Environmental Management and Sustainable Development
ISSN 2164-7682
2017, Vol. 6, No. 2

Effects of Lead and Cadmium on the Seedling of Plants, *Brassica Oleracea* L, and *Abelmoschus Esculentus* L.

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Received: April 21, 2017 Accepted: May 10, 2017

doi:10.5296/emsd.v6i2.11285 URL: https://doi.org/10.5296/emsd.v6i2.11285

Abstract

In this study we tested the negative effects of lead (Pb) and cadmium (Cd) at the concentrations of 5 – 500 µg/L and 1 – 100 µg/L, respectively, on the germination, fresh weight, root and shoot length of broccoli, *Brassica oleracea* L., and okra, *Abelmoschus esculentus* L. over the period of 7 days. Two other tests were also conducted as control in which the seeds of these two plants were watered with distilled water only. For each concentration of exposures, 10 seeds were put into a plastic container and nine replicates (n = 9) for each treatment were prepared. The results revealed slight effects of the metals on the germination of *B. oleracea* and *A. esculentus*. However, fresh weight of the sprouts was decreased upon metal exposures especially the significant reduction of *A. esculentus* fresh weight in the Pb incubations. Pb slightly affected on the root prolongation of both plants. There was no significant difference on the shoot length of *B. oleracea* in control and Cd treatments. Furthermore, Cd tended to stimulate the prolongation of root and inhibit the extension of shoot of *A. esculentus*. Besides, Cd (100 µg/L) and Pb (500 µg/L) caused the leaf abnormality in *B. oleracea*. Our results contribute more understanding on the toxicity of Pb and Cd at environmentally relevant concentrations on the seedling of plants. Further investigations on the toxicity of other pollutants as well as their mixture on seedling of plants are recommended.

Keywords: Trace metals, Toxicity, Fresh weight, Root and shoot length, Abnormality

1. Introduction

In nature, the occurrence of heavy metals is extremely varied in many habitats. They exist in the soil, atmosphere and water environment. The emission of heavy metals into the environment happens through various processes and different paths upon the naturally geographic processes and human activities. Specifically, heavy metals are emitted into the environment through combustion, mining and processing (Jarup 2003). Heavy metals contaminate the ground and surface water through the transportation process in the water currents. The inappropriate process management and treatment of industrial wastewater are also sources of pollution (Tiwaria *et al*. 2015). Leaking heavy metals into the soil could be occurred through the geochemical processes of weathering of parent materials, mining waste, leaded gasoline and paint, animal manure, sewage sludge, fertilizers, pesticides, irrigation wastewater and atmospheric deposition (Wuana and Okieimen 2011).

Among the heavy metals, lead (Pb) and cadmium (Cd) have been widely monitored and found in water bodies. The concentration of Cd in the Moon and Shi Rivers, Thailand, exceeded the limits of WHO whereas that of Pb did not (Wongklom, 2016). Jabłońska-Czapla *et al*. (2016) reported that the Przemsza Biała River in Poland was heavily polluted by Pb, Cd, and Zn, with the highest concentration of 510 µg Pb/L, more than 1500 µg Zn/L and 6 µg Cd/L. Similarly, Pb pollution (218 – 330 µg/L) and Cd contamination (8 – 12 µg/L) were also found in Mahrut River, Iraq (Obaidy *et al*. 2014). In the surface water in a mining area at Shandong Province, China, the Cd and Pb concentrations reached over 190 and 430 µg/L, respectively (Ning *et al*. 2011). In Vietnam, Cd contamination in water from the canal, river, estuary, and swamp from Ca Mau Province was reported and considered as the source of metal bio-accumulation for aquatic organisms (Nguyen and Bui 2009). The activities of agriculture, aquaculture and the rapid urbanization in Southern Vietnam have caused surface water become contaminated with metals such as Pb, Cu, Cr, As (Strady *et al*. 2017).

