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Avicennia marina (Forssk.) Vierh. as Phytoaccumulator of Sediment Heavy Metals in the Las Piñas – Parañaque Critical Habitat and Ecotourism Area (Philippines)

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ABSTRACT

The aim of the study is to determine the efficiency of *Avicennia marina* in phytoaccumulating the heavy metals in the Las Piñas-Parañaque Critical Habitat and Ecotourism Area (LPPCHEA). Inductively Coupled Plasma-Optical Emission Spectrometry (ICP/OES) was used to determine the chromium (Cr), copper (Cu), and lead (Pb) concentrations of the ten composite samples of sediments and plant organs, namely barks of stems and pencil roots, in the area. The bioconcentration factors (BCF) of each plant organ for each heavy metal were calculated to quantify the efficiency of phytoaccumulation. Results showed that Cu (37.41 ppm) had the highest concentration in the plant, followed by Pb (15.37 ppm) then Cr (4.94 ppm).However, taking into account the BCF values of the heavy metals, it was observed that Pb (1.09) was the highest phytoaccumulated heavy metal, followed by Cu (0.44) then Cr (0.28). Moreover, it was also observed that the average concentrations of Pb (13.55 ppm), Cu (27.27 ppm), and Cr (4.22 ppm) in the sediments were below the standard sediment concentrations. The study suggests that *A. marina* is efficient in phytoaccumulating Pb, having a BCF value greater than one, in LPPCHEA. Furthermore, the root and bark tissues of the mangrove contribute equally to its phytoaccumulating capacity.
Keywords: heavy metals, bioconcentration factor, phytoaccumulation, *Avicennia marina*

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INTRODUCTION

Las Piñas–Parañaque Critical Habitat and Ecotourism Area (LPPCHEA) is recognized as the last coastal frontier of Metro Manila. It serves as a sanctuary for 82 species of migratory birds – two of which are included in the list of threatened species by IUCN – and covers a 36-hectare mangrove forest composed of nine species [1]. Aside from its important role in wildlife conservation, LPPCHEA also serves as a sink to many coastal pollutants due to the presence of its mangrove swamp.

Mangrove ecosystems receive extensive amounts of waste products from different tributaries [2] and among these wastes are heavy metals – prevalent urban contaminants notorious for their adverse health effects. Mangroves function as a sink as they can also alleviate the environmental concentration of these pollutants through phytoremediation [3]. Phytoremediation is a technique of utilizing plants to mitigate environmental pollutions, more often than not, combined with their associated microorganisms. A type of phytoremediation is phytoaccumulation, i.e. the uptake of pollutants by the plants and its subsequent translocation to their different plant parts such as their roots, stems, and leaves.

Numerous studies have shown that certain mangrove species can carry out phytoaccumulation [4-6]. While there are existing studies in LPPCHEA that have indicated the presence of certain concentrations of heavy metals in the mangroves, there are no conclusive evidences proving the phytoaccumulating capacity of these resident mangroves. Thus, in order to assess such capacity, the concentration levels found in these plants must be related to that of their environment. This can be done through the calculation of bioconcentration factor (BCF) values of the mangroves. BCF values greater than one (>1) indicates efficiency of an organism to phytoaccumulate.

The present study aims to find out the phytoaccumulation efficiency of one of the dominant mangrove species, *Avicennia marina*, in the Long Island of LPPCHEA by calculating the BCF values, for certain heavy metals, of the said mangrove species.

MATERIALS AND METHODS

Study area

The study was conducted in the Long Island of Las Piñas – Parañaque Critical Habitat and Ecotourism Area. It is located at the western side of the Manila-Cavite Express Highway and bordered on the north by the Parañaque River and on the south by the Las Piñas River. One time collection of samples was done on October 23, 2013, 11:00 AM – 1:00 PM, low tide (0.01 m) (Figure 1).

Sampling Point	Geographical Position	
1	14° 29' 02" N	120° 58' 44" E
2	14° 29' 02" N	120° 58' 44" E
3	14° 29' 03" N	120° 58' 45" E
4	14° 29' 03" N	120° 58' 45" E
5	14° 29' 04" N	120° 58' 45" E
6	14° 29' 05" N	120° 58' 46" E
7	14° 29' 05" N	120° 58' 46" E
8	14° 29' 05" N	120° 58' 47" E
9	14° 29' 06" N	120° 58' 47" E
10	14° 29' 07" N	120° 58' 48" E

Figure 1: Geographical map of the sampling area

Sampling was done using the Point-Centered Quarter Method (PCQM). A 100-m transect line parallel to the roadside was randomly laid at the forest. Along the transect line, 10 sampling points were made with an interval of 10 meters each.

