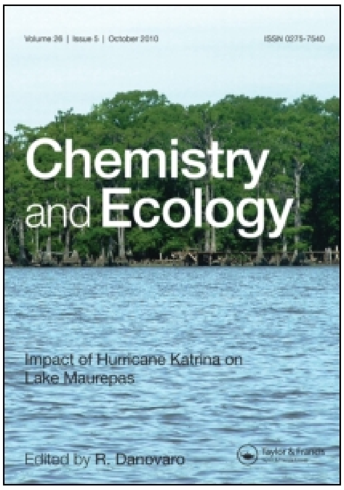

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Some Preliminary Observations On Zinc and Cadmium Accumulative Capacity in *Arum Italicum* Mill

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SOME PRELIMINARY OBSERVATIONS ON ZINC AND CADMIUM ACCUMULATIVE CAPACITY IN *ARUM ITALICUM* MILL

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Pot and hydroponic trials as well as tests on samples collected in a mining area and in two different urban sites of Tuscany were carried out on *Arum italicum* Mill (Italian arum) plants. Zinc and cadmium contents were determined by differential pulse anodic stripping voltammetry (DPASV) in different organs of about 180 samples. After 65 days treatment, zinc and cadmium median contents in leaves of plants grown hydroponically in solutions containing both metals at different concentrations fell within the range of 281-2022 and 8.3-45.1 $\mu g\ g^{-1}$ (dry weight), respectively. Some Italian arum plants were also cultivated in pots in the presence or absence of malate or citrate in order to evaluate the role of these ligands in zinc and cadmium accumulation. Citrate enhanced cadmium uptake compared to malate.

Keywords: *Arum italicum*; Heavy metals; Cadmium and zinc accumulative indicator; Phytoremediation

I. INTRODUCTION

Utilisation of plants in extracting, sequestering and/or detoxifying inorganic (heavy metals and radionuclides) or organic pollutants, is generally described by the term "phytoremediation". Thus, phytoremediation is a "green" technology, applicable to restore polluted soil, water or air, which represents an ecological and economical alternative

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to classical clean-up methods. Salt *et al.* (1998) divide phytoremediation into several areas such as phytoextraction (organic and inorganic pollutants concentration by plants in their harvestable parts), phytodegradation (organic pollutants degradation by plants and associated micro-organisms), rhizofiltration (pollutants absorption and adsorption from liquid substrates by plant roots), phytostabilisation (decrease of bioavailability of toxins in the environment by plants), phytovolatilisation (pollutants volatilisation by plants) and removal of pollutants from air by plants. Phytoextraction of metals has been developed on the basis of two different strategies that Salt *et al.* (1998) call "*induced phytoextraction*" and "*continuous phytoextraction*": the former implies the enhanced utilisation of metal following chelation, the latter is only represented by the use of hyperaccumulator plants.

For effective application of this promising and attractive technology it is necessary to screen a large number of plants, and to understand mechanisms effecting the phytoremediation of toxins. A useful strategy to select plants with metal accumulating capacities is to investigate the endemic flora of mining areas (Brooks, 1998). For this reason, we collected different vegetal species in a mineralised area of Tuscany and on the basis of their metal contents decided to study our initial attention on *Ilex aquifolium* (Cellini Legittimo *et al.*, 1995; Cellini Legittimo *et al.*, 1998) and subsequently on *Arum italicum* Mill. This is an herbaceous tuberous-rooted perennial plant of the Araceae family, that is typically grown in woodland conditions, both in sun and dry shade. The leaves appear in autumn and the plant stays green all winter (Baroni, 1984; USDA plant database; available on the Internet at http://plants.usda.gov).

The aim of the present study was to investigate the accumulative behaviour of *Arum italicum* with special emphasis in the zinc and cadmium uptake by plants grown in a pot or in hydroponic culture.

II. SAMPLING AREAS AND VEGETAL GROWTH CONDITIONS

In order to establish the possible utilisation in phytoremediation of *Arum italicum*, plants of this species are sampled for analysis in mining and urban areas of Tuscany.

