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Heavy Metal Bioaccumulation and Effects on Waterhyacinth Weevils, *Neochetina eichhorniae,* Feeding on Waterhyacinth, *Eichhornia crassipes*

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Both aquatic and terrestrial habitats frequently are subject to contamination by toxic heavy metals, yet very little is known about the influence of heavy metals absorbed by plant tissues upon the phytophagous insect fauna feeding upon these plants. As early as 1936 Hurd-Karrer and Poos (1936) reported that selenium was toxic to aphids. Byers and Zeiders (1976) indicated that the feeding of the frit fly, *Oscinella frit*, and the cereal leaf beetle, *Oulema melanopus*, were less upon reed canarygrass which was spray-irrigated with sewage effluent than upon controls grown without spray-irrigation. Cooley *et al.* (1979) suggested a relationship between high iron content of waterhyacinths and resistance to feeding by waterhyacinth weevils (*Neochetina eichhorniae*). Quimby *et al.* (1979) have shown that alligatorweed flea beetles, *Agasicles hygrophila*, feeding upon alligatorweed exposed to 1.0 mg•ℓ^{-1} cadmium in hydroponic culture were sensitive to leaf accumulations of 8.7 ug•g^{-1} cadmium, whereas nutsedge moths, *Bactra verutana*, feeding either upon diet containing from 0 to 18.0 ug•g^{-1} cadmium or upon purple nutsedge containing 6.5 ug•g^{-1} cadmium were not affected adversely. Weismann and Skrobak (1980) reported toxicity of lead chloride and lead acetate in a "semi-synthetic food" to a caterpiller, *Scotia segetum*. Weismann and Svatarakova (1981) indicated morphological abnormalities in pupae and reduced emergence of the imago of *Scotia segetum* when third instar caterpillars were fed diets containing 50 μg lead •g^{-1} or when fifth instar caterpillars were fed diets containing 400 μg lead •g^{-1}. Imagoes that hatched had disturbed synchronization of movements, and females did not oviposit. Zelenayova and Weismann (1983) reported necrosis and vacuolization of testes and vacuolization of ovaries of adult *Scotia segetum* moths reared from caterpillars fed on a diet containing 50 μg cadmium •g^{-1}.

The objectives of this study were to determine the influence of plant-absorbed metals upon the feeding, mortality, and body burdens of lead, cadmium, and copper in the waterhyacinth weevil, *Neochetina eichhorniae*, imported for the biological control of waterhyacinths (*Eichhornia crassipes*).

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MATERIALS AND METHODS

Waterhyacinths were propagated from plants grown from seed in an outdoor cage during the summer of 1979. Progeny from these plants were maintained in hydroponic culture in 70-ℓ containers in a greenhouse during the winter of 1979-80 and were transferred to outdoor cages after all danger of frost was past. Plant material was cultured hydroponically as described previously (Kay *et al.* 1984), except that well water was used for preparing the nutrient solutions and that the plants were placed inside cages to prevent natural infestation by *Neochetina eichhorniae*. After a five-week growth period, the metal solutions and fresh nutrients were added to the culture medium. Treatments were established so as not to exceed the aqueous metal concentrations which previously were found to be toxic to waterhyacinths (Kay *et al.* 1984): controls without added metals, 0.5 and 1.0 $mg \cdot \ell^{-1}$ cadmium, 1.0 and 2.5 $mg \cdot \ell^{-1}$ copper, and 1.0 and 5.0 $mg \cdot \ell^{-1}$ lead. The nitrate salts were used to prepare solutions containing lead or cadmium, and the sulfate salt was used for copper solutions. The 1.0 $mg \cdot \ell^{-1}$ treatment was selected for comparing the effects of similar levels of exposure to the different metals. Plants were given a four-week uptake period prior to the initiation of feeding studies.

