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Phytoremediation of Copper and Zinc Contaminated Soil around Textile Industries using *Bryophyllum pinnatum* Plant

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ABSTRACT

Phytoremediation is an acceptable, economical, and eco-friendly way to remediate the metal contaminated soils beside the industrial zone. Like other industries, the textile industries generate the effluent containing several types of pollutants such as metal conjugated dyes, several inorganic and organic substances, etc. When discharged to the environment, metals - specifically heavy metals - exert an adverse impact on soil and other biotas through the food chain. In this study, *Bryophyllum pinnatum* was used for phytoremediation in the contaminated soil sample collected from the area located around textile industries in Kaliakair, Bangladesh. The experiment was carried out by *ex-situ* in earthen pots. The concentration of six heavy metals including Zn, Cu, Ni, Cr, Pb, and Cd was analyzed before applying phytoremediation. Two heavy metals, Cu (28.57 µg/g) and Zn (143.88 µg/g) were found and others were not detected in that soil. After planting of *Bryophyllum pinnatum*, the concentrations of Cu and Zn in the contaminated soil were analyzed at three intervals of 45 days (S_3), 90 days (S_4), and 135 days (S_5) in three replications. The experiment revealed that there was a decline in the concentration of Cu in soil (27.08 µg/g for 45 days and 13.19 µg/g for 90 days) except for the 3rd replication of 135 days (S_5). However, the concentration of Zn (mean 103.09 µg/g) in soil was measured at 45 days and then remained within nearer values of concentration for other replications. The amounts of heavy metals uptake for both Cu and Zn by plants can be presented as leaves> stem >root which indicated that heavy metals were transferred from root to shoot over time. *Bryophyllum pinnatum* can, therefore, be considered as a good hyperaccumulator plant having BCF>1 and TF>1 values as well as possessing a better capacity of phytoextraction of metals.

Keywords: contaminated soil, heavy metal, phytoremediation, *Bryophyllum pinnatum*

INTRODUCTION

Pollution of the environmental segments due to anthropogenic activities is inevitable. Different physical, chemical, and other approaches were suggested and also applied to improve the condition of the contaminated soil with mixed results. Contamination of heavy metal exposes a crucial health concern as metals might be transferred to human beings along with livestock (Dubey et al., 2018). Heavy metals such as Cd, Cu, Pb, Cr, and Hg are considered as prime contaminants in the environment, specifically, where there is a vast amount of anthropogenic load (Nagajyoti et al., 2010). The heavy metals readily found in the textile effluent are As, Cd, Cu, Fe, Hg, Cr, and Ni (Nagajyoti et al., 2010). Heavy metals like cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and chromium (Cr) are extensively used for the manufacturing of textile dyes. Different heavy metals are linked with the configuration of dyes. When textile effluents arrive in the soil; there are possibilities of soil to come into contact with metal. Watering the agricultural lands by using different industrial wastewaters may alter the chemical, physical and biological properties of soil (Mani et al., 2019). Cu, Pb, and Cr were the prominent heavy metal from the dyeing, glass, and textile industry, at Tangail, Bangladesh (Tusher et al., 2018).

Phytoremediation refers to a combination of plant-based technologies that utilize the naturally occurring plants as well as genetically engineered plants for removing pollutants from environment (Prasad and De Oliveira Freitas, 2003). It is considered an environmentally friendly, appealing, soothing, non-harmful, energy-efficient, and beneficial mechanism to remediate the area with low-to-moderate intensities of heavy metal(loid) s (Sabir et al., 2015). It is based upon several methods such as phytodegradation, phytovolatilization, phytoaccumulation, and phytoextraction (Muthusaravanan et al., 2018). Cu, Zn, Cd, Ni, Cr, and Pb can be accumulated by various plants like *Calandulaofficinalis, Arabidopsis thaliana, Cynodondactylon, Brassica juncea, Bidenspilosa, Helianthus annuus* (Alaboudi et al., 2018; Goswami and Das, 2016; Wei et al., 2018). The greatest number of plants (more than 300 species) take up Zn, Ni, and Cu preferably; hence, these heavy metals are the most appropriate choice for the method of phytoextraction (Gupta and Balomajumder, 2015; Muthusaravanan et al., 2018). Metal accumulator plants possess the capacity of gathering heavy metals in their tissues remaining above ground without any indication of toxicity emerging (Srivastava, 2016). The plant tissues can accumulate the existing heavy metals from the soil**;** moreover, these metals can gather in the trophic levels of the food web by entering the biosphere (Shah and Daverey, 2020).

