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Alkali and Transition Metals in Macrophytes of a Wetland System

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Introduction

Wetlands, common receivers of wastewater and urban run off, are also an effective filter, sink and transformation system for pollutants. These functions are very important among the several other ecological services wetlands offer (Angeler et al., 2003; Hansson et al., 2005). Metals are well-known pollutants that get into wetlands, and in due course of time are likely to affect species of various trophic levels. Some of them (among the alkali, alkaline earth, and transition metals) are persistent and accumulate in water, sediments, and tissues of living organisms (Lambou and Williams, 1980; Chaphekar, 1991; Ramadan, 2003). Macrophytes, occupying a vital position in the structure and functioning of aquatic ecosystems (Boston and Perkins, 1982), are among the most affected groups and serve as a polishing system (Matagi et al., 1998; Deng et al., 2004). Macrophytes are known to function as nutrient pumps (Davis and van der Valk, 1983), extracting nutrients from the sediment and immobilizing them for various periods in tissues. The present paper reports levels of select alkali, alkaline earth, and transition metals in macrophytes and water in the wetland system of Keoladeo National Park (KNP), Bharatpur, India.

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Materials and Methods

The present investigation was conducted in Keoladeo National Park (Fig. 1) one of the early World Heritage and Ramsar sites in India. The details of the study area are reported elsewhere (Azeez et al., 1991, 2000). The area falls under the semi-arid hot dry zone of India (Pal et al., 2000), with temperatures ranging from 1 to 49°C.

Seven macrophyte species (*Paspalum distichum*, *Paspalidium punctatum*, *Cyperus alopecuroides*, *Pseudoraphis spinescens*, *Ipomoea aquatica*, *Neptunia oleracea*, and *Hydrilla verticillata*) predominating the aquatic vegetation of the park were selected for the study. The 29 km^2 park is divided physically into 15 blocks, designated by the letters A to O, by dykes and sluices. Of these blocks, the central eight are wetlands while the rest are terrestrial wooded areas and grasslands. Of the wetland blocks, only the two central blocks D and E (Fig. 1) were considered for the present investigation, as these remain inundated through out the year, whereas the others become dry during summer. Plants were collected from these two blocks within the same week, after the peak growth and just before flowering. The shoots and leaves of the plants were harvested, air-dried, put together, and cut into 3 to 4 cm long bits. The macrophyte samples were oven-dried to constant weight at 60°C (Vesk and Allaway, 1997) for about 24 h, to determine dry weights. The oven-dried shoot and leaf samples were powdered using an agate mortar and pestle and mixed in equal proportion to obtain composite sample of each species. Representative samples from each plant in triplicate were analyzed for alkali, alkaline-earth metals (Na, K, Ca and Mg), and transition metals (Fe, Mn, Zn, Cu, Ni, Cr and Pb) after appropriate digestion using a nitric – perchloric acid mixture and required pre-concentration. All

Fig. 1 Study area map

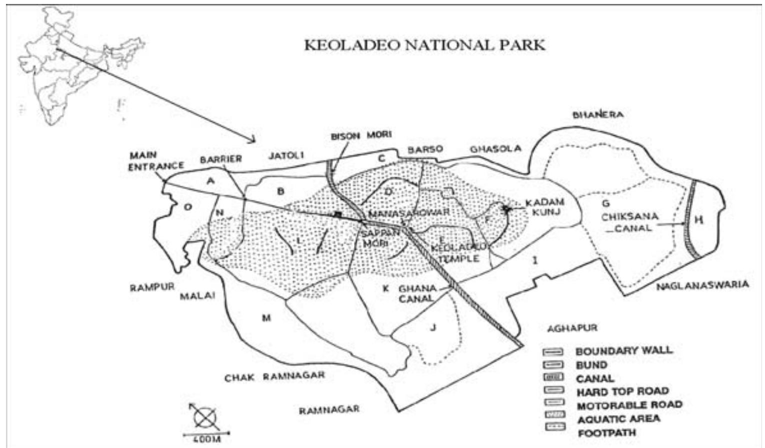

Table 1 Detection limits for transition metals

Metal	Fe	Mn	Zn	Cu	Pb	Cr	Ni
Detection level in plants (μg/g)	0.1	0.1	0.1	0.01	0.01	0.05	0.05
Detection level in water (μg/ml)	0.08	0.06	0.05	0.007	0.005	0.03	0.02
Recovery rate (%)	96.2	95.1	96.7	94.3	92.9	92.4	94.1

