isotopes in plant ecology", *Annual Review of Ecology and Systematics*, **33(1)**, pp.507-559.
- [7] N.T. Thao, T. Kashiwagi, M. Sawamura (2007), "Characterization by GC-MS of Vietnamese Citrus Species and Hybrids Based on the Isotope Ratio of Monoterpene Hydrocarbons", *Biosci. Biotechnol. Biochem.*, **71(9)**, pp.2155-2161.
- [8] A. Satake, A. Une, T. Ueno, H. Ukeda, M. Sawamura (2003), "Isotope ratio analysis by HRGC-MS of monoterpene hydrocarbons from citrus essential oils", *Biosci. Biotechnol. Biochem.*, **67(3)**, pp.599-604.
- [9] M. Sawamura, T. Ito, A. Une, H. Ukeda, Y. Yamasaki (2001), "Isotope ratio by HRGC-MS of citrus *Junos tanaka* (yuzu) essential oils: *m/z* 137/136 of terpene hydrocarbons", *Biosci. Biotechnol. Biochem.*, **65(12)**, pp.2622-2629.
- [10] M. Sawamura (2000), "Volatile components of essential oils of the *Citrus* genus", *Agricultural & Food Chem.*, **4(1)**, pp.131-164.
- [11] M. Sawamura (1994), "Citrus flavour", *Kagaku to seibutsu*, **32**, pp.114-119.
- [12] S.M. Njoroge, H. Koaze, P.N. Karanja, M. Sawamura (2005), "Volatile constituents of redblush grapefruit (*Citrus paradisi*) and pummelo (*Citrus grandis*) peel essential oils from Kenya", *J. Agric. Food Chem.*, **53(25)**, pp.9790-9794.
- [13] F.W. McLafferty, and F. Turecek (1993), *Interpretation of mass spectra*, University Science Books, p.371.
- [14] A. Satake, K. Furukawa, T. Ueno, H. Ukeda, M. Sawamura (2004), "MS fragment isotope ratio analysis for evaluation of citrus essential oils by HRGC-MS", *Biosci. Biotechnol. Biochem.*, **68(2)**, pp.312-316.

Immobilization of ZnO nanoparticles on fluorinated perlite granules for the photocatalytic degradation of methylene blue

Nguyet Anh Pham, Thi Huynh Nhu Nguyen, Tuan Ngoc Tran, Quy Tu Nguyen, Tien Khoa Le*

University of Science - Vietnam National University, Ho Chi Minh City

Received 15 June 2017; accepted 11 September 2017

Abstract:

The fluorination of perlite granules and the immobilization of ZnO nanoparticles on the perlite surface were carried out at the same time by a simple impregnation method to create new highly photocatalytic materials which are easily separated from reaction solutions after treatment. The influence of perlite fluorination on the crystal structure, morphology, UV-visible absorption, and surface functional groups of ZnO, as well as on the ZnO content on perlite, was respectively characterized by XRD, FE-SEM, UV-Visible diffuse reflectance, FTIR and atomic absorption spectrometry. The photocatalytic activity was evaluated via the extent of degradation of methylene blue under UVA irradiation. According to the results, the fluorination of perlite leads to numerous effects on ZnO, such as the decline of ZnO cell parameters, the increase of ZnO content on perlite granules (resulting in the enhancement of light absorption in UVA range), and the decrease of ZnO particle size, which can effectively improve its photocatalytic performance. The photocatalysts were also found to be able to stay afloat on water, allowing for easy separation from the reaction solution.

Keywords: coating, fluorination, perlite granules, photocatalytic activity, ZnO nanoparticles.

Classification number: 2.2

Introduction

Due to their chemical structure and stability, most of the dyestuffs used in the textile industry, including methylene blue, are resistant to solvents and are difficult to eliminate by conventional wastewater treatment methods such as cost-effective biological techniques [1-3]. Although it is not a strongly toxic compound, exposure to methylene blue can cause rapid pulse, shock, cyanosis, and tissue necrosis in humans [4]. Hence,

the release of waste water containing methylene blue can lead to harmful environmental effects and damages to human health. Over the past few decades, the use of semiconductor photocatalysts based on TiO₂ nanopowders has proven to be a promising method of waste water treatment since various organic pollutants including methylene blue and other organic dye molecules can be completely degraded under UV irradiation in the presence of these photocatalysts [5-9]. Besides TiO₂, ZnO

is another semiconductor which has been investigated in recent years as an excellent material for photocatalytic processes owing to its photosensitivity, high stability, and low toxicity [10, 11]. In some photodegradation experiments, ZnO nanopowders exhibit activity superior to that of TiO₂ for the treatment of dye wastewater [12, 13]. However, there are two major drawbacks for the application of these suspended particles in practical wastewater treatment procedures: (i) the scattering of UV light by nanoparticles can limit photocatalytic activity and (ii) the catalytic nanoparticles are difficult to separate from the reaction solution [14]. Therefore, it is necessary to immobilize semiconductor photocatalysts on solid substrates in order to solve these problems.

Many materials have been studied for the immobilization of photocatalytic TiO₂ particles, such as glass [14, 15], stainless steel plate [16, 17], polymers [18, 19], alumina [20], and ceramics [21]. Recently, Hosseini, et al. [22] investigated the immobilization of TiO₂ on perlite granules for the photocatalytic degradation of phenol. Granular perlite is an amorphous volcanic glass with high porosity, making it suitable as a potential substrate for TiO₂ nanopowder. The second advantage of perlite granules is that they are very light, which allows

*Corresponding author: Email: ltkhoa@hcmus.edu.vn

them to stay afloat on the surface of wastewater [22]. As a result, the TiO_2 photocatalyst coated on perlite granules is easily exposed to the available radiation source to ensure efficient light absorption [22, 23]. Unfortunately, the coating of TiO_2 particles is mostly distributed on the external surfaces of perlite granules [23] which usually consist of smooth flat layers. This may limit the mechanical adhesion between photocatalytic particles and the perlite surface and then hinder the immobilization of nanopowders. Thus the surface of perlite granules still needs to be modified in order to make it an effective substrate for photocatalysts.

However, so far, up to our best knowledge, no research has been conducted on the modification of perlite surfaces for the immobilization of ZnO catalysts. Since perlite granules are mainly composed of SiO_2 (73%) [24], it is suggested that fluoride ions can modify the perlite surface by slowly corroding its silica components. Therefore, in this work, we have prepared new photocatalytic materials based on ZnO nanoparticles coated on fluoride-modified perlite granules by a simple one-step impregnation method in order to improve the bonding between ZnO particles and perlite substrate and then enhance their photocatalytic activity. The influence of fluoride contents used to modify perlite surfaces on the coating of ZnO and the photocatalytic performance were also investigated.

Experimental section

Sample preparation

The starting materials $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ and KF (99%, extra pure grade) were purchased from Sigma Aldrich. Methylene blue (MB) (analytical grade) was purchased from Merck. These chemicals were used as received without further purification. Perlite granules

obtained from Ninh Binh province (Vietnam) were treated with H_2SO_4 solution (2 mol/l) at 80 - 100°C for 30 minutes. Distilled water was used in all the experiments.

For the preparation of ZnO coated on fluoride-modified perlite granules, firstly, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was separately dissolved in water to obtain 250 ml of Zn^{2+} solution (1 mol/l). Then 5 g of perlite granules were added to this Zn^{2+} solution under regular stirring at room temperature. After that, 250 ml of a solution containing both $\text{K}_2\text{C}_2\text{O}_4$ (1 mol/l) and KF (the KF concentration varied from 1 to 3 mol/l) was added to the Zn^{2+} solution to fluorinate the surface of the perlite granules and to create the white ZnC_2O_4 precipitate deposited on their surface. The slurry containing perlite granules was regularly stirred for 30 minutes for the fluorination. Next, these perlite granules were separated from the slurry, washed with distilled water, dried at 150°C for two hours, and heated in air at 500°C for two hours. In the following manuscript, these samples were labelled as PZnOF-X (X is equal to 1, 2, and 3 corresponding to the KF concentration of 1, 2, and 3 mol/l, respectively).

ZnO was also coated on bare perlite granules (labelled as PZnO) by the same process without using KF. Moreover, fluorinated perlite granules without ZnO immobilization were prepared by stirring 5 g of granular perlite in 500 ml of KF solution (1 mol/l) and then dried at 150°C during two hours in order to investigate the effects of fluorination on granular perlite.

Characterization

The surface morphology and particle size of PZnO and PZnOF-X catalysts were studied by field emission scanning electron microscopy (FE-SEM) using a HITACHI S-4800 with an

acceleration voltage at 10 kV. FE-SEM micrographs of bare perlite granules and fluorinated perlite granules without ZnO immobilization were also taken. Their specific surface area was measured with a NOVA 1000e instrument and calculated using the BET (Brunauer-Emmett-Teller) equation.

The crystalline structure and phase composition of PZnO and PZnOF-X samples were characterized by powder X-ray diffraction (XRD) measurements, which were carried out by a BRUKER-Binary V3 X-ray diffractometer using $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). The accelerating voltage and the applied current were 40 kV and 40 mA, respectively. The Rietveld refinements were carried out using Fullprof 2009 structure refinement software [25].

In order to investigate the surface functional groups of prepared catalysts, their FT-IR spectra were recorded in the 4000-400 cm^{-1} frequency range at room temperature using a Bruker VERTEX 70 spectrometer.

The quantity of ZnO coated on the surface of different perlite samples was evaluated by atomic absorption spectrometry using a Shimadzu AA-6300 spectrometer. The ZnO/perlite and FZnO/perlite samples were separately ground into fine powder with a mortar and pestle and then stirred in HCl solution (6 mol/l) for 24 hours. Then the quantity of Zn^{2+} ions was measured at a wavelength of 213.9 nm.

UV-Visible diffuse reflectance spectra of the catalysts were measured using a Perkin-Elmer Lambda 850 Spectrophotometer which is equipped with a 15 cm diameter integrating sphere bearing the holder in the bottom horizontal position and calibrated with a certified Spectralon white standard (Labsphere, North Sutton, USA). The spectra were recorded at room

temperature in steps of 1 nm, in the range of 300–400 nm with a bandwidth of 2 nm.

Photocatalytic tests

The photocatalytic activities of PZnO and PZnOF-X samples were evaluated via the degradation of MB. The photocatalytic reactor consists of a glass beaker containing 250 ml of MB solution (10^{-5} mol/l) with 2.0 grams of catalysts, cooled by continuous water flow and stirred continuously by a mechanic agitator. The outer wall of the reactor is covered with an aluminium layer to block out any exterior light. The pH of suspensions was fixed at 7 and the reaction temperature was maintained at 30°C during the experiments. Prior to the irradiation, the solution containing catalysts was stirred for 60 minutes in the dark in order to obtain the MB adsorption equilibrium. Then the reaction solution was irradiated by an 8-W UV Philips light lamp placed about 10 cm above the solution surface. During the illumination, 5 ml of suspension was sampled and analyzed with an SP-300 Optima spectrophotometer.

Results and discussions

Figures 1A and 1B present the FE-SEM micrographs of perlite granules before and after fluorination, respectively. It was observed that the surface of bare granular perlite is composed of relatively smooth terraces which are randomly oriented and superimposed on each other. When the perlite sample was fluorinated, the surface was clearly corroded with increased roughness and the appearance of various shallow holes. BET measurements also indicated the increase of the specific surface area of perlite granules from 0.415 to 0.498 m^2/g when fluorinated, confirming the corrosion role of KF on perlite which may improve the immobilization of ZnO nanoparticles on the perlite surface. For

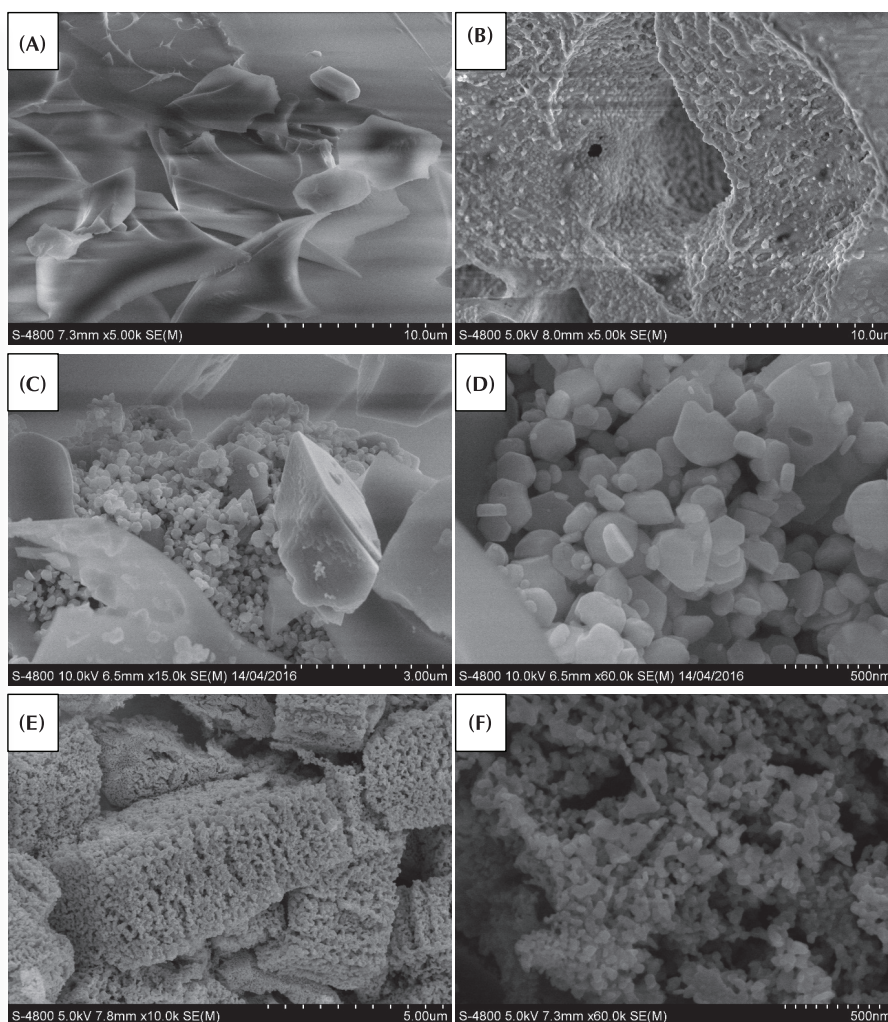


Fig. 1. FE-SEM micrographs at different magnifications of perlite granules (A), fluorinated perlite granules (B), PZnO (C, D) and PZnOF-2 (E, F).

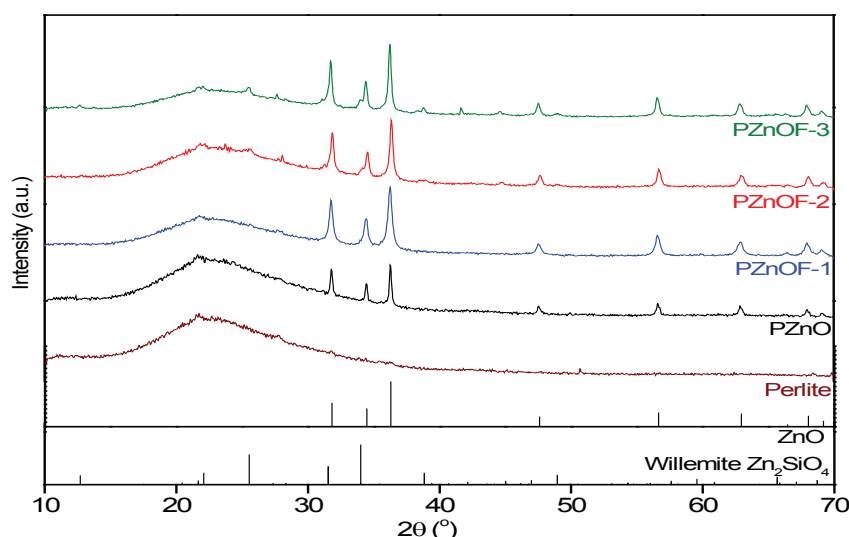


Fig. 2. XRD patterns of perlite, PZnO and fluorinated PZnO samples.

the PZnO sample, Fig. 1C and Fig. 1D display the presence of ZnO polyhedral particles, which demonstrates the successful immobilization of ZnO on the surface of perlite. These particles were found to be non-uniform in size (with a diameter of 100 - 300 nm) and tended to agglomerate. When ZnO was coated on fluorinated perlite (Fig. 1E and Fig. 1F for PZnOF-2 sample), the ZnO particles also appeared in large agglomerates but their particle size was reduced to around 50 nm. It should be noted that in this sample, nearly all the surfaces of fluorinated perlite granules were covered with ZnO nanoparticles whereas in the PZnO sample, the perlite surface was only partially covered with ZnO. Therefore, it seems that the fluorination of perlite does not only reduce the ZnO particle size but also increases the ZnO content on the perlite surfaces.

Powder XRD was used to follow the effects of perlite fluorination on the crystallite structures and phase compositions of ZnO coated on perlite granules. From Fig. 2, the perlite sample shows the XRD pattern in an arc-shaped baseline without any diffraction peak, confirming the amorphous structure of these granules, which is in agreement with Hosseini's findings [22]. For the pattern of PZnO powder, we observed a series of characteristic peaks at 31.77° ((100) line), 34.43° ((002) line), 36.26° ((101) line), 47.55° ((102) line) and 56.60° ((110) line). These diffraction peaks are in accordance with the zincite

phase of ZnO (space group P6₃mc, JCPDS No. 36-1451), which confirms that ZnO was successfully deposited on the surface of perlite granules. Moreover, when coating ZnO on fluorinated perlite with increasing KF concentration from 1 to 2 mol/l, no peaks of impurity were observed, suggesting that the fluorination did not modify the phase composition of ZnO. However, for the PZnOF-3 sample, the XRD pattern showed the apparition of willemite Zn₂SiO₄ phase (space group R-3, JCPDS No. 37-1485), identified by the diffraction peaks at 21.68°, 25.31° and 41.60°. The formation of this additional crystallographic phase may be attributed to the reaction between ZnO and the silicate components in the perlite composition, which was promoted by the addition of KF. Furthermore, it was observed that the cell parameters of ZnO were modified by the fluorination (Table 1). When ZnO was immobilized on fluorinated perlite with increased fluoride content, the cell parameters and the cell volume were decreased.

Table 2 represents the ZnO content in our samples measured by calculating the Zn concentration via atomic absorption spectrometry. The PZnO sample only contains 8.08 mg Zn g⁻¹ product. For ZnO nanoparticles immobilized on fluorinated perlite, the ZnO content was strongly increased, which is consistent with the observation in the FE-SEM study. The highest ZnO content was found in the PZnOF-2 sample with 71.44 mg Zn g⁻¹ product - nearly 9 times higher

than that found in the PZnO sample. This result suggests that KF may corrode the silicate component of perlite surfaces during the fluorination to improve the coating of ZnO. However, when KF concentration was increased to 3 mol/l, the ZnO content dramatically decreased (28.45 mg Zn g⁻¹ product), indicating that a high KF amount is capable of damaging the surface of perlite granules and thus lowering the bonding between ZnO nanoparticles and perlite granules.

Figure 3 presents the FT-IR spectra of PZnO and PZnOF-2 samples. From these spectra, two broad absorption peaks were observed around 1053.06 and 789.04 cm⁻¹, which are attributed to the stretching vibrations of Si-O and Si-O-Si bonds on the surface of perlite granules [26]. These spectra also display a sharp peak at 459.74 cm⁻¹ due to the stretching vibration of Zn-O [27], confirming the presence of ZnO deposited on the perlite surface. For the PZnO sample, another weak peak was detected at 1384.13 cm⁻¹. This peak may be ascribed to the C-O vibration originated from the adsorption of CO₂ on the surface of perlite granules [28, 29]. Nevertheless, this peak disappeared when the ZnO-perlite system was fluorinated. It should be noted that the fluorination of perlite promotes the coating of ZnO onto perlite granules, which may cover all the perlite surface and then hinder its adsorption of CO₂.

The optical responses of PZnO and PZnOF-2 samples were analyzed using UV-visible diffuse reflectance spectroscopy (Fig. 4). The spectrum of PZnO material shows a broad absorption band in the UV range below 400 nm (maximum absorption at the wavelength of 200-300 nm). When ZnO was immobilized on fluorinated perlite, the intensity of the absorption band in the visible zone slightly decreased whereas the absorption peak of the UV region

Table 1. Cell parameters and cell volumes of ZnO in PZnO and PZnOF samples.

Sample	Cell parameters		Cell volume (Å ³)
	<i>a</i> (Å)	<i>c</i> (Å)	
PZnO	3.25097	5.20838	47.67154
PZnOF-1	3.25031	5.20688	47.63846
PZnOF-2	3.24954	5.20495	47.59824
PZnOF-3	3.2493	5.20428	47.58509

Table 2. ZnO content and rate constant of MB bleaching under UVA light illumination on PZnO and PZnOF samples.

Sample	ZnO content determined by AAS (mg Zn g ⁻¹ sample)	Rate constant of MB bleaching under UVA illumination (h ⁻¹)
PZnO	8.08	0.33
PZnOF-1	21.31	0.87
PZnOF-2	71.44	1.23
PZnOF-3	28.44	0.90

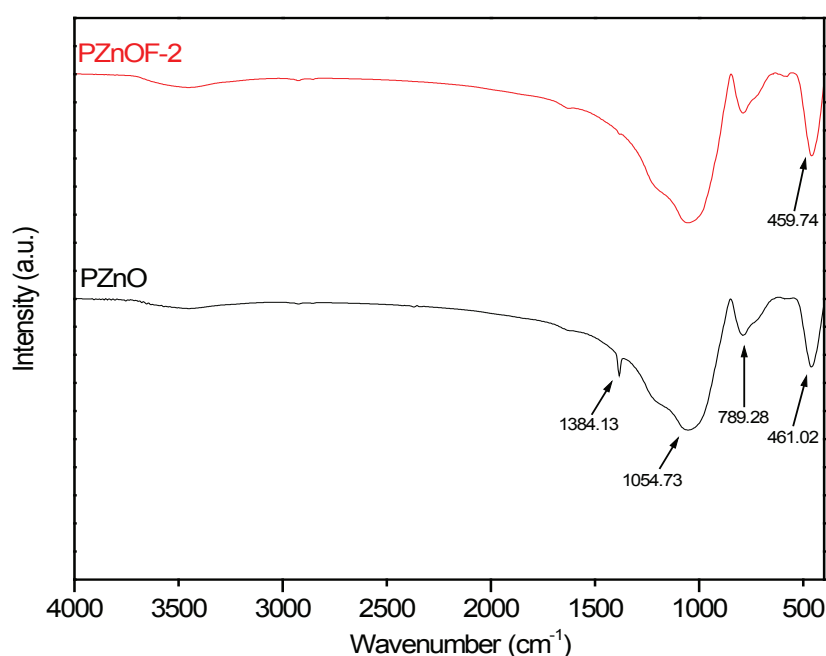


Fig. 3. FTIR spectra of PZnO and PZnOF-2 samples.

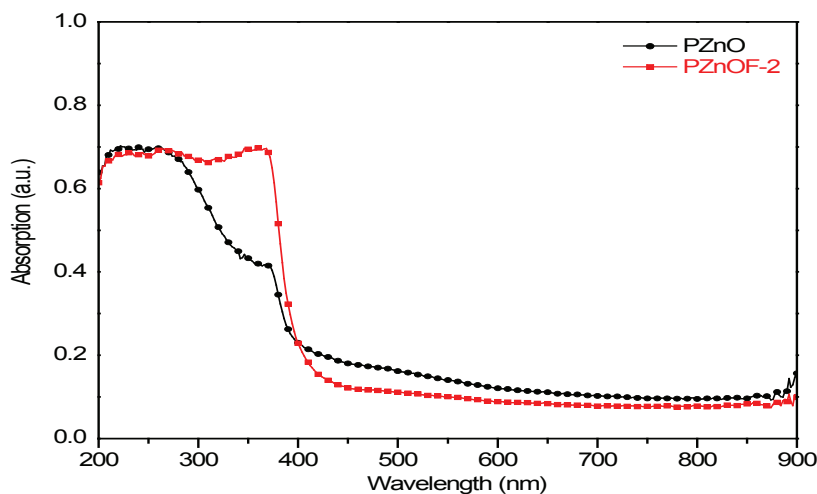


Fig. 4. UV-visible absorption spectra of PZnO and PZnOF-2 samples.

strongly rose in the range of 300-400 nm. This enhanced UV absorption of the PZnOF-2 sample can also be explained by the increase of ZnO content owing to the fluorination of perlite.

The UVA light induced photocatalytic activity of PZnO and PZnOF samples was evaluated via the photocatalytic MB degradation. The time-dependent profiles of MB degradation in the presence of our catalysts under UVA light irradiation (Fig. 5) prove that the net decomposition of MB in the aqueous solution followed the pseudo-first-order Langmuir-Hinshelwood kinetic model. Hence, the rate constant of this reaction was determined by plotting $\ln(C/C_0)$ versus time (C is the MB concentration at time t and C_0 is the initial MB concentration) and presented in Table 2. The catalytic tests indicated that the fluorination of perlite effectively improved the photocatalytic performance of ZnO supported on the perlite granules. In fact, the rate constant (k) of MB degradation in the presence of the PZnO sample only reached 0.33 h⁻¹ whereas the PZnOF-2 catalyst showed the best performance with $k = 1.23$ h⁻¹, which was about four times higher than that of the PZnO catalyst. The increase of photocatalytic activity in our samples can be explained by two factors. Firstly, based on the atomic absorption spectra and UV-visible reflectance diffuse spectra, the fluorination of perlite was found to successfully modify the surface of perlite granules, which increased the ZnO content on the perlite surface and then enhanced the UVA absorption of catalysts. It has been reported that the high photon absorption can promote the formation of photogenerated electrons and holes and then improve the photocatalytic activity [7, 30]. Secondly, the fluorination of perlite also decreased the particle size of ZnO. As a result, the active sites of photocatalytic ZnO were enhanced by the perlite fluorination,

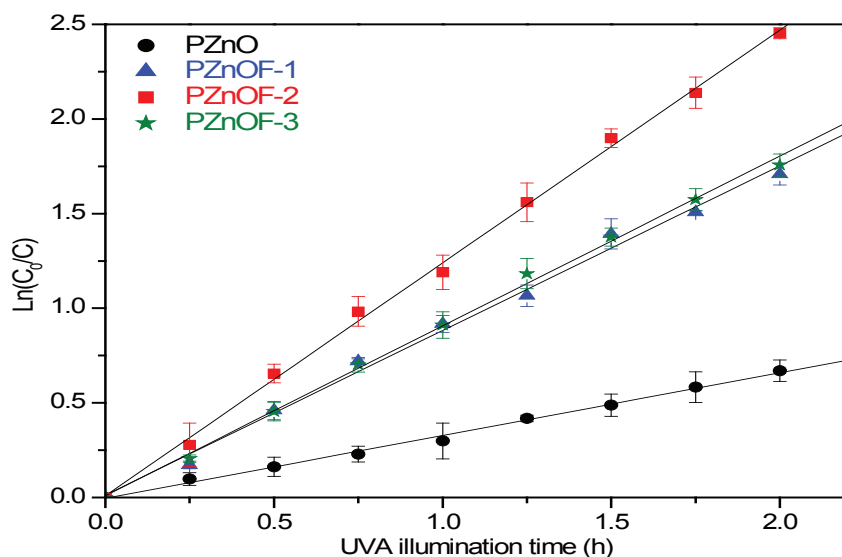


Fig. 5. $\ln(C_0/C)$ versus time plot of MB bleaching under UV irradiation on PZnO and PZnOF samples. C is the MB concentration (mol/l) at time t and C_0 is the initial MB concentration (mol/l).

leading to the rise of photocatalytic properties. However, when the perlite surface was fluorinated more strongly, with a KF concentration of 3 mol/l, the ZnO content was decreased on the PZnOF-3 sample, resulting in a decline in photocatalytic performance.

These results showed that the immobilization of ZnO nanoparticles supported on fluorinated perlite granules may be a simple and efficient method to obtain highly photocatalytic materials which are easily separated from solutions after treatment.

Conclusions

In this study, ZnO nanoparticles were developed on fluorinated perlite granules with various KF concentrations by a simple one-step impregnation method in order to study the effects of perlite fluorination on the crystal structure, morphology, optical properties, ZnO content, and photocatalytic activity of ZnO/perlite. The experimental results showed that the fluorination of perlite does not only enhance the ZnO content on perlite surfaces, increasing the UVA

absorption, but also decreases the particle size of ZnO. These modifications strongly improved the photocatalytic performance of our materials. The fluorinated sample prepared with KF concentration of 2 mol/l was found to be the optimal photocatalyst. When the KF concentration was further increased, the ZnO content on perlite granules dramatically decreased, leading to the reduction of photocatalytic activity.

ACKNOWLEDGEMENTS

The authors would like to thank the University of Science - Vietnam National University, Ho Chi Minh City for their technical support.

REFERENCES

[1] R. Asahi, T. Morikawa, T. Ohwaki, K. Aoki, Y. Taga (2001), "Visible-light photocatalysis in nitrogen-doped titanium oxides", *Science*, **293**, pp.269-271.

[2] E. Khelifi, H. Gannoun, Y. Touhami, H. Bouallagui, M. Hamdi (2008), "Aerobic decolourization of the indigo dye-containing textile wastewater using continuous combined bioreactors", *J. Hazard. Mater.*, **152**, pp.683-689.

[3] R.S. Dave, A.R. Patel (2010),

"Photochemical and photocatalytic of cypermethrin under UV radiation", *Der pharma chemica*, **2**, pp.152-158.

[4] H. Ma, Q. Zhuo, B. Wang (2009), "Electro-catalytic degradation of methylene blue wastewater assisted by Fe_2O_3 -modified kaolin", *Chem. Eng. J.*, **155**, pp.248-253.

[5] Y. Zhang, T. Oyama, A. Aoshima, H. Hidaka, J.C. Zhao, N. Serpone (2001), "Photooxidative N-demethylation of methylene blue in aqueous TiO_2 dispersions under UV irradiation", *J. Photochem. Photobiol. A*, **140**, pp.163-172.

[6] H. Lachheb, E. Puzenat, A. Houas, M. Ksibi, E. Elaloui, C. Guillard, J.M. Herrmann (2002), "Photocatalytic degradation of various types of dyes (Alizarin S, Crocein Orange G, Methyl Red, Congo Red, Methylene Blue) in water by UV-irradiated titania", *Appl. Catal. B*, **39**, pp.75-90.

[7] A. Vijayabalan, K. Selvam, R. Velmurugan, M. Swaminathan (2009), "Photocatalytic activity of surface fluorinated TiO_2 -P25 in the degradation of Reactive Orange 4", *J. Hazard. Mater.*, **172**, pp.914-921.

[8] H. Shin, T.H. Byun, S. Lee, S.T. Bae, H.S. Jung (2013), "Surface hydroxylation of TiO_2 yields notable visible-light photocatalytic activity to decompose rhodamine B in aqueous solution", *J. Phys. Chem. Solids*, **74**, pp.1136-1142.

[9] T.K. Le, D. Flahaut, H. Martinez, H.K.H. Nguyen, T.K.X. Huynh (2015), "Study of the effects of surface modification by thermal shock method on photocatalytic activity of TiO_2 P25", *Appl. Catal. B*, **165**, pp.260-268.

[10] A. Segura, J.A. Sans, F.J. Manjon, A. Munoz, M.J. Herrera-Cabrera (2003), "Optical properties and electronic structure of rock-salt ZnO under pressure", *Appl. Phys. Lett.*, **83**, pp. 278-280.

[11] L. Jing, D. Wang, B. Wang, S. Li, B. Xin, H. Fu, J. Sun (2006), "Effects of noble metal modification on surface oxygen composition, charge separation and photocatalytic activity of ZnO nanoparticles", *J. Mol. Catal. A*, **244**, pp.193-199.

[12] K. Gouvea, F. Wypych, S.G. Moraes, N. Duran, N. Nagata, P. Peralta-Zamora (2000), "Semiconductor-assisted photocatalytic degradation of reactive dyes in aqueous solution", *Chemosphere*, **40**, pp.433-440.

[13] J. Han, Y. Liu, N. Singhal, L. Wang, W. Gao (2012), "Comparative photocatalytic degradation of estrone in water by ZnO and TiO_2 under artificial UVA and solar irradiation", *Chem. Eng. J.*, **213**, pp.150-162.

[14] M.A. Behnadjady, N. Modirshahla, M. Mirzamohammady, B. Vahid, B. Behnadjady (2008), "Increasing photoactivity of titanium

dioxide immobilized on glass plate with optimization of heat attachment method parameters", *J. Hazard. Mater.*, **160**, pp.508-513.

[15] S. Gelover, P. Mondragon, A. Jimenez (2004), "Titanium dioxide sol-gel deposited over glass and its application as a photocatalyst for water decontamination", *J. Photochem. Photobiol. A*, **165**, pp.241-246.

[16] J. Yu, H. Yu, C.H. Ao, S.C. Lee, J.C. Yu, W. Ho (2006), "Preparation, characterization and photocatalytic activity of in situ Fe-doped TiO₂ thin films", *Thin Solid Films*, **496**, pp.273-280.

[17] S.W. da Silva, J.B. Bortolozzi, E.D. Banús, A.M. Bernardes, M.A. Ulla (2016), "TiO₂ thick films supported on stainless steel foams and their photoactivity in the nonylphenol ethoxylate mineralization", *Chem. Eng. J.*, **283**, pp.1264-1272.

[18] Z. Niu, F. Gao, X. Jia, W. Zhang, W. Chen, K. Qian (2006), "Synthesis studies of sputtering TiO₂ films on poly(dimethylsiloxane) for surface modification", *Colloids Surfaces A: Physicochem. Eng. Aspects*, **272**, pp.170-175.

[19] J. Zeng, S.L. Liu, J. Cai, L. Zhang (2010), "TiO₂ immobilized in cellulose matrix for photocatalytic degradation of phenol under weak UV light irradiation", *J. Phys. Chem. C*, **114**,

pp.7806-7811.

[20] S. Sakthivel, M.V. Shankar, M. Palanichamy, B. Arabindoo, V. Murugesan (2002), "Photocatalytic decomposition of leather dye - Comparative study of TiO₂ supported on alumina and glass beads", *J. Photochem. Photobiol. A*, **148**, pp.153-159.

[21] S. Ke, X. Cheng, Q. Wang, Y. Wang, Z. Pan (2014), "Preparation of a photocatalytic TiO₂/ZnTiO₃ coating on glazed ceramic tiles", *Ceram. Int.*, **40**, pp.8891-8895.

[22] S.N. Hosseini, S.M. Borghei, M. Vossoughi, N. Taghavinia (2007), "Immobilization of TiO₂ on perlite granules for photocatalytic degradation of phenol", *Appl. Catal. B*, **74**, pp.53-62.

[23] Y. Shavisi, S. Sharifnia, S.N. Hosseini, M.A. Khadivi (2014), "Application of TiO₂/perlite photocatalysis for degradation of ammonia in wastewater", *J. Ind. Eng. Chem.*, **20**, pp.278-283.

[24] D. Herskovitch, I.J. Lin (1996), "Upgrading of raw perlite by a dry magnetic technique", *Mag. Elect. Sep.*, **7**, pp.145-161.

[25] J. Rodriguez-Carvajal (2001), "Recent developments of the program FULLPROF. Commission of Powder Diffraction", *IUCr*

Newsletter, **26**, pp.12-19.

[26] W.R. Taylor (1990), "Application of infrared spectroscopy to studies of silicate glass structure: Examples from the melilite glasses and the systems Na₂O-SiO₂ and Na₂O-Al₂O₃-SiO₂", *Proc. Indian Acad. Sci. (Earth Planet Sci.)*, **99**, pp.99-117.

[27] K.S. Babu, A.R. Reddy, C. Sujatha, K.V. Reddy, A.N. Mallika (2013), "Synthesis and optical characterization of porous ZnO", *J. Adv. Ceram.*, **2**, pp.260-265.

[28] A.C. Tas, P.J. Majewski, F. Aldinger (2002), "Synthesis of gallium oxide hydroxide crystals in aqueous solutions with or without urea and their calcination behavior", *J. Am. Ceram. Soc.*, **85**, pp.1421-1429.

[29] Y. Zhao, R.L. Frost, J. Yang, W.N. Martens (2008), "Size and morphology control of gallium oxide hydroxide GaO(OH), nano- to micro-sized particles by soft-chemistry route without surfactant", *J. Phys. Chem. C*, **112**, pp.3568-3579.

[30] H. Zhang, C. Hu (2011), "Effective solar absorption and radial microchannels of SnO₂ hierarchical structure for high photocatalytic activity", *Catal. Commun.*, **14**, pp.32-36.

Integrating indigenous knowledge for participatory land use planning (PLUP) in Tra Hat hamlet, a climate-smart village in the Mekong River Delta, Vietnam

Thanh Vu Pham^{1*}, Hieu Trung Nguyen¹, Tuan Huy Vuong¹,
Pham Dang Tri Van¹, Quang Tri Le²

¹College of the Environment and Natural Resources, Can Tho University, Vietnam

²DRAGON Intitude - Mekong - Can Tho University, Vietnam

Received 29 May 2017; accepted 7 September 2017

Abstract:

Agriculture is a primary source of livelihood in the Vietnamese Mekong River Delta with different land use activities. There are different potential conflicts in agricultural land use, including: water resources use and interests. Therefore, establishing feasible land use planning (LUP) in an area needs an in-depth participation and discussion among stakeholders (including: governments at different levels, local farmers, and national and international scientists). Indigenous knowledge of local farmers plays an important role in making land use decision, which can support solving land-use conflicts to achieve the most sustainable management of land resources. In this study, we integrated local knowledge into a land use planning process to assess the suitability of land use types and to project potential impacts of climate change conditions on land use decisions in the Tra Hat hamlet, one of the climate-smart village (CSV) sites in the Vietnamese Mekong River Delta. A total of two consultation meetings, using simple tools (resources mapping, timeline, seasonal calendar, wealth ranking, Venn diagram, pair wise ranking, and key informants' interviews), were conducted, and 130 households were interviewed during a field visit in 2015. As a result, a participatory land use planning (PLUP) was implemented with active participation of the concerned communities to establish a suitable land use planning and identify the potential adaptation techniques in the near future (2020). Land use planning at the hamlet was discussed to get the agreement and the coordination among local governments, farmers and scientists on adaptation practices.

Keywords: *climate-smart villages, land use planning, participatory, the Vietnamese Mekong River Delta, Tra Hat hamlet.*

Classification number: 3.1

Introduction

CSV is a definition developed by the CGIAR research program on climate change, agriculture and food security (CAAFS), and Tra Hat hamlet is one of the CSV sites in Vietnam. These are sites where researchers, development partners, and farmers come together to test climate-smart agricultural interventions. The aim is to boost farmers' ability to adapt to climate change, to manage risks and, to build resilience. At the same time, the hope is also to improve livelihoods and incomes, and where possible, to reduce greenhouse gas emissions to ensure solutions are sustainable [1].

The villages are being set up in areas that have been identified as being high at risk from the effects of climate change, and where partners are already conducting work. Prior to the project start-up, a steering group made up of researchers and village representatives identify 'climate-smart' interventions best suited for that community. The list of activities is introduced to a group of farmers and village officials. The group prioritizes the different technologies based on yield and resilience potential, as well as other features they find useful. If accepted, the rest of the community gets to weigh in on the project. The process aims to be as participatory and inclusive as possible, especially to encourage women and more vulnerable groups to

*Corresponding author: Email: ptvu@ctu.edu.vn

participate. Researchers and farmers continuously monitor and evaluate the activities. These actions are to gain a full understanding of the benefits and effects they might have on farm production, income, resilience, equity, employment, adaptation, and mitigation. The aim is to scale-up the practices [1].

Indigenous knowledge of local communities can provide an important tool for early detection and understanding of the impacts of production condition changes [2]. Exploring Indigenous knowledge through group discussion is important to work to support the research group to understand the study area when the study began in local communities. Because field surveys can be time- and cost-intensive, early detection efforts are limited [2]. The benefits of integrating local ecological knowledge with the Western science are widely espoused. Examples demonstrate how this process can inform adaptive ecosystem management [3], facilitate effective co-management between resource users and land managers, and afford a more holistic understanding of a given system [4]. Local livelihoods are strongly influenced by the effects of human land use [5]. The competing land uses which are present in the river basin may further amplify the cumulative negative effects of land use changes

at the local and landscape scales [2]. PLUP is an iterative process based on the dialogue amongst all stakeholders aiming at the negotiation and decision for a sustainable form of land use in rural areas as well as initiating and monitoring its implementation [6]. PLUP is essentially bottom-up land-use planning, carried out with the active participation of the concerned communities [7]. Through the process of PLUP, the communities achieve a consensus on the sustainable management of their natural resources [6]. As one of the first steps of PLUP, a participatory rural appraisal (PRA) is carried out in every village, and a trained multidisciplinary team analyzes the situation on hand with the local beneficiary groups and stakeholders. A series of methods are applied to acquire and analyze information, including participatory surveillance, transect walk through the kebele, ranking and scoring as well as cropping calendars. Finally, the PRA survey leads to a number of group discussions, where the target groups identify and prioritize the problems, activities, and areas of intervention. As a result, a participatory village map is created during the discussion. The information collected from the PRA and field surveys forms the basis of local knowledge of a village [6]. Under the current and future climate change, PLUP must be intended to incorporate

possible climate change (and sea level rise, if relevant) into the identification and selection of land use options. Such participatory land use planning under climate change (PLUP-CC) should not only improve the livelihoods of the existing community but also sustain the resources development of the future generation [7]. In 2015, village baseline studies were conducted to analyze general local conditions. After that, a training and implementing for PLUP were conducted in Tra Hat hamlet to improve the capacity of staffs (CTU and PLUP) in PLUP.

The study aims at (1) assessing the utility of local indigenous knowledge in exploring basis problems in the study area and (2) integrating local indigenous knowledge with PLUP to map the suitable areas of land use types adapted to CSV purposes.