Plants can only use or uptake metals under the form of ions. The trace metals in ion forms are transferred from the soil solution or water into the plant cells through surface roots by root extension, mass-flow, and diffusion (Barber *et al*. 1963). The absorbance of trace metals could lead to the accumulation of those in plants and then the metals could be transferred into the animals and human beings through the food chain (Nazir *et al*. 2015). Many trace metals such as Co, Cu, Cr, Fe, Mn, Ni and Zn are essential for the growth and development of plants because they play important roles in the formation of enzymes, redox reactions. Therefore, they vitally have biochemical function and physiology of plants (WHO/FAO/IAEA 1996). However, when the metal concentrations exceed a certain threshold the metal becomes toxic to plant (Jaishankar 2014). Trace metals at high concentrations could cause the decrease of photosynthesis ability, cell-structural damage, reduction of physical and biochemical activities of plants consequently negative impacts on the growth, the root tip, the absorption of nutrients, the water uptake, and physiological and metabolic processes of plants. The toxic metals such as Cd and Pb are greatly toxic even at comparatively low concentration (Sardar 2013).

Li *et al*. (2005) studied the effects of toxic metals (Cd and Pb) on the germination and growth of *Arabidopsis thaliana* seeds. The authors reported that Pb did not strongly influence on the germination of seeds, while Cd (at 450 mg/L) severely inhibited the germination. Bautista *et al*. (2013) conducted the exposures of Cd and Cr (both at the concentrations of 2.8, 3.9 and 5.6 mg/L) to lettuce (*Lactuca sativa v. batavia*), Swiss chard (*Beta vulgaris v. cicla*) and spinach (*Spinacia olearcea*) and found that Cd caused strong impacts on those plants while Cr only slightly affected on some characteristics such as seed germination, root length and fresh weight. Besides, Pb and Cd negatively influenced on seed germination and development of *Leucaena leucocephala* (Shafiq *et al*. 2008) The authors observed that the germination significantly reduced when exposed to 75 mg Pb/L and 50 mg Cd/L. The length of stems and roots of the plant was shorter than the control after exposure to 50 mg/L of Pb or Cd. The dry weight decreased significantly compared with control samples when irrigated with Pb and Cd at the concentration of 25 mg/L (Shafiq *et al*. 2008).

The issues of heavy metal pollution are becoming popular in the world, especially in developing countries. Heavy metals are likely to cause high toxicity to plants. Investigations on heavy metal pollution in different environments and the effects of heavy metals to the plants have been conducted. However, the effects of heavy metals (e.g. Pb, Cd) in seedling of plant are not fully understood. The two plants *Brassica oleracea* and *Abelmoschus esculentus*, are among the very common vegetable in Southeast Asian. The sprouts of these plants are widely used as food for Vietnamese. Local farmers use surface water, now and then contaminated with trace metals, for watering their crops so there may be potentially negative effects to plants and risk to consumers. Therefore, this research aimed to observe the effects of Cd and Pb on the early life stage of *B. oleracea* and *A. esculentus*.

2. Materials and Methods

The seeds of *Brassica oleracea* and *Abelmoschus esculentus* for the bioassay were purchased from Trang Nong Store, located in District 6, Hochiminh City, Vietnam. The experiment was conducted in the Ecotoxicology Module, Laboratory of Environmental Analysis, Hochiminh City University of Technology. The two metals, Pb and Cd in stock solution of 1000 mg /L, for toxicity tests were purchased from the manufacturer, Merck (Germany). Metal concentrations in the experiments were chosen based on the Vietnam regulations 39: 2011 / MONRE – National Technical Regulation on the quality of water used for irrigation.

For experiment, the seeds were exposed to either Pb or Cd at four different concentrations: 0 (control), 5 µg Pb/L (Pb 5), 50 Pb µg/L (Pb 50), and 500 µg Pb/L (Pb 500); 1 µg Cd/L (Cd 1), 10 µg Cd/L (Cd 10) and 100 µg Cd/L (Cd 100). The experiment was implemented according to the description by Pflugmacher *et al*. (2007) and Dao *et al*. (2014) with minor modification. Briefly, for each concentration of exposures, 10 seeds were laid on tissue paper in a plastic container and nine replicates (n = 9) 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. During the experiment, the germination of seeds in each exposure was daily recorded. At the second, fourth and seventh 7 days of the experiment, the seeding in three replicates (n = 3) of each treatment was weighted, and its root and shoot were measured with a ruler, exactly to 0.1 mm.

