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Accumulation and Depuration of Metals by Duckweed (*Lemna Perpusilla*)

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Duckweed (*Lemna perpusilla*), inhabiting a heavy coal ash, secondary retaining basin from a coal-fired power plant, was the most abundant macrophyte sampled (occupying ~95% of the surface area) during the summer–fall period of 1979. *Lemna* in the basin accumulated the most abundant heavy and soft metals (Cd, Cu, Fe, Mn, Zn, Cr, Pb, Ni) affiliated with heavy ash to a greater extent than found in water or coal ash sediment. After a 14-day holding period under laboratory conditions of low metal influence, some duckweed metal concentrations declined to levels similar to those found in the ash basin sediments (Pb, Cr, Ni, and Cu), while other tissue concentrations (Cd, Zn, Mn, and Fe) remained higher. During the 10-day laboratory exposure bioassay (concentrations of 0.5, 1, 10, 25, and 100 times those measured in the basin for all metals) after the depuration period, high duckweed mortality was evident within 2 to 10 days at concentrations of 10× and higher, which were coupled with excessive pH reduction (3.6–2.6). Significant accumulation relative to conditions in the ash basin occurred at ≥10× concentration (≥0.50 and 0.10 mg/liter, respectively) for Pb and Cr and at the 1× level (0.10 mg/liter) for Ni. Depuration concentrations returned to control levels within 8 days. Cu and Cd showed similar high increases (~1200 and 100 mg/liter, respectively) in bioconcentration during the 0.5 to 1.0 × concentrations but failed to be significantly depurated during the 8-day recovery period. Exposures to 0.10 mg/liter Zn and 0.05 mg/liter Mn failed to show depuration to levels similar to the control concentrations. The capacity of duckweed to accumulate potentially toxic heavy metals in coal-ash-retaining systems may have an important role upon the displacement of these elements on a seasonal basis. Aquatic elemental releases of duckweed may be minimal during the growing season due to the bioconcentration capability but can be maximal in the fall during the process of natural mortality and the resulting depuration process into the receiving drainage system.

The sluicing of fly ash and heavy (bottom) coal ash residues into settling basins with the subsequent release of waste water into receiving drainage systems poses a serious potential for deleterious environmental impacts associated with coal-fired power plants (Dvorak, 1978). One area of concern has been the quantities of chemical elements which are released into aquatic ecosystems, including several highly toxic and bioaccumulative metals (Rohrman, 1971; Guthrie and Cherry, 1976; Patrick and Loutit, 1976; Gutenmann *et al.*, 1976; Cherry and Guthrie, 1977). The accumulation of metals in the biomass of aquatic organisms may facilitate the removal of toxicants from receiving waters (Guthrie and Cherry, 1976, 1979a; Cherry and Guthrie, 1979), increasing the potential for toxic responses or trophic level bioconcentration (Cherry and Guthrie, 1977, 1979; Guthrie and Cherry 1979a,b; Ryther *et al.*, 1979).

Aquatic macrophytes concentrate metallic elements above background or

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aqueous levels in unperturbed environments (Boyd, 1970; Cowgill, 1974) and stressed habitats (Ray and White, 1976; Rodgers *et al.*, 1978; Nakada *et al.*, 1979). While the ability of aquatic ecosystems to recover from pollutant impact recently has been reviewed (Cairns *et al.*, 1977; Cairns, 1980), the ability of macrophytes to depurate accumulated metals upon removal from stressed environments has not been investigated thoroughly. In this study, the accumulation and depuration of metals by duckweed, *Lemna perpusilla*, exposed to heavy ash basin effluents *in situ* and subjected to a mixture of metals associated with coal ash residues under controlled laboratory conditions, were investigated. The level of metal accumulation in the duckweed tissues relative to environmental and laboratory exposure levels was addressed along with the rapidity with which metals were released when *L. perpusilla* was removed from metal stress or during plant mortality.

