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Germination and shoot development of *Pisum sativum* L. under exposure to arsenic, lead, and copper in laboratory conditions

Thien-Trong-Nguyen Le, Thanh-Dat Dinh, Dinh-Dai Nguyen, Thi-My-Chi Vo, Thanh-Son Dao*

Ho Chi Minh city University of Technology

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Abstract:

Metal contamination and pollution are of human and environmental concern Phytoremediation is one of the suitable high-efficiency means to treat metal pollution. This study aims to observe the responses of *Pisum sativum* L. in its early life stage to three metals, arsenic (As), copper (Cu) and lead (Pb) in laboratory conditions. Seeds of *P. sativum* were treated with water containing 0, 50, and 500 µg/l of these metals over a period of 7 days. The results show that the germination of seeds is similar for the control and metal treatments, ranging from 90-100% after 4 days of watering. Shoot development of the seeds exposed to the metals and the control were not significantly different, except that the samples which had undergone the treatment with 500 µg Pb/l had longer shoots. Our results evidence a high capacity for metal tolerance in this plant in the early stages of its life. Therefore, *P. sativum* may be a promising candidate for the phytoremediation of metal contamination and pollution.

Keywords: **metals, phytoremediation, *Pisum sativum* L., tolerance.**

Classification number: **2.3**

Introduction

Heavy metals are wide distributed in many different habitats, such as the soil, atmosphere, and water, and have important functions in biota. The emission of heavy metals into the environment is due to two major causes, human activities and natural geology. Artificial metal emissions are mainly related to combustion, mining, and processing [1]. In addition, other applications such as fertilisers, pesticides, irrigation water, and atmospheric deposition also contribute to heavy metal emissions. Recently, there has recently been abundant proof of environmental pollution caused by trace metals, for example, in the Moon and Shi rivers in Thailand, which were polluted by cadmium (Cd) exceeding the WHO limit [2], and the Gali river in Malaysia, which was heavily polluted by iron (Fe) at a concentration of up to 14,400 µg/l [3]. In addition, heavy metal pollution in northern Vietnam, particularly in Hung Yen province, including Pb and Cd in the soil at concentrations of up to 3,809 µg/g of soil, exceeds the safety standards of Vietnam [4]. Additionally, soil in northern Vietnam is also contaminated with As at high concentrations of up to 31 µg/g [5]. The same authors recorded trace metals, such as As, Cu, Pb, Cd, and zinc (Zn), at high concentrations in the Red river. In southern Vietnam, enrichment by heavy metals, including Cd, chromiun (Cr), Cu, Ni, Pb, and Zn, in Thi Vai river and Can Gio mangroves has been noted [6]. In particular, Cu, Pb, Cr, nickel (Ni), and Zn concentrations were in excess of the Vietnamese safety guideline values. In the Mekong delta region, groundwater was contaminated with high concentrations of As, over 500 µg/l [7], presenting a serious health risk to local people.

In order to cope with such heavy metal contamination, physical and chemical methods of treating heavy metals in water and soil have been considered and applied. On the other hand, one safe and inexpensive method is to use plants as a means of absorbing heavy metals from the environment, a process referred to as phytoremediation.

*Corresponding author: Email: dao.son@hcmut.edu.vn

Phytoremediation removes environmental pollutants by means of a variety of mechanisms. The two most reliable mechanisms are phytoextraction and phytostabilisation [8]. Many plants can tolerate the toxicity of metals and reduce the mobility and bioavailability of metals in the roots and stems. Phytoremediation depends on the structure of the plant genome, as well as on the level of pollution and climatic conditions [9].

Thus far, there have been a number of studies on using plants to treat for heavy metals. While the plant *Psoralea pinnata* can accumulate up to 68% of Cr and 55% of Fe in its mass [10], another one, *Syngonium podophyllum*, was used to remove As from the soil; the treatment efficiency was 2.6 mg/m^2 of soil after 90 days [11]. In addition, the treatment of soil contaminated with 1,400 mg/kg of As with the fern (*Pteris vittata*) reached 18% after 6 months [12].

Green peas, *Pisum sativum*, are a member of the vine family, and can reach up to 2.7 m in length [13]. Green peas are grown around the world, the largest producers of green peans being China, India, Russia, France, and the United States [14]. The plant can thrive in many types of soil; however, the most suitable soil type is fertile and well-drained soil. The green pea plant can tolerate high heat amplitudes, withstand temperature from 12-25^{0}C and develop in soil with a pH of 5.5-7 [13].

