Chapter 8
A Multi-disciplinary Challenge for Phytoremediation of Metal-Polluted Pyrite Waste

Teofilo Vamerali, Marianna Bandiera, and Giuliano Mosca

8.1 The Phytoremediation Concept Evolves in Pyrite

After the discovery of *hyperaccumulators* (Raskin et al. 1994; Salt et al. 1995), plants which accumulate high above-ground levels of one or a few metals without evident symptoms of toxicity, the application of plant-based technologies to remedy metal-contaminated soils has received huge attention. Metalliferous soils provide several hyperaccumulators, but their application must be verified carefully in terms of biomass—generally very small—and uptake when plants are cultivated out of their native environment (Brooks et al. 1977).

Phytoextraction consists of removing toxic elements through the harvestable biomass, after sufficient translocation from roots has occurred. Although promising, the method has some limitations due to difficult plant establishment, possible limited soil metal availability, insufficient root uptake (exclusion), symplastic mobility and xylem loading, as well as the great energy costs required for detoxification and storage (Meagher 2000; Clemens et al. 2002).

The use of biomass species (trees and crops) may represent a realistic alternative to hyperaccumulators for removing trace metals (Vamerali et al. 2010). Biomass species can absorb a wider range of metals but at low concentrations, a feature compensated by higher biomass productivity. The application of cultivated species is facilitated by the easy availability of seeds and cuttings on the market, but their adaptability and method of cultivation should be verified in each specific site. The extended root system of trees is suitable for remediating especially deep polluted layers (Pulford and Dickinson 2005) and short-rotation coppices of poplar (*Populus*

T. Vamerali (✉)
Department of Food Sciences, University of Parma, Viale G.P. Usberti 11/A, 43100 Parma, Italy
e-mail: teofilo.vamerali@unipd.it

M. Bandiera • G. Mosca
Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (Padova), Italy

D.K. Gupta (ed.), *Plant-Based Remediation Processes*, Soil Biology 35,
DOI 10.1007/978-3-642-35564-6_8,

spp.) (Laureysens et al. 2004a, b) and willow (*Salix* spp.) (Rosselli et al. 2003; Dickinson and Pulford 2005) may provide an efficient and cost-effective decontamination method. Herbaceous species produce a denser vegetation cover which is effective against erosion and may create an aerobic environment in the rhizosphere, increasing soil aggregation and binding contaminants through the release of organic matter (Pulford and Watson 2003; Robinson et al. 2006). Roots can also efficiently act in phytostabilisation by sequestering metals, especially those with limited mobility such as Pb and Cu (Marmiroli et al. 2005; Yoon et al. 2006) and favouring precipitation with root exudates (Heim et al. 1999; Yang et al. 2005). Various means have been successfully tested in the last 10 years to improve phytoextraction efficiency in biomass species, but mainly in agricultural or forest soils. Assisted phytoextraction with low toxic organic chelators, like NTA (nitrilotriacetic acid) and EDDS (ethylene-diamine-disuccinic acid), positively increase metal uptake in Ethiopian mustard (*Brassica carinata* A. Braun) (Quartacci et al. 2007). Exogenous applications of growth regulators may also result in higher growth and metal uptake in alfalfa (*Medicago sativa* L.) and sunflower (*Helianthus annuus* L.) (Lopez et al. 2005; Liphadzi et al. 2006), whereas mycorrhization facilitates metal acquisition in maize (*Zae mays* L.) (Shen et al. 2006; Wang et al. 2007). However, investigations are often conducted in the laboratory or in microcosms, thus making transferral of results to the open field ineffective. Only few experiments have been carried out *in situ* and limited information is available on particular substrates, such as sediments and industrial wastes. In this framework, a summary of results on the phytoremediation of pluri-metal-contaminated pyrite cinders is presented here, focusing on plant responses to several agronomic practices at pot and field level. As a single green technology may fail in this context, the traditional concept of phytoremediation should be reviewed in the light of a multidisciplinary approach.

8.2 What the Literature States on Phytoremediation of Pyrite

Among metal-polluted media, great concern focuses on industrial waste or sediments, the unusual composition of which may further limit plant establishment and growth. Among these, we considered pyrite waste, which remains after sulphur extraction from pyrite ore roasting at extremely high temperatures (~800 °C). The waste presents itself as red cinders, mainly composed of fine particles of pyrite (FeS_2) and other minerals and devoid of organic matter (Vidal et al. 1999). Oxidation of metal sulphides from pyrite residues can release soluble metals and increase soil acidity (Clemente et al. 2006), with consequent hazardous metal movements. Phytomanagement of pyrite waste is an interesting and inexpensive option to reduce wind erosion and metal leaching, but little information is available in the literature on this issue, particularly at field scale. In recent years, some authors have found that cultivation of soybean (*Glycine max* (L.) Merr.), sorghum (*Sorghum bicolor* L.), maize and sunflower is possible at various rates of pyrite dilution, but only at pot level (Fellet et al. 2007). In the open field, the establishment and phytoremediation of Indian mustard (*Brassica juncea* (L.) Czern) in a site affected by the toxic spill of pyrite residues at Aznalcóllar (Spain) was effective only after the addition of organic matter (cow manure and compost) and amendment with lime to increase the pH (Clemente et al. 2006). Substantial biomass increases have also been achieved with sunflower and sorghum after organic or mineral fertilisation of pyrite waste (Marchiol et al. 2007), suggesting that attention should be paid to improvements in the physical and chemical properties of the substrate.