Methods

Study area: Tra Hat hamlet located in Chau Thoi commune, Vinh Loi district, Bac Lieu province, Mekong River Delta is one of the CSV sites in Vietnam of CGIAR program - CCAFS. The area is also in the highly vulnerable agricultural ecosystems zone (AEZ) of the Vietnamese Mekong River Delta due to sea level rise and climate change (Fig. 1). The hamlet has 301 households with

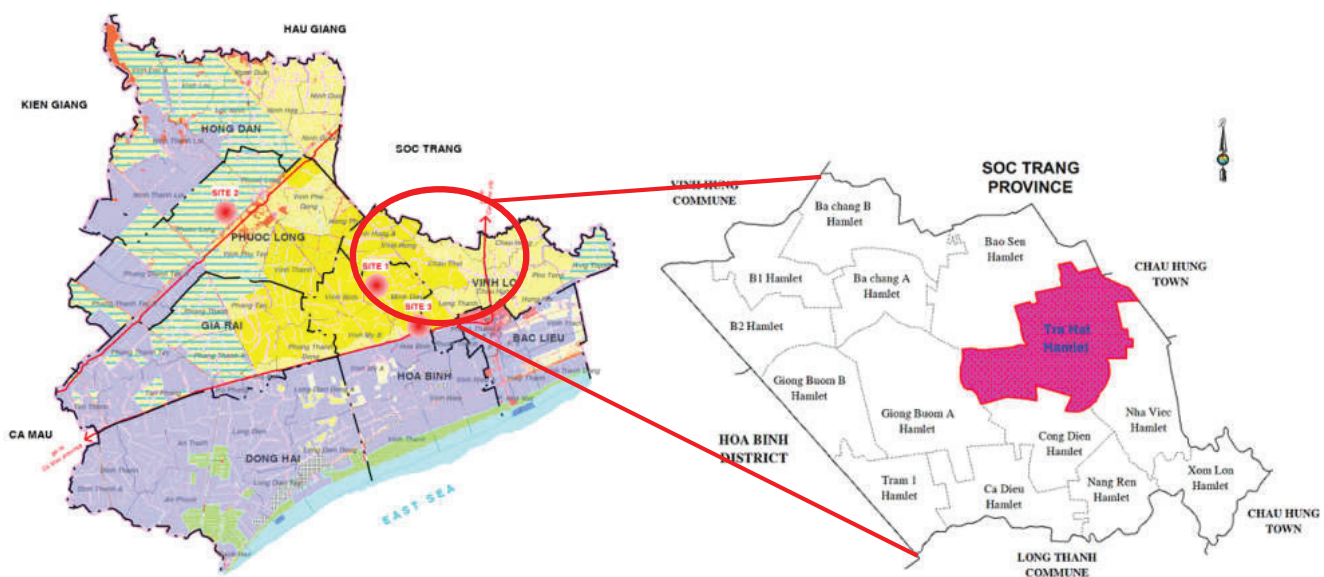


Fig. 1. The study area of Tra Hat hamlet, Vinh Loi district, Bac Lieu province.

main livelihoods depending on rice and home-garden (including upland crops, small-scale livestock and aquaculture). Tra Hat hamlet is inside Quan Lo Phung Hiep - a salinity control project area, and its agriculture is facing lack of fresh water and salinity intrusion during the dry season. Moreover, in the rainy season, low-lying areas of the hamlet are inundated, and the situation would be more serious in the future under the impacts of climate change and sea level rise (CC&SLR) [8]. Besides, the income of villagers is fluctuating due to not only the unpredictable water condition, such as surface water salinity intrusion and change of rainfall pattern but also unstable prices [9].

Data collection: Two consultation meetings were organized (20 participants). The list of participants was compiled by the head of village and gender was balanced among the participants (10 males and 10 females). 240 randomly selected household interviews were conducted to identify the impact factors on local agriculture and the interviewees were asked to rank these selected parameters on a scale of 1 to 5, in which 5 refers to high impact (severe), and 1 refers to very low impact (Table 1). The perceptions (scale) of relevant indicators were summed to estimate the overall scores of factors.

Framework

The PLUP process is mainly based on FAO methods including land evaluation and land use planning [11, 12], and the community agro-ecosystem analysis [13]. The process consists of five steps.

The process of PLUP-CC and sea level rise (SLR) consists of four steps.

In this study, the participatory land use planning session completed four tasks:

1. To conduct PRA workshops to identify and map the farm types based on land use systems and locations, and to review the past, current and near future (five years) of bio-physical and socio-economic changes.

Table 1. Impact categories and their range [10].

Rank	Impact categories	Magnitude	Level impact
5	Severe	4.1-5	> 20%
4	High	3.1-4	15 - < 20%
3	Moderate	2.1-3	10 - < 15%
2	Low	1.1-2	5 - < 10%
1	Very low	1 and below	< 5%

2. To quantify existing farm-type land uses' inputs/outputs via farm surveys and interviews.

3. To assess impacts of biophysical and socio-economic changes on the current and future land uses patterns.

4. To conduct workshops for screening potential adaptation strategies (organizing participatory consultation meetings between experts, planners and villagers to provide information and discuss potential adaptation measures based on the past and current experiences).

Results and discussions

Indigenous knowledge in identifying local resources

Land resources, water resources, and climate factors which impact on local land uses were specified. The hamlet's topography is rather stable. The ground elevation in low areas is not much different from in high areas. Local farmers assess the soil condition of Tra Hat as being in good condition (Fig. 2). Thanks to the well-built irrigation system and sluice gates, the soil's salinity and acidity are kept at a low level. However, in the dry season, when



Fig. 2. Training indigenous knowledge of integration in the PLUP process to local farmers. (A) A group of farmers focused on inspecting local resources, land use changes, climate issues and land use planning complement; **(B)** Identifying land qualities via participatory field survey in each AEZ; **(C)** Local farmers identified local resources during participatory mapping activities; **(D)** Group discussion with stakeholders on achieved results.

less water available, there are risks of acidity in some local areas.

The hamlet is located in freshwater ecological zones where closed dyke and sluice gates system are completely constructed. Both surface water and groundwater are the main sources of domestic water supply and agricultural production. Rainwater is used during the rainy season. In other periods, the main sources of water are provided by local canals (at rice producing area) and groundwater (at upland crop area). River flood hardly happens in the hamlet because it is located closely to the East Sea. However, water shortage in the dry season is still a problem in the area.

Tra Hat hamlet can be divided into four Agro-ecosystem zones (AEZs) (Fig. 3) that is characterized by high levels of topographic, soil fertility and land-use types. These zones share a boundary which is the local canals system.

Present land use assessment

The historical land use change (Table 2) shows that Tra Hat had rather a stable land use; most of the farmers cultivate traditional rice. Land use change process in the hamlet depended on the changes of physical conditions (salinization, irrigation system) and was driven by desires to increase income.

However, the improvement of rice production (increasing crops) has resulted in different problems. Soil becomes swampy because of intensive cropping. The farmers need to supply more fertilizer and pesticide. Therefore, the capital and price are increased, but the products' quality is decreased. Besides, fertilizer and pesticide residue also leaves negative effects on land and water environment.

Local farmers do not want to change their cropping pattern; they are afraid of facing incurred risks. Rice monoculture is a disadvantage since rice market and its

price fluctuate overtime. Moreover, they have not had experience in producing other cropping patterns (except rice production). Thus, they found it difficult to change into new cropping patterns, which could adapt to climate change and market fluctuation in the future.

In Tra Hat hamlet, agriculture is the primary source of livelihood, in which, the rice production occupies a large area while the rest is of the upland crop production. There are 310 households in the hamlet, in which 97.1% produces rice (including 93.3% double-rice crops and 6.7% triple-rice crops) and only 2.9% produce upland crops. The hamlet also has other agricultural activities but they keep a small percentage of the total area of the village such as small-scale livestock, orchard and aquaculture. These kinds of production did not get the interest of local farmers because of low (existing) farming techniques, limited labour and limited capital capability as well as farmers' risk aversion to land-use changes. Therefore, these kinds of production do not lead to an increase in household income as they should. In general, the farming patterns in Tra Hat are still intensive. Currently, three main land-use types are distributed in four AEZs in the hamlet (Fig. 4) including Triple-rice crops (at AEZ I); Double-rice crops (at AEZ II & III); Upland crops (AEZ 4).

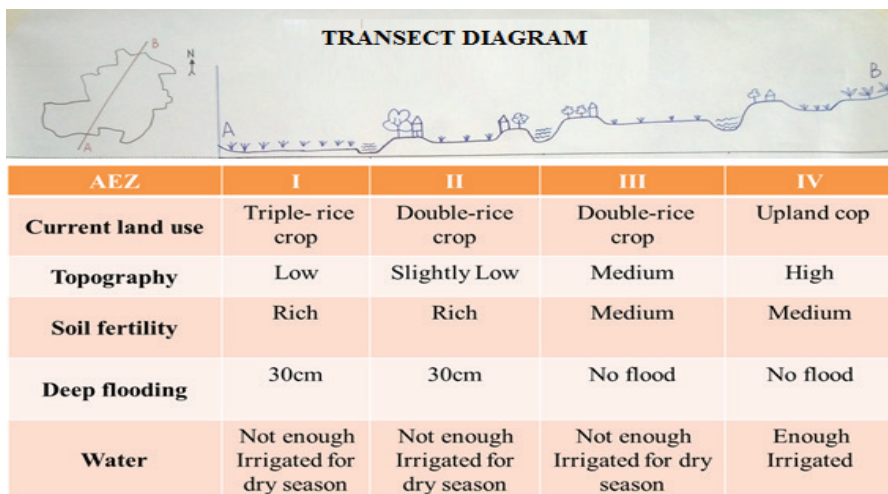


Fig. 3. Tra Hat transect diagram drawn based on topography, ascending from the South-West to North-East.

Table 2. Historical land use change.

Event history	Before 1990	1990-1994	1994-2015
Land use change	Paddy rice (Mono-rice crop)	Double-rice crops	Triple-rice crops
Rice varieties		IR42, OM545, Tai Nguyen	Short-time varieties, Tai Nguyen
Reason of transform	Salinized	invested sewer systems	increase income

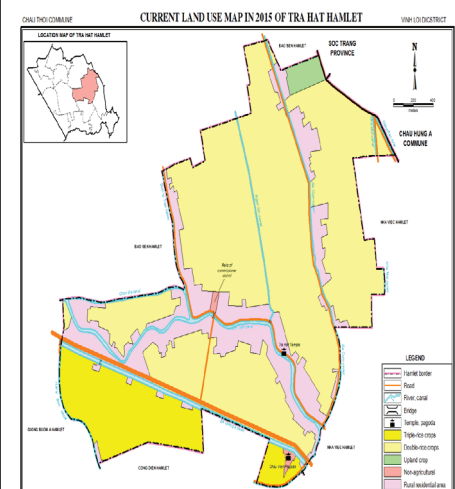


Fig. 4. Present land use map of Tra Hat hamlet in 2015.

Table 3. Inputs and outputs of existing land use systems.

Indicators	Double-rice crops (Million VND/ha/ year)	Triple-rice crops (Million VND/ ha/year)	Upland crops (day/ha/year)	Orchard (Million VND/1000 m ² / year)	Aquaculture (Million VND/1000m ² / year)	Livestock (Pig) (Million VND/1000m ² / year)
Cost	57.58	102.99	163.76	1.60	14.05	4,106.62
Revenue	49.39	43.10	152.91	0.54	15.07	1,215.46
B/C	0.86	0.42	0.93	0.34	1.07	0.30
Labour	129	163	690	21.00	126.00	4,166.67

Note: the majors included double-rice crops, triple-rice crops, and upland crops.

Table 4. Pair-wise ranking.

	Double-rice crops	Triple-rice crops	Upland crops
Double-rice crops			
Triple-rice crops	Double-rice crops		
Upland crops	Double-rice crops	Triple-rice crops	
Ranking	1	2	3

Table 5. PLUP suitability and selection.

AEZ	Current land use	PLUP suitability			Selected
		LUT 1 (Double-rice crops)	LUT 2 (Triple-rice crops)	LUT 3 (Upland crops)	
I	Triple-rice crops	3	2	2	Double-rice crops
II	Double-rice crops	2	2	0	Double-rice crops
III	Double-rice crops	3	1	2	Double-rice crops
IV	Upland crops	3	1	3	Double-rice crops/ Upland crops

Note: levels score - 0: not suitable; 1: marginally suitable; 2: moderately suitable; 3: highly suitable.

Table 6. Current exposure (in 2015) to extreme weather events.

Events	Duration	Description
Heavy rain	September-October	More rainy than usual
Drought	March- April	Acidification, cracked soil, dried up river, absence of rain
Rainstorm /high wind	May-October	Strong wind, collapse cultivation
Hot spell	March-April	Hotter (following weather forecast are 38-39°C)
Saline intrusion (forecast)	February - April November - December	Saltwater, withered crops

Local land-use types are distributed differently periodically (Fig. 5). Regarding to the double-rice drop, it is cultivated in the Summer-Autumn season (June - September), using short-day varieties, and in the Winter-Spring season (October - March), using local long-day varieties (Tai Nguyen). Therefore, when farmers produce double-rice crops, they have about three months (March - June) considered as leisure time and they can use this leisure time to increase their income with others activities. Triple-rice crops (Summer-Autumn, Autumn-Winter, Winter-Spring; using short-day varieties) and upland crops have the cultivating time of all year round. From surveys and interviews with 130 households, the inputs/outputs data of land-use types are shown in Table 3.

Formulation of LUT options, and selecting preferable and feasible land-use types (LUTs) in AEZs in present condition

Promising land-use types (LUTs) are chosen by local farmers. They only recommend current land-use types which are considered suitable in local condition. Consequently, it is not diversified in selection. Priority of land-use types is presented in Table 4, including: (1) Double-rice crop, (2) Triple-rice crop, (3) Upland crop.

PLUP suitability, selection and mapping

The land suitability classification is performed by the local land users, which is based on the participatory land evaluation (FAO, 1976). Depending on land use demand, land-use types' suitability is assessed by local land users (through four levels of score) to define whether it is potential or not. The results of current land suitability evaluation are presented in Table 5, Fig. 6.

Identifying Climate change and sea level rise (CC-SLR) issues

Agricultural activities are seriously affected by changes in climatic pattern.

Table 7. Local adaptation strategies in 2020-2025.

	Heavy rain	Drought	Rainstorm/ Strong wind	Hot spell	Saline intrusion
Before	Preparing land and checking drainage system	Embankment or storage water, preparing tools and diesel to pump water	Spraying pesticides	-	Warning for production, saving fresh water
During	Opening sluice, sewerage in fields	Pumping water from river, killing hamsters	-	-	Testing water before irrigation, finding fresh water resources
Immediately After	Keeping the dry fields, Fertilizing and spraying pesticides	Fertilizing	-	Replanting when cultivation is wilted dead	-
Future	Reinforcing and building irrigation system and pumping system	Dredging canal, lakes; finding, planting and breeding	Breeding strong stems, potassium fertilizer and spraying "Lun" pesticides	-	Using short-day varieties, Changes of season calendar, Land use changes

Local farmers struggle with extreme weather events, including: Heavy rain, drought, rainstorm/strong wind, and hot spell. In addition, salinity intrusion is also another significant problem in the future. List of exposures to extreme weather events is described by local farmers (Table 6).

Revising promising land use types (LUTs) to adapt to CC-SLR

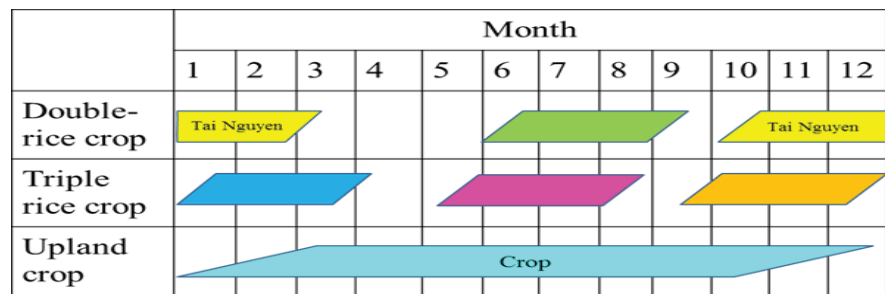
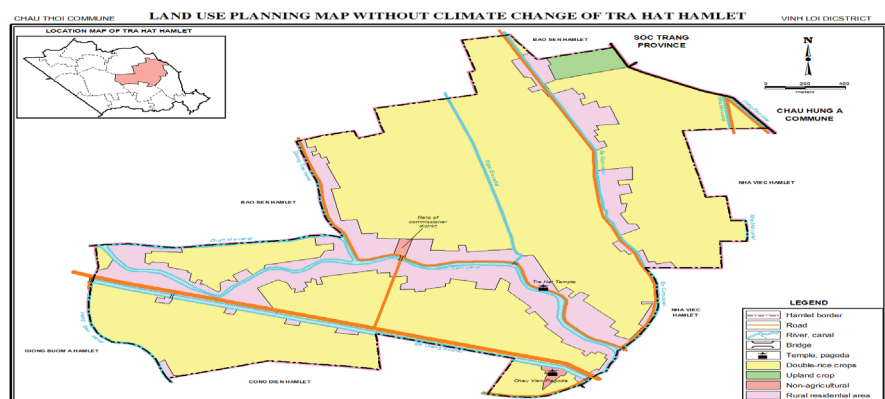
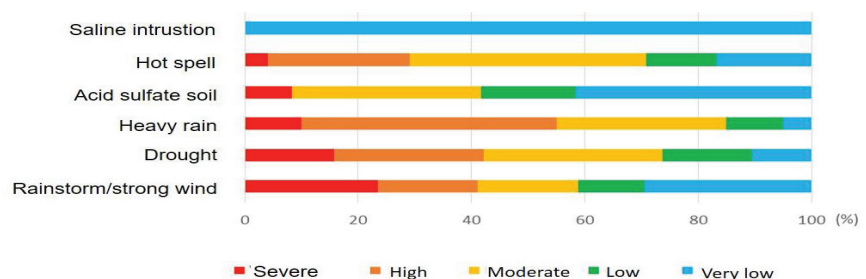
To deal with the effects of climate change, the farmers use indigenous knowledge effectively to adapt to changes and reduce risks and damages for agriculture and Table 7 presents strategies of local farmers. There are different solutions which would only be beneficial in a short term but bring unexpected risks in a long run (e.g. saline intrusion and drought).

Impact factors on agricultural production

The results of data analysis show that six factors were identified which impact local agricultural production (Fig. 7). These factors were assessed by indigenous knowledge with levels of effect ranging from 5 (severe) to 1 (very low).

LUP in CC-SLR conditions

Under the assumption that salinization would happen in Tra Hat in the future (2025), according to local farmers, salinization is a vital factor which affects the adapting capacity of land-use types. Potential land-use types are evaluated again by land user's knowledge. In salinization condition,

**Fig. 5. Seasonal calendar of main land-use types.****Fig. 6. Land use planning map without climate change of Tra Hat hamlet in 2020.****Fig. 7. Impact factors on agricultural production.**

there is no suitable land use type in AEZ I (lowest area, the area which is the most heavily affected by salinization). However, using some adaptable solutions (using short-time varieties,

storing freshwater), the double-rice crop is still chosen to cultivate. In AEZ II and AEZ III (medium and high areas, less affected by salinization), the double-rice crop pattern is also selected. The

Table 8. Participatory land use plan with CC-SLR adaptation.

LUs	Current land uses	PLUP suitability			Selected
		LUT 1 (Double-rice)	LUT 2 (Triple-rice)	LUT 3 (Upland)	
1a	Triple- rice crops	0	0	0	Double-rice crops (short-duration)
1b	Double-rice crops	0	0	0	Double-rice crops (short- duration)
2	Double-rice crops	2	0	2	Double-rice crops (short- duration)
3	Upland crops	1	0	2	Double-rice crops / Upland crop

Note: levels score - 0: not suitable; 1: marginally suitable; 2: moderately suitable; 3: highly suitable.

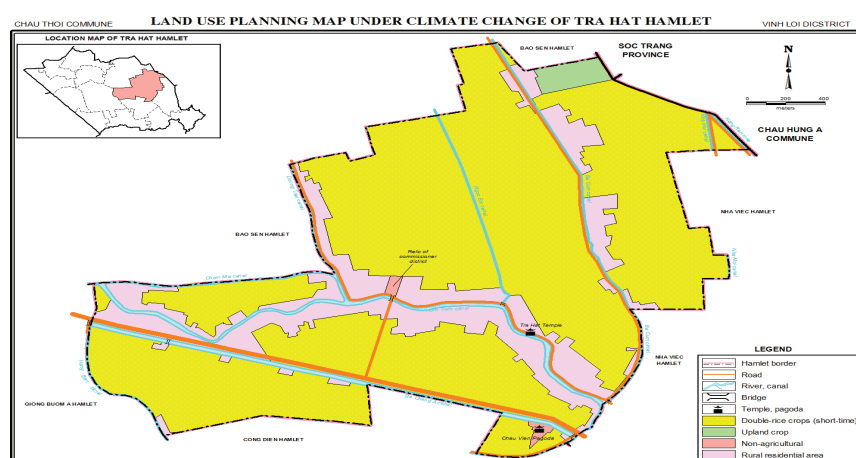


Fig. 8. Land use planning map in 2020 under the impacts of climate change and sea level rise of Tra Hat village.

upland crop pattern is chosen in AEZ IV. Especially, triple-rice crops are not suitable in all AEZs (Table 8, and Fig. 8).

Conclusions

Participatory data collection and mapping via group discussions with farmers revealed the detailed and highly accurate knowledge of local community members regarding natural and land use conditions. Local knowledge can contribute as an important early warning system to understand the resources distribution, ecosystem, and impacts of climate change and sea level rise and potentially provide a needed edge to a more effective management of production in the context of changes.

Differences on results of the PLUP with climate change-sea level rise adaptation compared with the PLUP without climate change adaptation demonstrated the identification and cognition ability of farmers prior to events of vulnerabilities and risks from climate change have considerable effects during and after the events and land use demands and agricultural production level which is suitable for market demands.

ACKNOWLEDGEMENTS

The authors would like to thank CCAFS project “Participatory integrated land use planning at Tra Hat climate-smart village under the context of climate change and sea level rise, Bac Lieu province, Vietnam” supported the information and financial resources.

REFERENCES

- [1] CGIAR (2013), *Climate-Smart Villages*, <http://www.seachangecop.org/sites/default/files/documents/2013%2007%20CGIAR%20-%20Climate-Smart%20Villages.pdf>.
- [2] M.V. Luizza, T. Wakie, P.H. Evangelista, C.S. Jarnevich (2016), “Integrating local pastoral knowledge, participatory mapping, and species distribution modeling for risk assessment of invasive rubber vine (*Cryptostegia grandiflora*) in Ethiopia’s Afar region”, *Ecology and Society*, **21**(1), p.22.
- [3] M.E. Fernández-Giménez and F.F. Estaque (2012), “Pyrenean pastoralists’ ecological knowledge: documentation and application to natural resource management and adaptation”, *Human Ecology*, **40**(2), pp.287-300.
- [4] C.A. Gagnon and D. Berteaux (2009), “Integrating traditional ecological knowledge and ecological science: a question of scale”, *Ecology and Society*, **14**(2), p.19.
- [5] J.D. Allan (2004), “Landscapes and riverscapes: the influence of land use on stream ecosystems”, *Annual Review of Ecology, Evolution, and Systematics*, **35**, pp.257-284.
- [6] GIZ (2012), *Land use planning, concept, tools and applications*, Bonn and Eschborn, Germany.
- [7] Chu Thai Hoanh, Bui Tan Yen, Nguyen Hieu Trung (2015), *Guidelines: Participatory land use planning at climate-smart villages*, CCAFS-SEA.
- [8] Nguyen Hieu Trung, Chu Thai Hoanh, To Phuc Tuong, Nguyen Xuan Hien, Le Quang Tri, Vo Quang Minh, Dang Kieu Nhan, Pham Thanh Vu, Van Pham Dang Tri (2015), *Theme 5: Integrated adaptation assessment of Bac Lieu Province and development of adaptation master plan*, CLUES project technical report, IRRI.
- [9] Ngo Dang Phong, Ngo Thi Thanh Truc, Nguyen Thanh Binh, Truong Thi Ngoc Chi, Le Minh Duong (2015), *Village baseline study - site analysis report for Tra Hat CSV-Vinh Loi, Bac Lieu, Vietnam*, CGIAR research program on climate change, agriculture and food security (CCAFS), Copenhagen, Denmark.
- [10] A. Dixit, M. Upadhyay, A. Pokhrel, K.M. Dixit, D.R. Rai (2007), *Flood Disaster Impact and Responses in Nepal Tarai’s Marginalised Basins Nepal*.
- [11] FAO (1976), *A framework for land evaluation*.
- [12] FAO (1993), *Guidelines for land-use planning*.
- [13] Department of Agricultural Extension (DAE), Ministry of Agricultural, Forestry and Fisheries (MAFF), Cambodia and CGIAR Challenge Program on Water and Food (CPWF) (2012), *Commune Agro-ecosystem Analysis in Cambodia: A Guidance Manual*.

Genome-wide identification and annotation of the Nuclear-factor YA gene family in cassava (*Manihot esculenta* Crantz)

Duc Ha Chu^{1*}, Thi Thuy Tam Do^{1,2}, Xuan Dac Le³, Thi Ly Thu Pham¹

¹Agricultural Genetics Institute, Vietnam Academy of Agricultural Sciences

²University of Science and Technology of Hanoi

³Institute of Tropical Ecology, Vietnam-Russia Tropical Center

Received 5 May 2017; accepted 6 September 2017

Abstract:

Nuclear factor Y (*NF-Y*) is a transcription factor which plays an important role in the regulation of various developmental processes and stress responses in plants. By using various bioinformatics tools, the identification and analyses of the *NF-YA* subunit of cassava (*Manihot esculenta* Crantz) have been attempted in this study. A total of 12 members of the *NF-YA* gene family were identified in the cassava genome. They were located on the 18 cassava chromosomes with different frequencies. Several initial structural analyses of the *NF-YA* family were also performed. Among them, the typical gene organization of the *MeNF-YA* gene family contained 5 exons/4 introns. Interestingly, the conserved region of *NF-YA* was characterized by the interaction of *NF-YB/C* domain and the DNA binding domain. This study provided information on *NF-Y* in plants.

Keywords: cassava, gene, in silico, *NF-YA*, transcription factor.

Classification number: 3.1

Introduction

NF-Y (Nuclear factor Y) is known as one of the most important transcription factor groups in all eukaryotes. This family has evidently played the key roles in the regulation of diverse genes [1]. *NF-Y* has three subunits (*NF-YA*, *NF-YB*, and *NF-YC*), which are connected with a range of biological processes, from the signalling pathways to stress responses in plants. Thus, it would be essential to study these subunits in order to expand our knowledge on plant's responses to adverse biotic/abiotic stresses.

To date, the *NF-Y* gene family has been found and characterized in many plant species such as rice (*Oryza sativa*) [2], canola (*Canola napus*) [3, 4],

soybean (*Glycine max*) [5], and foxtail millet (*Setaria italica*) [6]. Recently, the family has also been recorded in tomato (*Solanum lycopersicum*) [7], grape (*Vitis vinifera*) [8], and sorghum (*Sorghum bicolor*) [9]. Many *NF-YA* genes were reported to function in biological processes, especially in stress response in plants. For example, *Arabidopsis thaliana* transgenic plants overexpressing *AtNF-YA5* have shown a reduction of leaf water loss and a better resistance to drought stress than the wild-type plants, thus revealing that the *AtNF-YA5* might function in drought resistance through transcriptional and posttranscriptional regulatory mechanisms [10]. Additionally, *Arabidopsis AtNF-YA3* and *AtNF-YA8* were also found as redundant genes

required in early embryogenesis of plants [11]. In soybean, overexpression of *GmNF-YA3* conferred the reduction of leaf water loss and enhanced drought tolerance in transgenic *Arabidopsis* plants [12].

In this study, the *NF-YA* gene family in cassava (*Manihot esculenta*) was identified and annotated. The identifier, which was the chromosomal location of each gene encoding *NF-YA* subunit, was provided based on various available databases. Gene organization of *NF-YA* gene family in cassava was also analyzed by using bioinformatics approaches. Finally, protein features and conserved domains of *NF-YA* subunits were involved.

Materials and methods

Materials

The cassava genome database of "AM560-2" cultivar [13] is available in Phytozome v12.0 [14].

Methods

Identification and annotation of genes encoding NF-YA in cassava genome: Members of *NF-Y* family in cassava from the Phytozome v12.0 [14] were identified. Their identifiers and chromosomal locations were then confirmed by blasting (BLASTP) against the cassava genome database [13] in NCBI server.

Analysis of gene structure of NF-YA genes: The genomic sequence and CDS (coding DNA sequence) of each

*Corresponding author: Email: hachumser@yahoo.com

member of *NF-YA* genes were obtained from the cassava genome database [13] in the Phytozome v12.0 [14]. The GSDS (Gene Structure Display Server) v2.0 was used to analyze the exon/intron organization of *MeNF-Y* genes [15].

Multiple alignments and phylogenetic analysis of *MeNF-Y* proteins: The protein sequence of each member of *NF-YA* subunits was obtained from the Phytozome v12.0 [14]. The MEGA (Molecular Evolutionary Genetics Analysis) software v7.0 [16] was utilized for multiple alignments of *MeNF-YA* proteins. The parameters of sequence alignments were composed of a gap open penalty of 10 and a gap extension penalty of 0.2. An unrooted phylogenetic tree of all full-length *NF-YA* proteins was constructed with the Neighbor Joining Method as previously studied [17].

Analysis of protein features of *NF-YA* subunit: The general information, including the isoelectric point (pI) and molecular weight (mW), was collected through the ExPASy tool [18]. The subcellular localization of proteins was predicted via the TargetP v1.1 web-based tool [19, 20].

Results and discussions

Genome-wide identification of the *NF-YA* gene family in the cassava genome

In order to identify the *NF-YA* family in cassava, a comprehensive search of all proteins containing typical *NF-YA* conserved domain [1] was performed against the family in cassava from the Phytozome v12.0 [14]. As a result, a total of 12 members of the *NF-YA* family were found in the cassava genome (E-value < 1×10^{-6}). The gene annotation and nomenclature of *NF-YA* gene family were harvested by searching against the NCBI database (Bioproject: PRJNA86123) (Table 1).

The *NF-YA* subunit found in cassava genome was also encoded by a gene belonging to a multigene family

Table 1. Annotation of *NF-Y* gene family in cassava genome.

#	Gene name	Transcript name ^{1,2}	Alias name ¹	Locus name ²
1	<i>MeNF-YA1</i>	Manes.04G142600.1	cassava4.1_014256m.g.v4.1	OAY53185
2	<i>MeNF-YA2</i>	Manes.06G163900.1	cassava4.1_010819m.g.v4.1	OAY48517
3	<i>MeNF-YA3</i>	Manes.06G054900.1	cassava4.1_016099m.g.v4.1	OAY47137
4	<i>MeNF-YA4</i>	Manes.07G006600.1	cassava4.1_010627m.g.v4.1	OAY44802
5	<i>MeNF-YA5</i>	Manes.08G034700.1	cassava4.1_011620m.g.v4.1	OAY43008
6	<i>MeNF-YA6</i>	Manes.09G025200.1	cassava4.1_012382m.g.v4.1	OAY40472
7	<i>MeNF-YA7</i>	Manes.09G044200.1	cassava4.1_012637m.g.v4.1	OAY40725
8	<i>MeNF-YA8</i>	Manes.10G141400.1	cassava4.1_011264m.g.v4.1	OAY40005
9	<i>MeNF-YA9</i>	Manes.11G022300.1	cassava4.1_013364m.g.v4.1	OAY36452
10	<i>MeNF-YA10</i>	Manes.14G003100.1	cassava4.1_007505m.g.v4.1	OAY30095
11	<i>MeNF-YA11</i>	Manes.14G123000.1	cassava4.1_017907m.g.v4.1	OAY31569
12	<i>MeNF-YA12</i>	Manes.16G097900.1	cassava4.1_011576m.g.v4.1	OAY27078

Information obtained from ¹Phytozome v12.0 and ²NCBI databases.

as observed in other higher plants' genomes [1]. In comparison with recent annotated dicot species, a total of 21 *GmNF-YA* genes were identified in soybean [5], while 10 *NF-YA* genes were computationally predicted in tomato [7]. More recently, the genome-wide identification of eight *NF-YA* genes has been reported in grape [8].

The chromosomal locations of 12 *NF-YA* genes were identified based on the cassava genome database [13]. As manually illustrated in Fig. 1, these 12 members of *MeNF-YA* genes were mapped on the 18 cassava chromosomes with different frequencies. Among them, chromosomes 6, 9, and 14 contained two *MeNF-YA* genes, whereas only one *MeNF-Y* gene was distributed on each of the chromosomes 4, 7, 8, 10, 11, and 16 (Fig. 1).

Analysis of the structure of *MeNF-YA* genes

To analyze the structures of *NF-YA* genes in cassava, the genomic sequence and CDS of each *NF-YA* member were obtained from the cassava genome [13]. They were then used as query sequences

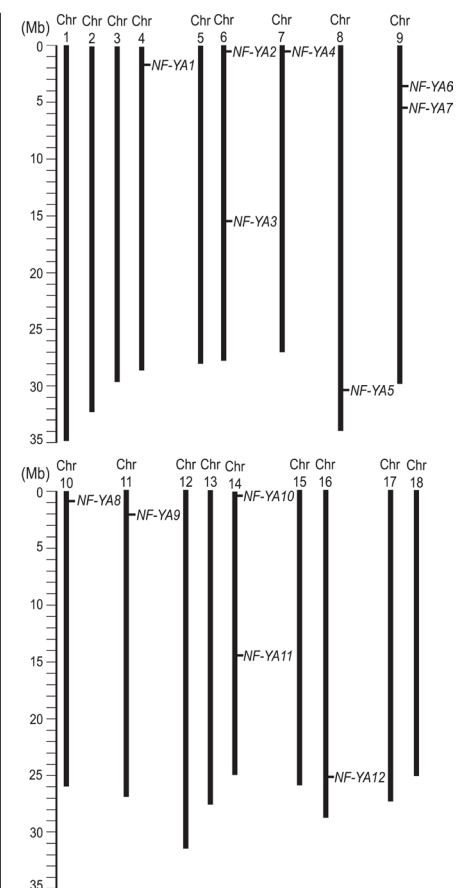


Fig. 1. Chromosomal distributions of *MeNF-YA* genes in cassava genome.

in the GSDS web-based tool to explore the structures of *NF-YA* genes in cassava (Table 2).

As provided in Table 2, the genomic regions of *NF-YA* genes had a variable length of from 4042 (*MeNF-YA4*, Manes.07G006600.1) to 16084 nucleotides (*MeNF-YA11*, Manes.14G123000.1). Previously, the genomic length of a gene was evidentially associated with the transcription level of this gene [21]. Hence, it would be proposed that all *MeNF-YA* genes were highly expressed in the cells, thus they might function in various biological processes and stress response in cassava plants.

Interestingly, the CDS of *NF-YA* genes varied from 645 (*MeNF-YA11*) to 1065 nucleotides (*MeNF-YA10*, Manes.14G003100.1) (Table 2). The structures of *MeNF-YA* genes commonly consisted of 5 exons/4 introns. Only *MeNF-YA6* (Manes.09G025200.1) had 4 exons/3 introns (Fig. 2). Our results clearly indicated that *NF-YA* gene family was completely conserved in cassava as well as in other higher plant species [1]. Furthermore, the introns in the CDS region of a gene might cause the structural diversity and complexity. Consequently, the presences of introns in *MeNF-YA* genes might be directly related to the evolution of *NF-YA* gene family in cassava.

Analysis of protein features of *MeNF-YA*

General features of *MeNF-YA* members of cassava were also figured out by analyzing the protein sequence of each member obtained from the Phytozome v12.0 [14] in the ExPASy tool [18]. The lengths of *MeNF-YA* proteins in cassava ranged from 212 (*MeNF-YA3*) to 354 amino acids (*MeNF-YA10*). The mW values of *NF-YA* family also reached from 23.34 (*MeNF-YA3*) to 38.30 kDa (*MeNF-YA2*)

Table 2. The structures of *NF-YA* genes in cassava.

#	Gene name	Chromosomal location	Genomic length	CDS length
1	<i>MeNF-YA1</i>	Chr04R:26895916..26901232	5317	780
2	<i>MeNF-YA2</i>	Chr06R:26629560..26634297	4738	1053
3	<i>MeNF-YA3</i>	Chr06F:15795526..15804280	8755	639
4	<i>MeNF-YA4</i>	Chr07F:807124..811165	4042	1029
5	<i>MeNF-YA5</i>	Chr08R:3126251..3131957	5707	993
6	<i>MeNF-YA6</i>	Chr09F:3779466..3789354	9889	930
7	<i>MeNF-YA7</i>	Chr09F:5927016..5931630	4615	996
8	<i>MeNF-YA8</i>	Chr10R:25259719..25266401	6683	1020
9	<i>MeNF-YA9</i>	Chr11F:2064501..2068703	4203	852
10	<i>MeNF-YA10</i>	Chr14F:401483..406287	4805	1065
11	<i>MeNF-YA11</i>	Chr14R:10921305..10937388	16084	645
12	<i>MeNF-YA12</i>	Chr16F:25396313..25401918	5606	996

Information was obtained from the Phytozome v12.0; Chr: Chromosome; F: Forward; R: Reverse; Genomic and CDS length were measured by nucleotides.

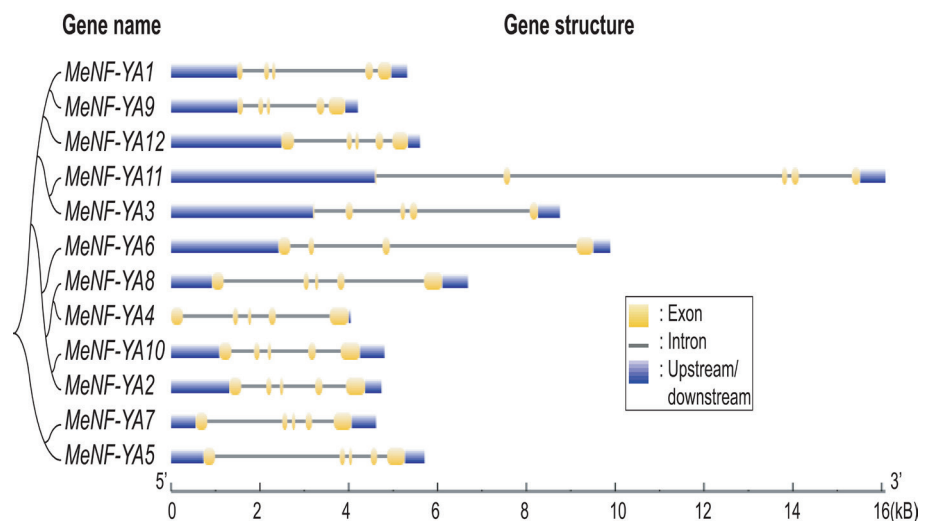


Fig. 2. Gene structure of *NF-YA* family in cassava.

(Table 3). Previously, eight members of *NF-YA* subunit were also identified in sorghum (*Sorghum bicolor*). Among them, SbNF-YA2 (ABXC01000113.1) was found to be the smallest member (90 amino acids, 10.21 kDa), whereas the size of SbNF-YA3 was 305 amino acids and 33.37 kDa [9].

Additionally, a majority of *MeNF-YA* proteins were the basic proteins,

from 8.53 (*MeNF-YA2*) to 9.61 (*MeNF-YA5*). The pI of four remaining *NF-YA* members approximately reached 7, thus indicating that they were likely neutral proteins (Table 3). As mentioned above, all *NF-YA* members in sorghum were also shifted towards basicity [9]. It is understood that the pI value of a protein was directly linked with its subcellular localization. Here, it was observed

Table 3. General features of *NF-YA* proteins in cassava.

#	Gene name	Transcript name	Protein length	pI	mW
1	<i>MeNF-YA1</i>	Manes.04G142600.1	259	8.97	28.83
2	<i>MeNF-YA2</i>	Manes.06G163900.1	350	8.53	38.30
3	<i>MeNF-YA3</i>	Manes.06G054900.1	212	7.16	23.34
4	<i>MeNF-YA4</i>	Manes.07G006600.1	342	8.76	37.32
5	<i>MeNF-YA5</i>	Manes.08G034700.1	330	9.61	36.03
6	<i>MeNF-YA6</i>	Manes.09G025200.1	309	8.77	34.78
7	<i>MeNF-YA7</i>	Manes.09G044200.1	331	9.13	36.30
8	<i>MeNF-YA8</i>	Manes.10G141400.1	339	8.99	36.56
9	<i>MeNF-YA9</i>	Manes.11G022300.1	283	6.95	31.11
10	<i>MeNF-YA10</i>	Manes.14G003100.1	354	6.67	38.05
11	<i>MeNF-YA11</i>	Manes.14G123000.1	214	6.75	23.44
12	<i>MeNF-YA12</i>	Manes.16G097900.1	331	9.01	36.37

Data were obtained from the Expasy tool; Protein length (amino acid); pI: Isoelectric point; mW: Molecular weight (kDa).

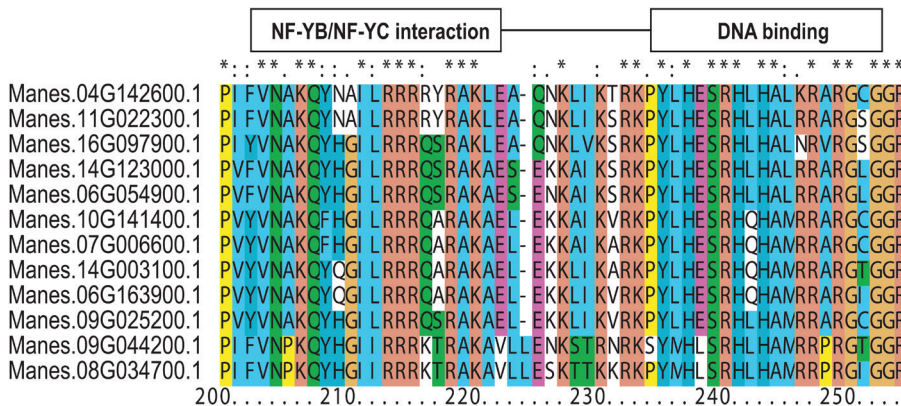


Fig. 3. The conserved domain of *NF-YA* subunit in cassava.

that basic *MeNF-YA* proteins seemed to belong to an integral membrane proteome.

The conserved domain of the *NF-YA* family in cassava was analyzed by using the MEGA software [16]. As shown in Fig. 3, the *MeNF-YA* proteins in cassava could be characterized by two conserved

regions, including a protein interaction and DNA binding domains. A twenty-amino-acid-domain could be bound to the combined surface of NF-YB/NF-YC complex [22] that was clearly observed in the alignment of *MeNF-YA* proteins. Interestingly, most of yeast and mammals functionally required amino

acids [23, 24] that were also obviously found in *NF-YA* family in cassava. These findings highlighted that the *NF-YA* family was completely conserved during the evolution.

Conclusions

A total of 12 members of the *NF-YA* gene family have been found in the cassava genome. The identified *MeNF-YA* genes were distributed on the 18 cassava chromosomes with different frequencies. The analysis of gene structure showed that the genomic regions of the *MeNF-YA* genes ranged from 4042 to 16084 nucleotides, while the CDS varied from 645 to 1065 nucleotides. The most common motif of *NF-YA* genes in cassava was 5 exons/4 introns.

Most of *MeNF-YA* members were basic proteins. This strongly suggested that they belonged to the integral membrane proteome in the cells. In addition, the *MeNF-YA* proteins could be recognized by two conserved regions, including NF-YB/NF-YC interaction and DNA binding domains.

This research provided an initial description of the *NF-YA* gene family in cassava plants. In further studies, the expression profiles of these identified *MeNF-YA* genes under various conditions should be analyzed.

REFERENCES

- [1] T. Laloum, S. De Mita, P. Gamas, M. Baudin, A. Niebel (2013), "CCAAT-box binding transcription factors in plants: Y so many?", *Trends Plant Sci.*, **18**(3), pp.157-166.
- [2] T. Thirumurugan, Y. Ito, T. Kubo, A. Serizawa, N. Kurata (2008), "Identification, characterization and interaction of HAP family genes in rice", *Mol. Genet. Genomics*, **279**(3), pp.279-289.
- [3] L. Xu, Z. Lin, Q. Tao, M. Liang, G. Zhao, X. Yin, R. Fu (2014), "Multiple NUCLEAR FACTOR Y transcription factors respond to abiotic stress in *Brassica napus* L.", *PloS One*, **9**(10), p.e111354.
- [4] M. Liang, X. Yin, Z. Lin, Q. Zheng, G. Liu, G. Zhao (2014), "Identification and characterization of NF-Y transcription factor families in canola (*Brassica napus* L.)", *Planta*, **239**(1), pp.107-126.

