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# Accumulation and Toxicity of Iron and Manganese in *Spirodela polyrrhiza* (L.) Schleiden

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Most of the existing water resources in rural India are contaminated with toxic metals and are unpotable because of high amounts of iron and manganese.The average concentrations of Fe and Mn in fresh water bodies have been estimated to be 0.5 and 1.0 $\mu g\ cm^{-3}$, respectively (Mitra 1989). Both $Fe^{2+}$ and $Mn^{2+}$ are essentially required by plants and animals at low concentrations, however, excess of these metal ions causes alteration in plant metabolism and poses potential health hazards.

Increased awareness of the long term effects of metals in aquatic ecosystems has necessitated the search for a suitable biological system for removal of these pollutants.In this context, duckweeds have been found effective in removal of a number of metals (Nasu et al.1984 ; Kwan and Smith 1988) and subsequently have been used for treatment of wastewater (Culley and Epps 1973; Zirschky and Reed 1988). Besides,they have been shown to be a sensitive phytoassay system to assess toxicity of metals using growth of fronds as the measured parameter.This has been mainly due to their genetically homogeneous population,small size and rapid growth under laboratory conditions (Landolt and Kandeler 1987).

Studies under our project on Water Technology Mission in "Rural Drinking Water and Related Water Management" have shown very high accumulation of Fe and Mn by aquatic macrophytes including S. polyrrhiza in different climatic zones (Chandra et al. 1993). Chromium

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accumulation by S. polyrrhiza under the influence of metal chelators and pH has been reported (Tripathi and Chandra 1991).This plant has been shown to remove heavy metals including Fe and Mn from pond water (Rai and Tripathi 1992).However, the metal accumulating potential varied under various environmental conditions which makes the assessment of the metal removing potential of the plant difficult.

In view of the above, it was considered desirable to assess the Fe and Mn removing potential of S. polyrrhiza under laboratory and field conditions.The metal concentrations used ranged from minimum level(Ø.Ø1 mM) which is highly toxic for drinking water and up to maximum level (Ø.2mM) which are phytotoxic for plants. The toxic effects of these metals on chlorophyll, multiplication rate and biomass production were also studied.

## MATERIALS AND METHODS

Kalyani Devi (KD) pond, Unnao, (U.P.,India) was selected for the field study. Pond water was found to be contaminated with several industrial effluents from tannery,foundaries,electroplating, domestic discharges and agricultural run offs.Water and plant samples were collected from several locations in the pond where S. polyrrhiza was found growing.

Plants of S. polyrrhiza collected from an unpolluted waterbody from the same location were cultured in 1Ø% Hoagland's nutrient solution under standard growth condition providing 16 h light (using day fluorescent tube light, 114 $\mu$ mol $m^{-2}$ $s^{-1}$ at 25±2 C) and 8 h dark period. Newly grown fronds were selected from stock culture based on the criteria that the plants were healthy and had four fronds of approximately equal size. Eight such exponentially growing colonies were transferred into 8Ø ml solution in 15Ø ml conical flasks. Fronds of S. polyrrhiza were treated with five concentrations of Fe and Mn separately (Ø.Ø1, Ø.Ø25, Ø.Ø5, Ø.1, Ø.2 mM) using salts $FeCl_3$ and $MnCl_2$. $2H_2O$ in 1Ø% Hoagland's solution . Nutrient medium without metal served as control. All the experiments were carried out at pH 7.5 in triplicates for a period of 3,7 and 14 d. The number of fronds in each

flask were counted periodically and multiplication rate (MR) was calculated by the increase in number of fronds as under :

$$\text{Multiplication Rate} = \frac{(N + N') - No}{No \times t} \times 100$$

Where;

N = healthy population , N'= morbid population
No= starting number of plants,t = time in days

Biomass was determined by drying fresh plants in an oven at 80 C to a constant weight.Total chlorophyll content was measured in 80 % chilled acetone extract following the method suggested by Arnon (1949). Both dried plant material and water samples were digested in $HNO_3$:$HClO_4$ (4:1,v/v) at 80 C.Metals were estimated using a Perkin Elmer 2380 Atomic Absorption Spectrophotometer. Uptake values were determined in each case after deducting the metal contents of control plants on dry wt basis.Variability of data and validity of results were checked employing statistical analysis (Schefler 1969).

