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Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds

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Abstract

The effects of Cd, Ni and Pb on the growth, chlorophyll (Chl) and protein contents, and content of proteases of potted weed plants *Cyperus difformis, Chenopodium ambrosioides* and *Digitaria sanguinolis* were determined. The three heavy metals inhibited the shoot growth but were less suppresive to root growth. They also lowered leaf Chl content. The changes in root and shoot protein and proteases contents of weeds were interrelated. The heavy metal additions to soil increased their contents in both roots and shoots, several times more in roots than in shoots.

Additional key words: *Chenopodium ambrosioides, Cyperus difformis, Digitaria sanguinolis,* heavy metals.

Introduction

Cadmium and lead occur naturally in all soils in at least trace quantities but their concentration in soils can be greatly increased by human activities. The uptake and integration of Cd, Ni and Pb into plants are affected by almost all environmental factors. Their contents in plants generally reflect the biological availability of these metals. Increased concentrations of Cd, Ni and Pb caused detrimental effects to plants, especially they altered growth (Eleiwa and Naguib 1986 Becerril *et al.* 1989, Metwally and Rabie 1989, Aidid and Okamoto 1992).

The adaptation mechanisms of plants to toxic concentrations of heavy metals restricted uptake and/or translocation of metals, their compartmentation within the cell, and formation of complexes with proteins or peptides (Grill *et al.* 1985, Tomsett and Thurman 1988, Brown and Brinkmann 1992). The aim of the present work was to study the bioaccumulation of Cd, Ni and Pb and their effects on the growth and some metabolic pools of three promising heavy metal tolerant weeds.

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Materials and methods

Seeds of *Cyperus difformis* L., *Chenopodium ambrosiodes* L. and *Digitaria sanguinolis* L. were obtained from the Ministry of Agriculture, Cairo. The plants were grown in pots (30 cm in diameter) filled with clay soil and irrigated as and when required. One month after sowing, 1000 cm^3 of solution of cadmium chloride (5, 10 or 20 mg kg^{-1}), nickel sulphate (50, 100 or 200 mg kg^{-1}) or lead acetate (50, 100 or 200 mg kg^{-1}) were applied to each pot. Control pots received the same amounts of pure water. One week later, samples of the shoot system were collected, and plant height, root length and dry mass of shoot and roots were determined. Contents of chlorophyll (Chl) were estimated according to Vernon and Seely (1966), contents of proteins according to Lowry *et al.* (1951), and contents of proteases according to Ong and Gaucher (1973). The three metal contents were determined by atomic absorption spectroscopy (*Perkin-Elmer* 560). The Student *t*-test was used to compare the differences between control and experimental plants.

Results and discussion

The three plant species are weeds common in moist or waste places, marshes and along roads. Their shoot heights and dry mass were not affected by the lowest applied doses of metals, but they were highly significantly lowered by the highest metal doses (Table 1). Inhibition of shoot growth was least in *Cyperus* followed by *Digitaria* and *Chenopodium*. Lead was more suppressive than Cd or Ni, in *Cyperus* and *Chenopodium*, but Ni was most effective in lowering shoot growth of *Digitaria*. These results are in conformity with those of Aidid and Okamoto (1992) working with *Impatiens balsamina*, where Pb (0.5 mM) and Cd (0.1 mM) suppress stem cell elongation. Also, Eleiwa and Naguib (1986) found that Ni (10^{-4} or 10^{-7} M) drastically suppressed the fresh and dry masses of soybean leaves.

With respect to root growth, the three elements showed mostly insignificant action (Table 1). The decrease in root dry mass was significant at high metal doses in *Cyperus* and *Chenopodium*. *Digitaria* roots did not significantly react to any dose of any heavy metal applied.

Wilkins (1957) proposed a simple, but effective way of measuring metal tolerance by comparing rates of root elongation in toxic and non-toxic solutions. This method yields useful results with grasses (Brown and Brinkmann 1992). Our results showing a small reduction in root growth of weeds grown in soil supplemented with the three heavy metals point to a heavy metal tolerance of the tested plant species.

The lowest applied heavy metal doses did not significantly affect contents of Chl *a* and *b* in the tested weeds (Table 2). The higher metal doses severely attenuated the Chl contents in *Chenopodium* followed by *Cyperus* and *Digitaria*. Cadmium was more effective (at 20 mg kg^{-1}) than Pb or Ni (at 200 mg kg^{-1}). 100 mg kg^{-1} Pb was less effective than Ni at the same concentration. A similar reduction in Chl content induced by heavy metals has also been observed by Naguib *et al.* (1982), Eleiwa and Naguib (1989), and Greger and Ogren (1991).