MATERIALS AND METHODS

L. perpusilla plants were collected on October 10, 1979, from a secondary, heavy ash settling basin at the Appalachian Power Company's coal-fired, 350 MW, Glen Lyn Plant, Glen Lyn, Giles County, Virginia. The duckweed plants were transferred to a laboratory holding facility at VPI&SU and maintained at 20°C under a 16/8, light/dark photoperiod in 300 liters of dechlorinated tap water. The plants were allowed to depurate accumulated metals for 14 days prior to initiation of the laboratory bioassay studies.

The accumulation and depuration of eight metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) by duckweed were investigated through a series of bioassay experiments. In several 30-liter aquaria, metal mixtures were constructed to represent approximately 0.0 (control), 0.5, 1.0, 10.0, 25.0, or 100 times the mean concentrations of the respective metals found in the ash basin water (Table 1). At the end of a 10-day exposure period, duckweed plants were placed in 30-liter aquaria of dechlorinated tap water and allowed to depurate accumulated metals. The alkalinity, hardness, dissolved oxygen, pH, and sulfate were monitored (APHA, 1976) for the ash basin and test aquaria water (Table 1). These water quality parameters, as measured in the test aquaria, were within the range of values recorded for the ash basin.

At 2-day intervals during the initial 14-day depuration period, followed by the 10-day exposure bioassay and the subsequent 8-day depuration period, triplicate duckweed samples (50–100 mg dry wt) were collected from the stock or test groups. The samples were rinsed with distilled water, dried at 65°C for 24 hr, weighed, and then ashed for 2 hr at 550°C. The ash residues were acidified with 10 ml of a 1:1:2 mixture of nitric acid, sulfuric acid, and water, respectively, then analyzed on a Perkin–Elmer Model 460 atomic absorption spectrophotometer. Metals were measured in water and sediment samples from the coal ash settling basin and in laboratory test aquarium water. Concentrations of the eight metals were computed as milligrams of metal per kilogram of dry plant weight or milligrams of metal per liter of water. Mean plant tissue concentrations for duckweed sampled from the various test aquaria were compared using Duncan's new multiple range procedure with significant differences reported at the $P = 0.05$ level.

RESULTS AND DISCUSSION

Duckweed was the predominant macrophyte found in the heavy ash basin, having covered 95% of the water surface area from July to September 1979. Rooted

TABLE 1

METAL CONCENTRATIONS[a] AND WATER QUALITY DATA FOR LABORATORY STUDIES AND NOMINAL CONCENTRATION DESIGNATION WITH RESPECT TO ASH BASIN METAL ABUNDANCES

	Laboratory exposure conditions						Ash basin concn
	Control	0.5×	1×	10×	25×	100×	(maximum)
	Accumulation Period in Laboratory						
Metal							
Cadmium	0	0	0.01	0.10	0.25	1.0	0.01
Chromium	0	0	0.01	0.10	0.25	1.0	0.01
Nickel	0	0	0.10	1.00	2.50	10.0	0.09
Lead	0	0	0.50	0.50	1.25	5.0	0.05
Copper	0	0.05	0.10	1.00	2.50	10.0	0.10
Iron	0	0.50	1.00	10.0	25.0	100.0	0.94
Manganese	0	0.025	0.05	0.50	1.25	5.0	0.04
Zinc	0	0.05	0.10	1.00	2.50	10.0	0.08
	Maximum aqueous concentration—Depuration period						
Cadmium	0	0.02	0.02	0.02	0.03	—	—
Chromium	0	0.03	0.13	0.39	0.13	—	—
Nickel	0	0	0.04	0.04	0.01	—	—
Lead	0	0	0.05	0.05	0.40	—	—
Copper	0	0.02	0.05	0.07	0.02	—	—
Iron	0	0	0.10	0.10	0.10	—	—
Manganese	0	0.01	0.01	0.01	0.01	—	—
Zinc	0	0.31	0.09	0.10	0.03	—	—
	Accumulation Period in Laboratory						(ash basin)
Water quality parameter							
pH	6.8	7.1	7.0	3.6	3.0	2.6	7.5
Alkalinity (mg $CaCO_3$/l)	47	46	45	45	42	42	41
Hardness (mg $CaCO_3$/l)	80	80	80	84	92	100	85
Conductivity (μmhos/cm)	100	94	105	120	115	1050	95
Sulfate (mg/l)	13.3	6.9	10.5	6.8	12.2	25.2	15.0
	Depuration Period in Laboratory						
pH	6.9	6.7	7.0	6.9	6.9	—	—
Alkalinity (mg $CaCO_3$/l)	46	46	48	48	47	—	—
Hardness (mg $CaCO_3$/l)	80	80	80	80	80	—	—
Conductivity (μmhos/cm)	100	97	120	120	115	—	—
Sulfate (mg/l)	12.4	11.7	6.8	6.8	4.2	—	—

