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Research Article

Bioaccumulation of heavy metals by macrophytes in Ghodaghodi Lake, a Ramsar site in Nepal

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Abstract

This research investigates the heavy metals (Zn, Cd, Cu, and Pb) concentration in water, sediments, and macrophytes in Ghodaghodi Lake during the winter and summer seasons of 2019 to find out the bioaccumulation of metals and phytoremediation potential of locally available macrophytes. An atomic absorption spectrophotometer with an acid digestion method was used for the analysis of heavy metals. The mean heavy metals concentration during the summer and winter seasons were in the order of Zn > Cu > Pb > Cd in water and sediments. The mean concentrations of Cd, Cu, and Pb were significantly higher in winter (0.79±0.71, 12.99±3.16, and 11.39±6.88 mg/g, respectively) than in summer season (0.01±0.00, 9.13±5.21, and 2.16±6.64 mg/g, respectively) in sediments which is also supported by geo-accumulation index. Zinc had also higher concentrations in winter (65.29±13.13 mg/g) compared to summer (44.00±21.08 mg/g), though not significant. *Ludwigia* sp. was more capable of accumulating heavy metals than the *Nymphaea* sp. in the winter season. On the other hand, *Nelumbo* sp. was more effective in accumulating heavy metals (except Zn) compared to *Ludwigia* sp. in the summer season. The highest bioaccumulation factor (macrophyte/sediment) for *Ludwigia* sp. (common in both seasons) was observed for Cd in winter and Pb in summer season. Thus, although there were some variations in the potentiality of bioaccumulation among the species, these macrophytes are capable of accumulating heavy metals providing scope in the bioremediation field.

Keywords Bioaccumulation; heavy metals; lentic ecosystem; macrophytes

Introduction

Wetlands act importantly as sinks, i.e., filter heavy metals having toxic effects on biota (Keller et al., 1998). Human-induced activities as well as naturally available chemicals are major causes of heavy metals in water and biota (Förstner & Wittman, 1981). Studies have specified that heavy metals such as Pb, Cd, Zn, and Cu are some of the chief pollutants in lentic ecosystems because of their environmental persistence, toxicity, bioaccumulation, and biomagnification ability in food webs (Zhang & Shao, 2013; Yang et al., 2014). When heavy metals are above the limit values, they could be a source of damage to the environment (Sierra et al., 2017).

Metals get settled out in the water column, but in lakes, they are likely to be re-suspended and cause secondary contamination to the water environment (Tao et al., 2011). Pollution of natural water bodies can be revealed by increased concentrations of heavy metals in sediments and aquatic plants (Borovec, 1996). Surface sediments normally trap considerably higher amounts in comparison to water bodies which defines the need for higher exploration of heavy metals in the case of surface sediments (Sundaray et al., 2011). Aquatic plants can absorb heavy metal elements via roots and/or shoots resulting in their bioaccumulation in the plant tissues. Various species of aquatic plants show different behavior regarding their ability to accumulate elements in roots, stems, and/or leaves (Pip & Stepaniuk, 1992; Jackson, 1998). For this study, dominant macrophytes in the study area were used for the analysis as representative as they were ubiquitous, easily collected and identified (Franzin & McFarlane, 1980), and as being an unexplored plant species for such studies.

Lentic ecosystems are more prone to the problem of heavy metal pollution due to low capacity of self-purification and pollutant dispersal as compared to lotic systems (Rai et al., 2007). The present study area is one of the important wetlands (Ramsar site) in the western part of Nepal that is a habitat for important aquatic organisms making it a most productive ecosystem. Additionally, the threat from heavy metal pollution entering the food chain of an aquatic ecosystem and its severity is still a limited subject in Nepal that needs to be addressed. The present study attempted to quantify some heavy metals in water, surface sediments, and macrophytes to find out the bioaccumulation of metals and phytoremediation potentials of two major macrophytes in the Ghodaghodi Lake. The study will help to estimate and make reference baseline data for

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long-term monitoring of the lentic ecosystem and trace elements relationship.

Materials and Methods

Study Area

The study was conducted in Ghodaghodi Lake (Fig. 1) which is a Ramsar site in the lowland of Western, Nepal (28°41'00" N and 80 °56' 45" E). It was established in August 2003 with an area of 2,563 ha (6,330 acres) in Kailali district at an altitude of 205 meters above sea level on the lower slopes of the Siwalik. It consists of 13 large and shallow oxbow lakes and ponds with other connected marshes and meadows (Bhuju et al., 2007), and Ghodaghodi is the major lake in the system. The lake area is surrounded by deciduous mixed forest dominated by *Shorea robusta* (Sal). The lake does not have direct input through the stream but receives water from atmospheric inputs and runoff. There are two outlets on the southern part near the Mahendra highway (along the sampling site G1 in Fig. 1). Some anthropogenic activities in the lake area include overexploitation of wetland resources by the people depleting the ecosystem services (Karki & Thomas, 2004; Lamsal et al., 2015) that still exists.

