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Accumulation of heavy metals in the metal-tolerant fern, *Athyrium yokoscense*, growing on various environments

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Abstract

The accumulation of copper, zinc and cadmium in *A. yokoscense* collected from Ashio (copper-contaminated area), Bandai (zinc- and cadmium-contaminated area) and Tama (non-contaminated area), has been investigated. Copper and zinc were accumulated most highly in the root, whilst cadmium was accumulated more in the leaf. The root of *A. yokoscense* growing in areas contaminated with metals contained maximum amounts of Cu (5,989 $mg.kg^{-1}$ dry weight) and Zn (6,384 $mg.kg^{-1}$ dry weight), while in the leaf from the Bandai area 164.8 mg $Cd.kg^{-1}$ dry weight was accumulated. These amounts are far greater than those found in *A. yokoscense* growing on the non-metalliferous habitat (Tama). Twenty five times more zinc and three times more cadmium were found in the dead leaf than in the living leaf. In *A. yokoscense* growing on soils containing more than 1,000 mg Cu or $Zn.kg^{-1}$ dry weight, the uptake of copper by the root increased considerably with increasing copper content in the soils, while the uptake of zinc increased only slightly compared with the increase of zinc in the soils.

Introduction

Heavy-metal contamination of soil in areas around mines and smelters gives rise to remarkable vegetational adaptations of the original plant population, leading to the development of a specific vegetation consisting of several metal-tolerant species. A fern, *Athyrium yokoscense* (aspideaceae) is a typical species in this specific vegetation and is used as an indicator in prospecting for metal-contaminated soils in Japan (Tasaki, 1979). It is not only predominant over various metal contaminated areas but is also distributed widely in non-metalliferous habitats, fulfilling the role of pseudometallophyte (Antonovics *et al.* 1971). It is reported that the fern accumulates large quantities of heavy metals which contaminate certain soils (Honjo *et al.* 1984; Ishizawa *et al.* 1980; Tasaki and Ushijima, 1972; Tasaki, 1979; Usui *et al.* 1975). However, there have been very few studies on differences of accumulation of heavy metals by ferns and some possible mechanisms of metal tolerance are still unclear. This paper reports on the levels of copper, zinc and cadmium in *A. yokoscense* growing in various environments on soils providing metalliferous and non-metalliferous habitats. Samples of the fern were taken from the following areas: Ashio-machi, in Tochigi prefecture, where copper was produced from 1877 to 1970 and where a copper smelter has been situated; Bandai-machi, in Fukushima prefecture, where a zinc smelter has been located; and Tama Hill which is a non-contaminated area.

Materials and methods

Plant and soil materials

Samples of *A. yokoscense* and soils were collected in May, June and October from the heavy-metal-contaminated areas of Ashio-machi and Bandai-

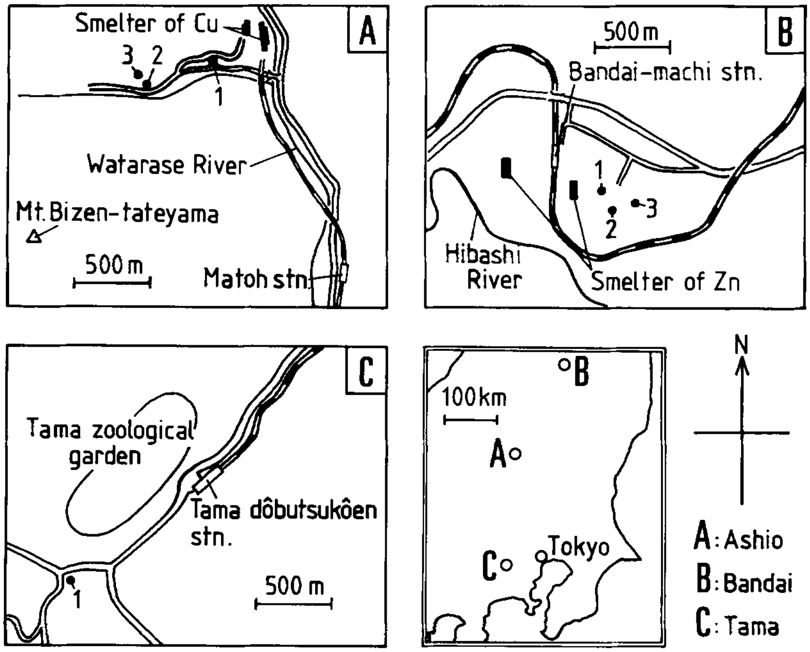

Fig. 1. Maps showing the location of sampling sites.

machi and also from the uncontaminated (normal), Tama Hill area. The location of the sampling sites is given in Fig. 1. One to three samples were collected from each site.