In both pyrite and other metal-contaminated wastes, plant growth is limited not only by contamination but also by several environmental variables, such as unsuitable pH, high salinity, insufficient aeration and low water and nutrient availability (Robinson et al. 2006). In these conditions, extensive root colonisation is essential for plant establishment and metal acquisition, but root responses under metal contamination have been investigated in a narrow range of species. For instance, roots of the hyperaccumulator *Thlaspi caerulescens* J. & C. Presl. were found to colonise predominantly Zn-polluted soil regions (Saison et al. 2004), whereas little information is available for most biomass species. The root system of non-metallophyte species is expected to be very sensitive to the presence of metals, with serious damage and growth reduction (Ubi and Osodeke 2007; Rascio et al. 2008). For instance, disruption of the root cuticle, reduced root hair proliferation and severe deformation of root structures are caused by copper in *Chloris gayana* Kunth (Sheldon and Menzies 2005). According to a general rule, which recommends thorough analysis of polluted sites before the application of any phytoremediation strategy (Wiegleb and Felinks 2001), the area contaminated by pyrite waste and metals which we studied was initially characterised for soil stratigraphy, contaminant distribution and floral analysis.

8.3 Site Characterisation

We focused attention on a contaminated area at Torviscosa (Udine—NE Italy, 45°49′23″N, 13°16′40″E, 3 m a.s.l.), near an abandoned chemical factory and within the polluted site 'Lagoon of Grado and Marano and adjacent rivers', which is included in the Italian priority site list for remediation (Fig. 8.1). Pollution was due to As- and metal-contaminated pyrite cinders, discharged between the 1940s and the late 1970s as by-products of pyrite ore roasting for sulphur extraction. Largely devoid of organic matter, with high bulk density (1.65 g cm^{-3}), poor in nutrients, pH 7.3 (Table 8.1) and relatively low electrical conductivity (0.3 S m^{-1}), over the years the cinders had been colonised by sparse spontaneous flora (Coletto et al. 2006). Within a confined area of 2,000 m^2 of the site, total metal concentrations in the substrate were identified in 2004 in 33 soil samples (2 m deep, 10 m apart); soil stratigraphy was monitored by the digging of six exploratory ditches (Fig. 8.2). Metals were detected in substrate samples and plant tissues by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) after microwave-acid digestion. The cinders extended for a depth of 0.7 m over a deep

Fig. 8.1 Polluted site at Torviscosa (Udine, NE Italy). (**a**) Yellow deposit of sulphur on soil surface near abandoned factory. (**b**) Exploratory ditch (1.5 m) for studying soil/pyrite stratigraphy. (**c**) Detail of gravelly capping soil with aged spontaneous vegetation (in winter). (**d**) Dense vegetation of *Pyracantha coccinea* M.J. Roemer (in winter) close to old factory

clay horizon and were capped with ~0.15 m of unpolluted gravelly soil. In detail, analysis of soil stratigraphy showed five different layers with the following characteristics:

a. 0–0.15/0.20 m: carry-over soil with vegetation, rich in gravel, with sand and silt
b. 0.15/0.20–1.20/1.75 m: wet pyrite cinders
c. 1.20/1.75–1.40/2.35 m: wet black–brown silty clay, rich in organic matter
d. 1.40/2.35–1.85/3.20 m: wet clay with grey–green silt
e. 1.85/3.20–2.90/3.80 m: white sandy soil with fine gravel, very wet (groundwater)

The presence of the almost impermeable clay horizon (layer *d*) has prevented significant downward metal leaching until recently, as confirmed by groundwater analysis. The high soil moisture along much of the profile, together with extended waterlogging during rainy periods, was indeed partly due to poor water infiltration in the cinders.

Table 8.1 Main chemical properties and level of metal contamination ($n = 3$) of pyrite waste and *in situ* capping soil, in comparison with a silty-loam uncontaminated reference soil (Legnaro—University of Padova)

		O.M.[a]	N[b]	P[c]	K[d]	As[e]	Cd[e]	Co[e]	Cu[e]	Pb[e]	Zn[e]
	pH	%	$g\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$
IGV[f]						20	2	20	120	100	150
Unpolluted capping layer	8.1	1.87	0.73	11.8	17.3	19.9	0.83	<0.01	72.8	22.4	96.5
Pyrite waste	7.3	<0.01	n.d.	n.d.	n.d.	**892**	**5.09**	**102**	**2,726**	**459**	**2,410**
Mixed soil-pyrite											
Ploughing[g]	7.6	2.52	5.33	12.7	153	**243**	**2.67**	**41.6**	**1,719**	**118**	**860**
Ploughing[h]						n.d.	0.31	n.d.	291	15.7	30.2
Ripping[g]	7.5	2.85	6.67	7.90	131	**292**	1.67	**29.5**	**1,103**	89.8	**674**
Ripping[h]						n.d.	0.16	n.d.	190	10.3	24.1
Reference soil at Legnaro[g]	7.8	2.17	5.67	33.1	62.8	15.5	0.38	7.95	31.1	17.9	79.5
Reference soil at Legnaro[h]						n.d.	0.09	n.d.	4.51	2.96	1.21