The plant and sediment samples were all collected using composite sampling technique. At each sampling point, an imaginary line was drawn such that four quadrants were made. One tree per imaginary quadrant was sampled such that one composite sample is derived from four different trees. A total of ten composite samples were collected each for the bark, roots, and sediments.

Collection of samples

Bark of stem and pencil roots (with a length of five to seven inches) were carefully cut and handpicked from the trees. At least twenty grams of each plant part per tree were collected. The plant parts were washed with distilled water. The composite samples of barks and root samples in each sampling point were then separately stored in properly labeled clean plastic bags [7].

Sediment sampling was done where the plant parts were obtained for each of the trees. The methods for acquiring the samples were in accordance with a reported protocol [7].

A stainless steel dipper was used to collect the samples by pushing it firmly downward into the sediment, and then quickly lifting it upward to reduce the amount of fine-grained sediment lost. Then, the sediment samples from each tree were transferred to a stainless steel bowl. These were homogenized using stainless steel spoon and transferred to clean and properly-labeled glass containers. Since there are ten sampling points, ten sediment samples were also collected. The glass containers with sediment samples were then stored in an ice chest filled with bagged ice for transport back to the laboratory.

Digestion of samples

The collected plant and sediment samples were oven-dried at 80°C until completely dried. Afterwards, they were homogenized and pulverized into powder using mortar and pestle and were placed in separate clean Ziplock plastic bags.

Both plant and sediment samples were subjected to acid digestion prior to its heavy metal analysis by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP/OES). The protocol for acid digestion as previously reported [8] was employed. In a 50 mL beaker, ten milligrams of dried sample was mixed with 600 µL of 30% hydrogen peroxide and 1200 µL 60% nitric acid. The mixture was heated at 140 ºC for four hours. Afterwards, the digested samples were diluted with distilled water and then filtered using a 0.22-µm filter paper.

Data analyses

BCF calculation. BCF was calculated as follows [9]:

$$BCF = \frac{C_{biota}}{C_{soil}}$$

where C_{biota} is the concentration of a particular heavy metal in the plant organ (ppm) and C_{soil} is the corresponding heavy metal concentration in the soil (ppm).

Statistical analysis

One-way Analysis of Variance (ANOVA) was carried out using the statistical software, SPSS 20, to determine if there were significant differences in heavy metal accumulation among the samples.

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RESULTS AND DISCUSSION

The Las Piñas-Parañaque Critical Habitat and Ecotourism Area (LPPCHEA) is being surrounded by highly urbanized cities – Pasay City in the northeast; by Bacoor, Cavite in the southwest; a main road (Manila-Cavite Expressway) in the west; and bodies of water – Manila Bay in the east, Parañaque River in the north, and Las Piñas River in the south. Being directly accessible to eight coastal barangays [1] makes it a catch basin for various pollutants including heavy metals. These pollutants pose great threat especially in highly urbanized areas because of their health and environmental hazards; hence the roles of the mangrove forest in LPPCHEA both as sink and phytoaccumulator of heavy metals are highly important.

Phytoaccumulation of heavy metals can be assessed by evaluating their Bioconcentration Factor (BCF) values wherein the ratio of the heavy metal concentration of the plant tissue against that of its environment is calculated. BCF values greater than one indicate that the concentration in the organism is greater than that of the medium (e.g., soil or water), indicating efficient phytoaccumulating activity. In this study, the heavy metals accumulated by the barks of stems and pencil roots of *A. marina* were compared against that of the sediment in LPPCHEA to assess the phytoaccumulating efficiency of the aforementioned dominant mangrove species.

Heavy metal concentration

Based on the results, it was proven that Pb, Cu, and Cr were all accumulated in the different plant parts studied. These results were similar to previous studies [10-13]. *A. marina* showed higher Cu accumulation in root tissues compared to bark tissues, but lower than the surrounding sediment level. The average concentration of Cu in the bark, root, and sediment, respectively, were 3.81 ppm, 6.33 ppm, and 27.27 ppm. This is also the same for Pb and Cr. The average concentration of Pb in the bark, root, and sediment were 0.75 ppm, 1.07 ppm, and 13.55 ppm, respectively. Cr had concentrations of 0.20 ppm, 0.52 ppm, and 4.22 ppm in the bark, root, and sediment respectively (Figure 2).

