Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

ACCUMULATION AND DISTRIBUTION OF HEAVY METALS IN *Leucaena leucocephala* Lam. AND *Bougainvillea spectabilis* Willd. PLANT SYSTEMS

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Received – December 02, 2015; Revision – December 21, 2015; Accepted – January 21, 2016
Available Online – February 15, 2016

DOI: http://dx.doi.org/10.18006/2015.4(1).01.06

KEYWORDS

Bio-monitoring

Bougainvillea spectabilis

Leucaena lecocephala

Heavy metals

ABSTRACT

This study was conducted to determine the degree of heavy metal contaminations in the soils around the perimeter of an industrial park located in the city of Sta. Rosa, Laguna, Philippines that houses light-to-medium scale manufacturing industries, through accumulation of heavy metals in two plant systems viz. *Bougainvillea spectabilis* (bougainvillea) and *Leucaena lecocephala* (ipil-ipil). Results of study revealed that the soil samples collected from the study site contained higher concentrations of Cu and Zn compared to a residential site as non-polluted source, some amount of nonessential mineral like Cd and Pb was also found from the sample collected from the study area. Findings of the study suggested that Cu is an immobile element, was highly accumulated in the roots of *B. spectabilis*, while highest concentration of Zn was accumulated in the leaves. Moreover, the leaves of *L. leucocephala* collected from the study site accumulated significantly higher concentrations of both Cu and Zn as compared to the leaves of the same plant species collected in a residential site. The non-essential metals, Cd and Pb, exhibit no significant difference in their accumulation and distribution to different plant parts and between the industrial and residential sites.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher (http://publisher.jebas.org/index.html).

1 Introduction

Environmental pollution by heavy metals as a result of increasing industrial activities has become a main global concern. One of the most predominant environmental pollution caused by various productions from industries is heavy metals contamination in the air and soil (Gaur & Adholeya, 2004). Although living organisms can tolerate numerous ranges of heavy metals, still at excessive levels several body systems of organism could be damaged (Chronopoulos et al., 1997). Because of this hazardous nature of heavy metals to human health, monitoring of the environmental burden of heavy metals is an important ecological interest (Onianwa & Ajayi, 2002; Peng et al., 2006). There are two different methods in order to monitor or assess the extent of pollution caused by heavy metals, i.e. direct method that measures metal concentrations in the substrate and indirect method that studies the presence of metal in some living organisms such as plants (Hervada-Sala et al., 2003).

Plants can be described as solar driven pumping stations for those that degrade pollutants or accumulate them from their immediate environment (Cunningham et al., 1995). Use of plants in removing toxins from the environment is known as phytoremediation and is an important means of cleaning up these toxins. Many plants species were used and have been reported successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from the soil (Wang et al., 2002; Sekara et al., 2005; Yazaki et al., 2006; Ching et al., 2008). There are also plants that used in bio-monitoring; these plants can be grouped into two *viz.* bio-indicator plants and bio-accumulator plants. Bio-indicator are those plants which are more sensitive to pollutants and shows visible symptoms of contamination on the leaf and other plant systems, these plants are generally used as pollution marker, whereas bio-accumulator plants have built resistance against these pollutants; they can store pollutants without any visible damage on their morphology and physiology (Radnai, 1997).

Burhan et al. (2001) suggested that there are about 50 metals which are of special interest with respect to the toxicological importance to human health, plants and animals. Essential elements such as Fe, Zn and Cu are useful to plants at low concentration but playing a detrimental role in plant development at higher levels. While trace metals present in the environment are not only hazardous to ecosystems but can also cause hazard to human health and plant growth (Shafiq & Iqbal, 2006). Because of such problems, it was deemed necessary to determine the accumulation of heavy metals such as Cu, Zn, Cd, and Pb. Present study was formulated for accessing the presence of these heavy metals in the soil samples collected from the perimeter of an industrial park situate in the city of Biñan, Laguna, Philippines soils. Two bio-accumulator common plant species *viz. Bougainvillea spectabilis* (bougainvillea) and *Leucaena lecocephala* (ipil-ipil) were used for the study. Further, this study determined the contamination level of the industrial area soil and the degree of heavy metal accumulation in the roots, stem, and leaves of *B. spectabilis* and in the leaves of the *L. leucocephala* collected around the industrial park.