Heavy metals are released into the soil and become accumulated because of the use of agricultural chemicals, municipal wastages, and contaminated industrial wastewater (Kumar et al., 2019). Indiscriminate discharge of industrial effluents, waste of mines, community litters along with heavy metal polluted sludge are the major reasons for soil contamination (Mao et al., 2015; Ye et al., 2017). Conversely, the delivery of industrial effluents helps to enrich the irrigated lands by storing nutrients for microbes (Jain et al., 2005; Li et al., 2015). Microbial diversity and their associated activities are adversely affected by the heavy metals due to contamination for a longer period (Chen et al., 2014). Toxic metals are non-degradable and accumulated in nature, as a result they consequently enter into the food chain (Patra et al., 2020). The model plant which is to be applied for improvement of heavy metal-contaminated soils needs to possess some special features like fast-growing capacity in several climatic situations and easily cultivable which facilitate the diminishing of significant quantities of toxicants (Pinto et al., 2015). For instance, *N. mucronata*-acts as the best accumulator for several heavy metals such as Pb, Zn, Cu, Ni, and Cd (Chehregani et al., 2009). In terms of heavy metal removal, hyperaccumulator plants do not preclude the metals to reach into the roots, instead they permit the accretion of metals into the biomass of plants (Patra et al., 2020).

MATERIALS AND METHODS

Study site

The soil sample used in the study was collected from Kaliakair Upazila of Gazipur, Bangladesh. Gazipur is an industrial area with having a total area of 1,1741.53 square kilometers and a total population of 3403912, an annual average temperature maximum of 37 °C and a minimum of 10 °C; average rainfall of 642.06 mm. The location of Kaliakair is specified by latitude: 24° 10’ 0” N and longitude: 90° 10’ 0” E. There are many industries established in Gazipur, such as chemicals, textile, knitting, dyeing, finishing, garments, etc.

Collection of soil and plantation of *Bryophyllum pinnatum* in ex-situ

The contaminated soil was collected on 17th March 2020 around Textile industries, Bangladesh (Figure 1). The soil sample weighing a total of ninety kg was collected randomly from nine different points at a single spot of Kalaikair from a depth of 0 cm to 15 cm. An equal amount of soil was taken of the above-mentioned depth from each point of the sampling site. Finally, soils were mixed scientifically to make one composite sample. The pH of the soil sample was measured by a pH meter (Jenway 3305) using a 1:5 soil to water ratio. The composite soil sample was spread on a sheet of paper and the loose aggregated soil was broken gently. Then the soil sample was sun-dried for 5 days in the presence of air. The dried weight of the composite soil was approximately 50 kg.

A total of nine earthen pots were prepared to accommodate three replications of the plantation (3 pots in each replica) for three different intervals of 45 days (S_3), 90 days (S_4) and 135 days (S_5) in *ex-situ*. Each pot was filled with 5 kg of soil. An additional replica was made and further prepared to measure the percentage of organic matter and soil texture of contaminated