The test area used was the Bottino mine, located in the Apuane Alps, near Seravezza (Lucca, Italy), an interesting site for environmental researches because the high heavy metal pollution (Cellini Legittimo *et al.*, 1995; Mascaro *et al.*, 2000). The Bottino mine had been extracting silver and lead since at least Renaissance times up to a few decades ago. Several mineral species were reported from this resource, which is characterised by a complex polymetallic sulphide assemblage in quartz-siderite gangue, formed during Apenninic metamorphism by mobilisation of metals mainly from the associated tourmalinites. Galena (PbS), sphalerite (ZnS) with cadmium contents between 0.6 and 1.2 weight percent CdS, pyrrhotite (FeS), pyrite (FeS_2), chalcopyrite ($CuFeS_2$), meneghinite ($CuPb_3(SbS_3)_7S_3$), arsenopyrite (FeAsS), and silver-rich tetrahedrite ($Cu_{12}(SbS_3)_4S$) are the most abundant ore minerals (Benvenuti *et al.*, 1989; Lattanzi *et al.*, 1992; Benvenuti *et al.*, 1992; Benvenuti *et al.* 1993a; Benvenuti *et al.*, 1993b). Several species of plants were collected on Bottino outcrops and zinc, cadmium, lead and copper contents of their leaves were measured (Tab. I). Among these plants, *Arum italicum* was the species with the highest zinc and cadmium contents. Italian arum was found on the outcrops of Bottino, up to the superior limit of its altitudinal vegetation zone. Samples from this site were compared to others collected from urban areas

TABLE I Mean content of zinc, cadmium, lead and copper ($\mu g\, g^{-1}$ dry weight) in leaves of some vegetal species collected on Bottino outcrops. Range of pH and mean metal contents found in samples of soil from the same site are reported. Values with asterisks are considered here to be the detection limits for cadmium and lead, respectively

Species	*Zn* $\mu g\, g^{-1}$	*Cd* $\mu g\, g^{-1}$	*Pb* $\mu g\, g^{-1}$	*Cu* $\mu g\, g^{-1}$
Arbutus unedo (strawberry tree)	46	0.20	1.5	2.5
Arum italicum (Italian arum)	1641	25.5	0.76	6.7
Castanea sativa (chestnut)	38	0.22	0.90	5.7
Daphne laureola (spurge laurel)	575	9.0		
Dryopteris Filix-mas (fern)	168	0.45	9.7	2.4
Erica arborea (tree heath)	12	0.005*	5.9	1.9
Fraxinus ornus (manna ash)	64	0.17	0.63	3.1
Ilex aquifolium (holly)	590	7.8	0.01	3.1
Lilium spp. (lily)	445	16.4		
Luzula spp. (woodrush)	48	0.84	4.4	4.5
Orchis spp. (orchis)	139	3.0	3.6	4.5
Rubus fruticosus (bramble)	146	0.90	19	2.2
Quercus ilex (holm oak)	34	0.10	0.010*	2.1
Soil (pH = 5.4 – 5.8)	89.5	1.2	66.5	28.5

(Lucca and Florence), where metal abundance in soil can be mainly ascribed to human activities. In each of the three areas of study, six sampling sites were chosen. Three plants were collected from each sampling site. As *Arum italicum* has large arrow-shaped leaves (50–100 cm^2), it was possible to analyse separately margins, petiole and main vein of the leaf to investigate a possible different accumulation of metals in the different parts of the same organ and identify the zone where metal accumulation was more significant.

Further small central cubic portions of collected tubers were analysed. All samples were collected from mature plants.

Some tubers of Italian arum, collected in the mining area of Bottino, were utilised for the hydroponic culture. Experiments were conducted in the laboratory, under artificial light (10 h day^{-1}) using a halogen lamp (mod. OSRAM Powerstar HQI-TS 70 W/NDL). Each plant was grown in a 250 ml Pyrex glass beaker containing 50 g of light extended clay aggregate (LECA, with size gradation of 8–16 mm). According to a previous study (Kumar *et al.*, 1995), nitrogen, potassium and calcium nutrients were added as 0.4 g l^{-1} potassium nitrate and 0.6 g l^{-1} calcium nitrate tetrahydrate solution with the resulting specific conductivity K_{20} was equal to 980 $\mu S\,cm^{-1}$. Three groups of 6 plants each were grown for two weeks. After this period, each group was treated with one of the following solutions:

- solution A containing 10 mg l^{-1} of zinc, 0.2 mg l^{-1} of cadmium and having K_{20} equal to 57 $\mu S\,cm^{-1}$
- solution B containing 40 mg l^{-1} of zinc, 0.8 mg l^{-1} of cadmium with K_{20} equal to 138 $\mu S\,cm^{-1}$
- solution C containing 100 mg l^{-1} of zinc, 2 mg l^{-1} of cadmium with K_{20} equal to 315 $\mu S\,cm^{-1}$.

After a first addition of 20 ml of treatment solution, in the course of the experiment each group of plants received different aliquots of the same solution so that the volume of the liquid phase was maintained constant in each beaker.

The zinc and cadmium amounts supplied to the three groups of plants are reported in the Table III.

Solutions exhibited a pH equal to about 5.7 and a specific conductivity (K_{20}) lower than 1500 $\mu S\,cm^{-1}$. Concurrently, six plants in the same cultural conditions and without metal treatments were grown as control samples.

Pot culture experiences were carried out on seven groups of 15 plants each, including control samples. A 500 ml polyethylene pot containing 250 g loam (70% in weight of sphagnum peat, 30% in weight of LECA) was used for each plant. In particular, sphagnum peat has been enriched with nitrogen, phosphorus as phosphorus oxide (P) and potassium as potassium oxide in amounts of 125, 110 and $250\,mg\,l^{-1}$ respectively. Its pH value in water ranged between 5.5 and 6 and cation exchange capacity resulted to be equal to $350\,meq\,l^{-1}$. Plants were grown in a cold greenhouse and during the period of growth were watered with a hydrosoluble fertiliser having a N:P:K ratio of 1:2:3 and containing magnesium and microelements (commercial product FLORY 4 Special manufactured by Planta, Germany).

The zinc and cadmium solutions added to the plants in 50 ml amounts for nine times were:

- solution C containing $100\,mg\,l^{-1}$ of zinc and $2\,mg\,l^{-1}$ of cadmium
- solution D containing $100\,mg\,l^{-1}$ of zinc and $10\,mg\,l^{-1}$ of cadmium

The solutions were added separately or with the addition of 50 ml of 5 mM ammonium malate or citrate solution, buffered at pH 5.7.

III. ANALYTICAL PROCEDURE

Cultivated plants were sampled during the vegetative period to monitor the possible absorption during the more physiologically active period.

Leaf samples of cultivated plants, both in hydroponic and in pot culture, were collected at fixed intervals of 10 and 30 days respectively, for a period of 2 months in the first case and 4 months in the second one. In the both experiments on cultivated plants the whole leaf was analysed.

Latex gloves and steel scissors were used for sampling operations.

In the laboratory, following a careful mechanical cleaning with moistened filter paper and washing with deionised water (Milli-Q), the plant samples were desiccated at 60°C for 48 hours and, finely ground; a carefully weighted aliquot of about 150 mg was subsequently digested using a 4:1 mixture of concentrated nitric acid and per chloric acid, both Suprapur grade (Merck), up to 200°C.

After mineralisation and addition of acetic buffer at pH 4.7, the concentrations of metals were determined by the technique of differential pulse anodic stripping voltammetry DPASV with a polarographic analyser (AMEL model 473).

Standard calibration curves were utilised for each metal in the same supporting electrolyte and in the same range of concentrations of the samples. Similar analytical results with respect to the use of calibration curves were obtained through the addition of known amounts of heavy metals to the significant number of samples before the mineralisation.

Metal contents were also determined in a BCR Reference Material (olive leaves, BCR number 62). Ten replicate determinations were used to estimate the precision of the method. In a previous paper (Cellini Legittimo *et al.*, 1998) it has already shown that the obtained data agree with the certified values.

