Assessment of Spatial and Temporal Variations of Heavy Metals Levels at Bhal Region of Gulf of Khambat - India

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ABSTRACT

Salt marsh areas are recognized as important natural sinks for metals. Bhal region one selected for this investigation can be classified as a 15 km wide coastal wetland comprising of marshy areas towards the Gulf and of either freshwater-salt marsh or freshwater bodies in landward margin of 10 km, which remains flooded during monsoon. Hence in this study attempts were made to evaluate the levels of heavy metals viz., Fe, Cu, Zn, Mn, Ni and Cd of habitats located at such interesting ecoregion. 54 soil samples collected from 18 twin belt transects during monsoon, winter and summer at 6 different coastal locations viz., Mingalpur, Mundi Bridge, Rahtalav, Khoon, Kamatalav and Bhogavo were analyzed by atomic absorption spectrophotometer. Furthermore results obtained were subjected to 2-way ANOVA. From results obtained, it can be said that metal concentrations in soil samples supporting coastal flora in Bhal ecoregion could possibly be arranged in a decreasing order as follows: Fe > Mn > Ni > Zn > Cu > Cd. Also coastal regions of Bhal are comparatively less contaminated by heavy metals than marshy locations of other countries. 2-way ANOVA assessing seasonal variations showed that concentrations of Fe, Mn and Cu in 2 marshy locations varied significantly during monsoon, winter and summer. In contrast, Ni, Zn and Cd did not show such trend of temporal observations for all the 6 selected locations. Furthermore, metal content in soil samples collected from 3 belt transects at each location did not fluctuate significantly, indicating a fact that one may chose any one sample area for any specific type of botanical investigations.

Keywords: Coastal wetland, salt marsh, freshwater, heavy metals.

INTRODUCTION

Heavy metals are extremely toxic and they are present in our immediate environment. They occur in soil, surface water and plants, and are readily mobilized by human activities that include mining and discarding industrialized waste materials in natural eco-systems comprises forests, rivers, lakes and ocean (Larison et al., 2000).
Consequently, heavy metals pose a potential threat to various terrestrial and aquatic organisms including human health (Hsu et al., 2006).

Salt marshes constitute the most productive systems of the world (Odum, 1971; Pomeroy and Wiegert, 1981) and accumulation of heavy metals at high concentration in the upper layers of such habitats has become a serious environmental concern, because it is associated to health risks to plant, animals and humans. Estuarine salt marshes are frequently highly contaminated with metals, due to human and industrial activities occurring in the estuaries and adjacent areas. Sedimentation of polluted particles results in high metal contents in the soil of tidal marshes, which are generally considered as a sink (Hart, 1982). The high historical input of metals into a marsh soil may still pose a risk to ecosystem functioning (Vandecasteele et al., 2002; Vandecasteele et al., 2004).

With increasing urbanization and industrialization, coastal areas of all tropical littoral countries in Asia, especially India, have been subjected to considerable environmental stress due to domestic sewage, industrial effluents, heavy metals and other toxic waste (Agoramoorthy and Hsu, 2005; Hsu et al., 2006). Furthermore, human impact on environment can be scaled by the measurements of heavy metals in soil, plants and animals because metal pollution adversely affects the density and diversity of biotic communities including human (Bengtsson et al., 1981; Mountouris et al., 2002; Hsu et al., 2006).

Bhal region one selected for this investigation can be classified as a 15 km wide coastal wetland comprising of marshy areas towards the Gulf and of either freshwater-salt marsh or freshwater bodies in landward margin of 10 km, which remains flooded during monsoon. Hence in this study attempts were made to evaluate the levels of heavy metals viz., Fe, Cu, Zn, Mn, Ni and Cd of marshy, fresh water-salt marsh and freshwater habitats located at such interesting ecoregion.

MATERIALS AND METHODS

Study site: The geographic region of 'Bhal' is situated at 21º55’S to 72º15’E on the west margin of the Gulf of Cambay (now Khambbhat) in Gujarat state, India and covers parts of two districts namely, Ahmedabad and Bhavnagar. The region consists of landmass having length of approximately 100 km and breadth of 25 km and stretches along the coast from Gulf of Cambay in Anand district to creek of Bhavnagar district. It receives an average annual rainfall about 650 to 700 mm.