the chemicals were of analytical grade procured from Qualigens Fine Chemicals division of GlaxoSmithKline Pharmaceuticals Limited, Mumbai and double-distilled water prepared using quartz distillation assembly was used to prepare the reagents. With each set of samples prepared for analysis, two acid blanks with no plant samples were also processed. A set of three previously analyzed and standardized plant samples was also processed to assess the recovery rate. Na and K were estimated using a flame photometer (Systronics 126), and Ca and Mg by EDTA titration. Transition metals (Fe, Mn, Zn, Cu, Ni, Cr and Pb) were estimated using a Perkin-Elmer atomic-absorption spectrophotometer (model AA 1100). The estimated detection limits of the metals in the plants (μg/g), water (μg/ml) and the recovery rate of all the metals are given in Table 1. Analysis of standardized plant samples showed an average recovery rate of 94.5%. Water samples in triplicate were also collected from the D and E blocks and analyzed for physico-chemical characteristics and metal content following standard procedures (American Public Health Association, 1985). The results were presented as the metal level in the wetland water (mean of the two blocks) as a whole. Least-significant difference (LSD) and two-way analysis of variance (ANOVA) were performed on the results of the chemical analysis using MEGASTAT, to assess the variations among the metals and among the macrophytes, setting the significance level as $P < 0.05$.

Table 2 Chemical properties of wetland water*#

Parameter	Maximum	Minimum	Mean@
pH	8.8	7	7.7
Phenolphthalein alkalinity	19	0	8.9
Total alkalinity	317.3	218.5	266.5
Total hardness	226.2	116.7	182.4
Chloride	266	44.2	137.5
Chemical oxygen demand	294.6	16.3	62.2
PO_4	3.5	0.1	1.3
SO_4	2167.1	299.4	752.1
Ca	153.8	52.3	80.6
Mg	38.8	5.8	24.8
Na	88.49	28.51	58.8
K	97.1	3.5	53.9
Fe	11.89	0.54	3.9
Mn	0.17	0.02	0.08
Cr	0.02	ND	0.02
Cu	0.02	ND	0.01
Ni	0.04	ND	0.02
Pb	0.08	ND	0.04
Zn	0.2	ND	0.11

* All concentrations except for pH are in milligrams per liter. ND = not detectable.

\# samples were analyzed in triplicate.

@ The means were calculated considering the ND values as equivalent to 50% of the minimum detectable levels.

Results and Discussion

The chemical characteristics of the water are presented in Table 2. The pH of the water varied between 7.0 and 8.8 with the average being 7.7. The phenolphthalein alkalinity

Table 3 Metals in select macrophytes from the wetlands of KNP

Metals	Macrophytes							
	CYP	HYD*	IPO	NEP	PDM	PLM	PSU	Mean**
Na	0.2	0.1	0.24	0.05	0.08	0.05	0.1	0.12 ± 0.07
K	1.56	1.63	1.61	1.54	1.55	1.61	1.47	1.57 ± 0.05
Ca	1.22	0.36	0.7	1	1.17	1.3	1.02	0.97 ± 0.33
Mg	0.13	0.19	0.15	0.16	0.1	0.12	0.1	0.14 ± 0.03
Fe	169	287	170	112	116	106	111	153 ± 65
Mn	230	1277	370	1007	160	280	130	493 ± 457
Zn	13	15	8	15	9	10	14	12.0 ± 2.9
Cu***	3	1	1	ND	ND	0.5	0.5	0.86 ± 1.03
Pb***	ND	0.02	ND	0.01	ND	ND	ND	0.008 ± 0.006
Cr***	0.3	0.2	0.1	ND	0.2	0.1	0.1	0.14 ± 0.09
Ni***	ND	0.2	0.1	0.1	ND	0.1	0.1	0.09 ± 0.07