IV. RESULTS AND DISCUSSION

IV.1. Wild Plants

Table II shows zinc, cadmium, lead and copper content in different organs and in different part of the leaf of *Arum italicum* plants grown in the mining site of Bottino and in urban areas of Florence and Lucca. Each single value found was representative of three plants, thus the data reported in Table II are calculated on the basis of six values. A capacity to accumulate cadmium to much higher concentrations than that for *Ilex aquifolium* (Cellini Legittimo *et al.*, 1995; Cellini Legittimo *et al.*, 1998) was observed for this species. Considerable differences were also obtained for zinc and cadmium concentrations between the samples collected from Bottino and those collected from urban areas. This behaviour reflects the higher amounts of metal in soil of the mining area. The low zinc and cadmium contents in leaves of Italian arum plants collected in urban areas, where these metals occur in air because of vehicular traffic (Ducceschi *et al.*, 1999), suggest direct foliar metal uptake was negligible. Individual portions of the leaf showed a different accumulation rates with the margin areas appearing to show the greatest accumulation although this was not

TABLE II Zinc, cadmium, lead and copper distribution ($\mu g\,g^{-1}$ dry weight) in different organs and different parts of the leaf of *Arum italicum* plants, collected in mining (Bottino outcrops) and urban (Florence and Lucca) areas

			Zn $\mu g\,g^{-1}$		*Cd* $\mu g\,g^{-1}$		*Pb* $\mu g\,g^{-1}$		*Cu* $\mu g\,g^{-1}$	
Site	*Organ*		*Range*	*Median*	*Range*	*Median*	*Range*	*Median*	*Range*	*Median*
Bottino (Apuane Alps)										
	Leaf	*margin*	1404–1586	1517	19–36	28	0.90–2.4	1.1	4.2–6.3	4.7
		main vein	1105–2444	1688	21–36	26	0.49–2.7	0.91	3.7–6.6	4.7
		petiole	861–1800	1315	25–54	39	0.98–6.7	2.5	0.57–4.9	3.0
	Spathe		650–913	799	19–33	27	0.64–0.91	0.78	2.6–5.8	3.5
	Tuber		53–130	85	0.95–1.7	1.4	0.94–6.1	2.8	0.57–2.9	1.3
Florence										
	Leaf	*margin*	34–109	65	0.13–0.24	0.15	0.51–0.60	0.54	2.0–3.6	2.4
		main vein	31–84	46	0.09–0.28	0.13	0.49–0.76	0.65	0.57–2.5	2.2
		petiole	34–86	43	0.10–0.20	0.13	0.16–0.20	0.19	0.25–1.3	0.77
	Tuber		18–38	23	0.01–0.04	0.02	0.13–0.31	0.24	0.57–0.90	0.67
Lucca										
	Leaf	*margin*	30–56	50	0.08–0.12	0.10	0.29–0.46	0.40	2.0–2.4	2.1
		main vein	23–46	39	0.06–0.11	0.09	0.32–0.41	0.35	2.3–3.5	2.7
		petiole	24–41	36	0.06–0.10	0.09	0.24–036	0.31	1.5–3.7	3.0
	Tuber		11–29	25	0.03–0.11	0.07	0.16–0.22	0.20	0.78–2.3	1.1

consistent for all metals. As for many other plants (Ernst, 1993), the tuber showed a capacity to accumulate metals with concentrations being similarly low for samples collected at Bottino mine as well as in urban areas.

IV.2. Hydroponic Culture

The addition of zinc and cadmium solutions effectively increased the contents of these metals during the course of the experiment to values distinctly higher than control samples. The amounts of metal taken up were not in the same proportions as those that were supplied (Tab. III). For each group of plants, zinc content shows a profile in

TABLE III Zinc and cadmium amounts supplied (columns 3 and 4) and subsequently found (columns 5, 6, 7 and 8) in leaves of Italian arum plants grown in hydroponic culture, during the 65 days treatment with different solutions in which zinc/cadmium ratio was fixed at the value of 50. Solution A: $Zn = 10\,mg\,l^{-1}$, $Cd = 0.20\,mg\,l^{-1}$; Solution B: $Zn = 40\,mg\,l^{-1}$, $Cd = 0.80\,mg\,l^{-1}$; Solution C: $Zn = 100\,mg\,l^{-1}$, $Cd = 2\,mg\,l^{-1}$

