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Interaction between Essential Elements—Zinc and Iron and Metal Pollutants—Cadmium and Lead on Cell Division and Chromosome Aberrations in *Vallisneria spiralis* L.

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Increasing industrial use of toxic heavy metals such as cadmium and lead has become a global problem resulting in water pollution by these metals in industrial waste effluents. Toxicity of such heavy metals may be altered by antagonistic interactions between toxic and essential cations (Bunn and Matrone 1966, Anke *et al.* 1970, Lepp 1977, Petering 1978, Jana *et al.* 1987). Antagonism between cadmium and zinc has been reported in *Euglena gracilis* (Falchuk *et al.* 1975) and in *Daphnia magna* (Attar and Maly 1982). Extensive studies have been carried out on the antagonisitc action of iron on lead toxicity in animals (Finley and Dieter 1978, Anders and Hill 1982, Stone and Fox 1984). Interaction studies in plants, however, are limited. Information on cytotoxicity of heavy metal pollutants in higher aquatic plants is meagre and no information is available on the action of Pb+Fe or Cd+Zn on plants.

In the present investigation, the effects of toxic heavy metals lead and cadmium and the interaction with essential elements iron and zinc respectively, have been observed on the common aquatic weed *Vallisneria spiralis*, with respect to cell division, chromosomal abnormalities and metal uptake.

Materials and methods

Plant material

Vallisneria spiralis L. (Hydrocharitaceae) plants were collected from the tank of the Departmental Experimental Botanical Garden where the colony has been maintained over the last five years.

Treatment with the test solutions

Aqueous solutions of lead nitrate and cadmium chloride were applied individually and in different dose combinations with essential metals iron (ferric chloride) and zinc (zinc chloride) respectively, to *Vallisneria spiralis* in the following doses:

Single metal sets		*Double metal sets*	
$Pb(NO_3)_2$	1 mgl^{-1} and 10 mgl^{-1}	$Pb(NO_3)_2+FeCl_3$	(1+1) mgl^{-1}
$CdCl_2$	1 mgl^{-1} and 10 mgl^{-1}	$Pb(NO_3)_2+FeCl_3$	(10+1) mgl^{-1}
$FeCl_3$	1 mgl^{-1}	$CdCl_2+ZnCl_2$	(1+1) mgl^{-1}
$ZnCl_2$	1 mgl^{-1}	$CdCl_2+ZnCl_2$	(10+1) mgl^{-1}

Healthy growing plants of *Vallisneria spiralis* L. (10 plants per set of experiment) were collected from the Departmental aquaria and transferred in glass jars containing the chemical solutions. *Vallisneria* plants were submerged in the test solutions by planting them in sand which was previously washed and autoclaved. The test solutions were artificially aerated,

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maintained at neutral pH in Hoagland's solution (control) or graded buffered Hoagland's solution (pH 7) and temperature of 30±2°C, and the plants exposed to a photoperiod cycle of 14 h dark and 10 h light to source of light intensity 10,000 lux m^{-2}.

After 24 h and 72 h, leaf tips were squashed after overnight fixation in acetic acid: ethyl alcohol (1 : 3) followed by acetic orcein squash technique (Sharma and Sharma 1980). The slides were coded and observed blind to eliminate observer bias. 10,000 cells were scanned per experimental as well as control sets for mitotic index, and 1000 cells per set for study of chromosomal aberrations. The latter were subdivided into three groups: Group A included anomalies arising from spindle disturbances leading to stickiness, lagging, anaphase bridge, early separation and diplochromatids; Group B consisted of aberrations arising from direct effect on the chromosome, such as breaks and gaps; Group C included gross aberrations such as pycnosis, clumping and fragmentation.

Duncan's multiple range test was used to determine the significance at 95% confidence limits. Accumulation of Cd and Pb in leaf tissue was measured by an Atomic Absorption Spectrophotometer. Fresh leaf tissue was dried in oven at 60 to 80°C. To 1 g of the dried sample, 5 ml of triacid mixture (conc. HNO_3, H_2SO_4 and 60% $HClO_4$; 9: 2: 1) were added. The mixture was heated until a clear solution was obtained. The digested material was then diluted to 100 ml with glass distilled water and prepared for observation in Perkin-Elmer 303 AAS. The mean values with standard deviation are given in Table 3.