[a] In mg/liter.

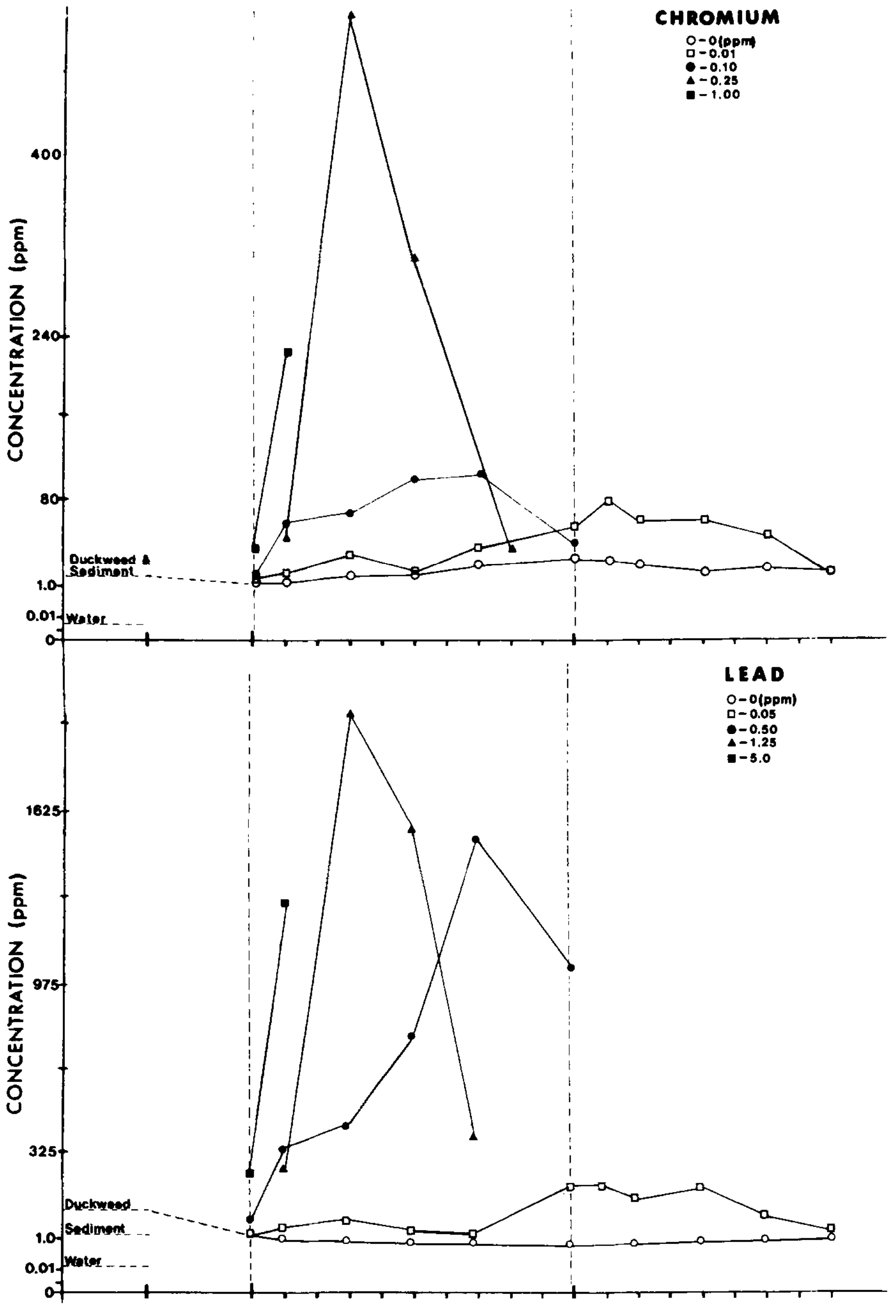

FIG. 1. Elemental concentrations of chromium, lead, and nickel in water, sediment, and duckweed in the heavy ash settling basin, followed by a 14-day laboratory holding period with subsequent exposures for 10 days and an 8-day period of depuration.

macrophytes were only occasionally found along the basin edge, which fluctuated according to power production demand. Duckweed