Adult *Neochetina eichhorniae* were collected in the field from Camp's Canal and the River Styx in Alachua County, Florida, and were held indoors for four to six days in 3.8-ℓ glass jars containing control waterhyacinth leaves. Ten adult *Neochetina* weevils were selected randomly for each experimental unit. One mature waterhyacinth leaf was placed into a 3.5 x 7 x 9.5 cm plastic box with ten adult weevils to constitute one replicate of one treatment. Boxes were then placed inside individual zip-loc plastic bags and were sealed to prevent moisture loss. Each treatment was replicated five times. Feeding studies were conducted indoors at 25 ± 2°C with natural daylight (approximately 12 hr) from an adjacent window. After 24 h feeding, the leaves were removed and replaced with fresh leaves. All leaf tissue was dried and saved for metals analysis. The number of feeding lesions and insect mortality were recorded daily for the first ten days. For the purpose of eliminating some of the variation due to lesion size, a standardized feeding spot size (2 mm) was estimated. Large lesions were evaluated by how many standard lesions they represented, and several unusually small lesions were counted as one. Any insect mortality during the first 24 h of the experiment was assumed to be the result of old or unhealthy insects or injury due to handling. Consequently, insects which died during the first 24 h were not counted and were replaced immediately. Mortality counts were continued for an additional ten days, and the test was terminated at the end of 20 days. Dead insects were removed daily and frozen. All insects remaining at the end of the experimental period were killed by freezing, combined with the other dead insects, and dried for 48 h at 105°C. All replicates (total of 50 insects) of a treatment were pooled for a single analysis for metals due to the small insect biomass. Insects and waterhyacinth leaves were ashed for 5 h at 450°C in a muffle furnace and digested with 2 N HCl. Digested samples were analyzed for lead, cadmium, and copper by flame atomic absorption spectrophotometry. Metal concentrations in leaves, mortality, and mean cumulative feeding lesions per insect were subjected to an analysis of variance, and means were compared with the Duncan's multiple range test.

RESULTS AND DISCUSSION

Figure 1 shows the cumulative feeding of the waterhyacinth weevil, *Neochetina eichhorniae*, when fed waterhyacinth leaves containing different levels of lead, cadmium, or copper. Plants exposed to the metals had higher levels of insect feeding (i.e., more lesions) than the control plants, but the overall analysis of variance indicated that these differences were not significant ($\alpha = 0.05$). When analyzed separately for each metal, however, there were significant differences between feeding on the controls and feeding on plants exposed to approximately 2.5 mg$\cdot\ell^{-1}$ copper. Cumulative feeding in the lead and cadmium experiments was not significantly different from the controls, regardless of level of exposure.

Table 1 shows that cadmium and copper accumulations in waterhyacinth leaves increased in proportion to the concentrations in the culture solutions. Lead in the leaves also increased, but not proportionally to the levels in solution. The bioaccumulation of metals by the weevils increased with metal concentrations in the leaves, except copper, which remained essentially constant in spite of significantly increasing copper in leaf tissues. There were no significant differences ($\alpha = 0.05$) in insect mortality during the first ten days in which feeding was monitored (Table 1). After 20 days, however, insects feeding on plants exposed to approximately 5.0 mg$\cdot\ell^{-1}$ lead, 1.0 mg$\cdot\ell^{-1}$ cadmium, or 2.5 mg$\cdot\ell^{-1}$ copper had significantly ($\alpha = 0.05$) lower mortality than insects feeding upon control plants.

The concentrations of lead, cadmium, or copper in waterhyacinth leaf tissues fed to the weevils were similar or substantially lower than those reported in previous laboratory studies with waterhyacinths but were substantially higher than those reported previously for field collected plants. Metal concentrations varied from about 20 to 80 percent of those reported for a 3-wk exposure in hydroponic culture (Kay *et al.* 1984). An earlier study (Sutton and Blackburn 1971) reported similar copper concentrations in leaves of control plants and slightly lower levels in leaves of plants exposed for 2 wks to 1 or 2.5 mg copper$\cdot\ell^{-1}$. Lead and cadmium concentrations observed in the present study were about 1 to 2 orders of magnitude lower than those reported by Muramoto and Oki (1983) for a 16-day exposure at similar solution concentrations. Two recent reports indicated substantially lower concentrations of copper (Cooley *et al.* 1979) and cadmium (Cooley and Martin 1979) in field-collected waterhyacinths than in the present study, and a 7-day laboratory exposure to 1 mg