Values in bold exceed IGV[f]

n.d. not determined

[a] Organic matter: Walkey and Black method

[b] Total N: Kjeldhal method

[c] Available P: Olsen method

[d] Exchangeable K: $BaCl_2$ TEA (ISO 11260)

[e] USEPA 3051 method

[f] Italian Guideline Values for ‘Green public, private and residential areas’ (Italian Legislative Decree 152/2006)

[g] Total metal concentration after tillage (0–0.15 m of depth)

[h] DTPA-extractable metal concentration after tillage (0–0.15 m of depth)

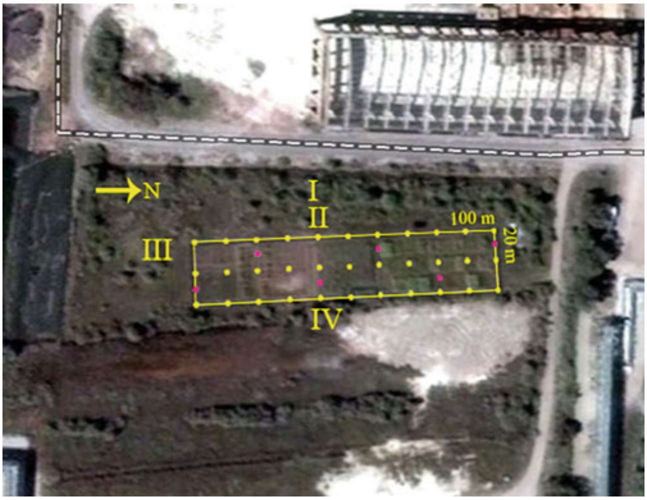

Fig. 8.2 Aerial view of study area. *Yellow dots*: soil sampling (33); *red dots*: exploratory ditches (6); *Roman numbers* (I–IV): areas for floral analysis. *Arrow*: north

The carry-over topsoil (layer *a*) generally had low metal contents, below the Italian Guideline Values (IGV) for 'Green public, private and residential areas' (Italian Legislative Decree 152/2006). Metal concentrations in cinders were heterogeneous across the sampling area, but on average very high, exceeding the IGV by as much as 45 times for As, 23× for Cu, 16× for Zn, 5× for Co and Pb, and 2.5× for Cd. Arsenic and Cu levels were particularly high and exceeded the less restrictive IGV for 'Industrial sites' (i.e., 50 and 600 mg kg^{-1} DW, respectively) (Table 8.1). The total amounts of Fe and S in the cinders were about 10 and 5 times higher than in cultivated soil, with concentrations of 97 % and 0.39 %, respectively. In spite of this, bioavailable Fe was not very high, comparable with the agricultural silty-loam soil at the experimental farm of the University of Padova (24 vs. 18 mg kg^{-1}).

The particular stratigraphy, together with abundant precipitation—the historical mean annual value of the site is 1,000 mm—led to the selection of a specific spontaneous flora. Analysis of the vegetation cover by visual evaluation (Pignatti and Mengarda 1962) during spring 2004 in four buffer zones identified in the surroundings of the soil-sampled area (Fig. 8.2) was believed to be useful in providing criteria for species selection for the planned phytoremediation setting. Buffer zones I and IV were colonised by both herbaceous and woody species, whereas the vegetation was mainly herbaceous in buffer zones II and III. For species with an appreciable degree of cover (>5 %), shoot samples (young branches for trees) were collected in early spring, washed and oven-dried (105 °C, 24 h) to determine metal concentrations. Our hypothesis was that a correlation exists between the extent of species diffusion and their metal accumulation.

In the buffer zones, more than 80 different species were classified, mainly herbaceous and only 10 woody. In zone I, close to the old factory, the latter were mainly represented by *Pyracantha coccinea* M.J. Roemer (27.5 %, i.e., percentage of the sum of all detected species), *Salix* spp. (21.6 %) and *Populus alba* L. (8 %). The most widespread grasses were *Solidago gigantea* Aiton (8 %) and *Dorycnium pentaphyllum* Scop. (8 %). Dominant species in zones II and III were *Poa pratensis* L. (24 % and 11.4 %, respectively), *Ambrosia artemisiifolia* L. (18 % and 34 %), *Medicago lupolina* L. (6 % and 9 %) and *Bromus arvensis* L. (11 %, only in zone III). Lastly, in zone IV the prevailing species were *Phragmites australis* (Cav.) Trin. ex Steud. (15 %), *Solidago gigantea* Aiton (10 %), *Dactylis glomerata* L. (7 %) and *Populus alba* L. (10 %).

Zinc, Mn and Cu were the three most frequently accumulated elements in the shoot tissues of all species, both herbaceous and woody (Fig. 8.3). The highest values of Zn and Cu were found in *Taraxacum officinale* Weber (360 and 96 mg kg^{-1}, respectively) and Mn in *Carex hirta* L. (393 mg kg^{-1}). The overall metal concentrations (summation of various elements) were highest in *Asteraceae* species, i.e., *T. officinale*, *Eupatorium cannabium* L. and *A. artemisiifolia* L., the latter being the most widespread. Interesting accumulations were also found in the hydrophyte *C. hirta* (family *Cyperaceae*), whereas trees and shrubs seemed to be less efficient than herbs, except for the *Salicaceae Salix alba* L. and *Populus nigra* L., which have been found to accumulate Zn efficiently in this and in other contaminated sites (Rosselli et al. 2003; Pietrini et al. 2010). These preliminary investigations confirmed the importance of species selection in phytoremediation. Although a particular relationship between metal accumulation and kind of root apparatus does not seem to exist (Fig. 8.3), the ability of our *Asteraceae* may partially depend on their deeper tap roots. The application of spontaneous species still raises the problem of seed supply and, with this in mind, screening of cultivated species was considered necessary.