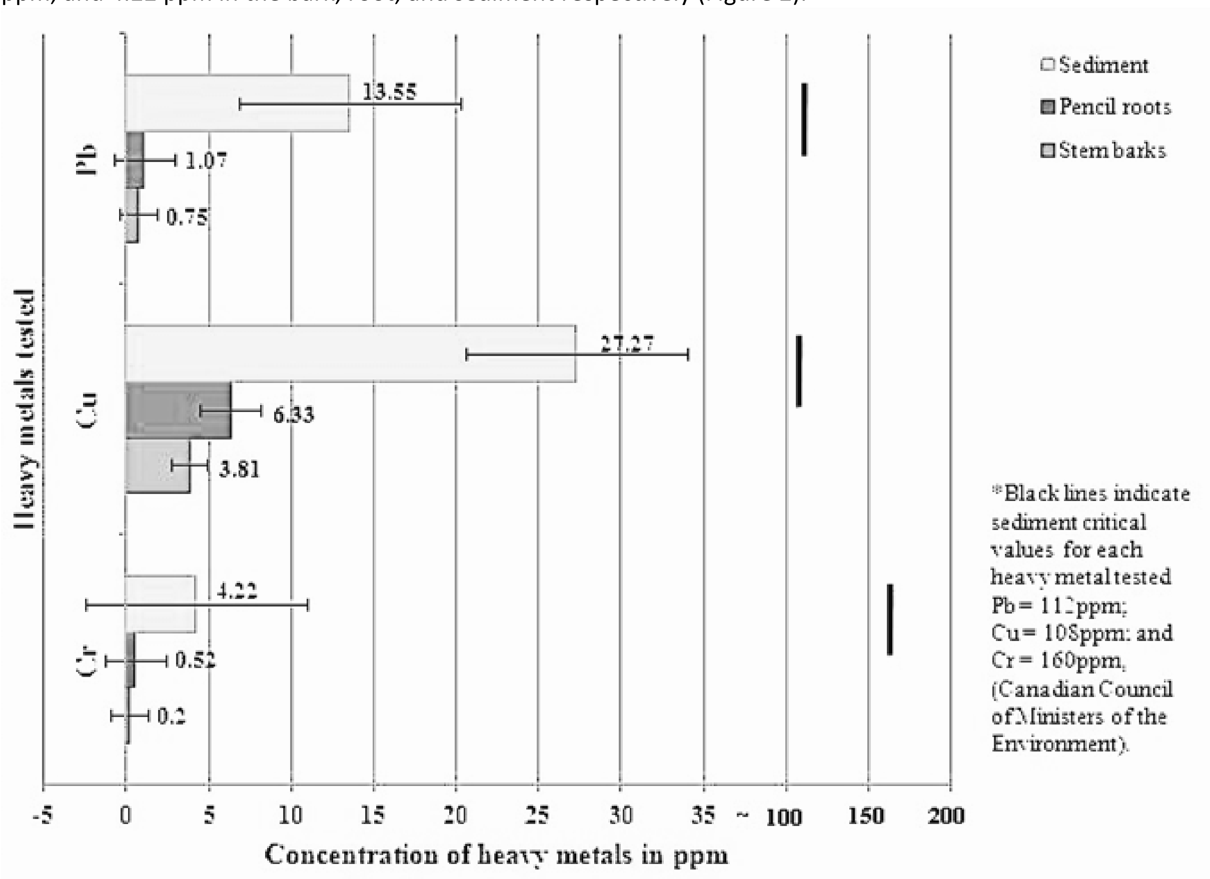

Figure 2: Mean concentration of heavy metals (ppm) in *A. marina* and in the sediments of the Long Island of LPPCHEA compared to the standard sediment heavy metal critical levels according to the Canadian Council of Ministers of the Environment

Based on Fig. 2, the total concentrations for all the heavy metals studied in LPPCHEA were below the critical soil concentrations. This might suggest that the heavy metals in the sediments are leached out of the sediments due to high salinity. Hence, the physico-chemical properties of the soil must also be taken into account in studying accumulation of heavy metals [14-15].