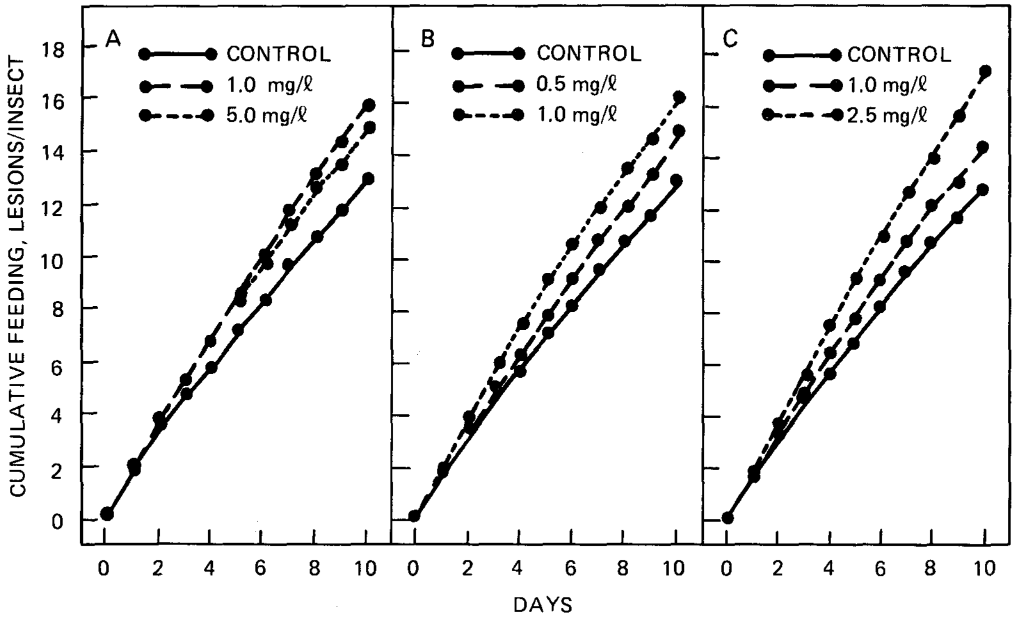

Figure 1. Cumulative feeding of *Neochetina eichhorniae* on waterhyacinth leaves: (A) lead; (B) cadmium; (C) copper.

cadmium $\cdot \ell^{-1}$ resulted in only 11.9 ± 5.9 ppb (ng $\cdot$ g^{-1}) cadmium (Cooley and Martin 1979).

The uptake of copper, an essential element, apparently was well regulated by the insects' metabolism. Lead and cadmium concentrations increased in the weevils when they increased in the leaves, but the increases were not directly proportional to the levels of the metals in the leaves. The lack of any significant effect of either lead or cadmium on insect mortality suggests that the metals were present in the insects' tissues in concentrations well below the toxicity threshold or possibly were bound by a metallothionein-like protein, thus preventing a toxic effect. The tolerance of the waterhyacinth weevils to cadmium implies that metal toxicity to insects may be highly species specific, as similar leaf concentrations (8.7 $\mu g \cdot g^{-1}$) of cadmium in alligatorweed produced similar (17 $\mu g \cdot g^{-1}$) cadmium accumulations in insects and caused significant mortality of alligatorweed flea beetles during a 10-day feeding study (Quimby *et al.* 1979). The presence of cadmium and lead in waterhyacinth weevils may be more important environmentally as a source of metals in insectivorous birds and predatory arthropods. Cadmium accumulation from plant material has been demonstrated in brown crickets (*Acheta domesticus*) and field crickets (*Pteronemobius fasciatus*) and has been shown to accumulate in wolf spiders (*Lycosa* spp.) fed on cadmium-contaminated field crickets (van Hook and Yates 1975). Cheng