8.4 Experience in an *On-Site* Pilot Phytoremediation Plant

The sparse natural vegetation cover of the site meant that difficulties in plant establishment and growth were predicted, but the presence of the capping unpolluted layer seemed useful for the vegetation. In a preliminary pot trial, we verified whether some crops like sunflower, alfalfa and fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.) could take advantage of a 7- or 15-cm top unpolluted soil layer (Fig. 8.4). Indeed, mimicking site stratigraphy, regardless of the thickness of the capping layer, all species showed regular growth both above- and below-ground over a 60-day period of cultivation, comparable with that of the uncontaminated reference soil of the University of Padova. However, roots tended to colonise mainly the uncontaminated layer (length: 90 % vs. 80 % of pyrite alone and 50 % of controls). The general marked reduction in plant growth with pyrite alone was evident, i.e., −77 % in shoots and −63 % in roots (length) on average.

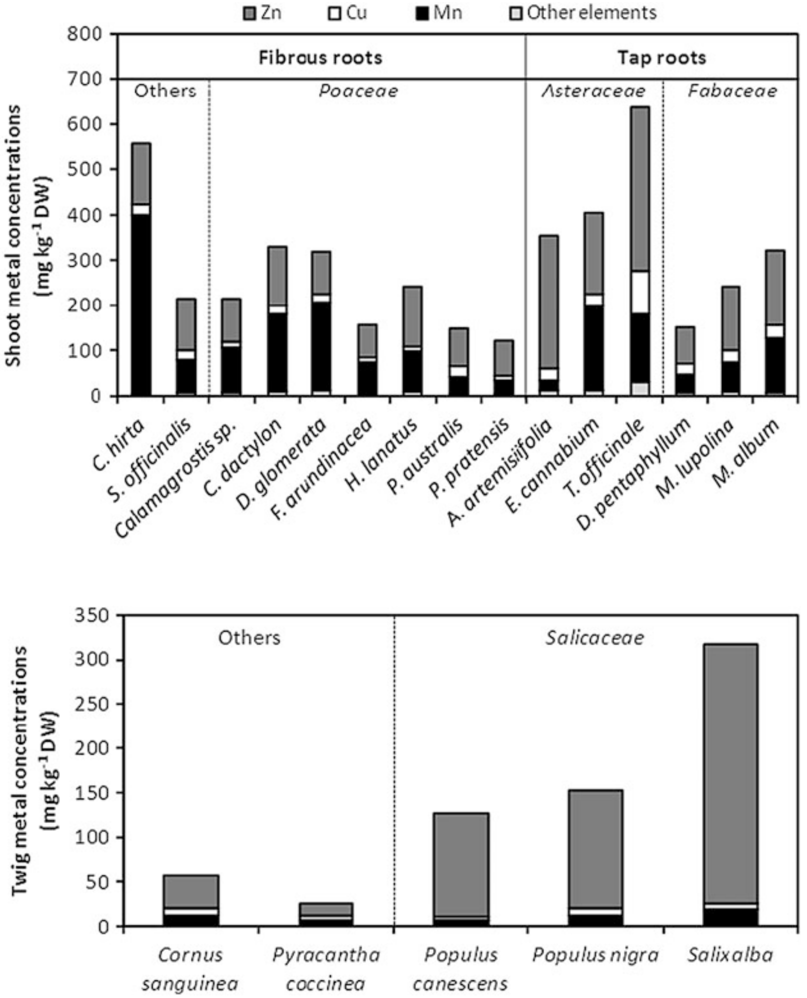

Fig. 8.3 Metal concentrations (mg kg^{-1} DW) in shoots of various spontaneous herbs (*above*) and wood of trees (*below*) collected at pyrite-contaminated site, sorted by root system type and botanical family. Herbs studied: *Carex hirta* L., *Sanguisorba officinalis* L., *Calamagrostis* sp., *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Holcus lanatus* L., *Phragmites australis* (Cav.) Trin., *Poa pratensis* L., *Ambrosia artemisiifolia* L., *Eupatorium cannabium* L., *Taraxacum officinale* Weber., *Medicago lupolina* L., *Melilotus album* Desr. Other elements: As + Cd + Co + Cr + Ni + Pb

Setting up the phytoremediation plant at Torviscosa in 2005 gave us the opportunity of testing various soil management strategies, by comparing unaltered stratigraphy with mixed layers, i.e., ripping vs. ploughing tillages, both at a depth of 0.3 m. Ploughing entailed more thorough mixing of soil than ripping, which simply broke up the surface. Ploughing was intended to dilute the waste with the unpolluted top soil, and ripping to allow roots to encounter a clean habitat, at least

