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# Accumulation of Chromium and Copper in Three Different Soils and Bioaccumulation in an Aquatic Plant, *Alternanthera philoxeroides*

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Environmental contamination of soils by heavy metals is practically inevitable due to natural leaching and mining activities. There is a concern for heavy metal toxicity to humans and their accumulation potential in soil (Merry et al. 1983) as well as magnification through the aquatic and terrestrial ecosystems. Alligator weed, Alternanthera philoxeroides co-exists abundantly in natural and man-made impoundments of Louisiana and other Southeastern United States. In some developing countries these plants have also been used for bio-gas (methane) production.

Chromium accumulates in aquatic macrophytes Smith (1989, Kahkonen 1998) and terrestrial plants, e.g., Sphagnum papillosum Gstoettner (1997). It is widely distributed in nature and different soils may contain as little as 5 mg/kg to several percent chromium which may be transported to plants in trace to toxic amounts (Shewry and Peterson 1976). Accumulation of Cu in aquatic and terrestrial plants in laboratory conditions was reported by (Pip 1990; Bagatto and Shorthouse 1991; Mullins and Burmester 1993). Two major objectives of our investigations were: (1) to investigate possible Cu and Cr accumulation in clay, humus and sand, and (2) uptake of these metals by alligator weed, Alternanthera philoxeroides.

## MATERIALS AND METHODS

Three types of top soils from A horizon (20-30 cm depth) were collected from different localities (10 mile radius ) of Baton Rouge, LA. Collections were made within a week period. Soil characteristics for percent clay, sand or silt were not analyzed but observed visually for texture and color.

Humic soil was collected from Ben Hur Experiment Station, Louisiana State University; clay from 8601 Block of Seigen Lane, Baton Rouge, and sandy soil from the bank of Mississippi River. Soil characteristics at the time of collection were: humus - gooey, blackish with abundant

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decaying leaves of alligator weed and other plants; clay - extremely sticky, yellowish, with no visible sign of organic matter; sand - basically granular, brownish. Soil was dried in an oven (110°C), ground to a powdered form and passed through a sieve to remove solid objects.

Nine all-glass aquaria (5 L) were prewashed with soap and water, rinsed with acetone and rewashed thoroughly with tap water to remove acetone. Two thousand grams of humus, clay and sand were transferred to each aquarium. Three controls received untreated soil and 2.5 L of aged tap-water, similarly the remaining 6 treatment aquaria received either 1 mg/kg or 10 mg/kg of chromium trioxide or copper sulfate solutions. The entire experiment was replicated thrice and a total of 27 aquaria were used. Test solutions were prepared by diluting a freshly prepared 1% stock solution of Cr03 or CuSO4, to desired concentrations. Proper photoperiod was maintained by 12 hr light and 12 hr dark regimen. The light source was incandescent tube lights in 2 rows (16 bulbs, 40 watts) provided in the laboratory.

Soil and water samples were removed each week for 6 wks. One hundred ml of control water and test solutions were collected in glass vials and stored in a freezer. Two gm sample of each soil was wrapped in aluminum foil and refrigerated each week. After 3 wks, 3 plants in each aquarium were introduced, their leaves remaining above the water level. After 4th, 5th and 6th wks plants were frozen (root and shoot separately). The pH of control and treated water was measured each week using a Beckman pH meter.

Soils and plant samples were dried at 110°C for 3 hrs and passed through a 2 mm sieve prior to digestion in concentrated nitric acid (10 ml for each 0.5 g sample). They were transferred to a 150 ml Teflon microwave digestion vessel. Twelve such samples were digested simultaneously in a microwave digester for 45 minutes, cooled, vented and redigested for an additional 25 minutes ensuring complete digestion. Contents of the vessels were transferred to a 100 ml volumetric flask and the volume adjusted to the mark with deionized water. Plants were similarly digested for 60 minutes.

Cr and Cu in water, soil and plants was quantitated by a Leehman Inductively Coupled Emission Spectrophotometer at Feed & Fertilizer Laboratory of Louisiana State. Parameters for the soil samples were: pressure 20-80 lbs/sq inch; ramp time 5-15 min; hold time O-10 min; for plants: pressure 20-85 lbs/sq inch; ramp time 6-10 min.