		Zn μg Total	*Cd μg Total*	*Zn $\mu g\,g^{-1}$*		*Cd $\mu g\,g^{-1}$*	
Solution	*Days*	*supplied*	*supplied*	*Range*	*Meidan*	*Range*	*Median*
A	0			91–153	114	2.2–5.3	3.7
	15	600	12	121–183	147	5.9–9.0	7.1
	25	1000	20	290–350	325	6.8–8.2	7.5
	35	1200	24	311–349	327	4.7–5.9	5.2
	45	1400	28	307–341	326	5.3–6.7	6.0
	55	1600	32	269–315	289	7.6–8.7	8.1
	65	1700	34	257–313	281	9.8–18.3	15.3
B	0			103–188	140	2.2–4.9	3.2
	15	1200	24	115–293	176	2.0–10.5	5.1
	25	2800	56	123–394	242	5.2–9.9	7.7
	35	3600	72	153–371	251	5.5–7.2	6.6
	45	4400	88	221–380	300	3.0–8.4	6.3
	55	5200	104	316–522	393	5.4–8.7	6.9
	65	5600	112	303–713	480	6.2–10.9	8.3
C	0			93–198	130	1.6–3.9	2.6
	15	4800	96	117–250	163	3.0–4.6	3.8
	25	8800	176	396–851	661	8.3–10.2	9.7
	35	10800	216	592–806	669	9.6–14.3	11.6
	45	12800	256	594–650	614	17.1–21.2	19.1
	55	14800	296	1082–1450	1234	24.8–27.8	26.2
	65	15800	316	1900–2102	2022	31.8–71.0	45.1
Control	0			88–168	130	1.7–4.5	3.3
Samples	65			102–236	164	1.9–4.2	3.2

which different temporal phases can be distinguished (Fig. 1):

- until the 25th day, zinc content increases for all plants even if in a different way,
- from the 25th to the 45th day, zinc content in plants remains almost constant for each group,
- from 45th to 65th day, zinc content enhances for plants treated with solution C more rapidly than for plants treated with solution B, whereas it decreases slightly for plants treated with solution A.

The decrease of zinc content observed in the last 20 days of the experiment for plants treated with the least concentrated solution, could depend either on a dilution effect associated with the growth of plants or on a redistribution of metal within the plants. Whether it depends on the former cause or the latter one, this trend is exhibited only by plants treated with solution A just because of its lower zinc content.

Related cadmium contents had much more irregular profiles.

The results of a comparison between zinc percent and cadmium percent contents and their relative amounts supplied are more interesting, as shown in Figure 2. Observing so that the three sections

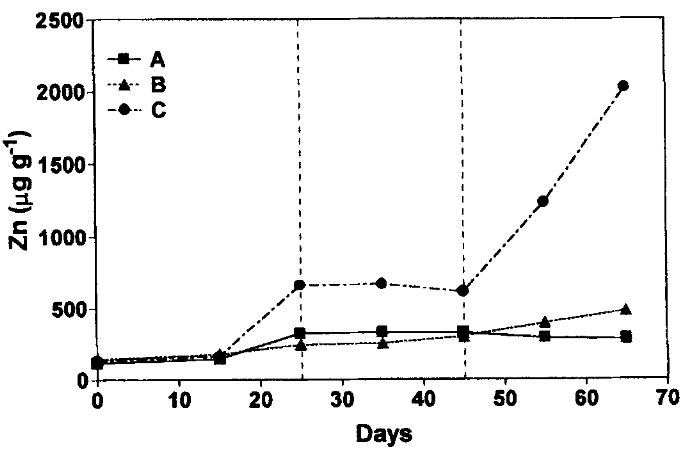

FIGURE 1 Zinc content *versus* time in leaves (page leaf) of *Arum italicum* samples treated with three different zinc and cadmium concentration solutions A, B and C (see text for details) having zinc/cadmium ratio fixed at the value of 50.