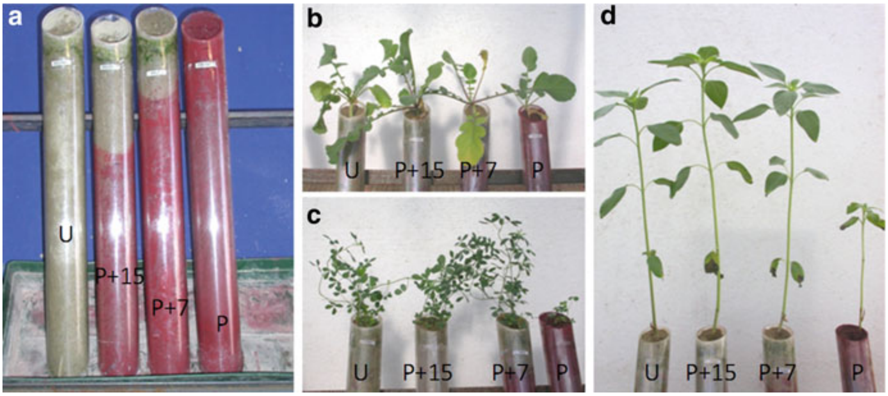

Fig. 8.4 Aspect of pyrite (**a**) and crop species (**b**, **c**, **d**) after 60 days of cultivation in rhizoboxes under various treatments. U: uncontaminated reference silty-loam soil; P+15: pyrite cinders capped with 15 cm of unpolluted soil; P+7: pyrite cinders capped with 7 cm of unpolluted soil; P: pyrite cinders

initially. In this way, in the sowing bed (top 0.15-m layer) pollution was roughly halved by ploughing and, despite some upward cinder movement, ripping led to lower contamination than ploughing (total and DTPA-extractable: ~−30 %) (Table 8.1).

Given the low fertility of pyrite (Marchiol et al. 2007, personal comunication in 2005), before sowing 100 kg ha^{-1} of each N, P_2O_5 and K_2O as chemical fertilisers were incorporated into the soil by harrowing. Four crop species, i.e., sunflower, Italian ryegrass (*Lolium multiflorum* Lam.), alfalfa and fodder radish (Vamerali et al. 2011b), and four woody species, i.e., white poplar (*Populus alba* L.), black poplar (*P. nigra* L.), European aspen (*P. tremula* L.) and white willow (*Salix alba* L.) (Vamerali et al. 2009), were grown under the two soil tillages and compared with the ploughed uncontaminated soil reference of the experimental farm of the University at Legnaro (45°21′N, 11°58′E, 12 m a.s.l.). Sowing of crops and transplanting of 2-year-old bare rooted cuttings of woody species took place in May, and shoot (biomass) and root investigations (RLD, volumetric root length density, by auger sampling) at the end of July and in mid-September, in both groups of species respectively.

Pyrite waste was an inhospitable substrate for all plants, as also reported by Fellet et al. (2007) and Marchiol et al. (2007), at the same site for other species. The anomalous physical properties (high bulk density and low water infiltration), together with high Fe and S, and multiple contamination of pyrite, greatly limited plant growth, almost regardless of tillage system. Only fodder radish profited by the lower contamination of ripping (Fig. 8.5). Improvements in the habitat should involve soil drainage and adequate irrigation, as we accomplished by digging shallow drains and setting up a low-intensity sprinkling system in summer.

The lower contamination due to ripping seemed to be less favourable for metal concentration in plants but more useful for growth, especially in fodder radish.