All concentrations in µg/g dry weight

ND = not detectable, CYP = *Cyperus alopecuroides*, HYD = *Hydrilla verticillata*, IPO = *Ipomoea aquatica*, NEP = *Neptunia oleracea*, PDM = *Paspalidium punctatum*, PLM = *Paspalum distichum*, and PSU = *Pseudoraphis spinescens*

* After 60 days

** mean ± standard deviation

*** The means were calculated considering the ND values as equivalent to 50% of the minimum detectable levels

ranged between 0.0 mg/l and 19 mg/l while the total alkalinity varied between 218.5 mg/l and 317.3 mg/l. The average hardness of the water samples was 182.4 mg/l. The high chloride content probably suggests slight intrusion of underground salt water into the wetland. According to Bhushan and Sharma (1987) the water in the phreatic aquifer in the area is predominantly saline. A saline tract with a brine of resistivity 20–30 Ω^{-1} and sodium chloride as the major salt is also present underneath. The phreatic aquifer in close interaction with wetland water may contribute to the chloride content (Azeez et al., 2000). The chemical oxygen demands of the samples were relatively low. Certain samples had a high sulfate content (2167.1 mg/l). The highest phosphate concentration recorded in the samples was 3.5 mg/l. The concentrations of metals in the water are also presented in Table 2. In the wetland water, among the alkali metals the concentration of Ca was found to be the highest and Mg the lowest. Of the transition metals, Fe was found be the highest in concentration.

The levels of alkali metals and transition elements in the macrophytes are presented in Table 3**.** Among these metals, K was the highest in concentration and Mg the lowest. The distributions of these elements were almost similar across all the macrophytes. In the case of Na, the highest concentration was 0.24 µg/g (*Ipomoea*) and the lowest 0.05 µg/g (*Neptunia* and *Paspalum*). K ranged between 1.47 and 1.63 µg/g with *Pseudoraphis* and *Hydrilla*, respectively. Ca was seen varying between 0.36 µg/g (in *Hydrilla*) to 1.30 µg/g (in *Paspalum*). Mg ranged between 0.10 µg/g (*Paspalidium* and *Pseudoraphis*) and 0.19 µg/g (*Hydrilla*). Unlike alkali and alkaline earth metals, transition metals have a high degree of variability among metals as well as macrophyte species. Among the transition metals, Mn was found in highest concentrations among all macrophytes, followed by Fe and Zn. Mn concentrations in the plants ranged between 130 µg/g (*Pseudoraphis*) and 1277 µg/g (*Hydrilla*). Fe concentration ranged between 106 µg/g (*Paspalum*) and 287 µg/g (*Hydrilla*). Fe, Mn, Zn and Cu are essential elements for plants and animals, with widely varying requirements. Zn plays an important role in the biosynthesis of enzymes and plant growth (Lepp, 1981; Aksoy and Öztürk, 1996). High uptake of these metals may lead to toxicity. Cu is known to cause toxic effects when plants accumulate levels exceeding 20 µg/g (Borkert et al., 1998). In the present study Cu, Pb Cr and Ni were not detectable (ND) in some of the plants. The highest level of Cu found in the macrophyte under the present study was 3.0 µg/g (in *Cyperus*).