## RESULTS AND DISCUSSION

Field populations of S. polyrrhiza were analysed for their metal contents(Table 1). Data revealed that the accumulation of some of the metals was fairly high (Fe, 71.0 ; Mn, 22.7 μ moles $g^{-1}$). However, the concentration of these metals in the water was quite low with the exception of Fe (30.8 μM).

Table 1. Metal content of water (μM)and S. polyrrhiza (μ moles $g^{-1}$) collected from KD pond, Unnao (pH 7.5)

| Metals | Pond Water | S. polyrrhiza |
|---|---|---|
| Fe | 30.8±2.15 | 71.0±1.19 |
| Mn | 2.5±0.07 | 22.7±0.58 |
| Cu | 2.2±0.05 | 1.8±0.09 |
| Cr | 6.6±0.62 | 3.3±0.37 |
| Pb | 3.3±0.37 | 3.0±0.08 |
| Cd | 0.026 | 0.029 |

Values expressed as mean ±SD (n=3)

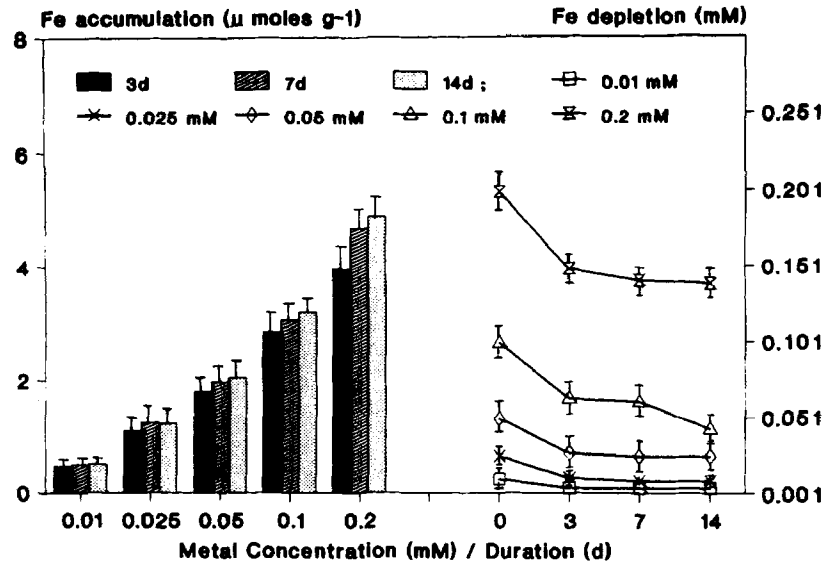


Figure 1.Iron accumulation as a function of concentration ($p<0.01$) and time [3d, $y=0.67+17.67x$ ($r=0.97$); 7d, $y=0.67+20.96x$ ($r=0.98$); 14d, $y=0.66+22.08x$ ($r=0.98$)] and removal from water by S.polyrrhiza as a function of time and biomass. Mean ±SE (n=3).

The internal Fe content (Figure 1) of S. polyrrhiza significantly increased from 0.467 to 3.97 µ moles $g^{-1}$ in response to ambient metal concentration within 3 d and further increased (4.88 µ moles $g^{-1}$) up to 14 d. Results revealed that plants reduced Fe level below maximum permissible limit (0.3 µg $ml^{-1}$) at the lowest concentration (0.01 mM).However,plants removed 50% iron at 0.05 mM Fe within 14 d.Manganese accumulation (Figure 2) increased linearly ($r= 0.98$) in response to increase in metal concentrations in the medium and increased slightly with treatment duration. Plants accumulated maximum amount of metal (4.74 µ moles $g^{-1}$) at 0.2 mM Mn after 14 d. Removal below maximum permissible limit could not be achieved even at lowest concentration of Mn (0.01mM), while plants removed 50% of metal at this concentration within 3 d.