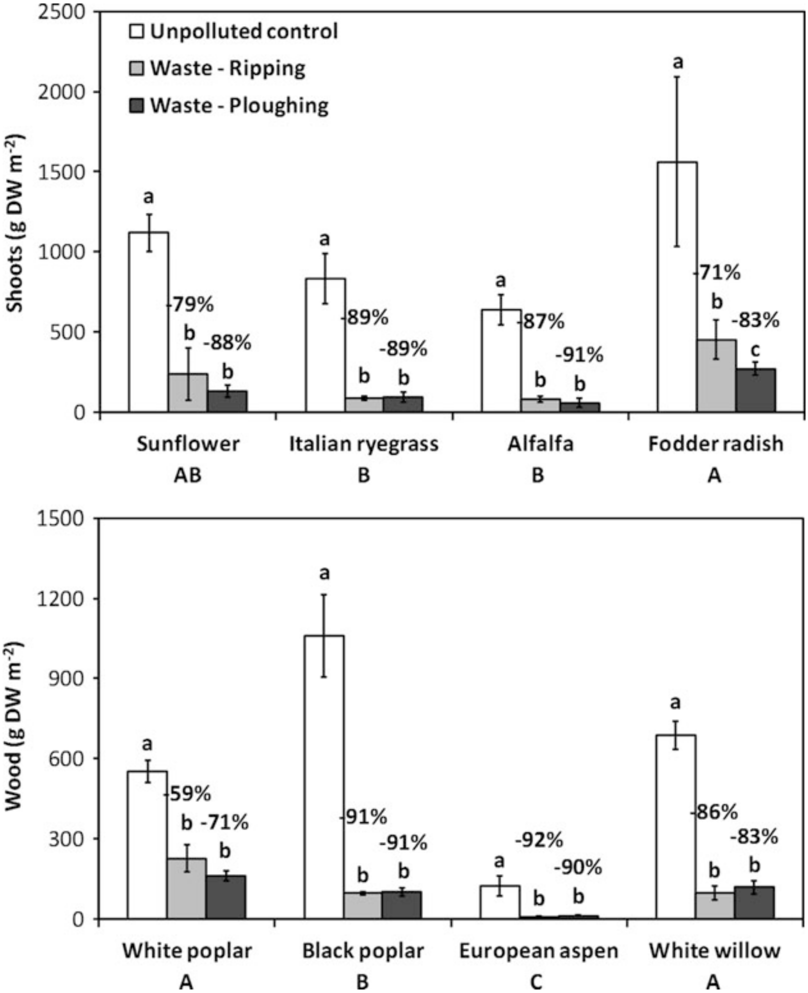

Fig. 8.5 Above-ground biomass (± S.E., $n = 3$) of crops at flowering (*above*) and trees (without leaves) 6 months after transplant (*below*) in pyrite waste under two tillages, in comparison with uncontaminated ploughed controls. Percentage of reduction for ploughing and ripping against control shown above bars. *Small letters*: statistically significant differences between treatments within same species (Newman-Keuls test, $P \leq 0.05$). *Capital letters*: statistically significant differences between species for pyrite only (main effect)

Overall, tillage choice was not critical in terms of mass balance of phytoextraction (Table 8.2), although we do recommend ripping to guarantee a better canopy cover against pollutant dispersion and for easier mechanical management of biomasses, e.g., cutting and harvesting operations. Among crops, fodder radish and sunflower were the highest biomass-yielding species, the former reaching the greatest but still poor metal removals (330 g ha^{-1} of metals). Fodder radish belongs to the

Table 8.2 Metal concentrations ($n = 3$) and removals in crop shoots and tree twigs of species under two tillage systems (Newman-Keuls test, $P \leq 0.05$)

		As		Cd		Co		Cu		Pb		Zn		9 HM[(a)]		Removals[(b)]	
Treatment	Species	(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mmol kg^{-1})		(9 HM)	
Crops																	
Ploughing	Sunflower	0.15	c	0.42	a	0.19	c	14	b	0.48	c	89	a	1.9	a	175	a
	Italian ryegrass	3.9	b	0.42	a	0.41	ab	30	a	1.7	a	160	a	3.4	a	243	a
	Alfa-alfa	6.9	a	0.45	a	0.52	a	30	a	2.2	a	119	a	2.7	a	93	a
	Fodder radish	3.3	b	0.55	a	0.29	bc	25	ab	1.1	b	75	a	1.8	a	327	a
	MEAN	3.6	A	0.46	A	0.35	A	25	A	1.4	A	111	A	2.5	A	210	A
Ripping	Sunflower	n.d.		0.15	b	0.05	a	11	a	0.13	b	34	b	0.94	b	156	a
	Italian ryegrass	5.4	a	0.58	a	0.58	a	37	a	2.3	a	126	a	3.0	a	180	a
	Alfa-alfa	4.2	a	0.33	ab	0.37	a	23	a	1.42	ab	64	b	1.7	ab	90	a
	Fodder radish	3	a	0.27	ab	0.21	a	18	a	0.58	ab	48	b	1.2	b	334	a
	MEAN	3.2	A	0.33	A	0.30	A	22	A	1.1	A	68	B	1.7	B	190	A
Trees																	
Ploughing	White poplar	–		0.41	a	0.07	a	4.9	bc	–		62	b	1.2	b	12	a
	Black poplar	–		1.2	a	0.02	a	4.0	c	–		77	b	1.4	b	9.1	a
	European aspen	–		1.2	a	0.15	a	9.1	a	–		106	a	2.1	a	1.6	b
	White willow	–		0.52	a	0.09	a	7.6	ab	–		57	b	1.1	b	7.9	a
	MEAN			0.82	A	0.08	A	6.4	A			75	B	1.5	A	7.7	A
Ripping	White poplar	–		0.51	b	0.09	a	4.4	b	–		65	b	1.3	b	18	a
	Black poplar	–		1.6	a	0.09	a	4.3	b	–		103	a	2.0	a	12	ab
	European aspen	–		1.1	b	0.21	a	6.6	a	–		116	a	2.2	a	1.3	b
	White willow			0.77	b	0.14	a	7.5	a	–		94	ab	1.8	ab	11	ab
	MEAN			1.0	A	0.13	A	5.7	A			94	A	1.8	A	10	A

Small letters: differences among species within same tillage. Capital letters: differences between tillages (main effect) within each metal

[(a)] As + Cd + Co + Cr + Cu + Mn + Ni + Pb + Zn

[(b)] g ha^{-1} for crops and mg per plant for trees

Brassicaceae, a botanical family which should be exploited in phytoremediation as it also includes several hyperaccumulators (Krämer 2010). The biomass reached by radish in our waste was obviously poor when compared with the uncontaminated reference. Its productivity was similar to that of the small-biomass yielding hyperaccumulator *T. caerulescens* (Anderson et al. 1999), but it is expected to have greater efficiency in pluri-contaminated sites. Indeed, fodder radish has been used to good effect in our past experiments in a pluri-contaminated agricultural soil near Milan, showing better shoot and root growth than controls and better phytoextraction than other *Brassicaceae*, such as Indian mustard, oilseed rape (*B. napus* L. var. oleifera D.C.) and garden cress (*Lepidium sativum* L.) (Mosca et al. 2004). The behaviour of fodder radish below-ground was favourable; its RLD in the 0.3-m layer being similar to that of controls. There was no significant 'species × tillage' interaction and, unfortunately, much of the root length was confined to the top 0.1-m; root distribution was similar between tillages in sunflower and alfalfa, whereas fodder radish and Italian ryegrass positively moved a greater fraction of length (~15 %) downwards (below 0.1 m depth) with ripping.