biomass peaked in late summer from the formation of a thick mat, extending below the surface. After the first frost in fall, duckweed density was reduced to ~5% of the surface area within a 1-week

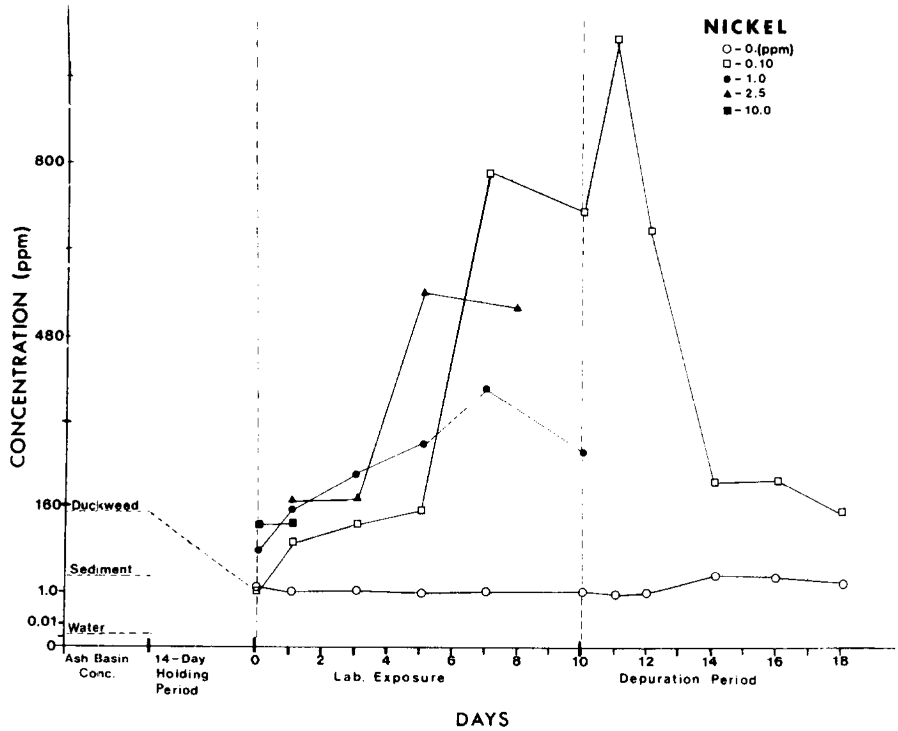

FIG. 1.—*Continued*

period. The duckweed plants were generally restricted to the basin shoreline for the rest of the fall season until complete eradication from consistent temperatures below 0°C.