- [5] Truyen N. Quach, Hanh T.M. Nguyen, Babu Valliyodan, Trupti Joshi, Dong Xu, Henry T. Nguyen (2015), "Genome-wide expression analysis of soybean NF-Y genes reveals potential function in development and drought response", *Mol. Genet. Genomics*, **290**(3), pp.1095-1115.
- [6] Z.J. Feng, G.H. He, W.J. Zheng, P.P. Lu, M. Chen, Y. Gong, Y.Z. Ma, Z.S. Xu (2015), "Foxtail millet NF-Y families: Genome-wide survey and evolution analyses identified two functional genes important in abiotic stresses", *Front. Plant Sci.*, **6**, doi: 10.3389/fpls.2015.01142. eCollection 2015.
- [7] S. Li, K. Li, Z. Ju, D. Cao, D. Fu, H. Zhu, B. Zhu, Y. Luo (2016), "Genome-wide analysis of tomato NF-Y factors and their role in fruit ripening", *BMC Genomics*, **17**, doi: 10.1186/s12864-015-2334-2.
- [8] C. Ren, Z. Zhang, Y. Wang, S. Li, Z. Liang (2016), "Genome-wide identification and characterization of the NF-Y gene family in grape (*Vitis vinifera* L.)", *BMC Genomics*, **17**, doi: 10.1186/s12864-016-2989-3.
- [9] N. Malviya, P. Jaiswal, D. Yadav (2016), "Genome-wide characterization of Nuclear Factor Y (NF-Y) gene family of sorghum [*Sorghum bicolor* (L.) Moench]: a bioinformatics approach", *Physiol. Mol. Biol. Plants*, **22**(1), pp.33-49.
- [10] W.X. Li, Y. Oono, J. Zhu, X.J. He, J.M. Wu, K. Iida, X.Y. Lu, X. Cui, H. Jin, J.K. Zhu (2008), "The *Arabidopsis* NFYA5 transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance", *Plant Cell*, **20**(8), pp.2238-2251.
- [11] M. Fornari, V. Calvenzani, S. Masiero, C. Tonelli, K. Petroni (2013), "The *Arabidopsis* NF-YA3 and NF-YA8 genes are functionally redundant and are required in early embryogenesis", *PLoS One*, **8**(11), p.e82043, doi: 10.1371/journal.pone.0082043. eCollection 2013.
- [12] Z. Ni, Z. Hu, Q. Jiang, H. Zhang (2013), "*GmNFYA3*, a target gene of miR169, is a positive regulator of plant tolerance to drought stress", *Plant Mol. Biol.*, **82**(1-2), pp.113-129.
- [13] J.V. Bredeson, J.B. Lyons, S.E. Prochnik, G.A. Wu, et al. (2016), "Sequencing wild and cultivated cassava and related species reveals extensive interspecific hybridization and genetic diversity", *Nat. Biotechnol.*, **34**(5), pp.562-570.
- [14] D.M. Goodstein, S. Shu, R. Howson, R. Neupane, R.D. Hayes, J. Fazo, T. Mitros, W. Dirks, U. Hellsten, N. Putnam, D.S. Rokhsar (2012), "Phytozome: A comparative platform for green plant genomics", *Nucleic Acids Res.*, **40**, pp.D1178-1186.
- [15] B. Hu, J. Jin, A.Y. Guo, H. Zhang, J. Luo, G. Gao (2015), "GSDS 2.0: An upgraded gene feature visualization server", *Bioinformatics*, **31**(8), pp.1296-1297.
- [16] S. Kumar, G. Stecher, K. Tamura (2016), "MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets", *Mol. Biol. Evol.*, **33**(7), pp.1870-1874.
- [17] C.V. Ha, M.N. Esfahani, Y. Watanabe, U.T. Tran, S. Sulieman, K. Mochida, D.V. Nguyen, L.S. Tran (2014), "Genome-wide identification and expression analysis of the CaNAC family members in chickpea during development, dehydration and ABA treatments", *PLoS One*, **9**(12), p.e114107.
- [18] E. Gasteiger, A. Gattiker, C. Hoogland, I. Ivanyi, R.D. Appel, A. Bairoch (2003), "ExPASy: The proteomics server for in-depth protein knowledge and analysis", *Nucleic Acids Res.*, **31**(13), pp.3784-3788.
- [19] O. Emanuelsson, S. Brunak, G. Von Heijne, H. Nielsen (2007), "Locating proteins in the cell using TargetP, SignalP and related tools", *Nat. Protoc.*, **2**(4), pp.953-971.
- [20] O. Emanuelsson, H. Nielsen, S. Brunak, G. Von Heijne (2000), "Predicting subcellular localization of proteins based on their N-terminal amino acid sequence", *J. Mol. Biol.*, **300**(4), pp.1005-1016.
- [21] H.N. Lim, Y. Lee, R. Hussein (2011), "Fundamental relationship between operon organization and gene expression", *Proc. Natl. Acad. Sci. USA*, **108**(26), pp.10626-10631.
- [22] D. Hackenberg, Y. Wu, A. Voigt, R. Adams, P. Schramm, B. Grimm (2012), "Studies on differential nuclear translocation mechanism and assembly of the three subunits of the *Arabidopsis thaliana* transcription factor NF-Y", *Mol. Plant*, **5**(4), pp.876-888.
- [23] S.N. Maity, S. Sinha, E.C. Ruteshouser, B. De Crombrughe (1992), "Three different polypeptides are necessary for DNA binding of the mammalian heteromeric CCAAT binding factor", *J. Biol. Chem.*, **267**(23), pp.16574-16580.
- [24] Y. Xing, J.D. Fikes, L. Guarente (1993), "Mutations in yeast HAP2/HAP3 define a hybrid CCAAT box binding domain", *EMBO J.*, **12**(12), pp.4647-4655.

Molecular detection and identification of a phytoplasma associated with cinnamon (*Cinnamomum cassia* B.) witches' broom disease in Quang Ngai province, Vietnam

Thi Nguyen Duong*, Thanh Van Dao

College of Agriculture and Forestry - Thai Nguyen University

Received 30 May 2017; accepted 5 September 2017

Abstract:

Cinnamon (*Cinnamomum cassia* B.) is an important crop in Vietnam for domestic consumption and exportation. In recent years, a disease known as cinnamon witches' broom (CinWB) has been discovered on cinnamon grown in Tra Bong district - Quang Ngai province. The typical symptoms of CinWB were the formation of small tumors on the stems, branches, petioles, and veins of plants. The tumors become long squid-like tassels giving the appearance of a witches' broom. Infected plants are stunted, with delayed growth and development, causing a high reduction in the yield and quality of farmed cinnamon. In the present study, nested-PCR was applied with the universal primer pairs P1/P7 and R16F2n/R16R2. PCR products of an approximate size of 1200 bp were amplified from the twelve CinWB-showing samples collected from Tra Bong district, Quang Ngai province. All PCR products were directly sequenced in both directions using R16F2n and R16R2 primers. A BLAST search indicated that DNA sequences of all 12 PCR products were identical and show 99% identity with phytoplasma sequences of the 16SrXIV group. And the CinWB phytoplasma isolated from the CinWB-showing cinnamon from Tra Bong district - Quang Ngai province (QQNVN) was deposited in GenBank under an accession number JX413793.

Keywords: cinnamon witches' broom, nested-PCR, phytoplasma.

Classification number: 3.1

Introduction

Chinese cinnamon (*Cinnamomum cassia* B.) is among the oldest spices, reaching ancient Egypt, by the seventeenth century B.C. [1]. Cinnamon is grown wild and is also cultivated in South-East Asia, south China (Kwangxi and Kwangtong provinces), Burma (Myanmar), Laos and Vietnam. It was introduced into Indonesia, Sri Lanka, South America and Hawaii. In Vietnam, it is found in many provinces from the North to the South, but is concentrated in the provinces of Quang Ninh, Yen Bai,

Tuyen Quang, Ninh Binh, Thanh Hoa, Nghe An, Thua Thien - Hue, Quang Nam, Quang Ngai and in the Western Highland plateau. In Quang Ngai province, cinnamon is widely grown in mountainous districts of Tra Bong, Tay Tra, Son Ha, and Son Tay. The total area of land used for cultivating cinnamon is 3,000 ha in this region, with Tra Bong accounting for about 1,000 ha.

In a poor mountainous district such as Tra Bong, cinnamon is a very important crop helping small farmers to overcome their difficulties in life. However, in recent years, cinnamon

production has been affected by disease and insect infestations, among them the CinWB was one of the most important factors causing the severe yield loss. The disease significantly reduced the quality and yield of cinnamon, directly taking from the livelihood of growers. More than 30% of farm land has been infected by the disease, with 3-year-old plants in the Tra Son, Tra Hiep and Tra Thuy communes of Tra Bong district most affected.

A significant amount of CinWB was found in nurseries, and on both young and old trees in Tra Giac hamlet (Tra Mi district, Quang Nam province) - considered a hot spot for this disease [2]. It has been found that CinWB is caused by a phytoplasma which can be combated with preventive measures including soaking cinnamon seeds in warm water (70°C) containing an antibiotic before sowing [1]. However, the mode of transmission, vector(s) and other aspects of this disease are not fully understood; and only a single measure, phytosanitation, has been identified as effective at reducing its incidence. In addition, no studies on methods of detection and identification of a CinWB phytoplasma on a molecular scale have been conducted in Vietnam.

Nested-PCR techniques in combination with DNA sequencing and phylogenetic analysis are currently the best methods for differentiation, characterization and classification of

*Corresponding author: Email: nguyentuaf1@gmail.com

phytoplasmas associated with plant diseases [3-5]. The 16S rDNA gene, 16S-23S rDNA intergenic spacer region and 23S rDNA gene are the targets for detecting and identifying different phytoplasmas [6, 7]. In Vietnam, nested-PCR and phylogenetic analysis have also been used for detection and identification of many other phytoplasmas associated with plant diseases in recent years [8-11].

In this paper, a DNA-based approach and phylogenetic analysis based on 16S rDNA gene sequencing confirmed a phytoplasma strain of 16SrXIV group is associated with CinWB in Tra Bong district, Quang Ngai province.

Materials and methods

Plant materials

CinWB - infected samples were collected from different fields in the Tra Bong district - Quang Ngai province of Vietnam by the Quang Ngai plant protection sub-department and one sample was collected from an asymptomatic cinnamon plant in the North of Vietnam as the first negative control. Another sample devoid of DNA template was used as the second negative control.

DNA extraction and nested-PCR assay

Total genomic DNAs were extracted from 1 gr of CinWB-showing plant tissues and an asymptomatic sample using DNeasy plant mini kit (QIAGEN) according to the manufacturer's instructions. The extracted DNAs were quantified with a UV-Vis Spectrophotometer Optima SP-3000 nano (Indonesia) and subjected to nested-PCR assays.

Fifty nanograms of the extracted DNA were used for PCR amplification using P1 (5'-AAG AGT TTG ATC CTG GCT CAG GAT T-3')/P7(5'-CGT CCT TCA TCG GCT CTT-3') primers [12, 13] in a 25 µl reaction. The PCR reaction included 0.4 µM



Fig. 1. Typical symptoms of cinnamon witches' broom disease collected from Tra Bong district, Quang Ngai province, Vietnam (Photo source: Quang Ngai plant protection sub-department).

of each primer, 0.2 µM of each dNTP, 1.25 U DreamTaq DNA polymerase (Fermentas, Vilnius, Lithuania) and 1×Dream Taq polymerase buffer. The first round of PCR assays were 35 cycles of: 95°C for 1 min, 55°C for 2 min, and 72°C for 3 min in a Mastercycler Pro (Eppendorf, Germany). In the nested-PCR assay, 1 µl of the first PCR product was used as the DNA template in a mixture containing R16F2n (5'-GAA ACG AGT GCT AAG ACT GG-3') and R16R2 (5'-TGA CGG GCG GTG TGT ACA CCC G-3') primers [6] and other PCR components - as in the first round PCR assay. Water and DNA extracted from the symptomless cinnamon plant were used as negative controls in all PCR reactions. Six microliters of the nested-PCR products were separated in 1% agarose gel containing 0.5 µg/ml ethidium bromide and visualized with GelDoc-It® 310 Imaging System (United Kingdom).

Phylogenetic analysis

The nested-PCR products were purified and directly sequenced with both R16F2n and R16R2 primers with an ABI3100 sequencer. The DNA sequences were subjected to a BLAST search tool <https://blast.ncbi.nlm.nih.gov/Blast.cgi> [14] to identify the closest match. The 22 phytoplasma 16S rDNA

sequences were obtained from GenBank (Table 1). Phylogenetic analysis was conducted using the Neighbor-Joining method in MEGA 6.0 [15] with default values and 1,000 bootstrap analysis replications, and *A. laidlawii* was used as an outgroup.

Results and discussion

CinWB symptoms

CinWB usually affects cinnamon seedlings in nurseries, and both young and old trees in the field (Fig. 1). Symptoms usually appear on the stems, branches, petioles and veins of cinnamon. Firstly, tumors appear on the stems and branches of plants. These tumors develop long squid-like tassels. The infected plants become stunted, and their development is delayed; if plants are infected at an early stage of development, there is a significant risk of death, leading to yield loss for farmers. The disease damages plants throughout the year; however, the new infection starts developing from September to March of the next year; and the growth rate of tassels then increases rapidly from November to December.

Detection and identification of CinWB phytoplasma

In the first round of PCR assay using P1/P7 primers, there was no DNA

Table 1. Phytoplasma strains, associated diseases and accession numbers of their 16S rDNA sequences used for phylogenetic analysis.

Phytoplasma strain	16 SrDNA group/sub-group	Associated disease	Geographical location	GenBank accession No.	Reference
LDN	XXII-A	Awka wilt of	Nigeria	Y14175	Tymon, et al. (1998)
LYJ-C8	IV	Coconut lethal yellowing	Jamaica	AF498307	Harrison, et al. (2002)
SorBS	XXIV-A	Sorghum bunchy shoot	Australia	AF509322	Blanche, et al. (2003)
Pin127S	XXI-A	<i>Cand.</i> Phytoplasma pini	Spain	AJ632155	
CnWB	VI	Japanese chestnut trees witches broom	South Korea	AB054986	Namba, et al. (2002)
LfWB	VIII	Loofah witches broom	Taiwan	AF353090	Dally, et al. (unpublished)
EY1	V-A	Elm yellows	USA	AY197655	Lee, et al. (2004)
CPR	VI	Clover proliferation	USA	AY390261	Hiruki, Wang (2004)
AshY1	VII	Ash yellows	USA	AF092209	Griffiths, et al. (1999)
RYD-Th	XI	Rice yellow dwarf	Thailand	AB052873	Jung, et al. (2003)
BGWL	XIV	Bermuda grass white leaf	Iran	EF444485	Salehi, et al. (unpublished)
BGWL-C1	XIV	Bermuda grass white leaf	Italia	AJ550984	Marcone, et al. (2004)
CinWB	XIV	Cinnamon witches broom	Vietnam	JX413793	This study
CYD	XIV	Coconut yellow decline	Malaysia	EU328159	Nejat, et al. (2009)
BGWL	XIV	Bermuda grass white leaf	Malaysia	EU294011	Nejat, et al. (2009)
CYD	XIV	Coconut yellow decline	Malaysia	EU636906	Nejat, et al. (2009)
BGWL	XIV	Bermuda grass white leaf	Thailand	AF248961	Davis, Dally (unpublished)
<i>Cand.</i> Phytoplasma phoenicium	IX	Lethal disease of almond trees	Lebanon	AF515636	Verdin, et al. (2003)
WX	III	Western X-disease	-	L04682	Schneider, et al. (unpublished)
WTTWB	XXV-A	Weeping tee tree witches broom	Australia	AF521672	Davis, et al. (unpublished)
WBDL	II	Lime witches broom	United Arab Emirates	U15442	Zreik, et al. (1995)
HibWB	XV	Hibiscus witches broom	Brazil	AF147708	Montano, et al. (2001)
<i>A. laidlawii</i>		-	-	M23932	

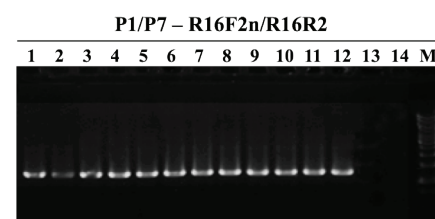


Fig. 2. Nested-PCR assay of phytoplasma isolated from the CinWB-showing cinnamon plants collected from Tra Bong district, Quang Ngai province, Vietnam (M: 1 kb DNA ladder; lanes 1 to 12: CinWB-infected samples collected from Tra Bong district, Quang Ngai province; lane 13: symptomless cinnamon plant as the first negative control; lane 14: No DNA as the second negative control).

observed in electrophoresis (data not shown). This was due to the fact that phytoplasma distributes un-uniformly in infected plant tissue therefore low DNA volume was amplified from the first round of PCR.

In the second round of PCR (nested-PCR), amplicons of about 1.2 kb in length were obtained from all 12 DNA templates isolated from the CinWB-showing cinnamon plants (Fig. 2 lanes 1-12), but the two negative controls produced no amplification (Fig. 2 lanes 13-14).

The twelve nested-PCR products were directly sequenced from both directions using two primers, R16F2n and R16R2, which were used in the second round of PCR. All 12 DNA sequences were identical. The consensus sequence of CinWB phytoplasma obtained in cinnamon grown in Quang Ngai province, Vietnam (QQNVN) was deposited in GenBank under accession number JX413793 (Fig. 3, shown in bold letters). A phylogenetic tree was constructed using 22 phytoplasma 16S rDNA sequences (Table 1). The QQNVN strain shared a high sequence similarity with a number of phytoplasmas classified in the 16SrXIV group and the phylogenetic tree confirmed this homology (Fig. 3).

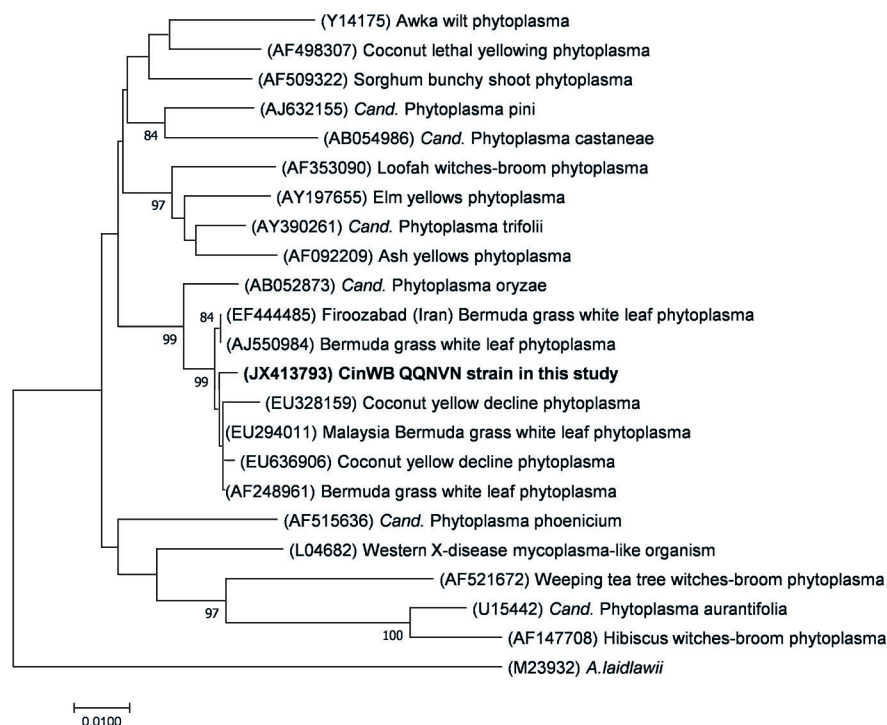


Fig. 3. Phylogenetic distance tree constructed by the neighbour-joining method, comparing the 16S rDNA sequence of QQNVN with other phytoplasmas from GenBank. Accession numbers are shown in parentheses. The number of branches is confidence percentages obtained from 1,000 bootstrap replicates (only values above 80% are shown). *A. laidlawii* is an outgroup.

Conclusions

In the present study, a combination of nested-PCR assays, DNA sequencing and phylogenetic analysis was applied. The results have confirmed the association of a phytoplasma strain with cinnamon plants showing witches' broom symptoms grown in Tra Bong district, Quang Ngai province. Through these approaches, for the first time the presence of a phytoplasma that belongs to a 16SrXIV group was demonstrated. Further studies are needed for fully understanding the causal agent(s) and their transmission manners - basic information for accurate management of such a disease.

ACKNOWLEDGEMENTS

Many thanks to the Quang Ngai plant protection sub-department for their support and assistance in collection of samples.

REFERENCES

- [1] CRC (1999), *Cinnamon and Cassia: The genus Cinnamomum*, Medicinal and Aromatic Plants - Industrial Profiles, CRC Press.
- [2] P.V. Luc (1999), *Study results on witches broom disease in Cinnamon and Cassia of South Vietnam, and prevention measures*, Vietnam National Centre for Natural Science & Technology, Hanoi.
- [3] Y. Arocha, O. Antesana, E. Montellano, P. Franco, G. Plata, P. Jones (2007), "Candidatus *Phytoplasma lycopersici*, a phytoplasma associated with hoja de perejil disease in Bolivia", *International Journal of Systematic and Evolutionary Microbiology*, **57**(Pt8), pp.1704-1710.
- [4] I.M. Lee, R.W. Hammond, R.E. Davis, D.E. Gundersen (1993), "Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasma-like organisms", *Molecular Plant Pathology*, **83**, pp.834-842.
- [5] D. Valiunas, J. Staniulis, R.E. Davis (2006), "Candidatus *Phytoplasma fragariae*, a novel phytoplasma taxon discovered in yellows diseased strawberry, *Fragaria ananassa*", *International Journal of Systematic and Evolutionary Microbiology*, **56**(Pt1), pp.277-281.
- [6] D.E. Gundersen, I.M. Lee, D.A. Schaff, N.A.

Harrison, C.J. Chang, R.E. Davis, D.T. Kingsbury (1996), "Genomic diversity and differentiation among phytoplasma strains in 16S rRNA group I (aster yellows and related phytoplasmas) and III (X-disease and related phytoplasmas)", *International Journal of Systematic and Evolutionary Microbiology*, **46**(1), pp.64-75.

[7] J. Hodgetts, N. Boonham, R. Mumford, N. Harrison, M. Dickinson (2008), "Phytoplasma phylogenetics based on analysis of secA and 23S rRNA gene sequences for improved resolution of candidate species of Candidatus *Phytoplasma*", *International Journal of Systematic and Evolutionary Microbiology*, **58**(Pt8), pp.1826-1837.

[8] D.T. Nguyen, T.X. Hoat, M.V. Quan (2016a), "Molecular detection and characterization of 16SrI phytoplasma associated with rice orange leaf symptom in Vietnam", *Phytopathogenic Mollicutes*, **6**(1), pp.29-32.

[9] D.T. Nguyen, T.X. Hoat, M.V. Quan (2016b), "Molecular detection and identification of a phytoplasma associated with maize bushy stunt in Vietnam", *Phytopathogenic Mollicutes*, **6**(2), pp.102-105.

[10] T.X. Hoat, N.G. Bon, M.V. Quan, V.D. Hien, N.D. Thanh, M. Dickinson (2012), "Detection and molecular characterization of sugarcane grassy shoot phytoplasma in Vietnam", *Phytoparasitica*, **40**(4), pp.351-359.

[11] T.X. Hoat, L.T.T. Nhung, D.V.T. Thanh, N.G. Bon, C.A. Dường, T.N. Ha, C.K. Nimal (2013), "Molecular detection and identification of sugarcane white leaf phytoplasma in Vietnam", *International Sugar Journal*, **115**(1357), pp.505-511.

[12] S. Deng, C. Hiruki (1991), "Amplification of 16S rRNA genes from culturable and nonculturable mollicutes", *Journal of Microbiological Methods*, **14**(1), pp.53-61.

[13] B. Schneider, E. Seemuller, C.D. Smart, B.C. Kikpatrick (1995), "Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas", *The Molecular and Diagnostic Procedures in Mycoplasmaology*, pp.369-380.

[14] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman (1990), "Basic local alignment search tool", *Journal of Molecular Biology*, **215**(3), pp.403-410.

[15] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar (2013), "MEGA6: Molecular Evolutionary Genetics Analysis version 6.0", *Molecular Biology and Evolution*, **30**(12), pp.2725-2729.

Promising rice mutants with high yields, good grain quality, and disease resistance due to irradiation technology

Duy Duong Tran¹, Manh Ha Nguyen², Duy Quy Tran^{3*}, Thi Dung Quan⁴, Hoang Dung Tran⁵

¹Institute of Agricultural Genetics (IAG)

²Technical Economic Vocational College To Hieu

³Institute for Asian - Pacific science technology research cooperation

⁴Tan Trao University

⁵Nguyen Tat Thanh University

Received 20 February 2017; accepted 28 June 2017

Abstract:

Three rice genotypes of NPT4, NPT5, and QP-5 were successfully selected through mutation breeding using Co⁶⁰ gamma rays with 250-300 Gy. Of the three lines, two genotypes, NPT4 and NPT5, obtained high yields of 8.9-9.2 tonnes/ha, had growth duration of 103-110 days in the season of autumn and 130-135 days in the spring season. They exhibited dark green leaves, which were found to be adapted to intensification. They were resistant to major pests and diseases, and tolerant to major abiotic stress due to unfavourable conditions, as compared to the popular rice hybrids of Nhi Uu 868, Thien Nguyen Uu 9, and HYT100. The grain quality properties of NPT4 and NPT5 genotypes were also found to be good, identified as such due to their light aroma and amylose content of 15-16%. The genotype QP-5 exhibited a high grain quality and high yield of 6.5-7.0 tonnes/ha, with a growth duration of 105-110 days, and 125-135 days in autumn and spring seasons, respectively. The aromatic rice genotypes offered 10-12% amylose content and became more delicious as compared to the Bac Thom 7 variety.

Keywords: amylose contents, gamma ray, irradiation technology, mutation.

Classification number: 3.1

Introduction

Ever since the epoch-making discoveries made by Muller and Stadler eighty years ago, the application of mutation techniques using different agents of physical and chemical nature has generated a vast amount of genetic variability and has played a significant role in modern plant breeding and genetic studies. The use of induced mutations over the past five decades has played a major role in the development of smart crop varieties all over the world. The widespread use of induced mutants in plant breeding programmes across the globe has led to the official release of 3,222 plant mutant varieties from more than 170 different plant species in more than 60 countries throughout the world; the developed varieties increase biodiversity and provide breeding material for conventional plant breeding, thus directly contributing to the conservation and use of plant genetic resource [1].

Before and up through 2016, Vietnam had developed 78 mutant rice varieties, according to the IAEA database. Vietnam is ranked 8th for mutant breeding in the world [2, 3]. The list includes 32 mutant rice varieties accordingly identified as: DT10, DT11, DT13, DT33, A20, DT21, DV2, DCM1, Khang Dan Dot Bien, DT37, DT39, VND-95-20, VND-99-3, Tai Nguyen Dot Bien, Tam Thom Dot Bien, P6DB, ST3DB, DB5, and DT39 [4]. Three rice genotypes NPT4, NPT5, and QP-5 were created and selected from leading rice cultivars as ST19 and DH18 using Co⁶⁰ gamma rays.

According to the Department of Phytosanitary VIII across several regions and ranging from 1/10/2015 to 2/12/2015, department procedures completed nearly 70 batches of imported hybrid rice seeds from China in spring 2014, with the total amount of seeds being 5,616 tonnes, including 4,496 tonnes of Nhi Uu 868 hybrid seeds; 376 tonnes of Nhi Uu 63; 350 tonnes of Nhi Uu 7; 271 tonnes of San Uu 63, and some varieties including: Nghi Huong 305, Xuyen Huong 178, Kinh So Uu 1588, Thinh Du 4, Thinh Du 11, and Hoa Uu 2. Although dominant rice hybrids usually produce high yields, in fact, no pest resistant rice hybrids is as pure as rice with *Xanthomonas* bacteria, and as far as pests of rice hybrids, they were

*Corresponding author: Email: duyquyvaas@gmail.com

great, especially when combined with China's rice hybrids. Furthermore, the average yield of rice hybrids cultivated in Vietnam is higher than the amount cultivated of pure rice by only about 10-15%. So if Vietnam does not actively study the creation of rice hybrids and pure rice varieties that produce high yields of high-quality rice, we will always spend foreign currency to import rice hybrids and continue our dependence on China and other countries [5].

The objectives of this study aim at: (1) Releasing new rice genotypes with short growth durations of 105-110 days in the summer season, and 120-135 days in the spring season, (2) and enhancing the highest yields by 7-10 tons/ha with resistance to blast, sheath blight, brown plant hopper, and being widely adaptable. These new genotypes could partly replace the proportion of cultivable area used for rice hybrid varieties, including in Khang Dan 18 and Q5 introduced from China.

Materials and methods

Materials

Rice seeds of two varieties: DH18 and ST19 were collected and stored in IAP.

Methods

Dry seeds of two varieties DH18 and ST19 with 13% grain moisture were irradiated by gamma ray Co^{60} at 25-30 Krad in the summer season of 2009.

After treatment, the normal seeds were collected, germinated, and planted in the IAP experimental field; this happened through individual selections of M2, M3, M4, and M5 from 2010 to 2012 during two different seasons.

The selection criteria emphasised the growth duration, plant height, panicle length, number of filled grains per panicle, grain shape, 1,000-grain weight, and grain yield. Biotic stress evaluation was done through IRRI-SES score with 9 levels.

- Fertiliser (NPK/ha) was applied at a rate of 100N: 90P: 80K kg/ha.

- Data is processed using IRRISTAT and Excel.

- DUS and VCU were conducted at the National Center for Yield Trial and Seed Certification - MARD.

- Quality test: TCVN 1643:1992.

- VCU test: Area of at least 1,000 m² per each site was required with local control varieties.

Results and discussions

Breeding schedule of new mutants NPT4, NPT5, and QP-5

Dry seeds of two varieties, DH18 and ST19, had 13% moisture and were irradiated with gamma ray Co^{60} at 250-300 Gy during the 2009 autumn season. Individual selection was done from the M2 up to M7 (Fig. 1).

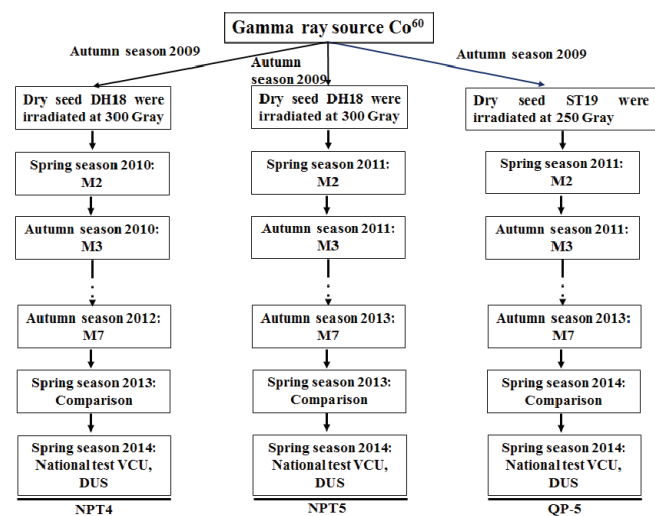


Fig. 1. Breeding schedule of three rice mutants viz. NPT4, NPT5, and QP-5.

Agronomical traits of NPT4, NPT5, and QP-5 mutants

The growth duration of the NPT4 genotype occurred for between 130 to 135 days in spring, and between 103 to 110 days in autumn as well as Khang Dan and also 5-7 days earlier than DH18. The NPT4 genotype exhibited as a compact plant type with pale green leaves, and it adapted to intensive practices performed to obtain high yields. Its grain amount per panicle offered 500-600 long, slender grains at a 1,000-grain weight of 20-22 g. Its grain quality property addressed a desirable amylose content of 15.8% to fit the consumer preference; with its gray husk colour as well as Bac Thom 7 (Table 1, Fig. 1).

Table 1. Agronomical traits of NPT4 rice genotype growing in IAP, Binh Xuyen District, Vinh Phuc Province, 2015.

Trait	NPT4	DH18	KD18	Nhi Uu 86b	HYT100	Thien Nguyen Uu 9
Growth duration (days)						
+Spring season	130-135	135-140	130-135	135-145	130-135	130-135
+Autumn season	103-105	110-115	100-105	110-120	105-110	110-115
Plant height (cm)	121-125	115-125	105-110	115-120	105-112	110-115
Plant type	V	V compact	V compact	V compact	V compact	V compact
Leaf angle	Horizontal	Horizontal	Straight	Straight	Straight	Straight
Grain no./ pan.	500-600	200-450	180-200	130-160	190-220	200-250
Unfilled grain (%)	15-20	20-25	12-14	10-12	12-15	12-15
1,000-gr.wgt. (g)	20-22	16-17	19-20	27-28	23-24	27-29
Yield (100 kg)	90-100	75-85	50-65	70-75	80-85	80-85
Amylose (%)	15.8	16.5	26-27	16-17	20-21	16-17
Alkaline digestion	4.7	4.8	4.9	4.2	3.9	4.3
Brown rice (%)	78.5	75.5	79.8	78.2	79.6	72.0
Milled rice (%)	70.5	69.5	70.2	70.4	70.8	65.6
Grain length (mm)	6.5	6.1	5.7	6.8	7.0	6.4
L : l ratio	2.90	2.69	2.70	2.85	3.05	2.30

The growth duration of NPT5 occurred at between 130-135 days in spring and between 105-110 days in autumn (105-110 days) as well as Khang Dan and 3-5 days earlier than DH18. The NPT5 exhibited a compact plant type with pale green leaves and adapted to intensive practices utilised to obtain high yields. Its grain number per panicle offered 400-550 long, slender grains and had a 1,000-grain weight of 20-23 g. Its grain quality addressed the desirable amylose content of 15.9%, which is enough to fit with consumer preference for a bright yellow husk (Table 2, Fig. 2).

Table 2. Agronomical traits of NPT5 rice genotype growing in IAP, Binh Xuyen District, Vinh Phuc Province, 2015.

Trait	NPT5	DH18	KD18	Nhi Uu 86b	HYT100	Thien Nguyen Uu 9
Growth duration (days)						
+ Spring season	130-135	135-140	130-135	135-145	130-135	130-135
+ Autumn season	105-110	110-115	100-105	110-120	105-110	110-115
Plant height (cm)	120-128	115-125	105-110	115-120	105-112	110-115
Plant type	V	V compact	V compact	V compact	V compact	V compact
Leaf angle	Straight	Horizontal	Straight	Straight	Straight	Straight
Grain no./ pan.	400-550	200-450	180-200	130-160	190-220	200-230
Unfilled grain (%)	13-15	20-25	12-14	10-12	12-15	12-15
1,000-gr.wgt. (g)	20-23	16-17	19-20	27-28	23-24	27-29
Yield (100 kg)	95-100	75-85	50-65	70-75	80-85	80-85
Amylose (%)	15.9	16.5	26-27	16-17	20-21	16-17
Alkaline digestion	4.8	4.8	4.9	4.2	3.9	4.3
Brown rice (%)	79.7	75.5	79.8	78.2	79.6	72.0
Milled rice (%)	72.4	69.5	70.2	70.4	70.8	65.6
Grain length (mm)	6.7	6.1	5.7	6.8	7.0	6.4
L : l ratio	2.85	2.69	2.70	2.85	3.05	2.30

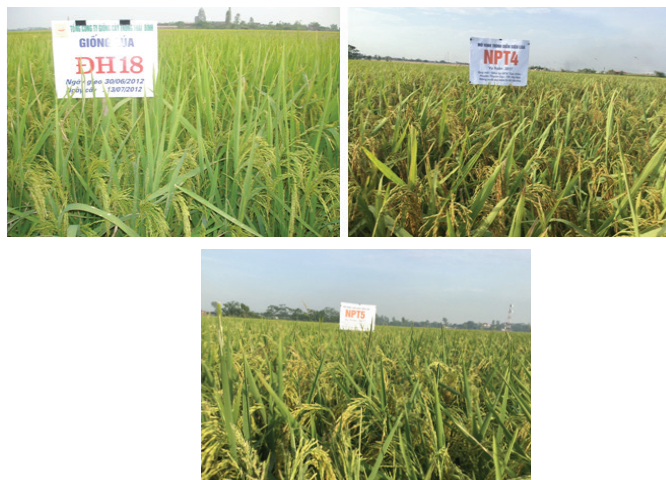


Fig. 2. NPT4, NPT5 varieties compared with DH18 varieties.

QP-5 rice mutants exhibited shorter growth duration than ST19 (5-8 days), which was used as a control because of its plant height of 95-100 cm, bright yellow grain husks, and 1,000-grain weigh of 24.8-25.4 g. Its grain quality addressed the desirable amylose content of 10.4-12% as compared to the control of ST19 at 28.7% with a slight aroma (Table 3).

Table 3. Agronomical traits of the Q5 rice genotype grown in IAP, Cam Giang district, Hai Duong province, 2015.

Trait	QP-5	ST19
Growth duration (day)	105-135	115-145
Plant height (cm)	95-100	105-108
Trunk diameter (mm)	0.4-0.5	0.4-0.5
Husk color	Bright yellow	Gold - yellow
1,000-grain weight (g)	24.8 -25.4	22.5-23.8
Amylose content (%)	10.4-12.0	28.7
Aroma (SES score from 0 to 2)	1	1
Grain length (mm) [L]	6.46	5.8
L : l ratio	3.01	2.87

Yield trial of NPT4, NPT5, and QP-5 mutant rice varieties

The yield trials were conducted over three years continuously at the IAP (2013-2015). The results indicate that NPT4 and NPT5 offered higher yields than the checks of DH18, Khang Dan 18, and the leading hybrid rice varieties as Nhi Uu 86b, Thien Nguyen Uu, and HYT100 (Table 4).

Table 4. Yield trials of NPT4 and NPT5 as compared to checks at IAP (tonnes/ha).

Designation	2013 Spring	2013 Autumn	2014 Spring	2014 Autumn	2015 Spring	Average yield
NPT4	9.65	8.27	9.88	8.72	9.75	9.25
NPT5	9.05	8.14	9.56	8.54	9.57	8.97
DH18	8.41	6.78	8.54	7.28	8.63	79.2
Khang Dan 18 (C)	6.85	5.85	6.65	5.67	6.52	6.30
Nhi Uu 86b	8.15	7.25	8.03	7.14	8.15	7.75
HYT100	8.26	7.35	8.34	7.38	8.52	7.95
Thien Nguyen Uu 9	8.44	7.46	8.47	7.46	8.5	8.07
CV (%)	4.90	6.60	7.50	5.80	7.40	
LSD 0.05	2.30	2.80	1.90	2.30	2.40	

Table 4 shows that the average yield of NPT4 and NPT5 mutants offered higher yields than hybrid rice varieties. NPT4 average yield obtained 9.25 tonnes/ha, which is more than NPT5, at 8.97 tonnes/ha, which was higher than the hybrid rice varieties *viz.* Nhi Uu 86b, HYT100, and Thien Nguyen Uu 9 at 7.75, 7.95, and 8.07 tonnes/ha, respectively.

Table 5 indicates that QP-5 yielded higher than the control (BT7) with an average yield of 6.94 tonnes/ha as compared to BT7, which yielded 5.92 tonnes/ha.

Table 5. Yield trial of QP-5 as compared to checks at IAP (tonnes/ha).

Designation	2013 Spring	2013 Autumn	2014 Spring	2014 Autumn	2015 Spring	2015 Autumn	Average yield
QP-5	7.02	6.58	7.38	6.76	7.25	6.67	6.94
BT7 (C)	6.55	5.27	6.43	5.37	6.52	5.35	5.92
CV (%)	4.80	6.60	6.30	6.20	7.10		
LSD 0.05	3.10	3.77	2.90	2.40	2.50		

Disease response by NPT4, NPT5, and QP-5 mutants

Under field conditions, NPT4 and NPT5 mutants exhibited a resistance to blast, brown planthopper, and sheath blight; even though it was lightly infected by bacterial leaf blight (NPT4).

Table 6. Reaction to major stresses from NPT4 and NPT5 mutants in two autumn seasons (2013-2014).

Designation	Scirpophaga incertulas	Plant hopper	Sheath blight	Bacterial leaf blight	Lodging
NPT4	1-3	1-3	1-3	3-5	1-3
NPT5	1-3	1-3	1-3	1-3	1-3
DH18	1-3	3-5	3-5	3-5	3-5
Nhi Uu 86b	1-3	1-3	1-3	3-5	1-3
Thien Nguyen Uu 9	1-3	1-3	1-3	3-5	1-3
HYT100	1-3	1-3	1-3	3-5	1-3
QP-5	1-2	1-3	1-3	1-3	1-3
Bao Thai Hong	1-3	3-5	3-5	1-3	3-5
Khang Dan 18	3-5	3	3	3-5	3-5
BT7	3-5	3	3	5-7	3-5

Sources: IAP, Plant protection research Institute.

Table 6 shows that NPT4, NPT5, and QP-5 mutants highly responded to major stresses as compared to wild rice types and Khang Dan 18 (a leading variety). They also expressed the better reaction as compared to some hybrid rice cultivars in the north.

Grain quality properties of NPT4, NPT5, and QP-5 mutants

Table 7 shows that NPT4 and NPT5 offer a higher milled rice percentage than hybrid rice varieties offer. Its amylose content was lower (15-17%) than with BC15, Khang Dan 18, Thien Nguyen Uu 9, and Nhi Uu 86b. Their productivity and quality exhibited better than some rice hybrids. In particular, the QP-5 grain quality was as good as Bac Thom 7 to meet the demand of consumers, with its soft, sticky, and transparent milled rice (Table 8, Fig. 3).

Table 7. Grain appearance quality of NPT4, NPT5, and QP-5 mutants in Cam Giang district, Hai Duong province in the 2015 autumn season.

Designation	Brown rice (%)	White rice (%)	Grain length (mm)	L : l ratio	Amylose contents (%)
Khang Dan 18	79.8	70.2	5.68	2.72	26-27
BC15	83.3	54.1	5.70	2.9	18-20
BT7	78.18	69.06	5.56	2.7	11.2-12.4
NPT4	78.9	70.5	6.12	2.90	15-16.2
NPT5	79.7	72.4	6.75	2.85	15-17
QP-5	80.2	69.6	6.46	3.01	11.5-12.5
Nhi Uu 86b	79.1	67.5	6.20	2.3	23-25
HYT100	80.0	65.5	6.70	2.9	18-20
Thien Nguyen Uu 9	79.5	64.1	6.90	3.0	20-22

Sources: IAP Institute.

Table 8. Grain cooking quality of rice genotypes.

Season	Designation	Aroma	Softness	Stickiness	Whiteness	Gloss	Exquisite
2014 Spring	Huong Thom 1	2	4	4	5	4	3
	Bac Thom 7	2	4	4	5	3	3
	Nang Tien	2	4	4	5	3	2
	QP-5	1	4	4	4	3	3
	AGPPS 151	3	4	4	5	3	3
	HQT6 (MB68)	1	4	4	5	3	3
	KTH08	1	4	4	5	4	3
2014 Autumn	Huong Thom 1	2	4	4	5	4	3
	Bac Thom 7	3	4	4	5	4	4
	Nang Tien	2	4	4	5	3	4
	QP-5	2	4	4	5	4	3
	HQT6 (MB68)	2	4	4	5	3	2
	ADI30	1	4	4	5	3	2

(Assessing by 10TCN-590-2004 Standard; Source: Data from Center for national testing 2014).