Kruskal-Wallis test, Sigmaplot, version 12 was used for evaluation the significant difference on the fresh weight (FW), root and shoot length of the seedling of plants between control and metal exposures.

3. Results

3.1 Influences on the Germination

Results showed that the germination rate of *B. oleracea* in the control and Pb exposures was 92% (control), 91% (Pb 5), 92% (Pb 50) and 89% (Pb 500) after 2 days of incubation. Similarly, after 4 days of experiment, the germination rates of *A. esculentus* in the control, Pb 5, Pb 50 and Pb 500 were 95%, 93%, 93% and 88%, respectively (Table 1). In the tests with Cd, the germination rate of *B. oleracea* was 93% in both control and Cd 1, but it was a little decreased to 79% and 89% in the exposures to the higher Cd concentrations, Cd 10 and Cd 100. Interestingly, the concentrations of 1 – 100 µg/L of Cd did not reduce the germination rate of *A. esculentus* compared to the control (Table 1).

Table 1. Seed germination ratio (%) from the exposures after the first two days for *Brassica oleracea* and 4 days for *Abelmoschus esculentus*

Seeds/ plants	control	Pb 5	Pb 50	Pb 500
B. oleracea (2 days)	92	91	92	89
A. esculentus (4 days)	95	93	93	88
	control	Cd 1	Cd 10	Cd 100
B. oleracea (2 days)	93	93	79	89
A. esculentus (4 days)	95	95	95	95

3.2 Influences on the Fresh Weight

In the experiment on *B. oleracea* exposed to Cd, the average FW of the seedling after 2 days in the control, Cd 1, Cd 10 and Cd 100 were 13 mg, 12 mg, 10 mg and 9 mg, respectively. There was a significant difference between the FW in the control and Cd 10 or Cd 100 according to the Kruskal-Wallis test ($p < 0.05$, Fig. 1). Similar situation was recorded for the FW of the *B. oleracea* after 4 days of treatment. However, by the end of the test, there was no statistical difference in relation to the FW between the control and Cd exposures (Fig. 1).

In the tests with Pb, the detrimental impact of Pb 50 and Pb 500 on FW of *B. oleracea* was only observed after two days of experiment ($p < 0.05$). The average FW of one *B. oleracea* in the control and Pb exposures ranged 17 – 20 mg, and 40 – 50 mg after 4 and 7 days of incubation, respectively (Fig. 1). The negative effect of Pb on FW of *A. esculentus* was apparently showed at the highest Pb concentration (Pb 500) and after 7 days of incubation in which the FW from the metal exposures was significantly decreased compared to the control (Fig. 1).