Table 1. Mean concentrations (dry weight basis) of metals in waterhyacinth leaves and waterhyacinth weevils and the 10- and 20-day mortality in the *Neochetina* feeding tests.*

Metal	Metal Concentration in Solution, $mg \cdot \ell^{-1}$	Metal Concentration in Leaves, $\mu g \cdot g^{-1}$	Metal Concentration in Insects, $\mu g \cdot g^{-1}$**	10-day Cumulative Mortality	20-day Cumulative Mortality
Lead	0.0	0.00a	0.00	0.8a	2.8a
	1.0	5.89ab	0.00	0.6a	2.4a
	5.0	9.84b	44.45	0.0a	0.8b
Cadmium	0.0	0.06a	6.52	0.08a	2.8a
	0.5	8.00b	14.46	0.8a	1.0a
	1.0	17.20c	36.67	0.0a	0.0b
Copper	0.0	15.35a	30.42	0.8a	2.8a
	1.0	21.62b	38.37	0.2a	1.4a
	2.5	44.77c	32.77	0.0a	0.8b

* Means in the same column (within each metal) followed by the same letter are not significantly different ($\alpha = 0.05$) according to Duncan's Multiple Range procedure.

** Pooled data for all 5 replicates (50 insects).

et al. (1984) reported that sea skaters (*Halobates sericeus*) appeared to be a significant source of cadmium for seabird predators of this insect.

There appears to be no precedent to explain the enhanced level of feeding in the presence of high accumulations of potentially toxic metals in leaves. Previous work has shown significantly depressed feeding of adult alligatorweed flea beetles in the presence of $8.7\ \mu g \cdot g^{-1}$ cadmium in leaves of alligatorweed (Quimby *et al*. 1979). Differences in cumulative feeding noted in these experiments may have been caused by several factors. An experiment in which feeding is assessed by the counting of feeding lesions is subject to considerable variation from differences in sizes of feeding spots. Difficulties in such a technique are apparent, especially when leaves have numerous feeding lesions. Even with such inherent errors in measurement, the error in estimation would not likely cause bias in the data that so consistently favored one treatment over another. The presence of high plant levels of lead, cadmium, or copper may have altered the plant chemistry sufficiently to produce enhanced levels of some feeding stimulant or attractant, such as the kairomone reported by Del Fosse and Perkins (1976). The presence of high concentrations of metals also may have decreased the plant content of one or more essential dietary components, thus enhancing feeding on leaves with high metal content. The appearance of slight chlorosis in plants exposed to copper and cadmium suggests such a potential metabolic disturbance and has been observed previously (Kay *et al.* 1984; Tatsuyama *et al.* 1979). This might be sufficient to explain the enhanced feeding in the presence of high cadmium or copper but not lead, as chlorosis did not appear in plants exposed to lead. Another possible explanation is that plant tissues may have contained more water when exposed to high levels of metals, thus causing enhanced insect feeding to obtain the same amount of food. Relative leaf water content was not measured, however.

Lead, cadmium, and copper in waterhyacinths had no biologically significant effect upon the feeding or mortality of adult waterhyacinth weevils. At the levels and lengths of exposure in this study, lead, cadmium, and copper apparently were not sufficiently accumulated by the insects to cause either acute or chronic toxicity. This suggests that waterhyacinth weevils, *Neochetina eichhorniae*, are not likely to be affected adversely by environmental contamination with lead, cadmium, or copper. These studies were done with adult weevils. The effects upon larval and pupal development, emergence of the adult, fecundity, and oviposition have not been studied. Further experimentation is needed before a decisive conclusion can be made concerning the impact of environmental contamination by toxic heavy metals upon the waterhyacinth weevil, *Neochetina eichhorniae*. The weevils potentially could be a substantial source of lead and cadmium for predatory arthropods and insectivorous birds feeding in areas of heavy weevil infestation on waterhyacinths.

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