Metal pollution is becoming a serious problem in the world in general, and in Vietnam in particular. Studies have been conducted to counter this situation, and phytoremediation has been shown to be an efficient treatment model, showing feasibility with some plants. However, to our knowledge, no studies have been conducted with green peas. Hence, this study was conducted to investigate the germination, growth ability, and potential resistance of *Pisum sativum* in an environment exposed to As, Cu, and Pb.

Materials and methods

The seeds of *Pisum sativum* L. used for the investigation were purchased from Trang Nong Store, located in District 6, Ho Chi Minh city, Vietnam. The experiment was implemented in the Ecotoxicology Module, Laboratory of Environmental Analysis, Ho Chi Minh city University of Technology. The metals As, Pb, and Cd (for ICP/MS, Merck, Germany) used for the test were in stock solution of 1,000 mg/l.

For the experiment, the seeds were exposed to metals (As, Pb, and Cu) at concentrations of 0 (control), 50 µg/l, and 500 µg/l. The metal concentrations in the experiments were selected based on the Vietnamese regulation 39:2011/MONRE - a national technical regulation on the quality of water used for irrigation [15]. For each concentration of exposure, 10 seeds were laid on tissue paper in a plastic container and three replicates (n=3) for each treatment were prepared at the start of the tests. The seeds were watered daily (~ 6 ml) with distilled water only (control) or water containing trace metals at the concentrations mentioned above. The tests lasted for 7 days. During the first four days of the experiment, the germination of the seeds in each exposure was observed and recorded. When the tests terminated, the seedling in each treatment was weighed, and its shoots were measured exactly with a ruler, to 0.1 mm.

The Kruskal-Wallis test, Sigmaplot version 12, was used for evaluating the significant differences on in the fresh weight (FW) and shoot length of the control and metal-exposed seedlings.

Results and discussion

Effects of metals on the germination rate of Pisum sativum

The results demonstrated that the germination rate of *Pisum sativum* in the control sample reached 100% after 4 days of incubation. In addition, the rate of germination of the peas was relatively high in all the exposure samples in the same period of time. Specifically, in the first four days, 97% of the seeds sprouted in the As50 plot and 94% in the As500 plot (Table 1). For those exposed to Cu, the peas' germination rate was 91% and 93%, respectively, in Cu50 and Cu500 (Table 1). Finally, in the plots exposed to Pb, the germination rate was 90% in the Pb50 and - notably - 100% in the Pb500 (Table 1).

Table 1. Seed germination ratio (%) of *Pisum sativum* after 4 days of incubation.

Control	As50	As500	Cu50	Cu500	Pb50	Pb500
100	97	94	91	93	90	100

Pisum sativum had similar germination rate when exposed to all three metals. In a study by Kunjam, et al., the rate of germination of *P. sativum* exposed to Cu at 20,000 µg/l still reached 100%, and there were only certain negative effects when the Cu concentration exceeded 60,000 µg/l [16]. Unfortunately, the data on the germination rates of seeds exposed to Pb and As could not be compared to academic references due to the lack of published studies. In excess concentrations, as in this study, the *P. sativum* germination rate conclusively indicated normal germination. It was also demonstrated that the resistance of *P. sativum* in the first four days was extremely stable for the individually exposed concentrations of As, Cu, and Pb.

Effects of metals on the fresh weight of Pisum sativum

Regarding the fresh weight of the peas after 7 days of exposure to the three metals, the results showed no statistically significant differences, although there was generally a slight decrease in the fresh weight of the exposed peas compared to the beans in control plot. In the control plot, the mean fresh weight of the beans was 0.69 g, while the mean fresh weight of the beans in the As50 exposure plot was 0.62 g, and in the As500 exposure plot it was 0.63 g (Fig. 1). For the plots exposed to Cu, the mean fresh weight of the peas was 0.62 g and 0.59 g for the plots of Cu50 and Cu500, respectively (Fig. 1). The beans exposed to Pb had a mean fresh weight of 0.61g in the Pb50 plot and 0.68 g in the Pb500 plot (Fig. 1). Compared to the control plots, the p values of these fresh weight mean values was always greater than 0.05; as a result, there is no statistically significant difference.