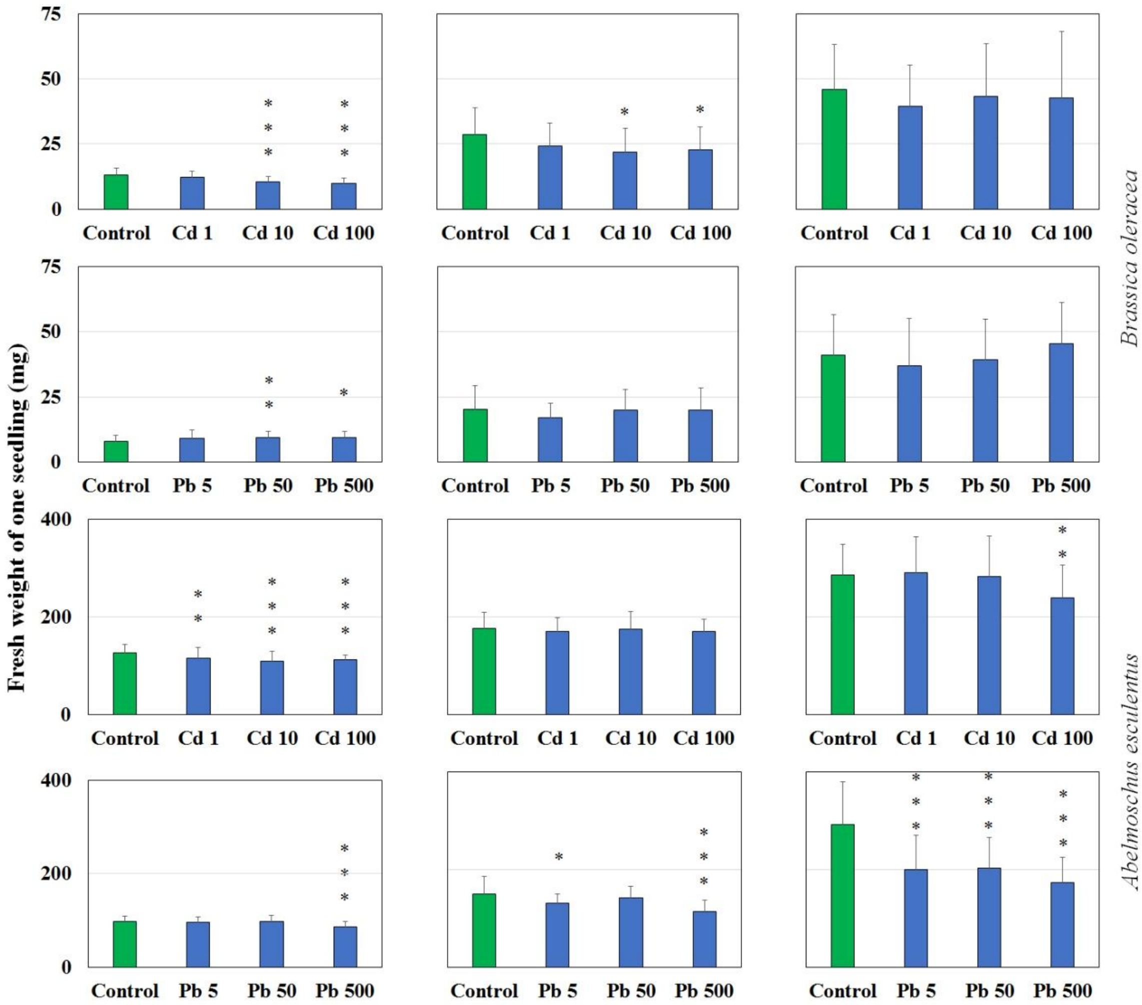

Figure 1. Fresh weight of *Brassica oleracea* and *Abelmoschus esculentus* during the experiment. Asterisks indicate the significant difference between the control and metal exposures by Kruskal-Wallis test (*, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.001$)

3.3 Influences on the Root Length

The root of the seedling was measureable after 4 days of incubation. In the exposures to Cd, the root length of *B. oleracea* in the three concentrations was not significantly different with the control after 4 or 7 days of experiment. The Cd 10 and Cd 100 caused the root length of *A. esculentus* prolong to 30 – 31 mm, significant longer than the control, 24 mm, after 4 days of testing. However the average length of the root was similar among the control or the three Cd treatments, 38 – 46 mm, after 1 week of testing (Fig. 2).

The average root length of *A. esculentus* after 4 days of experiment in the control, Pb 5 and Pb 50 were 19 mm, whereas that in the Pb 500 was 16 mm. After 7 days, the average root length in the control, Pb 5, Pb 50 and Pb 500 was 37 mm, 27 mm, 34 mm and 26 mm, respectively (Fig. 2). Statistical results showed that Pb 500 caused the root length of *A. esculentus* significantly shorter than the control after 7 days of experiment (Fig. 2). There was no statistically different of the root length of *B. oleracea* in control and Pb exposures.