2 Materials and Methods

2.1 The Study Site

The study site is a 224-hectare industrial park located in the city of Sta. Rosa, Laguna, Philippines. This industrial park is an estate houses for light-to-medium scale manufacturing industries like garments, foods and papers, plastics, ceramics, paints, electronics, rubber, home appliances and car parts.

2.2 Collection of Soil Samples and Plant materials

Two most common plant species of study area are *B. spectabilis* and *L. lecocephala* selected for the present study. Plant samples i.e. roots, stems and leaves of *B. spectabilis* and leaves of *L. lecocephala* were collected from the plant found within 5 meters range around the perimeter of the study site. Simultaneous to the collection of plant samples, about 0.5 kg soil samples were also collected from the upper 2 -10 cm of the surface soil (Ochotorena, 1994). Likewise, soil and plant samples of the same species were collected from a residential site in the city of Biñan, Laguna, more than 20 km away from the study site to serve as basis of comparison from a non-polluted source (Tsikritzis et al., 2002).

2.3 Processing of Samples and Concentration Analysis

Prior to determination of heavy metal concentration, samples collected from the different plant parts were oven dried at 150°C, ash of the dried samples were made in the furnace at 450°C (Ochotorena, 1994). One-half gram of dry samples was digested with 4 ml of 65% HNO_3, and 1 ml of 37% HCl for 20 min. After digestion, the remaining soil and sand particles were removed by filter paper. The digested and filtered samples were diluted with 0.2% nitric acid. At the same time, blank solutions of 1 ml hydrochloric acid and 4 ml nitric acid was also prepared (Tsikritzis et al., 2002).

Soil samples were also oven-dried at 100-105°C. Representative sample was taken by quartering technique and was ground to pass a 60-mesh sieve. About 0.5 g of the sample was weighed into a porcelain crucible and ignited at 450°C in furnace to destroy the organic matter. It was decomposed twice with 10 ml of a 1:1 mixture of concentrated HNO_3 and HF in a 100 ml polypropylene beaker and was evaporated to dryness over a water bath. The residue was dissolved in a 20 ml of 2M HNO_3 and was diluted in a 100-ml volumetric flask (Mitra, 2003).

Table 1 Average Concentrations of Heavy Metals (mean ± SD) in the Soils from Industrial and Residential Sites.

Heavy Metal	Average Metal concentration (mg/kg)	
	Industrial	Residential
Cu	0.847 ± 0.01[a]	0.793 ± 0.01[b]
Zn	3.464 ± 0.04[a]	2.869 ± 0.04[b]
Cd	0.690 ± 0.05[a]	0.688 ± 0.08[a]
Pb	1.390 ± 0.02[a]	1.334 ± 0.02[a]

Metal concentrations are average of three replicates; mean ± SE values followed by the different letter in same horizontal row are significantly different

Aliquots of the plant and soil solutions were taken for the concentration analysis of copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) using a graphite furnace atomic absorption spectrophotometer (AAS). The analysis was performed at the Chemistry Research Center of De La Salle University-Dasmariñas in the city of Dasmariñas, Cavite, Philippines.

2.4 Data Analysis

The degree of heavy metal concentrations for each of the plant sample collected from the study site was measured by comparing it to the heavy metal concentrations of the same plant species collected from the residential site. To determine the significant difference in the heavy metal concentrations among the collected plant species and the pattern of variations in the heavy metals content accumulated in the different plant parts, two-way analysis of variance (ANOVA) was employed. Whenever there is significant difference, Tukey test was used as post-statistical treatment. All statistical analyses were done at 95% level of significance.

