THE EFFECT OF PLUMBUM AND CADMIUM ON THE SEEDS GERMINATION AND GROWTH OF *C. odorata, T. paniculatum* AND *C. pallida*

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THIS DISSERTATION IS SUBMITTD IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

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ABSTRACT

Heavy metals pollution has been a concern and is getting worse each day which could bring adverse effects toward organisms such as plants and animals. Plants will uptake the heavy metals that are present in the surrounding environment and either die due to intolerance or accumulate in edible parts which will cause heavy metals poisoning to organisms that consume the plants such as humans. This study is carried out to determine the effect of heavy metals such as lead (Pb) and Cadmium (Cd) on the germination of various seeds of *Chromolaena odorata, Talinum paniculatum* and *Crotalaria pallida* and the growth of shoot and root at day 14. The seeds were grown in the Petri dish with cotton and treated with different concentrations (0, 1, 5, 10, 50, 100, 200, 400, 600, 800, 1000 mg/L) of lead (Pb) and (0, 0.1, 0.5, 1, 5, 10, 50, 100, 150, 200, 300 mg/L) cadmium (Cd) solution. The germination percentage and the length of the shoot and root were being observed and measured for a duration of 14 days. The increasing Pb and Cd concentrations resulted in the reduction in the germination percentage, length of shot and root. *C. odorata* had the highest germination percentage of 42.31% when compared to both different concentrations of Pb and Cd. The highest germination percentage of 33.85% was obtained when *T. paniculatum* was grown in Pb at 0 mg/L whereas the highest germination percentage of 35.38% was obtained at 50 mg/L of Cd. For *C. pallida*, the highest germination percentages of 55.38% at 1 mg/L of Pb and 45.38% at 0 mg/L of Cd were obtained. When the seeds were grown on cotton containing Pb, *C. odorata* had the highest length of shoot and root of 2.2 cm and 1.4 cm respectively at 0 mg/L; *T. paniculatum* had the highest length of shoot and root of 2.3 cm at 1 mg/L and 1 cm at 5 mg/L respectively whereas *C. pallida* had the highest length of shoot and root of 5.2 cm at 0mg/L and 2.3 cm at 10 mg/L respectively. Moreover, when the seeds were grown in Cd condition, *C. odorata* had the highest length of shoot of 2.2 cm and root of 1.5 cm at 0 mg/L; *T. paniculatum* had the highest length of shoot of 1.3 cm at 0 mg/L and root of 1.3 cm at 0.1 mg/L whereas *C. pallida* had the highest length of shoot of 5.2 cm at 0 mg/L and root of 1.7 cm at 0.5 mg/L. In conclusion, *C. pallida* displayed high overall germination percentage, length of shoot and root when exposed to Pb and Cd as compared to other plant species tested. Hence, *C. pallida* might serve as a suitable plant that could be used to remediate Pb and Cd contaminated area.

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
As	Arsenic
Cd	Cadmium
Ca	Calcium
Cr	Chromium
Cu	Copper
d	days
DNA	Deoxyribose Nucleic Acid
Fe	Iron
Pb	Lead
$Pb(NO_3)_2$	Lead Nitrate
mg	milligram
mg/L	milligram per litre
mL	millilitre
NADH	Nicotinamide adenine dinucleotide
%	percentage
ROS	Reactive oxygen species
Zn	Zinc