Methods

The study was conducted in the winter (January) and summer (June) seasons of 2019. Surface water, sediments, and macrophytes were randomly sampled based on different land use patterns at 13 sites (Fig. 1, right). *In-situ* determination of water physico-chemical, which includes temperature, pH, dissolved oxygen (DO), conductivity, and total dissolved solids, was done before collections of sediments and macrophytes.

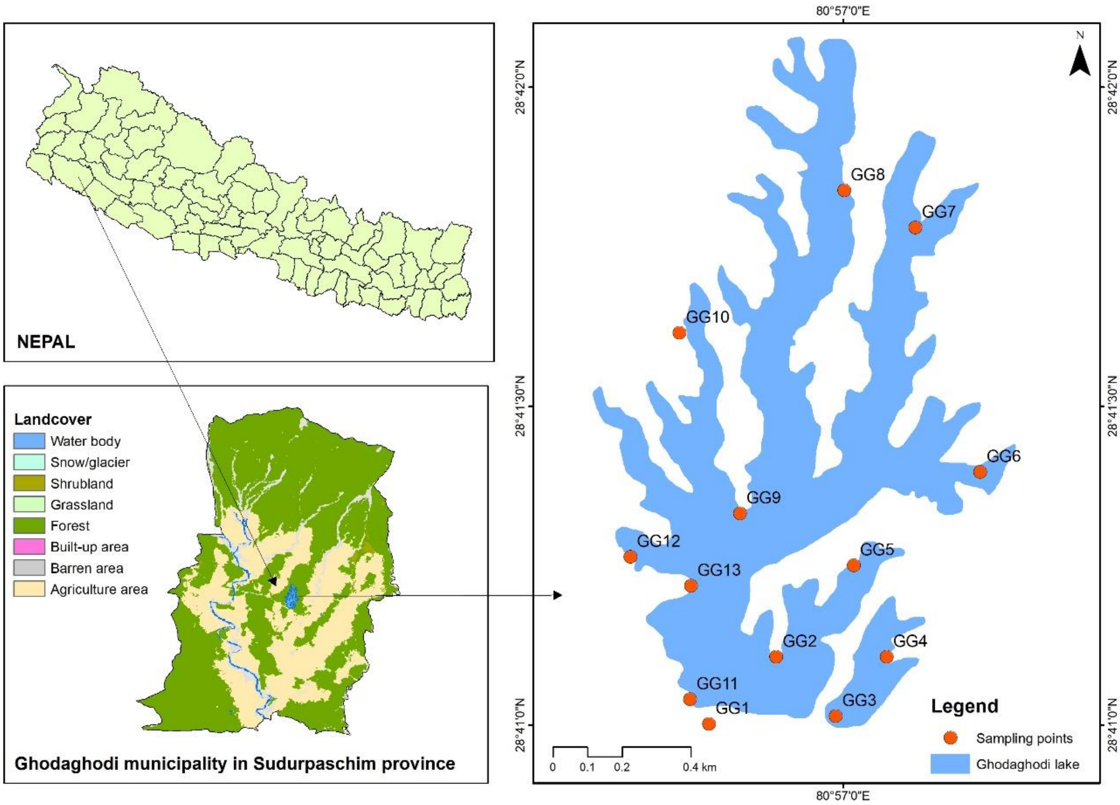

Figure 1 Location of the study area in Nepal map (upper-left), administrative map (lower-left), and Ghodaghodi Lake with sampling sites (right)

At each sampling site, a 500 mL water sample was collected in a plastic bottle at a depth of about 0.25 m and was preserved with nitric acid-pH below 2 (USEPA,1986). For surface sediments, around half a kg of sample was collected manually in a zip lock bag from each sampling site. At the same time, two dominant macrophytes (Franzin & McFarlane, 1980) were also collected manually from each sampling site in both seasons and were rinsed with the lake water to remove sediments and other substrates. Finally, they were stored in a sterilized zip lock bag. To maintain a temperature of 4^{o}C in the field and during transportation, the samples (water, sediments, and macrophytes) were packed in sterilized bottles and bags and put in an ice box.