After sampling, the plants were washed in a large volume of deionized water and blotted dry with paper towels. The roots and leaves were cut into small pieces, lyophilized and ground in a mill.

Soils were dried, to constant weight, at 105 °C and were sieved to pass a 60 mesh (0.250 mm).

Table 1. Content of Cu in the soils and the roots, leaves and dead leaves of *Athyrium yokoscense* of the plots in Ashio, Bandai and Tama

Plot no.	Sampling date	Content of Cu (μg.g^{-1} dry weight)			
		Soils	Roots	Leaves	Dead leaves
Ashio-3	9 May '85	3589	3846	94	518
Ashio-2	16 Jun. '84	3303	5989	–	–
Ashio-3	16 Jun. '84	1780	3811	169	–
Ashio-3	16 Jun. '84	1574	3177	119	–
Ashio-3	9 May '85	1464	2602	–	–
Ashio-2	16 Jun. '84	1382	2491	90	–
Ashio-1	16 Jun. '84	913	3387	275	1990
Ashio-3	16 Jun. '84	744	735	123	–
Ashio av.		1844	3255	145	1254
Bandai-1	19 May '84	235	44	–	–
Bandai-1	16 May '85	120	70	24	305
Bandai-3	16 May '85	80	127	32	178
Bandai-2	16 May '85	76	51	–	–
Bandai-2	16 May '85	69	26	19	154
Bandai av.		116	64	25	212
Tama-1	3 Oct. '84	49	10	11	–

Analysis of plant and soil for heavy metals

A 0.5-g of dried sample was refluxed with 10 ml of 61 % HNO_3 for 60 min. After cooling, the digested mixture was diluted with 10 ml of distilled water and 10 ml of 30 % H_2O_2 and refluxed for a further 30 min. The mixture was filtered through Toyo 5A filter paper and the filtrate made up accurately to 100 ml with distilled water. Copper, zinc and cadmium were determined by atomic absorption spectroscopy (Shimadzu AA-620).

Cultivation of A. yokoscense *in nutrient solution containing heavy metals*

Four well-grown, individual plants of *A. yokoscense* from Tama Hill were grown singly, for 7 days, in an aerated, full-strength, Hoagland solution made up with deionized water, with iron at 0.245 mmol/l added as ferrous tartrate, and the pH adjusted to 5.0, in 12-l Wagner-pots (1/2000 acre). The ferns were grown indoors at 23 to 25 °C with a regulated cycle of 12 h dark and 12 h light provided by daylight fluorescent lamps, after this period the nutrient solution was replaced with a fresh nutrient solution, but containing additionally either 2 mg $Cu.l^{-1}$, 2 mg $Zn.l^{-1}$, or 0.2 mg $Cd.l^{-1}$ as chlorides and the ferns were grown on for a further 8 or 9 days. A control fern was grown in the nutrient solution without the addition of heavy metals.

Results and discussion

Heavy metal content of soils and A. yokoscense

Tables 1–3 show the amounts of copper, zinc and cadmium in soils and *A. yokoscense* collected from the Ashio, Bandai and Tama sampling areas.

The soils from Ashio and Bandai contained greater amounts of these heavy metals than that from Tama. It has been reported by various authors (Swaine, 1969; Vinogradov, 1962) that normal soils contained 2–100 mg $Cu.kg^{-1}$ dry weight and 10–300 mg $Zn.kg^{-1}$ dry weight and the average concentrations are 20 mg $Cu.kg^{-1}$ dry weight, 80 mg $Zn.kg^{-1}$ dry weight and 0.5 mg $Cd.kg^{-1}$ dry weight. It is clear from these data that the soils are contaminated particularly with copper in Ashio and with zinc and cadmium in Bandai.