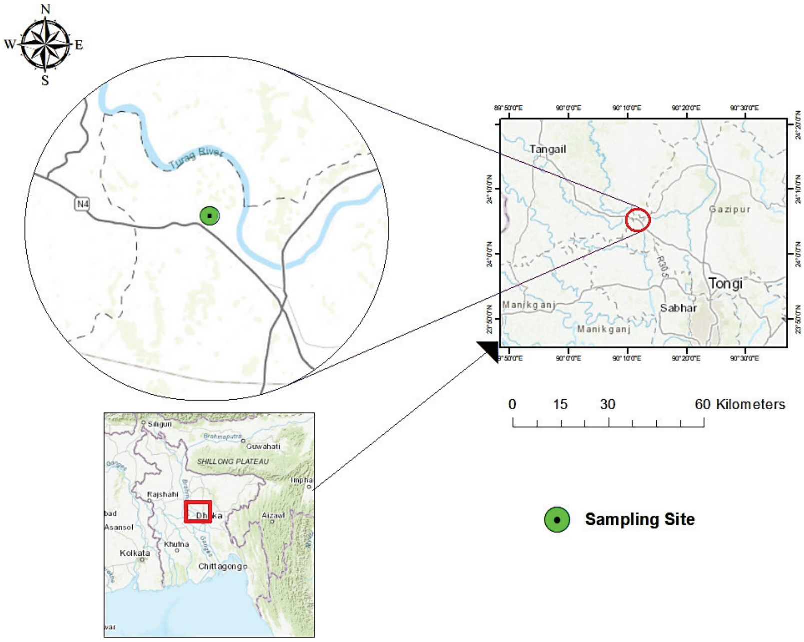

Figure 1. Soil collection site (Kaliakair, Gazipur, Bangladesh)

soil before planting seedlings. Two seedlings of *Bryophyllum pinnatum* (approximate age of 15 days) were planted in each earthen pot following the regular watering was done on the plants. Finally, after completion of the specific intervals, soils were collected from the earthen pot and stored in the labeled jar. A periodic analysis was performed to obtain the result of the phytoremediation of heavy metal.

Sample preparation and chemical analysis of soil and plant

The textural class of the soils was measured by using a triangular diagram following the USDA hydrometer method. The organic matter content (%) was experimented by Walkley and Black's wet oxidation method. After uprooting the plants from the different pots having various intervals (45 days, 90 days, and 135 days), the different parts of the plants such as root, stem, and leaves were separated for each replication. The plant parts were washed mildly by distilled water for about 2.5 minutes to remove the adhered soil particles from the plants. Then those plant parts were dried in the microwave oven at 80ºC and ground by using a grinding machine to facilitate subsequent testing. The dried plant samples were filtered with a 1 mm sieve. The soil samples were also sieved in the same way. The respective soil and plant samples were digested using the hot-block digestion method (USEPA 3050B) for measuring the total concentration of various metals such as Ni, Cr, Pb, Cu, Zn, and Cd that were analyzed by atomic absorption spectrophotometer (Varian AA240).

Statistical analysis and graphical presentation

Three replications were made and three values of metal concentrations were taken from three pots under each replication for soil, root, stem, and leaves to obtain the average value. The statistical analysis was carried out using one-way ANOVA (analysis of variance) followed at ($p<0.05$) significance level. The data summary and calculations were performed using Microsoft Excel, and the graphical representation was done using R Studio v.1.3.1093. The map of the soil collection site was made by means of ArcGIS 10.4.1 software.

RESULTS AND DISCUSSION

Physicochemical properties of soil

The observed result of pH 5.65 indicates that the raw contaminated soil is acidic. In the case of metal cations, lower pH resembles the higher mobility and availability for plants (Shah and Daverey, 2020). Contrarily, at higher pH, these ions show the tendency to be absorbed in the soil, resulting in the reduction of such movement and further its availability as well as this phenomenon is correct for the anions of metals (Kader et al., 2016; Shaheen et al., 2013). Tusher et al., (2017), found the value of soil pH of 4.4, 6.1, and 6.4 around the dyeing, glass, and textile industries respectively in Tangail, Bangladesh. A higher value of pH than in the present study was found from the sampling sites of Padaeng zinc mine area, Thailand within the range of 7.1–7.6 (Phaenark et al., 2009). This result indicated that the soil pH around industrial zone remained within the range of acidic to neutral value.

