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# Interactive Metal Accumulation and Its Toxic Effects under Repeated Exposure in Submerged Plant *Najas indica* Cham

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Among aquatic macrophytes, the role of submerged plants in relation with accumulation of metals and its toxicity has been well documented (Guilizzoni 1991; Sinha et al. 1994; 1997, Gupta et al. 1996; Rai et al. 1995a, b; Vajpayee et al. 2001; Sinha et al. 2002). These authors have reported that submerged plants attain equilibrium with their surroundings as they do not migrate and have shown better accumulation due to large exposed surface area. This may be a viable alternative for the remediation of metals if proper disposal of the spent plants can be employed. Iron and chromium are distributed unevenly and their high concentrations have been reported in the aquatic systems (Chandra et al. 1993). Iron is an essential metal of pivotal importance in free radical defense mechanisms of living cells, yet excessive concentrations of iron and its compounds have demonstrated the potential to induce free radical production, which plays an important role in the onset of peroxidation of membrane lipids (Halliwell and Gutteridge 1993). Among the stable oxidation states of Cr (trivalent and hexavalent), Cr (VI) is considered to induce toxic effects on growth and development of the plants (Sinha et al. 1993; Rai et al. 1995a, Suseela et al. 2002). Biological interest in Cr and Fe arose due to their significant presence in industrial effluents, particularly tannery industry and their toxic effects on plants (Vajpayee et al. 2001; Sinha et al. 2002; Halliwell and Gutteridge 1993). In aquatic ecosystems, the plants are seldom exposed to single metal and in most of the cases the stress of pollution may be attributed to the effect of metals in combination. Thus, the study of interactive effect of metals played a significant role on the accumulation and toxicity in the plants which may be synergistic and/ or antagonistic (Sinha and Chandra 1990; Sinha et al. 1993; Rai et al. 1995a). The effect of repeated exposure of metals may also influence its accumulation (Sinha 1999). However, the information about the accumulation and toxicity of metals in submerged plant, *Najas indica* Cham. is very meager. Lee et al. (1998) reported the use of dry biomass of *Najas graminea* Del., *Myriophyllum scabratum* and *Myriophyllum elatinaides* for the removal of metals. Rout and Shaw (2001) reported the involvement of antioxidant enzymes in salt tolerance in the plants of *Najas indica*. Recently, Sinha et al. (2002) reported the accumulation of Cr in the submerged plants of *Najas indica* and *Vallisneria spiralis* under laboratory conditions. In view of the above, the effect of iron singly and in combination with chromium was examined in the plant, *Najas indica* Cham. with respect to metal accumulation, photosynthetic pigments, protein, cysteine and malondialdehyde content under laboratory conditions.

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## MATERIALS AND METHODS

The plants of *Najas indica* Cham. were maintained in the field laboratory for experimental studies. Healthy plants (6" long) were cut off from mother plants and acclimatized in 10% Hoagland's solution for 6 weeks under laboratory conditions. Using AR-grade $FeCl_3$ and $K_2Cr_2O_7$, different concentrations of Fe and Fe+Cr (0.5, 2.0, 4.0 and 8.0 μg $ml^{-1}$) were prepared in 10% Hoagland's solution. The test concentrations were based on the metal and measured by GBC Avanta Σ Atomic Absorption Spectrophotometer (AAS) to check the actual exposure level while setting the experiments. The metal concentrations are the mean of five replicates and the standard deviation ranges from 0.04 to 0.06. Three sets of each concentrations (served as three replicates) were kept in 250 ml beaker (200 ml solution) containing different concentrations (0.5, 2.0, 4.0 and 8.0 μg $ml^{-1}$) of Fe and equal concentrations of Fe and Cr under mixed metal treatment in 10% Hoagland's solution along with one set of control in 10% Hoagland's solution. The plants with almost the same biomass (3.0 g fresh weight) were kept in all the treatments and control sets. The experiments were performed under standard physiological conditions providing 16 h light (114 μ moles $m^{-2}$ $s^{-1}$) and 8 h dark photoperiod using fluorescent tube lighting (Philips) at 26 ± $2^0$ C temperature. The plants were harvested after 3, 6 and 9 days of exposure and the solutions were changed on every 3rd day. The blotted plants were used for the estimation of photosynthetic pigments, protein, cysteine, and malondialdehyde contents. The remaining part of the plants were dried in the oven at 80°C for one week and kept for metal analysis. Photosynthetic pigments in treated and control leaves (100 mg) were estimated by the method of Arnon (1949) using GBC Cintra $10_e$ spectrophotometer. Protein content was estimated by the method of Lowry et al. (1951), using bovine serum albumin as the standard. The level of lipid peroxidation was measured in terms of malondialdeyde (MDA), a product of lipid peroxidation in the plant samples estimated by thiobarbituric acid (TBA) reaction (Heath and Packer 1968). Cysteine content was measured in fresh plants by the method of Gaitonde (1967). Dried tissues were digested in $HNO_3$ (70%) using microwave digestion system MDS 2000 and metal contents were estimated using AAS. Detection limit of Cr and Fe are 0.003 and 0.005 μg $ml^{-1}$, respectively. Chromium content in the control plants was found below detection limit and Fe 59.3 μg $g^{-1}$ dw. Iron content of control plant has already been subtracted from the data of accumulation of Fe, shown in Table 1.