## RESULTS AND DISCUSSION

Aged tap-water used for preparing test solutions had undetectable amount of any of the metals. The actual amount of Cr and Cr metal in 1 mg/kg solutions measured 0.8 and 0.66 mg/kg ; in 10 mg/kg solutions the actual amount was 8.16 and 6.47 mg/kg, respectively. The mean pH values of water (control) was 7.2 and for field-collected humus, clay and sand were: 7.5, 7.8 and 9.2, respectively. There was no visible morphological change in alligator weed exposed to 1 and 10 mg/kg CrO3. However, in 10 mg/kg CuSO4, there was a considerable amount of necrosis in root and shoot tissue and loss of chlorophyll. Mean Cr and Cu amounts present in different soils and standard deviations are given in Tables 1 and 2.

**Table 1.** Accumulation of Cr in 1 and 10, mg/kg Cr03 treated soils (6 wks) mean of replications and standard deviations (in parentheses).

| | HUMUS | | CLAY | | SAND | |
|---|---|---|---|---|---|---|
| Wks | 1 | 10 | 1 | 10 | 1 | 10 |
| 1 | 20.9<br>(0.71) | 31.6<br>(0.68) | 17.4<br>(0.33) | 25.5<br>(0.65) | 7.6<br>(0.33) | 8.8<br>(0.65) |
| 2 | 31.3<br>(0.77) | 60.0<br>(0.71) | 16.4<br>(0.73) | 26.4<br>(0.43) | 9.9<br>(1.20) | 11.6<br>(0.70) |
| 3 | 31.3<br>(0.88) | 77.7<br>(0.51) | 27.0<br>(0.55) | 31.5<br>(0.26) | 10.1<br>(0.82) | 11.7<br>(0.44) |
| 4 | 33.5<br>(0.42) | 79.9<br>(0.31) | 27.4<br>(0.68) | 36.1<br>(0.14) | 10.5<br>(0.81) | 13.5<br>(0.55) |
| 5 | 35.3<br>(0.66) | 95.9<br>(0.71) | 28.4<br>(0.51) | 37.7<br>(0.64) | 10.7<br>(0.66) | 13.8<br>(0.77) |
| 6 | 38.7<br>(0.28) | 132.0<br>(0.55) | 33.1<br>(0.98) | 39.6<br>(0.50) | 10.8<br>(0.32) | 14.5<br>(0.58) |

Based on these data presented in Tables 1 and 2, results may be summarized as follows:

(1) After 6 wks, maximum Cr accumulation occurred in 10 mg/kg treated humus: 132 mg/kg and least in sand (14.5 mg/kg). The same was true for 1 mg/kg Cr treatment.The maximum values for Cu in 10 mg/kg for humus were much less, 56.0 and about the same for sand (14.0 mg/kg), respectively.

(2) Considering the overall maximum and minimum accumulation of Cr and Cu accumulation in all soils exposed to 1 or 10 mg/kg metal, in soils after 6 wks exposure to 10 or 1 mg/kg metal, the trend was: humus>clay>sand.

(3) Cr and Cu accumulation in treated soils was generally time-dependent.

(4) Statistical analyses of the data revealed that Cr and Cu accumulation in 10 mg/L treated humus was highly significant ($P<0.01$), and significant in clay and sand ($P<0.05$) .

**Table 2.** Accumulation of Cr in 1 and 10, mg/kg CuSO4 treated soils (6 wks) mean of 3 replications and standard deviations (in parentheses).

| Wks | HUMUS | | CLAY | | SAND | |
|---|---|---|---|---|---|---|
| | 1 | 10 | 1 | 10 | 1 | 10 |
| 1 | 16.5<br>(0.26) | 26.8<br>(1.42) | 8.5<br>(0.70) | 9.8<br>(0.30) | 2.4<br>(0.46) | 3.8<br>(0.81) |
| 2 | 19.7<br>(0.68) | 34.2<br>(1.79) | 17.5<br>(0.73) | 19.3<br>(0.78) | 3.0<br>(0.75) | 6.2<br>(0.65) |
| 3 | 20.3<br>(0.64) | 44.9<br>(1.56) | 17.6<br>(0.92) | 27.8<br>(1.05) | 3.2<br>(0.26) | 11.0<br>(1.65) |
| 4 | 18.8<br>(1.15) | 46.4<br>(0.72) | 16.5<br>(0.46) | 35.4<br>(1.34) | 3.5<br>(0.43) | 12.5<br>(0.45) |
| 5 | 18.7<br>(0.72) | 41.7<br>(1.34) | 16.6<br>(0.96) | 38.4<br>(1.10) | 3.2<br>(0.26) | 13.2<br>(0.78) |
| 6 | 19.8<br>(0.91) | 56.0<br>(1.01) | 19.1<br>(0.79) | 39.4<br>(0.81) | 5.5<br>(0.87) | 14.0<br>(0.96) |

Time-dependent accumulation of these two heavy metals in all soils tested and their maximum amount in humic soil is supported by the following studies: Lund and Fobian (1991) reported time-dependent accumulation of Cr, As and Cu in humus and clay containing soils in field-collected samples during a chemical spill in Denmark. Zhu and Alva (1993) concluded that As, Cu, Cr accumulation is variable in different soils and depends upon the amount of organic matter in the soil. This was further confirmed by Holmgren et al. (1993) who reported significantly higher quantities of metals in soils of Florida, Oregon and Great Lakes which had higher amount of organic matter.