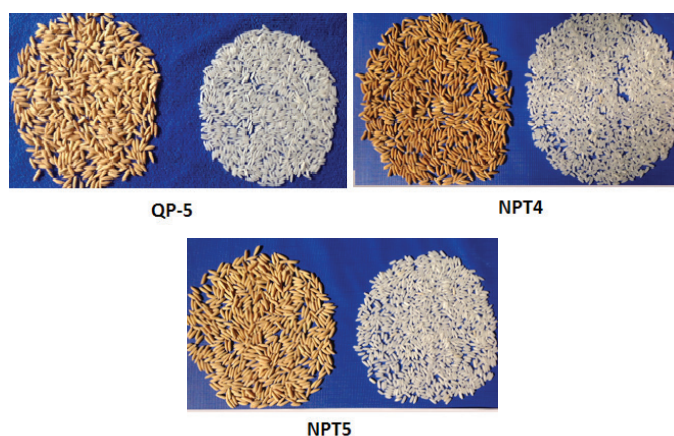


Fig. 3. Paddy and milled rice of QP-5, NPT4, and NPT5 varieties.

Conclusions

Three mutant rice genotypes NPT4, NPT5, and QP-5 were successfully selected and released through Co^{60} induction at 250-300 Gy.

NPT4 and NPT5 yielded an impressive form from between 8.9 to 9.2 tonnes/ha, with shorter growth durations (103-110 days in autumn and 130-135 day in spring), and good phenotypical acceptability as dark green leaves that are compact plant types. This is suitable for intensive practice as

compared to Nhi Uu 86b, Thien Nguyen Uu 9, and HYT100, which are the leading cultivars.

NPT4 and NPT5 exhibited their good grain quality properties as lightly scented grain, with an amylose content of 15-16%, and able to meet consumer demand in Northern Vietnam.

The QP-5 rice mutant exhibited high quality and yielded 6.5-7.0 tonnes/ha over a short growth duration of 105-110 days in autumn, and 125-135 days in spring. QP-5 is considered as an aromatic rice genotype with an amylose content of 10-12% to be recommended to commercialise as Bac Thom 7 cultivar.

REFERENCES

- [1] A. Raina, A.L. Rafiul, K. Shah Nawaz, A. Ruhul, R.T. Younas, P. Kouser, K. Samiullah (2016), "Role of Mutation Breeding in Crop Improvement - Past, Present and Future", *Asian Research Journal of Agriculture*, **2**(2), pp.1-13.
- [2] IAEA/FAO (2012), *Plant mutation breeding and biotechnology*.
- [3] H. Saika, A. Oikawa, F. Matsuda, H. Onodera, K. Saito, S. Toki (2011), "Application of gene targeting to designed mutation breeding of high-tryptophan rice", *Plant Physiology*, **156**(3), pp.1269-1277.
- [4] Duy Quy Tran, Duy Duong Tran, Huy Thuy Bui (2015), *Proceedings of the National Conference on Science and Nuclear Technology XIth*, pp.135-144.
- [5] Wang, X.C. Zhuang, Y. Jia (2014), "Development and Characterization of Rice Mutants for Functional Genomics Studies and Breeding", *Rice Science*, **21**(4), pp.1-13.

Establishment of ultrasensitive PCR assay targeting *cell-free* EBV DNA for early detection of nasopharyngeal carcinoma

Huu Tho Ho^{1*}, Nguyen Quynh Anh Vu¹, Tram Anh Do², Dinh Ung Nguyen¹, Thi Thu Hang Dinh¹,
Tien Sy Bui³, Dao Chinh Hoang⁴, Minh Ky Le⁵, Truong Phong Vu⁵, Thanh Tung Ngo⁶,
Kim Luu Nguyen⁷, Truong Sinh Luu¹, Van Ba Nguyen⁷, Anh Son Ho¹, Van Luong Hoang¹,
Duc Thuan Nghiem²

¹Biomedical & Pharmaceutical Applied Research Centre,
Vietnam Military Medical University

²ENT Department, 103 Hospital, Vietnam Military Medical University

³Department of Molecular Biology, 108 Military Central Hospital

⁴Department of Oncology and Radiation, 108 Military Central Hospital

⁵Oncology and Head&Neck Surgery Centre, Vietnam National ENT Hospital

⁶Department of Head&Neck Cancer and Radiation Oncology, Vietnam National K Hospital

⁷Oncology and Nuclear Medicine Centre, 103 Hospital, Vietnam Military Medical University

Received 5 July 2017; accepted 6 September 2017

Abstract:

In Vietnam, nasopharyngeal carcinoma (NPC) is the eighth most common cause of death from cancer. *Cell-free* Epstein Barr virus DNA (*cf*-EBV DNA) was reported to be present in almost all NPC patients. However, currently available assays in Vietnam can detect *cf*-EBV DNA in only 67.6% of NPC patients, thus leaving 32.4% of cancer cases undetected. Therefore, in this study, we aim to develop a highly sensitive quantitative PCR (qPCR) assay that measures the load of *cf*-EBV DNA for the purpose of early detection of NPC, and then evaluate the sensitivity and the specificity of the developed qPCR assay on the clinical samples. The major methods used in this study include primer/TaqMan probe design, *cf*-DNA extraction, optimization of qPCR assay and statistical analysis. Using an international standard panel from the Chinese University of HongKong, the linear range of developed qPCR assay is from 50-150,000 copies/ml ($R^2 = 0.99613$) and the detection limit has been shown to be 25 copies/ml. The developed assay could detect *cf*-EBV DNA with a sensitivity of 96.9% (31/32 NPC patients) and *cf*-EBV DNA has not been detected in 103 out of 105 healthy controls, which corresponds to a specificity of 98%. Consequently, the performance of the optimal assay has achieved remarkably high sensitivity and specificity. Moreover, the detection limit of our optimal qPCR assay is 25 copies/ml of plasma, which is at least ten times better than other assays tested in recent studies in Vietnam. This developed qPCR assay will also form the basis for further studies in Vietnam and will open many new applications in management of NPC.

Keywords: *cell-free Epstein-Barr virus DNA, nasopharyngeal carcinoma, quantitative PCR.*

Classification numbers: 3.2, 3.5

Introduction

Nasopharyngeal carcinoma (NPC) is malignant tumors that arise from epithelium cells of the nasopharynx - the upper part of the throat that is situated behind the nasal cavity and near the base of the skull [1, 2]. Globally, there were an estimated 84,400 cases of NPC and 51,600 deaths in 2008. In 2012, it was reported that there were 86,700 cases and 50,800 deaths respectively [3, 4]. However, the prevalence and the distribution of NPC varies throughout the world. NPC shows a low prevalence, and is even rare in some parts of the world such as Western Europe, North America and Japan, where it accounts for as little as 1% of total cases per year. Conversely, Southern China (especially Guangzhou) and Hong Kong are the regions with the highest incidence across the globe; while some parts of Southeast Asia and Asian countries show intermediate rates of this cancer [5-7]. In Vietnam, NPC is the eighth most common cancer with an annual incidence rate of about 7 per 100,000 people [5].

Due to the fact the patients often lack specific symptoms, NPC is often diagnosed at a late stage, leading to a 5-year survival rate of only 41%. However, if this disease is diagnosed at an early stage, this survival rate can be improved to more than 95% [8]. Unfortunately, owing to the hidden anatomic area of the nasopharynx, the occurrence of occult primary tumors in this area increases the difficulty of diagnosing the disease early and accurately. Meanwhile, imageological examinations are not effective for early detection of NPC tumors [9]. For these reasons, the development of reliable methods to detect NPC at an early stage is critical to improve the survival rate of patients.

Epstein-Barr virus (EBV) is known as human ubiquitous γ -herpesvirus 4 (HHV-4) and is one of eight known viruses in

*Corresponding author: Email: huuthottncydqs@yahoo.com

the herpesviridae family. This virus is one of the most common viruses in humans with greater than 90-95% of worldwide adult human population latently infected [10, 11]. Morphologically, EBV has the same structure of virion as other members of the herpesviridae family [12]. The genome of the EBV virus is a linear, double-stranded DNA molecule, varying in size from 168 kilo base pairs (kb) to 192 kb, that encodes more than 85 genes, most of these protein-encoding genes [13, 14].

A strong association between NPC and the Epstein-Barr virus has been documented in many studies. *Cf*-EBV DNA has been detected in the plasma and serum of patients with NPC and correlated with EBV status in tumors. In contrast, *cf*-EBV DNA has not been detected in healthy control subjects. In 1998, Mutirangura, et al. detected *cf*-EBV DNA in peripheral blood of NPC patients while no viral load was detected in healthy controls. Although this study only had a sensitivity of 31% [15], it was still an important breakthrough in early detection of NPC patients using a *cf*-EBV DNA assay. Later, in 1999, Lo, et al. developed a qPCR assay that could detect *cf*-EBV DNA in 96% of NPC patients (55 of 57) and 7% of controls (3 of 43), showing a high sensitivity and specificity [16]. Realtime qPCR for detecting *cf*-EBV DNA is a highly sensitive, specific and quantitative diagnostic approach. Since its advent, it has become a promising tool for early detection of NPC.

The quantification of *cf*-EBV DNA was demonstrated as a useful tool for early detection of NPC. However, its clinical utility is still not routinely applied in Vietnam. There are few studies following this approach in our country. Quantitative PCR assays for detection of *cf*-EBV DNA in peripheral blood in our country have not been described in detail and have low sensitivity, with a LoD of 300 copies/ml plasma [17]. Meanwhile, the procedure applied at research centers around the world has reached the LoD of 60 copies/ml plasma [18]. As a consequence, studies in Vietnam are able to detect DNA of EBV in peripheral blood in only 67.6% of NPC patients, leaving more than 30% of cancer cases undetected - a sensitivity much lower than that in other studies around the world. Consequently, we aim to develop a qPCR assay that is able to detect *cf*-EBV DNA in peripheral blood with high sensitivity in order to make early detection of NPC possible, therefore lengthening the survival rate of patients. We further aim to evaluate the sensitivity and specificity of the developed qPCR assay on clinical samples. This development would also form the basis for further studies and open many new applications to improve management of NPC patients and increase their survival rate.

Methods and materials

Study subjects

This study involved two groups of samples: clinical samples and healthy samples. Frozen and fresh human plasma samples were obtained from the archives of our clinical and research

laboratories. In clinical groups, a total of 32 biopsy-proven NPC patients were recruited for this study with informed consent. In another group, plasma samples from 105 volunteers were used as healthy controls, and follow up examinations of healthy volunteers were conducted for 6-12 months.

International standard panel

In this study, we have evaluated the performance of the optimal qPCR assay using the international standard panel that was provided by Prof. Allen Chan, at the Chinese University of Hong Kong. The international standard panel includes the series of nine DNA standard samples with known concentrations including: 15,000 - 6,000 - 600 - 120 - 60 - 30 - 15 - 5 - 2.5 copies/5 μ l and a standard plasma sample positive with EBV DNA.

EBV DNA extraction from plasma

Peripheral venous blood (5 ml) was taken from each patient and control, and placed in an EDTA tube. Within 6 hours of collection, all blood samples were transported to the research laboratory and were centrifuged at 1,500 g for 10 min. The plasma was then carefully removed from the EDTA tubes and transferred into 1.5 ml microtubes. The clotted blood samples and the plasma samples were stored at -80°C for further processing or gene analysis as needed.

DNA was extracted from the plasma by using ANAPURE VIRAL DNA/RNA Mini Kits (Anabio, Vietnam), with a modification regarding the volume of plasma utilized for isolating DNA, to increase the concentration of final eluted DNA by approximately 3 times (Fig. 1). This modification partly contributes to the enhancement of the sensitivity of the real-time PCR assay that measures the loads of *cf*-EBV DNA in peripheral blood.

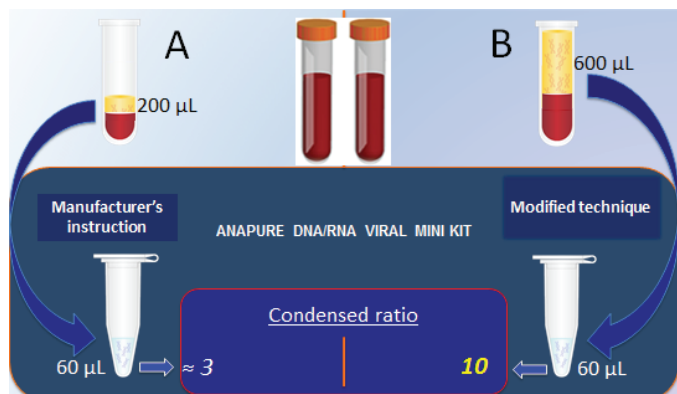


Fig. 1. Modified procedure for extracting DNA from plasma. The manufacturer's instruction (A) uses 200 μ l of plasma and elutes 60 μ l DNA, so the sample is concentrated about 3 times. Meanwhile, the eluted DNA was concentrated 10 times with the modified procedure (B) using 600 μ l of plasma.

Quantitative PCR

Design of primer/Taqman probe for quantitative PCR assay:

The Primer and Taqman probe used for the qPCR assay in this study were automatically designed and validated by Oligo Primer Analysis Software (Molecular Biology Insights, USA). EBV genomic sequences were downloaded from the database of GenBank (www.ncbi.nlm.nih.gov, accession: V01555).

One of the most important factors determining a high detection sensitivity of the plasma/serum qPCR test for circulating EBV DNA is sensitive target selection. In our study, we have designed three sets of primers/probes targeting three different regions of the EBV genome with characteristics: *BamHI*-W, EBNA-1, and LMP-1. *BamHI*-W is the region containing a large number of short repeated sequences (W repeats) and the EBNA-1 region also has glycine alanine sequence repeats [19]. LMP-1 was reported as the major oncogenic factor of NPC development and these were detected in 80-90% of NPC tumors [13], so this region was selected as one of the target genes for designing primers/probes in the qPCR assay. Moreover, during the study, a designation of primer/probe sets targeting the BZLF1 gene of the EBV genome from University of North Carolina was referenced [20].

The procedure of qPCR assay:

The quantitative PCR assay was performed and products were detected using a Rotor-Gene Q instrument system which is essentially a combined thermal cycler/fluorescence detector with the ability to optically monitor the progress of individual PCR reactions. The DNA template volume used in each 20 µl-qPCR reaction was a 10 µl DNA sample which was extracted from plasma. The designed primer and conventional dual labelled probes conjugated with FAM fluorophore and BHQ1 non-fluorescent quencher were used as the components of real-time PCR reaction. All the data was collected and analysed using Rotor-Gene Q software.

In qPCR experiments specific error is seen due to the quality of DNA after extraction from plasma. In order to minimize this error, a housekeeping gene, in this case β-actin, was simultaneously amplified in the same run with the sample, which served as an internal control template for all DNA samples with primer and probe set which were described in previous studies [21].

Statistical analysis

For plasma samples, *cf*-EBV DNA was expressed in copies per ml of plasma. Plasma results were considered negative for EBV when the spiked internal positive control sequence was amplifiable while EBV DNA was not detected. For purposes of data analysis, samples with no measurable EBV DNA were reported as having a viral load of zero.

The concentration of *cf*-EBV DNA in a plasma sample was calculated by the equation:

$$C = Q \times (V_{\text{DNA}}/V_{\text{PCR}}) \times (1/V_{\text{ext}})$$

where:

C = target concentration in plasma (copies per milliliter);

Q = target quantity (copies) determined by sequence detector in PCR;

V_{DNA} = total volume of DNA obtained after extraction, typically 50 µl per Anabio extraction;

V_{PCR} = volume of DNA solution used for PCR, typically 8,6 µl;

V_{ext} = volume of plasma extracted, typically 600 µl.

All the collected data was used to calculate the sensitivity and the specificity of the qPCR assay on the clinical samples.

Results and discussions

Optimization of quantitative PCR assay

Design of primer/probe used for qPCR assay:

After designing three primer/Taqman probe sets, we simultaneously validated these three sets (I), (II), (III) in one run using the same DNA template which was extracted from the standard EBV DNA positive plasma sample from the Chinese University of Hong Kong. In the initial experiment, the primer/Taqman probe set (III) targeting the *BamHI*-W region showed the earliest amplification signal (the red line) and highest specificity (no amplification signal in the “no template” control) (Fig. 2). Therefore, the primer/Taqman probe set (III) was chosen for further optimization of the qPCR assay.

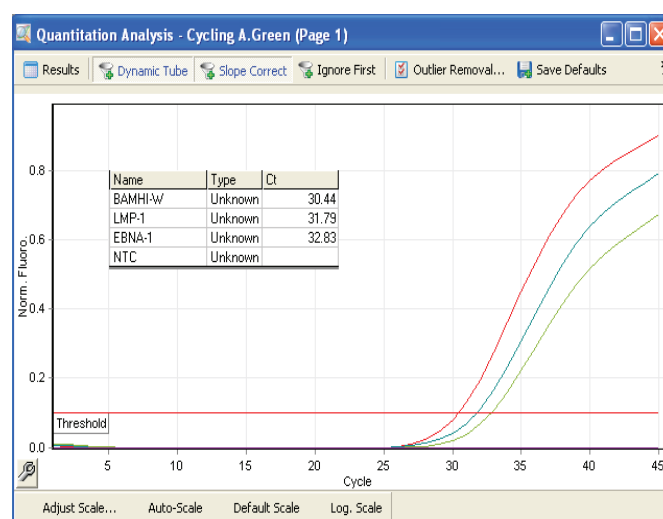


Fig. 2. The evaluation of three sets of primer/Taqman probes. EBNA-1, LMP-1 and BamHI-W indicated for three sets of primer/Taqman probes (I), (II), and (III), respectively.

Optimization of the primer concentration:

To optimize the qPCR assay, we optimized several components in the real-time quantitative PCR reaction, starting with primer concentration. The range of primer concentrations to test in the primer optimization as well as the concentrations of the probe to use needed to be determined separately. In our study, we utilized SYBR® Green dye-based assays to optimize the primer concentration without using a Taqman probe.

Five different concentrations of primer pairs in set (III) which was chosen in previous experiments were investigated, from 0.1 to 0.5 μM , leaving all the other reaction conditions unchanged. A primer concentration was considered to be optimal when the amplification resulted in an amplicon of the expected size, and when it showed a better performance than other concentrations.

As it was shown in Fig. 3, the real-time PCR analytical result revealed no amplification signal in reactions with primer concentration of 0.1 μM and “no-template” control, which means no contamination occurs in this experiment. The amplification signals of the remaining reactions with the primer concentration ranging from 0.2 to 0.5 μM were equivalent with the oscillation interval of Ct value from 30.89 to 31.90. The result from melting curve analysis (only for SYBR® Green dye-based assays) indicated the specificity of the amplification products.

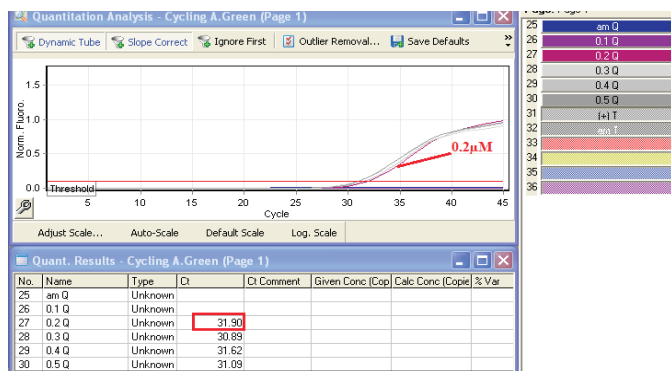


Fig. 3. Optimization of the primer concentrations for qPCR assay. 0.1Q, 0.2Q, 0.3Q, 0.4Q, 0.5Q indicated for the qPCR reactions with the same volume of positive DNA template and different primer concentrations, 0.1 μM , 0.2 μM , 0.3 μM , 0.4 μM and 0.5 μM , respectively. The primer concentration of the negative control or “no-template” control qPCR reaction (am Q) was 0.2 μM . As a result, the optimal primer concentration for the qPCR assay was determined to be 0.2 μM .

Optimization of the Taqman probe concentration:

Using the optimal primer concentration of 0.2 μM , we performed an experiment to determine the optimal concentration for the Taqman probe used in the qPCR assay among 3 different concentrations: 0.05, 0.1 and 0.2 μM .

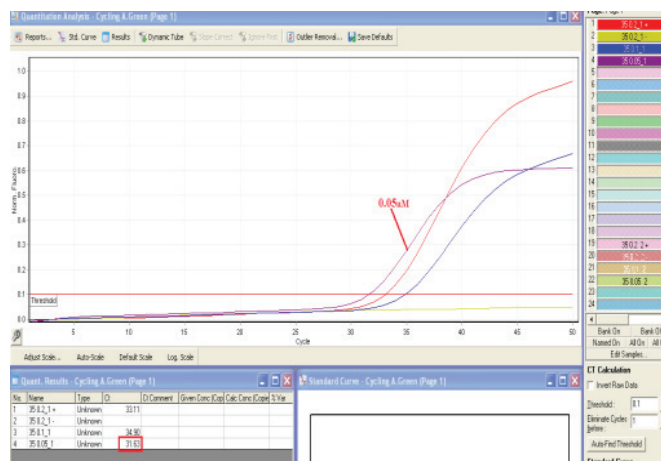


Fig. 4. Optimization of the Taqman probe concentration. (35 0.2_1+), (35 0.1_1), and (35 0.05_1) indicated for the qPCR reactions with the same volume of DNA template, optimal concentration of primer (0.2 μM), and of the Taqman probe at three different titres - 0.2, 0.1 and 0.05 μM , respectively. (35 0.2_1-) was the “no-template” control reaction with a primer concentration of 0.2 μM and probe concentration of 0.2 μM .

As it is shown in Fig. 4, the concentration that showed the earliest amplification signal was 0.05 μM . Consequently, 0.05 μM was determined as the optimal concentration of the Taqman probe for the qPCR assay.

Optimization of the DMSO concentration:

Following optimization of the primer and probe concentration in several previous experiments, we realized that non-specific products appeared in some real-time quantitative PCR reactions. Therefore, we tried to add to the qPCR reaction a small amount of DMSO as an additive to decrease the non-specific products generated in the qPCR reactions.

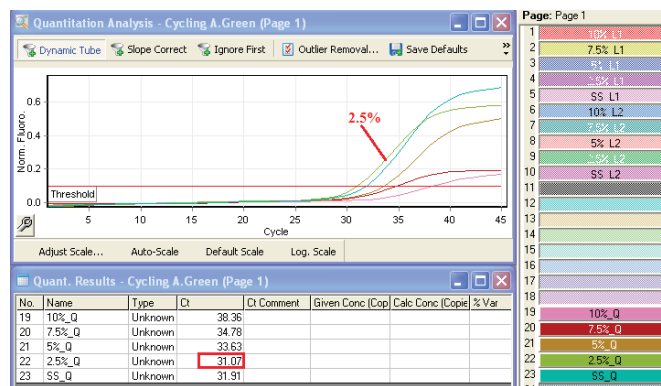


Fig. 5. Optimization of the DMSO concentration. (10%_Q), (7.5%_Q), (5%_Q), (2.5%_Q) indicated for the qPCR reactions with the same conditions of primer, probe, DNA template but different DMSO concentrations - 10%, 7.5%, 5% and 2.5%, respectively. (SS_Q) was the positive control reaction without DMSO addition (0% DMSO).

The concentrations of DMSO that were used in this experiment were: 2.5%, 5%, 7.5%, 10% and 0% (no addition of DMSO). The reaction with 0% DMSO was used as positive control to compare with other concentrations. As it is shown in the Fig. 5, the (2.5%_Q) sample gave the earliest amplification signal, even earlier than the positive control (SS_Q). The (2.5%_Q) sample was proven to amplify specific PCR products by agarose electrophoresis. Consequently, the DMSO concentration of 2.5% is optimal for the qPCR assay.

Evaluation of qPCR assay on international standard panel

Upon achieving an optimal qPCR assay, we evaluated this assay using an international standard panel from the Chinese University of Hong Kong. Nine DNA standard samples with concentrations of 150,000 - 60,000 - 6,000 - 3,000 - 1,200 - 600 - 300 - 150 - 50 - 25 copies/ml (equivalent to 15,000 - 6,000 - 600 - 300 - 120 - 60 - 30 - 15 - 5 - 2.5 copies/5 μ l) were used as a template of an optimal qPCR assay to investigate the sensitivity of the optimal qPCR assay. Reactions with high and medium concentrations of the template were run in duplicate and those reactions with low titers were run in triplicate (Fig. 6).

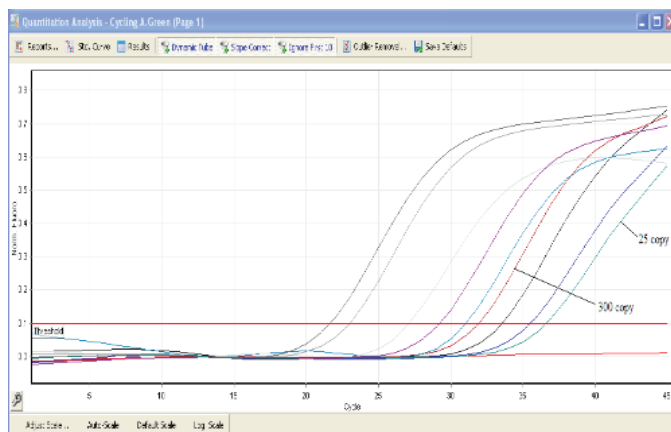


Fig. 6. The analytical sensitivity of the qPCR assay on standard panels. Amplification signals have been detected in all reactions (9/9) with standard DNA samples at different titers as template signals. The lowest concentration of DNA template corresponds to 25 copies/ml, which is ten times lower than the current detection limit in Vietnam. The negative control without a DNA template (red line below the threshold) did not show an amplification signal, indicating high specificity of the qPCR assay.

No amplification signal was detected in the “no-template” control, which indicated high specificity of the optimal qPCR assay. All the concentrations of DNA templates in the standard panels were detected in six out of the six replicates except the lowest standard concentration (25 copies/ml) that was detected in 17/18 replicates (95%). Therefore, 25 copies/ml was determined as the LoD of the optimal qPCR assay. This LoD is

more than ten times lower than that of qPCR assays published so far in Vietnam with an LoD of 300 copies/ml [17].

Simultaneously, the performance of the optimal qPCR assay for quantification of DNA EBV was also assessed on the international standard panels with a high correlation coefficient of $R^2 = 0.99613$ (>0.99) (Fig. 7). Thus, the optimal qPCR assay has a linear range from 50 to 150,000 copies/ml with an LoD of 25 copies/ml and highly reliable quantification of the *cf*-EBV DNA concentration.

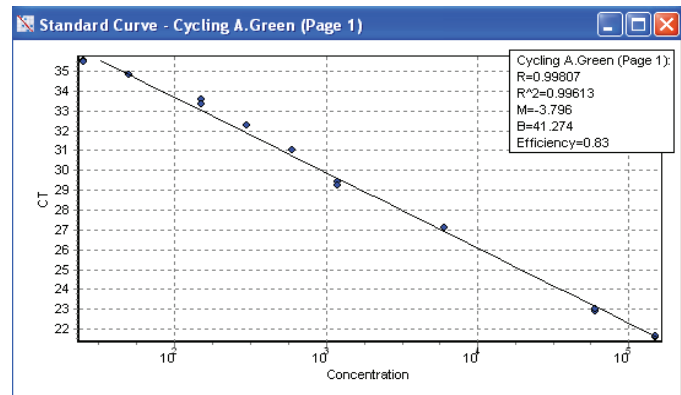


Fig. 7. Standard curve of qPCR assay based on international standard panel. The log of each known concentration in the dilution series of the standard panel (x-axis) is plotted against the corresponding Ct value for that concentration (y-axis).

Evaluation of qPCR assay on clinical samples

Using the optimal qPCR assay and the standard curve established using an international standard panel, we evaluated the qPCR assay on clinical samples to determine its sensitivity and specificity. The plasma samples from NPC patients ($n=32$) and healthy controls ($n=105$) were collected and quantified using the optimal qPCR assay to measure the *cf*-EBV DNA load in each sample (Fig. 8).

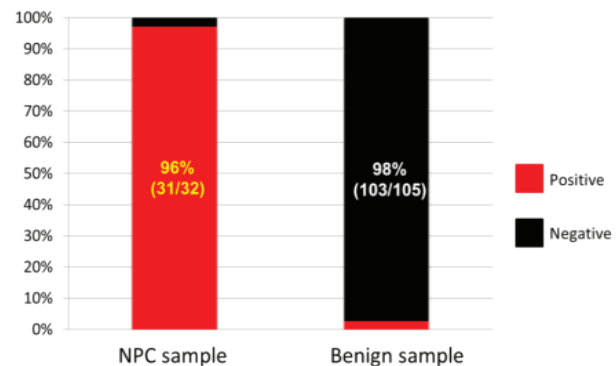


Fig. 8. The sensitivity and the specificity of the qPCR assay based on clinical samples.

Figure 8 illustrates the sensitivity and the specificity of the optimal qPCR assay based on two clinical sample groups: blood samples of NPC patients and those of healthy controls. In the first group, the optimal qPCR assay detected *cf*-EBV DNA in 31 of 32 NPC patients, which corresponds to a sensitivity of 96.9%. The concentration of *cf*-EBV DNA in these samples varied from 53 to 3.8×10^5 copies/ml. In the group of healthy controls, *cf*-EBV DNA was detected in only 2 cases and was not detected in 103 out of 105 healthy controls which corresponds to a specificity of 98% (103/105). The *cf*-EBV DNA loads detected in two blood samples derived from healthy controls were lower than 40 copies/ml. However, no *cf*-EBV DNA was detected by the optimal qPCR assay with the blood drawn from these two healthy controls after two weeks. All the extracted DNA samples have been shown to be amplifiable with the internal control qPCR assay (β -actin) (Fig. 9).

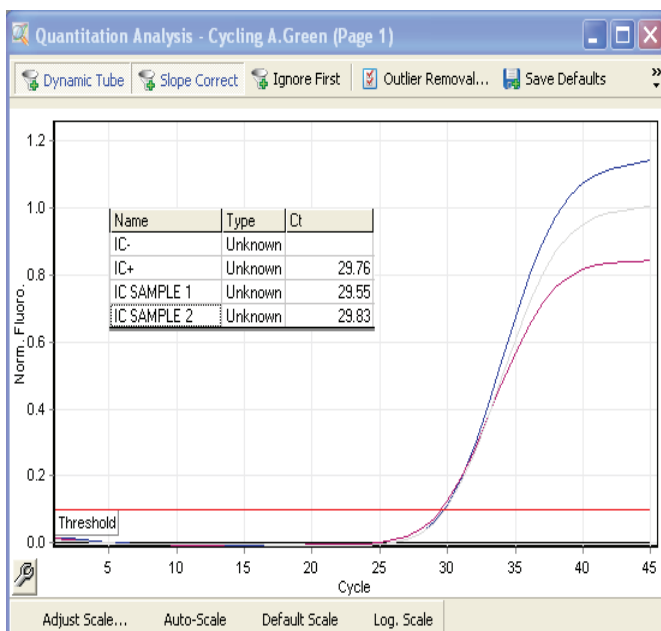


Fig. 9. Illustration of internal control qPCR assay. IC+ indicated for positive control of internal control, IC- indicated for no template control and IC SAMPLE 1 and IC SAMPLE 2 indicated for the evaluation of extracted DNA samples using internal control qPCR assay targeting β -actin gene.

Compare to recent studies in Vietnam

Based on the data we have so far, optimal qPCR assay targeting *cf*-EBV DNA for early detection of NPC has achieved high sensitivity of 96.9% and specificity of 98%. Currently available qPCR assays in studies published so far in Vietnam (2015) have a sensitivity of 68% at most, and unknown specificity, which is not sufficient for early detection of NPC. Moreover, the LoD of our optimal qPCR assay is 25 copies/ml plasma, which is ten times lower than other qPCR published in recent studies in Vietnam.

Conclusions

This study has shown the successful establishment of an ultrasensitive qPCR assay for detection of *cf*-EBV DNA with the detection limit of 25 copies/ml, which is more than ten times better than currently available assays in our country (300 copies/ml).

Evaluation on clinical samples has proven a remarkably high sensitivity of 96.9% and high specificity of 98%.

Furthermore, the optimal qPCR assay was evaluated using the international standard panel from the Chinese University of Hong Kong, thus resulting in high reliability.

Our novel qPCR assay enables detection of NPC at an early stage, thus contributing to an improved survival rate of patients. It will also form a basis for further studies in Vietnam and open many new applications in the management of NPC.

ACKNOWLEDGEMENTS

This work was supported by the Hanoi Department of Science and Technology. The standard panel of EBV DNA and positive control were kindly provided by Professor Allen Chan, of the Chinese University of Hong Kong. We would like to thank Pham Van Quyen for his excellent technical assistance and Pham The Tai, Hoang Xuan Su, Duong Thuy Linh, and Do Lan Huong for their helpful input.

REFERENCES

- [1] American Cancer Society (2015), *Nasopharyngeal Cancer*.
- [2] P. Busson, C. Keryer, T. Ooka, M. Corbex (2004), "EBV-associated nasopharyngeal carcinomas: from epidemiology to virus-targeting strategies", *Trends Microbiol.*, **12**(8), pp.356-360.
- [3] L.M. Sri Ganesh Muthiah (2016), "Epstein-barr virus (EBV) and its association with nasopharyngeal carcinoma (NPC): a systematic review", *International Journal of Public Health and Clinical Sciences*, **3**(4), p.149.
- [4] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal (2015), "Global cancer statistics, 2012", *CA Cancer J. Clin.*, **65**(2), pp.87-108.
- [5] M.D. Cheng Her (2001), "Nasopharyngeal Cancer and the Southeast Asia Patient", *American Family Physician*, **63**(9), pp.1776-1783.
- [6] Everett E. Vokes, David N. Liebowitz, Ralph R. Weichselbaum (1997), "Nasopharyngeal carcinoma", *Lancet*, **350**, pp.1087-1091.
- [7] K.F.T. Kwok Wai Lo, Dolly P. Huang (2004), "Focus on nasopharyngeal carcinoma", *Cancer Cell*, **5**(5), pp.423-428.
- [8] S.J. Chai, K.C. Pua, A. Saleh, Y.Y. Yap, P.V. Lim, S.K. Subramaniam, C.L. Lum, G. Krishnan, W.R. Mahiyuddin, N.P.C.S.G. Malaysian, S.H. Teo, A.S. Khoo, L.F. Yap (2012), "Clinical significance of plasma Epstein-Barr Virus DNA loads in a large cohort of Malaysian patients with nasopharyngeal carcinoma", *J. Clin. Virol.*, **55**(1), pp.34-39.
- [9] J. Zhang, C. Shu, Y. Song, Q. Li, J. Huang, X. Ma (2016), "Epstein-Barr virus DNA level as a novel prognostic factor in nasopharyngeal carcinoma: A meta-analysis", *Medicine (Baltimore)*, **95**(40), p.e5130.
- [10] M.S. Chang and W.H. Kim (2005), "Epstein-Barr virus in human malignancy: a special reference to Epstein-Barr virus associated gastric

carcinoma", *Cancer Res. Treat.*, **37(5)**, pp.257-67.

[11] I. Anagnostopoulos, M. Hummel, Cornelia Kreschel, Harald Stein (1995), "Morphology, immunophenotype, and distribution of latently and/or productively Epstein-Barr virus-infected cells in acute infectious mononucleosis: implications for the interindividual infection route of Epstein-Barr virus", *Blood*, **85(3)**, p.744-750.

[12] O.A. Odumade, K.A. Hogquist, H.H. Balfour Jr. (2011), "Progress and problems in understanding and managing primary Epstein-Barr virus infections", *Clin. Microbiol. Rev.*, **24(1)**, pp.193-209.

[13] A.S. Ali, M. Al-Shraim, A.M. Al-Hakami, I.M. Jones (2015), "Epstein- Barr virus: clinical and epidemiological revisits and genetic basis of oncogenesis", *Open Virol. J.*, **9**, pp.7-28.

[14] S.D. Hudnall (2014), "Epstein-Barr virus: pathogenesis and host immune response", *Viruses and Human Cancer*, pp.7-24.

[15] A. Mutirangura, W. Pornthanakasem, A. Theamboonlers, V. Sriuranpong, P. Lertsanguansinchi, S. Yenrudi, N. Voravud, P. Supiyaphun, Y. Poovorawan (1998), "Epstein-Barr viral DNA in serum of patients with nasopharyngeal carcinoma", *Clin. Cancer Res.*, **4(3)**, pp.665-669.

[16] Y.M. Lo, L.Y. Chan, K.W. Lo, S.F. Leung, J. Zhang, A.T. Chan, J.C. Lee, N.M. Hjelm, P.J. Johnson, D.P. Huang (1999), "Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma", *Cancer Res.*, **59(6)**, pp.1188-1191.

[17] Huy Tan Pham, Huy Thinh Tran, Thi Thuy Hang Tran, Dinh Phuc Nguyen, Van Khanh Tran (2015), "Quantitative analysis of cell - free EBV-DNA in plasma of patients with nasopharynx cancer and correlation to Tumor Nodes Metastasis diagnosis", *Journal of Medical Research*, **95(3)**, pp.24-31.

[18] Q.T. Le, Q. Zhang, H. Cao, A.J. Cheng, B.A. Pinsky, R.L. Hong, J.T. Chang, C.W. Wang, K.C. Tsao, Y.D. Lo, N. Lee, K.K. Ang, A.T. Chan, K.C. Chan (2013), "An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma", *Clin. Cancer Res.*, **19(8)**, pp.2208-2215.

[19] T.T. Yip, R.K. Ngan, A.H. Fong, S.C. Law (2014), "Application of circulating plasma/serum EBV DNA in the clinical management of nasopharyngeal carcinoma", *Oral Oncol.*, **50(6)**, pp.527-538.

[20] J.L. Ryan, H. Fan, S.L. Glaser, S.A. Schichman, N. Raab-Traub, M.L. Gulley (2004), "Epstein-Barr virus quantitation by real-time PCR targeting multiple gene segments: a novel approach to screen for the virus in paraffin-embedded tissue and plasma", *J. Mol. Diagn.*, **6(4)**, pp.378-385.

[21] Y.M. Lo, M.S. Tein, T.K. Lau, C.J. Haines, T.N. Leung, P.M. Poon, J.S. Wainscoat, P.J. Johnson, A.M. Chang, N.M. Hjelm (1998), "Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis", *Am. J. Hum. Genet.*, **62(4)**, pp.768-775.

Neuroprotective effects of *Diospyros kaki* and *Gardenia jasminoides* against ischemic neuronal injury in mice

Thi Xoan Le*, Thi Nguyet Hang Pham, Van Tai Nguyen, Viet Dung Le

National Institute of Medicinal Materials

Received 8 May 2017; accepted 15 September 2017

Abstract:

This study aims to clarify the neuroprotective effects of ethanol extracts of *Diospyros kaki* (DK) leaves and *Gardenia jasminoides* (GJ) fruit on cerebral ischemia injury using middle cerebral artery occlusion (MCAO) model in mice. *Swiss albino* mice were daily treated with DK extract (125-500 mg/kg b.w) and GJ extract (250-1,000 mg/kg b.w) for 1 week before being subjected to MCAO. The administration of edaravone (6 mg/kg, i.v), which was a reference drug, was started immediately after MCAO. DK and edaravone treatment improved neurological deficits and reduced infarct volume of MCAO mice compared to that of a vehicle-treated one. GJ treatment improved neurological deficits, but did not affect the infarct volume of MCAO mice. These results suggested that the DK and GJ treatment might be beneficial for protecting the neuronal system against cerebral ischemic injury.

Keywords: cerebral ischemia, *Diospyros kaki*, *Gardenia jasminoides*, middle cerebral artery occlusion, neuroprotection.

Classification number: 3.3

Introduction

Stroke is the major cause of disability and the fourth leading cause of death worldwide. Ischemic stroke accounts for approximately 80% of all strokes. Ischemic injury is associated with vascular leakage, inflammation, tissue injury, and cell death [1]. Cellular changes associated with ischemia include impairment of metabolism, energy failure, free radical production, excitotoxicity, altered calcium homeostasis, and protease activation. All

these events affect the brain's functions and contribute to long-term disabilities [2]. The advantage of herbal medicines with diverse chemical components and multi-targeted effects may bring breakthroughs for the complicated and closely related diseases like cerebral ischemia. Therefore, searching for the potential drugs from plants to treat ischemic cerebrovascular diseases will be a worthy direction to explore.

DK is a deciduous tree belonging to the family Ebenaceae. Pharmacological

studies have shown that DK leaf flavonoid has extensive pharmacological actions, including dilation of blood vessels, a lipid-reducing effect, a glucose-lowering effect, and antioxidant properties. DK leaf flavonoid can elevate ischemic tolerance by reducing inflammatory reactions and vascular endothelial injury [3]. Moreover, DK leaves possess an antithrombotic activity. A 10,000 D anticoagulant fraction, which was purified from the leaves of DK, inhibited thrombin-catalyzed fibrin formation with a competitive inhibition pattern [4].

GJ is an evergreen flowering plant of the family Rubiaceae. The fruit of GJ is traditionally used due to its homeostatic, antiphlogistic, analgesic, anti-inflammatory, and antipyretic effects. GJ has the obvious effect of preventing and treating atherosclerosis and thrombosis in the cardiovascular system [5]. It also has central sedative, analgesic, anti-diabetes, anti-depression and anti-inflammatory effects [6]. Moreover, Haiyan, et al. [7] reported that GJ extract had the functions of learning and memory improvement and neuroprotective effect on chronic cerebral ischemia in rat models.

In this study, the neuroprotective effects of DK and GJ extracts against cerebral ischemia were investigated by using middle cerebral artery occlusion

*Corresponding author: Email: xoanle@gmail.com

(MCAO) model in mice.

Materials and methods

Extract preparation

DK leaves were collected in Yen Bai province and GJ fruit were collected in Bac Ninh province. These herbs were identified by Dr. Pham Thanh Huyen, Department of Medicinal Plant Resources, National Institute of Medicinal Materials (NIMM).

For the DK extract preparation, the leaves of DK were dried in a hot-air oven and ground. The DK powder (100 g) was extracted with 70% ethanol under reflux for two hours and then filtrated. The extraction was repeated three times. After filtration, the combined extract was concentrated at 50°C under reduced pressure and dried in vacuum oven at 50°C to obtain 18.4 g of DK extract. The total flavonoid content of this DK extract was estimated to be 7.99% using spectrophotometric analysis. For the GJ extract preparation, the fruit of GJ was dried in a hot-air oven and ground. 100 g of GJ powder was extracted with 50% ethanol under reflux for two hours and filtrated. This step was repeated three times and the filtrate was combined, concentrated at 50°C under reduced pressure, and then dried in a vacuum oven at 50°C. The yield of the extraction from the dried fruit was calculated to be 32.4%. The GJ extract was estimated to contain 11.79% of geniposide (HPLC analysis).

Animals

Male *Swiss albino* mice (National Institute of Hygiene and Epidemiology, Hanoi, Vietnam) were purchased at the age of 6-7 weeks old. The animals were housed in the laboratory animal room maintained at 25±1°C with 12-hour light/dark cycle for at least one week before the commencement of the experiments. Animals were given access to food and water *ad libitum*. The behavioural

experiments were conducted during the light phase from 9:00 to 18:00.