It can also be noted that the highest heavy metal concentrations in the plant were recorded in its root tissues. This coincides with the studies of [3-4, 12]. Plants possess specific co-transport mechanisms in the plasma membrane of their roots, allowing for the acquisition of inorganic metals from the soil [16]. However, because of their toxicity, the transport of these metals is hindered by various structural and physiological plant defense mechanisms. A previous study [16] described the three-line defense mechanism of plants against heavy metal toxicity. The first response is to prevent or reduce uptake into the root cells by restricting metal ions to the apoplast, either by binding them to the cell wall or to extracellular exudates. Moreover, according to a previous study [13], aside from the accumulation of heavy metals in the cell walls of the root tissues, the presence of Casparian strips in the endodermis also hinders the translocation of heavy metals. The Casparian strips serve as barrier to the entry of the heavy metals into the steel, thus inhibiting long distance transport to other plant parts. With the prevention of further conduction of heavy metals into other tissue parts, accumulation is expected to be highest in the roots. Meanwhile, the metals that were able to escape the plant's first line of defense were translocated to other plant tissues, one of which is the bark of the stem. Thus the plant makes use of the second line of defense which is metal chelation in the cytoplasm using organic acids, amino acids, and phosphate derivatives [13]. Moreover, sequestration of excess heavy metals in the vacuole is also employed to relieve their high cytosolic concentration. This is made possible through the use of specific intracellular transporters present in the tonoplast [12]. The secondary phloem component of the bark might have been the site where the heavy metals were accumulated since they function mainly for storage [14].

Copper had the highest total accumulation, 10.14 ppm, followed by Pb, 1.83 ppm, and then Cr, 0.72 ppm, in *A. marina*. Similar results were noted in previous studies [10-11]. This is expected since Cu, compared to Pb and Cr, is part of the micronutrient requirement of plants. Cu is needed by plants in various redox reactions in photosynthesis and respiration; hence it has the highest concentration [11].

However, this does not immediately indicate that Cu is the most phytoaccumulated heavy metal because as an essential element, it is more retained by the plant for its nutritional value. Comparison with sediment heavy metal concentration is still needed to determine the phytoaccumulating capacity of the plant, hence the BCF values were calculated.

Bioconcentration factor

Results of the Bioconcentration Factors (BCF) for each plant tissue are shown in Figure 3. It was observed that the root tissue showed the highest BCF values for all the heavy metals tested, while the bark tissue exhibited the least accumulation based on its BCF. The BCF values for the root tissue were 0.1972 for Cr, 0.2828 for Cu, and 0.9543 for Pb. Meanwhile, the BCF values for the bark tissue were 0.0834, 0.1575, and 0.1370 for Cr, Cu, and Pb, respectively. Furthermore, it was found out that *A. marina* accumulates Cr, Cu, and Pb, 0.2806, 0.4403, and 1.0913 times greater than the sediment levels.

A comparison among the different total BCF values obtained from other studies and the present study was also done in order to validate the experimental values acquired (Table 1).

This study suggests that *A. marina* is efficient in phytoaccumulating Pb in LPPCHEA, having a BCF value higher than one. This finding also coincides with the previous studies [3-4, 6].

Based on Fig. 3, root tissues had the highest mean BCF value compared to bark tissue for all heavy metals. However, using statistical analysis, the difference among the BCF values for root and bark tissues were evaluated to be insignificant ($p > 0.05$). Hence, it can be inferred that the root and bark tissues of the mangrove contribute equally to its phytoaccumulating capacity.