The highest concentration of Pb detected in the present study was 0.02 µg/g in *Hydrilla* while it was undetectable in other plants except *Neptunia*. Pb concentration reported in uncontaminated wetland plants ranged between 6.3 and 9.9 µg/g (Table 4, Outridge and Noller, 1991) while the reported concentration toxic to plants is higher (approximately 27 µg/g, Beckett and Davis, 1977). The Cr concentration was highest in *Cyperus* (0.3 µg/g). The Ni level was found at highest concentration in *Hydrilla* (0.2 µg/g). The metals in order of decreasing concentration in each macrophyte species are given in Table 6. In all the macrophyte species, Mn was found to be in highest concentration followed by Fe and Zn. K followed Zn in all the plants except *Cyperus*. In the macrophytes, the variation among the metals was highly significant. Similarly with respect to the metal content, the macrophyte species also differed significantly (Table 5, LSD, $P < 0.05$). The macrophyte species in order of decreasing tissue metal residues are given in Table 6. Of the 11 metals examined, *Hydrilla* had the highest concentration for seven metals. The general concentration range for the metals detected in the present study is low compared to the available information from elsewhere (Table 4), except in the case of Mn. Mn ranged between 16 and 1277 µg/g against the reported range of 50 to 1000 µg/g. Mn reportedly tends to accumulate to a higher degree in shoots (Filho et al., 2004), the part of the plant analyzed in the present study.

Concentrations of metals in the water from the wetland system (average of both the blocks) are given in, fig. 2 as the two-way ANOVA shows that the wetland blocks do

Table 4 General concentration (μg/g dry weight) range for the metals in plants

Metal	Present observation #	Reported concentration	
		Range	Authors
Na	0.12	0.2–3.0	Allen (1989)
K	1.57	5.0–50.0	Allen (1989)
Ca	0.97	3.0–25.0	Allen (1989)
Mg	0.14	1.0–5.0	Allen (1989)
Fe	153	40–500	Allen (1989)
Mn	493.43	50–1000	Allen (1989)
Zn	12	10–100	Aksoy and Öztürk (1996)
Cu	1.2	1.6–10.7	Breteler et al. (1981)
Ni	0.015	–	–
Cr	0.17	0.76–4.25	Breteler et al. (1981)
Pb	0.12	6.3–9.9	Outridge and Noller (1991)

Mean values

Table 5 LSD of metals and plants

Source of variation	*SS*	*df*	*MS*	*t*	*P*-value	LSD
Metals	1590057	10	159005.7	2	<0.05	148.7
Total for metals	2866816	76				
Macrophytes	137826	6	22971	2	<0.05	168.4
Total for macrophytes	2866816.161	76				

Table 6 List of metals and macrophytes in decreasing order

Macrophytes	Metal residues
CYP	Mn < Fe < Zn < Cu < K < Ca < Cr < Na < Mg < Pb = Ni
HYD	Mn < Fe < Zn < K < Cu < Ca < Cr = Ni < Mg < Na < Pb
IPO	Mn < Fe < Zn < K < Cu < Ca < Na < Mg < Cr = Ni < Pb
NEP	Mn < Fe < Zn < K < Ca < Mg < Ni < Na < Pb < Cu = Cr
PDM	Mn < Fe < Zn < K < Ca < Cr < Mg < Na < Cu = Pb = Ni
PLM	Mn < Fe < Zn < K < Ca < Cu < Mg < Cr = Ni < Na < Pb
PSU	Mn < Fe < Zn < K < Ca < Cu < Na = Mg = Cr = Ni < Pb
Metal	Macrophyte species
Na	IPO < CYP < HYD = PSU < PDM < NEP = PLM
K	HYD < IPO = PLM < CYP < PDM < NEP < PSU
Ca	PLM < CYP < PDM < PSU < NEP < IPO < HYD
Mg	HYD < NEP < IPO < CYP < PLM < PDM = PSU
Fe	HYD < IPO < CYP < PDM < NEP < PSU < PLM
Mn	HYD < NEP < IPO < PLM < CYP < PDM < PSU
Zn	HYD = NEP < PSU < CYP < PLM < PDM < IPO
Cu	CYP < HYD = IPO < PLM = PSU < NEP = PDM
Pb	HYD < NEP < CYP = IPO = PDM = PLM = PSU
Cr	CYP < HYD = PDM < IPO = PLM = PSU < NEP
Ni	HYD < IPO = NEP = PLM = PSU < CYP = PDM