Table 1. Effect of various concentrations [mg kg^{-1}] of cadmium, nickel and lead on plant height [cm] and shoot dry mass [g] and length and dry mass of roots of *Cyperus difformis*, *Chenopodium ambrosioides* and *Digitaria sanguinolis*. Each value is a mean of 10 measurements ± standard error (S.E.). * $P = 0.05$, ** $P = 0.01$.

Treatment		*Cyperus*				*Chenopodium*				*Digitaria*			
		plant height	shoot mass	root length	root mass	plant height	shoot mass	root length	root mass	plant height	shoot mass	root length	root mass
Cont.	0	16.31±1.12	1.25±0.03	6.34±0.32	0.51±0.03	12.50±0.81	1.85±0.03	4.53±0.22	0.60±0.03	18.21±1.08	1.12±0.05	7.02±0.036	0.43±0.03
Cd	5	16.02±0.81	1.23±0.02	7.00±0.41	0.45±0.04	11.30±0.91	1.79±0.03	4.50±0.36	0.55±0.05	15.38±0.93	1.07±0.03	6.87±0.31	0.47±0.03
	10	13.12±1.02*	1.16±0.03*	6.30±0.36	0.43±0.05	9.40±1.10*	1.31±0.04**	4.33±0.29	0.53±0.03	13.50±0.73**	0.93±0.06*	6.77±0.35	0.44±0.02
	20	10.21±1.15**	1.02±0.04**	5.34±0.40	0.41±0.03*	7.30±1.33**	1.02±0.05**	4.01±0.31	0.47±0.03**	9.31±1.15**	0.75±0.05**	6.31±0.41	0.40±0.03
Ni	50	16.21±0.62	1.31±0.03	6.50±0.37	0.48±0.03	11.80±0.89	1.91±0.05	4.46±0.41	0.57±0.03	17.31±0.93	1.10±0.03	7.05±0.38	0.45±0.05
	100	12.31±1.21*	1.12±0.04*	5.58±0.41	0.40±0.04*	9.60±0.92*	1.51±0.03**	4.11±0.31	0.50±0.02*	12.11±0.84**	0.94±0.05*	6.87±0.30	0.43±0.03
	200	9.37±1.62**	0.91±0.03**	5.01±0.35*	0.37±0.05*	7.70±1.31**	1.24±0.05**	4.21±0.33	0.48±0.03*	8.19±1.05**	0.81±0.03**	6.50±0.41	0.39±0.03
Pb	50	15.31±0.51	1.20±0.05	6.13±0.40	0.47±0.04	11.70±0.81	1.73±0.04*	4.51±0.31	0.55±0.04*	16.31±0.85	0.98±0.05	6.75±0.35	0.45±0.04
	100	10.31±0.91**	1.12±0.03**	5.09±0.31*	0.43±0.05	9.50±0.84**	1.41±0.05**	4.42±0.27	0.51±0.03*	14.59±0.93*	0.89±0.03**	6.35±0.40	0.44±0.03
	200	8.51±1.59**	0.90±0.06**	4.81±0.35**	0.39±0.04*	8.30±1.31**	1.03±0.06**	4.05±0.30	0.50±0.03*	9.11±1.26**	0.75±0.05**	6.22±0.31	0.41±0.05

Table 2. Effect of various concentrations of cadmium, nickel and lead on chlorophyll (Chl) contents [g kg^{-1} (fresh mass)] of *Cyperus difformis*, *Chenopodium ambrosioides* and *Digitaria sanguinolis*. Each value is a mean of 3 readings ± S.E. $*P = 0.05$, $**P = 0.01$.

Treatment [mg kg^{-1}]		*Cyperus* Chl *a*	Chl *b*	Chl (*a*+*b*)	*Chenopodium* Chl *a*	Chl *b*	Chl (*a*+*b*)	*Digitaria* Chl *a*	Chl *b*	Chl (*a*+*b*)
Cont.	0	3.57±0.09	2.73±0.12	6.30±0.20	4.14±0.07	4.37±0.13	8.52±0.20	3.61±0.10	2.72±0.11	6.32±0.21
Cd	5	3.30±0.04	2.80±0.05	6.10±0.09	4.06±0.11	4.01±0.06	8.07±0.17	3.33±0.07	2.80±0.12	6.13±0.19
	10	2.61±0.09**	2.90±0.15	5.51±0.24	3.51±0.13*	3.05±0.09**	6.56±0.21**	3.00±0.09*	2.51±0.11	5.51±0.20*
	20	2.07±0.08**	2.11±0.07**	4.18±0.15**	2.90±0.21**	2.22±0.18**	5.12±0.39**	2.33±0.11**	2.04±0.09*	4.37±0.20**
Ni	50	3.42±0.06*	2.73±0.10	6.15±0.16	4.23±0.15	3.91±0.08	8.14±0.23	3.71±0.09	2.31±0.20	6.02±0.29
	100	3.07±0.09**	2.01±0.21*	5.08±0.30*	3.23±0.18**	3.11±0.19*	6.34±0.37	3.11±0.08	2.02±0.12	5.13±0.20
	200	2.27±0.07	2.00±0.11*	4.27±0.18**	3.01±0.15**	2.81±0.15**	5.82±0.30**	2.35±0.10**	2.10±0.12*	4.45±0.22**
Pb	50	3.41±0.07	2.80±0.08	6.01±0.15	4.20±0.12	4.01±0.10	8.21±0.22	3.70±0.09	2.40±0.08	6.10±0.17
	100	2.95±0.11**	2.41±0.09	5.36±0.20*	3.95±0.09	3.00±0.10**	6.95±0.19**	3.20±0.10*	2.50±0.10	5.70±0.20
	200	2.61±0.09**	2.23±0.08*	4.84±0.17**	3.05±0.13**	3.10±0.10**	6.15±0.23**	2.85±0.10**	2.30±0.09*	5.15±0.19*