Table 1. Effects of concentrations of Cd, Pb, Fe and Zn on leaf tip cell division of *Vallisneria spiralis* L.

Treatment with concentration (mgl^{-1})	Dividing cell (%)*	
	24 hr exposure	72 hr exposure
Control (0)	4.105±0.922a	4.126±1.05a
Cd (1)	4.299±1.405a	3.770±1.321a
Cd (10)	2.791±0.980b	2.086±1.044b
Zn (1)	4.327±2.654a	3.890±1.360a
Cd (1)+Zn (1)	3.055±1.276b	2.687±1.348b
Cd (10)+Zn (1)	2.081±0.864b	1.754±0.784b
Pb (1)	2.377±0.637b	2.209±0.647b
Pb (10)	1.068±0.158c	0.973±0.220c
Fe (1)	4.290±1.313a	4.021±1.264a
Pb (1)+Fe (1)	4.739±1.452a	4.305±1.914a
Pb (10)+Fe (1)	0.974±0.108c	0.883±0.172c

* Values in a vertical column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test. Each value is the mean of 10 samples±S.D.

Results and discussion

The frequency of dividing cells decreased significantly ($P<0.05$) from that of control data with increasing concentrations of Pb, with the higher concentration of Cd, as well as with increasing periods of exposure, of singly administered Pb and Cd salts (Table 1). The percentage of aberrant cells in the single metal treatment sets were significantly higher than that of control and related to the doses used and the period of exposure to these salts (Table 2). The group A type of aberrants were predominant over group B or C types: Chromosome breaks were relatively less frequent, and effects of spindle disturbance resulting in stickiness, anaphase sticky bridge, metaphase arrests, diplochromatid formations and unequal separation of the chromosomes was predominant. At 72 h, for both Pb and Cd, group C type was significantly higher than that observed in control group.

Following combined treatment with:

(i) Pb (1 mgl^{-1}) and Fe (1 mgl^{-1});
(ii) Pb (10 mgl^{-1}) and Fe (1 mgl^{-1});
(iii) Cd (1 mgl^{-1}) and Zn (1 mgl^{-1}) and
(iv) Cd (10 mgl^{-1}) and Zn (1 mgl^{-1})

the frequencies of dividing cells were significantly lower ($P<0.05$) and the aberrations induced were significantly higher ($P<0.05$) in the combined treatments (ii), (iii) and (iv) than that recorded in control and in the corresponding concentrations of single Pb or Cd treated sets (Table 1). The percentage of aberrant cells increased with increasing period of exposure (Table 2). The types of aberrations noted mainly involved the spindle disturbance type (*i. e.* group A); however, a higher frequency of breaks (group B) was observed in these combination sets than that of single Pb or Cd treated sets. In the combined treatment (i), the frequency of dividing cells was higher and that of aberrant cells lower than the single Pb-treated set.