CHAPTER 1

INTRODUCTION

As the advancement of technology and industrialization occurs, heavy metals pollution has become a concern and poses threats to environment and organisms including humans. Heavy metals are elements that have density of 5 g/cm^3 which is 5 times higher than the density of water (Duruibe, Ogwuegbu & Egwurugwu, 2007). There are five heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr) and mercury (Hg) that are classified as carcinogenic towards human due to their high toxicity effects which cause DNA damage even when the exposure amount is low (Tchounwou, Yedjou, Patlolla & Sutton, 2012; Järup, 2003). Heavy metals can be spread through air, water, ground and even food which will then accumulate in the organ of the organisms exposed because they are not biodegradable (Duruibe, Ogwuegbu & Egwurugwu, 2007). Heavy metals are being used in myriad areas such as agriculture, industry, and so on. Heavy metals contamination can occur both naturally and man-made. Phenomenon such as the eruption of volcano can also contribute to the pollution of heavy metals whereas activities such as mining and use of herbicide can lead to the distribution of heavy metal in environment. Some heavy metals are the crucial micronutrients involve in biological function for instance, copper (Cu), chromium (Cr), et. cetera (Tchounwou, et al., 2012).

Lead (Pb), one of the heavy metals, is present in the crust of Earth and can be dispersed into air, food, soil and water. There are various applications of Pb that contribute to the pollution such as in industry and agriculture. The creation of petrol in the past decades has led to the emission of Pb into air which will then deposit in water and soil and accumulate in blood (Kinder, 1997). In the ancient time, Pb was used as sweetener in wine by Romans (Järup, 2003). High amount of Pb is used to produce lead-acid battery and it can also be found in paint, pipes, glass and so on. It is reported that Pb poisoning occurs among children that were in contact with lead-contaminated soil and the chips of paint from homes (Tong Schirnding, & Prapamontol, 2000). The symptoms of exposure to lead are headache, memory loss and more that are associated with the nervous system. Studies show that Pb poisoning is carcinogenic due to its interaction with tumour suppressing proteins and causes mutation in genes (Tchounwou, et al., 2012).

Cadmium (Cd) can be found within an ore together with zinc (Zn), Cu and Pb. Cd is commonly used in industries that produce alloys, batteries and acts as a stabilizer in products such as polyvinyl chloride (PVC) which is the concern of industrial employee for its adverse effects (Godt et al., 2006). Recycle of these products seldom takes place which leads to the contamination of Cd in the environment when tossed with domestic wastes or dispersed into air when incinerated (Järup, 2003). The amount of Cd present in phosphate fertilizer is high which in turn become the source of soil contamination. When the Cd is being absorbed by plants, it will lead to the accumulation of Cd in human's organ after being consumed. Besides crops, Cd poisoning can also occur among people that smoke cigarette which the level of Cd present in blood is four times higher than non-smokers. In 1950s, itai-itai disease which caused damage to bone and kidney had been discovered in Japan due to the high exposure to Cd (Bernard, 2008).

Seed is being produced by two types of plants which are angiosperm and gymnosperm as a mean of reproduction. Seeds will eventually fall onto ground and germinates by utilizing the nutrients present in seed itself and soil. The presence of contaminants in the soil will or will not affect the germination of seeds depending on the ability of seeds or plants to secrete enzymes to degrade or utilize the contaminants. It is reported that heavy metals such as Pb has adverse effect by inhibiting the germination of seed, elongation of root, uptake of nutrients, et. cetera. The expression of ACBP1 in *Arabidopsis* has shown to enable the plant to tolerate Pb contamination which enable seedling to germinate (Hsia, et al., 2014). Hence, the growth of seeds with the presence of heavy metals can be observed for the selection of potential plants that can be used in phytoremediation of heavy metals. However, there is no research conducted to test the effect of heavy metals such as Cd and Pb on the growth of *Chromolaena odorata*, *Talinum paniculatum* and *Crotalaria pallida*.

Thus, the aims of this study are (a) to determine the effect of cadmium (Cd) and lead (Pb) on the seeds germination of *C. odorata*, *T. paniculatum* and *C. pallida* and (b) to determine the effect of cadmium (Cd) and lead (Pb) on the growth of the seedlings of *C. odorata*, *T. paniculatum* and *C. pallida* seeds.