Total chlorophyll content of the plants treated with Fe and Mn as a function of concentration and time is presented in Table 2. Results indicate an increase in chlorophyll content with the increase in background metal concentrations.An increase of 18.2% over control

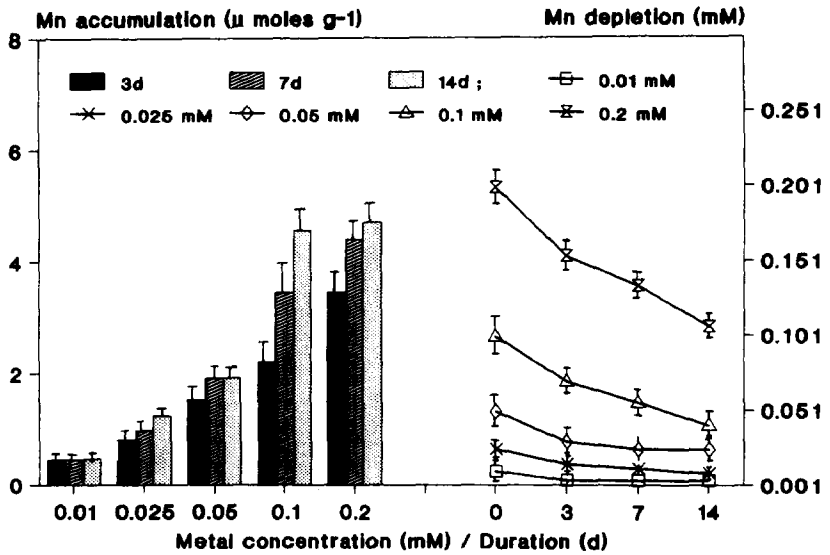


Figure 2. Manganese accumulation as a function of concentration ($p < Ø.Ø1$) and time [3d, y= Ø.51+15.32x (r=Ø.98);7d, y=Ø.52+23.82x (r=Ø.98);14d, y=Ø.31+35.94x (r=Ø.99)] and removal from water by S. polyrrhiza as a function of time and biomass. Mean ± SE (n=3).

($p < Ø.Ø1$) was found at Ø.2 mM Fe after 3 d, while at Ø.1 mM Fe,the chlorophyll increased to 48.7% ($p <Ø.Ø1$) after 7 d and 53.Ø% ($p<Ø.Ø1$) at Ø.Ø5 mM Fe after 14 d of traetment.Chlorophyll content in Mn treated plants increased up to a level of Ø.Ø5 mM Mn after 3 and 7 d and upto Ø.Ø25 mM after 14 d. An increase of 29.5% ($p<Ø.Ø1$) over the control was recorded at this concentration after 3 d of treatment. However, a decrease (8.9%) in chlorophyll content was recorded at 2.Ø mM Mn after 14 d of treatment ($p < Ø.Ø5$).

Fronds of S. polyrrhiza were found tolerant to high concentrations of Fe and Mn (Table 3). However, with increasing metal concentration in the medium, biomass and multiplication rates (MR) were affected progressively. Biomass decreased to 23.2% ($p < Ø.Ø5$) and 29.4% ($p < Ø.Ø1$) over control in Fe and Mn treated frond, respectively at Ø.2 mM. Multiplication rates of Fe treated plants decreased steadily from 17.61 to a low of 12.5 at Ø.2 mM Fe after 7 d of treatment. Similarly, all Mn concentrations resulted in reduced MR (from 17.7 to 11.Ø4 at Ø.2mM Mn).

Table 2. Effect of Fe and Mn on Chlorophyll content (mg $g^{-1}$ fw) in S. polyrrhiza at different durations.