The ferns from Ashio and Bandai also contained higher concentrations of the heavy metals than ferns from Tama. The maximum concentrations of heavy metals in the ferns from metalliferous habitats were 5,989 mg $Cu.kg^{-1}$ dry weight in a root (Ashio), 6,384 mg $Zn.kg^{-1}$ dry weight in a root (Bandai) and 164.8 mg $Cd.kg^{-1}$ dry weight in a leaf

Table 2. Content of Zn in the soils and the roots, leaves and dead leaves of *Athyrium yokoscense* of the plots in Ashio, Bandai and Tama

Plot No.	Sampling date	Content of Zn ($\mu g.g^{-1}$ dry weight)			
		Soils	Roots	Leaves	Dead leaves
Ashio-3	16 Jun. '84	672	644	–	–
Ashio-3	9 May '85	658	634	–	–
Ashio-3	9 May '85	544	418	144	401
Ashio-1	16 Jun. '84	349	204	127	335
Ashio-2	16 Jun. '84	261	173	87	–
Ashio-3	16 Jun. '84	240	106	155	–
Ashio-3	16 Jun. '84	215	121	76	–
Ashio-2	16 Jun. '84	95	132	–	–
Ashio av.		379	304	118	378
Bandai-1	19 May '84	11430	4040	–	–
Bandai-3	16 May '85	11290	2231	803	18008
Bandai-1	16 May '85	9195	6384	802	25654
Bandai-2	16 May '85	3867	1869	–	–
Bandai-2	16 May '85	882	1120	692	14464
Bandai av.		7333	3123	766	19369
Tama-1	3 Oct. '84	35	41	60	–

Table 3. Content of Cd in the soils and the roots, leaves and dead leaves of *Athyrium yokoscense* of the plots in Ashio, Bandai and Tama

Plot No.	Sampling date	Content of Cd (μg.g^{-1} dry weight)			
		Soils	Roots	Leaves	Dead leaves
Ashio-2	16 Jun. '84	7.1	13.1	–	–
Ashio-1	16 Jun. '84	4.3	7.2	9.5	19.0
Ashio-3	16 Jun. '84	3.3	8.8	23.3	–
Ashio-3	16 Jun. '84	3.2	6.4	–	–
Ashio-3	9 May '85	3.1	5.8	–	–
Ashio-3	9 May '85	3.0	7.7	48.0	36.2
Ashio-2	16 Jun. '84	2.6	7.0	28.8	–
Ashio-3	16 Jun. '84	1.5	3.9	–	–
Ashio av.		3.5	7.5	27.4	27.6
Bandai-1	16 May '85	96.0	53.8	150.2	462.2
Bandai-3	16 May '85	61.7	10.8	164.8	275.3
Bandai-1	19 May '84	56.6	11.2	–	–
Bandai-2	16 May '85	38.1	17.4	–	–
Bandai-2	16 May '85	22.8	14.3	36.0	215.1
Bandai av.		54.8	21.5	117.0	317.7
Tama-1	3 Oct. '84	0.7	2.4	0.9	–

(Bandai). It has been reported that different species of ferns growing on non-contaminated soils generally contain 12 mg Cu.kg^{-1} dry weight, 92 mg Zn.kg^{-1} dry weight and 0 $\simeq$ mg Cd.kg^{-1} dry weight (Bojčenko, 1968). These values are the averages obtained by the analysis of whole fern plants. *A. yokoscense* from Tama showed heavy metal contents similar to the general averages of ferns. However, the copper content of *A. yokoscense* from Ashio, and the zinc and cadmium contents of the fern from Bandai were abnormally high compared with the general averages for ferns.

Plants which take up a high concentration of metal are classified into two groups, namely, habitual and non-habitual condensers. Members of the latter group take up low amounts of heavy metals when they grow on normal soils but high amounts of metals when they grow on soils contaminated with metals (Koval'skij and Petrunia, 1964). So, *A. yokoscense*, which shows the remarkable accumulation of the heavy metals only when growing on soils containing high concentrations of the heavy metals, is a non-habitual condenser.