In the local language, 'Bhal' means forehead. It stands for a topography that is as flat, as saline and as barren as forehead. The region suffers from a hostile geo-climatic environment, highly saline shallow ground water and erotic monsoon rains.

Six locations comprising of three different groups were selected i.e. 2 Marshy locations – Location 1 (L1) Mingalpur and Location 3 (L3) Rahtalav; 3 freshwater-salt marsh locations- Location 2 (L2) Mundi Bridge, Location 4 (L4) Khoon and Location 5 (L5) Kamatalav and a Freshwater location- Location 6 (L6) Bhogavo. 54 soil samples from these 6 locations were collected during monsoon, winter and summer and analyzed for estimation of heavy metals viz., Fe, Zn, Ni, Cd, Mn and Cu by atomic absorption spectrophotometry.

Sample preparation: About 1.00 ± 0.05g dried soil sample was taken into silica crucible, incinerated and was ignited in a muffle furnace at a temperature of 400°C before transferring to a 100 ml teflon beaker. 10 ml of 1:1 diluted hydrochloric acid was added to the sample and was kept on waterbath (60-80 °C) for 1 hour. The supernatant was decanted, while 10 ml of hydrofluoric acid and 10 ml of hydrochloric acid were added to the residue, which was evaporated to dryness on waterbath. The last step was repeated once. Later on 5 ml of both the acids was added and sample was evaporated to dryness. The residue was dissolved in 10-12 ml of hydrochloric acid and was combined with the supernatant separated earlier and final volume was made upto 250 ml with deionised water. This extract was further used for estimation of Fe, Zn, Ni, Cd, Mn, and Cu by atomic absorption spectrophotometry (Perkin Elmer, Analyst 200, Germany).

RESULTS AND DISCUSSION

Heavy Metals – Coastal Habitats

Data of heavy metal (Fe, Cu, Mn, Zn, Ni, and Cd) concentration of different habitats of ‘Bhal’ ecoregion of Ahmedabad district are presented here and results of these samples were considered for temporal and spatial variations.
Assessment of Spatial and Temporal Variations of Heavy Metals Levels

Location 1 Mingalpur
Findings of heavy metals composition in soils at this site (Table 1) showed the dominance of Fe in a range of 12.441 to 12.629 mg.g\(^{-1}\), followed by that of Mn (0.903 to 1.019 mg.g\(^{-1}\)). On the other hand, soils contained low amounts of Ni (0.115 to 0.131 mg.g\(^{-1}\)); Cu (0.100 to 0.110 mg.g\(^{-1}\)); and Zn (0.096 to 0.104 mg.g\(^{-1}\)). The Cd content was yet less and it fluctuated between 0.007 to 0.013 mg.g\(^{-1}\). Although seasonal variations primarily showed a close range of variations of all elements in this marshy habitat, maximum values of the majority of elements were recorded during summer reflecting their greater accumulation in dry season.

Location 2 Mundi Bridge
The Fe content ranging between 12.234 to 12.274 mg.g\(^{-1}\), indicated its greater concentration than any other heavy metals in this freshwater-salt marsh habitat (Table 1). Mn varied between 0.715 to 0.790 mg.g\(^{-1}\) followed by Ni (0.104 to 0.131 mg.g\(^{-1}\)); Zn (0.088 to 0.103 mg.g\(^{-1}\)) and Cu (0.074 to 0.080 mg.g\(^{-1}\)). A low amount of Cd (0.014 mg.g\(^{-1}\)) was the same during all 3 seasons. Leaching of heavy metals from soils during monsoon may be a reason for a moderate decrease in their concentration.

Location 3 Rahtalav
As noticed for preceding 2 habitats, Fe (12.430 to 12.598 mg.g\(^{-1}\)) in this marshy location far exceeded that of all elements (Table 1). Furthermore, the Mn content (1.013 to 1.179 mg.g\(^{-1}\)) in soils was almost ten times greater than that of Ni (0.140 to 0.144 mg.g\(^{-1}\)) and Zn (0.114 to 0.124 mg.g\(^{-1}\)). Cu (0.076 to 0.088 mg.g\(^{-1}\)) and Cd (0.010 to 0.012 mg.g\(^{-1}\)) were found in low amounts. Moreover, petty seasonal fluctuations of metals were noticed here.