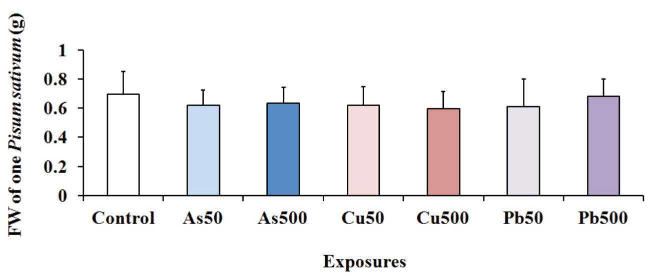

Fig. 1. Fresh weight of *Pisum sativum* after 7 days of incubation.

The results also showed that when distilled water and metal-exposed water was used, there was no significant difference in the harvesting parameters of the fresh weight of peas at 50 and 500 µg/l exposure concentrations. This leads to the conclusion that after a week of development, *P. sativum* shoots had an appreciable resistance to all three heavy metals. On the other hand, the resistence revealed the potential absorption of these metals into the shoot, which requires further investigation. It is important to note that studies of the fresh weight of *P. sativum* exposed to As, Cu, and Pb are not very popular, consequently there is no specific source reference.

Effects of the metals on the development of shoot length

The shoot length of *P. sativum* after one week of incubation showed a significant difference in the Pb500 plot ($p<0.05$), though there was no statistically significant difference in the other plots compared to the control. Specifically, the control plot resulted in a mean shoot length of 28.3 mm, which was not much different from the values of 28.2 mm and 25.9 mm in the As50 and As500 plots, respectively (Fig. 2). Where *P. sativum* was exposed to Cu, for the Cu50 plot, the average shoot length of the beans was 28.857 mm; while in the Cu500 plot, the value was 31.7 mm, which was slightly longer (Fig. 2).

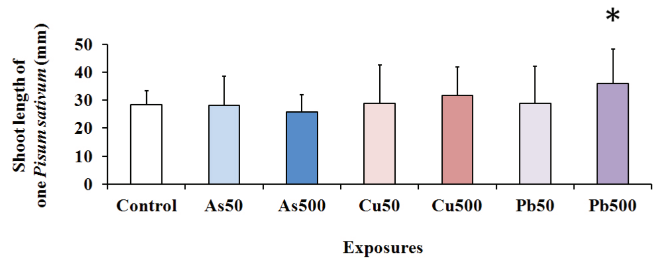

Fig. 2. Shoot length of *Pisum sativum* after 7 days of incubation. Asterisk indicates the significant difference between the control and exposures by means of the Kruskal-Wallis test (*$p<0.05$).

With regard to shoot development, previous studies on other plants such as *L. leucocephala*, *B. oleracea*, and *A. esculentus* have demonstrated that shoot growth was more or less influenced by metal exposure [17, 18]. However, for *P. sativum*, the toxic effects of all three heavy metals did not affect shoot prolongation. This is different from the results of another study of shoot extension, which showed that exposure to Cu at concentrations as high as 20,000 and 40,000 µg/l resulted on shoot stimulation [16]. The most likely causes for this may be due to the bound to active sites of enzymes, cell structure metabolism, or the cell division mechanism of *P. sativum* being highly adaptable to As, Cu, and Pb contaminants. It was also demonstrated the shoot development stimulation in the exposure to Pb500. To better understand this, investigations of the mechanisms of heavy metal absorption and processing in *P. sativum* are highly recommended. Moreover, further research ought to be conducted on the likelihood of metals and their concentrations stimulating the growth of shoots.

During the early stage of life, plants are usually highly sensitive to contaminants. The results showed that, at the concentration of three metals used in this study, there was almost no negative effect on the subjects. Therefore, it can be deduced that this plant is tolerant of these three metals, and thus can be considered potential resource for reducing metallic environmental pollution. Therefore, further research and empirical work on this are highly recommended, especially to explore the exploitation of the ability of *P. sativum* to overcome pollutants from contaminated sites by means of the phytoextraction mechanism. More specifically, future studies should include the exploration of the tolerance of *P. sativum* to high concentrations of metals, heavy biomass, and metal accumulation, as well as its ability to grow rapidly and its profuse root system.

Conclusions

We found that the three metals, As, Cu and Pb, at concentrations of up to 500 µg/l did not negatively affect the germination and shoot development of *Pisum sativum* over a period of 7 days. This demonstrates the high capacity for tolerance of this plant to the metals even in its early stage of life. Therefore, the plant shows good potential for use as a candidate for the phytoremediation of metal contamination and pollution. Further investigations of the responses of this plant to a combination of metals are suggested.

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

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