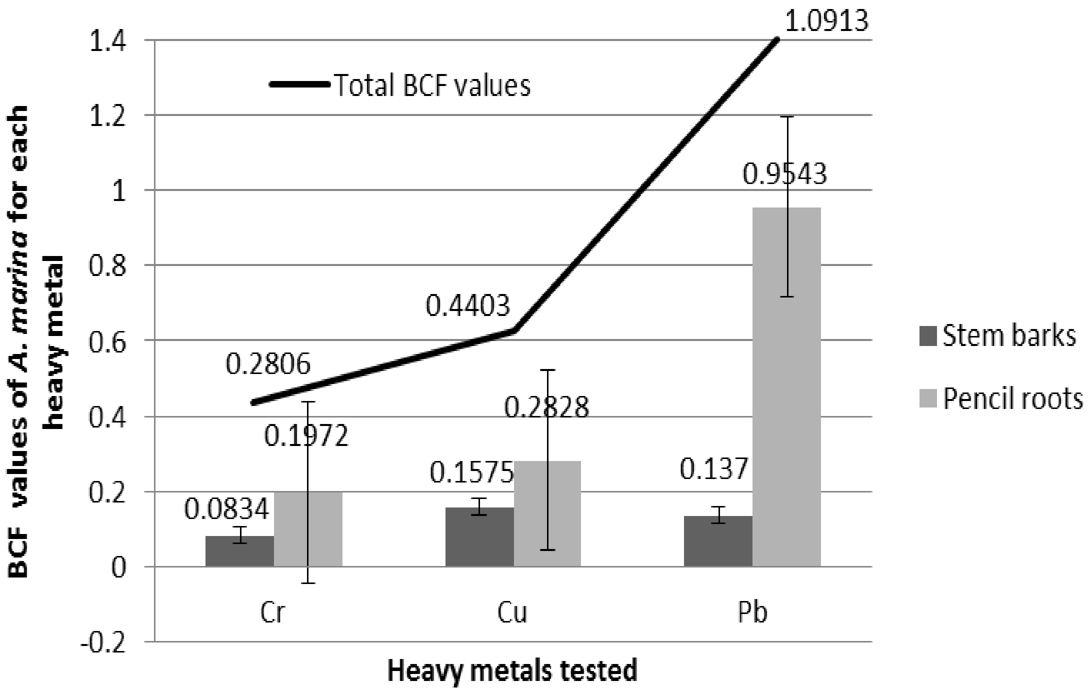

Figure 3: Total Bioconcentration Factor (BCF) values for each organ of *A. marina*

Table 1: BCF values for the plant parts of *A. marina* in the Long Island of LPPCHEA (present study) relative to other countries

Location	Reference	Total BCF		
		Pb	Cu	Cr
Long Island, LPPCHEA	present study	1.0913	0.4403	0.2806
Elephanta Island, India	Shete et al. (2007)	0.1627	-	-
Koparkhairane, India	Shete et al. (2007)	0.1424	-	-
Ghatkopar, India	Shete et al. (2007)	0.4304	-	-
Gujarat, India	Kumar et al. (2011)	1.9500	-	-
Sirik Creek, Iran	Keshavarz et al. (2011)	0.4413	-	-
Pahang, Malaysia	Kamaruzzaman et al. (2011)	0.9500	0.4036	-
Sundarbans, Bangladesh	Chakraborty et al.(2013)	0.2568	0.7619	0.3702

Mangroves have developed structural adaptations to littoral habitat [7]. *A. marina*, in particular, has a fairly complex root system that is of great importance for tolerating environmental stress such as heavy metal pollution. Since the pencil roots of *A. marina* are primarily for gaseous exchange, the presence of high heavy metal concentrations there suggests an adaptive response of the plant to heavy metal stress. Once the heavy metal-loaded water is absorbed by the plant through the underground roots, the metals are transported to the pneumatophores via the apoplastic pathway, thus preventing its subsequent translocation to the other plant parts. Although not included in the present study, the leaves of *A. marina* might also contribute to its phytoaccumulating ability [3, 5, 7, 11]. An adaptation of *A. marina* against heavy metal toxicity is through the use of glandular trichomes and salt glands in the leaves. According to a previous study [13], SEM X-ray microanalysis showed the presence of salt crystals in the glandular tissue on the leaf surfaces. This suggests that excess heavy metals are excreted by glands in order to minimize the risk of high heavy metal concentrations. Hence, these structural and functional adaptations to heavy metal stress clearly show the efficiency of *A. marina* in phytoaccumulating heavy metals from the environment.

CONCLUSION

This study found that Cu, Cr and Pb are accumulated in the tissues of *A. marina*, with Cu having the highest concentration. Accounting the total BCF values of each heavy metal, Pb, having a value greater than one, is the most phytoaccumulated heavy metal. This suggests that *A. marina* is effective in phytoaccumulating

Pb compared to the other heavy metals. The roots stored the highest concentration of the heavy metals and yielded the highest BCF values. However, statistical analysis showed that the differences in the values between the roots and bark are not significant; hence it suggests they both contributed equally to the phytoaccumulating capacity of *A. marina*.

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