Location 4 Khoon
Concentration of Fe (12.411 to 12.583 mg.g\(^{-1}\)) was also high in this freshwater-salt marsh habitat (Table 1); whereas that of Mn fluctuated between 0.961 to 1.021 mg.g\(^{-1}\). These results further indicated a low and narrow range of fluctuations of Zn (0.132 to 0.155 mg.g\(^{-1}\)), Ni (0.137 to 0.143 mg.g\(^{-1}\)) and of Cu (0.075 to 0.080 mg.g\(^{-1}\)). Noticeably low amount of Cd (0.010 to 0.012 mg.g\(^{-1}\)) was recorded for this site. Except Cd remaining all elements showed low values in summer.

Table 1. Heavy metal concentration in soils of 6 coastal habitats of Bhal wetland.

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<tbody>
<tr>
<td>Fe</td>
<td>Location 1 Mingalpur</td>
<td>M***</td>
<td>12.441</td>
<td>12.234</td>
<td>12.596</td>
<td>12.533</td>
<td>12.405</td>
<td>12.114</td>
</tr>
<tr>
<td>Mn</td>
<td>Location 2 Mundi Bridge</td>
<td>M***</td>
<td>0.932</td>
<td>0.715</td>
<td>1.179</td>
<td>1.021</td>
<td>0.985</td>
<td>0.939</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W***</td>
<td>0.903</td>
<td>0.790</td>
<td>1.085</td>
<td>0.961</td>
<td>0.946</td>
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<tr>
<td></td>
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<td>S***</td>
<td>1.019</td>
<td>0.774</td>
<td>1.013</td>
<td>0.991</td>
<td>0.945</td>
<td>0.844</td>
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<tr>
<td>Ni</td>
<td>Location 3 Rahtalav</td>
<td>M***</td>
<td>0.115</td>
<td>0.104</td>
<td>0.140</td>
<td>0.141</td>
<td>0.153</td>
<td>0.131</td>
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<tr>
<td></td>
<td></td>
<td>W***</td>
<td>0.124</td>
<td>0.121</td>
<td>0.141</td>
<td>0.143</td>
<td>0.143</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>S***</td>
<td>0.131</td>
<td>0.131</td>
<td>0.144</td>
<td>0.137</td>
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<tr>
<td>Zn</td>
<td>Location 4 Khoon</td>
<td>M***</td>
<td>0.104</td>
<td>0.088</td>
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<td>0.155</td>
<td>0.113</td>
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<tr>
<td></td>
<td></td>
<td>W***</td>
<td>0.096</td>
<td>0.096</td>
<td>0.114</td>
<td>0.148</td>
<td>0.108</td>
<td>0.130</td>
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<tr>
<td></td>
<td></td>
<td>S***</td>
<td>0.099</td>
<td>0.103</td>
<td>0.124</td>
<td>0.132</td>
<td>0.095</td>
<td>0.093</td>
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<tr>
<td>Cu</td>
<td>Location 5 Bhogavo</td>
<td>M***</td>
<td>0.100</td>
<td>0.074</td>
<td>0.088</td>
<td>0.080</td>
<td>0.070</td>
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<tr>
<td></td>
<td></td>
<td>W***</td>
<td>0.106</td>
<td>0.080</td>
<td>0.088</td>
<td>0.076</td>
<td>0.056</td>
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<tr>
<td></td>
<td></td>
<td>S***</td>
<td>0.110</td>
<td>0.076</td>
<td>0.076</td>
<td>0.075</td>
<td>0.053</td>
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</tr>
<tr>
<td>Cd</td>
<td>Location 6 Kamatalav</td>
<td>M***</td>
<td>0.013</td>
<td>0.014</td>
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<td>0.010</td>
<td>0.014</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>W***</td>
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<td>0.014</td>
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<td></td>
<td></td>
<td>S***</td>
<td>0.007</td>
<td>0.014</td>
<td>0.012</td>
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<td>0.014</td>
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</table>

*(L1-Mingalpur; L2- Mundi Bridge; L3- Rahtalav; L4- Khoon; L5- Kamatalav; L6- Bhogavo) **(SM- Salt marsh; FWSM- Fresh water salt marsh; FW-Freshwater) ***{(M- Monsoon; W- Winter; S- Summer)}
Table 2: 1-way ANOVA assessing temporal variations in heavy metals in soils at each of 6 habitats in 'Bhal' region