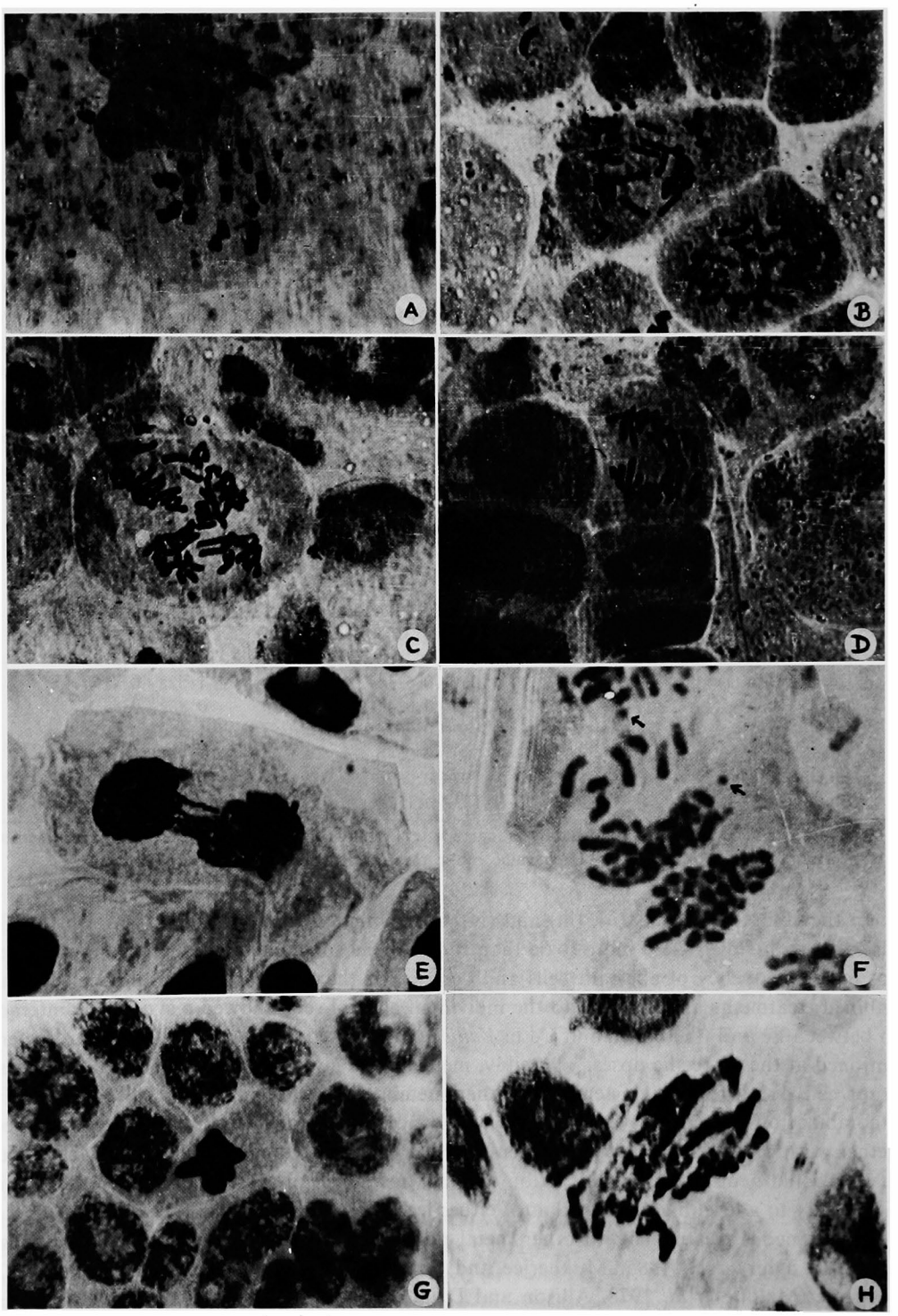
Table 2. Effects of concentrations of Cd, Pb, Fe and Zn on mitotic cells of *Vallisneria spiralis* L.

Treatment with concentration (mgl^{-1})	Total aberrant cell (%)*	
	24 hr exposure	72 hr exposure
Control (0)	0.138±0.041a	0.147±0.05a
Cd (1)	0.516±0.054b	0.771±0.365b
Cd (10)	0.721±0.430b	0.893±0.437b
Zn (1)	0.472±0.216b	0.763±0.247b
Cd (1)+Zn (1)	0.878±0.230c	1.409±0.373c
Cd (10)+Zn (1)	1.062±0.511c	1.528±0.415c
Pb (1)	0.843±0.024c	0.882±0.551b
Pb (10)	1.059±0.257d	1.352±0.295c
Fe (1)	0.509±0.054b	0.747±0.393b
Pb (1)+Fe (1)	0.597±0.097b	0.704±0.410b
Pb (10)+Fe (1)	1.183±0.157d	1.408±0.385c

* Values in a vertical column followed by the same letter are not significantly at 5% level as determined by Duncan's multiple range test. Each value is the mean of 10 samples±S.D.

In the combination sets (ii), (iii) and (iv), the combined effects of $FeCl_3$ with Pb $(NO_3)_2$ and $ZnCl_2$ with $CdCl_2$ were more toxic than those of the single metals. Significant enhancement in the frequency of aberrant cells and decrease in the frequency of dividing cells in the combined treatments as compared to the individual control sets, showed a synergistic interaction between Fe and Pb and Zn and Cd in *Vallisneria spiralis* L. with respect to the parameters mentioned at these specific doses. Possibly, metals when given in combination at these doses, disrupt cell division through acting on other metabolites or through ionic disbalance. In the combination set (i) however, when a lower concentration of Pb $(NO_3)_2$ was administered together with $FeCl_3$, antagonism between the two salts was apparent. It seems therefore, that iron could neutralise the toxic action of small doses of lead, leading to an increase in mitotic activity to a level higher than that of either lead or iron given alone.