Duckweed in the ash basin accumulated all elements to either a slightly greater (Cr, Pb, Ni) or an appreciably higher (Cd, Cu, Fe, Mn, Zn) extent than found in the heavy ash basin sediment (Figs. 1–3). Aquatic concentrations were always lower than duckweed or sediment measurements for the eight metals measured in the system. During the 14-day holding period after duckweed was returned to the laboratory and held in control water, concentrations of Pb, Cr, and Ni declined to levels similar to those measured in the ash basin sediment (Fig. 1). The other elements also had a decline in tissue concentrations with Cu and Cd levels approaching sediment concentrations (Fig. 2) while Zn, Mn, and Fe remained considerably higher (Fig. 3).

During the 10-day laboratory exposure bioassay, bioaccumulation of Pb and Cr in duckweed was significantly greater than control and 0.5× test populations for the 10×, 25×, and 100× exposures, with rapid mortality observed by the end of the 10-day period (Fig. 1). No significant bioaccumulation of Pb or Cr was observed (although general uptake was evident) for duckweed at the 1× exposure. After the 8-day laboratory depuration period, the accumulated metals were reduced to the same levels as controls. Depuration data were not available at the higher exposure levels as a result of test population mortality. Trends in the elemental concentrations of Ni in duckweed were similar to those of Pb and Cr except that the 1× test concentration (0.10 mg Ni/liter) resulted in an unusually high rate of