In the laboratory, 50 mL of water samples were acid digested (HNO_3) and it was digested slowly until white fumes were obtained and were cooled down. Then, the volume was made to 50 mL with distilled water. The turbid digested samples were filtered through Whatman filter paper no. 40. The samples were aspirated in an atomic absorption spectrophotometer (AAS) using method no. 311 c by APHA (2017). The same method was used for the 4 replicate samples from two sampling sites (two samples each from GG2 and GG7). The detection limits (DL) of the instrument for water samples were 0.01 mg/L for Cu, Zn, and Pb and 0.003 mg/L for Cd. Similarly, DL for sediment and macrophyte samples was 0.02 μg/g for all heavy metals. The heavy metal concentrations that were not detected by the instruments were given half of the DL values for analysis (Beal 2001; Weinke & Biddanda, 2017).

Additionally, collected sediment samples were air dried and were sieved through a 0.8 mm sieve (<2 mm) to remove large debris, stones, and pebbles. The macrophytes were thoroughly washed with distilled water, oven-dried for 48 hours at 80 °C, ash-dried at 105 °C for 24 hours and cooled in a desiccator for further analysis. Dried plant samples were grounded by using a hand mortar following methods based on Xing et al. (2013). About 0.5 g of macrophytes as well as sediment samples were weighed and transferred to a 250 mL beaker with the addition of 25 mL of tri-acid of HNO_3:H_2SO_4: $HClO_4$ in the ratio of 9:4:1. Then, the beaker was covered with watch glass and was digested over hot plate at low temperature until white fumes was obtained and was cooled down. After that, the digested sample was filtered through Whatman filter paper no. 41 and the volume was made to 100 mL by adding distilled water. Finally, the blank solution, standard, and samples were aspirated in an atomic absorption spectrophotometer (AAS) using wet digestion EPA method 3050 to find out the metal concentration (USEPA, 1986).

Data Analysis

An Independent sample t-test was used to find out the seasonal differences in water, sediment, and macrophytes. Pearson correlation coefficients were calculated to observe an association between sediments and macrophytes as well as water and macrophytes (*Ludwigia sp.*) seasonally by using R studio 3.5.1. Similarly, the Geo-accumulation index (I_{geo}) was calculated by a formula originally stated by Muller (1969) to define metal contamination in sediments by comparing current concentrations with preindustrial levels by using the formula:

$$I_{geo} = \log_2 [C_n/1.5B_n].$$

Where C_n is the measured concentration in the sediment for the metal n, B_n is the background value for the metal n (Turekian & Wedepohl, 1961), and the factor 1.5 is used because of possible variations of the background data due to lithological variations. The I_{geo} value was calculated using the global average shale data from Turekian and Wedepohl (1961). Moreover, the bioaccumulation factor (BAF) was calculated to understand the efficiency of plant species in accumulating metals into their tissue from the surrounding environment (Ladislas et al., 2012, Ghosh & Singh, 2005). The following formula was used to calculate BAF as given by Wilson and Pyatt (2007).

$$BAF = C\ (\text{plant tissue})/C\ (\text{sediment})$$

Where C (plant tissue) is metal concentration in plant tissue and C (sediment) is metal concentration in sediment.

Results and Discussion

Heavy Metals in Water

Metals concentrations in both summer and winter seasons in lake water were found in the following order: Zn > Cu > Pb > Cd (Tables 1). The mean value of Zn was higher in the summer season (0.512±0.873 mg/L) than the winter season (0.293±0.551 mg/L) and was highest among all metals in both seasons. Nevertheless, there were no significant differences between Zn and Cu. The average concentrations of these metals in both seasons were lower than the permissible level given by the Nepal Drinking Water Quality Standard (CBS, 2019) and WHO (2017). Zn had the highest concentration in water among the four metals analyzed in the present study Table 1); this is probably the reason for the highest concentration in plants and sediments (Núñez et al., 2011; Flefel et al., 2020).