At this point, the question which arose was: which are the criteria allowing selection of efficient herbaceous species in pyrite? A partial answer comes from the morphological features of roots. For instance, better translocation of metals from roots to shoots were related to high specific root length ($R^2 = 0.21$), whereas the maximum root length (Italian ryegrass), although correlated with shoot metal concentrations ($R^2 = 54$ %), is probably an invalid criterion when considered alone. The greater relative RLD and above-ground productivity with respect to the reference soil, as occurred in fodder radish, seem to provide better all-round criteria. The response of woody species was also variable in terms of growth and metal accumulation. Compared with controls, the wood production of white poplar was much less affected than that of black poplar (−65 % vs. −91 %), with small differences between tillages (Fig. 8.5). In general, As and Pb had not accumulated in twigs, Cu was low and Co was just above the detection limit (Table 8.2). Our tree species confirmed initial observations on spontaneous *Salicaceae*, only Zn having interesting levels, especially after ripping, i.e., 94 vs. 75 mg kg^{-1} of ploughing (means). We detected a negative correlation between biomass and concentrations of metals, so that white poplar yielded the best biomass and European aspen the highest metal concentrations. Also for trees, the expected removals were basically poor, although more precise phytoextraction balances depend on plant density of short-rotation coppices and rotation cycle.

When working on pyrite improvement, the first requirement is undoubtedly increased yields and, secondly, increased metal concentrations and translocation. Substantial productivity gains can be achieved with abundant fertilisation (Marchiol et al. 2007) and small plant sizes suggest that plant density could be increased, at least for wide-spaced crops like sunflower. Grasses could not tolerate frequent cutting in the contaminated area because of insufficient growth after the first harvest, as in Italian ryegrass and alfalfa. Undoubtedly, the latter was disadvantaged by the absence of root nodulation with *Rhizobium* and by particularly high shoot As, like *Salicaceae,* which lacked mycorrhization (data not shown).

Generally the roots of crops were less restricted in their growth than shoots, although roots had much higher metal concentrations, i.e., Cd 4-fold, Zn 7×, Co 28×, As 33×, Pb 51× and Cu 77×. They acted as a substantial accumulation sink for most trace elements, particularly Cu, Pb and As—a result also found in coarse and particularly fine roots of poplars and willow. Fine root biomass was quite modest, estimates from soil cores in crops not exceeding 20 g m^{-2}, and their contribution to long-term metal stabilisation is probably negligible because of fast turnover (Goins and Russelle 1996). For the coarse roots of trees and tap roots of annual species, degradation is probably slower, but this is an issue to be further investigated in phytostabilisation processes. From several aspects, root systems may hold the key to understanding the possibilities and options for phytomanagement of pyrite waste, although the maximum rooting depth (0.3 m) still remains to be greatly enhanced. In this context, the very high number of spontaneous species with shallow fasciculate roots is likely the result of severe selection of tap-rooted ones. Phytoextraction enhancement through increased metal concentrations in biomasses (e.g., by soil ploughing) turned out to reduce species differences, a strategy contrasting with many other agricultural practices which are instead addressed to yield improvements. The main information obtained from this trial was the need to reduce soil contamination through soil amendment and to facilitate plant establishment—for instance with the application of growth regulators.

8.5 Improving Pyrite Hospitality and Plant Metal Uptake

In order to improve the phytoextraction in pyrite, some pot trials with fodder radish were set up in 2006 and 2007, the aim being to improve the environment for roots and enhance above-ground productivity. In all experiments, plants were grown for 3 months in cylindrical 52-cm high pots (1.3 L volume), filled with a pyrite cinder–sand mixture and regularly watered with 50 % diluted Hoagland solution. Sand was added to attenuate contamination and improve water drainage, but leachates were collected in order to check whether our treatments had environmental counter-indications. Treatments were compared with untreated controls with five replicates.

We first thought of humic acid treatment for plants and pyrite directly. Humic acids (HA) are characterised by acidic groups which play an important role in enhancing the solubility, bioavailability, uptake and transport of metals (Evangelou et al. 2004), and are known for their auxin-like effect (Delfine et al. 2005). HA came from a commercial product (Humic super, Tiller—Italy) as liquid formulation (10 % DW of HA) and were applied as follows: foliar spraying (0.1 g HA L^{-1} solution, once a week for 3 weeks), two doses mixed with the substrate before sowing (0.1 and 1 g HA kg^{-1}) in combination or not with foliar treatment and a low rate (0.1 g HA kg^{-1}) applied at sowing through irrigation. The highest amendant dose of 1 g kg^{-1} positively increased shoot metal concentrations (overall elements: +44 %) but, unfortunately, curbed shoot growth and worsened metal removals probably as consequence of high metal availability in pyrite (Bandiera et al. 2009). Improved translocation of all metals was the only positive effect of high HA dosages. Actually, only at the small dose of 0.1 g HA kg^{-1} were there significant increases in root length (+46 %) and—although with only slight above-ground yield improvements—substantial enhancement of plant metal removals (+35 %). These results confirm the auxin-like properties of humic substances, and their effectiveness at low rates is a premise for low-cost large-scale applications.