Analytical data quality of Cr and Fe were ensured through repeated analysis (n=6) of EPA quality control samples (Lot TMA 989) in water and the results were found to be within ±3.05% certified values. Recoveries of Fe and Cr from the plant tissues were found to be 99.1 ±4.05% and 98.9 ±3.58%, respectively as determined by digesting four samples each from untreated plant with known amount of metals. The blanks were run all the time and triplicate was carried out to check the precision of the method. Analysis of variance (ANOVA) at significance level $p<0.05$, in completely randomized block design and students t-test were performed to confirm the validity of the data (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

The accumulation of metal in single (Fe) and mixed metal (Fe+Cr) treatments at 0.5, 2.0, 4.0 and 8.0 μg $ml^{-1}$ for 3, 6 and 9 days by replacing the solution on every 3rd day is shown in Table 1. The accumulation of metals increased in a concentration-duration

**Table 1.** Accumulation of Fe and Cr (μg $g^{-1}$ dw) in *Najas indica* (mixed).

| Conc. (μg $ml^{-1}$) | Fe (μg $g^{-1}$ dw) | | | Cr (μg $g^{-1}$ dw) | | |
|---|---|---|---|---|---|---|
| | Exposure periods(d) | | | | | |
| | 3 | 6 | 9 | 3 | 6 | 9 |
| 0.5 | 95±12 (120±8) | 269±10 (222±1) | 410±16 (387±2) | 39±8 (97±9.1) | 113±12 (144±18) | 141±23 (163±20) |
| 2.0 | 113±23 (243±8) | 302±21 (272±2) | 735±20 (521±9) | 84±8 (178±11) | 166±13 (240±20) | 188±9 (178±17) |
| 4.0 | 151±25 (264±17) | 321±18 (301±3) | 596±19 (629±3) | 136±17 (299±30) | 303±25 (317±16) | 277±25 (229±17) |
| 8.0 | 206±24 (320±19) | 384±41 (300±3) | 460±61 (610±3) | 144±10 (430±32) | 405±21 (473±24) | 436±20 (458±28) |

Values in parenthesis are accumulation of Fe and Cr under single metal treatments. Metal solutions were replaced with initial metal concentrations on 3rd and 6th day. (Cr accumulation treated singly reported earlier, Sinha et al. 2002). Values are mean of triplicates±S.D. ANOVA, F (Conc.) =128.22* (Fe, single), 5.95* Fe, mixed); F (Expo.) =789.96* (Fe, single), non-significant (Fe, mixed). F (Conc.) =596.22* (Cr, mixed); F (Expo.) = 596.71* (Cr, mixed). *P < 0.05.