Middle cerebral artery occlusion

Transient cerebral ischemia in mice was induced as previously reported [8, 9] with slight modifications. Briefly, mice were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.). After disinfecting the fur and skin with 70% ethanol, the midline neck was incised to dissect the left common carotid artery (CCA) from surrounding tissues. The CCA was temporarily occluded by a temporary suture using 5-0 silk. A permanent suture is placed around the external carotid artery (ECA), and another temporary suture is placed on the ECA distal to the bifurcation. The left internal carotid artery (ICA) was clipped using reverse-action tweezers to avoid bleeding. After cutting a small hole into ECA between the permanent and temporary sutures, 12-mm long 6-0 silicon-coated (about 2 mm is coated with silicon) monofilament suture was introduced into the ECA and then inverted into the ICA. The suture was tightly tied around the monofilament to prevent bleeding and the reverse-action tweezers were removed. The occluder was introduced to occlude the origin of the MCA in the circle of Willis (9-10 mm insertion beyond the bifurcation of ECA and CCA). The suture on the ECA was tightly tied to fix the monofilament in position. The temporary suture was removed from the CCA. After 60 minutes of occlusion, the monofilament suture was withdrawn to allow reperfusion.

Neurological score

A neurological grading scale was used to assess neurological recovery after MCAO injury according to Menzies, et al. [10]: scale: 0 = no apparent deficits; 1 = right forelimb flexion, 2 = decreased grip of the right forelimb while tail pulled, 3 = spontaneous movement in all directions (right circling only if pulled

by tail), 4 = spontaneous right circling. The tests were performed daily for 6 days from day 0 (one hour after the reperfusion) and continued until the end of the experiment.

Estimation of brain infarct volume

Six days after reperfusion, the MCAO-subjected mice were killed to estimate the brain infarct volume. Brains were removed quickly from the skulls and chilled in ice-cold saline. The coronal tissue sections (2×5 mm) were obtained using a tissue slicer. The slices were immersed in a saline solution containing 0.8% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, St. Louis, MO, USA) for 10 minutes at 37°C. The area of the infarction was measured using Image J software (ver. 1.41, NIH; Bethesda, MD, USA). The total infarct volume of each brain was calculated by the summation of the infarct areas of all brain slices. The infarct area of each slice was calculated by subtracting the normal ipsilateral areas from the contralateral hemisphere to reduce errors due to cerebral edema and was presented as the percentage of the infarct to the area of the contralateral hemisphere [8, 11].

Drug administration

DK and GJ extracts were suspended in distilled water. The administration period of DK and GJ extracts was started one week before the surgery and continued until the decapitation day (day 6). DK extract at a daily dose of 125, 250, 500 mg/kg b.w or GJ extract at a daily dose of 250, 500, 1,000 mg/kg b.w were per-orally administered to mice. On the operation day, mice were received the DK and GJ extracts one hour before the test. The edaravone (≥ 98% purity) provided by Dr. Nguyen Van Tai, Department of Phytochemistry, NIMM was dissolved in 0.9% saline. The administration of edaravone (6 mg/kg b.w, i.v) was started from the day of operation immediately after the MCAO. Distilled water was

per-orally administered to MCAO vehicle group mice.

Data analysis

Statistical analyses were performed using SigmaPlot 12.0 (SYSTAT Software Inc., Richmond, CA, USA) (statistical analysis software). Data were presented as mean $\pm$ S.E.M. or as median (interquartile range). Neurological scores were analysed using Kruskal-Wallis and Mann-Whitney U-test. Infarct volume was analysed using one-way analysis of variance (ANOVA) followed by *post hoc* Student-Newman-Keuls test for multiple comparisons. Differences of $p < 0.05$ were considered significant.

Results

The effects of DK and GJ extracts on neurological score

MCAO mice showed neurological deficits after being subjected to MCAO for 60 minutes and reperfusion. The MCAO animals revealed the decrease in motor ability and ability to respond to stimuli on the side of the body contralateral to ischemia.

The animals treated with DK extract showed a significant improvement in neurological deficits induced by MCAO at a dose of 500 mg/kg on day 2, day 4, and day 6 after injury (Table 1). The effect of DK extract on the neurological score is in a dose-dependent manner. Treatment with GJ extract at the dose of 500 mg/kg reduced neurological deficits in MCAO mice observed on day 5 and day 6 after MCAO operation. At the dose of 1,000 mg/kg, the GJ extract treatment also reduced neurological deficits of MCAO mice on day 6 ($p < 0.05$). Edaravone-treated MCAO mice showed a significant decrease in neurological deficits compared to the vehicle-treated one on day 2 and day 6 ($p < 0.05$) (Table 1).

Table 1. The effects of DK and GJ extracts on the neurological score.

Treatment groups	Dose (mg/kg)	Day(s) after MCAO surgery						
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Vehicle	0	4 (3.25; 4)	2.5 (2; 3.75)	2 (2; 3)	2 (1.25; 3.75)	2.5 (1.25; 3)	2 (2; 3)	3 (2; 3)
DK	125	4 (3; 4)	3 (2; 4)	3 (1.5; 3)	3 (1.5; 3)	2 (2; 3)	2 (1.25; 3)	2.5 (0.5; 3)
	250	4 (4; 4)	3 (2; 3)	2 (1; 3)	2 (1; 3)	2 (1; 3)	1 (1; 3)	2 (0; 3)
	500	3 (3; 3.75)	2 (2; 2.75)	1.5* (1; 2)	2 (2; 2)	1* (1; 1.75)	1 (1; 1.75)	2* (1; 2)
GJ	250	4 (4; 4)	2.5 (2; 3)	2.5 (2; 3)	2.5 (1.75; 3)	2 (0.75; 2.25)	2 (0.75; 2.25)	1.5 (0.75; 3)
	500	4 (3; 4)	3 (2; 3)	3 (1.5; 3.5)	3 (1; 3)	2 (1.5; 3)	1* (1; 2)	2* (1.5; 2)
	1000	3.5 (3; 4)	2 (2; 3)	3 (2; 3)	3 (2; 3)	2.5 (1.75; 3)	2 (1.75; 2.25)	2* (1; 2)
Edaravone	6	4 (3; 4)	2 (0; 3)	1* (0; 2)	1 (1; 2)	1 (1; 2)	2 (0; 2)	2* (1; 2)

Data were presented as median (interquartile range). * $p < 0.05$ vs. vehicle-treated MCAO mice (Kruskal-Wallis and Mann-Whitney U-test).

The effects of DK and GJ extracts on infarct volume

TTC staining was employed to measure the cerebral infarcts in focal ischemia induced by MCAO. TTC acted as a proton acceptor for many pyridine nucleotide-linked dehydrogenases that, along with the cytochromes, formed an integral part of the inner mitochondrial membrane and made up the electron transport chain. The tetrazolium salt was reduced by the enzymes into a red, lipid-soluble formazan. Viable tissue, therefore, stained deep red while the infarct remained unstained [12].

Figure 1A showed the typical photographs of TTC-stained brain sections of MCAO mice 6 days after reperfusion. As shown in Fig 1B, 6 days after MCAO, the infarct volumes

of vehicle-treated MCAO animals were 40% higher in average. The treatment with DK extract dose-dependently and significantly reduced the brain infarction in the mouse brain. The effect of GJ extract on brain infarction in MCAO mice was insignificant while the treatment of edaravone (6 mg/kg) immediately after MCAO produced a significant reduction in brain infarct.

Discussions

The MCAO in rodents has been widely used to evaluate the effects of the potential treatment of cerebral ischemia. This model offers the advantage of inducing reproducible transient or permanent ischemia of the MCA territory in a relatively non-invasive manner. Intraluminal approaches interrupt the blood flow of the entire territory

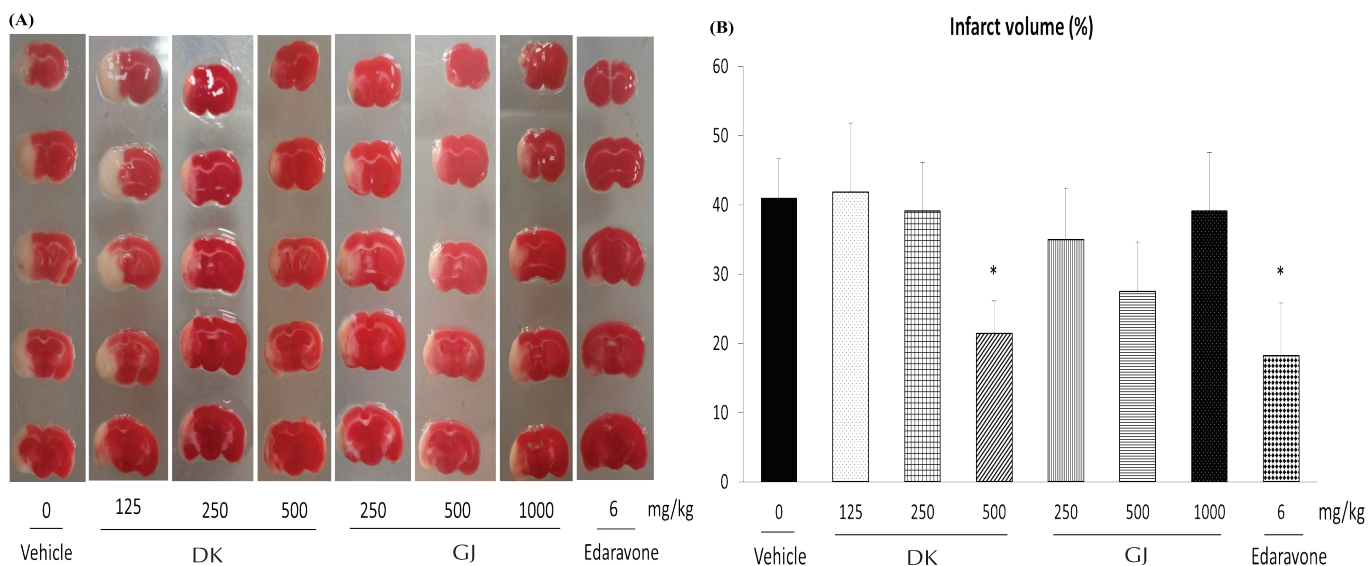


Fig 1. The effects of DK and GJ extracts on the infarct volume of MCAO mice 6 days after reperfusion. (A) Typical images of TTC-stained brain section; **(B)** Quantitative analysis of infarct volume (%) in the brain of MCAO mice. Each data column represents the mean $\pm$ S.E.M. (n=6-9). *p<0.05 vs. vehicle-treated MCAO mice (ANOVA followed by post hoc Student-Newman-Keuls test).

of this artery, result in reproducible lesions in the cortex and striatum, and can be either permanent or transient [13]. The reperfusion by removal of the occluding filament at least partially results in the restoration of blood flow after spontaneous or therapeutic lysis of a thromboembolic clot in human. In this study, the 60-minute MCA occlusion after reperfusion induced a remarkable infarct area along with motor function deficits in mice. These results are in agreement with our previous report [8]. Moreover, the administrations of DK and GJ extracts showed potential neuroprotective effects on brain ischemia injury induced by MCAO. These results suggest that the effects of DK and GJ extracts against ischemic brain injury are independent from the suppression of cerebral thrombosis mechanism.

The administration of DK extract significantly and dose-dependently reduced MCAO-induced injury. The effects of DK extract were quite similar to a reference drug, edaravone. Edaravone

has been approved for the treatment of acute ischaemic stroke in Japan and is still under clinical investigation in some countries [14]. Edaravone has been reported to reduce brain infarction and oedema after ischemic/reperfusion injury in animal models as well as in stroke patients. The possible mechanism of action of edaravone mainly encompasses the decrease in oxidative stress or lipid peroxidation, pro-inflammatory response, and protection of neurovascular tissues after ischemic stress [15]. It has been reported that flavonoid, which is the main therapeutic constituent in DK leaves, possesses anti-inflammatory and anti-apoptosis activities [16]. If the DK extract contains a total flavonoid content of 7.99%, it can be speculated that the anti-inflammatory and anti-apoptosis activities of DK flavonoids play a role in the neuroprotective action of the DK extract against ischemic neuronal injury.

The administration of GJ extract at the doses of 500 and 1,000 mg/kg showed the

improvement of neurological deficit in mice from day 5 and day 6, respectively. This action was different from those of DK extract and edaravone that could be observed from day 2 after ischemic injury. In contrast, these GJ treatments have no effect on the infarct volume of MCAO mice. The plausible explanation for these results is unclear. However, it may infer that the GJ extract may protect peripheral neuron from ischemic injury. This explanation is supported by the fact that the transient cerebral ischemia induces apoptosis in the peripheral neuron [17] and geniposide, which is an active component of GJ extract, possesses anti-apoptosis activity [18].

Conclusions

The present study demonstrated that the administrations of DK and GJ extracts enhanced the recovery of neuronal injury after cerebral ischemia in mice. This finding suggested that the DK and GJ treatment might be beneficial for protecting the neuronal system against ischemic injury.

ACKNOWLEDGEMENT

This study was supported by a grant from National Institute of Medicinal Materials, Ministry of Health, Vietnam.

REFERENCES

- [1] P. Lipton (1999), "Ischemic cell death in brain neurons", *Physiological Reviews*, **79**, pp.1431-1568.
- [2] R. Brouns, P.P. De Deyn (2009), "The complexity of neurobiological processes in acute ischemic stroke", *Clinical Neurology and Neurosurgery*, **111**, pp.483-495.
- [3] M. Mingsan, Z. Xuexia, B. Ming, W. Linan (2014), "Persimmon leaf flavonoid promotes brain ischemic tolerance", *Neural Regen. Res.*, **8**, pp.2625-2632.
- [4] Y.S. Sa, S.J. Kim, H.S. Choi (2005), "The anticoagulant fraction from the leaves of *Diospyros kaki* L. has an antithrombotic activity", *Arch. Pharm. Res.*, **28**, pp.667-674.
- [5] T. Hayashi, L. Zongyou (1993), "Stimulating the growth of cultured endothelial cells stimulated by *Gardenia*", *Chinese Archives of Traditional Chinese Medicine*, **15**, pp.51-53.
- [6] H. Liu, Y. Chen, F. Li, H. Zhang (2013), "Fructus *Gardenia* (*Gardenia jasminoides* J. Ellis), phytochemistry, pharmacology of cardiovascular, and safety with the perspective of new drugs development", *J. Asian Nat. Prod. Res.*, **15**, pp.94-110.
- [7] Z. Haiyan, L. Qiong, L. Yan, L. Yang, Y. Ming (2017), "Learning and memory improvement and neuroprotection of *Gardenia jasminoides* (Fructus *gardenia*) extract on ischemic brain injury rats", *Journal of Ethnopharmacology*, **196**, pp.225-235.
- [8] T.X. Le, T.P. Nguyen, N.T.T. Phuong, T.N.H. Pham, V.T. Nguyen, et al. (2016), "Neuroprotective effect of *Panax notoginseng* against ischemic neuronal injury in mice", *Journal of Medicinal Materials*, **21**(3), pp.344-348.
- [9] E. Rousselet, J. Kriz, N.G. Seidah (2012), "Mouse Model of Intraluminal MCAO: Cerebral Infarct Evaluation by Cresyl Violet Staining", *J. Vis. Exp.*, **69**, p.e4038.
- [10] S.A. Menzies, J.T. Hoff, A.L. Betz (1992), "Middle cerebral artery occlusion in rats: a neurological and pathological evaluation of a reproducible model", *Neurosurgery*, **31**(1), pp.100-106.
- [11] H.Y. Son, H.S. Han, H.W. Jung, Y.K. Park (2009), "*Panax notoginseng* Attenuates the Infarct Volume in Rat Ischemic Brain and the Inflammatory Response of Microglia", *J. Pharmacol. Sci.*, **109**, pp.368-379.
- [12] C.N. Joshi, S.K. Jain, P.S. Murthy (2004), "An optimized triphenyltetrazolium chloride method for identification of cerebral infarcts", *Brain Research Protocols*, **13**, pp.11-17.
- [13] O. Engel, S. Kolodziej, U. Dirnagl, V. Prinz (2011), "Modeling Stroke in Mice - Middle Cerebral Artery Occlusion with the Filament Model", *J. Vis. Exp.*, **47**, p.2423.
- [14] C.X. Wang, A. Shuaib (2007), "Neuroprotective effects of free radical scavengers in stroke", *Drugs & Aging*, **24**, pp.537-546.
- [15] K. Kikuchi, N. Miura, Y. Morimoto, T. Ito, S. Tancharoen, et al. (2011), "Beneficial Effects of the Free Radical Scavenger Edaravone (Radicut) in Neurologic Diseases", *J. Neurol. Neurophysiol. S1.*, doi: 10.4172/2155-9562.S4171-4001.
- [16] S. Lijun, Z. Jianbao, F. Kun, D. Yan, Z. Liyu, et al. (2014), "Flavonoids from persimmon (*Diospyros kaki*) leaves (FPL) attenuate H₂O₂-induced apoptosis in MC3T3-E1 cells via the NF- κ B pathway", *Food & Funct.*, **5**(3), pp.471-479.
- [17] P. Deb, S. Sharma, K.M. Hassan (2010), "Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis", *Pathophysiology*, **17**, pp.197-218.
- [18] L.X. Gua, J.H. Liu, Z.N. Xia (2009), "Geniposide inhibits CoCl₂-induced PC12 cells death via the mitochondrial pathway", *Chin. Med. J.*, **122**, pp.2886-2892.

An overview of petroleum potential and investment opportunities in Southeast Asia

Minh Hoang Cu^{1*}, Khắc Hoan Phung¹, Hai An Le²

¹PVEP Overseas Co., Ltd

²Hanoi University of Mining and Geology

Received 2 June 2017; accepted 1 September 2017

Abstract:

Southeast Asia has 11 countries: Brunei, Cambodia, East Timor, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam. Southeast Asia is considered one of the most active economic development regions in the world, with high demand for energy development, cultural diversity, and language. These countries and Vietnam have good relationships in diplomacy and cooperation and in other fields; therefore, it is very convenient for Vietnam to invest in oil and gas exploration and exploitation in the region. On the basis of evaluating the criteria of natural conditions, geological features, sedimentary basins, exploration and exploitation status as well as investment environment of each country in Southeast Asia, this article will introduce the most comprehensive information about the potential and the overall picture of the oil and gas industry in Southeast Asia and in individual countries, thereby providing information and orientation for investment opportunities of each country in the region.

Keywords: hydrocarbon reserves, investment opportunities, petroleum potential, Southeast Asia.

Classification number: 4.2

Southeast Asia Petroleum Potential Overview

There are 11 countries in the Southeast Asia, including Philippines, Malaysia, Brunei, Indonesia, East Timor, Singapore, Myanmar, Thailand, Laos, Cambodia, and Vietnam. The rapid economic growth is being recorded in some countries such as Indonesia, Malaysia, Thailand, Singapore, and Vietnam.

The proven oil reserves of Southeast Asia are around 16 billion barrels, approximately 1% of the world reserves and 39% of the Asia-Pacific reserves (Fig. 1) [1]. In which, Malaysia has the largest amount of the proven reserves of around 5.9 billion barrels, approximately 36% reserves of the region. The follow-up proven reserves belong to Vietnam, Indonesia, and Brunei, respectively, in which Indonesia owns ~ 4 billion barrels, and Brunei owns ~ 1.1 billion barrels, (Fig. 2).

The proven gas reserves of Southeast Asia are around 6.8 Tcm (trillion cubic meter), approximately 3% of the world reserves and 40% of the Asia-Pacific reserves (USGS, 2012 - Fig. 3). In which, Indonesia has the greatest amount of the proven reserves of around 3 Tcm. Malaysia takes the second

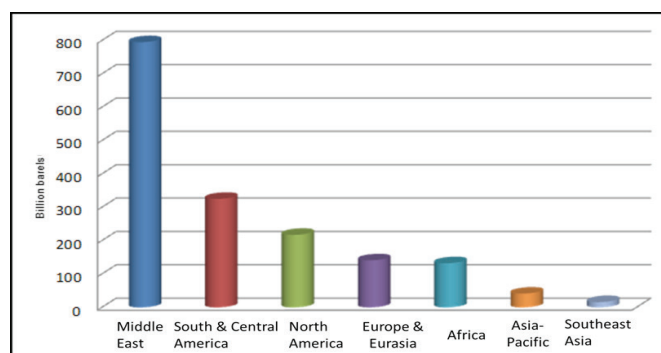


Fig. 1. Worldwide oil reserves distribution (USGS, 2012).

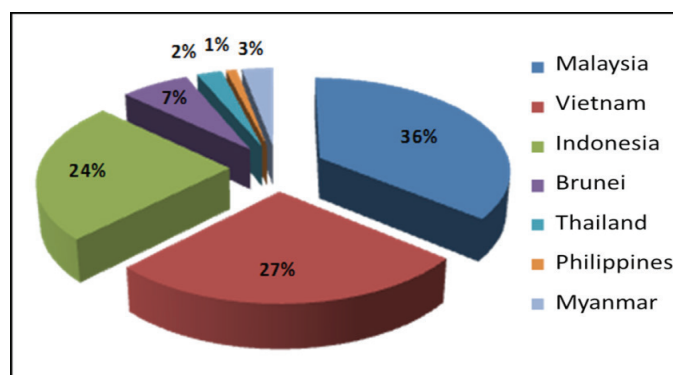


Fig. 2. Oil reserves distribution of Southeast Asia (USGS, 2012).

*Corresponding author: hoangcm@pvep.com.vn

position with 2.4 Tcm and is followed by Vietnam. The gas reserves of remaining countries in the region are very small or limited (Laos, Cambodia...) (Fig. 4).

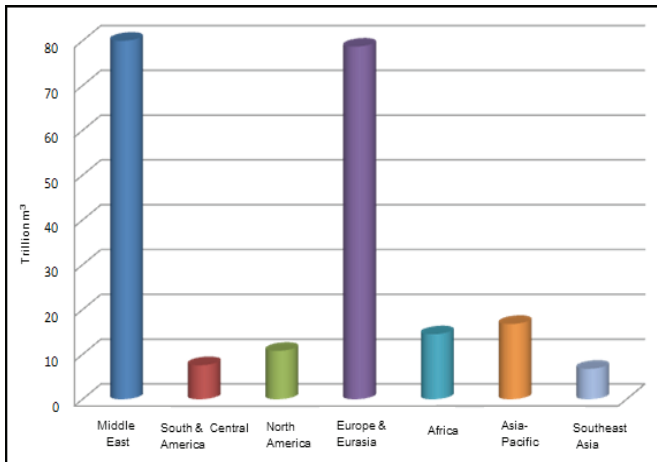


Fig. 3. Worldwide gas reserves distribution (USGS, 2012).

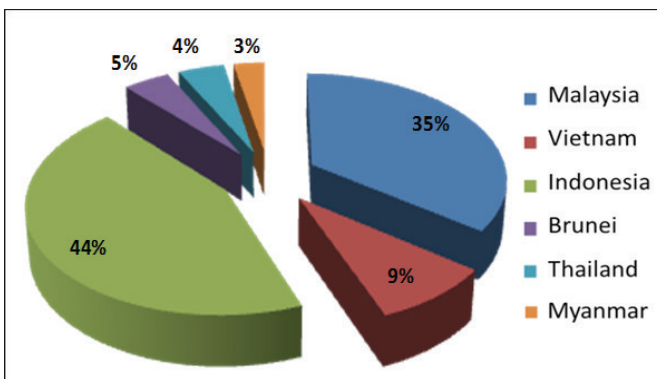


Fig. 4. Gas reserves distribution of Southeast Asia (USGS, 2012).

Referring to the analysis of USGS in the year 2012, the remaining potential of oil and gas in Southeast Asia is around 22 billion barrels (~3.6% of worldwide total reserves - 595 billion barrels) and 8.5 Tcm (~5.3% of worldwide total reserves - 159 Tcm), respectively. Generally, the remaining potential of the region is distributed mainly in Indonesia (41% of oil and 36% of gas potential), Malaysia (25% of oil and 27% of gas potential), and Vietnam (12% of oil and 16% of gas potential). The attractive basins for exploring the remaining oil potential in the Southeast Asia are Baram Delta Basin (Brunei) - Sabah Basin (Malaysia), Kutei and East Java Basins (Indonesia), Cuu Long Basin (Vietnam), offshore basins of Myanmar, and the Gulf of Thailand. The attractive basins for exploring the remaining gas potential are Kutei and East Java Basins (Indonesia); Baram Delta Basin (Brunei), Sarawak and Sabah Basins (Malaysia), and Nam Con Son Basin (Vietnam).

Southeast Asia geological characteristic overview

Southeast Asia recently is a part of the south-eastern Eurasia plate. To the east, it is bounded by the Pacific Plate and Philippines Microplate. To the west, southwest and the south, it is bounded by Indian-Australia Plate. These boundaries are defined by Arakan - Nicoba - Java - Timo subduction zone (1), Sorong strike-slip zone (2) and East Philippines subduction zone (3), (Fig. 5).

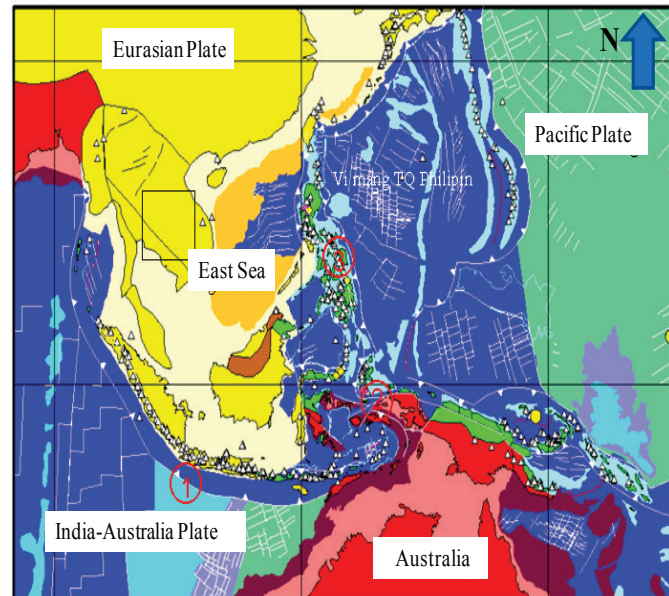


Fig. 5. Southeast Asia tectonic map (Hall, 2004 [2]).

The subduction of India-Australia Plate beneath Eurasia Plate formed the Sunda active margin with five main elements including Java Trench, Nias Accretionary Complex, Northeast Nias fore-arc basin, Sumatra-Java volcanic arc, and Ace back-arc basin. The subduction of the Pacific Plate and the Philippines Microplate beneath the Western Eurasian plate resulted in forming Philippines active continental margin elongating from Taiwan - Philippines to East Hal Mahera.

The crust of Southeast Asia has generally been formed since Cambrian. Only some areas, such as the center of East Sea, Sulu Sea, Sulawesi Sea, Banda Sea, and Andaman Sea, have typical oceanic crust formed by sea spreading during the Cenozoic. Recently, in the Southeast Asia, there are five main geotectonic blocks, namely South China (I), Indonesia (II), Sibumasu (III), West Myanmar (IV), India (V), and Borneo (VI). The suture zones between these geotectonic blocks are: Ma River (1), Utaradi-Bentong-Rup (2), Sittang-Bham (3), East Aracan (4), Kuching-Lupa (5), Sumatra-Java subduction zone (6), Mae Ping-Hau River Fault (7), Three-Pagoda Fault (8) (Fig. 6).



Fig. 6. Main geotectonic blocks of Southeast Asia: I) South China; II) Indosinia; III) Sibumasu; IV) East Myanmar; V) India; VI) Borneo; Suture zones: 1) Ma River 2) Utaadi; 3) Sittang-B. Ham; 4) East Aracan; 5) Kuching-Lupar; 6) Sumatra-Java subduction zone; 7) Mae Ping-Hau River; 8) Three-Pagoda Fault (PVEP Mekong, 2008 [3]).

Petroleum investment opportunities in the Southeast Asia

In the Southeast Asia, hydrocarbon has been discovered and produced mainly in Cenozoic basins which distributed in margin shelves. A minority of hydrocarbon production is from Paleozoic-Mesozoic basins. Vietnam, Malaysia, and Indonesia are known as the top countries in the region for their petroleum potentials. The Potential of Myanmar is smaller, but its opening may give a chance of investment. Thailand and Brunei are recorded as small potential countries while Laos, Cambodia, East Timor, and Singapore are very limited or not proven of the potential.

In fact, countries with high potentials have high success ratio. However, exploration investment in these countries is very difficult, because of their long histories of exploration and production resulting in fewer areas available for exploration. Remaining open blocks in these countries are located in very deep water causing very high exploration cost. Thus, to invest in these countries, companies need to have suitable technologies and be ready to take risks.

PVEP/PVEP Overseas has experiences in investing in the Southeast Asia with contracts signed in Indonesia, Malaysia, Myanmar, Laos, Cambodia and East Sea. Based on our evaluation, there is very little opportunity for investment in

Laos, Cambodia, Philippines, East Timor, and Singapore because of their limited petroleum potentials. Brunei and Thailand that have small potentials, a limited number of open blocks, high risk of geology and geography (deep water sea)... should not be considered for investment. Although Malaysia has the biggest potential in the region, the opportunity is not much because the open blocks are located in areas either very risky in terms of geology or with very deep water. In this country, all attractive blocks/areas belong to Petronas (Malaysia national petroleum company) and giant worldwide oil companies. Thus, it is very difficult for new investors to find success in remaining unattractive blocks.

Generally, referring to the conditions of potential and investment environment, only Myanmar and Indonesia in the Southeast Asia should be considered for studying and seeking for new ventures. In these countries, especially Indonesia, the potential in remaining open blocks is still expected. These countries have quite flexible environments for investment, high security and available infrastructure. Vietnam has close relationships with these countries for a long time. Petrovietnam has some experiences in making contracts with Indonesia and Myanmar. Investing in these countries could be conducted by joint studying, signing new petroleum contracts or farming in existing contracts...

Myanmar

Myanmar is a country with an area of 678,500 square kilometers and a long history of oil and gas industry and rather good petroleum infrastructure. The potential of Myanmar is at average level in the region, with gas reserves of about 50,200 billion cubic feet (mainly distributed in three offshore areas of Myanmar with a total reserve of 46,900 BCF) and oil reserves of 4,814 million barrels (located in onshore basins, of which 75% are located in the central basin). At present, Myanmar's total oil and gas productions are about 22,000 barrels per day and 1,300 million cubic feet per day, respectively.

By the present time, Myanmar area has divided into 105 contract blocks, of which 53 blocks are onshore, and 52 blocks are offshore. The number of onshore blocks which were discovered for oil and gas is 49 while discovered offshore blocks are 26 (in the Rakhine, Tanintharyi and Mottamma basins).

Myanmar has totally 17 sedimentary basins (Fig. 7), including 14 Tertiary basins (11 onshore, 3 in shallow marine) and 3 Pre-Tertiary basins. Out of 14 Tertiary basins, there are 6 inland basins producing oil, gas, and condensate (Chindwin, Center, Prome (Pyay) Embayment, Rakhine Coastal, Ayeyarwaddy, and Bago Yoma); 3 basins on the continental shelf are producing gas and condensate (Moattama, Rakhine Offshore, and Tanintharyi); and 5 basins are still in exploration with no discovery yet (Hukawng, Shwebo-Monywa, Sittaung Valley, Mepale, and Mawlamyine). Currently, neither oil or gas has been discovered in Pre-Tertiary basins, which are small,

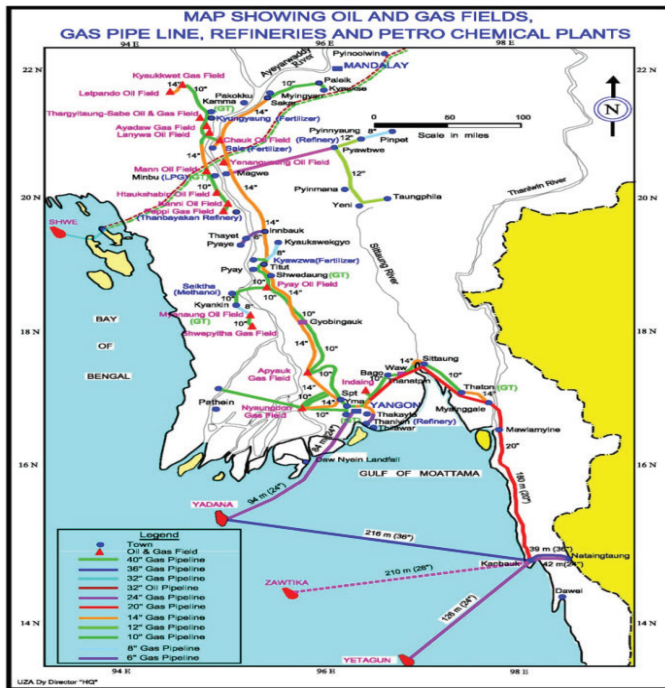


Fig. 7. Myanmar oil and gas infrastructure (MOGE, 2011 [4]).

low exploration level, and being offered (Namyau, Hsipaw-Lashio, Kalaw).

The regional assessment shows that the Center-Prome, Rakhine Offshore and Moattama sedimentary basins have been discovered and exploited for oil and gas. Significant oil reserves have been found, open blocks are available, and the ability to buy assets and tendering in open blocks is high (Table 1). Therefore, the Central-Prome, Rakhine Offshore, and Moattama basins are priorities when selecting a basin for

investing in oil and gas industry in Myanmar.

Currently, the negotiation of new contracts in Myanmar is relatively favourable because there are still open areas/blocks. Operators in Myanmar are also willing to call on investors to participate in exploratory risk sharing. Therefore, investment opportunities in Myanmar are quite feasible.

Indonesia

Indonesia is a country with an area of 1,919,440 km² and over 60 sedimentary basins, in which 30 basins had oil and gas exploration activities, and 14 basins have been under production activities, while other 22 basins still have little or no petroleum production activities. Proven reserves are 4.2 billion barrels of oil and 108 trillion cubic feet of gas. Indonesia also has a long history of oil and gas exploration for over 130 years. Commercial discoveries take place in 10 basins (Fig. 8), (Pertamina 2003 [5], PVEP Overseas, 2013 [6]).

Due to the differences in geological conditions, the sedimentary basins in Indonesia can be divided into two groups: the western group and the eastern group. The western Indonesian basins are assessed with denser and earlier exploration history than the eastern basins. Most of the western basins have been explored, whereas 20 of 38 basins in the eastern Indonesia have not been explored. That limited exploration activity in the eastern Indonesia reflects difficulties in terms of infrastructure and logistics, wild areas, and deep-water (> 200 m).

Western Indonesia basins:

The group of basins with large oil and gas potential in the western part of Indonesia is mainly distributed in continental or shallow water areas. The basins generally exhibit similar

Table 1. The potential blocks for investments (MOGE, 2015).

Basin	Area (km²)	Number of Blocks	Block with Operato	Open Blocks	Oil and Gas Fileds		Oil and Gas Reserves				Cumulative Production		Selected Basins
					Discovered	Producing	Oil (MMbb)		Gas (TCF)		Oil (MMbb)	Gas (BCF)	
							P1	P2+3	P1	P2+3			
Onland		53	18					3405.6		2.95	504.1	757.6	
Center	34320	17	8	9		8	1793.33	2243.81	0.678	0.279	516.55	318.63	v
Prome	16027	15	5	10	18	4	144.46	627.15	0.416	1.38	36.03	289.55	v
Irrawaddy	33996	5	2	3	8	1							
Chindwin	35198	3	1	2	3	0							
Bago & Sittaung	55200	4	1	3	1	0							
Hukawnd	24237	1	1		1								
Rakhine Coastal	25175	2	1	1		0							
Shwebo - Monywa	27333	1	1		1								
Mepale	1259	1		1									
Namyaw	5110	1		1									
Hsipaw Lashio	2126	1		1									
Kalaw	3320	1		1									
Mawlamvine	11494	1		1									
Offshore Coast		53							19.52	37.68			
Rakhine Offshore	29546	28		22		3			3.37	10.64			v
Moatama	46070	13		3	3	5			11.8	25.8			v
Tanintharyi	171297	14			7	1			4.35	1.24			
					2								

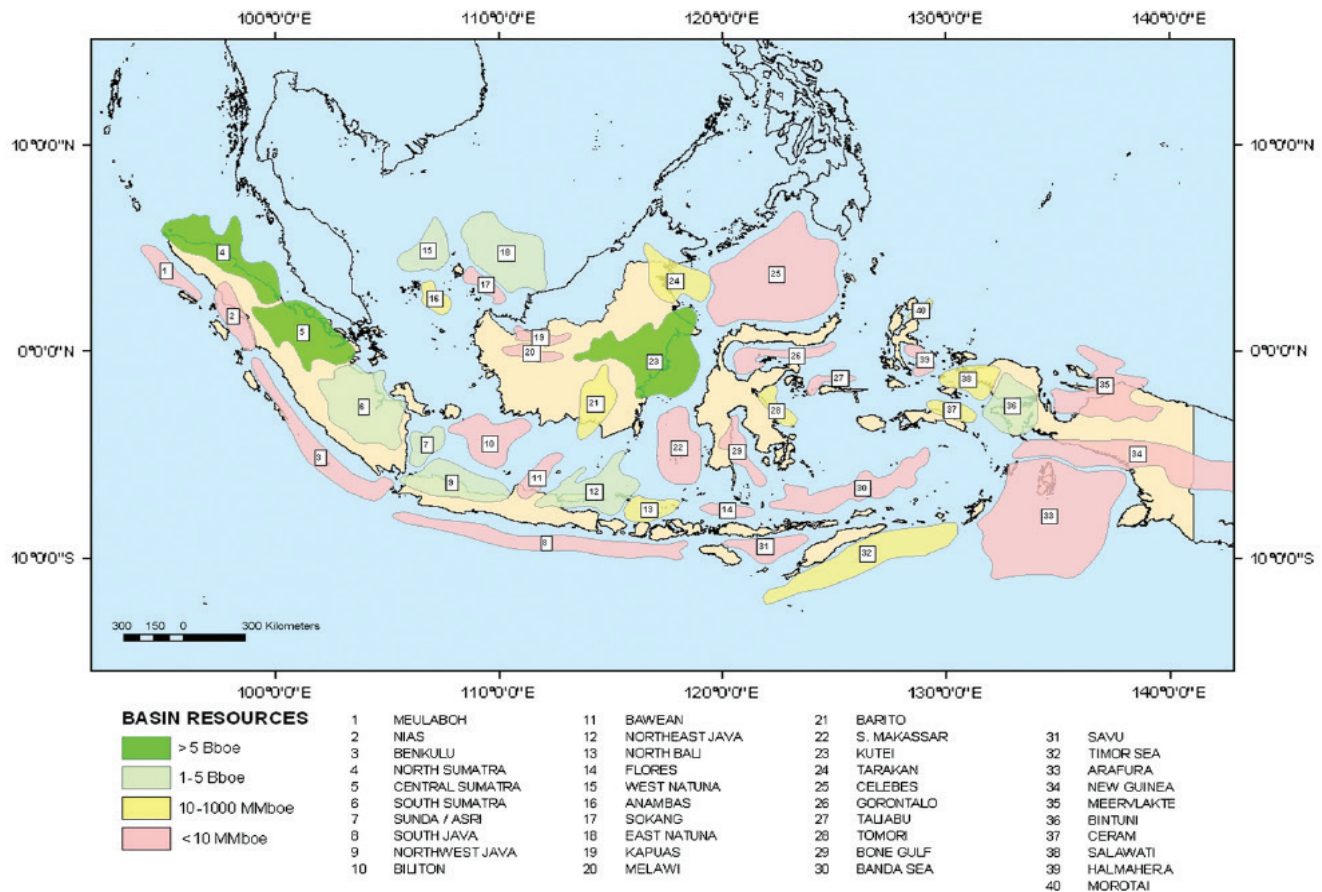


Fig. 8. Indonesian sedimentary basins & potential (Doust & Noble, 2008 [7]).

structural and stratigraphic similarities, reflecting the similar control regime of tectonic throughout the history of Cenozoic development. The basins with similar geological setting lie in the southeast of the Sunda Shelf, undergoing general tectonic effects related to the movement of the Indo-Australasian, Eurasian Plates and the global change of sea level, which influence the filling of sedimentary basins.

Some of the similarities of the western Indonesia basins: (1) the initial rifting phase occurs in mid-late Eocene. This phase is filled by river-lake sediments, which can form an important oil and gas source rock; (2) The sea progradation proceeds from Middle Oligocene to Middle Miocene, which is characterized by changes in sediment- deposit-environment, from river, delta to shallow waters and shallow seas, forming terrigenous reservoirs of river and delta facies and carbonate reservoirs. The regional seal related to the maximum sea level in the Middle Miocene is formed; (3) The late of Miocene-Pliocene compression related to the collision between Australian Continental Plate with Euro-Asia Plate.

The fore-arc basins are generally considered to have lower prospect due to limited source rocks, poor reservoir quality and low heat. Most of the recoverable reserves (85% of Indonesia's

reserves) are mostly back-arc basins, which have large potential reserves, such as North Sumatra Basins, Sumatra Centers, South Sumatra, Sunda-Asri, West Java, East Java, Kutei, East and West Natuna (Table 2).

Table 2. Proven reserves in major Indonesian sedimentary basins (USSG, 2012).

No	Sedimentary Basin	Oil Reserves(MMbbbl)	Gas Reserves(TCF)
1	North Sumatra	3,111.89	21.28
2	Central Sumatra	3,790.48	8.56
3	South Sumatra	909.80	17.90
4	West Java	556.52	3.7
5	East Java	1,022.35	6.4
6	Natuna	383.35	51.46
7	East Kalimantan (Kutai)	670	18.33
8	Sulawesi	49.78	4.23
9	Matuku	48.07	15.22
10	Papua	94.93	24.32

Eastern Basins in Indonesia:

The eastern Indonesian sedimentary basins are geologically distinguishable from the western basins, they locate in a very complex area geologically affected by collision of continental plates, arcs, and remnant oceans. Sedimentary basins in eastern part are small, mostly undeveloped, and located in deep water, remote areas, or forested areas with limited infrastructure.

Although the petroleum potential of the eastern Indonesian basins is estimated less than that of the western Indonesian basins, the eastern basins are currently attracting the interest of oil and gas companies; as exploration activities are limited in the area, some of the new commercial discoveries in the Mesozoic reservoirs, even very large discovered field (Tangguh gas field ~ 20 Tcf, Paleogene and Jura objects, Bintuni basin).

It is clear that oil and gas exploration activities, as well as important commercial discoveries, are concentrated in some areas such as Sumatra, Java, East Kalimantan, Sulawesi, Natuna, and Papua (the contiguous part of Papua New Ghine). Most of the best hydrocarbon systems can be found mainly in the western region, including Sumatra, Java, Natuna, and East Kalimantan (Kutei) basins. There are some gas discoveries in the eastern region of Tanggu, West of Irian Jaya. In the eastern part, only Salawati Basin on Irian Jaya's Bird's Head is considered to have an active hydrocarbon system. With the development of geological understanding and technological advances, exploration costs in remote and deep-water areas are reduced; recent exploration has been shifted from west region to east region. Indonesia has a long and relatively well-developed oil and gas infrastructure, which is very favourable for oil and gas exploration and exploitation.

In Indonesia, investors can purchase assets or sign contracts as the country annually maintains international tenders for new blocks or relinquished areas. With the remaining relatively large potential - especially deep-water prospects, many operators are also willing to share interests in the exploration and production blocks; therefore, investment possibilities in Indonesia are quite attractive.

Conclusions

Southeast Asia is an active economic development zone with high demand for energy development and has favourable conditions for investment in oil and gas exploration and production.

Oil and gas are explored and produced in Southeast Asia in both Paleo-Mesozoic and Cenozoic sedimentary basins, with various types of sedimentary basins and complex geological features. The great oil and gas potential is concentrated mainly in the Cenozoic basins, with many large fields discovered and distributed on the margin of the continental shelf of Southeast Asia.