Table 1 Heavy metal concentration (mg/L) in water sample of Ghodaghodi Lake and maximum permitted concentration in water (mg/L)

Heavy metals	Summer Mean±SD	Winter Mean±SD	NDWQS (CBS, 2019)	WHO (2017)	t-test value	p-value
Zn	0.512±0.873	0.293±0.551	3	0.01-3	0.7617	0.454
Cu	0.005±0.01	0.007±0.003	1	2	-1.2081	0.239
Cd	0.0015±0.00	0.0015±0.00	0.003	0.003	NA	NA
Pb	0.005±0.00	0.005±0.00	0.01	0.01	NA	NA

Note: SD, standard deviation; NDWQS, National Drinking Water Quality Standard (CBS, 2019); WHO, World Health Organization (2017)

Heavy Metals in Sediments

Concentrations of heavy metal in sediments are summarized in Table 2. Lake sediment metal concentrations in both seasons were found in the order Zn > Cu > Pb > Cd (Tables 2, A2). Additionally, the mean concentrations of all four metals in sediments were higher in winter than in summer season. For the winter season, the maximum values of Zn, Cu, Cd, and Pb are 76.23, 16.95, 1.98, and 21.59 μg/g, respectively. For the summer season, the maximum values of Zn, Cu, Cd, and Pb are 73.02, 16.41,0.01, and 23.94 μg/g, respectively (Table 2).

Heavy metal concentrations of sediments in Ghodaghodi Lake differ among seasons for Cu, Cd, and Pb but for Zn, it showed insignificant value (Table 2). Mean concentrations for Zn, Cu, and Pb were lower than the Average shale value (ASV), Toxicity reference value (TRV), Lowest effect level (LEL), and Severe effect level (SEL) in both seasons except for Cd in winter seasons.

Table 2 Heavy metal concentration (µg/g) in sediment sample of Ghodaghodi Lake and maximum permitted concentration in sediment(µg/g)

Parameters	Period of year	Mean±SD	Max	Min	t-test	p-value	ASV[a] (Turekian & Wedepohl, 1961)	TRV[b] (USEPA, 1999)	LEL[c] (Persaud et al., 1993)	SEL[d] (Persaud et al., 1993)
Zn	Winter	56.29±13.13	76.23	0.01	1.78	0.09	95	110	120	820
	Summer	44.00±21.08	73.02	33.9						
Cu	Winter	12.99±3.16	16.95	5.98	2.28	0.03*	45	16	16	110
	Summer	9.13±5.21	16.41	0.01						
Cd	Winter	0.79±0.71	1.98	0.01	3.95	0.001*	0.3	0.6	0.6	10
	Summer	0.01±0.00	0.01	0.01						
Pb	Winter	11.39±6.88	21.59	0.01	3.48	0.002*	20	31	31	250
	Summer	2.16±6.64	23.94	0.01						

*****: Significant at 0.05, **ASV[a]**, average shale value ; **TRV[b]**, toxicity reference value; **LEL[c]**, lowest effect level; **SEL[d]**, severe effect level

Sediments consist of very high metal concentrations compared with water. This is facilitated as sediment particles attract the metals which accumulate in the sediment over time (Sancer & Tekin-Özan, 2016). Metals concentrations in sediment were higher in winter than in summer due to the lower water flow during winter which could assist in accumulating the heavy metals in sediment, particularly in non-residua phases such as Cd and Pb (Islam et al., 2015). Cd, Cu, Pb, and Zn concentration in sediment, when compared with the average shale values given by Turekian and Wedepohl (1961), showed that they are primarily derived from pollution sources except for Zn (Korfali & Davies, 2005). The major sources are probably atmospheric dispersion, vehicular combustion from the nearby roads, and other anthropogenic activities. Additionally, the formation of smog in the Gangetic Plain (the study area is a part of GP) during winter contributes to the scavenging of pollutants that may increase their concentrations in the sediments (Mishra & Kulshrestha, 2020).

Geo-accumulation Index

The mean values of the geo-accumulation index in sediment were greater in winter than summer season (Fig. 2). Among four metals, Cd in the winter season has the highest I_{geo} value (0.53±0.47). When compared to the geo-accumulation index (I_{geo}) value given by Muller (1969), the mean value of all metals falls under the uncontaminated to moderately contaminated category with Class 1 although the Cd maximum value in the winter season showed the moderately contaminated category value with Class 2 of I_{geo} class.

The increase in the concentration of Cd in the winter season could be due to industrial processing and intensive agricultural practices resulting in the contamination of forage feed and water which are sources of Cd exposure for farmed ruminants (Lane et al., 2015). Furthermore, the eventual mixing of feces with the water source might have increased the Cd concentration in the winter season which was prominent in the study area but was absent in the summer season. Some other research found a low impact of highway traffic on the content of other heavy metals in soils and found out moderately polluted category of geo-accumulation index of cadmium near highway (Różański et al., 2017). These findings are also consistent with our results, as some sampling sites were near highway roads with higher traffic concentrations, and Nepal's lowlands are blanketed in smog during the winter season causing atmospheric deposition of these pollutants could have escalated Cd concentrations along the sampling sites.