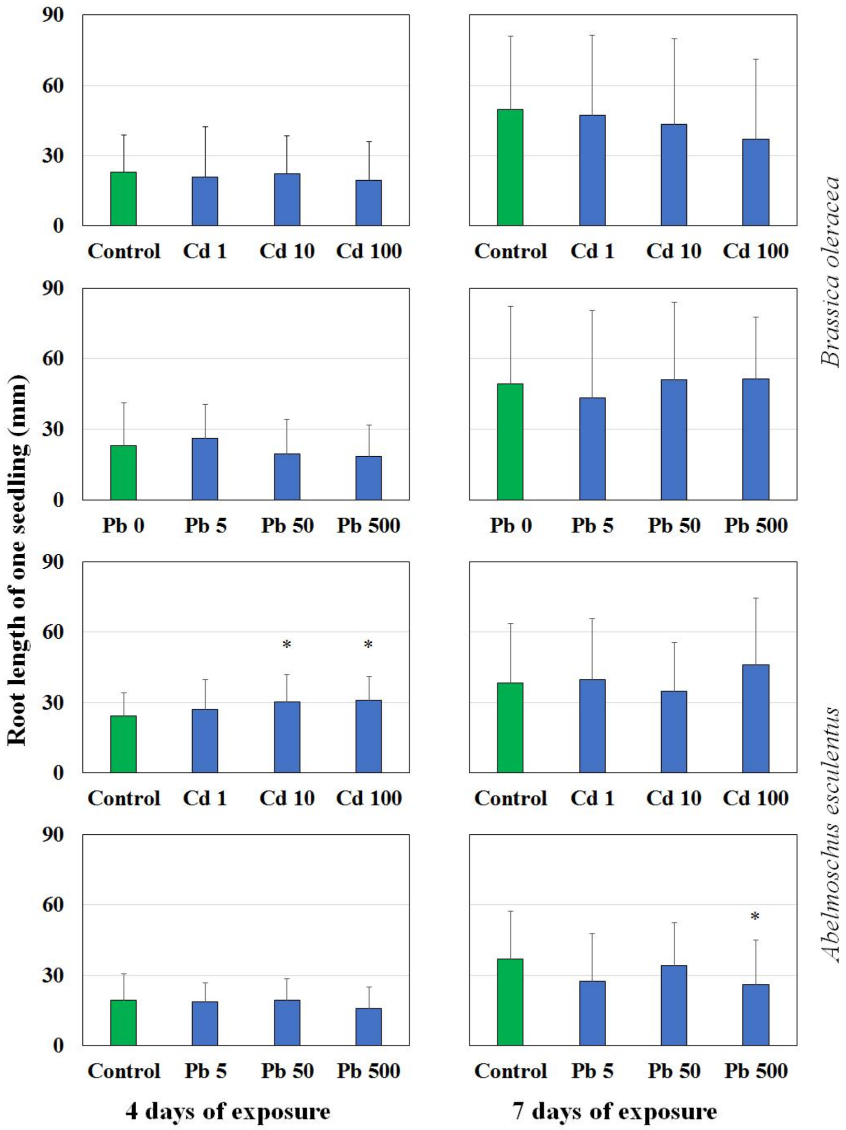

Figure 2. Root length of *Brassica oleracea* and *Abelmoschus esculentus* during the experiment. Asterisk indicates the significant difference between the control and metal exposures by Kruskal-Wallis test (*, $p < 0.05$)

3.4 Influences on the Shoot Length

The shoot length of *B. oleracea* was measureable after 4 days of experiment whereas that of *A. esculentus* was able to be recorded after 7 days of incubation. After 4 experimental days, average shoot length of *B. oleracea* in the control was 13 mm, while the shoot length in the Cd 1 and Cd 10 was 12 mm, and that in the Cd 100 was 10 mm (Fig 3).

By the end of experiment the average shoot length of *B. oleracea* in the control, Cd 1, Cd 10 and Cd 100 was 34 mm, 28, 26 and 23 mm, respectively. The among the metal treatments, only shoot length of *B. oleracea* Cd 100 was significantly shorter than the control at 4 and 7 days of exposures (Fig. 3). In the experiment with *A. esculentus* Cd 1 and all three Pb exposures inhibited the prolongation of shoot. However, Cd 10 and Cd 100 stimulated the prolongation of the shoot (Fig. 3). Therefore, the shoot length of the two plants, *B. oleracea* and *A. esculentus,* was regulated differently by the two metals and the concentrations of the metals.