dependent manner, more in Fe treated plants. At lowest metal concentration (0.5 μg $ml^{-1}$), the treated plants accumulated 95 μg $g^{-1}$ dw of Fe and 39 μg $g^{-1}$dw of Cr under single metal treatment, which increased with increase in Fe and Cr concentrations after 3 days of exposure. The accumulation of metal further increased by the addition of metal under mixed metal treatment. At 9 days of exposure period, maximum accumulation of Fe, 629 μg $g^{-1}$ dw ($p< 0.01$) was found at 4 μg $ml^{-1}$ in single metal treatment, however, the accumulation of Fe increased under mixed metal treatment upto 2 μg $ml^{-1}$ followed by decrease at 4 and 8 μg $ml^{-1}$, compared to single metal treatment. The maximum accumulation of Cr (436 μg $ml^{-1}$, $p< 0.05$) was found in Fe+Cr treated plants at 8 μg $ml^{-1}$ on day 9 and there was non-significant difference in Cr accumulation compared to single metal treatment. The effect of different metal concentrations, both Fe and Fe+Cr on protein content at different exposure periods is shown in Fig 1. Significant increases of 15.77 % ($p<0.01$) and 14.40% ($p<0.02$) in protein content of Fe and Fe+Cr treated plants were found at 2 μg $ml^{-1}$, respectively after 3 days. However, the decrease of 42.52% ($p< 0.02$) was found more under mixed metal treatment than iron treated plants (35.78%, $p< 0.05$) at 8 μg $ml^{-1}$ after 9 days of exposure period as compared to control.

The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents (Table 2) increased with an increase in concentration of metal up to 3 days of exposure at 2 μg $ml^{-1}$ followed by decrease at higher metal concentrations, as compared to control. However, an increase in photosynthetic pigments was recorded during all the exposure periods at lowest metal concentration (0.5 μg $ml^{-1}$). The maximum increases (%) of 51.94 (Fe, $p< 0.02$), 44.27 (Fe+Cr, $p< 0.01$) in total chlorophyll and 42.31 (Fe, $p < 0.05$), 32.07 (Fe+Cr, $p< 0.02$) in carotenoid contents were found at 2 μg $ml^{-1}$ after 3 days as compared to controls. At 9 days, the maximum decreases (%) of 34.59 ($p< 0.01$), 35.05 ($p< 0.01$), 59.46 ($p< 0.01$), 40.74 ($p< 0.05$) in Fe treated plants and 46.27 ($p< 0.02$), 42.71 ($p< 0.02$), 71.79 ($p< 0.02$), 56.36 ($p< 0.01$) in Fe+Cr treated plants were found in total chlorophyll, chlorophyll a, chlorophyll b and carotenoid

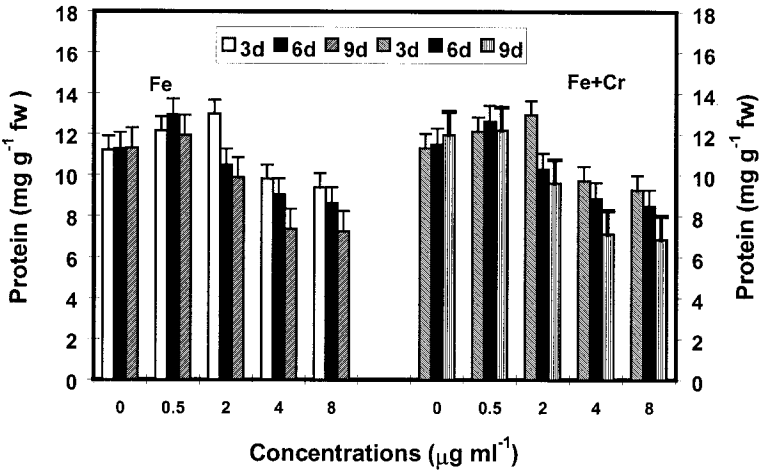


**Figure 1**. The effect of Fe and Fe+Cr on protein content (mg $g^{-1}$ fw) of *Najas indica*. Values are mean of triplicates±S.D. Standard deviations are represented by vertical bars. The metal solutions were replaced with initial metal concentrations on 3rd and 6th day. ANOVA, F (Conc.) = 96.70* (Fe), 179.85* (Fe+Cr); F (Expo.) = 33.90* (Fe), 51.58* (Fe+Cr). *$P < 0.05$.

contents, respectively at 8 μg $ml^{-1}$, compared to their respective controls.