Heavy Metals in Macrophytes

The concentrations of heavy metals are compared in two dominant submerged macrophytes in both seasons (Table 3). Heavy metal concentrations were found in the following order: Zn > Pb > Cu > Cd for *Nymphaea* sp. and *Ludwigia* sp. in winter and for *Nelumbo* sp. and *Ludwigia* sp. in the summer season.

Significant difference for *Ludwigia* sp. was found only in Cd and Pb ($p<0.05$; Table 3). The maximum values of Zn, Cu, Cd, and Pb concentrations in the winter season were 87.48, 14.53. 1.31 and 53.9 µg/g, respectively.

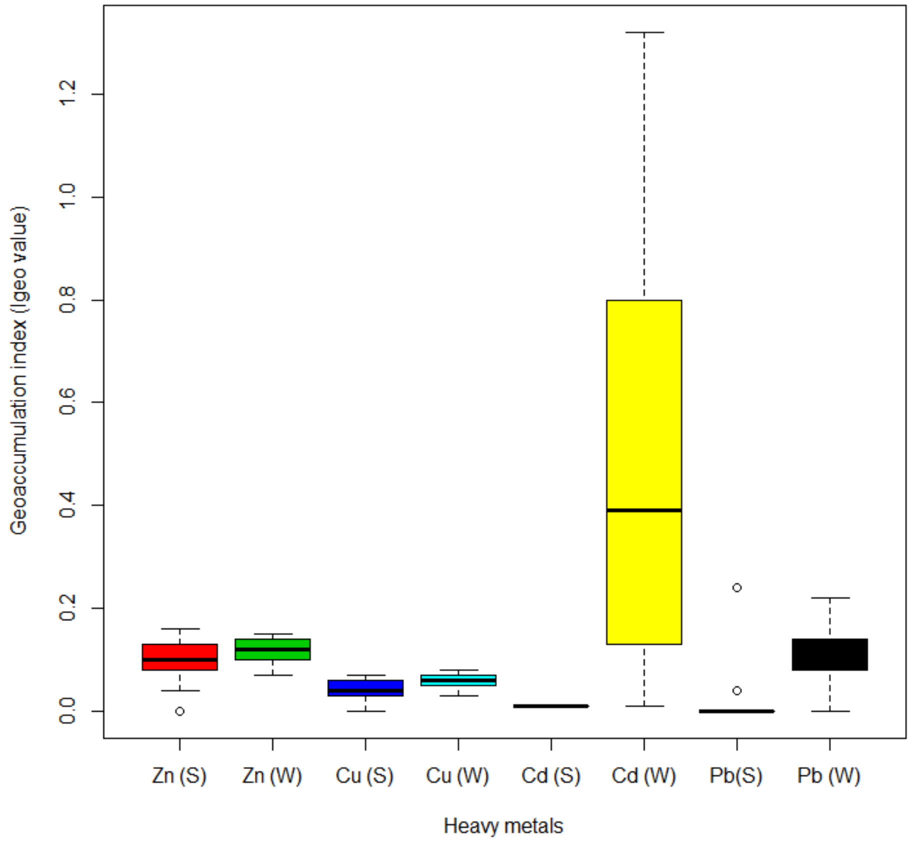

Figure 2 Geo-accumulation index (I_{geo}) in lake sediments of Lake Ghodaghodi in winter and summer season

Table 3 Heavy metal concentration (µg/g) in macrophytes sample of Ghodaghodi Lake

Parameters	**Period of year**	**Species**	**Mean±SD**	**Max**	**Min**	**t-test value**	**p-value**
Zn	Winter	*Ludwigia* sp.	59.18±24.8	87.48	20.25	1.21	0.2978
	Summer		40.37±6.86	49.84	30		
Cu	Winter		6.58±5.5	14.53	0.77	-0.001	0.99
	Summer		6.10±2.72	10.6	2.4		
Cd	Winter		1.01±0.3	1.31	0.63	5.27	0.003*
	Summer		1.89±0.98	3.39	0.01		
Pb	Winter		16.70±21.0	53.9	3.77	6.97	0.0006*
	Summer		24.08±9.11	33.96	3.99		
Zn	Winter	*Nymphaea* sp.	58.34±21.6	89.81	37.82		
Cu			4.00±1.4	5.22	1.73		
Cd			0.90±0.5	1.38	0.2		
Pb			4.22±4.2	10.08	0.01		
Zn	Summer	*Nelumbo* sp.	32.94±14.82	62.87	14.89		
Cu			8.51±4.50	16.19	4.29		
Cd			2.17±0.43	2.79	1.6		
Pb			27.96±5.72	36	17.99		

Note: *Ludwigia* sps. is dominant in both seasons; *Correlation is significant at the 0.05 level.