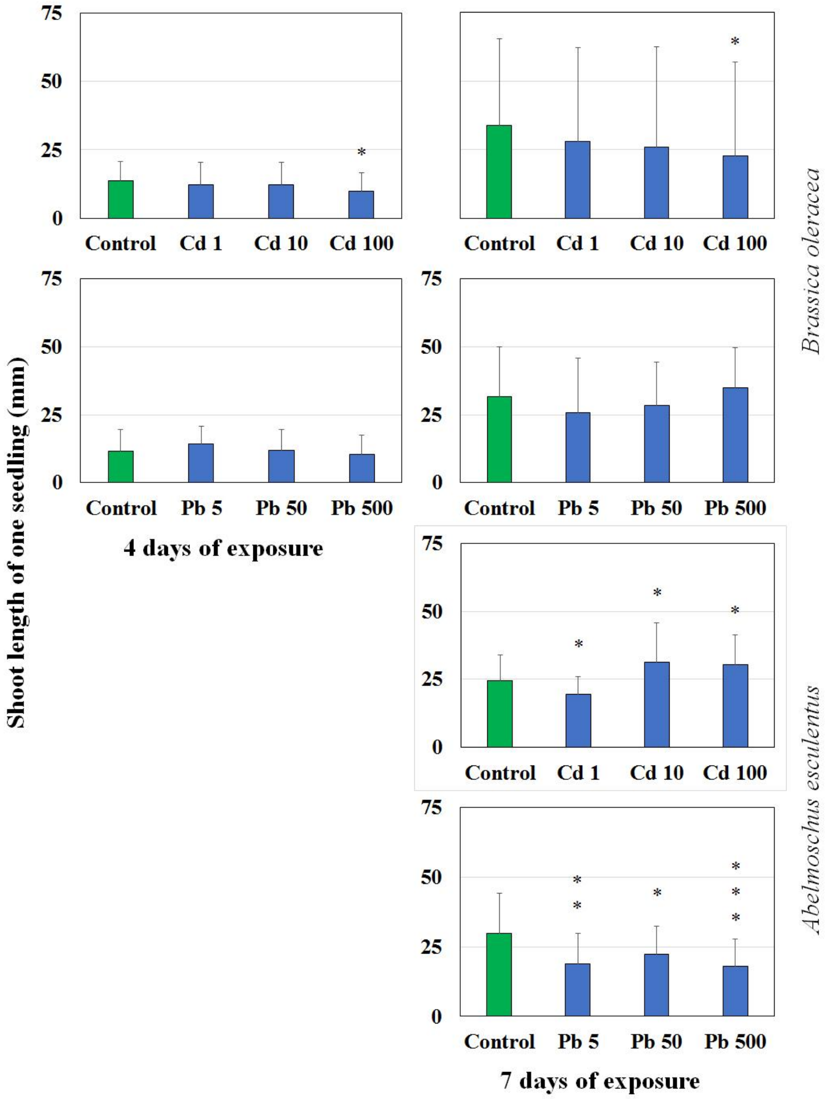

Figure 3. Shoot length of *Brassica oleracea* and *Abelmoschus esculentus* during the experiment. Asterisks indicate the significant difference between the control and metal exposures by Kruskal-Wallis test (*, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.001$)

3.5 Influences on Leaf Morphology

There was no abnormal appearance occurring on the color leaves, shoot and root of *A. esculentus* during the both metal exposures. However, in some of the *B. oleracea* seedlings, manifestations of black and brown spots on the young leaves from the Pb and Cd treatment were observed after 7 days of incubation (Figure 4).

Figure 4. The normality (A, control) and abnormality of young leaves (arrows) of *Brassica oleracea* upon exposures to Cd 100 (B) and Pb 500 (C)

4. Discussion

Generally, the test concentrations of Pb and Cd did not cause significant difference on the germination of *B. oleracea* and *A. esculentus*. Shafiq *et al*. (2008) reported the negative effects of Pb and Cd on the germination of *L. leucocephala*. However, the metal concentrations used in their study, 75,000 µg Pb/L and 50,000 µg Cd/L, were much higher than those in the current investigation, 500 µg Pb/L and 100 µg Cd/L. Because the metals at high concentrations can prevent the water uptake consequently germination inhibition, the higher Pb and Cd concentrations (75,000 and 50,000 µg/L, respectively) would properly decrease the germination of *B. oleracea* and *A. esculentus* which needs further investigations.