The root and leaf of *A. yokoscense* from both Ashio and Bandai accumulated different quantities of the heavy metals, though there were no differences in the amounts of heavy metals found in the roots and leaves of ferns from Tama. Greater amounts of copper and zinc were accumulated in the root than in the leaf. The copper content of the root from Ashio was 22 times that of the leaf and the zinc content of the root from Bandai was 2 times that of the leaf. By contrast, cadmium was accumulated more in the leaf than in the root and the concentration of cadmium in the leaf from Bandai was *ca* 5 times that of the root. This result showed that excess copper in the root was transported with difficulty to the leaf, but cadmium was transported easily to the leaf and accumulated highly in it.

Moreover, the dead leaf of the fern also contained large quantities of the heavy metals, namely, 1,990 mg Cu.kg^{-1} dry weight, 25,654 mg Zn.kg^{-1} dry weight and 462.6 mg Cd.kg^{-1} dry weight as maximum. In addition, the accumulations of zinc and cadmium in the dead leaf was 25 and 3 times respectively that of the living leaf. It is not clear why high concentrations of metals are accumulated in the dead leaf. A proportion of the heavy metals which are taken up by the root may be transported to the dead leaf and removed from the living fern.

Fig. 2 shows the relationship between the soil and the root heavy-metal content in *A. yokoscense*. The contents of the heavy metals in the roots were significantly correlated with the soil concentrations. The root of the fern showed a variation in the uptake patterns of these heavy metals. There was

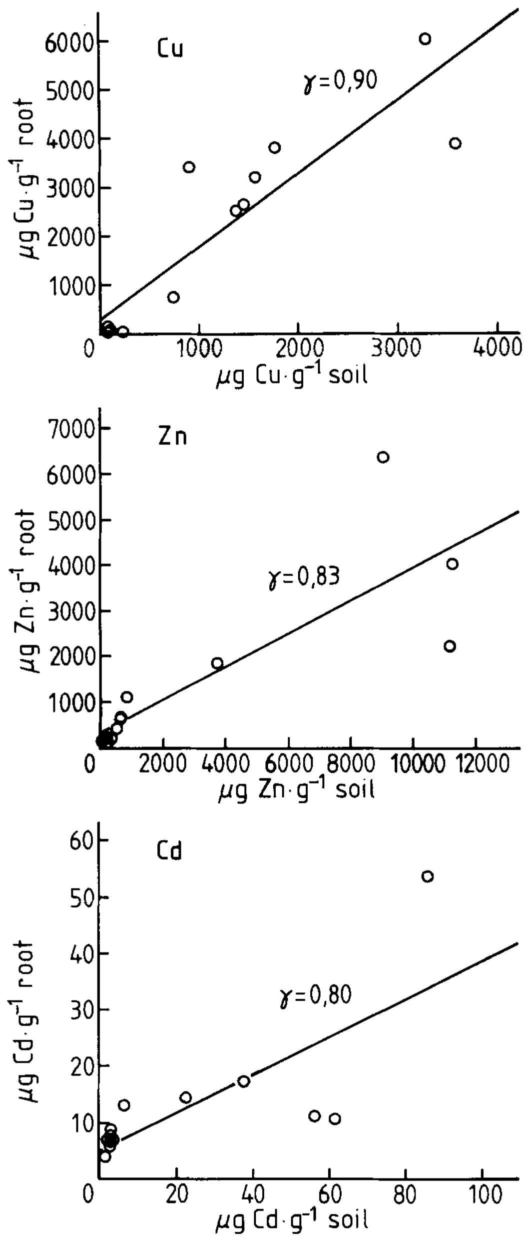

Fig. 2. Relationships between the amounts of Cu, Zn and Cd in the soil and in the root of *Athyrium yokoscense*.

little uptake of copper below a soil concentration of 1,000 $mg.kg^{-1}$ dry weight. When the soil-copper value was above 1,000 $mg.kg^{-1}$, copper uptake by the root increased steeply, reaching a concentration about twice that in the soils.

The uptake of zinc by the root of the fern growing in the soil with the lower zinc level increased in proportion to the concentration of zinc in the soil. However, uptake by the root increased less steeply from soil containing a zinc concentration greater than 1,000 $mg.kg^{-1}$ dry weight and the zinc content in the root was below one half of that in the soils.