Some countries including Vietnam, Indonesia, Malaysia, Brunei, Myanmar, and Thailand have great petroleum potentials and well-developed infrastructures. However, Thailand, Malaysia, and Brunei have no open blocks or limited access. Meanwhile, Laos, Cambodia, Philippines, East Timor, and Singapore have very limited or no oil or gas potential. Therefore, investment in these countries should not be considered.

Looking for investment opportunity is currently most feasible in Indonesia and Myanmar, ranging from new exploration contracts to asset purchase. In Myanmar, Central, Prome, Rakhine Offshore, and Moattama basins should be priorities for investment. Whereas, in Indonesia, the most preferred basin groups are the western Indonesian basins such as Sumatra, Java, East Kalimantan (Kutai), Sulawesi and Natuna. For Eastern Indonesian basin group, the basin in the Papua Islands region and most notably the Bintuni basin may attract interest.

REFERENCES

- [1] USGS (2012), *Hydrocarbon Reserves of Southeast Asia*.
- [2] Hall (2004), *Tectonic Evolution of Southeast Asia*.
- [3] PVEP Mekong (2008), *Characteristics of Indochina Structural Geology*.
- [4] MOGE (2011), *Annual Magazine of July 2011*.
- [5] Pertamina (2003), *Petroleum Geology of Indonesia*.
- [6] PVEP Overseas (2013), *Petroleum Potential Review of Southeast Asia*.
- [7] Doust and Noble (2008), *Petroleum System of Indonesia*.

Assessment of landfill leachate in Nam Son, Hanoi using 3D electrical imaging and self-potential data

Van Giang Nguyen^{1*}, Quang Dung Nguyen², Ba Duan Nguyen¹

¹Institute of Geophysics, VAST

²Ho Chi Minh City Institute of Resources Geography, VAST

Received 10 March 2017; accepted 25 August 2017

Abstract:

The main objective of this study was to assess the environmental impact of the Nam Son landfill on the subsurface geological structure studied using 3D electrical imaging and self-potential investigations. The map of the equipotential was based on self-potential data and was indicative of concentrations of redox-active components, and the map of the electrical conductivity provided information about the mineralization of the groundwater. The electrical conductivity of the subsurface was obtained from 3D-electrical imaging analysis conducted using a Wenner-based array configuration. An inversion of the data was carried out using 3D regularized least-squares optimization methods with robust model constraints. Three profiles of 3D electrical imaging and 180 self-potential data points, scattered within the study area near the disposal site, were implemented. The self-potential method supports the results of the geoelectrical method relating to a contaminated zone in the survey area with a large negative self-potential anomaly of ~ 260 mV with respect to the reference of a contaminant plume. Interpretations of geophysical data showed a low resistivity zone ($<10 \Omega \text{ m}$), which appeared to be a fully saturated zone with leachate from the Nam Son open dumpsite. The correlation between geophysical investigations and hydrochemical analysis proved to show high conductivity zones related with landfill leachate.

Keywords: contaminant plume, electrical imaging, fluid conductivity, landfill leachate, self-potential.

Classification number: 4.3

Introduction

One of the major causes of the pollution problem for municipal solid waste landfills is landfill leachate, which is generated from precipitation, surface run-off, and the infiltration or intrusion of groundwater percolating through a landfill, biochemical processes in waste cells and the inherent water content of wastes themselves [1].

The landfill leachate has the potential to contaminate groundwater and surface water supplies, and threaten human health as it migrates from landfills and

contaminates surrounding lands and water. As the population grows and industries develop, the landfill leachate problem becomes increasingly serious [2, 3].

Landfill leachate consists of a number of complex factors, including the composition of the solid waste composition, the age of the waste, the operation of the landfill, the hydrogeological conditions in the vicinity of the landfill site, the rate of water movement through the waste, the temperature of the landfill, the moisture content, the pH, the landfill chemical and

biological activities, and the seasonal variations in weather [4].

Nam Son is the biggest landfill in Hanoi and receives more than 4,000 tons of domestic waste per day. landfill leachate is a serious problem there. During the last decade, some research projects have been conducted to compare landfill leachate treatment systems which have been used across Vietnam and in the world at large, including at the Nam Son landfill site in Hanoi [5], and studies investigating the recovery ability of N, P in leachate from the Nam Son landfill, Hanoi for pretreatment of ammonium have also been carried out [6].

During the past 20 years, geophysical methods have proven to be efficient and effective means for characterizing the subsurface geology and hydrology associated with landfills [7-9]. As a result, the geophysical characterization of landfills is now routine practice. Even so, due to the inherent uniqueness of each site, it is necessary to carefully select a method and appropriately design survey parameters in order to derive adequate benefits. In many cases, particularly when multiple objectives are desired, it is best to use complimentary geophysical methods [1, 2, 10]. A variety of techniques can be applied to define landfill properties, leachate circulation, or regional contamination from offsite seepage [11]. A combination of various geophysical methods, as well as 3D electrical imaging and self-potential, were used for shallow geological structure investigation in the north of the Nam Son landfill. These techniques were applied in the lowest altitude

*Corresponding author: Email: giangnv@igp-vast.vn

terrain around the landfill to monitor the volatility of the soil and water environment; that may be caused by the landfill operation.

Geological features of the study area

The terrain of the study area is hilly, but the typography is fairly simple. The general form terrain is a very soft transition from 10 to 20 m. The study area is located within a region of moist, tropical monsoon heat. Average annual rainfall is 1,670 mm and average air humidity is 84%. The aquifers are deposits of Holocene (qh) and Pleistocene (qp) [11, 12]. The water layer deposit of the Triassic Rift (t), is exposed in the north of the Soc Son district, and is characterized by slightly better water quality in the quaternary sediments. The silver-coloured soil in some parts of the research area is the result of the ancient alluvial ferralitic products. According to a geological-mineral map (scale 1:50,000) published in 2015 by the Hanoi Institute of geology and environment, the structure of Soc Son district is mainly in the upper Triassic system and the Jura conglomerate [13]. There are two stratigraphic formations: the Khon Lang (T_{2a} kl) and the Na Khuat (T_2 nk). There are three boreholes at 30 m of depth there, where, in general, the stratigraphic column consists of:

- From the ground surface to 1.5/2 m - the class covered ruins in a sepia-brown golden colour, the composition of clay and gravel in a semi-hard state.
- From 1.5/2 m to 14/15 m - lightning weathered siltstone rock was made perfectly golden brown, semi-hard hard status.
- From 14/15 m to 30 m: grey-black shale weathering to soft weathering, medium to the hard state.

Methodology of research

The research area covers 180 x 300 m (Fig. 1) which allowed for the convenient use of many geophysical methods, e.g. 3D electrical imaging and self-potential (SP) methods to assess subsurface environmental contamination.

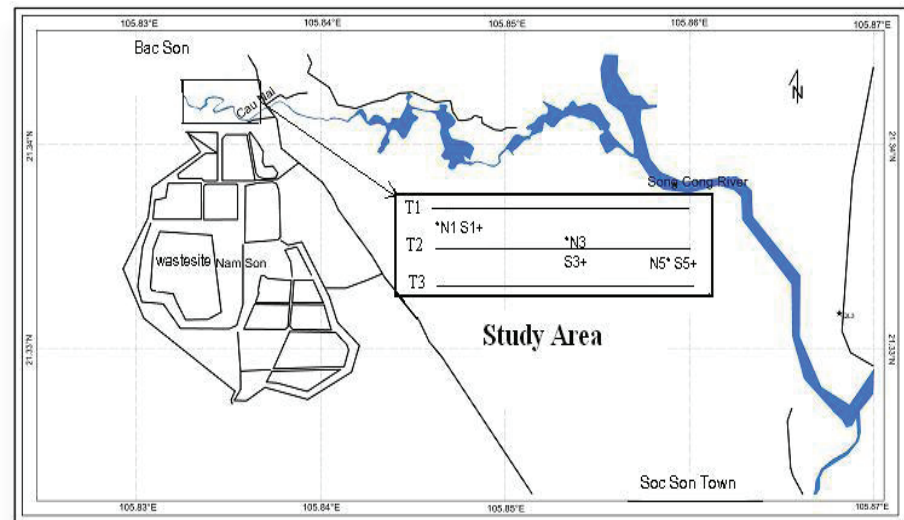


Fig. 1. The location of the Nam Son landfill and study area of the measurements profiles: T1, T2, T3, and samples surface water sites: N1, N3, N5, and samples groundwater sites: S1, S3, and S5.

3D electrical imaging surveys method

Overview of electrical surveys:

The purpose of the electrical surveys was to determine the subsurface resistivity distribution by making measurements of the ground surface. From these measurements, the true resistivity of the subsurface was estimated. Ground resistivity is related to various geological parameters such as the mineral and fluid content, porosity and degree of water saturation in the rock [10, 14].

Resistivity measurements were normally made by injecting current into the ground through two current electrodes and measuring the resulting voltage difference at two potential electrodes. From the current (I) and voltage (V) values, an apparent resistivity (ρ_a) value was calculated [10].

$$\rho_a = k V / I \quad (1)$$

where k is the geometric factor which depends on the arrangement of the four electrodes.

Resistivity meters normally gives a resistance value of $R = V/I$, so in practice, the apparent resistivity value was calculated by:

$$\rho_a = k R \quad (2)$$

To interpret the data from such a survey, it is normally assumed that the subsurface consists of horizontal layers. However, in this case, the subsurface resistivity changed only with depth, but did not change towards the horizontal direction. It was a one-dimensional model (1D) of the subsurface structure. Another classical survey technique is the profiling method. However, in this case, the spacing between the electrodes remained fixed, and the entire array was moved along a straight line. This gave some information about lateral changes in the subsurface resistivity, but it did not detect vertical changes in the resistivity. An interpretation of the data from profiling surveys was mainly qualitative. Nowadays, however, 3D electrical surveys are practical commercial techniques with the relatively recent development of multi-electrode resistivity surveying instruments [3] and fast computer inversion software [15].

Before dealing with the 2D and 3D resistivity surveys, we will briefly look at the resistivity values of some common rocks, soils, and other materials. Resistivity surveys give a picture of the subsurface resistivity distribution. To convert the resistivity picture into a

geological picture, some knowledge of typical resistivity values for different types of subsurface materials and the geology of the surveyed area, is important.

3D electrical imaging surveys:

Since all geological structures are 3D in nature, a fully 3D resistivity survey using a 3D interpretation model gives the most accurate results [16]. At the present time, 3D surveys are a subject of active research. Many of the early 2D surveys were carried out using the Wenner Array because the sensitivity plot of the Wenner Array had nearly horizontal contours beneath the centre of the array. As well, the Wenner Array was relatively sensitive to vertical changes in the subsurface resistivity below the centre of the array. In general, the Wenner is good for resolving vertical changes (i.e. horizontal structures). Compared to other arrays, the Wenner Array has a moderate depth of investigation. With the Wenner Array, the geometric factor is $2\pi a$, ('a' is for the electrode spacing) which is smaller than the geometric factors of other arrays. Among the most common arrays is the Wenner Array as it has the strongest signal strength. This can be an important factor if the survey is carried out in areas with high background noise. One disadvantage of this array, however, is that 2D surveys have relatively poor horizontal coverage because the electrode spacing is increased. This could be a problem if you use a system with a relatively small number of electrodes.

Figure 2 shows one possible arrangement of the electrodes from a 3D survey using a 25 electrodes system. For convenience the electrodes were usually arranged in a square grid with the same unit electrode spacing in the x - and y -directions. To map slightly elongated bodies, a rectangular grid with different numbers of electrodes and spacing in the X - and Y - directions was used. In this case, each electrode was, in turn, used as a current electrode, and the potential of all the other electrodes was measured.

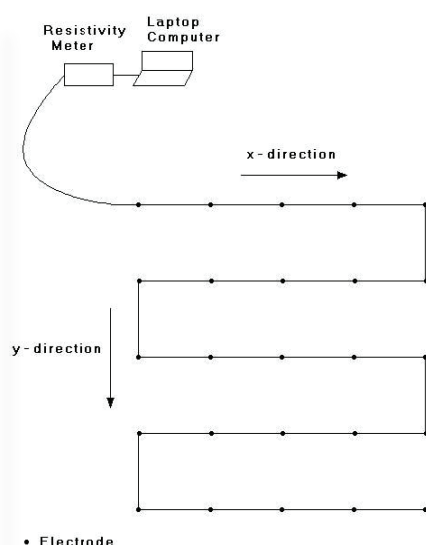


Fig. 2. The arrangement of the electrodes for a 3D survey [16].

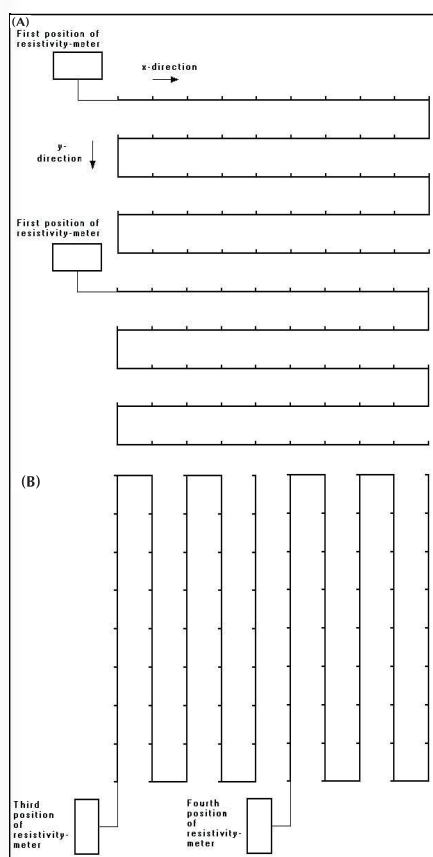


Fig. 3. Using the roll-along method to survey a 10 by 10 grid with a resistivity meter system with 50 electrodes. (A) Survey using a 10 by 5 grid with lines orientated in the x -direction. (B) Surveys with lines orientated in the y -direction [16].

3D roll-along techniques: The nearly 3D surveys involved grids of at least 16 by 16 in order to cover a reasonably large area. A 16 by 16 grid required 256 electrodes, which was more than what was available on many multi-electrode resistivity meter systems. A method used to survey such large grids with a limited number of electrodes was to extend the roll-along technique used in 3D surveys [4, 16]. The traditional survey showed an example of a survey using a multi-electrode resistivity-meter system with 50 electrodes to survey a 10 by 10 grid. Initially, the electrodes were arranged in a 10 by 5 grid with the longer lines orientated in the x -direction (Fig. 3). Measurements were then made primarily in the x -direction, with some possible measurements made diagonally. Next, the entire grid was moved in the y -direction so that the 10 by 5 grid then covered the second half of the 10 by 10 grid area. The 10 by 5 grid of electrodes was then orientated to the y -direction (Fig. 3).

The 3D forward modeling program: The free 3D resistivity forward modeling program, RES3DMOD.EXE, enables user to calculate the apparent resistivity values for a survey of a rectangular grid of electrodes over a 3D structure. We calculated resistivity values using the resistivity model, we also calculated the apparent resistivity values using the Calculate option. The RES3DMOD program also has an option to save the apparent resistivity values into a format that can be accepted by the RES3DINV inversion program. For our case, to save the apparent resistivity values as a RES3DINV data file for one of the models, and later carry out an inversion of this synthetic data set [16].

Data inversion: A model used to interpret the 3D data set is shown in Fig. 4. The subsurface is divided into several layers and each layer is further subdivided into a number of rectangular blocks. A 3D resistivity inversion program, RES3DINV, was used to interpret the data from 3D surveys. This

program attempted to determine the resistivity of the blocks in the inversion model that most closely reproduced the measurements' apparent resistivity values from the field survey. Within the RES3DINV program, the thickness of the layers was modified by the user [4, 16].

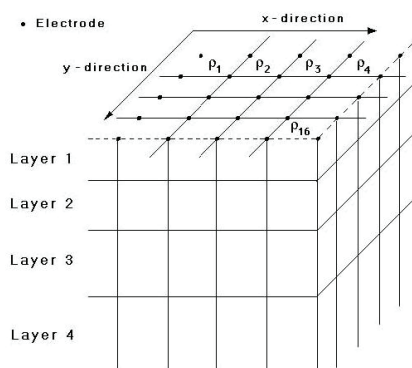


Fig. 4. The model used in 3D inversion where the widths of the rectangular blocks are equal to the unit electrode spacing in the x- and y-directions [16].

Self-potential (SP) method:

The basic concept: Various potentials are produced in native ground and within the subsurface which is altered by our actions. Natural potentials occur in dissimilar materials, at near varying concentrations of electrolytic solutions, and are due to the flow of fluids which may be mapped to determine information about the subsurface. SP can be produced using mineralization differences, electro-chemical action, geothermal activity, and bioelectric generation of vegetation. Four different electrical potentials are also recognized. Electrokinetic potential, or the streaming potential, is caused by the flow of fluids with certain electrical properties that passes through pipes or porous mediums with different electrical properties [17]. Liquid-junction potential, or diffusion potential, is caused by the displacement of ionic solutions of dissimilar concentrations. Mineralization potential, or electrolytic contact potential, is produced at the surface of conductors

with other media. Shale potential occurs when similar conductors have solutions that are of differing concentrations about them. Telford, Geldart, and Sheriff (1990) provided equations for these differing potentials [10]. Recognition of different SP sources is important in order to eliminate noise and lower background voltages. Some engineering and environmental occurrences may be mapped by contouring surficial voltages between base electrodes and mobile electrodes.

Data acquisition: A simple SP survey consists of a base electrode position and a roving electrode to determine potential differences on a gridded survey or along profile lines. The required equipment merely includes electrodes, wires, and a precise millivolt meter. The electrodes are in contact with the ground surface and should be of the nonpolarizing type, which are also called porous pots. Millivolt Meters are used to read the potentials in the millivolt range using a resolution from 0.1 to 1.0 mV [18]. Measurements using the electrodes may require systems to reverse the electrode position to resolve contact potentials at the electrodes. Previously measured locations may need to be remeasured on a systematic or periodic basis. Reoccupation of stations is necessary when very accurate surveys are being conducted and for sites with temporal potential changes or spatial variations of electrode potential. Changes are temporally in the electrodes and are due to the self-potential of the field require the survey to be conducted in a gridded or looped array [19].

Data interpretation: Most SP investigations use a qualitative evaluation of the profile amplitudes, or grid contours, to evaluate self- and streaming-potential anomalies. Flow sources produce potentials in the direction of the flow. Fluid inflow produces negative relative potentials, as would greater distance from the flow tube; outflow of the fluid results in positive potentials [20]. The self-potential due to water flow is a direct

parameter for a grouting remediation of reservoir leakage. SP methods can be very useful for karst groundwater regimes identified in quick surveys of sites or in long-term surveys during rainy seasons. There can also be a qualitative evaluation of the flow volume in different subsurface routes if the ground surface may be assumed parallel to the surface through the irregular flow paths.

Results of investigations

3D electrical imaging surveys

We used Terrameter SAS 4000 (four channel) Resistivity and IP instrument, with an integrated PC for full control of the data acquisition process and storage of data, and 64 electrodes [21]. The data collection was carried out along three profiles T1, T2, and T3 using the Wenner Array with 5 m electrode spacing and using integrated roll-along surveys. (Fig. 2). The raw data was interpreted using the software RES3DINV to build 3D inversion models. The RMS error for iterations of resistivity data varied by 9.46% in the inverse resistivity models. The results suggest delineation of geological formations such as various sub-surface lenses like clays and in situ-saturated sands and clays, which were up to the depth of several meters. The principal aquifer was formed in the unconsolidated alluvial formations, deposited under various sedimentary environments. The 3D electrical imaging showed resistivity values that varied laterally with the depth; two levels were distinguished in accordance with their obtained resistivity values.

Figure 5 shows the distribution of resistivity values. In the resistivity value less than 10 Wm for a majority on the surface, in the position of a 50 meter to 190 meter, with a width of about 20 m and a depth of about 5 m, which tended to penetrate deep down at nearly 10 m and spread out towards the N-canal Lai Son. Meanwhile, the resistivity value block > 30 Wm occupied most of the lower portion of the model that corresponded with the medium grain sand and clay

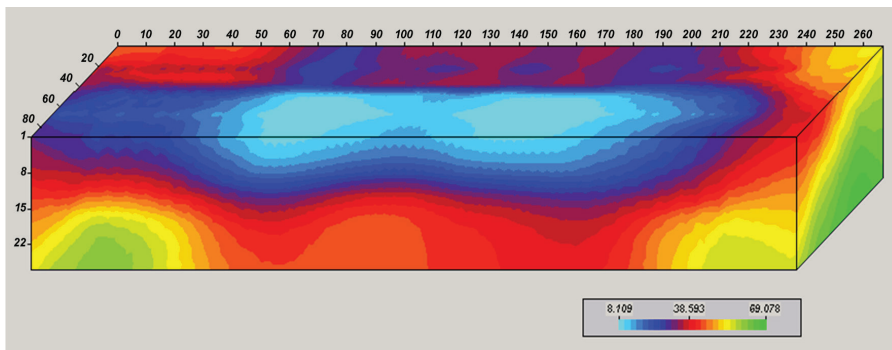


Fig. 5. The distribution of resistivity values by 3D model inversion for the study area.

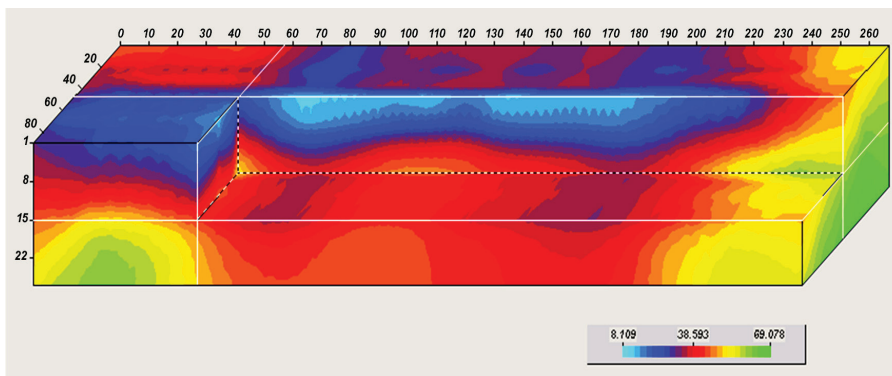


Fig. 6. The distribution of resistivity values by 3D model inversion after cutting a part of structures.

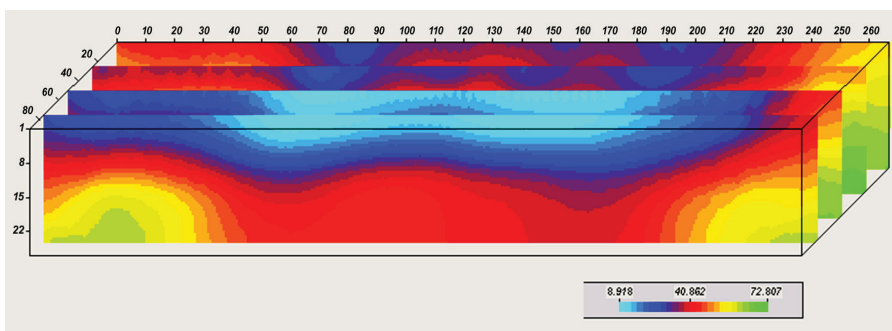


Fig. 7. The distribution of resistivity values by 3D model inversion by y-direction.

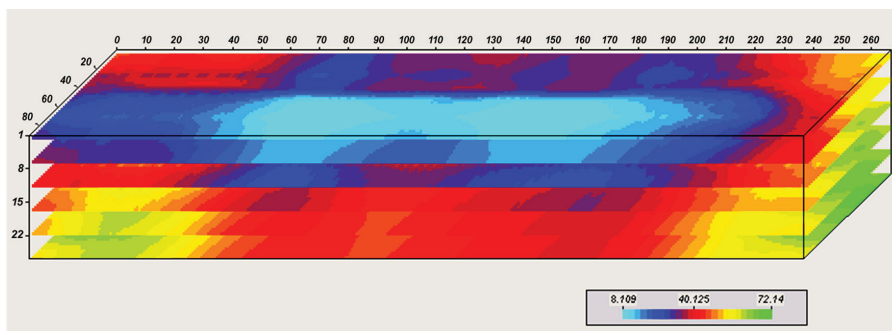


Fig. 8. The distribution of resistivity values by 3D model inversion by z-direction.

shale lithology.

To more clearly observe the distribution of blocks down to a deep resistivity was low, and we had to cut off parts in places from 60 to 270 metres according to the x-direction, from 50 to 90 metres according to y-direction, and from the ground down the 15 meter according to the z-direction (Fig. 6). We can see the block of low resistivity distribution only down about 10 m deep, which tends to expand at the N-canal of Lai Son. Below a depth greater than 10 m, the resistivity values were from 30 to 70 Wm corresponding to the lithology and are medium sand and clay shale (water layer).

To review the distribution of resistivity value according to the y-direction, we divide the model into the sections along the local y-direction; each cut was from 10 m, resulting in showing the resistivity zone at less than 10 Wm (blue colour) on the surface to the depth of 10 m (Fig. 7).

We can divide the blocks into six sections and vertical sections, each separated by 5 m. In Fig. 8, resistivity values less than 10 Wm appear on the surface quite broadly and as deep down as this value is possible, shrinking to a depth of about 10 m, then disappearing. Meanwhile, high resistivity values of over 30 Wm rise when more down deep.

The 3D electrical imaging shows the spatial distribution of waste and leachate concentrations within the study area of the landfill. Accordingly, a 3D survey was undertaken to determine the pattern of leachate drainage within the waste so that a more refined contaminant transport model could be developed. A full 3D survey was undertaken by sub-dividing the landfill into a number of discrete rectangular blocks and acquiring data on multiple parallel lines. The line data were merged into a single x-y matrix file and then inverted using a 3D finite element algorithm. The results were then presented as a 3D volumetric

tomograms in order to show the inferred waste distribution and leachate flow. The resistivity model indicated that leachate had accumulated at several discrete localities within the landfill at high conductivities. A narrow leachate plume was indicated in one part of the landfill, migrating down-gradient to the north. The inferred leachate concentrations in the study area were subsequently confirmed by drilling down and making hydrochemical samples which showed elevated solute levels that are coincident with distinct resistivity lows of $<10 \Omega\text{m}$.

This survey shows that surface electrical resistivity imaging can be a suitable method to study resistivity distribution (3D) at a large scale (ten to several tens of meters wide and down to 30 m deep). 3D electrical imaging is becoming a common tool to study recirculation experiments in landfills, as well as focusing on electrical resistivity changes in the subsurface by pollution of groundwater or location of shallow or deep infiltration/recharge zones or delineation of leachate plume in landfills.

Self-potential survey

The data collected by the SP method was processed to eliminate noise exemplified by low background voltages for recognition of different spontaneous-potential sources. After processing, the SP data was in the range of -260 to $+30$ mV for three measured profiles (T1, T2, and T3). We aimed to assess the relationship of the near-surface geological formation with environmental occurrences, and we used the processed SP data to map the contouring surficial voltages between base/reference electrode(s) and the mobile electrodes. On the map made from an equipotential based on SP data collected in March 2016 (Fig. 9), one can distinguish three positive anomalies (marked in red/brown) and two zones of negative anomaly (in dark blue). The values of the positive SP anomalies were within the range $+10$ to $+25$ mV

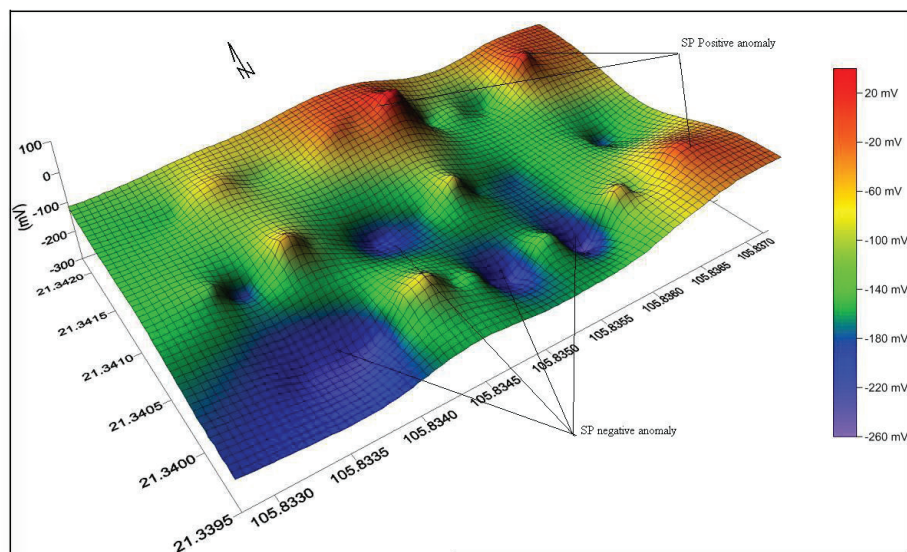


Fig. 9. A map of equipotential based on self-potential (SP) data carried out in March 2016.

and negative was in the range from -150 to -260 mV. The SP anomalies were positive (resurgence) where the hydrostatic pressure decreased (i.e. in the direction of the water flux), and the SP anomaly was negative for the infiltration case. Other occurrences produce spontaneous potentials, which were mapped to determine information about the subsurface. Spontaneous potentials can be produced by differences in the mineralization of the ground, electrochemical actions, geothermal activities, and bioelectric generation of vegetation.

The notable thing here are the SP positive anomalies at three points (Fig. 9 -red/brown colour), and the SP negative anomaly at the back which expands out into two distinct zones that the position of the two zones on the measured profile, T3, located near the Nam Son landfill. So, we much ask if it was due to the landfill leachate that caused this effect? As a blanket statement, this phenomenon has the related zone with the high electrical conductivity in the structure according to 3D electrical imaging stated above.

There is an increasing interest in the use of the SP method for non-invasive characterizing of subsurface

groundwater flow and contamination. The interpretation of SP signals is underlined by the understanding of the main physical process involved in their origin. The equipotential map based on the SP data demonstrates the direction of the water.

Hydrochemical analysis

There are many sources of water in the landfill, for example, precipitation, groundwater infiltration, surface runoff, and the disposal of liquids or sludges. The amount of water entering the landfill depends on such factors as the seasonal distribution of the water balance, the topography, the geology, and the hydrogeological properties of the site, soil type, land cover, human activity, and other similar factors. [1]. Water plays three major roles in land filling: first, as a medium essential for bacterial activity; second, as a medium for chemical reactions in organic matter; and third, as a medium for the movement of contaminants and solvents.

In this research project, to conduct analysis, we sampled surface water in three locations (N1, N3, and N5) on the Lai Son canal and groundwater in three boreholes, drilled in the area of research

Table 1. Hydro-geochemical analysis of surface water (N1, N3, and N5) and groundwater (S1, S3, and S5) samples affected by the Nam Son Landfill for the 2016 year by Institute of Chemistry and Institute of Geophysics, VAST.

Parameter	Surface water name of samples and content for date			Groundwater name of samples and content for date			Standard values QCVN-08/09-MT(B1-2015)
	N1	N3	N5	S1	S3	S5	
pH	7.86	7.71	7.52	7.22	7.14	7.08	5.5÷8.5
EC (μS/cm)	2,507	2,646	2,659	1,645	1,778	1,601	1,000
Water level (m)				4.2	4.7	5.5	
Temperature (°C)	21.3	22.5	22.8	24.8	25.0	25.1	
TDS (ppm)	1,630	1,720	1,702	1,051	1,156	1,025	1,500
DO (mg/l)	2.1	2.7	2.2	3.7	3.4	3.3	≥4
COD (mg/l)	156	162	157	44	56	53	40
BOD ₅ (mg/l)	112	124	105	28	26	33	25

Legend: COD: chemical oxygen demand; EC: electrical conductivity; TDS: total dissolved solids; DO: dissolved oxygen; BOD₅: Biochemical or Biological Oxygen Demand during 5 days at 20°C; QCVN-08/09-MT (B1-2015): Vietnam Technical Regulation on surface water or groundwater quality by Ministry of Natural Resources and Environment (MONRE).

(S1, S3, and S5) (Fig. 1). The water samples were collected and analyzed in March 2016. The results of the analysis are presented in Table 1 for surface water and groundwater, respectively.

The environmental indicators of water quality can be roughly divided into the physical indicators of temperature, EC, and TDS; and the chemical indicators of pH, BOD, COD, and DO. Because direct measurements of water quality can be expensive, we used on-site test kits and water samples analyzed at laboratories using real-time monitoring. The values of the parameters are presented in Table 1. The data shows that the values of most parameters in 2016 are of a higher concentration by comparison of their standard values. Most of the water samples indicated a slightly alkaline nature with pH 7.08/7.86. The values of DO, for all sites,

are lower than regular, but the values of COD and BOD₅ exceed permissible limits. High concentration of TDS and EC were observed in all sites along Lai Son canal and all wells, which means that the surface water and groundwater in the landfill areas are of very poor quality. The results of the hydrochemical analysis show that the values of TDS and EC for surface and groundwater are very high. Although generally TDS is not considered a primary pollutant, it is used as an indication of aesthetic characteristics of domestic water and as an aggregated indicator of the presence of a number of chemical contaminants [2]. Primary sources of the TDS in waters are soil contaminants and polluted water from the landfill. Bear in mind, however, that the concentration of some naturally occurring dissolved solids increases as a result of weathering and dissolution of

rocks and soils and influences the results of analyses.

The BOD is widely used as an indicator of water quality. In this case, BOD values ranged from 26 to 33 mg/l for groundwater and from 105 to 124 mg/l for surface water. These values are too high for natural water to acceptable.

Conclusions

Integrated, geophysical surveys methods, as well as 3D electrical imaging and self-potential (SP) were employed to determine the subsurface geological structure, and assess the effect of landfill leachate at the Nam Son waste site. The case study area was generally close to the ground surface and was characterized by the non-homogenous formations which were largely responsible for the transfer of contaminated water into the underlying aquifers.

Interpretations of 3D resistivity geoelectrical data showed a low resistivity zone (<10 Ωm), which appeared to be a zone that is fully saturated with leachate from an open dumpsite. The SP method supported the results of the geoelectrical method relating to a contaminated zone in the survey area from a largely negative SP anomaly of ~-260 mV with respect to the reference with the contaminant plume. These electrical anomalies could be the signature of oxido-reduction phenomena which occur at various depths in the contaminated groundwater. A comparison with the available hydrochemical data suggests that this SP anomaly could be attributed to high redox potential gradients in this area.

The results of our research clearly indicate the need for regular monitoring of groundwater and surface water quality in the vicinity of the Nam Son landfill. Also, we recommend installing a waste water treatment plant that could diminish pollution from seeping waters flowing directly from the Lai Son canal. The

management of the landfill should also pay more attention to the environmental protection of groundwater resources from domestic wells in the areas around the landfill area.

ACKNOWLEDGEMENTS

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 105.05-2014.28.

REFERENCES

- [1] R. Guerin, Ph. Begassat, Y. Benderitter, J. David, A. Tabbagh, M. Thiry (2004), "Geophysical study of the industrial waste land in Mortagne-du-Nord (France) using electrical resistivity", *Near Surf. Geophys.*, **2**(3), pp.137-143.
- [2] N. Abu-Zeid, G. Bianchini, G. Santarato, C. Vaccaro (2004), "Geochemical characterization and geophysical mapping of landfill leachates: the Marozzo canal case study (NE Italy)", *Environ. Geol.*, **45**(4), pp.439-447.
- [3] A. Ahmed, W. Sulaiman (2001), "Evaluation of groundwater and soil pollution in a landfill area using electrical resistivity imaging survey", *Environ. Manag.* **28**(5), pp.655-663.
- [4] E. Martinho, F. Almeida (2006), "3D behavior of contamination in landfill sites using 2D resistivity/IP imaging: case studies in Portugal", *Environ. Geol.*, **49**(7), pp.1071-1078.
- [5] N.H. Khanh (2007), *Research and comparison of landfill leachate treatment system in Vietnam and the world to choose suitable one to meet level of Vietnamese standard for landfill site Nam Son in Hanoi*, Report of the project, Institute of Environmental Technology, VAST.
- [6] N.M. Khai, H.T. Quynh Trang (2012), "Chemical precipitation of ammonia and phosphate from Nam Son landfill leachate, Hanoi", *Iranica Journal of Energy & Environment (Special Issue on Environmental Technology)*, **3**, pp.32-36.
- [7] T.U. Andrea, R.E. Vagner, M. Giulliana, V.Z. Lazaro, L.G. Heraldo (2012), "Case study: a 3D resistivity and Induced polarization imaging from downstream in waste disposal site in Brazil", *Environ. Earth Sci.*, **66**(3), pp.763-772.
- [8] E. Cardarelli, F. Fischanger (2006), "2D data modeling by electrical resistivity tomography for complex subsurface geology", *Geophys. Prospect.*, **54**(2), pp.121-133.
- [9] R. Frederico, W. Phygeo, M. Márcio, M. Guilherme, K.N.A. Paulo (2013), *The self-potential (SP) method applied for investigating the contamination in the vicinity of the structural city landfill, in Brasília-DF*, In 13th international congress of the Brazilian Geophysical Society, Rio de Janeiro, Brazil, pp.1-4.
- [10] W.M. Telford, L.P. Geldart, R.E. Sheriff (1990), *Applied Geophysics*, 2nd edition, Cambridge University Press, 792p.
- [11] N.V. Giang, N.B. Duan, L.N. Thanh, N. Hida (2013), "Geophysical techniques to aquifer locating and monitoring for industrial zones in North Hanoi, Vietnam", *Acta Geophysica*, **61**(6), pp.1573-1597.
- [12] N. Hida and N.V. Giang (2012), "Decline in groundwater levels in Thang Long Industrial Park within the area of Northwest Hanoi, Vietnam", *J. Japan Assoc. Hydrol. Sci.*, **42**(4), pp.167-172 (in Japanese).
- [13] D.X. Sam (eds) (2010), *The natural conditions of the natural resources and the environment in the development of space-oriented Hanoi capital*, Hanoi Published House, 590p (in Vietnamese).
- [14] G. Gupta, J. Patil, S. Maiti, V.C. Erram, N. Pawar, S. Mahajan, R. Suryawanshi (2015), "Electrical resistivity imaging for aquifer mapping over Chikotra Basin, Kolhapur District, Maharashtra", *Environ. Earth Sci.*, **73**(12), pp.8125-8143.
- [15] M.H. Loke, R.D. Barker (1996a), "Rapid least squares inversion of apparent resistivity pseudosections by a quasi-Newton method", *Geophys. Prospect.*, **44**(1), pp.131-152.
- [16] M.H. Loke, R.D. Barker (1996b), "Practical techniques for 3D resistivity surveys and data inversion", *Geophysical Prospecting*, **44**(3), pp.499-523.
- [17] L. Jouniaux, A. Mainault, V. Naudet, M. Pessel, P. Sailhac (2009), "Review of self-potential methods in hydrogeophysics", *Comptes Rendus Geoscience*, **341**(10-11), pp.928-936.
- [18] M. Weigel (1989), "Self-potential surveys on waste dumps. Theory and practice in detection of subsurface flow phenomena Lect", *Notes Earth Sci.*, **27**, pp.109-120.
- [19] T. Arora, N. Linde, A. Revil, J. Castermant (2007), "Non - intrusive characterization of the redox potential of landfill leachate plumes from self-potential data", *J. of Contaminant Hydrology*, **92**(3-4), pp.274-292.
- [20] R.F. Corwin (1990), "The self - potential method for environmental and engineering applications", *Geotech. Environ. Geophys.*, **1**, pp.127-145.
- [21] SAS 4000 instrument manual introduction (2010), *ABEM Sweden*.

Synthesis, characterization and up-conversion luminescence properties of α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOFs multilayer nanocrystals

Thi Kieu Giang Lam^{1,2*}, Lukasz Marciniak³, Quang Huy Tran⁴, Manh Tien Dinh¹, Duc Chinh Vu¹, Vu Nguyen¹, Thu Huong Tran¹, Thi Khuyen Hoang¹, Thanh Huong Nguyen¹, Duc Roan Pham⁵, Thanh Binh Nguyen⁶, Quoc Minh Le^{1,2}

¹Institute of Materials Science, Vietnam Academy of Science and Technology

²Graduate University of Science and Technology, Vietnam Academy of Science and Technology

³Institute of Low Temperature and Structural Research, Polish Academy of Sciences, Poland

⁴National Institute of Hygiene and Epidemiology (NIHE)

⁵Faculty of Chemistry, Hanoi National University of Education

⁶Laboratory of Photochemistry Imaging and Photonics, Institute of Applied Physics and Scientific Instrument, Vietnam Academy of Science and Technology

Received 1 June 2017; accepted 5 September 2017

Abstract:

The precursor PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres were used as the templates for preparing the α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOFs multilayer nanocrystals with a self-template method. By using iron (III) carboxylate and zeolitic imidazolate frameworks dissolved in dimethylformamide (DMF) solution containing 25% of diethylene glycol (DEG), the spherical-shaped α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100 and α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 multilayer nanocrystals were successfully prepared with the sizes of 300-500 nm at 100°C for one hour. Under a 976 nm laser excitation at room temperature, the α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100 and α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 multilayer nanocrystals exhibited strong up-conversion luminescence with three emission bands centered at around 520 nm, 540 nm, and 655 nm corresponding to ²H_{11/2} → ⁴I_{15/2}, ⁴S_{3/2} → ⁴I_{15/2}, and ⁴F_{9/2} → ⁴I_{15/2} transitions of Er³⁺ ions, respectively.

Keywords: metal-organic frameworks, multilayer nanocrystals, self-template method, up-conversion, α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100, α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8.

Classification numbers: 5.2, 5.5

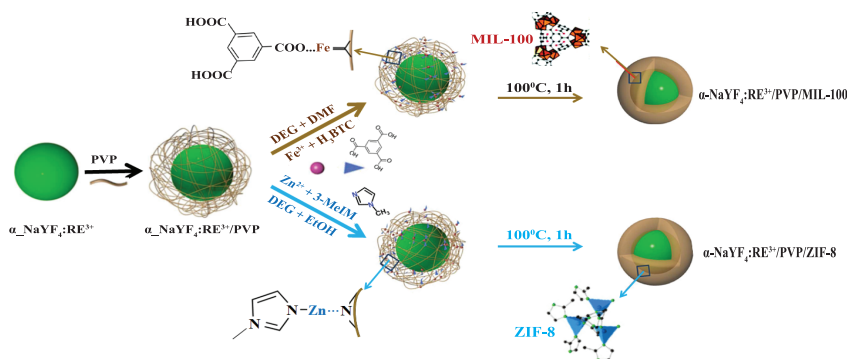
Introduction

Metal-organic frameworks (MOFs) are considered as the new classes of hybrid porous materials assembled with metal cations and organic ligands. Due to their unique physical and chemical characteristics, they have been widely investigated for various applications such as biosensors, gas storage, catalysis, and separation, etc. [1-5]. Particularly, MOFs based on iron (III) carboxylate materials (MIL-100) and/or zeolitic imidazolate framework (ZIF-8) have recently attracted a great deal of attention owing to their prospective applications in drug delivery, diagnostics, and therapy of cancer [6-8].

Recently, rare earth doped NaYF₄ nanoparticles (NPs) have been proven to have excellent near-infrared (NIR) excited up-conversion luminescence (UCL) properties, making the new generation of bio-probes in diagnostics and therapy of cancer [9-12]. Stimulated by this discovery, many research groups have been paying their attention to fabricate and study UCL@MOFs nanocrystals for applications in bioimaging, diagnosis, and targeted drug delivery [13, 14].