In both the treatments, the plants have shown significant increase in cysteine content (Fig. 2) at 2 μg $ml^{-1}$ after 3 days and at 0.5 μg $ml^{-1}$ after 6 days of exposure followed by decrease with increase in metal concentrations. In Fe treated plants, the maximum increase in cysteine content (14.75%, $p< 0.01$) was found at 2 μg $ml^{-1}$ after 3 days of exposure and maximum decrease of 44.27% ($p< 0.01$) was found at 8 μg $ml^{-1}$ after 9 days of exposure period as compared to their respective controls. In mixed metal treatment, the maximum increase in cysteine content (12.93%, $p< 0.01$) was found at 2 μg $ml^{-1}$ after 3 days of exposure and maximum decrease (48.44%, $p< 0.001$) was found at 8 μg $ml^{-1}$ after 9 days of exposure as compared to their respective controls. There was insignificant difference in cysteine content in both the treatments. The MDA content (Table 3) has shown significant increase with increase in metal concentrations and treatment durations except at 0.5 μg $ml^{-1}$ in Fe treated plants and 2 μg $ml^{-1}$ in Fe+Cr treated plants after 3 days. At 8 μg $ml^{-1}$, the maximum increase in MDA content, 47.06 % ($p< 0.01$) in Fe treated plants and 43.75% ($p< 0.01$) in Fe+Cr treated plants was found after 9 days as compared to their respective controls.

The submerged plants possess significant potential to bioconcentrate metals relative to their environment (Guilizzoni 1991; Rai et al. 1995a; Sinha et al. 1994, 1997). In the present study, an increase in metal accumulation in the plants of *N. indica* by replacing the metal solutions is in agreement with other reports (Sinha 1999; Sinha et al. 2002). Further, the accumulation of Fe increased in the presence of Cr at all the metal concentrations after 6 days of exposure and upto 2 μg $ml^{-1}$ after 9 days of exposure, however, the accumulation of Cr decreased in presence of Fe. These findings are in agreement with earlier reports of Sinha and Chandra (1990), where the accumulation of essential metal (Cu) enhanced and non-essential metal (Cd) inhibited under mixed metal treatment.

| Conc. ($\mu g\ ml^{-1}$) | Exposure periods (d) | | | Photosynthetic pigments |
|---|---|---|---|---|
| | 3 | 6 | 9 | |
| 0.0 | 1.29±0.12 | 1.31±0.12 | 1.33±0.05 | Total chlorophyll |
| | (1.31±0.05) | (1.33±0.07) | (1.34±0.05) | |
| | 0.95±0.06 | 0.96±0.09 | 0.97±0.05 | Chlorophyll a |
| | (0.95±0.10) | (0.96±0.06) | (0.96±0.03) | |
| | 0.35±0.08 | 0.36±0.03 | 0.37±0.02 | Chlorophyll b |
| | (0.35±0.04) | (0.36±0.02) | (0.39±0.01) | |
| | 0.52±0.03 | 0.53±0.03 | 0.54±0.07 | Carotenoid |
| | (0.53±0.03) | (0.54±0.02) | (0.55±0.03) | |
| 0.5 | 1.59±0.09 | 1.47±0.09 | 1.42±0.08 | Total chlorophyll |
| | (1.47±0.15) | (1.41±0.04) | (1.37±0.04) | |
| | 1.18±0.11 | 1.12±0.07 | 1.08±0.06 | Chlorophyll a |
| | (1.05±0.11) | (1.01±0.02) | (0.99±0.04) | |
| | 0.52±0.05 | 0.47±0.02 | 0.44±0.02 | Chlorophyll b |
| | (0.46±0.04) | (0.43±0.01) | (0.41±0.03) | |
| | 0.69±0.07 | 0.65±0.03 | 0.61±0.03 | Carotenoid |
| | (0.64±0.01) | (0.60±0.02) | (0.58±0.06) | |
| 2.0 | 1.96±0.01[b] | 1.39±0.03 | 1.28±0.08 | Total chlorophyll |
| | (1.89±0.07)[c] | (1.25±0.08) | (1.22±0.07) | |
| | 1.39±0.01[c] | 0.98±0.03 | 0.83±0.03 | Chlorophyll a |
| | (1.24±0.05)[a] | (0.85±0.05) | (0.69±0.04) | |
| | 0.59±0.02[a] | 0.39±0.01 | 0.32±0.02 | Chlorophyll b |
| | (0.51±0.02)[a] | (0.35±0.02) | (0.30±0.02) | |
| | 0.74±0.05[a] | 0.54±0.02 | 0.49±0.04 | Carotenoid |
| | (0.70±0.02)[b] | (0.51±0.04) | (0.41±0.03) | |
| 4.0 | 1.21±0.03 | 1.17±0.01 | 1.04±0.05 | Total chlorophyll |
| | (1.18±0.36) | (1.1±0.04) | (0.99±0.05) | |
| | 0.91±0.15 | 0.85±0.11 | 0.76±0.04 | Chlorophyll a |
| | (0.82±0.24) | (0.74±0.02) | (0.60±0.04) | |
| | 0.30±0.08 | 0.26±0.07 | 0.21±0.02 | Chlorophyll b |
| | (0.25±0.12) | (0.22±0.02) | (0.18±0.01) | |
| | 0.48±0.08 | 0.44±0.03 | 0.41±0.03 | Carotenoid |
| | (0.42±0.13) | (0.38±0.02) | (0.32±0.06) | |
| 8.0 | 1.11±0.01 | 1.01±0.11 | 0.87±0.03[c] | Total chlorophyll |
| | (1.06±0.45) | (0.88±0.01) | (0.72±0.11)[b] | |
| | 0.87±0.03 | 0.78±0.09 | 0.63±0.02[c] | Chlorophyll a |
| | (0.75±0.32) | (0.69±0.06) | (0.55±0.07)[b] | |
| | 0.25±0.07 | 0.21±0.03 | 0.15±0.01[c] | Chlorophyll b |
| | (0.20±0.13) | (0.17±0.01) | (0.11±0.05)[b] | |
| | 0.40±0.03 | 0.37±0.02 | 0.32±0.04[a] | Carotenoid |
| | (0.36±0.09) | (0.31±0.03) | (0.24±0.03)[c] | |