CYP = *Cyperus alopecuroides*, HYD = *Hydrilla verticillata*, IPO = *Ipomoea aquatica*, NEP = *Neptunia oleracea*, PDM = *Paspalidium punctatum*, PLM = *Paspalum distichum*, and PSU = *Pseudoraphis spinescens*

not differ significantly. However, it was found that the variation among metals in these blocks was significant ($F = 74.059$, $P < 0.05$). The concentration factors, the ratio of a metal's concentration in macrophytes to that in the surrounding water, showed wide variation among the metals (Table 7). Transition metals concentrated more in the macrophytes unlike alkali and alkaline earth metals. The concentration factors vary significantly ($P < 0.05$) among metals, while macrophytes do not vary significantly with respect to the concentration factors. Macrophytes grown in the wetland take up metals in varying degrees. Variations in the accumulation of metals with increasing concentration in the medium (Sinha et al., 2003) and even among individual plants of the same species have been reported (Vestk and Allaway, 1997). Given that many macrophytes have a high potential to accumulate metals, phytoremediation has become an emerging strategy to deal with metal contamination in recent years (Chandrasekhar et al., 2004, 2005; Thangavel and Subbhuraam, 2004). The metal uptake by plants is largely influenced by the bioavailability of the metals, which in turn is determined by both external (matrix-associated) and internal (plant-associated) factors (Caussy et al., 2003), respectively known as external bioavailability and internal bioavailability. External bioavailability is determined by the ambient conditions and the physico-chemical characteristics of the microenvironment of the organism. Organisms are also known to change their microenvironment to control the chance of a toxicant entering their body or to selectively harvest required elements (Wood and Wang, 1985; Reichman, 2002).

The present study indicates that alkali metals are restricted from accumulating in the tissue or are actively excreted while transitional elements are considerably accumulated. In the present investigation, all the metals were within the general concentration range. Nevertheless, there is the likelihood of elevated levels in the root parts, as reported elsewhere (Deng and Wong, 2004; Vesk and Allaway, 1997). Most plants, save those that can hyperaccumulate metals (Thangavel and Subbhuraam, 2004), restrict the metal mobility to the photosynthetic (Weis et al., 2004) and other active tissues.

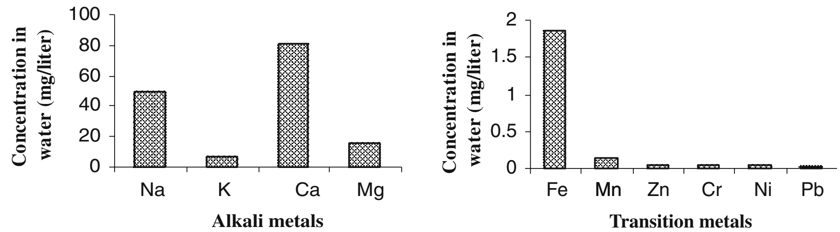

Fig. 2 Metal level (mg/l) in water from the wetland system of Keoladeo National Park

Table 7 Concentration factors of metals in macrophytes

Species	Metals									
	Na	K	Ca	Mg	Fe	Mn	Zn	Cu	Pb	Cr	Ni
CYP	0.004	0.237	0.015	0.008	91.4	766.7	288.9	NA	NA	7.5	NA
HYD	0.002	0.248	0.004	0.012	155.1	4256.7	333.3	NA	0.4	5.0	4.4
IPO	0.005	0.244	0.009	0.010	91.9	1233.3	177.8	NA	NA	2.5	2.2
NEP	0.001	0.234	0.012	0.010	60.5	3356.7	333.3	NA	0.2	NA	2.2
PDM	0.002	0.235	0.015	0.006	62.7	533.3	200.0	NA	NA	5.0	NA
PLM	0.001	0.244	0.016	0.008	57.3	933.3	222.2	NA	NA	2.5	2.2
PSU	0.002	0.223	0.013	0.006	60.0	433.3	311.1	NA	NA	2.5	2.2

NA = not applicable as the metals were below detectable level in plants or water

CYP = *Cyperus alopecuroides*, HYD = *Hydrilla verticillata*, IPO = *Ipomoea aquatica*, NEP = *Neptunia oleracea*, PDM = *Paspalidium punctatum*, PLM = *Paspalum distichum*, and PSU = *Pseudoraphis spinescens*

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