According to the soil texture triangle, the collected soil from around textile industries comprises clay, silt, and sand having an amount of 14.075%, 40.375%, and 45.55% respectively. Therefore, the soil has loamy characteristics. Herlina et al., (2020) found the properties of soil containing clay 11.67%, silt 45.78%, and sand 42.55%. This study observed nearly similar results of the soil texture from the contaminated soil in Gedang Anak village, Ungaran Timur district Semarang, Indonesia. The texture of soil plays a crucial role in the availability of metals in the soil. For instance, the soil having fine texture holds about eight times higher quantity of heavy metal - such as Pb - compared to the soil having coarser texture (Sherene, 2010). Volk and Yerokun, (2016), observed the presence of 1.7 times higher bioavailable concentration of Cobalt and Chromium in clay loam soil compared to sandy loam soil while studying the route of these metals in different soil fractions.

Table 1. Physicochemical properties of contaminated soil

Parameters	Value	Remark
pH	5.65	Acidic
Organic content (%)	0.11%	Presence of a slight amount of carbon
Soil texture		
Clay	14.075%	Loam
Silt	40.375%	
Sand	45.55%	

Moreover, the amount of organic matter content (%) in the soil of the study area around textile industries at Kaliakair, Gazipur showed the result of 0.11%. This result resembles the lower organic matter content than the previously done work in Konabari, Gazipur having the range 0.6-1.19 % (Islam, 2012). The average organic matter content (%) in the soil was 6.45, 4.52, and 1.31% in surface soil around the dyeing, textile, and glass industry in Tangail, respectively (Tusher et al., 2017). Therefore, in the present study, there is a presence of a small amount of OM content in the soil around Kaliakair, Gazipur beside textile industries compared to the previous work in the Konabari and Tangail area. According to long-term fieldwork, it is observed that the addition of organic matter to soil impacts the immobilization of metal because of the holding capability OM and reveals the positive influences on the availability of metal (Cambier et al., 2014).

Concentration of metals in the control soil and plants

As shown in Table 2 and 3, there was presence of Cu (16.62 μg/g) and Zn (78.06 μg/g) in control soil. The control plants possess some amount of metals in the root, stem, and leaves already after 45 days. The reason behind this distribution of heavy metals into the different parts of the control plant from the soil might be because of the availability of some amount of metals in the soil generally.

Uptake of Copper by *Bryophyllum pinnatum* from soil

Figure 2 indicates that the concentration of Cu (μg/g) in the root tends to decrease with time. However, the stem and the leaves tend to show the accumulation of Cu in the increasing

Table 2. Concentration of Cu in the control soil, contaminated soil and plants

Sl. No.	Period (days)	Sample ID	Cu Concentration (μg/g)			
			Soil	Root	Stem	Leaves
01	–	S_0	16.62	–	–	–
02	–	S_1	28.57	–	–	–
03	45	S_2	–	40.39	15.1	10.18

Note: S_0 – control soil; S_1 – contaminated soil; S_2 – control plant (plant from uncontaminated soil).

Table 3. Concentration of Zn in the control soil, contaminated soil and plants

Sl. No.	Period (days)	Sample ID	Zn Concentration (μg/g)			
			Soil	Root	Stem	Leaves
01	–	S_0	78.06	–	–	–
02	–	S_1	143.88	–	–	–
03	45	S_2	–	385.35	246.91	102.36

Note: S_0 – control soil; S_1 – contaminated soil; S_2 – control plant (plant from uncontaminated soil).