In this work, a self-template method was used to prepare high-quality

*Corresponding author: Email: giangltk@ims.vast.ac.vn



Scheme 1. Schematic illustration of the synthesis of α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOF multilayer nanocrystals.

multilayer nanocrystals, in which PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres were used as the core, and MIL-100 (or ZIF-8) served as shell layers. It hypothesized that the spherical shape of α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100 and α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 multilayer nanocrystals would exhibit simultaneously both NIR optical property of UCL cores and the unique property of metal-organic frameworks (Scheme 1).

Experimental

Materials

Iron (III) chloride hexahydrate (FeCl₃·6H₂O, 99.0%), Zinc nitrate tetrahydrate (Zn(NO₃)₂·4H₂O, 98.5%), Trimesic acid (H₃BTC), Dimethylformamide (DMF, 99.5%), 3-Methylimidazole (3-MeIM, C₄H₆N₂), Diethylene glycol (DEG), Sodium acetate (CH₃COONa), Ammonium fluoride (NH₄F), Rare-earth chlorides (RECl₃·6H₂O, RE³⁺:Y³⁺, Yb³⁺, Er³⁺), polyvinylpyrrolidone (PVP, Mw ~20,000), and hydrogen chloride solution were purchased from Merck and Sigma-Aldrich. All the chemicals were of analytical grade.

Synthesis of PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres

The PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres were synthesized according to our previous report [15] as follows:

Firstly, three solutions of YCl₃·6H₂O, YbCl₃·6H₂O, and ErCl₃·6H₂O were mixed

by magnetic stirring for one hour (Y³⁺/Yb³⁺/Er³⁺ molar ratio of 79/19/2). Then, the solution of CH₃COONa dissolved in DEG was slowly added while being stirred for 30 minutes to obtain solution A. Simultaneously, a solution containing NH₄F was dissolved in DEG and slowly added to the solution A, then stirred until a homogeneous mixture was obtained. The resulting homogeneous mixture was poured into a 100 ml Teflon vessel and heated up at a temperature of 120°C for two hours in the argon atmosphere under vigorous magnetic stirring, and then cooled down to room temperature by ice water. The samples of α -NaYF₄:Yb³⁺/Er³⁺ nanopowders were cleaned by centrifugation with deionized water and isopropanol and dried at 70°C in air.

After that, the α -NaYF₄:Yb³⁺/Er³⁺ nanopowders were re-dissolved into 10 ml HCl (0.1 M) solution, washed three times by ultrasonic treatment and centrifugation. The products of 0.1 g α -NaYF₄:Yb³⁺/Er³⁺ nanoparticles were dissolved in 10 ml ethanol solution containing 0.5 g of PVP (M_w=20000) and vigorously stirred to obtain the homogeneous solution of PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres.

Synthesis of α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOF multilayer nanocrystals

Firstly, the mother solution B was prepared for the secondary growth to form a thick MIL-100 and ZIF-8 layers as follows:

- Mix the solution of 0.05 g of

FeCl₃·6H₂O and 0.04 g of H₃BTC into 20 ml solution containing 75% of DMF and 25% of DEG under stirring at 25°C for one hour (with MIL-100 layer).

- Mix the solution of 0.2 g of Zn(NO₃)₂·4H₂O and 0.3 g 3-Methylimidazole (3-MeIM) into 20 ml solution containing 75% of ethanol (EtOH) and 25% of DEG under stirring at 25°C for one hour (with ZIF-8 layer).

After that, 10 ml of the prepared solution of PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres was dropped into the mother solution B and gently stirred at room temperature for one hour to obtain the homogeneous solution C. The homogeneous solution C was heated up at 100°C for one hour in the argon atmosphere under vigorous magnetic stirring and cooled down to room temperature by ice water.

Finally, the obtained products of α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOF multilayer nanocrystals were cleaned three times with ethanol by centrifugation to remove redundant iron ions and acid, and then dried at 70°C for 24 hours.

Instrumentation

The crystalline phase structure was determined by using a PANalytical X'Pert Pro diffractometer with Cu K_α radiation (λ = 1.54060 Å) in the 2θ range of from 5° to 70°. The average grain size was calculated by using Scherrer's formula [16]:

$$D = k\lambda/(\beta \cos\theta) \quad (1)$$

where λ is the wave length of the X-ray diffraction, θ is the diffraction angle and β is full width at half maximum (FWHM).

The morphology of the nanocrystals was investigated by FE-SEM (S-4800, Hitachi). Fourier transform infrared spectroscopy (FTIR) analysis was carried out on the Thermo Nicolet NEXUS 670 FTIR (USA). Up-conversion luminescence measurements were performed at room temperature with a Jobin-Yvon HR1000 monochromator,

equipped with a charge-coupled device (CCD) camera using a 976 nm laser diode.

Results and discussions

The α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOF multilayer nanocrystals were successfully synthesized by using the self-template method at the temperature of 100°C for one hour. The evolution of the crystalline phase of α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOF multilayer nanocrystals compared with the single crystalline of PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres, MOF, and reference crystallographic data of α -NaYF₄ (JCPDS No. 77-2042) was confirmed by XRD measurements (Fig. 1).

With the samples of PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres (patterns P₁ and P₄), all diffraction peaks corresponding to pure cubic α phase of NaYF₄ (JCPDS No. 77-2042) with a calculated lattice constant are 5.447 Å, space group Fm-3m, and Z=4. Meanwhile, after being mixed with the mother solution for the secondary growth to form a thick MOF layer, the as-synthesized α -NaYF₄:Yb³⁺/Er³⁺/PVP/MOF multilayer nanocrystals (pattern P₃) have two group peaks which match the standard cubic NaYF₄ XRD pattern (JCPDS No. 77-2042) and MIL-100 crystalline phase (marked with “*”, pattern P₂) [17] or amorphous ZIF-8 (pattern P₅) [18]. In addition, the average grain size calculated by using Scherrer's formula with all samples is around 45 ± 5 nm, suggesting the formation of MOF layer onto the surface of the core α -NaYF₄:Yb³⁺/Er³⁺ nanospheres while maintaining the crystallographic phase of α -NaYF₄:Yb³⁺/Er³⁺ nanospheres.

Figure 2 shows the morphology of α -NaYF₄:Yb³⁺/Er³⁺/MIL-100 and α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 multilayer nanocrystals obtained at the temperature of 100°C for one hour. As it is shown in the inset of Fig. 2A (S₁), the core of PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres has the size of around 40 nm. After being mixed with the mother solutions for the secondary growth to form a thick MOF

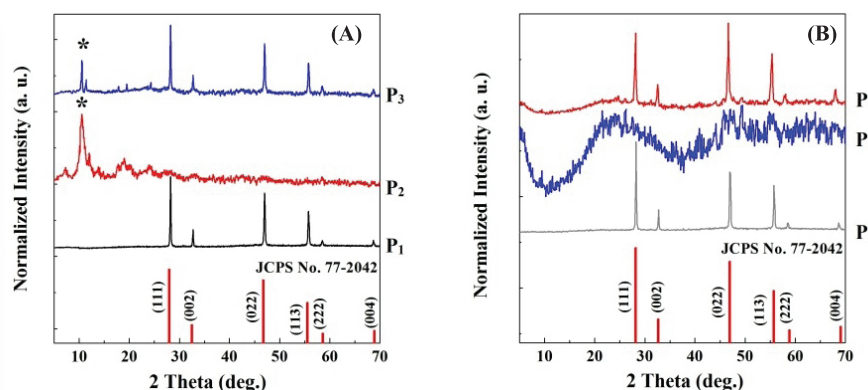


Fig. 1. The normalized XRD patterns of samples: (A) α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100 multilayer nanocrystals (pattern P₃) compared with PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres (pattern P₁) and MIL-100 (pattern P₂) and (B) α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 multilayer nanocrystals (pattern P₆) compared with PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres (pattern P₄) and amorphous ZIF-8 (pattern P₅). The JCPDS No. 77-2042 is the reference crystallographic data of α -NaYF₄ (JCPDS No. 77-2042) and the peaks of MIL-100 are marked with “*”.

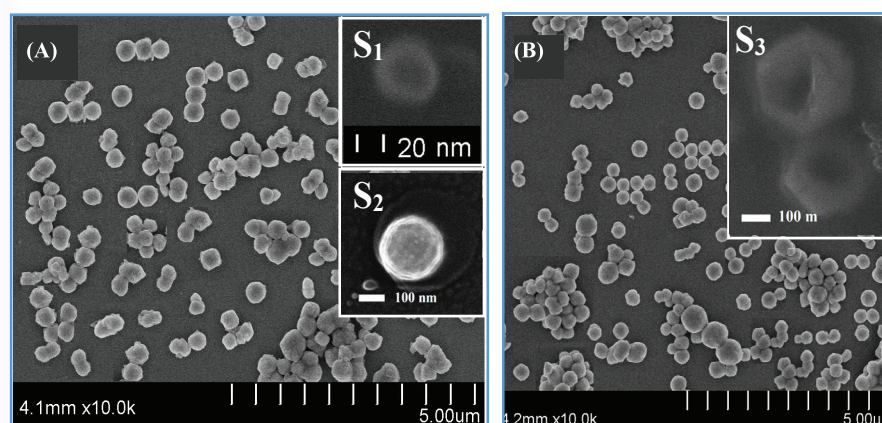


Fig. 2. The FE-SEM images of (A) α -NaYF₄:Yb³⁺/Er³⁺/MIL-100 and (B) α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 multilayer nanocrystals prepared by self-template method at the temperature of 100°C for one hour. The inset S₁ shows the core of PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres. The insets S₂ and S₃ give the high-magnification SEM image of the samples α -NaYF₄:Yb³⁺/Er³⁺/MIL-100 and α -NaYF₄:Yb³⁺/Er³⁺/ZIF-8 multilayer nanocrystals (scale bar: 100 nm).

layer, the α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100 and α -NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 multilayer nanocrystals obtained have the sizes of 300-400 nm (Fig. 2A, S₂) and 300-500 nm (Fig. 2B, S₃), respectively. The insets of Fig. 2A (S₂) and Fig. 2B (S₃) confirm the core/shell structures of α -NaYF₄:Yb³⁺/Er³⁺/MIL-100 and α -NaYF₄:Yb³⁺/Er³⁺/ZIF-8 multilayer nanocrystals.

Figure 3 presents the FTIR spectra of α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100 (curve a₂) and NaYF₄:Yb³⁺/Er³⁺/PVP/ZIF-8 (curve b₂) multilayer nanocrystals compared with PVP-capped α -NaYF₄:Yb³⁺/Er³⁺ nanospheres (curves a₁ and b₁), MIL-100 (curve a₃) and ZIF-8 (curve b₃). The characteristic infrared (IR) absorption bands and the corresponding organic functional groups of samples α -NaYF₄:Yb³⁺/Er³⁺/PVP/MIL-100 (NP/MIL100), MIL-100,

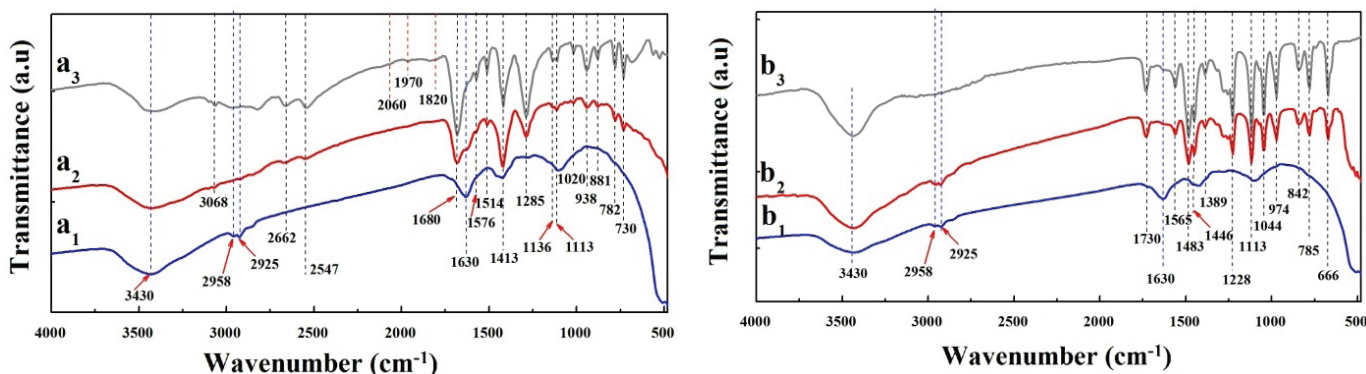


Fig. 3. FTIR spectra of α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{MIL-100}$ (curve a_2) and $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{ZIF-8}$ (curve b_2) multilayer nanocrystals compared with PVP-capped α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanospheres (curves a_1 and b_1), MIL-100 (curve a_3), and ZIF-8 (curve b_3).

and α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanospheres (NP), $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{ZIF-8}$ (NP@ZIF8), and ZIF-8 are illustrated in Table 1.

It can be seen in Fig. 3 and Table 1 that the broad absorption peak at around 3430 cm^{-1} corresponding to hydroxyl groups (-OH) was observed in all samples (curves a_1 - a_3 and b_1 - b_3). In addition, compared to the spectra of α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ (curve a_1), the spectrum of MIL-100 and α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{MIL-100}$ multilayer nanocrystals has adsorption bands which represent for MIL-100 structure. For example, the strong vibrational bands at around 1285 , 1413 , and 1680 cm^{-1} corresponding to the symmetric -COOH stretching and interaction between the deprotonated -COOH and the Fe ion indicate the growth of MIL-100 crystals on the surface of the PVP-capped α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanospheres [17]. Especially, in the MIL-100 and α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{MIL-100}$ multilayer nanocrystals, we can observe the weak signal in the range of 1820 - 2060 cm^{-1} bands corresponding to the traces of residual trimesic acid, which proves that the cleaning process by using centrifugation with ethanol is very effective in removing the residual trimesic acid. Furthermore, the characteristic peaks of ZIF-8 observed in both of ZIF-8 (curve b_3) and $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{ZIF-8}$ (curve b_2) samples suggest the existence of ZIF-8 layer on the surface of the PVP-capped α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanospheres [19].

Table 1. The characteristic IR absorption bands of samples α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{MIL-100}$ (NP/MIL100), MIL-100, and α - $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanospheres (NP), $\text{NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP}/\text{ZIF-8}$ (NP@ZIF8), and ZIF-8 [20].

Characteristic absorptions (cm^{-1})	Functional groups [21]	Intensities: vs-very strong; s-strong; m-medium; w-weak; vw-very weak				
		NP@MIL-100	MIL-100	NP	NP@ZIF8	ZIF-8
3430	-OH groups	vs	vs	vs	vs	vs
3068	C-H stretching	vw	vw	-	-	-
2958, 2925	C-H stretching	-	-	w	w	-
2662, 2547	-OH (of DMF)	w	w	-	-	-
2060, 1970, 1820	Residual H_3BTC	vw	vw	-	-	-
1730	-C=O stretching	-	-	-	m	m
1680	-COOH stretching	vs	vs	-	-	-
1630	-C=O stretching	w	-	s	w	w
1565	C=C stretching	-	-	-	m	m
1483	Aromatic stretching	-	-	-	vs	vs
1446	C-H stretching	-	-	-	m	m
1413	-COOH stretching	vs	vs	-	-	-
1285	-C=O stretching	s	vs	-	-	-
1228	C-N stretching	-	-	-	vs	vs
1113	C-N stretching	-	-	-	vs	vs
1044	C-N-C stretching	-	-	-	vs	vs
974	C-N stretching	-	-	-	s	s
938	$\delta(\text{O-H})$	m	m	-	-	-
842	C-N stretching	-	-	-	s	s
782	-CH	m	m	-	-	-
730	C-H stretching	m	m	-	-	-
666	C-N stretching	-	-	-	s	s

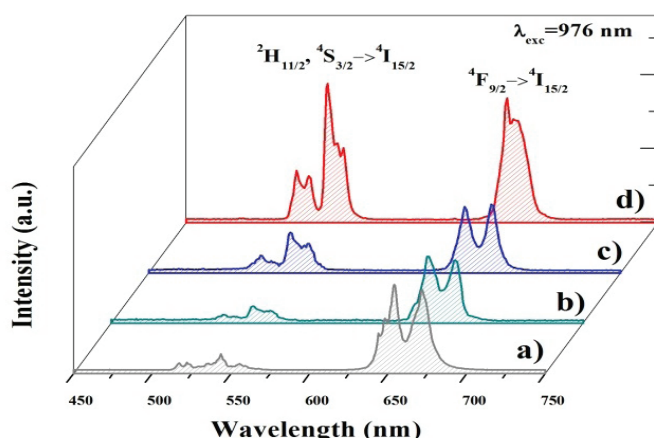


Fig. 4. The comparison of up-conversion luminescence spectra of the $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$ and $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals compared with the bare core $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanoparticles and PVP-caped $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanospheres upon 976 nm excitation at 800 mW.

Table 2. Integrated emission intensity ratio of the red to green regions of the as-synthesized samples

Sample	I_{Green}	I_{Red}	$I_{\text{Green}}/I_{\text{Red}}$
Bare core $\alpha\text{-NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanoparticles	0.38	2.82	~ 0.13
PVP-capped $\alpha\text{-NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanospheres	0.38	1.90	~ 0.20
$\alpha\text{-NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals	1.00	1.82	~ 0.55
$\alpha\text{-NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}/\text{PVP/MIL-100}$ multilayer nanocrystals	2.75	2.73	~ 1.01

The up-conversion luminescence spectra of the $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$ and $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals compare with the bare core $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanoparticles and PVP-caped $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanospheres upon 976 nm excitation at 800 mW are showed in Fig. 4.

The results revealed that the $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$ and $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals had three emission bands at around 520 nm, 540 nm, and 655 nm corresponding to $^2\text{H}_{11/2} \rightarrow ^4\text{I}_{15/2}$, $^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$, and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$ transitions of Er^{3+} ions, respectively. The integrated intensity of

UCL emission in green and red spectral regions of as-synthesized samples was shown in Table 2 with the note that all of the data shown in Table 2 were obtained for the same experimental conditions.

The obtained data show that the total integrated emission intensity of the $\text{NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$ multilayer nanocrystals is about 1.94 times higher than that of the $\text{NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals, and about 1.71 and 2.41 times higher than that of the PVP-caped $\alpha\text{-NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanospheres and bare core $\alpha\text{-NaYF}_4\text{:2\%Er}^{3+}, 19\%\text{Yb}^{3+}$ nanoparticles, respectively. Moreover, when adding the layers of PVP, PVP/ZIF-8 or PVP/MIL-100, the

integrated intensity ratio of green to red emissions increased from 0.13 to 1.01. This suggests that the efficiency of the up-conversion increases when increasing the particle size of bare core $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanoparticles, PVP-caped $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanospheres, $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$, and $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals [21]. The increase in the efficiency of the up-conversion could be speculated due to the porous structure of MIL-100 shells. This leads to the limitation of transferring photo-generated electron-hole pairs in the $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$ multilayer nanocrystals.

Conclusions

In summary, a self-template method was utilized to prepare high-quality $\text{NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$ and $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals. The UCL spectra studies demonstrated that the integrated intensity ratio of green to red emissions increased from 0.13 to 1.01 when adding the layers of PVP, PVP/ZIF-8 or PVP/MIL-100 on the surface of the $\alpha\text{-NaYF}_4\text{:Yb}^{3+}/\text{Er}^{3+}$ nanoparticles. This suggests that the efficiency of the up-conversion increases due to the decrease in contribution of the non-radiative processes when an increase in particle size affects the bare core $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanoparticles, PVP-caped $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}$ nanospheres, $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/MIL-100}$, and $\alpha\text{-NaYF}_4\text{:19\%Yb}^{3+}/2\%\text{Er}^{3+}/\text{PVP/ZIF-8}$ multilayer nanocrystals.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Professor Acad. Nguyen Van Hieu (VAST), Prof. Nguyen Quang Liem (VAST), and Prof. Vu Dinh Lam (VAST) for their great support and encouragement to promote the application research of new research directions for metal-organic frameworks

in Institute of Materials Science. This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 103.03-2016.60.

REFERENCES

- [1] R.J. Kuppler, D. J. Timmons, Q.R. Fang, J.R. Li, T.A. Makal, M.D. Young, D. Yuan, D. Zhao, W. Zhuang, H.C. Zhou (2009), "Potential applications of metal-organic frameworks", *Coordination Chemistry Reviews*, **253**, pp.3042-3066.
- [2] H. Furukawa, K.E. Cordova, M. O'Keeffe, O.M. Yaghi (2013), "The chemistry and applications of metal-organic frameworks", *Science*, **341**, doi:10.1126/science.1230444.
- [3] A.C.M. Kinlay, R.E. Morris, P. Horcajada, G. Férey, R. Gref, P. Couvreur, C. Serre (2010), "BioMOFs: Metal-Organic Frameworks for Biological and Medical Applications", *Angew. Chem. Int. Ed.*, **49**, pp.6260-6266.
- [4] T.B. Nguyen, M.T. Dinh, T.K.G. Lam, T.K. Hoang, T.H. Nguyen, T.H. Tran, D.L. Tran (2014), "Study on preparation and characterization of MOF based lanthanide doped luminescent coordination polymers", *Materials Chemistry and Physics*, **143**, pp.946-951.
- [5] T.B. Nguyen, T.T. Phung, T.H.L. Ngo, M.T. Dinh, T.K. Hoang, T.K.G. Lam, T.H. Nguyen, T.H. Tran, D.L. Tran (2015), "Study on preparation and properties of a novel photo-catalytic material based on copper-centred metal-organic frameworks (Cu-MOF) and titanium dioxide", *Int. J. Nanotechnol.*, **12**, pp.447-455.
- [6] S. Keskin and S. Kizilel (2011), "Biomedical Applications of Metal Organic Frameworks", *Industrial & Engineering Chemistry Research*, **50**, pp.1799-1812.
- [7] W. Cai, C.C. Chu, G. Liu, Y.X.J. Wang (2015), "Metal-Organic Framework-Based Nanomedicine Platforms for Drug Delivery and Molecular Imaging", *Small*, **11**, pp.4806-4822.
- [8] M. Zheng, S. Liu, X. Guan, Z. Xie (2015), "One-Step Synthesis of Nanoscale Zeolitic Imidazolate Frameworks with High Curcumin Loading for Treatment of Cervical Cancer", *ACS Applied Materials & Interfaces*, **7**, pp.22181-22187.
- [9] S. Jiang, Y. Zhang, K.M. Lim, E.K.W. Sim, L. Ye (2009), "NIR-to-visible up-conversion nanoparticles for fluorescent labeling and targeted delivery of siRNA", *Nanotechnology*, **20**, doi: 10.1088/0957-4484/20/15/155101.
- [10] C. Wang, L. Cheng, Z. Liu (2011), "Drug delivery with up-conversion nanoparticles for multi-functional targeted cancer cell imaging and therapy", *Biomaterials*, **32**, pp.1110-1120.
- [11] S. Chen, Q. Zhang, Y. Hou, J. Zhang, X.J. Liang (2013), "Nanomaterials in medicine and pharmaceuticals: nanoscale materials developed with less toxicity and more efficacy", *Eur. J. Nanomed.*, **5**, pp.61-79.
- [12] C. Wang, L. Cheng, Z. Liu (2013), "Up-conversion Nanoparticles for Photodynamic Therapy and Other Cancer Therapeutics", *Theranostics*, **3**, pp.317-330.
- [13] Y. Li, J. Tang, L. He, Y. Liu, Y. Liu, C. Chen, Z. Tang (2015), "Core-Shell Up-conversion Nanoparticle@Metal-Organic Framework Nanoprobes for Luminescent/Magnetic Dual-Mode Targeted Imaging", *Adv. Mater.*, **27**, pp.4075-4080.
- [14] K. Deng, Z. Hou, X. Li, C. Li, Y. Zhang, X. Deng, Z. Cheng, J. Lin (2015), "Aptamer-Mediated Up-conversion Core/MOF Shell Nanocrystals for Targeted Drug Delivery and Cell Imaging", *Scientific Reports*, **5**, doi: 10.1038/srep07851.
- [15] T.K.G. Lam, K.A. Tran, T.B. Nguyen, Q.M. Le, L. Marciniak, W. ojkowski (2015), "Fabrication and up-conversion emission processes in nanoluminophores NaYF₄: Er, Yb and NaYF₄: Tm, Yb", *Int. J. Nanotechnol.*, **12**, pp.538-547.
- [16] A. Patterson (1939), "The Scherrer Formula for X-Ray Particle Size Determination", *Physical Review*, **56(10)**, pp.978-982.
- [17] M. Wickenheisser, T. Paul, C. Janiak (2016), "Prospects of monolithic MIL-MOF@poly(NIPAM)HIPE composites as water sorption materials", *Microporous and Mesoporous Materials*, **220**, pp.258-269.
- [18] S. Cao, T.D. Bennett, D.A. Keen, A.L. Goodwind, A.K. Cheetham (2012), "Amorphization of the prototypical zeolitic imidazolate framework ZIF-8 by ball-milling", *Chem. Commun.*, **48**, pp.7805-7807.
- [19] J. Li, Y. Wu, Z. Li, B. Zhang, M. Zhu, X. Hu, Y. Zhang, F. Li (2014), "Zeolitic Imidazolate Framework-8 with High Efficiency in Trace Arsenate Adsorption and Removal from Water", *J. Phys. Chem. C*, **118**, pp.27382-27387.
- [20] B.H. Stuart (2004), *Infrared Spectroscopy: Fundamentals and Applications*, 244p.
- [21] R. Pazik, M. Maczka, M. Malecka, L. Marciniak, A. Ekner-Grzyb, L. Mrowczynska, R.J. Wiglusz (2015), "Functional up-converting SrTiO₃:Er³⁺/Yb³⁺ nanoparticles: structural features, particle size, colour tuning and in vitro RBC cytotoxicity", *Royal Society of Chemistry.*, **22**, pp.10267-10280.

Effects of ENSO on the intraseasonal oscillations of sea surface temperature and wind speed along Vietnam's coastal areas

Quoc Huy Le¹, Thuc Tran¹, Xuan Hien Nguyen^{1*}, Van Uu Dinh²

¹Vietnam Institute of Meteorology, Hydrology and Climate Change

²University of Science, Vietnam National University, Hanoi

Received 25 May 2017; accepted 1 September 2017

Abstract:

Our study applied the Ensemble Empirical Mode Decomposition (EEMD) method to analyze intraseasonal variability (ISV) of sea surface temperature (SST) and wind speed using a 22-year monitoring data set from 10 coastal stations. Results show that the El Niño and Southern Oscillation (ENSO) significantly affected the ISO Quasi-Biennial Oscillation (QBWO) 10-20 day periods and Madden-Julian Oscillation (MJO) 30-60 day periods of SST and wind speed at the coastal stations. As seen with MJO, the effects of ENSO on SST tend to increase from the north to south, whereas its impact on wind speed decreases from the north to south of Vietnam's coastal areas. In contrast, with QBWO, the effect of ENSO on SST reduces moving from the north to south, whereas its impact on wind speed increases from the north to south of Vietnam's coastal areas.

Keywords: EEMD, El Niño, ENSO, ISV, SST.

Classification number: 6.2

Introduction

The hydro-meteorological time series data collected around the world and most specifically collected at the South China Sea particularly contains the high to low-frequency signals, or from synop to interannual periods. These oscillation signals are due to the influences of processes varying from a planetary to regional scale, including: Seasonal oscillation with the monsoon (3-6 months), QBO (20-30 months), ENSO (3-5 years), Pacific Decadal Oscillation (PDO) (10-11 years), and others. ISO is the bridge between the synop scale and the seasonal scale, and directly affects the weather and climate in the region. Previous studies have shown that the South China Sea has two local ISOs including a 10-20-day period QBWO and a 30-60 day period MJO [1-5].

The ENSO is an oscillation phenomenon found on a global scale covering a period of 3-5 years. This oscillation significantly affects the large-scale circulations and others that are smaller scale, such as ISV, and seasonal oscillation; which, in turn, affects climate and weather in the region, including in the South China Sea. So far, the effect of ENSO on ISV is still an ongoing debate. Some studies suggested that the phases of ISV or MJO are strongly related to

the warm phases of ENSO (El Niño) [6, 7], but other studies have found no significant relationship between MJO and ENSO [8, 9]. However, most of the studies show a common agreement that the main effect of ENSO on ISV is limited to areas of the Pacific Ocean, while MJO tends to operate in the Central Pacific and does not operate in the Western Pacific Ocean during the warm phases of the ENSO [10, 11]. D.E. Waliser, et al. (1999) suggested that ISV is very sensitive to small changes from SST and the author also suggested that ISO may be related to ENSO [9]. Wen Zhou, et al. (2005) suggested that in the warm phase of ENSO, MJO switches to activate in the Central and Eastern Pacific, and is not active in the Indian Ocean nor the South China Sea. In the cold phases of ENSO, MJO is active in the South China Sea, but the author also noted that this hypothesis needs further study [12].

Thus, although a lot of studies on the ISV and its interactions with large-scale global oscillations have been conducted, the study of ISV in coastal areas of Vietnam is still very limited, especially studies using measured data from coastal stations.

This paper aims to study the ISV of marine hydro meteorological factors and its interaction with ENSO. To do that,

*Corresponding author: Email: nguyexuanhien79@gmail.com

we applied EEMD method to analyze ISV of SST and wind speed in Vietnam's coastal areas using a 22-year data set from ten coastal stations.

Method and data

Empirical Mode Decomposition (EMD) is a new and useful method used to separate and analyze a time series of data, particularly non-linear and non-stationary data. EMD decomposes data into different frequencies (from high to low) and different amplitudes. The data is analyzed based on characteristics of the data itself (adaptive analysis), which does not depend on the choices of the user [13].

From a time series $X(t)$, through the filtering process (sifting process), EMD decomposes $X(t)$ into a finite number of intrinsic mode functions (IMFs):

$$X(t) = \sum_{i=1}^n IMF_i + r \quad (1)$$

where: IMF_i represents mode i^{th} , and r is the residual of the data $X(t)$, which is then referred to the trend of data, and n is the number of IMFs, which depends on the length of data.

In order to apply EMD for decomposing data, the input data has to satisfy three conditions: (i) The signal must have at least two extremes, including one maximum and one minimum; (ii) The time scales must be determined for the time interval between two extreme points; and (iii) If the data does not have extreme values, only the bending point is recorded for the extreme values to be determined by taking their derivatives. The major steps of the EMD method are as follows:

1) Identify all extremes, connecting the high peak points by an upper boundary and the low peak points by a lower boundary, and then calculate the mean values of the upper and lower boundaries to get an average of $m_1(t)$.

2) Subtract the original data from $m_1(t)$, we get the first component of the sifting process $h_1(t)$:

$$h_1(t) = X(t) - m_1(t) \quad (2)$$

3) Assign $h_1(t)$ to a new time series, and step 1, step 2 is repeated:

$$h_2(t) = h_1(t) - m_2(t)$$

...

$$h_k(t) = h_{k-1}(t) - m_k(t)$$

The iteration process only stops when the Cauchy Convergence Criterion is satisfied [14]:

$$SD_k = \frac{\sum_{t=0}^T |h_{k-1}(t) - h_k(t)|^2}{\sum_{i=1}^n h_{k-1}^2} \quad (3)$$

In which, h_k is the sifting result in the k^{th} interaction, if SD_k is smaller than a given value (usually about 0.2-0.3), thus the filtering process can be stopped because the IMF has brought full physical meaning. The highest frequency of the $c_i(t)$ -component will be assigned using $h_k(t)$:

$$c_i(t) = h_k(t) \quad (4)$$

4) After the IMF component has the highest frequency value extracted- $c_i(t)$, the rest of the data is then determined:

$$r_i(t) = X(t) - c_i(t) \quad (5)$$

5) The remaining data- $r_i(t)$ continues to be used to extract IMF components with lower frequencies. When $r_i(t)$ becomes a monotonic function, or a function that has only one extreme, no IMF component is extracted further, and the decomposition stops. Finally the data is decomposed into the form (1).

However, the EMD method has a limitation that is the mixed frequencies problem (or mode mixing). That is, there is more than one frequency that exists in an IMF, or a frequency is present in two different IMF functions. This will lead to false results for the physical nature of each IMF received.

The EEMD method was improved

by Z.H. Wu and N.E. Huang (2009) using EMD to rectify the mode-mixing problem. Accordingly, the original data was added to a white noise series (Gaussian noise) with finite amplitude. Then, the data is decomposed into IMFs using the EMD method for new time series. The IMFs received from the EEMD method significantly reduced the mode-mixing phenomena [14]. Usually, the amplitude of white noise at 0.2-0.4 times the standard deviation of the original data and number of repetitions of the filtering process is several hundred times.

The steps of the EEMD method are as follows:

i) Add a white noise series to the original data

ii) Decompose the data with added white noise into IMFs by EMD

iii) Repeat steps 1 and 2 as many times as is required until the envelopes are symmetric with respect to zero (note that each time a different white noise series is added)

iv) Obtain the ensemble means of the corresponding IMFs of the decompositions as the final result.

To determine the average period of each IMF, the following formula is proposed [1]:

$$AC_k = n/Peaks_k$$

In which, Ac_k is the average period of k^{th} IMF, n is the sample size or the length of original data. $Peaks_k$ is the number of local extreme peak values of the k^{th} IMF.

SST and wind speed data have been measured at Vietnamese coastal stations from since the mid-20th century. However, until 1993, data measured synchronization was continuous and comprehensive. After analysis and quality assessment of data, SST and wind speed observed from 1993 to 2015 at 10 stations are used in the study, including: Bai Chay, Hon Dau, Hon Ngu, Con Co,

Son Tra, Quy Nhon, Phu Quy, Vung Tau, Con Dao, and Phu Quoc.

Oceanic Niño Index (ONI) is obtained from the National Oceanic and Atmospheric Administration (NOAA) [15]. ONI is running 3-month means of the SST anomaly across the Niño 3.4 area (5°N-5°S, 120°E-170°W). It is a standard that NOAA uses to determine the El Niño (warm phase) and La Nina (cold phase) in the tropical Pacific region.

Result and discussion

Determine ENSO winter events

The El Niño and La Nina events are determined from the ONI. A ENSO event occurs when ONI exceeds or equals the threshold of ± 0.5 in five consecutive months. The years in which ONI is greater than or equal to 0.5 is an El Niño year, and the years in which ONI is less than or equal to -0.5 is a La Nina year. ENSO winters are the years that ENSO occurs in winter (months 12, 1, and 2). December is the month of the previous year and January and February are the months of the following year. The neutral years are the years that ENSO does not occur throughout the year (Table 1).

There are seven El Niño winter events, seven La Nina winter events and nine neutral years.

Decompose SST and wind speed data of coastal stations

Decomposition by EEMD shows that, there are 13 components decomposed, in which intraseasonal oscillations is IMF4, IMF5 and IMF6 components (Table 2). IMF4 component is QBWD oscillation (10-20 days period). IMFs components have frequencies close together is IMF5 and IMF6 be combined into a single component to make sure of the physical meaning of the oscillation [14]. Taking the average of the IMF5 and IMF6, we obtained a 30-60 days period oscillation, called an MJO.

Table 1. The ENSO years and neutral years.

No	El Niño	La Nina	ENSO Winter		Neutral
			El Niño	La Nina	
1	1994	1995	1994	1995	1993
2	1997	1998	1997	1998	1996
3	2002	1999	2002	1999	2001
4	2004	2000	2004	2000	2003
5	2006	2007	2006	2007	2005
6	2009	2010	2009	2010	2008
7	2015	2011	2015	2011	2012
8					2013
9					2014
Total	7	7	7	7	9

Table 2. ISV of SST and wind speed (ws).

Station/IMF	IMF4		IMF5		IMF6	
	ws	SST	ws	SST	ws	SST
Bai Chay	14	16	27	30	41	67
Hon Dau	14	15	27	31	50	65
Hon Ngu	14	16	29	31	55	47
Con Co	15	15	29	32	48	53
Son Tra	14	16	27	31	56	70
Quy Nhon	14	17	23	32	48	63
Phu Quy	17	16	33	33	57	55
Vung Tau	14	16	30	35	49	68
Con Dao	16	16	33	32	66	48
Phu Quoc	15	16	21	34	53	38

Unit: days.

From here, ISV of SST in 10-20 days period is presented as SST QBWO; ISV of SST in 30-60 days period is presented as SST MJO; similarly for wind speed is WS QBWO and WS MJO.

Assessing the effect of ENSO to ISV

Correlation between ENSO and ISV:

Using lead/lag correlation analysis (SST Niño had a 3.4 lead of 60 months longer than ISV) between interannual variation (IAV) of SST Niño 3.4 and interannual variation of ISV, results show that at the time of ENSO activity (zero time), the effects of ENSO on ISV were not significant in most stations with low correlation coefficients (from -0.2 to

0.3). However, at the time of SST Niño 3.4 lead 40-50 months than ISV, the correlation between SST Niño 3.4 and ISV is significant at most stations (Fig. 1A, 1B, 1C, 1D, and Table 3):

- The IAV of SST Niño 3.4 has a negative correlation with the IAV of SST-QBWO (from -0.1 to -0.6) and have a positive correlation with IAV of SST-MJO (from 0.2 to 0.7) in at most of the stations.

- IAV of SST Niño 3.4 has a negative correlation with IAV of WS-QBWO (from -0.3 to -0.6) and has a negative correlation with IAV of WS-MJO (from -0.4 to -0.7) at most of the stations.

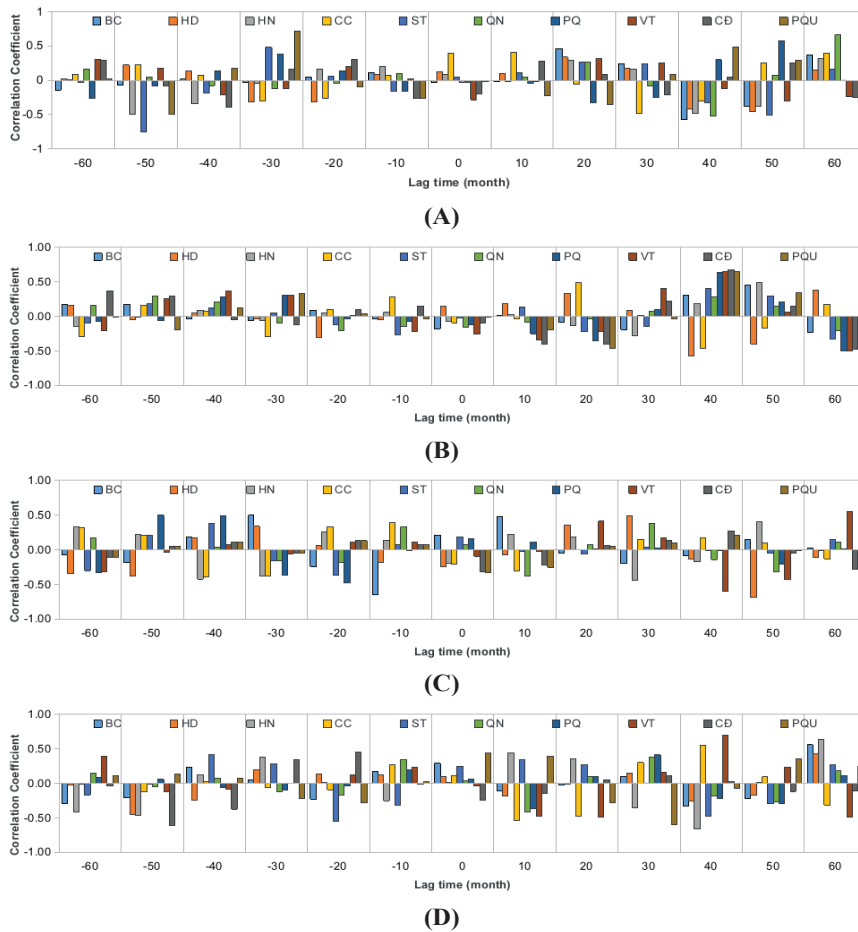


Fig. 1. The lead/lag correlation coefficient between the IAV of SST Niño 3.4 and the IAV of ISV. (A) IAV of SST Niño 3.4 and SST-QBWO; (B) IAV of SST Niño 3.4 and SST-MJO; (C) IAV of SST Niño 3.4 and wind speeds QBWO; (D) IAV of SST Niño 3.4 and WS-MJO.

Table 3. The correlation coefficient between the IAV of SST Niño 3.4 and the IAV of ISV at the time of SST Niño 3.4 lead 40-50 months than ISV (the 95% statistically significant correlation coefficient is marked by*).

Stations	Periods			
	10-25 days		30-60 days	
	SST	WS	SST	WS
Bai Chay	-0.58*	-0.09	0.31*	-0.33*
Hon Dau	-0.42*	-0.14*	-0.57*	-0.25*
Hon Ngu	-0.48*	-0.17*	0.19*	-0.66*
Con Co	-0.3*	0.18*	-0.47*	0.56*
Son Tra	-0.33*	-0.01	0.41*	-0.47*
Quy Nhon	-0.52*	-0.14*	0.28*	-0.18*
Phu Quy	0.3*	-0.01	0.64*	-0.22*
Vung Tau	-0.12	-0.6*	0.65*	0.70*
Con Dao	0.04	0.27*	0.68*	0.03
Phu Quoc	0.49*	0.21*	0.65*	-0.08

The average of the absolute value of the correlation coefficient between IAV of SST Niño 3.4 and IAV of ISV was calculated and presented in Table 4.

Table 4. The average of the absolute value of the correlation coefficient between IAV of SST Niño 3.4 and IAV of ISV.

IAV of ISO/ stations	Northern stations	Central stations	Southern stations
SST-QBWO	0.49	0.36	0.21
WS-QBWO	0.13	0.08	0.36
SST-MJO	0.35	0.45	0.66
WS-MJO	0.41	0.35	0.27

From Table 4, we could see that the effects of ENSO on SST-QBWO decrease from north to south, while the effects of ENSO on WS-QBWO at southern stations are higher than northern stations. In contrast, the effects of ENSO on SST-MJO increase from north to south, and the effects of ENSO on WS-MJO decrease from north to south, and this may be due to the influence of terrain and shoreline shape. In the following section, we assess the different levels of effect of ENSO to ISO from SST and wind speed in the El Niño and La Nina phases.

Effects of ENSO to ISV of SST and wind speed in El Niño and La Nina:

In order to research the changes of ISV on El Niño and La Nina conditions, multi-year monthly means of ISV over all stations were calculated over a full time period of 1993-2015 and for the El Niño and La Nina years. The result showed that SST-QBWO had phase transitions in mid-October when winter monsoons prevailed in the South China Sea. In the La Nina condition, SST-QBWO obtained positive values for the winter, with a peak in December; and negative values in the spring and fall, with a peak in July and an increasing trend held until the end of October (phase two). Under El Niño conditions, SST-QBWO changed the opposite with low

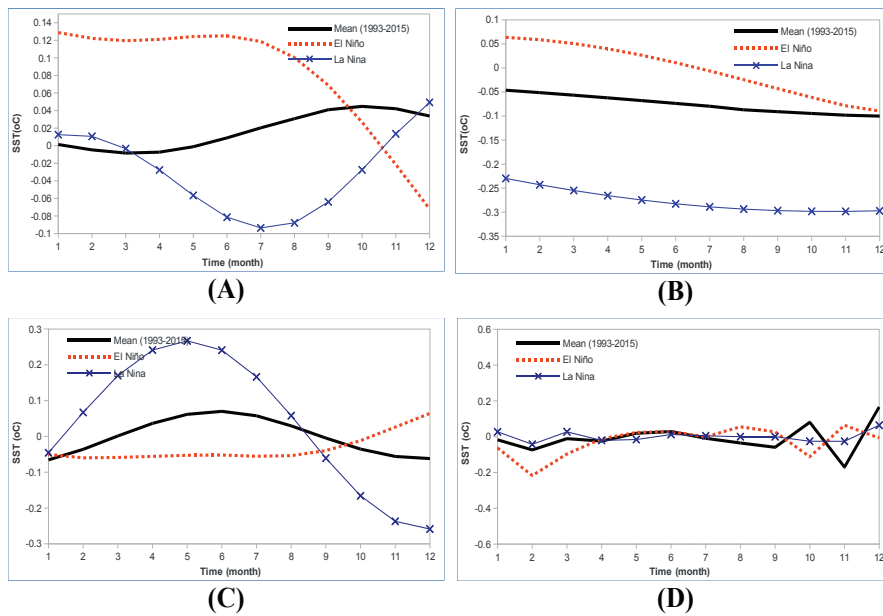


Fig. 2. Fluctuation of multi year monthly mean of ISV across all stations in a full time period 1993-2015 and El Niño, La Nina years. (A) SST QBWO, (B) SST MJO, (C) WS QBWO, (D) WS MJO.