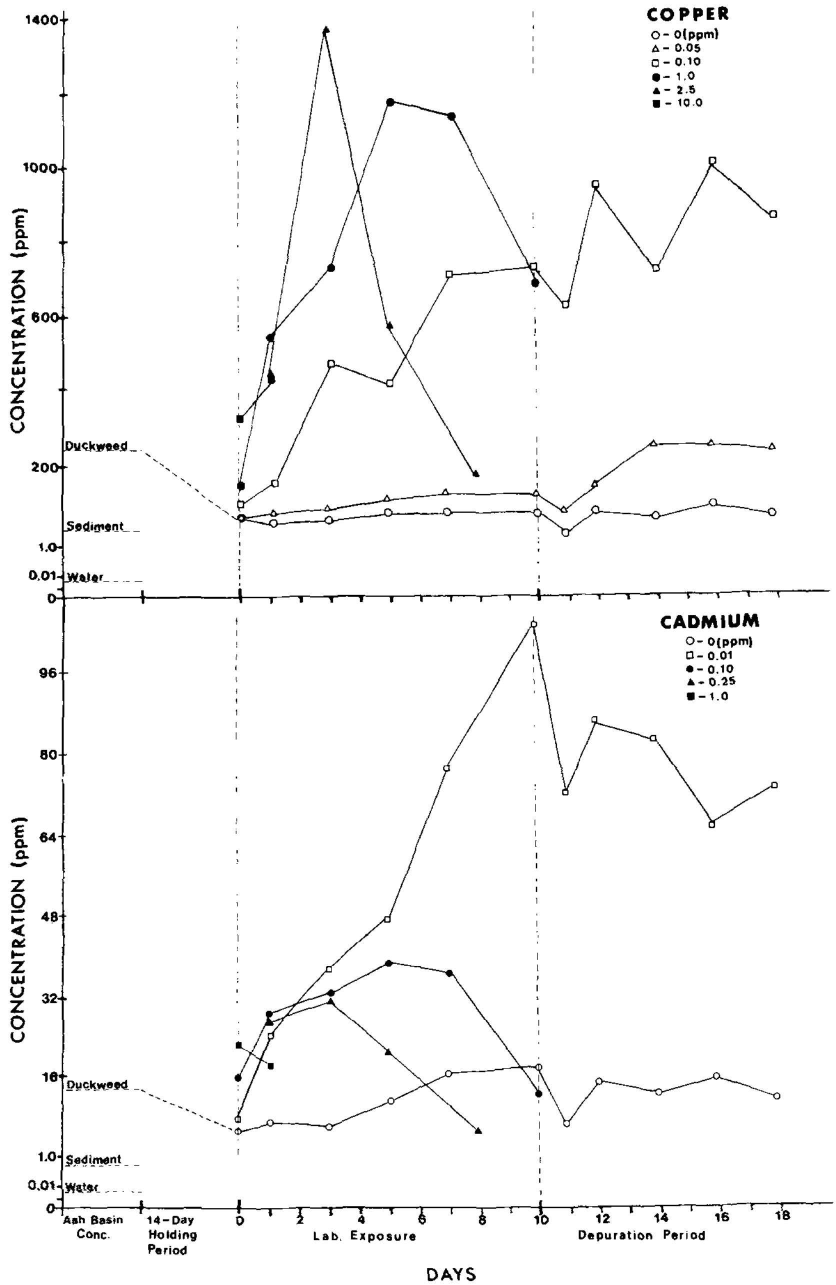

FIG. 2. Elemental concentrations of copper and cadmium in water, sediment, and duckweed in the heavy ash settling basin, followed by a 14-day laboratory holding period with subsequent exposures for 10 days and an 8-day period of depuration.

accumulation during the laboratory exposures, exceeding those recorded for the 10×, 25×, and 100× concentrations. Depuration of Ni for the 1× test population required a period of time longer than that of Cr or Cu. The peak bioaccumulation concentration of Ni occurred 2 days after the depuration period began with a great amount of elemental elimination from duckweed occurring 2 days thereafter (Fig. 1). The lower levels of accumulated Pb, Cr, and Ni for duckweed exposed to the higher test concentrations were a combined result of the metal toxicities and highly acidic pH conditions ($\bar{x}$ = 3.6, 3.0, 2.6) in the 10, 25, and 100× exposures which resulted in an inhibition of plant metabolic activities (Table 1).

Copper and cadmium measurements differed from the Pb, Cr, and Ni data in that duckweed populations failed to show significant depuration during the 8-day test period; however, some decrease in tissue concentrations for these metals was recorded over the initial 2-week holding period (Fig. 2). The 10×, 25×, and 100× test populations died during the 10-day exposure period. Duckweed accumulated Cu at the low exposure level (0.05 mg Cu/liter) although depuration did not occur after the exposure period. Exposure to 2.5 mg Cu/liter resulted in the highest accumulation in duckweed during the period of treatment (after 3 days), with rapid loss of Cu thereafter due to mortality. For Cd, bioaccumulation was greatest at the first treatment level (0.01 mg Cd/liter), with tissue levels increasing throughout the 10-day exposure period.

Exposures to Zn, Mn, and Fe were more difficult to categorize (Fig. 3). Low test concentrations for Zn (0.10 mg/liter) and Mn (0.05 mg/liter) failed to show depuration to levels similar to the control, although the lowest exposures (0.05 and 0.025 mg/liter for Zn and Mn, respectively) approximated control levels. Zinc accumulation in the plant tissues during the laboratory exposure was highest at the 0.10 mg/liter exposure level (10×). These tissue levels were similar to the highest measurements of zinc in duckweed during the depuration period. The same trend was evident for Mn where the greatest concentration in the depuration period was recorded for the 0.5× test population (0.025 mg Mn/liter). This population exposure was generally the highest in accumulated Mn during the laboratory bioassays. The highest duckweed tissue concentrations in the laboratory exposures to Fe occurred at a lethal test concentration (25×, 25 mg Fe/liter), with general elimination of Fe occurring during the depuration period at the lower (0.5 and 1.0×) test concentrations (0.5 and 1.0 mg Fe/liter).

The accumulation of Ni, Pb, Cr, Cd, and Cu in duckweed during the bioassays exceeded the concentrations recorded for duckweed sampled from the ash basin (Figs. 1 and 2). This uptake was evident even at the 1× exposure levels during the laboratory bioassay when the metal concentrations were similar to those recorded for the ash basin water. At all exposure levels, concentrations of Fe, Zn and Mn in the duckweed test populations were equal to or less than the accumulated levels occurring in duckweed from the ash basin.