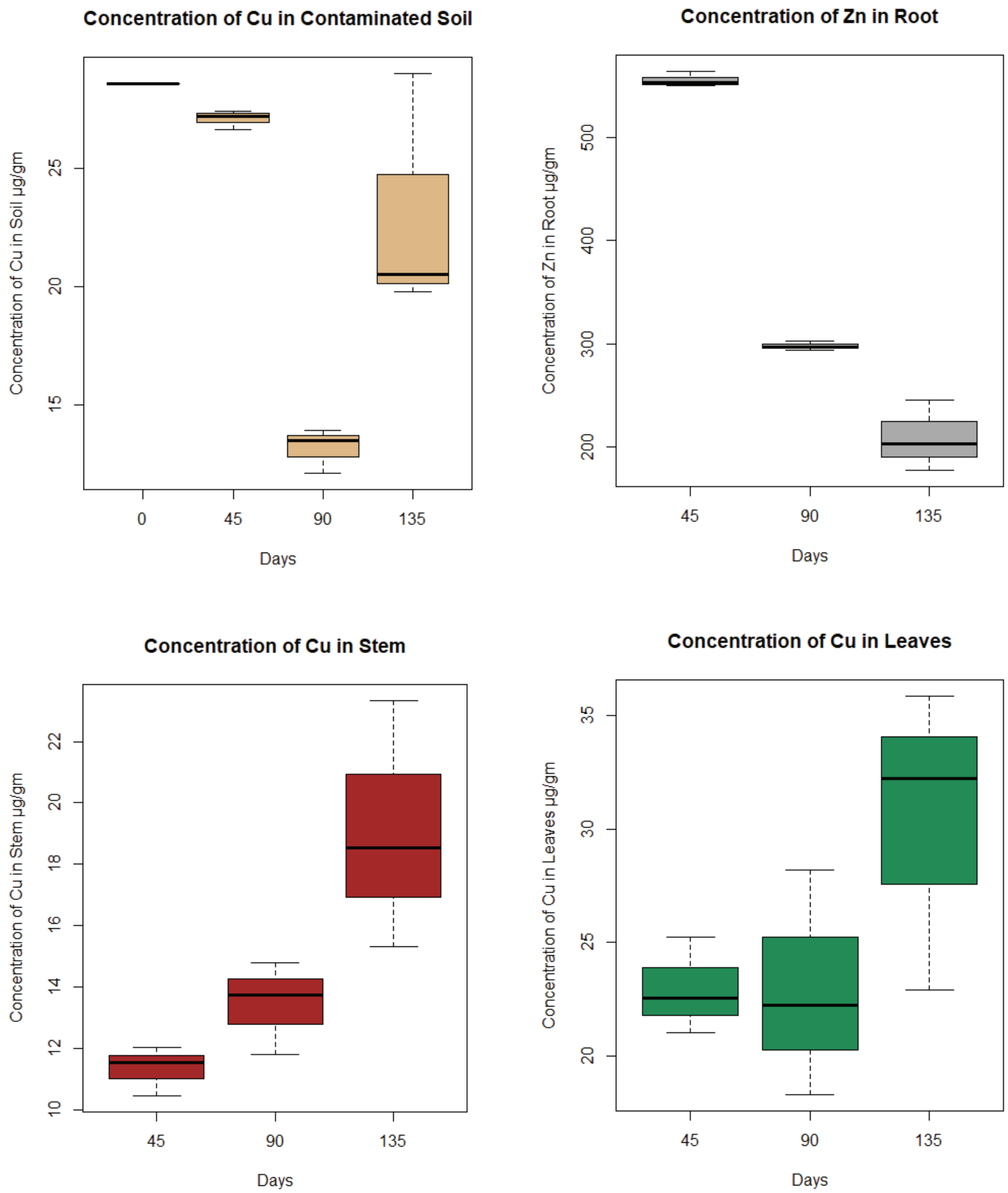

Figure 2. Concentration of Copper in contaminated soil, root, stem, and leaves at different intervals (45, 90, 135 days)

mode as time passes by. On the other hand, the soil does not show sequential results regarding the Cu accumulation. Here, the other mechanisms might go on due to the soil-microorganism interaction besides the accumulation of metals by plants at different intervals of the experiment. Most soil animals, including protozoa, nematodes, collembola, mites, earthworms, etc. achieve their resources through the consumption of bacteria, fungi, or plant roots (Morris and Blackwood, 2015). For the removal of heavy metals, Ekwumemgbo et al. (2013), collected the soil samples from an industrial layout in Kano State, Nigeria, and analyzed for the total concentration of Cd, Cr, Cu, Ni, Pb, V, and Zn. By applying *Bryophyllum pinnatum* for remediation of the above mentioned heavy metals and observed the reduction of heavy metals due to phytoextraction. In their study, the total uptake by plants might be expressed in the sequence of Zn > Pb > Co. The uptake of metals by different parts of the plant showed a direction of leaves > stems > roots that might be established by that study (Ekwumemgbo et al., 2013). According to ANOVA analysis at ($p<0.05$) significance level, the concentration of Cu in soil, root, and stem of the *Bryophyllum pinnatum* plant showed significant variation in the present study. Conversely, the concentration value of Cu in leaves did not show such variation.