Values in parenthesis are photosynthetic pigments of Fe+Cr treated plants. The metal solutions were replaced with initial metal concentrations on 3rd and 6th day. Values are mean of triplicates±SD. t- test (two tailed) as compared to control. [a]= $p<0.05$, [b]= $p<0.02$, [c]= $p<0.01$. ANOVA, For total chl., F (Conc.) = 112.09* (Fe), 20.22* (Fe+Cr); F (Expo.) = 53.77* (Fe), 10.11* (Fe+Cr); For chl. a, F (Conc.) = 47.61* (Fe), 16.92* (Fe+Cr); F (Expo.) = 36.59* (Fe), 12.44* (Fe+Cr); For chl. b, F (Conc.) = 59.9* (Fe), 171.43* (Fe+Cr); F (Expo.) = 20.65* (Fe), 28.57* (Fe+Cr); For carotenoid, F (Conc.) = 58.75* (Fe), 75.0* (Fe+Cr); F (Expo.) = 16.35* (Fe), 20.0* (Fe+Cr). *$P < 0.05$.

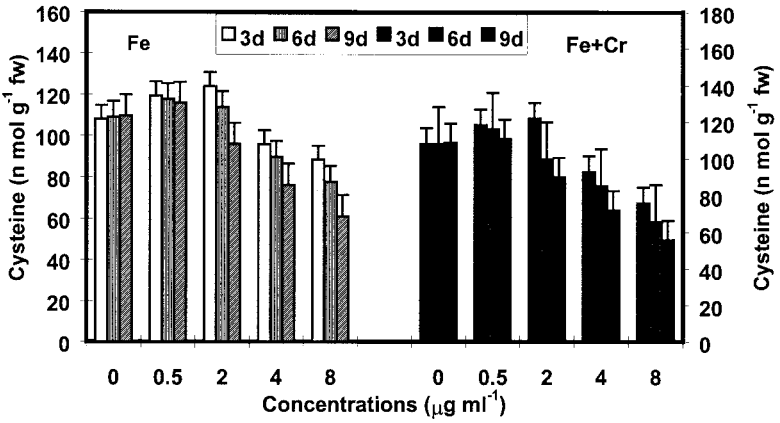


**Figure 2**. The effect of Fe and Fe+Cr on cysteine content (n mol $g^{-1}$ fw) of *Najas indica*. Values are mean of triplicates±S.D. Standard deviations are represented by vertical bars. The metal solutions were replaced with initial metal concentrations on 3rd and 6th day. ANOVA, F (Conc.) = 2186.82* (Fe), 1807.04* (Fe+Cr); F (Expo.) = 700.69* (Fe), 468.97* (Fe+Cr). *P < 0.05.