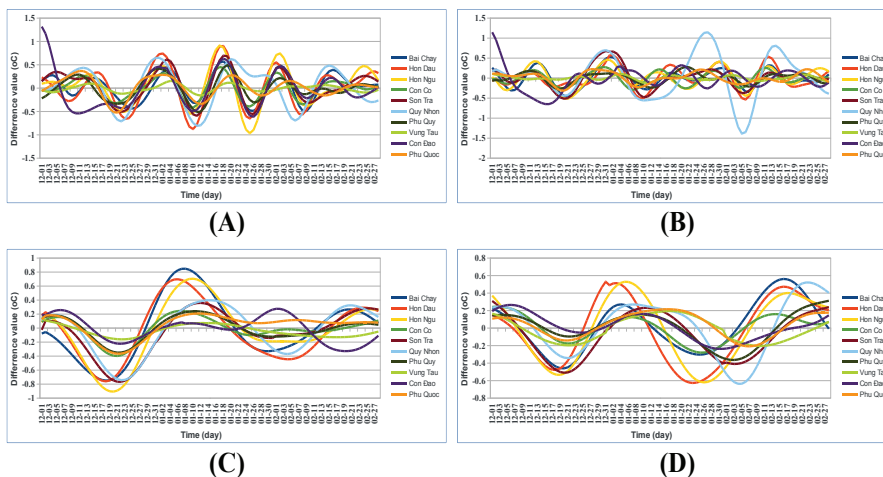


Fig. 3. Fluctuation difference value of SST ISO between ENSO winter and neutral winter at each stations. (A) SST QBWO between El Niño and neutral winter years, (B) SST QBWO between La Niña and neutral winter years, (C) SST MJO between El Niño and neutral winter years, (D) SST MJO between La Niña and neutral winter years.

peaks in December and was enhanced from January to September (Fig. 2A). WS-QBWO had phase transitions in February and September, when winter and summer monsoons began reducing. In La Niña condition, WS-QBWO obtained positive values in spring and summer with the high peak in May, and the obtained negative values in fall and winter at a low peak in December. In El

Niño condition, WS-QBWO changed opposite with negative values from January to October. The amplitude of WS-QBWO in the winter less than in summer and in El Niño condition less than La Niña condition (Fig. 2C).

In Neutral and La Niña conditions, SST-MJO obtained negative values across a full year. In all conditions, SST-MJO has a decreasing trend throughout

the year. The SST-MJO value for La Niña was strong, and more steadily decreasing than El Niño and Neutral conditions (Fig. 2B). ENSO has not significant effect to WS-MJO from January to the end June when WS-MJO-less change. WS-MJO only changes from July to December. The amplitude of WS-MJO in El Niño condition is less than La Niña condition (Fig. 2D).

Thus, ENSO's effect on SST-ISV was more significant than WS-ISV. There is the opposite phase of the effect of ENSO to SST-QBWO and WS-QBWO during El Niño and La Niña conditions.

The effect of ENSO to ISV from SST and wind speed in ENSO winter years:

Calculation was conducted to find the difference of ISV values between ENSO winter and neutral winter months at each station. Fluctuation of this difference showed that, there were four QBWO and two MJO occurrences in the three months of winter (Fig. 3). The next step was to calculate the standard deviation of the above differences. This standard deviation values reflect the amplitude of ISV during ENSO winter years. The standard deviation of the difference of SST-QBWO obtained high values at Hon Dau, Con Dao, Quy Nhon, and the lowest at Vung Tau (Fig. 4A). The standard deviation of the differences of SST-MJO decrease from northern stations to southern stations. Almost all stations had fluctuations of SST-MJO in La Niña winter months greater than in El Niño winter months (Fig. 4B). The standard deviation values of WS-QBWO at Son Tra, Quy Nhon, and Vung Tau stations were lower than the remain stations. Specially, Phu Quy station had the highest value (Fig. 4C). The standard deviation value of WS-MJO was highest at Phu Quy too (Fig. 4D). This due to Phu Quy Island is located in the sea area with strong winds stress compared to other stations.

Conclusions

ENSO's effects are significant to the

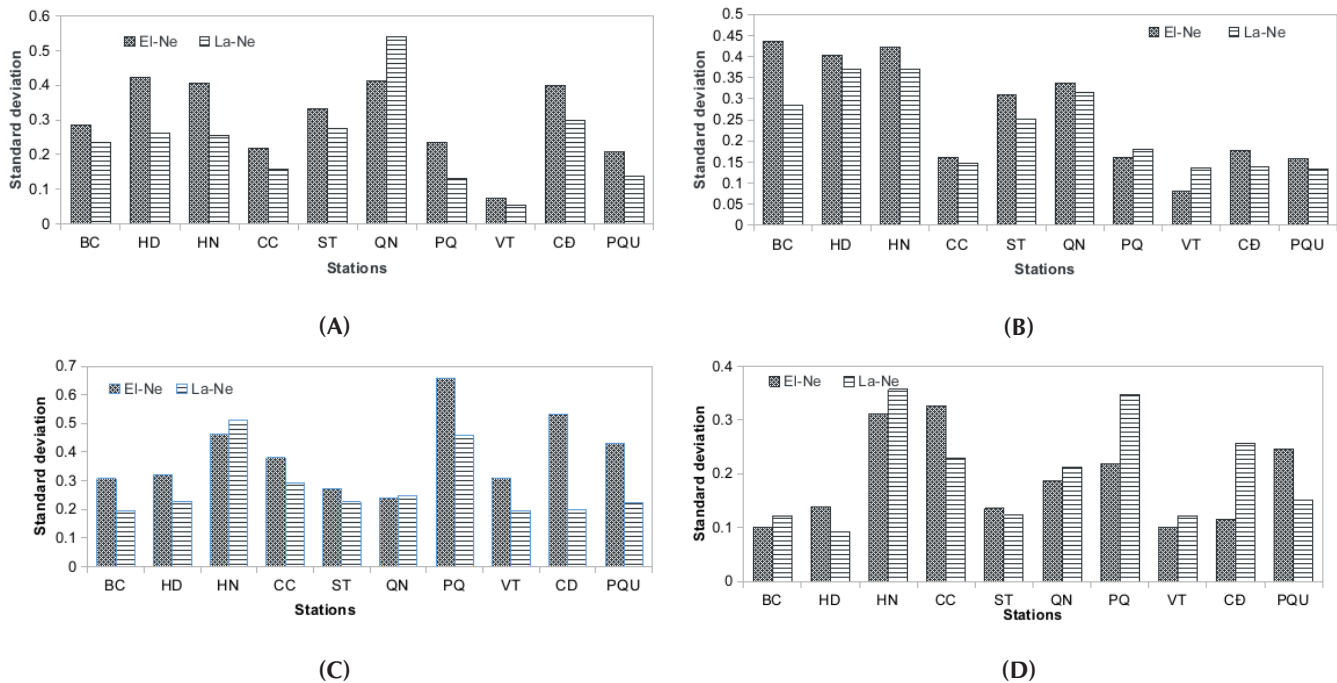


Fig. 4. The standard deviation of difference between SST ISV, WS ISV in ENSO winter and neutral winter at each stations. (A) SST QBWO in El Niño winter year, (B) SST MJO in La Nina winter year, (C) WS QBWO in El Niño winter year, (D) WS MJO; El-Ne is difference between El Niño and neutral year; La-Ne is difference between La Nina and neutral year.

intra-seasonal oscillation of SST and wind speed at the coastal stations in both the QBWO and the MJO. The effect of ENSO on SST-MJO tends to increase from north to south, while the effect of ENSO on WS-MJO tends to decrease from north to south. The effect of ENSO on SST-QBWO decreases from north to south, and the effect of ENSO on WS-QBWO at the southern stations are higher than that of the northern stations. ENSO effects are significant to SST-ISO than WS-ISO. There are the opposite phases of the effect of ENSO on SST-QBWO and WS-QBWO during El Niño and La Nina conditions. There are four QBWO and two MJO occurrences in the three months of winter every year.

REFERENCES

- [1] Johnny C.L. Chan, W. Ai, J. Xu (2002), "Mechanisms responsible for the maintenance of the 1998 South China Sea summer monsoon", *Journal of the Meteorological Society of Japan*, **80**(5), pp.1103-1113.
- [2] Tsing-Chang Chen, Jau-Ming Chen (1993), "The 10-20-day mode of the 1979 Indian monsoon: Its relation with the time variation of monsoon rainfall", *Mon. Wea. Rev.*, **121**, pp.2465-2482.
- [3] T-C. Chen, J-M. Chen (1995), "An observational study of the South China Sea monsoon during the 1979 summer: onset and life cycle", *Mon. Wea. Rev.*, **123**, pp.2295-2318.
- [4] T-C. Chen, M-C. Yen, S-P. Weng (2000), "Interaction between the summer monsoon in East Asia and the South China Sea: Intra-seasonal monsoon modes", *J. Atmos. Sci.*, **57**, pp.1373-1392.
- [5] K-M. Lau, G-J. Yang, S-H. Shen (1988), "Seasonal and intraseasonal climatology of summer monsoon rainfall over East Asia", *Mon. Wea. Rev.*, **116**, pp.18-37.
- [6] W.S. Kessler, M.J. McPhaden (1995), "Oceanic equatorial waves and the 1991-1993 El Niño", *Journal of Climate*, **8**(7), pp.1757-1774.
- [7] J.M. Slingo, D.P. Rowell, K.R. Sperber, F. Nortley (1999), "On the predictability of the interannual behavior of the Madden-Julian oscillation and its relationship with El Niño", *Quarterly Journal of the Royal Meteorological Society*, **125**, pp.583-609.
- [8] H.H. Hendon, C. Zhang, J.D. Glick (1999), "Interannual variation of the Madden-Julian oscillation during austral summer", *Journal of Climate*, **12**, pp.2538-2550.
- [9] D.E. Waliser, K-M. Lau, J.H. Kim (1999), "The influence of coupled sea surface temperature on the Madden-Julian Oscillation: A model perturbation experiment", *Journal of the Atmospheric Sciences*, **56**, pp.333-358.
- [10] A. Fink, P. Speth (1997), "Some potential forcing mechanisms of the year-to-year variability of the tropical convection and its intraseasonal (25-70-day) variability", *International Journal of Climatology*, **17**(4), pp.1513-1534.
- [11] D.S. Gutzler (1991), "Interannual fluctuations of intraseasonal variance of near-equatorial zonal winds", *Journal of Geophysical Research*, **96**, pp.3173-3185.
- [12] Wen Zhou, Johnny C.L. Chan (2005), "Intraseasonal Oscillations and the South China Sea summer monsoon onset", *Int. J. Climatol.*, **25**, pp.1585-1609.
- [13] N.E. Huang, Z. Shen, S.R. Long, M.C. Wu, H.H. Shih, Q. Zheng, N-C. Yen, C.C. Tung, H.H. Liu (1998), "The empirical mode decomposition and the Hilbert spectrum for nonlinear and nonstationary time series analysis", *Proc. R. Soc. London, Ser. A*, **454**, 903-993.
- [14] Z.H. Wu, N.E. Huang (2009), "Ensemble empirical mode decomposition: A noise-assisted data analysis method", *Adv. Adapt. Data. Anal.*, **1**(1), pp.1-41.
- [15] <http://ggweather.com/enso/oni.htm>.

Development of a methodological framework for calculation of carbon footprint of rice production in Vietnam

Minh Trang Dao*, Thi Lan Huong Huynh

Vietnam Institute of Meteorology, Hydrology and Climate Change

Received 5 May 2017; accepted 1 August 2017

Abstract:

Currently, there are various standards and guidelines to calculate product carbon footprints in the world such as the Greenhouse Gas (GHG) Protocol of the World Resources Institute/World Business Council for Sustainable Development (WRI/WBCSD), ISO 14067, and PAS 2050. Most of the studies on carbon footprints of rice production adopt the ISO Life Cycle Assessment (LCA) method while very few studies apply PAS 2050 and the Greenhouse Gas Protocol Agricultural Guidance of WRI/WBCSD. However, the above standards and guidelines do not provide a separate methodology for calculating carbon footprints of rice production. From that perspective, this research paper has developed a methodology to calculate the carbon footprints of rice production from the upstream processes, rice production process to post-farm stage. However, several sources of GHG emissions during the life cycle of rice have not been included in this methodological framework due to either the lack of data or complicated calculation methods.

Keywords: *product life cycle, rice carbon footprints.*

Classification number: 6.2

Introduction

The term “carbon footprint” is derived as an integral part of the “ecological footprint”¹, whereby “carbon footprint” is understood as the land area that absorbs the amount of CO₂ emitted by the humans during their lifetime. However, as climate change has gradually become a global challenge, the concept of “carbon footprint” has developed independently and in a different form from its origin [1] and defined as “the quantity of GHGs expressed in terms of CO₂-equivalent (CO₂e), emitted into the atmosphere by an individual, organization, process, product, or event from within a specified boundary” [2]. In addition, ISO 14040 defines that carbon footprint is the total amount of CO₂ and other GHGs (e.g., methane, nitrous oxide, etc.) emitted during the life cycle of the product.

The scope of the carbon footprint depends on the range of activities to be taken into account, including Tier 1 (on-site emissions), Tier 2 (emissions embodied in purchased energy), and Tier 3 (all other indirect emissions not covered under Tier 2) [3-5]. The choice of direct and indirect emissions is also incompatible with the different studies. In most cases, the inclusion of all indirect emissions is very complex; therefore, many studies on carbon footprint calculate only direct emissions or indirect emissions in Tier 2 [4, 6, 7]. However, indirect emissions can account for most of the carbon footprints of many activities.

Carbon footprint calculations can be carried out based on a product-based approach or an activity-based approach, i.e. GHG emissions from activities of individuals, groups or organizations. The carbon footprints of activities are the annual GHG emission inventories of individuals, groups, organizations, companies, and governments. One of the guidelines for calculating the carbon footprints of activities is IPCC Guidelines for National Greenhouse Gas Inventories [8]. The product carbon footprint (PCF) refers to the life cycle assessment of the whole/part of the product or service

¹Ecological footprint refers to the biologically productive land and sea area required to sustain a given human population expressed as global hectares.

*Corresponding author: Email: daominhtrang@gmail.com.

life cycle. Since 2009, government agencies and international organizations have made significant strides in developing standards and guidelines for calculating PCF [9]. At present, three PCF calculation guidelines are universally accepted, including PAS 2050 of the British Standards Institute (BSI), the GHG Protocol of the WRI/WBCSD, and ISO 14067. All the three standards are based on the LCA method specified in ISO 14040 and ISO 14044.

Methodological framework for calculating carbon footprint

The methodology of this study is based on the reference to the GHG Protocol Agricultural Guidance of WRI/WBCSD, the IPCC Guidelines for National Greenhouse Gas Inventories in 2006 (GL 2006), the Good Practice Guidance for Land Use, Land Use Change and Forestry (GPG LULUCF 2003), the Good Practice Guidance and Uncertainty Management in National GHG Inventories (GPG 2000), and other relevant studies. The calculation process of carbon footprints of rice production consists of five steps:

Step 1: Select the GHGs under the regulation of the Kyoto Protocol.

Step 2: Determine the scope of calculation: GHG emissions from upstream processes (production of electricity, fertilizer, lime and pesticides); rice production (rice cultivation, land use change, operation of agricultural machinery, groundwater extraction, fertilizer and lime use), and post-production of rice (straw burning on the farms).

Step 3: Collect activity data.

The activity data can usually be obtained from existing data such as bills, electricity meters, production records, and land registration records, etc. In general, data on energy purchase and production can commonly be collected with high quality. On the contrary, it is difficult to collect reliable data on land management and land use change [3].

Step 4: Calculate carbon footprint.

a) Calculate GHG emissions/removals

Specific calculation formulas will be presented in more detail later in the section “Calculation of GHG emissions and removals in the life cycle of rice”.

b) Calculate carbon footprint

Global warming potential (GWP) of all tiers is calculated individually using the conversion factor of IPCC (2007). The formula for calculating GWP of tier_i (i = 1, 2 or 3) is as follows:

$GWP(tier_i) = \text{emission/removal of } CH_4 \times 25 + \text{emission/removal of } N_2O \times 298 + \text{emission/removal of } CO_2$

where:

GWP is in kg CO₂e/ha.

The carbon footprint is calculated by summing the GWP of all tiers and its unit can be presented as spatial or yield-scaled

carbon footprints, which are calculated as follows:

$$CF_s = \sum_{i=1}^3 [GWP(tier_i)]$$

$$CF_y = \frac{CF_s}{\text{Grain yield}}$$

where:

CF_s: Spatial carbon footprint (kg CO₂e/ha)

CF_y: Yield-scaled carbon footprint (kg CO₂e/yield).

This study will use carbon footprint by yield, i.e. kgCO₂e/kg rice.

Step 5: Analysis of uncertainty (optional).

Two reasons for the uncertainty of the calculation results are the uncertainty of the model and of the data. The results of GHG emission calculation cannot avoid the uncertainty.

Calculation of GHG emissions and removals in the life cycle of rice

GHG emissions from the production of inputs for rice cultivation

CO₂ emissions from electricity generation for rice cultivation:

Emissions from the burning of fossil fuels such as diesel and natural gas during the operation of agricultural machinery are direct emissions. Meanwhile, emissions from the generation of electricity used in the operation of agricultural machinery are indirect due to the burning of fossil fuels during electricity production. GHG emissions from electricity generation for rice cultivation are calculated according to the formula given below:

$$\text{GHG emissions} = \text{electricity consumption} * EF_{\text{grid}} \quad (1)$$

where:

GHG emissions = GHG emissions from electricity generation (tCO₂e)

Electricity consumption = Amount of consumed electricity for the operation of agricultural machinery (MWh)

EF_{grid} = Emission Factor = 0.6612 tCO₂/MWh (According to Decision No. 605/KTTVBKD-KH-GSPT of the Department of Climate Change dated 19 May 2016 on emission factor (EF) of Viet Nam's electrical grid, 2014).

GHG emissions from the production of fertilizers and lime:

GHG emissions from fertilizer production depend on different production technologies and energy sources [10, 11]. This analysis includes emissions from three main nutrients (N, P, K) and agricultural lime (CaCO₃). CO₂ emissions from the production of the above substances are attributable to the use of energy during production and transportation. In

order to calculate indirect emissions from the production and transportation of fertilizers and lime, the mean emission factor is derived from [12] and multiplied by the amount of fertilizer application rate using the following formula:

$$\text{Emissions} = \text{application rate} * EF_{\text{fertilizer/lime}} \quad (2)$$

where:

Application rate = amount of fertilizer/lime application rate per hectare (kg/ha)

$EF_{\text{fertilizer/lime}}$ = emission factor for the production of fertilizer and lime (kg CO₂e/kg fertilizer/lime). Kool, et al. (2012) has provided $EF_{\text{fertilizer/lime}}$ for N, P, K and lime for global, Western Europe, Russia and Central Europe, North America, China, India and the other countries.

GHG emissions from the production of pesticides:

Energy consumption in pesticide production depends on the composition and the production process employed. The emission factor of 0.069 kg CO₂e/MJ from [13, 14] can be used to calculate emissions from pesticide production. If all electricity used to produce pesticides is generated from nuclear or hydropower, which emit less carbon, the above factor will be 0.049. Where the data on the application rate of pesticide are available, the CO₂e emissions are calculated using the following formula:

$$\text{Emissions} = \text{Input energy} * \text{Application rate} * EF_{\text{pesticides}} \quad (3)$$

where:

Input energy = energy used to produce 1 kg of pesticide (MJ/kg)

Application rate = the application rate of common pesticides (kg/ha)

$EF_{\text{pesticides}}$ = emission factor of energy for the production of pesticides (kgCO₂e/MJ).

Greenhouse gas emissions from rice cultivation

Methane emissions from rice cultivation:

Based on IPCC (2006), CH₄ emissions are calculated using formula (4), where CH₄ emissions are estimated by multiplying daily emission factors by means of rice cultivation period and annual harvest area.

$$CH_{4 \text{ rice}} = \sum_{i,j,k} (EF_{i,j,k} * t_{i,j,k} * A_{i,j,k} * 10^{-6}) \quad (4)$$

where:

$CH_{4 \text{ rice}}$ = Annual methane emissions from rice cultivation (Gg CH₄ per year)

EF_{ijk} = Daily emission factor under i, j, and k conditions (kg CH₄/m²/day)

t_{ijk} = Cultivation period of rice under i, j, and k conditions (days)

A_{ijk} = Annual harvested area under i, j, and k conditions

(ha/year)

i, j, and k = different ecosystems, water regimes, type and amount of organic amendments, and other conditions under which CH₄ emissions from rice may vary.

Emissions from different regions are adjusted by multiplying a baseline default emission factor. According to GPG 2000, the daily emission factor can be calculated using the following formula:

$$EF_i = EF_c * SF_w * SF_{pj} * SF_o * SF_{s,r} \quad (5)$$

where:

EF_i = Adjusted daily emission factor for a particular harvested area

EF_c = Baseline emission factor for continuously flooded fields without organic amendments

SF_w = Scaling factor to account for the differences in water regime during the cultivation period (continuously flooded = 1, error range = 0.79-1.26)

SF_{pj} = Scaling factor to account for the differences in water regime in the pre-season before the cultivation period (less than 30 days = 1.9, error range = 1.65 and 2.18 source)

SF_o = Scaling factor that accounts for differences in both type and amount of organic amendment applied

$SF_{s,r}$ = Scaling factor for soil type, rice cultivar, etc.

Emissions increase as the amount of organic material increases. Formula (6) and the default conversion factor for farm yard manure present an approach to vary the scaling factor according to the amount of manure used on the farm (IPCC, 2007) [15].

$$SF_o = (1 + \sum_i ROA_i * CFOA_i)^{0.59} \quad (6)$$

where:

SF_o = Scaling factor for both type and amount of organic amendment applied

ROA_i = Rate of application of organic amendment *i*, in dry weight of straw and fresh weight for others (tonnes/ha)

$CFOA_i$ = Conversion factor for organic amendment *i*. According to IPCC (2006) [16], the default conversion factor for farmyard manure is 0.14 with an error range of 0.07-0.2.

Carbon stock change in the living biomass due to land use change:

GPG LULUCF classifies the national land into six categories, i.e. Forest Land, Cropland, Grassland, Wetlands, Settlements, and Other land and subdivides each of them into two subcategories on the basis of whether or not land conversion has been occurred. The GHG emissions and removals in LULUCF include the carbon stock changes in living biomass (aboveground/belowground), litter, and soil. According to the

assumption of GPG LULUCF 2003, the carbon stock in the biomass of all land uses is zero after conversion. Formula (7) is used to calculate the biomass stock change associated with land use change, except for the conversion from Forest Land to Cropland:

$$\Delta C = A (\text{conversion}) * [(C_{\text{Before}} - C_{\text{After}}) + \Delta C_{\text{Growth}}] \quad (7)$$

where:

ΔC : Annual change in carbon stocks in living biomass in land converted from “before” to “after” (tonnes C/yr)

$A_{\text{Conversion}}$: Annual area of land converted from “before” to “after” (ha/yr)

C_{After} : Carbon stocks in biomass immediately after conversion (tonnes C/ha)

C_{Before} : Carbon stocks in biomass immediately before conversion (tonnes C/ha)

ΔC_{Growth} : Changes in carbon stocks from one year growth of land “after” (tonnes C/ha).

For the conversion from Forestland to Cropland, the decrease in carbon in living biomass will be calculated according to the following formula:

$$C_{\text{loss}} = L_{\text{wood-removals}} + L_{\text{fuelwood}} + L_{\text{other losses}} \quad (8)$$

$$L_{\text{wood-removals}} = H * BCEF_r * (1 + R) * CF \quad [8a]$$

$$L_{\text{fuelwood}} = FG * D * CF \quad [8b]$$

$$L_{\text{other losses}} = A_{\text{disturbance}} * B_w * (1 - f_{BL}) * CF \quad [8c]$$

where:

C_{Loss} : Annual decrease in carbon stocks due to biomass loss, tonnes C/yr

CF: Carbon fraction of dry matter (tonnes C/tonne d.m)

R: Ratio of below ground biomass to above ground biomass (root-to-shoot ratio), dimensionless

$BCEF_i (= D * BEF_i)$: Biomass conversion and expansion factor for expansion of annual net increment in volume (including bark) to aboveground biomass increment (tonnes d.m/m³), equivalent to basic wood density multiplied by biomass expansion factor

$L_{\text{wood-removals}}$: Annual carbon loss due to biomass removals (tonnes C/yr)

L_{fuelwood} : Annual carbon loss due to fuelwood gathering (tonnes C/yr)

$L_{\text{other losses}}$: Annual other losses of carbon (tonnes C/yr)

H: Annual wood removals, roundwood (m³/yr)

FG: Annual volume of fuelwood gathering (m³/yr)

$BCEF_r (= D * BEF_r)$: Biomass conversion and expansion factor for conversion of removals in merchantable volume to biomass removals (including bark) (tonnes d.m/m³), equivalent

to basic wood density multiplied by biomass expansion factor

D: Wood density (tonnes d.m/m³)

$A_{\text{disturbance}}$: Areas affected by disturbances (ha)

B_w : Average annual above-ground biomass of land areas affected by disturbance (tonnes d.m/ha/yr)

f_{BL} : Fraction of biomass lost in disturbance.

Formula (9) is used to calculate the emissions from biomass burning:

$$L_{\text{fire}} = A * B * C * D * 10^{-6} \quad (9)$$

where:

L_{fire} : Quantity of GHG released due to fire (tonnes of GHG)

A: Area burned (ha)

B: Mass of “available” fuel (kg d.m/ha)

C: Combustion efficiency (or fraction of the biomass combusted), dimensionless

D: Emission factor (g/kg d.m).

Greenhouse gas emissions from on-farm machinery use for field operation:

In farming, three types of fuel are commonly used, including diesel, natural gas and electricity. Diesel is used for rice production and machine operation in the field. Natural gas and electricity are used more often for farm operations such as underground water intake, machine maintenance, and drying. According to IPCC (2006), GHG emissions from diesel combustion for the operation of agricultural machines are calculated based on the following formula:

$$\text{GHG emissions} = \text{amount of used fuel} * EF_{\text{fuel}} \quad (10)$$

According to Table 2.5, p.2.2 of GL 2006, the default emission factor for stationary emissions of diesel in agriculture is 74528.8 kg CO₂/TJ.

Greenhouse gas emissions from the extraction of groundwater for irrigation:

GHG emissions from irrigation are calculated based on the energy required for extraction (pumping) and water application. Irrigation is the primary consumer of energy on farms especially when pumping is required. Therefore, any changes in irrigation methods can lead to a change in on-farm energy consumption. The direct energy inputs are mainly used for the operation of agricultural machinery and pumps, while indirect energy inputs refer to energy that is used to produce equipment and other products and services used on-farm. When groundwater is used, a lot of energy is required for pumping water.

CO₂ emissions from irrigation are calculated based on the energy needed for extraction and application of water. The calculation of CO₂ emissions from water absorption is based

on the assumption that the energy required to extract water from a surface source is negligible and only the amount of energy to extract groundwater is calculated. In addition, the study assumes that water source is in close proximity to the field and the water is conveyed to the farm by gravity.

The energy used for water extraction is the energy required to lift 1 m³ of water (1000 kg m³) up to 1 m at 100% efficiency of 0.0027 kWh [17]. GHG emissions are calculated by multiplying energy consumption by emission factor.

$$\text{Energy (kWh)} = \frac{9.8ms^{-2} * \text{Lift (m)} * \text{Mass (kg)}}{3.6 * 10^6 * \text{Efficiency (\%)}} \quad (11)$$

where:

Energy = Energy used to extract water from shallow and deep wells

Lift = Average depth value (m)

Efficiency = Efficiency ranges from 11-30% for electric pumps and 40-67% for diesel engines

Mass = Amount of groundwater used for irrigation (m³/year).

Then the CO₂ emissions from the use of diesel pumps will be calculated by taking the amount of energy consumed and the emission factor of the diesel engine. According to Table 2.5, p.2.2 of IPCC (2006), the default emission factor for stationary emissions of diesel burning in agriculture is 74528.8 kg CO₂/TJ.

For electric pumps, CO₂ emissions are calculated by multiplying the amount of energy consumed by the emission factor of Vietnam's electrical grid in 2014 (0.6612 tCO₂/MWh).

Greenhouse gas emissions from fertilizer application:

GHG emissions from the application of N, P and K fertilizers are calculated by multiplying the amount of applied fertilizer by the emission factor of fertilizer application by type derived from (12).

$$\text{Emissions} = \text{Application rate} * \text{EF}_{\text{fertilizer application}} \quad (12)$$

where:

Emissions = Emission level (CO₂e)

Application rate = Amount of applied fertilizer (kg)

EF = Emission factor of fertilizer application (CO₂e/kg fertilizer).

Greenhouse gas emissions from lime application to soil:

Lime is commonly used to manage soil and grasslands to reduce soil acidity. Lime is commonly applied as crushed limestone (CaCO₃) or crushed dolomite (CaMg(CO₃)₂). Adding lime to soil leads to CO₂ emissions as the carbonate limes dissolve and release bicarbonate (2HCO₃), which will decompose into CO₂ and water. The CO₂ emissions from the dissolution of carbonate rock do not include the emissions from fossil fuel used to crush, transport, and spread the crushed

rock on the field. The direct emissions of lime application to soil is calculated by multiplying the amount of lime application (kg) by the emission factor of crushed limestone or dolomite. According to GPG LULUCF (2003), the carbon emission factor of the crushed limestone is 0.12 (tC/ton) and that of crushed dolomite is 0.122 (tC/ton). Carbon emissions are converted to CO₂ emissions by using the following formula: CO₂e=44/12*C.

GHG emissions from on-farm straw burning:

Straw is the main by-product of rice production. In recent years, on-farm straw burning has been increasing and negatively affecting the environment, human health, and contributing to global climate change. This study assumes that GHG emissions in post-production of rice are mainly from the burning of straw on farm. The calculation of GHG emissions from straw burning is based on the methodology of similar studies such as Nam, et al. [18], which includes the following steps:

Step 1: Determine the straw-to-grain ratio

Straw-to-grain ratio is calculated according to the following formula:

$$R = \frac{W_r}{W_h} \quad (13)$$

where:

R: Straw-to-grain ratio

W_r: Dry weight of straw (kg)

W_h: Weight of rice (kg).

According to Le, et al. [19], the rate of on-farm straw burning in Thai Binh province is respectively 51% and 78.5% during the winter-spring and autumn-winter season. This is because in the winter-spring season, farmers often cut the tops of the rice, and due to high temperature most of the straw is plowed into the soil, thus significantly reducing the burning rate. In the autumn-winter season, farmers often cut the rice from the roots, then dry or burn, and hence the rate of straw burning is higher.

Step 2: Calculate the amount of straw generated after harvest

The amount of straw generated per crop is calculated by the following formula:

$$\text{Amount of straw generated} = \text{Rice yield} * \text{Straw/grain ratio} \quad (14)$$

Step 3: Estimate the quantity of burned straw on farm

The quantity of burned straw on the farm is calculated according to the following formula:

$$Q_{st} = Q_p * R * k \quad (15)$$

where:

Q_{st} : Quantity of burned straws on farm (tonnes)

Q_p : Quantity of rice yield (tonnes)

R : Straw-to-grain ratio

k : Ratio of straw burned on farm to total straw quantity.

Step 4: Calculate GHG emissions from burned straw

GHG emissions from straw burning are calculated by the following formula:

$$E_i = Q_{st} \times EF_i \times F_{co} \quad (16)$$

where:

E_i : Emissions of i into the environment due to burning straw on farm (tonnes)

EF_i : Emission factor of i emissions from on-farm straw burning (g/kg) (based on Gadde, et al. (2009) with $E_{CO_2} = 1464$; $E_{CO} = 34.7$; $E_{NO_x} = 3.1$)

F_{co} : Rate of conversion to gas when burning straw. $F_{co} = 0.8$ [20].

Conclusions

In conclusion, PAS 2050, the GHG Protocol of WRI/WBCSD, and ISO 14067 are commonly accepted standards and guidelines for calculating carbon footprints which are based on the process approach and LCA as regulated in ISO 14040/44. Most of the studies in the world have used the LCA method to calculate carbon footprints during the rice life cycle. Several studies have used both LCA method of ISO and GHG inventory guidelines. Very few studies used PAS 2050, the GHG Protocol Agricultural Guidance of WRI/WBCSD and ISO 14067. The purpose of the LCA is to assess the environmental impact of the entire life cycle of products/services; therefore, future studies should use standards, guidelines for calculating product carbon footprint. In addition, the above-mentioned guidelines for PCF calculation have yet to develop a separate methodology for calculating rice carbon footprints. Therefore, this study has developed a methodological framework for calculating rice carbon footprints, from upstream processes, rice production to post-farm stage. However, there remain sources of GHG emission in the life cycle of rice that have not been included in this methodological framework due to either the lack of input data or complicated calculation methods. They are GHG emissions from seed production and transportation of materials to the field, carbon stock changes in litter and soil due to land use changes, GHG emissions during rice distribution and consumption, HFC and PFC emissions from air conditioners and refrigerators, and other emissions apart from burning straw during the disposal process. These issues need to be further researched to refine the methodology in the future.

REFERENCES

- [1] A.J. East (2008), "What is a carbon footprint? An overview of definitions and methodologies", *Vegetable industry carbon footprint scoping study - discussion papers and workshop*, Horticulture Australia Limited, Sydney.
- [2] D. Pandey, M. Agrawal, J.S. Pandey (2011), "Carbon footprints: Current

methods of estimation", *Environmental Monitoring and Assessment*, **178**, pp.135-160.

[3] WRI/WBCSD (2013), *Greenhouse gas protocol agricultural guidance: Interpreting the corporate accounting and Reporting standard for the agricultural sector*, Geneva.

[4] Carbon Trust (2007), *Carbon footprint measurement methodology*, Version 1.1, The Carbon Trust, London, UK.

[5] BSI (2008), *PAS 2050:2008: Specification for the assessment of the life cycle greenhouse gas emissions of goods and services*, United Kingdom.

[6] T. Wiedmann, J.A. Minx (2007), *Definition of carbon footprint*, ISA-Research and Consulting, Durham.

[7] H.C. Matthews, C.T. Hendrickson, C.L. Weber (2008), "The importance of carbon footprint estimation boundaries", *Environmental Science and Technology*, **42**(16), pp.5839-5842.

[8] M.J. Franchetti and D. Apul (2013), *Carbon footprint analysis: Concepts, methods, implementation, and case studies*, Taylor & Francis Group, Boca Raton. London - NewYork.

[9] Cong Khanh Doan, Thi Thanh Huyen Truong, Huy Hoan Tran, Thi Kim Tuyen Vo, Van Thang Tran, Hong Thom Nguyen, Trung Thanh Ho, Ngoc Thinh Tran and Huu Lam Son Nguyen (2014), *Assessment of the current status and development trends of the market for low-carbon commodities in Vietnam and on the world and propose solutions to promote*, Summary report 04.14/CC, Ministry of Industry and Trade.

[10] F. Cherubini (2010), "GHG balances of bioenergy systems - Overview of key steps in the production chain and methodological concerns", *Renewable Energy*, **35**, pp.1565-1573.

[11] S. Wood and A. Cowie (2004), *A review of greenhouse gas emission factors for fertiliser production*, Research and Development Division, State Forest of New South Wales.

[12] A. Kool, M. Marinussen, and H. Blonk (2012), *LCI data for the calculation tool Feedprint for greenhouse gas emissions of feed production and utilization: GHG emissions of N, P, K fertilizer production*, Blonk Consultants, Netherlands.

[13] E. Audsley, K. Stacey, D.J. Parsons, A.G. Williams (2009), *Estimation of the greenhouse gas emissions from agricultural pesticide manufacture and use*, Cranfield University.

[14] A.G. Williams, E. Audsley, D.L. Sandars (2006), *Final report to Defra on project ISO 205*.

[15] IPCC (2003), *Good Practice Guidance for Land Use, Land-Use Change and Forestry*, IGES, Japan.

[16] IPCC (2006), *IPCC Guidelines for National Greenhouse Gas Inventories*, IGES, Japan.

[17] S. Rothausen and D. Conway (2011), "Greenhouse gas emissions from energy use in the water sector", *Nature Climate Change*, **1**, pp.210-219.

[18] Sy Nam Tran, Thi Huynh Nhu Nguyen, Huu Chiem Nguyen, Vo Chau Ngan Nguyen, Hoang Viet Le, Kjeld Ingvorsen (2014), "Estimation of straw yield and treatment measures in some provinces in the Mekong River Delta", *Journal of Science of Can Tho University*, **32**, pp.87-93.

[19] Le Hoang, Thi Thu Hanh Nguyen, Thuy Linh Le (2013), "Estimation of greenhouse gas emissions from straw burning on farms in Thai Binh province", *Journal of Science*, Vietnam National University, Hanoi, **29**(2), pp.26-33.

[20] B. Gadde, S. Bonnet, C. Menke, S. Garivait (2009), "Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines", *Journal of Environmental Pollution*, **157**(5), pp.1554-1558.

Vietnam Journal of Science, Technology and Engineering (VISTECH): Manuscript Template

General Instructions on using this template and submitting a manuscript to VISTECH: Thank you for preparing a manuscript for submission to VISTECH. Using this template, or following the guidelines below, will help us in processing your paper. Our goal is to be able to identify each section of your manuscript so that we can accurately record the title, authors, abstract, etc. and also be able to enrich it by including reference links and an accurate layout.

You can submit your paper at <http://vietnamscience.vn>

So that we can identify the parts of your paper, and even if you do not use our template, please begin each section with the specific key words listed below. Please do not use paragraph breaks in the title, author list, or abstract.

Title

Authors

Affiliations

Abstract

One Sentence Summary

Main Text

Acknowledgements

References

Several of these headings are optional, for example, not all papers will include a one-sentence summary, figures or tables.

Please use the .docx format (all version after Word 2007).

More specific formatting instructions are provided in the actual template, which follows.

Title: How to Format a Science Paper (replace with your real title)

Authors: The author list should be one single paragraph (no breaks). Authors should be listed by first name or initial followed by last name and separated by commas. Use superscript numbers to link affiliations, and symbols *†‡ for author notes. For example, X. Jones^{1*}, P. Smith^{1,2}

Affiliations:

¹Affiliations should be preceded by superscript numbers corresponding to the author list, and each affiliation should end with a period.

²Each affiliation should be a separate paragraph.

Correspondence to: include the email addresses or telephone number of the corresponding author(s). Please use the asterisk () symbol for the corresponding author information.

Abstract: The abstract should be about 100-150 words, and organized in this structure: An opening sentence that sets the question that you address and is comprehensible to the general reader, background content specific to this study, results, and a concluding sentence. It should be one paragraph only.

One Sentence Summary: A brief summary of the main result of your paper, without excessive jargon and less than 150 characters.

Main Text: In general, this should include a brief (1-2 paragraph) introduction, followed by a statement of the specific scope of the study, followed by results and then interpretations. Please avoid statements of future work or claims of priority, and avoid repeating the conclusions at the end.

All of the Figures and Tables should be cited in order (which should be cited as, for example, figure S1, and table S1). You can include page breaks if you would like to place the figures within the

text.

Subheads: These can be included in Research Articles or Reviews and should be brief.

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).

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. 2. You can place graphics above each equation as part of this file.

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.

Acknowledgments: The acknowledgments should include a statement about where the data reported in the paper are presented, archived, or available. If in an archive, include the accession number or a placeholder for it. Please also include relevant funding information such as grant numbers and funding agencies. You can also include a statement of author contributions here. The acknowledgements can be multiple paragraphs.

References:

References should be cited in bracket with a number [1]. Multiple reference citations are separated by commas [2, 3] or if a series, dashes [4-6].

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 paper.

Please include the above heading “References”.

Please do not mix in references with explanatory notes.

The reference consist of: Family name(s) and initials of all authors (year of publication), “Titles of journal articles” (optional), *Title of journal, book or other publication*, **volume(number)**, page numbers.

Example of a reference list:

[1] T. Binzoni, T.S. Leung, D. Boggett, D.T. Delpy (2002), “A new near infrared laser-Doppler flowmeter for deep tissue perfusion monitoring”, *MAGMA*, **14**, pp.74-75.

[2] T. Binzoni, T.S. Leung, D. Boggett, D.T. Delpy (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.

EDITORIAL COUNCIL

STANDING MEMBERS:

Bao Chau Ngo (*University Avenue Chicago, USA*)
 Gia Khanh Pham (*Chairman of Vietnam Organ Transplant, Vietnam*)
 Thuc Tran (*Vietnam Panel on Climate Change, Vietnam*)
 Van Hieu Nguyen (*Vietnam Academy of Science and Technology, Vietnam*)

MEMBERS:

Akos Redey (*University of Pannonia, Hungary*)
 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*)
 Henry Nguyen (*University of Missouri, Columbia, USA*)
 Hung Viet Pham (*University of Science - Vietnam National University, Hanoi, Vietnam*)
 John Nieber (*University of Minnesota, USA*)
 Pierre Darriulat (*European Organization for Nuclear Research; Vietnam Academy of Science and Technology, Vietnam*)
 Stefan Norra (*Institute of Applied Geosciences, Karlsruhe Institute of Technology, Germany*)
 Tran Binh Le (*University of Science and Technology of Hanoi, Vietnam*)
 Tri Nang Tran (*University of Minnesota, USA*)
 Truong Duc Pham (*University of Birmingham, UK*)
 Tu Bao Ho (*Japan Advanced Institute of Science and Technology, Japan*)
 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 in Ho Chi Minh City, Vietnam*)

EDITORIAL OFFICE

113 Tran Duy Hung Str., Cau Giay Dist., Hanoi
 Tel: (84.4) 39436793; Fax: (84.4) 39436794
 Email: khcnvn@most.gov.vn
 Website: vietnamscience.vn

EDITOR-IN-CHIEF

Ngoc Bao Dang

DEPUTY EDITOR

Thi Hai Hang Nguyen
 Thi Huong Giang Nguyen

HEAD OF EDITORIAL BOARD

Thi Minh Nguyet Pham

HEAD OF ADMINISTRATION

Ngoc Quang Hung Luong

ART DIRECTOR

Thi Luan Dinh

PUBLICATION LICENCE

No.1153/GP-BTTTT 26th July 2011

No.2528/GP-BTTTT 26th December 2012

No.592/GP-BTTTT 28th December 2016