3 Results and Discussion

3.1 Concentration of heavy metals in soil sample

Soil samples collected from the industrial site were found to contain significantly ($p<0.05$) higher Cu and Zn concentrations, both considered as essential metals, than those collected from a residential site (Table 1). Concentrations of metals in industrial sites have an average of 0.847 mg kg^{-1} for Cu and 3.464 mg kg^{-1} for Zn. While those collected in the residential sites, concentrations have an average of only 0.793 mg kg^{-1} and 2.869 mg kg^{-1} for Cu and Zn, respectively. However, for the non-essential metals, Cd and Pb, no significant difference was established between the metal concentrations in the soils of industrial and residential sites.

Although significantly higher concentration of Cu and Zn was reported from the samples collected from the industrial site but it did not exceed from the standards set by the Government of China, i.e. 250 mg kg^{-1} for Cu and Zn. These concentrations were also within the range from soils collected at polluted sites in China (Wang et al., 2003) but slightly higher than soils samples collected from agricultural land, pasture lands and forests of Belguim (Aydinalp & Marinova, 2003). High concentrations of metals in soil from the industrial site could be attributed to the industrial activities that pollute the environment with gases containing these heavy elements. Soil is contaminated by material from the air and by direct depositing of pollutants. Most of the industrial plants were operated without taking into consideration the problem of pollution and wastes, and consequently they have no technological ways to manage the problem (Wang et al., 2003; Ching et al., 2008). Areas near heavy industries, including smelters and mining sites, are exposed to the atmospheric deposition of heavy metals, so that such deposition may contribute significantly to the concentrations of metals in the soils (Wang, et al., 2003).

3.2 Accumulation of heavy metals in plant sample

Accumulation and distribution of heavy metals in the roots, stems, and leaves of *B. spectabilis* collected from industrial and residential sites are presented in Table 2. Roots were found to have significantly higher concentrations of Cu as compared to stems and leaves. While for the Zn, leaves were found to accumulate the highest concentration followed by stems and roots. However, Cd and Pb did not show any significant variations of in the distributions to the different plant tissues of the plant sample. Only Cu and Zn, the essential elements, showed significantly higher concentrations in *B. spectabilis* collected from industrial site as compared to the samples collected from the residential site but there was no visible damage or symptoms of contamination on the examined plant parts.

Roots worked as a primarily passageway for all fluids and nutrients spread to the plant tissues, thus it could accumulated higher concentration of metals. Johansson et al. (2005) reported that accumulation of Cu varied with plant species, these researchers reported that in *Pistacia terebinthus* and *Cistus creticus*, most of the Cu was found in the roots, while *Bosea cypria* accumulated most of the Cu in the leaves, in this manner, results of present study are in agreement with *P. terebinthus* and *C. creticus.* Zn is a mobile element and it primarily enters through the roots of the plant species and spread throughout the plant system. According to Herrero et al. (2003), plants have special Zn transporters mechanism to absorb this metal.

Table 2 Accumulation and distribution of heavy metals in plant tissues samples of *B. spectabilis* collected around the Perimeter of an Industrial Park and residential area

Heavy metals	Sites	Average Metal Concentration (mg/kg)			
		Roots	**Stems**	**Leaves**	**Total**
Cu	Industrial	1.256±0.02^{x}	0.142±0.004^{y}	0.835±0.02^{z}	2.233^{a}
	Residential	0.423±0.01^{x}	0.045±0.01^{y}	0.399±0.02^{x}	0.867^{b}
Zn	Industrial	1.208±0.02^{x}	1.544±0.01^{x}	2.150±0.01^{y}	4.902^{a}
	Residential	0.313±0.02^{x}	0.540±0.03^{x}	1.818±0.03^{y}	2.671^{b}
Pb	Industrial	0.629±0.1^{x}	0.676±0.03^{x}	0.677±0.05^{x}	1.982^{a}
	Residential	0.656±0.08^{x}	0.596±0.07^{x}	0.606±0.04^{x}	1.858^{a}
Cd	Industrial	1.490±0.04^{x}	0.964±0.006^{x}	1.484±0.03^{x}	3.938^{a}
	Residential	1.267±0.02^{x}	1.409±0.04^{x}	1.321±0.05^{x}	3.997^{a}