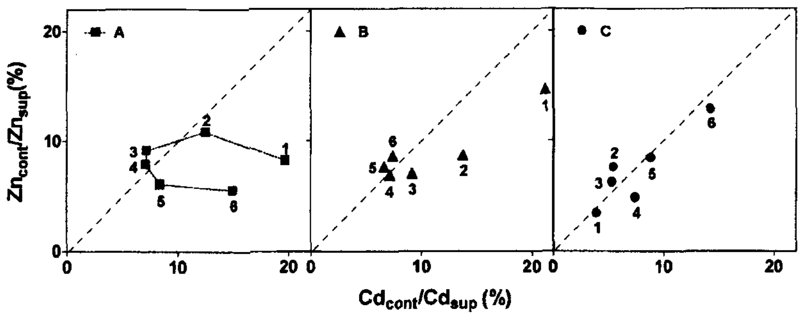

FIGURE 2 $Zinc_{content}/zinc_{supplied}$ percent ratio *versus* $cadmium_{content}/cadmium_{supplied}$ percent ratio in leaves (page leaf) of *Arum italicum* samples treated with three different zinc and cadmium content solutions A, B and C (see text for details) having the same zinc/cadmium ratio equal to 50. Numbers indicate the temporal sequence of measurements.

that are related to the three different treatments, we can see that:

- at low levels of metals supplied, zinc and cadmium concentrations in plants seem to be in competition,
- increasing the supplying of metals to medium levels, plants seem to avoid the take up zinc and cadmium probably as a result of a defence mechanism,
- at higher levels, the massive presence of metals forces the plants to take up indiscriminately high metal concentrations.

Table IV shows zinc and cadmium contents measured before and after 65 days treatments in different organs of *Arum italicum* and *Lilium bulbifer* L. samples. *Lilium bulbifer*, a wild lily growing in Italy, was used for a comparison, because of the high contents of zinc (445 $\mu g\, g^{-1}$) and cadmium (16.4 $\mu g\, g^{-1}$) found in leaves of some specimens collected on Bottino outcrop. *Daphne laureola* plants collected on the same site showed high zinc and cadmium contents (575 and 9.01 $\mu g\, g^{-1}$, respectively). However, this species was not studied further because its distribution to fluvial areas characterised by high humidity and shadow conditions and because it does not live well in the hydroponic culture.

Results for *Arum italicum* and *Lilium bulbifer* confirmed a low heavy metal accumulation in hypogean stock organs, with high zinc and cadmium contents in roots. Differences between the two species relative to metal contents in leaves are shown in Figure 3 where zinc/cadmium

TABLE IV Zinc and cadmium contents found after 65 days in different organs of *Arum italicum* and *Lilium bulbifer* plants grown in hydroponic culture and treated with solution C containing 100 mg l^{-1} zinc and 2 mg l^{-1} of cadmium. For control samples values found before treatment are reported (*)

Species	*Solution*	*Organ*	*Zn* $\mu g\,g^{-1}$ *Median*	*Cd* $\mu g\,g^{-1}$ *Median*	*Zn/Cd Median*
Arum	A				
Italicum		Leaf	281	15.3	18.4
		Tuber	105	2.2	47.7
		Root	219	11.5	19.0
	B				
		Leaf	480	8.3	57.8
		Tuber	110	2.2	50.0
		Root	325	11.4	28.5
	C				
		Leaf	2022	45.1	44.8
		Tuber	174	2.8	62.1
		Root	1999	127.5	15.7
Control Samples					
		Leaf*	130	3.3	39.4
		Leaf	164	3.2	51.3
		Tuber*	117	2.4	48.8
		Tuber	108	1.9	56.8
		Root*	54	1.8	30.0
		Root	48	1.6	30.0
Lilium	C				
bulbifer		Leaf	71	0.21	338.1
		Bulb	16	0.05	320.0
		Root	1880	32	58.8
Control Samples					
		Leaf*	43	0.15	286.7
		Leaf	51	0.15	340.0
		Bulb*	21	0.08	262.5
		Bulb	78	0.98	79.6
		Root*	40	0.67	59.7
		Root	56	0.71	78.9

ratio is plotted as a function of the cadmium content. The reported data are for both species the values observed before the beginning and at 15th, 25th, 35th, 45th, 55th and 65th days of the treatment. Data appear distributed in two distinct groups for the two species. Zinc/cadmium ratios for *Arum italicum* samples resulted lower with respect to *Lilium bulbifer*. By considering that zinc median values ranged between 163 and 2022 $\mu g\,g^{-1}$ for the former plant and between 41 and 88 $\mu g\,g^{-1}$ for the latter, a preferential cadmium accumulative capacity by *Arum italicum* leaves is put in evidence.