**Table 3.** Chromium and copper accumulation (mg/kg) in alligator weed, Alternanthera philoxeroides exposed to 1 and 10 mg/kg CrO3 and CuSO4 for 3 wks.

| Soil Type | 1 mg/kg Root | Shoot | 10 mg/kg Root | Shoot |
|---|---|---|---|---|
| | | **CHROMIUM** | | |
| Humus | 27.7<br>(0.70) | 5.1<br>(0.58) | 119.7<br>(0.70) | 5.7<br>(0.22) |
| Clay | 79.0<br>(0.61) | 8.4<br>(1.18) | 116.0<br>(0.91) | 11.7<br>(0.49) |
| Sand | 45.4<br>(0.55) | 4.9<br>(0.67) | 214.1<br>(0.91) | 39.1<br>(1.30) |
| | | **COPPER** | | |
| Humus | 56.9<br>(1.60) | 6.2<br>(0.43) | 231.9<br>(1.51) | 10.9<br>(0.35) |
| Clay | 80.1<br>(0.87) | 12.7<br>(0.69) | 235.5<br>(1.17) | 13.5<br>(1.37) |
| Sand | 190.0<br>(1.28) | 23.3<br>(0.79) | 1775.0<br>(0.96) | 73.8<br>(1.47) |

Mean of triplicate samples are written on the upper line and standard deviations in parentheses. Contrary to accumulation of Cr and Cu in 3 soil types per se, the maximum amount of Cr and Cu uptake occurred in the root of sand-grown plants exposed to 10 mg/L solutions (214 mg/L and 1775 mg/kg, respectively). One of the plausible reasons for this would be that Cr and Cu bind more tightly to humus and clay than sand, consequently, greater amounts were available in test solutions of sand-grown plants. The uptake of Cr and Cu was: sand>clay>humus. The least uptake of Cr occurred (4.9 mg/kg) in sand-grown shoot exposed to 1 mg/kg CrO3.

Invariably, greater amount of metals accumulated in root than shoot. Combining the total amount of metal in root of all plants exposed to Cr and Cu (both 1 and 10 mg/kg), root had 88.6 and 96.5% Cr and Cu, respectively. We have previously noticed (Naqvi et al. 1993) that alligator weed accumulated greater amount of Cd in root than shoot. Smith (1989) concluded that regardless of the form Cr presented to plants, it is largely retained in the roots, although the oxidation state, pH, presence of humate and fulvates as well as plant species might affect uptake and transport. Other investigators have also confirmed that primary site of metal (Cu,Cd, Pb) accumulation in the aquatic plants is root (Srivastav et al. 1994; Wolverton and McDonald 1975). The same was true for terrestrial plants (Pip 1990; Bagatto and Shorthouse 1991; Otte 1991; Minnich et al. 1983).

The uptake of Cu by alligator weed grown in different soils was significantly greater than Cr. This is exemplified by root and shoot of this plant (exposed to 1 and 10 mg/kg CuSO4) which accumulated a greater amount of Cu than Cr (Table 3). Possibly, this was due to greater water solubility of Cu than Cr.

Water hyacinths (Eichornia crassipes) have been used for removing Ag, Co, Sn, Pb, Cd, Ni and Hg from polluted waters (Muramoto and Oki, 1983; Wolverton and McDonald, 1975). We (Naqvi et al. 1993) have reported earlier that alligator weed accumulated 1.31 mg/kg Pb in root exposed to 0.5 mg/kg lead nitrate for 3 wks. Plants exposed to Cd (0.1 mg/kg) had 0.99 mg/kg Cd. Shi (1990) reported purifying efficiencies of several freshwater aquatic plants in China. Srivastav and co-workers (1994) also used aquatic plants to remove Cr and Ni from waste-water in India. It is clear from these studies that aquatic macrophytes are capable of removing certain heavy metals rapidly from water. We feel, alligator weed can also be suitably used for Cu or Cr abatement from contaminated waters. it is imperative, that the plants are harvested promptly after metal removal. However, they should then be incinerated to avoid environmental contamination.

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