The FW decrease of *B. oleracea* during the first 4 days on incubation in our study is in line with previous report by Bautista *et al*. (2013) testing with some plants like lettuce, Swiss chard, and spinach. Trace metals at high concentration could inhibit the water uptake and later the physiological and metabolic processes of plants (Sardar 2013) consequently FW reduction of the seedling in our study. Besides, it could be seen that the toxicity of Pb to *B. oleracea* and *A. esculentus* was higher than that of Cd. Also, the tolerance of *B. oleracea* to Pb was lower than that of *A. esculentus*. Hence different species would have different sensitivity or tolerance to a certain toxin.

The root of *B. oleracea* was slightly affected when exposed to Cd at the test concentrations. According to Shafiq *et al*. (2008), the reduction in the root length exposed to heavy metals may be attributed to the reduction of the root cell tissue. Properly, the used metal concentrations (1 – 100 µg Cd/L and 5 – 500 µg Pb/L) were not high enough to induce the strong impact on the root length of *B. oleracea* and *A. esculentus*. However, an investigation on the cell development of the plants upon exposure to Pb and Cd is highly suggested to confirm.

Reducing the shoot length of the seedling exposed to heavy metals may be attributed to the reduction of embryonic sprouts' cell tissue and some enzymes contained in the leaf and endosperm. When the activity of the hydrolyzed enzymes is affected, leading to the effects on shoot length of the seedling (Shafiq *et al*. 2008). Our results also showed that Cd was more toxic than Pb on the development of *B. oleracea* sprouts. The metals Pb and Cd are capable of inhibiting the activity of certain enzymes within the cell leading to disruption of protein synthesis. This may lead to the inhibition of the elongation of root in high metal concentration exposures (Pb, Cd). This meant the presence of the metals reduced the cell division in the tissue consequently inhibition of the growing process (Sardar 2013). Chemically, the toxic metals Pb and Cd have the same valency as Cu, Mn and Zn. Therefore, these two toxic metals may outcompete the essential trace metals (Cu, Mn and Zn) in the bound to active sites of enzymes or cell structures during metabolism processes in cells of plants. Consequently the development during early stage of *B. oleracea* and *A. esculentus* would be more or less disordered. Properly the effects of Pb and Cd on prolongation of the shoot should be similar to those of the root which needs further investigations on cellular levels to have the detail understanding on this.

Pb could inhibit the photosynthesis processes by degrading the absorption of essential elements such as Fe, Mg (Burzynski 1987). Besides, Cd altered the function of cell membrane by reducing the activity of enzymes involved in fixation of CO_2 (De Filippis and Ziegler 1993). These negative effects of the metals could lead to the appearance of brown spots on the leaves. Our observation on the abnormality of young leaves of *B. oleracea* is in agreement to a previous report by Dao *et al*. (2014) in which the plants were exposed to the cyanobacterial toxin, microcystin. The detrimental impacts of Pb and Cd on the seedling of *B. oleracea* and *A. esculentus* are evidenced. This means that the toxic metals entered the cells of the plants (during watering with metal-contaminated water). Hence further studies on bioaccumulation of heavy metals in these plants should be studied for the health risk evaluation of local residents who consume the sprout of these plants daily.

5. Conclusions

The toxic metals Pb and Cd at the test concentrations (5 – 500 µg Pb/L; 1 – 100 µg Cd/L) did not significantly reduce the germination of *B. oleracea* and *A. esculentus.* However, the toxic metals strongly affected on FW of the seedling especially potent impact of Pb on FW of *A. esculentus* after 7 days of incubation. Cd slightly influenced on root and shoot length of *B. oleracea* while Pb caused minor change of the root prolongation of the *A. esculentus*. On the other hand, Cd tended to stimulate the prolongation of root and inhibit the extension of shoot of *A. esculentus*. The highest concentrations of Cd (100 µg/L) and Pb (500 µg/L) induced the abnormality of the young leaves of *B. oleracea*. Further investigations on the toxicity of other pollutants as well as their mixture on seedling of plants are recommended.

Acknowledgement

This research is funded by Hochiminh City University of Technology – VNU-HCM under the granted project number SVOISP-2016-MTTN-15.

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