Table 3. Total soluble protein content [g kg^{-1}(d.m.)] and content of proteases [g kg^{-1}(d.m.)] in roots and shoots of *Cyperus difformis, Chenopodium ambrosioides* and *Digitaria sanguinolis* 7 d after treatment with cadmium, nickel or lead. Each value is a mean of 3 replicates ± S.E. * $P = 0.05$, ** $P = 0.01$.

Treatment [mg kg^{-1}]		Protein content						Protease content					
		Cyperus		*Chenopodium*		*Digitaria*		*Cyperus*		*Chenopodium*		*Digitaria*	
		root	shoots	root	shoots	root	shoots	root	shoots	root	shoots	root	Shoots
Cont.	0	1.83±0.51	26.41±0.55	21.03±0.63	31.42±0.71	19.00±0.50	25.31±0.42	2.05±0.15	4.11±0.17	3.20±0.16	5.51±0.15	2.31±0.15	4.51±0.13
Cd	5	18.20±0.45	25.91±0.48	23.00±0.31	32.00±0.52	20.07±0.48	25.50±0.55	2.11±0.10	4.00±0.21	3.21±0.15	5.33±0.20	2.25±0.10	4.33±0.15
	10	20.33±0.50*	28.00±0.63	24.50±0.62*	33.11±0.63	20.15±0.55	27.08±0.61	2.00±0.18	4.32±0.18	3.00±0.15	5.45±0.18	2.18±0.21	4.30±0.20
	20	21.90±0.62*	31.75±0.74**	25.11±0.64*	35.21±0.55*	23.18±0.62**	27.59±0.50*	1.82±0.15	4.50±0.22	2.81±0.11	5.62±0.15	2.00±0.16	4.01±0.15
Ni	50	20.12±0.49	25.11±0.50	21.21±0.50	31.00±0.81	19.50±0.50	25.01±0.50	1.95±0.11	4.11±0.10	3.10±0.15	5.61±0.23	2.20±0.15	4.49±0.20
	100	21.20±0.60*	25.00±0.63	23.57±0.61*	30.21±0.51	22.05±0.63*	23.11±0.65*	1.67±0.16	4.51±0.20	2.87±0.11	5.71±0.20	2.09±0.10	4.67±0.19
	200	22.10±0.55**	23.07±0.55*	25.13±0.55**	31.31±0.62	25.00±0.56**	22.00±0.55**	1.53±0.13	4.87±0.17*	2.75±0.16	5.50±0.23	1.95±0.20	4.95±0.15
Pb	50	18.91±0.46	23.90±0.61*	23.10±0.66	32.03±0.51	20.08±0.50	24.00±0.53	1.81±0.13	3.95±0.15	3.08±0.10	5.53±0.20	2.11±0.15	4.60±0.20
	100	20.50±0.63*	22.40±0.70**	25.07±0.53**	32.00±0.39	22.15±0.63*	23.08±0.62*	1.60±0.15	4.31±0.20	2.90±0.16	5.73±0.15	2.01±0.21	5.11±0.15
	200	23.70±0.50**	20.91±0.62**	26.00±0.60**	33.15±0.60	23.08±0.55**	22.75±0.55*	1.52±0.13	4.92±0.19*	2.85±0.19	5.85±0.20	1.93±0.18	5.35±0.21*

As concerns total soluble protein content and proteolytic activities, roots were more affected by heavy metals than shoots (Table 3). The lowest applied doses did never induce significant effects, but the higher metal doses in some cases increased the protein content in dry mass of roots. As concerns shoots, the effects of Cd on the protein content were positive and those of Ni and Pb negative. The increases of protein contents induced by Cd, Ni or Pb suggest that the plants operate a metabolic mechanism for channeling heavy metals within their cells. A similar conclusion was made by Grill *et al.* (1985), Leblová *et al.* (1986), Robinson *et al.* (1987) and Razak (1989). In contrast, the decreases in protein contents in shoots could be due to a metabolic disorder leading to inhibition in protein synthesis. In accordance to this hypothesis, Delhaize *et al.* (1989) observed a decrease in the rate of protein synthesis in *Datura innoxia* treated with Cd.