Uptake of Zinc by *Bryophyllum pinnatum* from soil

Figure 3 indicates that the concentration of Zn (μg/g) in the root of plant *Bryophyllum pinnatum* tends to decrease with time. However, the leaves tend to show the accumulation of Zn in the increasing mode for the time passes by; and the stem also tends to do so with the slight decreasing mode in the last observation of 135 days. In the case of the soil, the concentration of Zn in soil was decreased at 45 days (average103.09 μg/g) and then remained almost the same for other replications. Olegario et al., (2010) showed that the reduction or oxidation of metal occurred by microorganisms directly or by the reducing/oxidizing agents generated by those organisms. Redox reactions decrease the phytotoxicity of heavy metals by converting the mobile, toxic metals into non-mobile, non-toxic forms (Ma et al., 2016). According to ANOVA analysis at ($p<0.05$) significance level, the concentration of Zn in the root, and leaves of plant *Bryophyllum pinnatum* showed significant variation. However, the concentration of Zn in soil and stem did not show such variation. The roots take up metals by two processes known as symplastic transport or by apoplastic transport (Ling et al., 2017; Thakur et al., 2016). Furthermore, the texture of the soil is regarded as an important criterion for the phytoavailability of heavy metals and finally for the efficacy of phytoextraction (Antoniadis et al., 2017; Liu et al., 2018).

Table 4. Distribution of Cu in soil, root, stem, and leaf of *Bryophyllum pinnatum*

Sl. No.	Period in days	Sample ID	Cu Concentration (μg/g)			Accumulation factor		
			soil	root	stem	leaves	*BCF	*TF
Replica-1								
01	45	S_3	27.40	92.33	12.01	25.25	3.37	0.40
02	90	S_4	13.92	28.64	11.79	28.19	2.06	1.39
03	135	S_5	20.53	20.48	15.30	22.90	0.71	1.86
Replica-2								
04	45	S_3	26.63	53.62	10.45	21.04	2.01	0.59
05	90	S_4	12.14	36.23	14.78	18.29	2.98	0.91
06	135	S_5	19.78	22.76	23.34	35.87	1.15	2.60
Replica-3								
07	45	S_3	27.2	75.26	11.53	22.53	2.77	0.45
08	90	S_4	13.5	32.63	13.75	22.25	2.42	1.10
09	135	S_5	20.5	22.52	18.52	32.23	1.09	2.25

Note: S_3 – plant from contaminated soil (after 45 days); S_4 – plant from contaminated soil (after 90 days); S_5 – plant from contaminated soil (after 135 days).

*BCF, Bioconcentration Factor – metal concentration ratio of plant roots to soil (Herlina et al., 2020; Yoon et al., 2006)

*TF, Translocation Factor – metal concentration ratio of plant shoots to roots(Herlina et al., 2020; Yoon et al., 2006).