Lemna valiviana grown in medium containing mixtures of cadmium and zinc or copper and nickel at 0.10 ppm each (Hutchinson and Czyrska, 1975) accumulated these metals to levels similar to those of *L. Perpusilla* which had been subjected to similar metal concentrations in this study. Duckweed in the heavy ash basin accumulated cadmium and chromium to the same extent as *L. perpusilla* in a fly-ash-influenced drainage system (Rodgers *et al.*, 1978); however, the tissue levels of copper and zinc were 20 and 40 times greater, respectively, for plants in the heavy ash basin. The aqueous concentrations of these four metals in the heavy ash basin effluent were less than one-tenth of those reported by Rodgers *et al.* (1978).

Duckweed macrophytes have demonstrated a significant potential for accumulation of heavy metals with the potential for metal contamination of other ecosystem

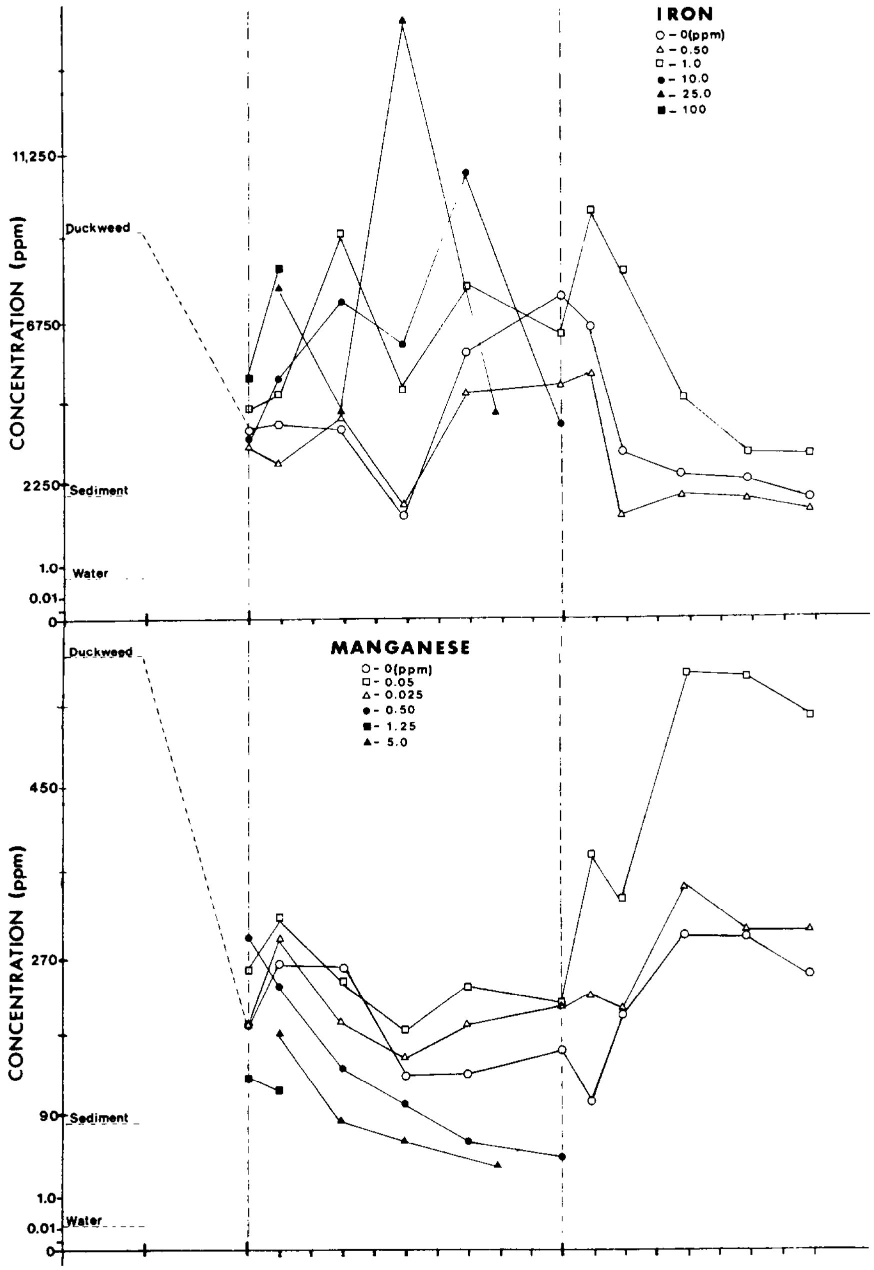

FIG. 3. Elemental concentrations of iron, manganese, and zinc in water, sediment, and duckweed in the heavy ash settling basin, followed by a 14-day laboratory holding period with subsequent exposures for 10 days and an 8-day period of depuration.

components through food web interactions (Hutchinson and Czyrska, 1975; Guthrie and Cherry, 1976, 1979a; Rodgers *et al.*, 1978). The accumulation of metals in the plant tissues provides a mechanism of biological removal of these potential toxicants from waste water systems. Analysis of water concentrations in the test