Generally, Cd, Ni and Pb did not affect the proteolytic activities characterized by the contents of proteases (Table 3). An insignificant effect of heavy metals on enzyme activities was also observed by Mizrahi and Achitur (1989). The exceptional significant increases in proteases (*Cyperus* shoots supplemented with Ni or Pb at 200 mg kg^{-1} and *Digitaria* shoots supplemented with 200 mg kg^{-1} Pb) may be related to the decreased shoot protein content. Increased proteolytic activities in response to heavy metals were found by Lee *et al.* (1976) who observed that 0.45 - 1.35 mM Cd increased activities of hydrolytic enzymes of soybean leaves.

Table 4. Bioaccumulation of cadmium, nickel and lead in roots and shoots [mg kg^{-1}(d.m)] of *Cyperus difformis, Chenopodium ambrosioides* and *Digitaria sanguinolis* 7 d after treatment with cadmium, nickel or lead [mg kg^{-1}]. Values represent the mean ± S.E. of three replicates. $^{*}P = 0.05$, $^{**}P = 0.01$.

Treatments		*Cyperus*		*Chenopodium*		*Digitaria*	
		roots	shoots	roots	shoots	roots	shoots
Cd	0	27.0±1.02	15.5±0.98	18.5±1.00	12.4±0.95	25.3±1.12	14.9±1.03
	5	130.0±6.81**	51.0±6.21**	135.0±8.51**	63.3±8.10**	185.0±10.5**	20.0±6.51**
	10	298.0±8.10**	75.3±8.03**	207.0±12.3**	95.4±10.5**	257.0±12.0**	51.5±8.52**
	20	650.0±11.5**	121.0±10.5**	551.0±15.3**	151.0±13.0**	730.0±11.5**	95.7±7.61**
Ni	0	20.3±2.07	12.5±1.50	25.3±2.50**	11.8±1.80	24.6±2.10	10.3±1.95
	50	285.0±10.5**	49.5±7.00**	273.0±9.51**	60.0±7.51**	195.0±9.88**	45.0±6.50**
	100	457.0±15.0**	105.0±8.50**	508.0±10.3**	93.5±8.03**	403.0±11.5**	93.5±9.00**
	200	855.0±13.4**	183.0±9.31**	817.0±8.12**	161.0±9.00**	953.0±12.0**	175.0±8.31**
Pb	0	28.6±3.03	14.5±5.05	21.4±3.50	10.5±1.37	25.1±3.00	11.5±1.50
	50	343.0±10.3**	53.3±5.31**	253.0±9.51**	40.5±5.53**	350.0±11.5**	58.7±5.51**
	100	683.0±10.5**	85.4±6.11**	461.0±8.31**	73.0±6.83**	773.0±15.3**	87.0±8.73**
	200	970.0±12.0**	115.0±7.81**	881.0±7.91**	105.0±9.55**	1207.0±15.5**	95.0±9.30**

Application of Cd, Ni and Pb caused highly significant increases of their contents in both roots and shoots of the studied weed species (Table 4). Their contents increased with increasing metal doses. Most of the heavy metals were accumulated in roots (81 % after Cd treatment, 83 % after Ni treatment, and 89 % after Pb treatment). Only 19, 17 and 11 % of Cd, Ni and Pb, respectively, were translocated

into the shoots. The order of metal accumulation in shoots was again Ni followed by Cd and Pb. This supports the results of Becerril *et al.* (1989) working on clover and lucerne, that Cd is more readily translocated than Pb. On the other hand, Table 4 shows that lead is more accumulated in roots than both Ni and Cd and that more heavy metals are accumulated in *Digitaria* roots than in roots of other weeds. In contrast, the rate of shoot metal accumulation was least in *Digitaria,* then in *Cyperus* and in *Chenopodium.* Our results are consistent with the conclusions of El-Kobbia and Ibrahim (1988) and Becerril *et al.* (1989).

Generally roots act as a barrier to movement of toxic heavy metals through the soil-plant system. According to El-Kobbia and Ibrahim (1988), Metwally and Rabie (1989) and Becerril *et al.* (1989), roots always contain more heavy metals than the aerial parts of plants. Cd is easily transferred to plant shoots while Ni or Pb are retained more in the root system. The presence of heavy metals is accompanied by an increase in cellular protein content (specially in roots). Such results indicate that the plant operates a metabolic mechanism for challenging heavy metals within their cells. In spite of the inhibitions in shoot growth and Chl contents of the studied weeds under the effect of Cd, Ni or Pb, the root growth and protease enzyme contents are only slightly affected.

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