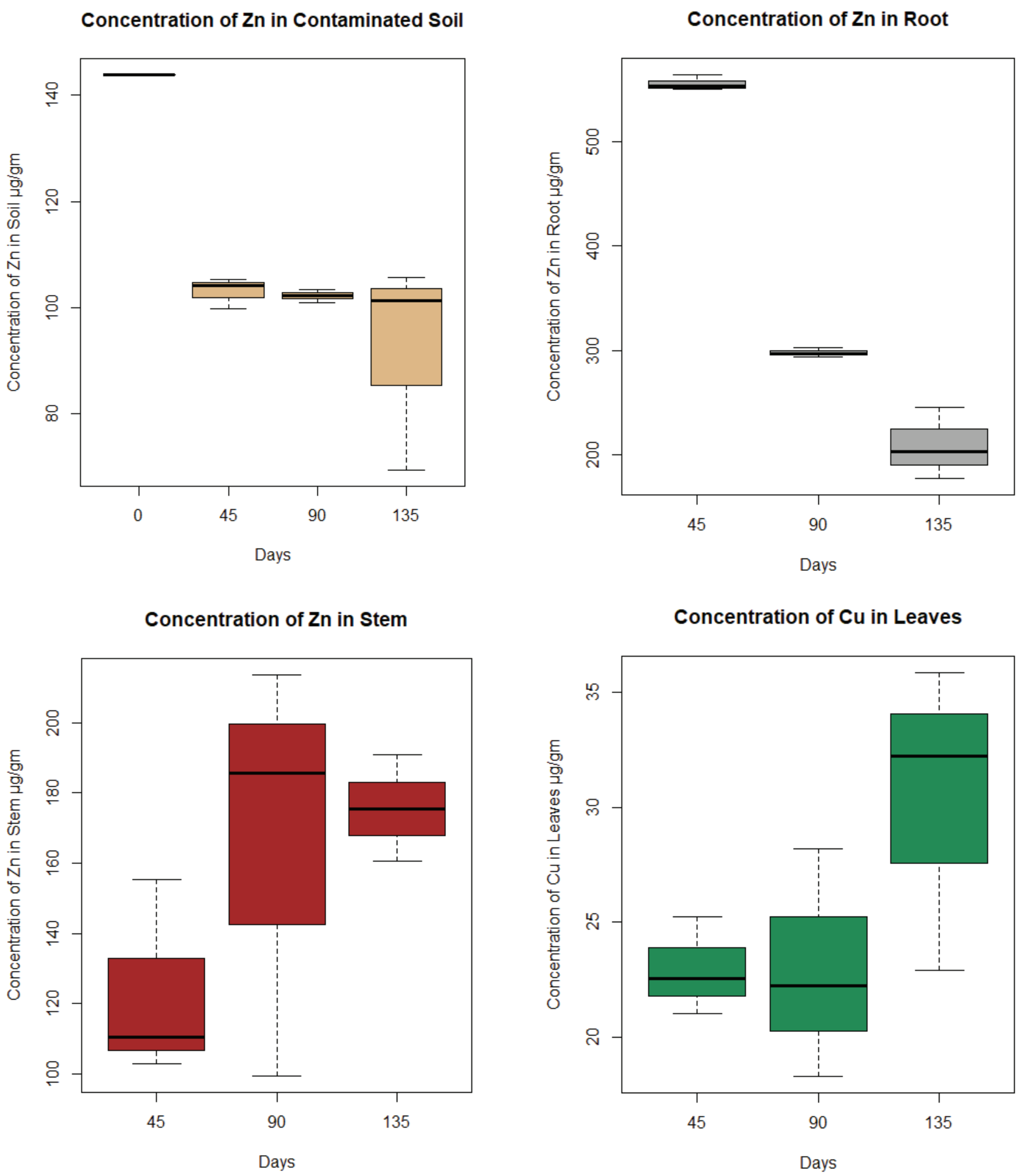

Figure 3. Concentration of Zinc in contaminated soil, root, stem, and leaves at different intervals (45, 90, 135 days)

Bioaccumulation and Translocation factor of *Bryophyllum pinnatum* for Cu and Zn

The bioconcentration factor of *Bryophyllum pinnatum* for Cu tends to show a decreasing trend with the passage of time maintaining the sequence 45 days>90 days>135 days. However, the translocation factor of *Bryophyllum pinnatum* for Cu tends to show an increasing trend with the passage of time maintaining the sequence of 45 days<90 days<135 days. Bioconcentration factor (BCF) was calculated as the metal concentration in root divided by the metal concentration in the soil; translocation factor (TF) was calculated as the metal concentration in shoots divided by the concentration in roots (Herlina et al., 2020; Yoon et al., 2006). Again, the bioconcentration factor of *Bryophyllum pinnatum* for Zn tends to decrease with the passage of time maintaining the sequence 45 days>90 days>135 days. Likewise, Yang et al.,