CHAPTER 2

LITERATURE REVIEW

2.1 HEAVY METALS POLLUTION

Heavy metals can be found naturally and categorized as metallic and non-metallic elements such as Arsenic (As). Heavy metals have greater density than water and with atomic number higher than 20 (Rajeswari & Sailaja, 2014). Arsenic (As), one of the metalloids that is classified as heavy metals due to its toxicity induced when exposed at low concentration is similar with heavy metals. Heavy metals can also be termed as trace elements because some of the heavy metals such as Cu and Zn are required in low concentration for metabolic process and the amount of heavy metals present in the environment naturally is negligible (Kibria, 2016). In the age of modern technology, industries have been expanding and human activities are worsening the environment in term of heavy metals pollution. Activities such as smelting, mining, the use of pesticide or herbicide, release of industrial sewage, disposal of gadgets and so on can contribute to the contamination of heavy metals (Tchounwou, et al., 2012). Heavy metals pollution can present in three forms which are air, soil and water. Heavy metals can be found in the atmosphere in the forms of particulate. The emission of heavy metal into the atmosphere occurs naturally or through anthropogenic activities such as combustion of heavy metal-containing waste (Agarwal, 2009). The estimation of heavy metals being emitted worldwide annually is shown in Table 2.1.

Table 2.1. The international emission of several heavy metals estimated annually (Postel, 1986).

Metal	Human activity (1000 tonnes)	Natural sources (1000 tonnes)	Ratio of human to natural contribution
Lead	2000	6	333
Zinc	840	36	23
Copper	260	19	14
Arsenic	78	21	4
Selenium	14	3	5

Airborne heavy metals can be found mainly in the area that involves emission of heavy metals and depend on the speed of the wind to transfer the pollutants. The pollutants can

reach the area that is 11 km from the industry that emits the pollutants (Agarwal, 2009). The heavy metals in the air can also contaminate the other sources such as soil and water by raindrops. Soil is the major depository of heavy metals which are difficult to be degraded by microbial and chemical means (Wuana & Okieimen, 2011). The heavy metals will be deposited to the organic matters or clay in the soil which will affect the bioavailability, toxicity and so on of the heavy metals (Su, Jiang & Zhang, 2014). The uptake of heavy metals in soil by microbes and plants at the site will lead to inhibition or death of the organisms. The soil microbes will only be inhibited when the concentration of heavy metals such as Pb and Zn is above the standard set of environment by the European Union whereas at low concentration, the growth of soil microbes can be stimulated (Su, Jiang & Zhang, 2014). The contaminant in soil will also cause the river or lake nearby to be polluted. The heavy metals will be deposited at the bottom due to their insolubility and accumulate in the ecosystem present in the water source. Eutrophication can occur due to algae blooming by utilizing the pollutant which will lead to high oxygen demand and eventually resulted the death of aquatic organisms such as fish. Since heavy metals can hardly be seen by our naked eyes and can cause chronic diseases, it is important to develop tools or methods to monitor and degrade these heavy metals.

2.1.1 Lead

In periodic table, Pb belongs to period six of group four which has atomic mass and number of 207.2 and 82 respectively. Pb is naturally occurring grey metal and is the fifth most used metal in the industrial production of pipes, paint, gasoline and more which is shown in Figure 2.1 (Wuana & Okieimen, 2011). The uses of Pb are well recognized in the production of lead-acid batteries that will be used for cars and so on which is shown in Figure 2.2. When ionic form of Pb such as Pb(II) is discarded into soil, it will interact with other molecules that are present in the soil to become a stable and insoluble compound such as $Pb(NO_3)_2$ (Shiomi, 2015). It can also form as an organic compound by the alkylation process carried out by the microbial biomass to become tetraethyl lead. The formation of lead compound will affect its solubility, bioavailability and toxicity (Wuana & Okieimen, 2011).