Similarly, the t-test for *Ludwigia* sp. for both seasons showed that Cd and Pb concentrations were significantly different ($t = 5.2719$, $p = 0.003$ and $t = 6.9722$, $p = 0.0006$, respectively). However, Zn and Cu were not significantly different in *Ludwigia* sp. seasonally. *Ludwigia* sp. could be proposed as Cu and Zn phytoremediators in Lake Ghodaghodi as has also been suggested by Núñez et al. (2011). *Ludwigia* sp. showed high metal removal efficiencies from water where Pb was accumulated fundamentally by roots, while Zn was accumulated more in the leaves (San Juan et al., 2018); and the present study also showed a similar pattern of result (Zn>Pb> Cu> Cd) in both seasons.

In a study, the roots and shoots of *Nelumbo nucifera* showed the pattern of Pb > Zn > Cu > Cd and Zn > Pb > Cu > Cd. respectively (Chabukdhara & Nema, 2012); and our study has shown the same order. Also, another study by Verma et al. (2018) in Dudh Talai, Udaipur, Rajasthan shows the accumulation of the heavy metals of *N. nucifera* in order Fe>Zn>Pb>Cd that was also similar to our study for common metals. The abundance patterns of heavy metals in leaf, petiole, and root were Cd>Cu>Pb>Zn (Kabeer et al., 2014) in *Nymphea* sp. which is in contrast to our study. Whereas both roots and stems based on a preliminary study of heavy metals in water lily plants around Kota Samarahan, Malaysia show the Zn accumulation capacity higher of all (Rahim, 2014) which matches our findings.

No seasonal differences in Zn and Cu concentrations in the two seasons in the present study might be because of the high bioavailability rate for Zn and Cu (Perin et al., 1997) around all seasons. However, significant seasonal differences of Cd and Pb between winter and summer may be due to vehicular engine combustion as its source, and other human activities because of nearby roadway and atmosphere as means of dispersion. This may cause the difference in the accumulation of heavy metals (Cd and Pb) seasonally in macrophytes based on the sources and their availability.

Relation between Macrophytes and Sediments

Table 4 shows a correlation between heavy metals in macrophytes (*Ludwigia* sp.) and sediments for winter and summer seasons. Overall, the heavy metal concentrations are negatively correlated for Zn and Cd while positively correlated for Cu and Pb in the winter season. Meanwhile, Zn and Cu were positively correlated and Cd and Pb were negatively correlated in the summer season.

Table 4 Relation between Macrophytes and Sediments

Winter	Zn	Cu	Cd	Pb
Zn	-0.244	-0.100	0.152	-0.731
Cu	0.162	0.386	0.453	-0.356
Cd	-0.507	-0.252	0.479	-0.567
Pb	-0.280	-0.022	0.879*	0.019
Summer	Zn	Cu	Cd	Pb
Zn	0.292*	0.331*	NA	-0.238
Cu	0.286*	0.299*	NA	-0.014
Cd	-0.517*	-0.537*	NA	-0.104
Pb	-0.492*	-0.518*	NA	-0.003

The uncommon correlations of metal concentration were observed between sediment and plant tissues. Such results indicate that metal concentrations in plants depend on several factors rather than on sediment concentration only. Moreover, when focused on the correlation between Cu and Zn in sediment showed an increment in metal concentrations of *Ludwigia* sp. probably because they have a higher tolerance and metal accumulation capacity than the other species. Therefore, *Ludwigia* sp. could be proposed as Cu and Zn bio-monitors (Núñez et al., 2011).

Bioaccumulation Factor

Bio-accumulation factor (macrophytes/sediment) followed the order of Cd> Cu> Pb> Zn for *Ludwigia* sp. and Cd>Zn>Cu>Pb for *Nymphaea* sp (Figs. 3 & 4) in the winter season. Cd by *Ludwigia* sp. shows the highest bioaccumulation factor among all metals in the winter season (Fig. 3).

Similarly, the bioaccumulation factor (macrophytes/sediment) for the summer season followed the order of Pb>Zn>Cd>Cu for *Ludwigia* sp. and Pb>Cd>Zn> Cu for *Nelumbo* sp. in the summer season (Figs. 5 & 6). Pb showed the greatest accumulation factor among all four metals in *Ludwigia* sp. in summer (Fig. 5).