**Table 3.** Effect of Fe and Fe+Cr on MDA contents (m moles $g^{-1}$ fw) of *Najas indica*. The metal solutions were replaced with initial metal concentrations on 3rd and 6th day.

| Conc. (µg $ml^{-1}$) | Exposure periods (d) | | | | | |
|---|---|---|---|---|---|---|
| | Fe | | | Fe+Cr | | |
| | 3 | 6 | 9 | 3 | 6 | 9 |
| 0.0 | 10.8±0.44 | 11.2±0.75 | 11.9±0.59 | 10.8±0.34 | 10.9±0.49 | 11.2±0.75 |
| 0.5 | 10.2±0.2[a] | 12.6±0.56 | 13.2±0.83 | 10.1±0.08 | 11.2±0.18 | 11.2±1.04 |
| 2.0 | 11.8±0.85 | 14.0±0.71 | 14.3±0.53 | 8.8±0.83[b] | 11.5±0.15 | 12.6±0.73 |
| 4.0 | 12.6±0.44 | 15.5±0.43 | 16.7±0.39 | 11.0±0.65 | 12.8±0.14 | 14.1±0.82 |
| 8.0 | 13.4±0.39 | 16.7±0.76 | 17.5±0.18[c] | 12.5±0.39 | 13.6±0.28 | 16.1±0.07[c] |

Values are mean of triplicates±SD. t- test (two tailed) as compared to control. [a]= p<0.05, [b]= p<0.02, [c]= p<0.01. ANOVA, F (Conc.) = 108.56* (Fe), 69.59* (Fe+Cr); F (Expo.) = 91.24* (Fe), 69.31* (Fe+Cr). *P < 0.05.

An increase in protein content under metal stress is well known due to the formation of stress proteins (Costa and Spitz 1997), however, the decline in protein content at higher metal concentrations may be due to the oxidation of proteins (Halliwell and Gutteridge 1993). Our study also showed an increase in protein content at lower metal concentration followed by decrease with increase in metal concentrations and exposure periods which conforms with the findings of earlier reports (Sinha et al. 1994; Vajpayee et al. 2001).

Reduction of chlorophyll biosynthesis in the presence of heavy metals is well known (Van Assche and Clijsters 1990), however, an increase in chlorophyll content on treatment with metals has also been reported at lower concentrations (Gupta et al. 1996; Sinha et al. 1997). In the present study, an increase in chlorophyll content was recorded at lower metal concentrations. In Fe treated plants, an increase in chlorophyll content may be due to the high requirement of Fe for chlorophyll biosynthesis. The mixed

**Table 4.** Regression analysis of various responses in the metal treated plant of *Najas indica* at different exposure periods.