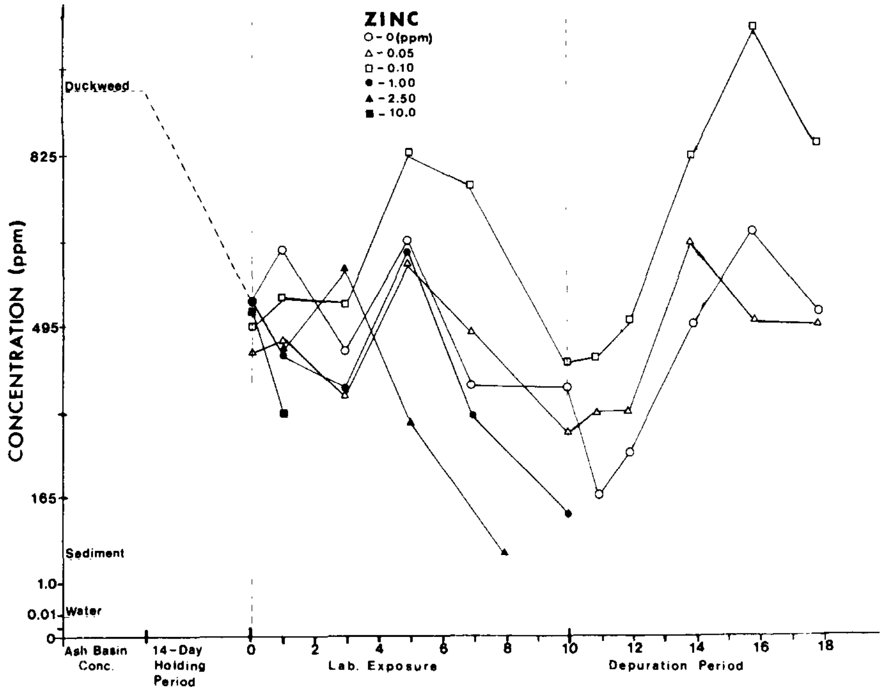

FIG. 3.—*Continued*

aquaria confirmed the release of heavy metals as duckweed populations died at high metal concentrations. The release of metals upon the death of the plant may contaminate sediments or produce a rapid influx of aqueous metal concentrations at the end of a growing season. This phenomenon may have been apparent from the rapid mortality observed after the first frost condition in fall. The capacity for depuration of metals by living plants may contribute to the recovery of duckweed populations after a brief exposure to metal toxicants. However, this potential condition may prolong the exposure of other ecosystem components to heavy metals after a spill of contaminants or if duckweed was released from a waste treatment system through overflow or flooding. The dynamics of metal bioconcentration, depuration, or release in biological components of ecosystems, as well as the complex aqueous chemistry of metals (e.g., proportion of dissolved and combined forms), need further study before the overall ramifications of disposing coal ash residues can be reliably predicted.

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