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<tbody>
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<td>Fe</td>
<td>9.66¹</td>
<td>1.44 ns</td>
<td>15.10¹</td>
<td>6.36 ns</td>
<td>3.36 ns</td>
<td>11.87¹</td>
</tr>
<tr>
<td>Mn</td>
<td>74.89¹</td>
<td>25.85²</td>
<td>40.12²</td>
<td>3.71 ns</td>
<td>1.76 ns</td>
<td>6.27 ns</td>
</tr>
<tr>
<td>Ni</td>
<td>1.79 ns</td>
<td>19.38²</td>
<td>0.11 ns</td>
<td>0.42 ns</td>
<td>5.00 ns</td>
<td>0.81 ns</td>
</tr>
<tr>
<td>Zn</td>
<td>1.33 ns</td>
<td>0.84 ns</td>
<td>1.67 ns</td>
<td>1.06 ns</td>
<td>51.80²</td>
<td>4.49 ns</td>
</tr>
<tr>
<td>Cu</td>
<td>14.30¹</td>
<td>3.19 ns</td>
<td>10.83¹</td>
<td>3.00 ns</td>
<td>5.79 ns</td>
<td>2.17 ns</td>
</tr>
<tr>
<td>Cd</td>
<td>18.05²</td>
<td>0.28 ns</td>
<td>0.81 ns</td>
<td>2.38 ns</td>
<td>4.55 ns</td>
<td>0.61 ns</td>
</tr>
</tbody>
</table>

*(L1-Mingalpur; L2- Mundi Bridge; L3- Rahtalav; L4- Khoon; L5- Kamatalav; L6- Bhogavo) **(SM- Salt marsh; FWSM- Fresh water salt marsh; FW-Freshwater) ¹ = significant, ² = highly significant, ³ = very highly significant and ns = non-significant.

Location 5 Kamatalav

Dominance amount of Fe (12.405 to 12.253 mg.g⁻¹) was noticed for this freshwater-salt marsh site (Table 1). Fluctuating amount of Mn (0.945 to 0.985 mg.g⁻¹) reflected its low content. Amount of Ni (0.135 to 0.153 mg.g⁻¹) was marginally greater than that of Zn (0.095 to 0.113 mg.g⁻¹). Cu accumulation ranged from 0.053 to 0.070 mg.g⁻¹; while Cd was found in low concentration (0.012 to 0.014 mg.g⁻¹). Seasonal changes primarily indicated maximum values of the metals in monsoon.

Location 6 Bhogavo

Freshwater location of Bhogavo also had a large Fe content (11.978 to 12.232 mg.g⁻¹) throughout the year (Table 1). Amount of Mn ranged from 0.815 to 0.939 mg.g⁻¹, followed by Ni (0.131 to 0.141 mg.g⁻¹) and Zn (0.093 to 0.151 mg.g⁻¹), whereas that of Cu and Cd showed a narrow range of 0.003 to 0.042 mg.g⁻¹ and 0.013 to 0.014 mg.g⁻¹. Fluctuations of these metals during 3 seasons did not reflect any specific trend.

Thus, the minimum and maximum values of heavy metals in 54 soil samples supporting coastal flora in 'Bhal' ecoregion, primarily showed that Fe having a concentration from 11.978 to 12.629 mg.g⁻¹ was a principal element, followed by Mn, amounts of which fluctuated from 0.715 to 1.179 mg.g⁻¹. Ni and Zn varying from 0.088 to 0.155 mg.g⁻¹ occupied the middle position. Though the Cu content varied from 0.034 to 0.110 mg.g⁻¹, its higher values were noted just at one location (Mingalpur). Cd in soils was found in low concentration (0.007 to 0.014 mg.g⁻¹).

Temporal and Spatial Variations

As results showed narrow range of variations for Fe, Mn, Ni, Zn, Cu and Cd in soils, additional attempts were made to examine whether fluctuations in concentration of heavy metals indicated by primary data were statistically significant or not from temporal and / or spatial points of view. Soil observations recorded for 18 different twin belt transects laid down during 3 seasons at 6 locations were subjected to 2-way ANOVA (Tables 2&3). Temporal changes in Fe (Table 2) were significant for 2 marshy locations of Mingalpur (L1), (F= 9.66; P≤0.05); and of Rahtalav (L3) (F= 15.10; P≤0.05); and for freshwater location of Bhogavo (L6) (F= 11.87; P≤0.05). However, Fe content...
was not significantly different during 3 seasons at freshwater-salt marsh locations of Mundi Bridge (L2); Khoon (L4) and of Kamatalav (L5).