Table 5. Distribution of Zn in soil, root, stem, and leaf of *Bryophyllum pinnatum*

Sl. No	Period (days)	Sample ID	Zn concentration (µg/g)				Accumulation factor	
			soil	root	stem	leaves	*BCF	*TF
Replica-1								
01	45	S_3	105.27	563.83	155.25	220.13	5.36	0.67
02	90	S_4	103.45	302.99	99.40	261.28	1.41	1.19
03	135	S_5	69.47	177.65	190.91	250.73	2.55	2.49
Replica-2								
04	45	S_3	99.78	549.45	102.80	180.49	5.51	0.52
05	90	S_4	101.02	293.95	213.62	233.41	2.91	1.52
06	135	S_5	105.79	245.80	160.44	271.55	2.32	1.76
Replica-3								
07	45	S_3	104.23	552.58	110.50	205.23	5.30	0.57
08	90	S_4	102.25	296.87	185.52	240.25	2.90	1.43
09	135	S_5	101.36	202.98	175.36	280.52	2.00	2.25

Note: S_3 – plant from contaminated soil (after 45 days); S_4 – plant from contaminated soil (after 90 days); S_5 – plant from contaminated soil (after 135 days).
*BCF, Bioconcentration Factor – metal concentration ratio of plant roots to soil (Herlina et al., 2020; Yoon et al., 2006)
*TF, Translocation Factor – metal concentration ratio of plant shoots to roots(Herlina et al., 2020; Yoon et al., 2006).

(2020) showed that the concentrations of Zn remained much higher in roots than in shoots. However, the translocation factor of *Bryophyllum pinnatum* for Cu tends to increase with the passage of time maintaining the sequence of 45 days<90 days<135 days. The study of Shi et al., (2016) identified that the two species, i.e. *R. chinensis* and *L. formosana* had noticeably higher translocation factor values for Pb (0.88) and Zn (1.78). The present study reveals a similar higher TF of *Bryophyllum pinnatum* for Zn (TF average 1.5; having a range from 0.52 to 2.49) and for Cu (TF average 1.28; having a range from 0.40 to 2.6). Therefore, this *Bryophyllum pinnatum* can be regarded as a hyperaccumulator plant (BCF>1) with better phytoextraction capacity because of the transfer of the metal to the stem and leaves from the roots over time. A translocation factor greater than 1 specifies favored segregating of metals to the shoots (Branquinho et al., 2007). On the other hand, the plants revealing TF and specifically BCF values less than one are unsuitable for phytoextraction (Fitz and Wenzel, 2002). Hypraccumulator plants take up toxic heavy metals in the tissues of the above-ground part of the plant, whereas non-hyperaccumulators receive the metals in the below-ground plant tissues (Sharma et al., 2016).

CONCLUSIONS

The presence of two heavy metals, i.e. Cu and Zn, was observed in the contaminated soil around the textile industries at Kaliakair, Gazipur, Bangladesh. The other metals Cd, Cr, Pb, and Ni were not found in that tested soil. The fresh plants initially possess some heavy metals because of the availability of metals in the contaminated soil. The plants extract these metals from the soil as nutrients. As *Bryophyllum pinnatum* is generally a non-edible plant, there is less chance of transfer of heavy metal to the human being through the food chain. The amount of heavy metals in the contaminated soil tends to be decreased over time after the planting of *Bryophyllum pinnatum*. Thus, this plant acts like a hyper-accumulator. Initially, the root takes up more metals than the stem and leaves of the plants, but when time goes on gradually, the stem and leaves accumulate more metals compared to the root. It can be decided that when the plants finish the growing stage, they are not likely to store nutrients in their root. Therefore, *Bryophyllum pinnatum* can be safely used as a phytoremediator.

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