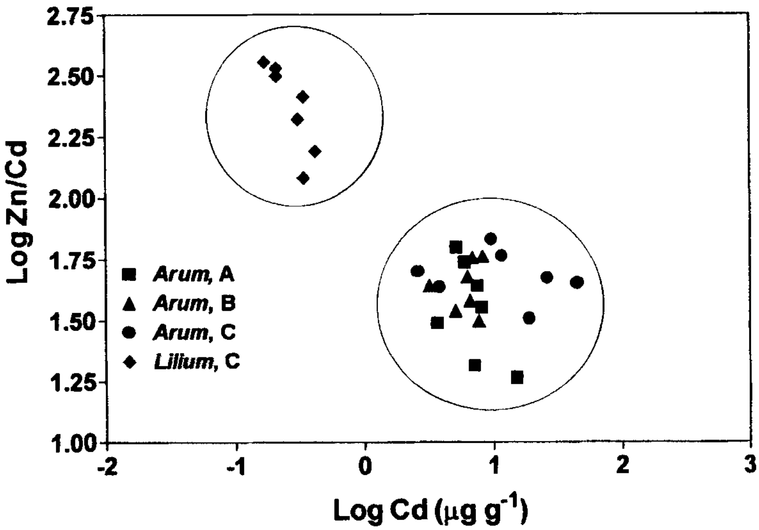

FIGURE 3 Zinc/cadmium content ratio (log) *versus* cadmium content (log) in leaves of *Arum italicum* and *Lilium bulbifer* samples grown in hydroponic culture and treated with three different zinc and cadmium content solutions A, B and C (see text for details) having the same zinc/cadmium ratio equal to 50.

IV.3. Traditional Pot Culture

Organic acids and peptides, involved in cell heavy metal detoxification, play an important role in translocation of metals between roots and leaves as well as in their following phloematic distribution (Stephan and Scholz, 1993; Krämer *et al.*, 1996). As a rule, in many plant species, the most abundant organic acids are malic and citric ones, whose contents in leaves range from 2 to 10 $\mu mol\, g^{-1}$ fresh weight (Clark, 1969).

Malic and citric acids are also the main organic acids present in xylem exudates and their capacity to bind zinc and cadmium has been demonstrated (White, 1981). Cataldo *et al.* (1988) showed that organic acid concentrations could change in the xylem exudate with the age of the plant. According to Mullins *et al.* (1986) the translocation of these metals in the xylem sap mainly occurs as metal citrate complex, and a positive correlation between higher malate and citrate contents in xylem sap and tolerance of genotypes to certain metals was shown (Harmens *et al.*, 1994). Many aspects of the mechanism linking the production of these organic acids to the presence of metals in the soil remain to be clarified.

For this reason, in order to increase zinc and cadmium accumulation in Italian arum, an experiment was carried out adding malate and citrate directly to the substrate.

The analytical data related to zinc and cadmium amounts supplied (with or without ammonium malate or citrate addition) and found in leaves of Italian arum grown in a pot, during 114 days of treatment are shown in Table V. Results show a reduced capacity to accumulate zinc

TABLE V Zinc and cadmium amounts supplied (with or without 5 mM ammonium malate or citrate addition) and found in *Arum italicum* leaves, during 114 days treatment. Solution C: $Zn = 100\,mg\,l^{-1}$, $Cd = 2\,mg\,l^{-1}$; Solution D: $Zn = 100\,mg\,l^{-1}$, $Cd = 10\,mg\,l^{-1}$