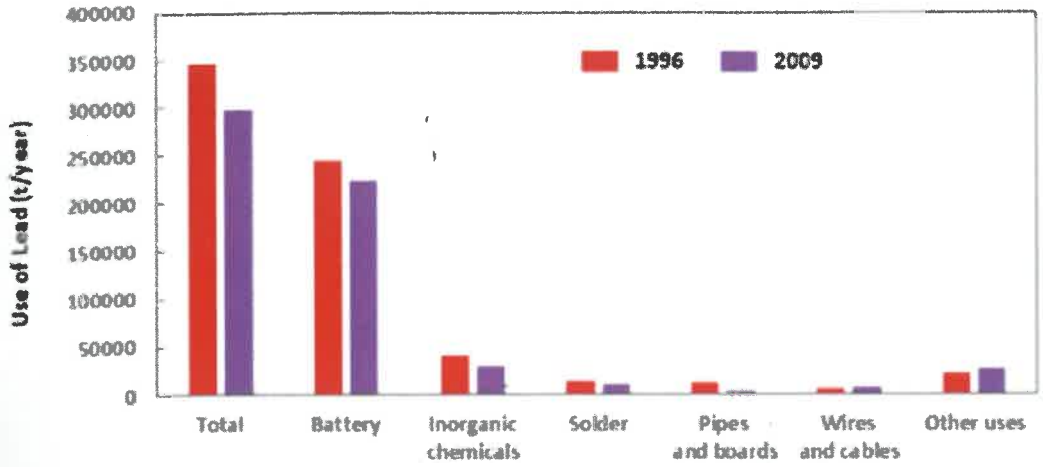

Figure 2.1. The uses of lead in Japan (Shiomi, 2015).

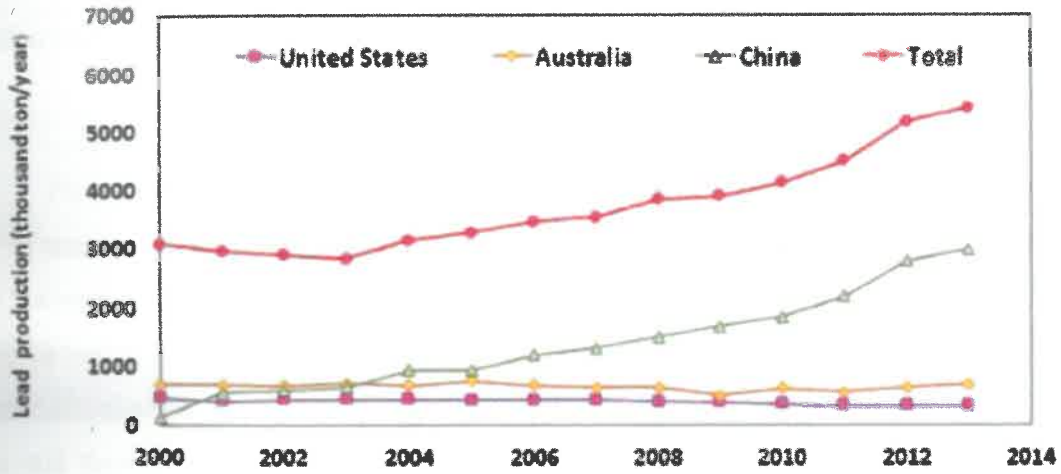

Figure 2.2. The production of lead in different countries (Shiomi, 2015).

There are several exposure routes toward Pb which can be through ingestion and inhalation. Pb can contaminate the food chain which when ingested will accumulate in the body and causes adverse health effects. Mostly, the Pb being consumed together with food is between 20 to 25 µg/kg and about 5 to 10% of the Pb will be absorbed by adults whereas 40% of Pb will be absorbed by children (Assi, Hezmee, Haron, Sabri & Rajion, 2016). Besides, the inhalation of Pb is caused by the presence of Pb in the ambient air which nearly 40 to 50% of Pb will be inhaled into the lung and led to the increase of Pb in the blood shown in Figure 2.3 (Shiomi, 2015).