Simultaneous treatment with other metal pollutants in plants gave either additive (Fiskesjo 1980, Dhir *et al.* 1985, Mukherjee and Sharma 1987, Mukhejee and Sharma 1988), synergistic (Malone *et al.* 1978, Allison and Dzialo 1981, Whitton and Snehlata 1982, Jana and Choudhuri 1984) or antagonistic effects (Pietilainen 1975, Keul *et al.* 1979). Certain metals like Hg, Cu, Zn, Cd and Pb were more toxic in combination than when present alone (Gachter 1976). Interactions between Pb and Cd on primary production of phytoplankton indicated antagonism when concentration of Pb exceeded that of Cd while synergism was



Figs. A–H. Microphotographs showing chromsomal aberrations in *Vallisneria spiralis* following Cadmium and Lead treatments. A, Normal metaphase showing 2n=20 (ca ×2500). B and C, Spindle disruption leading to multipolarity and laggards (ca ×2500). D and E, Anaphase sticky bridge (ca ×2500, ×4800). F, Chromosome breaks and laggards at anaphase (ca ×3500). G, Clumped mephase (ca ×1800). H, Pycnosis and gross pulverisation of chromatin matter (ca ×4800).

observed in solution where the concentration of Cd was greater than that of Pb (Pietilainen 1975).

The amount of toxic metals retained as observed by atomic absorption spectrophotometric (AAS) studies, attribute to the synergism observed between Cd and Zn or account for the antagonism observed between Pb and Fe. The concentration of Pb retained in the leaf tissues recorded after 24 h and 72 h of exposure, revealed that in the combination set (i) the amount of Pb decreased when administered in combination with Fe than when administered individually (Table 3). In combination sets (ii), (iii) and (iv), AAS studies showed that the amount of Pb or Cd retained in the leaf tissues was less when administered individually than when given in combination with Fe or Zn respectively. At these concentrations of toxic metals, the addition of ions of another metal further increased the toxicity. Hydroponic experiments with corn showed incresed toxicity of Cd by the addition of Zn to the nutrient solution (Malone *et al.* 1978).

Table 3. Accumulation of Pb and Cd in leaf tissue of *Vallisneria spiralis* L.

Treatment with concentration (mgl^{-1})	Cd accumulated ($mg\ kg^{-1}$ DW)*		Pb accumulated ($mg\ kg^{-1}$ DW)*	
	24 hr	72 hr	24 hr	72 hr
Cd (1)	0.001±0.0008	0.002 ±0.001		
Cd (10)	0.003±0.001	0.004 ±0.001		
Cd (1)+Zn (1)	0.004±0.001	0.0035±0.002		
Cd (10)+Zn (1)	0.005±0.002	0.004 ±0.002		
Pb (1)			0.012±0.001	0.014±0.001
Pb (10)			0.014±0.002	0.016±0.001
Pb (1)+Fe (1)			0.008±0.001	0.011±0.002
Pb (10)+Fe (1)			0.015±0.003	0.016±0.002

* Data represents mean values±S.D. for 10 samples.

Conclusion

An overall assessment thus confirms that with respect to the parameters studied, Fe could antagonise the cytotoxicity of low concentrations of Pb while Zn enhanced the cytotoxicity of low and higher doses of Cd in the submerged aquatic plant, *Vallisneria spiralis* L.

Summary

The cytotoxic effects of cadmium and lead: non-essential trace metals in combination with important trace elements–zinc and iron respectively were studied on cell division and chromosomal aberrations in leaf-tip cells of *Vallisneria spiralis* L., a submerged aquatic plant. The results showed that the toxic effects of lower concentrations of heavy metal lead, with respect to the parameters mentioned, could be antagonised by the essential metal iron. Zinc, on the other hand, further accentuated the effect of cadmium: the frequency of dividing cells decreased and the percentages of chromosomal aberrations were significantly high in the combination sets.

Key words

Cytotoxicity, metal-metal interactions, antagonism, synergism, heavy metals, essential elements, *Vallisneria spiralis* L.

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