Solution	*Days*	*Zn μg Total supplied*	*Cd μg Total supplied*	*Zn μg g⁻¹ Mean (n = 15)*	*Cd μg g⁻¹ Mean (n = 15)*
C	0			28.3	0.27
	30	20000	400	34.3	0.59
	57	30000	600	56.5	0.71
	79	40000	800	70.0	1.1
	114	45000	900	103.5	1.4
D	0			32.6	0.39
	30	20000	2000	62.3	1.3
	57	30000	3000	60.1	1.4
	79	40000	4000	62.4	2.5
	114	45000	4500	51.9	6.0
C + malate	0			35.5	0.41
	30	20000	400	55.4	1.2
	57	30000	600	70.3	1.7
	79	40000	800	84.1	1.7
	114	45000	900	168.5	3.6
D + malate	0			29.9	0.36
	30	20000	2000	77.8	1.1
	57	30000	3000	75.3	1.3
	79	40000	4000	58.1	1.6
	114	45000	4500	125.1	4.6
C + citrate	0			25.3	0.22
	30	20000	400	77.8	0.56
	57	30000	600	100.9	0.93
	79	40000	800	127.2	2.2
	114	45000	900	162.5	1.0
D + citrate	0			42.1	0.37
	30	20000	2000	65.3	0.50
	57	30000	3000	93.5	2.1
	79	40000	4000	122.0	5.0
	114	45000	4500	138.6	6.3
Control Samples	0			31.2	0.93
	30			33.2	1.1
	57			34.3	0.89
	79			36.4	0.38
	114			30.4	0.18

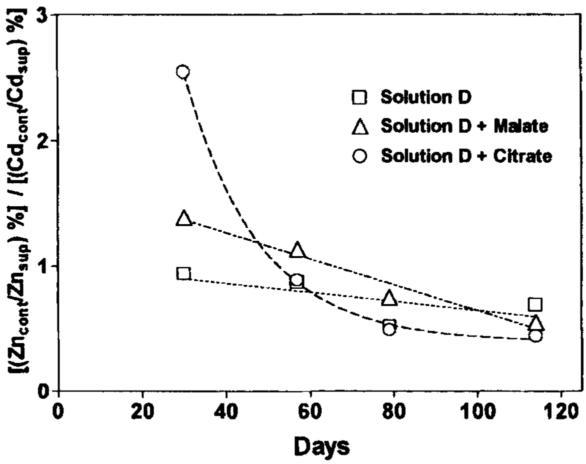

FIGURE 4 $Zinc_{content}/zinc_{supplied}$ percent over $cadmium_{content}/cadmium_{supplied}$ percent *versus* time in leaves of *Arum italicum* samples grown in pots and treated with solution D, containing $100\,mg\,l^{-1}$ of zinc and $10\,mg\,l^{-1}$ of cadmium, supplied with or without 5 mM ammonium malate or citrate solution.

and cadmium both as free ions or as complexes for plants grown in pots respect to plants grown in hydroculture or at the mining site of Bottino. Italian arum leaves collected at Bottino mine showed zinc and cadmium contents as one order of magnitude higher than those of leaves of plants cultivated in pots. Nevertheless, among the plants grown in pots those treated with ligands had higher metal concentrations than the others. Particularly, Figure 4 shows as citrate, more than malate, enhances cadmium content more than zinc content in *Arum* leaves.

On the other hand, a comparison among data relative to experiments on wild plants, plants grown in hydroponic culture and plants cultivated in pots can be hardly carried out because different growth conditions and time scales for accumulation have been involved.

V. CONCLUSIONS

Analysis carried out on plants collected in the mining area of Bottino (Tuscany), suggests that *Arum italicum* is an interesting plant potentially useful as accumulator of zinc and especially cadmium. Particularly cadmium taken up by the plant is enhanced by the use of a chelant such as malate or principally citrate. Further studies are needed to evaluate the growth and metal extraction potential under field conditions, to test the possible use of Italian arum and different *Arum spp.* in phytoextraction of cadmium and zinc from contaminated soils. Even if the accumulative capacity of this species seems to be lower if compared to that of other plants such as the *Thlaspi spp.*, the great adaptability of *Arum italicum* to marginal soils makes these plants an attractive useful alternative for phytoremediation in some particular situations.

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