Cadmium uptake by the root increased slightly with increasing levels of soil cadmium, rising from 4 to 17 $mg.kg^{-1}$ while soil content varied from 1 to 60 $mg.kg^{-1}$. The smallness of this increase may have been caused by the transport of excess cadmium from the root to the leaf.

The comparison of the uptake of heavy metals by the root shows that *A. yokoscense* takes up a larger quantity of copper, than the other two metals, from soils.

Uptake of heavy metals from the culture solution by A. yokoscense

A. yokoscense collected from Tama, an area not contaminated with metals, was exposed to the nutrient solution containing copper, zinc or cadmium for 8 or 9 days. Fig. 3 shows the result.

The root took up high concentrations of the heavy metals from the nutrient solution and accumulated far greater amounts of metals than the leaf. The ferns which were exposed to the metals died in about one week, though a control fern, which was not exposed to the metal, survived over the period of the cultivation. This experiment shows that *A. yokoscense* from Tama has an ability to take up a high concentration of heavy metals into the root but has no mechanisms for inactivating excess metals.

There are many reports with regard to the metal tolerance mechanism of plants but various points are still not clear. Some studies have shown that the cell wall plays an important role in metal tolerance. For example, in a tolerant strain of *Agrostis tenuis* (Turner and Marshall, 1971), zinc and copper are concentrated in the cell walls and thereby prevented from entering more sensitive sites of cell metabolism.

Another important mechanism is the presence in the cell of copper-binding substances such as cysteine- or thiol-based compounds. It is reported that copper-thioneine, consisting of 18 % cysteine, occurs in the root of *Agrostis gigantea* tolerant to excess copper and this may have a role in metal metabolism in plants (Rauser and Curvetto, 1980).

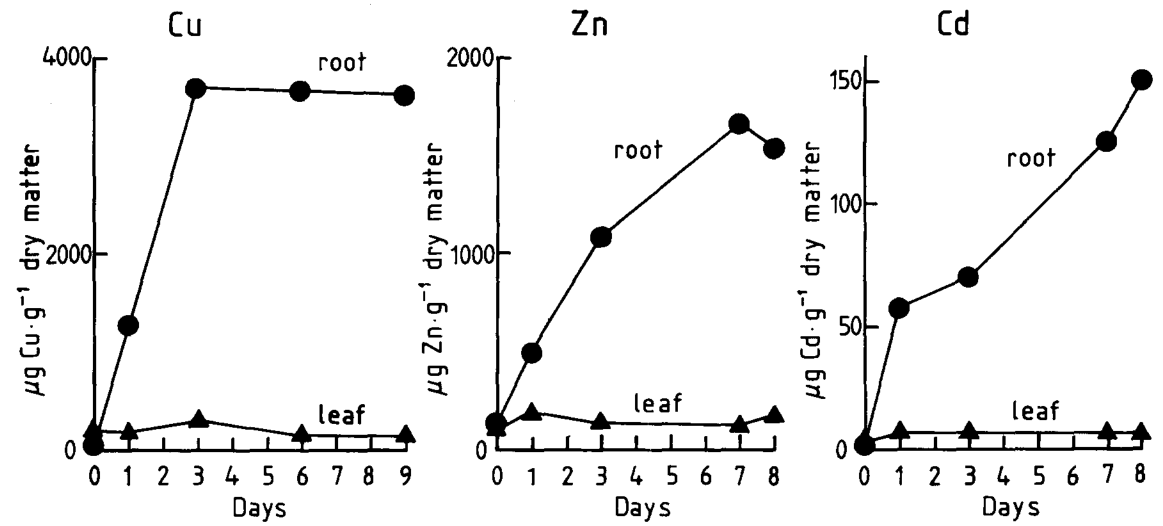

Fig. 3. Uptake of Cu, Zn or Cd from the culture solution by *Athyrium yokoscense.*

Recently, heavy metal complexing peptides, phytochelatins, (γ-glutamic acid-cysteine)$_n$-glycine (n = 3 to 7), were isolated from cell suspension cultures of *Rauvolfia serpentina* (Grill *et al.* 1985). These peptides appear upon induction of plant cell with heavy metals. *A. yokoscense* growing on heavy metal contaminated soils may have the mechanisms such as mentioned above. Further experiments are now in progress.

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