Plants with a bioaccumulation factor (BAF) greater than 100 have the potential to act as hyper-accumulators and indicators of pollution (Wilson & Pyatt, 2007). *Ludwigia* sp. and *Nelumbo* sp. in summer were hyper-accumulator macrophyte species indicating high efficiency of plants to accumulate these metals from sediment and water. The present study is in line with earlier reports indicating the immobile nature of lead from soil and sediments to aerial parts (Siedlecka et al., 2001). Some hyper-accumulator plants can accumulate metals in higher concentrations and evaluate the persistent and acute toxicity from the aquatic ecosystem (McGeer et al., 2003).

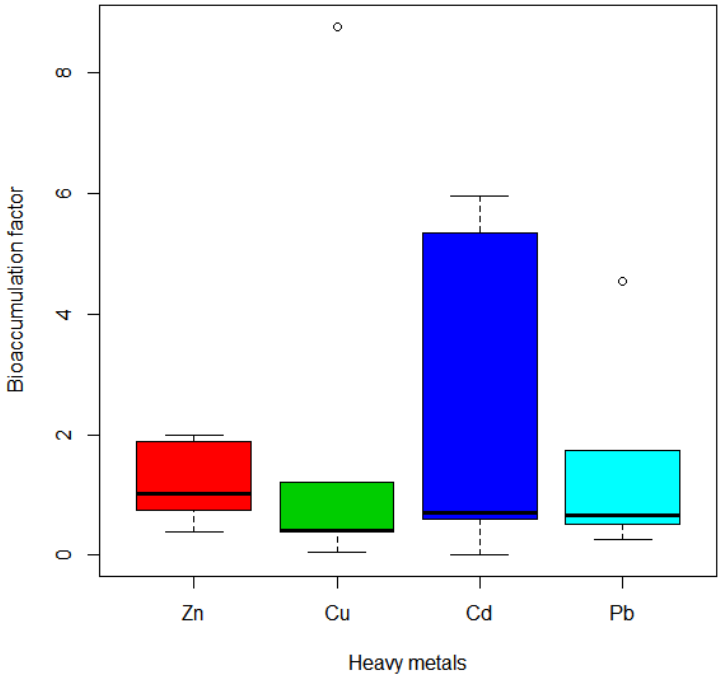

Figure 3 Bioaccumulation factor of heavy metals in sediment in winter (*Ludwigia* sp.)

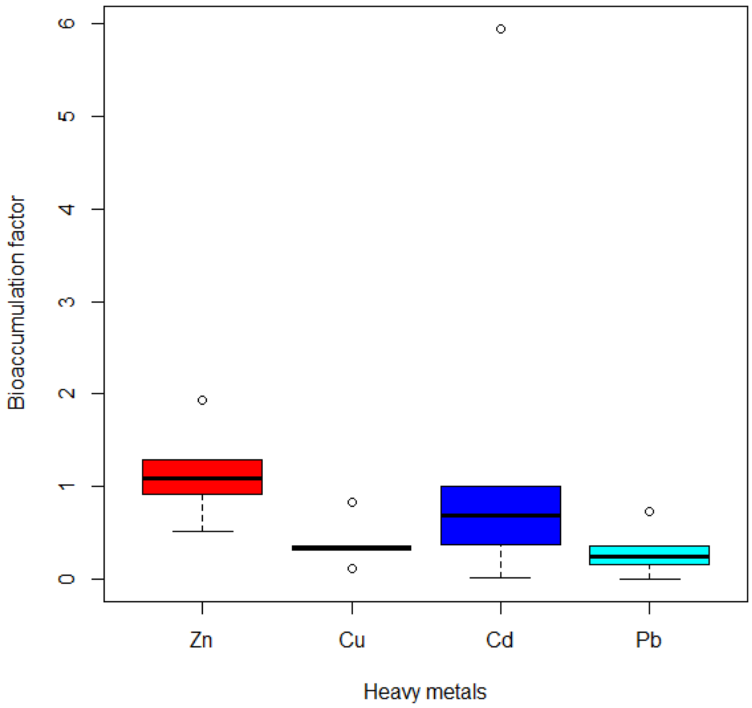

Figure 4 Bioaccumulation factor in sediment in winter (*Nymphaea* sp.)