Metal concentrations are average of three replicates; mean ± SE values followed by the different letter a/b shows significantly different between the study site and residential site while mean ± SE values followed by the different letter x/y/z shows significantly different between the various plant tissues

Similarly, Cd, is also a mobile element in the soil and is taken up by plants primarily through the roots. Cd and Pb strengthen the effect of each other's. Further, Cd promotes the accumulation of Zn, but this process decelerated the number of Cu and Pb in soil concentrations (Valizadehfard et al., 2012). Pb is one of the elements that could also be taken by plant through the aerial way. Since that it could pass through the air, there was a high accumulation in the leaves of the plant. Another factor that contributes to the high accumulation of lead in the leaves only was the slow mobility of the metal (Ogundiran & Osibanjo, 2008).

Heavy metal accumulation in the leaves of *L. leucocephala* collected from industrial and residential sites were presented in Table 3. Although there was no morphological symptoms of contamination observed but the concentrations of essential heavy metals, Cu and Zn, in the leaves of plant samples collected from the industrial site were reported as significantly higher ($p<0.05$) than those collected in the residential site. However, in case of non-essential metals, Cd and Pb, there was no significant difference was observed in the metal concentrations in leaves samples collected from both sites.

These results were congruent to the findings of Rehman & Iqbal (2009) in the study of metal transfer ratio in *L. leucocephala* by using soils of industrial areas of Korangi and Landhi, Karachi. Results of this study revealed that the presence of high concentrations of metals in the leaves of the plant could be attributed to other sources like aerial deposition. Non-essential metals, like Cd and Pb, have lesser accumulation as compared to the essential metals, i.e. Cu and Zn. This may be possible because of slower mobility of these metals (Yazaki, et al., 2006).

Conclusion

Soil samples collected from the industrial site were found to have significantly higher levels of essential metals, Cu and Zn, than those collected from the residential site. While for the non-essential metals, Cd and Pb, no significant difference was established between the metal concentrations in the soils of industrial and residential sites. However, heavy metal concentrations in the soils collected from the study site were found to be within the range of non-polluted soil. Higher concentrations of Cu and Zn was reported in the plant sample collected industrial site but this higher concentration is not making any morphological damage, so it can be conclude that these two plant species worked as a potential bio-accumulators. On the contrary, the non-essential elements Cd and Pb did not show any significant variations for both plant samples collected on both sites. Cu accumulated highest in the roots of *B. spectabilis* while its leaves accumulated the highest concentration of Zn. Heavy metals can also be distributed and accumulated by means of aerial deposition, thus metals could be transmitted to the leaves and stems of the plant. Cd and Pb are evenly distributed in all the tissues of *B. spectabilis* having no significant difference on their concentrations.

Table 3 Accumulation and distribution of heavy metals in leaves of *L. leucocephala* collected from the industrial and residential sites.

Heavy metals	Average Metal Concentration (mg/kg)	
	Industrial site	**Residential site**
Cu	3.418± 0.46^{x}	1.181 ± 0.01^{y}
Zn	3.203 ± 0.1^{x}	1.536 ± 0.07^{y}
Cd	0.679 ± 0.06^{x}	0.677 ± 0.06^{x}
Pb	1.487 ± 0.07^{x}	1.411 ± 0.05^{x}

Metal concentrations are average of three replicates; mean ± SE values followed by the different letter in same horizontal row are significantly different

The same pattern of accumulation was observed in the leaves of *L. leucocephala.*

Furthermore, researches must be carrying out in order to establish the phytoremediation capability of plants that are common in industrial sites. Likewise, studies on the interactions among several metal contaminants affecting the uptake mechanisms in plants must also be carried out along with establishing the transformation processes for metal tolerance of different plant species.

Acknowledgements

The researchers express their deepest gratitude to the Dr. Airill Mercurio, Ms. Chona Bandelaria and Ms. Jonnacar San Sebastian of the Biological Sciences Department under the College of Science and Computer Studies of De La Salle University-Dasmariñas for their unwavering support and intelligent inputs in all possible ways.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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