| Parameters | Regression analysis at different exposure periods | | |
|---|---|---|---|
| | 3 d | 6 d | 9 d |
| Accumulation | Y (Fe) = 153.34 + 23.01x, r = 0.89<br>Y(Fe mixed)=86.64 +15.07x, r =0.1<br>Y(Cr mixed)= 52.4 +13.34x, r =0.88 | Y(Fe) = 240.6 + 9.14x, r = 0.8<br>Y(Fe mixed)= 265.35 +14.8x, r = 0.99<br>Y(Cr mixed) = 102.3 +39.85x, r = 0.98 | Y(Fe) = 439.67 + 26.78x, r = 0.79<br>Y(Fe mixed)= 577.66 - 7.56x, r = -0.17<br>Y(Cr mixed)= 115.76 +39.92x, r = 0.1 |
| Protein | Y (Fe) =12.13-0.35x, r = -0.743<br>Y (Fe+Cr) = 12.15-0.37x, r = -0.77 | Y (Fe) =11.81-0.46x, R = -0.85<br>Y(Fe+Cr) = 11.72-0.47x, r = -0.09 | Y (Fe) =11.29-0.06x, r = -0.89<br>Y(Fe+Cr) = 11.6-0.70x, r = -0.90 |
| Total Chlorophyll | Y (Fe) = 1.59-0.06x, r = -0.52<br>Y(Fe+Cr) = 1.54-0.056x, r = -0.56 | Y (Fe)= 1.42-0.05x, r= -0.09<br>Y(Fe+Cr) = 1.38-0.063x, r = -0.98 | Y (Fe) = 1.38-0.07x, r = -0.96<br>Y(Fe+Cr) = 1.39-0.08x, r = -0.98 |
| Chlorophyll a | Y (Fe)= 1.16-0.033x, r = -0.49<br>Y(Fe+Cr) = 1.08-0.04x, r = -0.66 | Y (Fe)=1.04-0.34x, R = -0.85<br>Y(Fe+Cr) = 0.96-0.04x, r = -0.92 | Y (Fe)=0.99-0.05x ,r = -0.92<br>Y(Fe+Cr) = 0.92-0.055x, r = -0.87 |
| Chlorophyll b | Y(Fe) = 0.48-0.28x, r = -0.63<br>Y(Fe+Cr) = 0.44-0.03x, r = -0.75 | Y(Fe)= 0.42-0.03x, R = -0.86<br>Y(Fe+Cr) = 0.39-0.3x, r = -0.92 | Y(Fe)= 0.39-0.034x, R = -0.93<br>Y(Fe+Cr) = 0.39-0.04x, r = -0.96 |
| Carotenoid | Y(Fe)=0.65-0.03x, r= -0.68<br>Y(Fe+Cr) = 0.63-0.033x, r = -0.76 | Y(Fe)=0.59-0.03x, r= -0.88<br>Y(Fe+Cr)=0.57-0.35x, r = -0.94 | Y(Fe) = 0.57-0.032x, r = -0.95<br>Y(Fe+Cr) = 0.54-0.042x, r = -0.94 |
| Cysteine | Y(Fe) = 117.67-3.65x, r = -0.788<br>Y(Fe+Cr)= 118.26-5.13x, r = -0.88 | Y(Fe)= 115.69-4.89x, R = -0.925<br>Y(Fe+Cr)= 112.35-6.04x, r = -0.098 | Y(Fe) = 111.36-6.79x, r = -0.96<br>Y(Fe+Cr) = 107.86-7.04x, r = -0.97 |
| MDA | Y(Fe) =10.67+ 0.38x, r = 0.94<br>Y(Fe+Cr) = 9.8 +0.29x, r = 0.70 | Y(Fe)=12.12+0.64x, r=0.94<br>Y(Fe+Cr)= 11.01+ 0.35x, r = 0.99 | Y(Fe) = 12.77 + 0.67x, r = 0.94<br>Y(Fe+Cr) = 11.16+ 0.64x, r = 0.99 |

metal (Fe+Cr) treated plants have not shown much significant effect of Cr as compared to Fe alone treated plants. On considering the study of Sinha et al. (2002), it may be inferred that Fe has antagonistic effect on Cr in mixed metal treatment. In the present study, the decrease in chlorophyll content at higher metal concentrations and increase in exposure periods is in conformity with the findings of several other authors (Rai et al. 1995a; Gupta et al. 1996; Sinha et al. 1994, 2002). This is due to the interaction of heavy metals with the functional group of chlorophyll synthesizing enzymes. An increase in carotenoid content under metal stress may be attributed to the strategy of plants to overcome the metal induced oxidative stress (Kenneth et al. 2000; Vajpayee et al. 2001). Cysteine, a –SH containing amino acid, constitutes one of the amino acids of glutathione. Cysteine content is affected by the activity of cysteine synthesizing enzymes, adenosine 5-phosphosulphate sulfotransferase and ATP-sulfurylase (Nussbaum et al. 1988). The results of the current study showed an increase in cysteine

content at lower metal concentration and decrease at higher concentrations which conform with the findings of Sinha et al. (1994, 1997) and it may be due to depletion of metal in the solution. Heavy metal induced lipid peroxidation is measured in terms of concentration of MDA produced in cell membranes and isolated lipids (Halliwell and Gutteridge 1993). The metal induced membrane destabilization is mostly attributed to increased level of peroxidation of membranes via increased production of free radicals (Halliwell and Gutteridge 1993; Sinha et al. 1997). This results in an increase in malondialdehyde content with the increase in metal concentration. Similar to these reports excess metal concentration resulted in an increase in MDA content in *N. indica* in the present study.

We is intended to show that the accumulation and toxicity of Cr and/ or Fe in *Najas indica* Cham. can be altered by the ratio of the two metals in water. The values generated in this study should not be used to set the toxicity screening levels, nor should they be used to set remediation levels at hazardous waste sites. On comparing the results, it can be inferred that the metal toxicity is more pronounced in the Fe+Cr treated plants than single metal treatment, which may be due to a greater accumulation of metals.

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