| Fe/Mn(mM) | 3d | 7d | 14d |
|---|---|---|---|
| Control | Ø.690±Ø.Ø11 (Ø.678±Ø.Ø13) | Ø.726±Ø.Ø26 (Ø.711±Ø.Ø17) | Ø.729±Ø.Ø26 (Ø.779±Ø.Ø15) |
| Ø.Ø1Ø | Ø.732±Ø.Ø21 (Ø.733±Ø.Ø13)**a** | Ø.834±Ø.Ø27**a** (Ø.740±Ø.Ø21) | Ø.891±Ø.Ø31**a** (Ø.784±Ø.Ø26) |
| Ø.Ø25 | Ø.746±Ø.Ø11**a** (Ø.858±Ø.ØØ8)**c** | Ø.891±Ø.Ø11**c** (Ø.848±Ø.Ø12)**c** | 1.ØØØ±Ø.Ø11**c** (Ø.846±Ø.Ø34) |
| Ø.Ø5Ø | Ø.783±Ø.Ø12**b** (Ø.878±Ø.Ø23)**c** | Ø.959±Ø.Ø11**c** (Ø.861±Ø.Ø14) | 1.116±Ø.Ø11**c** (Ø.766±Ø.Ø48) |
| Ø.1ØØ | Ø.793±Ø.Ø12**c** (Ø.724±Ø.Ø17) | 1.Ø8Ø±Ø.Ø12**c** (Ø.723±Ø.Ø16) | Ø.9ØØ±Ø.Ø12**c** (Ø.71Ø±Ø.Ø56) |
| Ø.2ØØ | Ø.816±Ø.Ø21**b** (Ø.712±Ø.Ø22) | Ø.939±Ø.Ø67**a** (Ø.713±Ø.Ø18) | Ø.889±Ø.Ø21**b** (Ø.7Ø9±Ø.Ø17)**a** |

Values in parentheses are chlorophyll content of Mn treated plants. Values not in parentheses are Fe treated. **a**=p<Ø.Ø5;**b**=p <Ø.Ø2;**c**=p <Ø.Ø1. Mean ±SD (n=3)

Table 3. Effect of Fe and Mn on final biomass (mg dw) yield and multiplication rate in S.polyrrhiza.

| Conc. (mM) | Iron | | Manganese | |
|---|---|---|---|---|
| | Biomass | MR | Biomass | MR |
| Control | 38.2±1.5 | 17.61 | 39.2±1.2 | 17.7Ø |
| Ø.Ø1Ø | 38.3±1.3 | 16.Ø6 | 39.7±2.2 | 16.Ø2 |
| Ø.Ø25 | 33.3±2.4 | 15.18 | 31.5±1.4**b** | 17.54 |
| Ø.Ø5Ø | 33.2±1.4 | 14.27 | 29.5±1.2**b** | 16.7Ø |
| Ø.1ØØ | 33.2±1.7 | 13.82 | 29.Ø±1.2**c** | 13.1Ø |
| Ø.2ØØ | 29.3±2.3**a** | 12.48 | 27.6±1.3**c** | 11.Ø4 |

**a**=p <Ø.Ø5;**b**=p <Ø.2;**c**=p <Ø.Ø1. Mean ±SD (n=3)

Results showed significant accumulation of both the metals.However, the iron accumulation was more than that of manganese.Similar trends of metal accumulation was also evident from field populations of S.polyrrhiza.Such a high accumulation of Fe might be ascribed to be due to the greater requirement of iron as a constituent of many enzymes and the cytochromes of

certain porphyrins in early growth phases(Hewitt 1958). All the concentrations of Fe and Mn decreased MR, while concentrations > Ø.Ø25-Ø.2Ø mM reduced final biomass yield.In contrast, the chlorophyll content was least affected and a significant decrease was found only with Ø.2 mM Mn.This might be due to the high specificity of the plants for Fe which is required for chlorophyll biosynthesis. Results of Fe and Mn effects on MR agree with those of Nasu et al.(1984) who found inhibition of MR as a criterion for deciding the order of toxicity.However, increased MR and decreased biomass at very low levels of Fe in S. polyrrhiza as reported by Schreinemakers (1984) were not encountered. Plants exhibited differential toxic responses; Mn being more toxic than Fe. This could be mainly due to high affinity of Fe to form complexes with the organic components of the plants.This conforms to our previous report (Rai and Chandra 1992) on Fe and Mn toxicity and accumulation in Hydrodictyon reticulatum.

Various responses of plants like,visible injury to key morphological and physiological endpoints,growth characteristics and biochemical changes (Burton 1986, Van Assche et al.1988) have been used to monitor elevated environmental contamination. However,in the present study it was interesting to note that MR was more sensitive than biomass and chlorophyll. At the lowest concentration of metals, when there was no inhibition of biomass and chlorophyll,MR decline. This could be due to the greater sensitivity of cell differentiation than the biosynthesis of chlorophyll. The study suggests the possiblitiy of using MR as a bioassay of very low levels of Fe and Mn contamination in aquatic environments.Further research should be carried out using S.polyrrhiza in metal abatement studies.

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