It was further noticed from Table 2 that seasonal changes in Mn was very highly significant at one marshy location L1 (F= 74.89; P≤0.001); highly significant at freshwater-salt marsh location L2 (F= 25.85; P≤0.01) and another marshy habitat L3 (F= 40.12; P≤0.01); whereas they were not significant at P≤0.05 level at 2 freshwater-salt marsh locations L4 (F= 3.71) and L5 (F= 1.76) as well as at freshwater pocket L6 (F= 6.27).

Though 2-way ANOVA for Ni indicated that its seasonal concentrations were highly significant just at freshwater-salt marsh location L5 (F= 51.80, P≤0.01), but such changes for remaining 5 locations did not vary significantly at P≤0.05 (Table 2).

Concentrations of Cu were affected by climatic changes (Table 2) at 2 marshy locations (i.e., L1 – F = 14.30, P≤0.05 and L3 – F = 10.83, P≤0.05); no such impact was noticed for 3 freshwater-salt marsh locations L2, L4 and L5 and one freshwater location L6. It was further observed that amounts of Cd (Table 2) were also not affected by climatic changes at all locations, except L1 (F= 18.05, P≤0.01).

In contrast to temporal changes, spatial variations considered from of view point of 3 belt transects laid down at each of the habitats (Table 3) were non-significant at P≤0.05 level, except for Ni (F= 9.71, P≤0.05) in freshwater-salt marsh location L2.

**Heavy Metals – Coastal Habitats**

Heavy metals levels in soils often result from anthropogenic activities such as mining, smelting, electroplating, agriculture practices, and industrial and municipal waste disposal on land (Errasquin and Vazquez 2003; Ait-Ali et al, 2004; Yang et al, 2004). In recent years, speciation of metals in salt marsh sediments has also been studied in several estuaries and different geochemical association of metals depending on metal nature, pressure of roots, and local factors as sediment redox potential or grain size have been found (Allen et al, 1990; Cacador et al, 1996b; Mortimer and Rae, 2000; Otero et al, 2000; Saenz et al, 2003; Almeida et al, 2004). It is of interest to mention here that vegetated areas of the salt marsh concentrate more metals in sediments, at least in rooted depths than non-vegetated areas (Cacador, et al, 1996a; Cacador et al, 1996b; Doyle and Otte, 1997; Otero and Macias, 2002).

Results of heavy metals in 54 soil samples presently collected from 6 locations in ‘Bhal’ area showed that Fe and Mn were major elements and their concentrations varied between 12.430 to 12.629 mg.g⁻¹ and 0.903 to 1.179 mg.g⁻¹ in 2 marshy habitats. Their values for 3 freshwater-salt marsh locations ranged between 12.234 to 12.583 mg.g⁻¹ and 0.715 to 1.021 mg.g⁻¹, while for freshwater site (Table 1), Fe value fluctuated between 11.978 to 12.232 mg.g⁻¹ and that of Mn between 0.815 to 0.939 mg.g⁻¹.

Ramanathan et al (1999) reported very high amount of Fe (29.1 mg.g⁻¹) than that of Mn (0.385 mg.g⁻¹) in salt marsh habitat at Pichavaram on east coast of India. Likewise, Carrasco et al (2006) noticed yet greater concentrations of Fe (24.9 to 176.6 mg.g⁻¹) and that of Mn (1.53 to 4.48 mg.g⁻¹) for 4 different contaminated salt marsh sites in Spain.

In this study, concentrations of Ni, Zn and Cu at marshy site fluctuated between 0.115 to 0.144 mg.g⁻¹, 0.096 to 0.124 mg.g⁻¹ and 0.076 to 0.110 mg.g⁻¹, respectively (Table 1). In case of freshwater-salt marsh habitats, amounts of Ni were noted in a range of 0.104 to 0.153 mg.g⁻¹; that of Zn between 0.088 to 0.155 mg.g⁻¹; and Cu between 0.053 to 0.080 mg.g⁻¹ (Table 1). Moreover, 0.131 to 0.141 mg.g⁻¹ Ni; 0.093 to 0.151 mg.g⁻¹ Zn; and 0.034 to 0.042 mg.g⁻¹ Cu (Table 1) were found for a freshwater habitat.