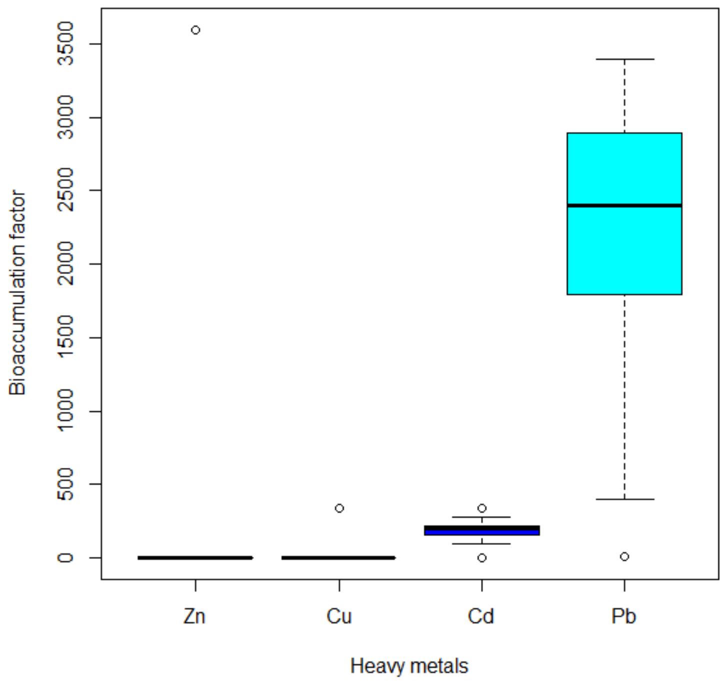

Figure 5 Bioaccumulation factor in sediment in summer (*Ludwigia* sp.)

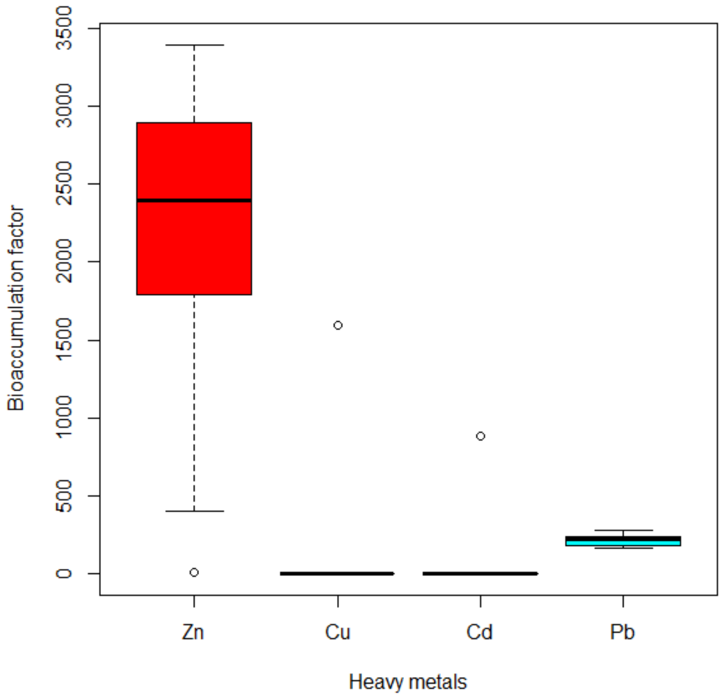

Figure 6 Bioaccumulation factor in sediment in summer (*Nelumbo* sp.)

Conclusions

In a nutshell, the summer season was dominated by zinc in water. In the case of sediment, the winter season is dominated more by all metals than the summer season supported by the geo-accumulation index. Among the four metals, Cd in the winter season has the highest I_{geo} value indicating concentration affected from pre-industrial levels along with other anthropogenic activities. *Ludwigia* sp. showed a greater trend of Zn accumulation in both seasons indicating *Ludwigia* sp. as the better phytoremediator for Zn metal. The highest bioaccumulation factor (macrophyte/sediment) among all metals was observed for Cd by *Ludwigia* sp. in winter and Pb by both macrophytes in the summer season. Thus, these macrophytes are capable of accumulating heavy metals providing scope in the bioremediation field.

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Author Contributions: DR: Conceived and designed the experiments, performed the experiment, analyzed and interpreted the data, wrote the paper; SG: conceived and designed the experiments, performed the experiment, analyzed and interpreted the data, wrote the paper; RB, ST, BMD, NR, KRK, RK, AP, LT, and DT: performed the experiment, analyzed and interpreted the data; CMS: conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, wrote the paper. All authors commented on previous versions of the manuscript, read, and approved the final manuscript.

Conflicts of Interest: The author declares no conflicts of interest.

Data availability: The datasets generated during and/or analyzed during the current study are available from the first and corresponding authors on reasonable request.

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