During their studies on 10 sites in Florida, Yoon et al (2006) found less Cu content (0.02 to 0.03 mg.g⁻¹) at 4 locations than presently noted for ‘Bhal’ region. Nevertheless, they recorded much greater amounts of Cu (0.30 to 0.99 mg.g⁻¹) at 6 remaining sites. The Florida sites also had noticeably high concentration of Zn (0.195 to 2.2 mg.g⁻¹) than that observed during present investigation. On the other hand, Carrasco et al (2006) reported 0.04 to 0.16 mg.g⁻¹ Cu and 1.25 to 14.51 mg.g⁻¹ Zn for four locations in Spain.

Ramanathan et al (1999) observed low content of Cu (0.024 mg.g⁻¹) as well as of Zn (0.05 mg.g⁻¹) in natural habitats of mangroves and salt tolerant plants. According to Luoma (1990) and De-Lacerda et al (1993), Zn often occurs in high concentrations in polluted estuarine sediments, typically up to 0.8 mg.g⁻¹.
Studies on coastal habitats in India indicate that marshy locations in Orissa contained 0.063 mg g\(^{-1}\) Cu and 0.015 mg g\(^{-1}\) Zn (Sarangi et al., 2002); while that in Kerala had 0.303 mg g\(^{-1}\) Cu and 0.764 mg g\(^{-1}\) Zn (Thomas and Fernandez, 1997). Recently Nirmal Kumar et al. (2006) recorded most abundant Zn (0.554 mg g\(^{-1}\)) and usual low concentration of Cu (0.033 mg g\(^{-1}\)) in sediments collected from Nal Sarovar in Gujarat.

It is of interest to add here that Zahir et al. (2004), who worked on Karachi coast in Pakistan, noted 0.012 to 0.056 mg g\(^{-1}\) Cu and 0.035 to 0.067 mg g\(^{-1}\) Zn in salt marshes. Similarly, salt marshes in Hong Kong had greater amounts of Cu (0.05 mg g\(^{-1}\)) and Zn (0.321 mg g\(^{-1}\)) (Ong Che, 1999); whereas Zheng and Lin (1996) observed 0.036 mg g\(^{-1}\) Cu and 0.099 mg g\(^{-1}\) Zn for salt marsh sites in China.

The present analyzed values for Cd fluctuated between 0.007 to 0.013 mg g\(^{-1}\) for 2 marshy habitats (Table 1); between 0.010 to 0.014 mg g\(^{-1}\) for 3 freshwater-salt marsh locations (Table 1); and from 0.013 to 0.014 mg g\(^{-1}\) for one freshwater site (Table 1). Although the concentration of Cd was quite low, it was present in all habitats selected for this study. Recently Reboreda and Cacador (2007) observed higher availability of Cd (0.575 to 0.970 mg g\(^{-1}\)) in the sediment of salt marsh site. However, the Cd content (0.008 mg g\(^{-1}\)) reported for a nearby Nal Sarovar Bird Sanctuary (Kumar et al., 2006) is in conformity with present results.

**CONCLUSION**

It becomes clear from forgoing discussion that marshy locations of other countries contain greater amounts of all metals, except that of Cu, than 'Bhal' coastal sites, perhaps because of their contaminated nature. As for Indian habitats, no consistency in the metallic composition appears to exist, as the value varies for different locations.

In sum, it can be said that although 'Bhal' area has low concentrations of the metals, they could possibly be arranged in a decreasing order as follows: Fe > Mn > Ni > Zn > Cu > Cd. 2-way ANOVA (Table 2) assessing seasonal variations showed that concentrations of Fe, Mn and Cu in 2 marshy locations varied significantly during monsoon, winter and summer. In contrast, Ni, Zn and Cd did not show such trend of temporal observations for all the 6 selected locations. Furthermore, metal content in soil samples collected from 3 belt transects at each location did not fluctuate significantly, indicating a fact that one may chose any one sample area for any specific type of botanical investigations.

**REFERENCES**


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