More complicated was management of exogenous application of IBA, one of the most powerful root-enhancing phytohormones. Starting from about 1 month after sowing, we tentatively applied IBA five times (at 10-day intervals) to fodder radish leaves at 10 mg L^{-1} or to the waste at 0.1 and 1 mg kg^{-1}, in association or not with foliar spraying. We obtained negative responses from this trial as—with the exception of foliar spraying alone—the hormone reduced shoot and root biomass (−60 % on average) when applied to the waste, probably due to unsuitable dosages and long persistence caused by low microbial activity (Vamerali et al. 2011a). The expected phytoextraction balance was thus greatly worsened, in spite of improvements in concentrations due to the chelating effect of this phytohormone.

Lastly, verification of the applicability of chelant-assisted phytoextraction was tested on pyrite, which is an uncommon substrate for this technique. We wished to ascertain whether the recently available EDDS (ethylene diamine disuccinic acid), characterised by higher degradability compared with EDTA (ethylene diamine tetracetic acid), could improve metal uptake without causing substantial phytotoxicity and leaching. The tested plants were fodder radish and Ethiopian mustard treated with [S,S]-EDDS at various doses and application times: 2.5 and 5 mmol kg^{-1} substrate applied through irrigation 1 week before harvest (common application time of chelators) and 1 mmol kg^{-1} soil repeated five times at 5- or 10-day intervals, respectively starting 48 or 28 days after sowing. At these doses, the chelator did successfully improve Cu, Co, Zn and Pb above-ground concentrations (Table 8.3), together with Cu translocation, but reduced plant biomass, especially with repeated applications and in radish (Bandiera et al. 2010). This may have a direct effect on leaching, as the drop in transpiration caused by diminished leaf area leads to significant losses of Cu, the metal with the greatest stability constant with EDDS (Tandy et al. 2004). Better metal phytoextraction (+31 %) together with minimal metal leaching was achieved with moderate (2.5 mmol kg^{-1}), traditional close-to-harvest chelator applications, but in Ethiopian mustard only. Certainly, these results on the use of EDDS and its management require on-site confirmation, but the generally unfavourable phytoextraction balance, associated with the uncertain fate of metal-EDDS compounds after plant harvest, gives rise to doubts about its use.

8.6 Conclusions

Phytoremediation of pyrite waste is complicated to manage because of multiple constraining factors which affect plant growth, beyond metal contamination. Removal of the most labile fraction of metals with field crops seems to be a feasible

Table 8.3 Shoot metal concentrations (mg kg^{-1}, $n = 5$) and translocation factor (shoot-to-root metal concentration ratio %) in two species under different EDDS treatments in pyrite

	As		Cd		Co		Cu		Pb		Zn	
	mg kg^{-1}	TF	mg kg^{-1}	TF	mg kg^{-1}	TF	mg kg^{-1}	TF	mg kg^{-1}	TF	mg kg^{-1}	TF
Ethiopian mustard												
C	4.6 a	8.38	0.28 b	74.7	0.14 c	3.02	29 c	11.6	-	-	80 c	16.2
2.5	5.4 a	7.70	0.43 ab	68.6	0.23 bc	3.03	49 b	19.2	-	-	99 ab	18.0
5	**5.9** a	6.67	0.48 ab	70.2	0.31 b	3.90	62 b	20.4	**0.84** a	0.23	**114** a	19.1
1 × 5–5d	5.6 a	5.90	**0.62** a	84.7	**0.62** a	8.93	**100** a	21.0	0.51 ab	0.09	103 ab	16.9
1 × 5–10d	5.1 a	7.26	0.58 a	131	0.33 b	6.34	55 b	15.7	0.25 ab	0.02	96 bc	15.3
Fodder radish												
C	13 a	14.8	0.76 a	85.9	0.28 b	2.67	39 b	9.58	2.2 b	1.12	91 a	17.4
2.5	16 a	28.8	1.30 a	167	0.47 b	7.53	90 b	17.6	3.8 b	1.96	102 a	8.75
5	12 a	23.0	0.80 a	150	0.73 ab	12.9	94 b	24.8	**8.1** a	1.11	**112** a	19.7
1 × 5–5d	**20** a	24.8	**1.60** a	202	**1.30** a	22.7	**161** a	28.5	2.5 b	0.30	107 a	15.9
1 × 5–10d	15 a	19.2	0.77 a	99.6	0.79 ab	12.7	87 b	19.2	1.3 b	0.05	95 a	10.2

C: untreated controls; 2.5 and 5: 2.5 and 5 mmol EDDS kg^{-1} substrate applied 1 week before harvest; 1 × 5–5d and 1 × 5–10d: 1 mmol kg^{-1} substrate repeated five times at 5- or 10-day intervals. Letters: differences among treatments within same species (Newman-Keuls test, $P \leq 0.05$). Highlighted values (bold) are the highest concentrations for a particular treatment in each species phytomanagement option with some species and for some trace elements only, but is probably only effective over a long-term period. Among a narrow range of crops, we found the *Brassicacea* fodder radish showed substantial Zn and Cu removals, whereas management of the most toxic metals, such as As and Pb, still remains problematic. The much larger variability in shoot metal concentrations of the crops tested here compared with woody species suggests exploiting the potential of other herbaceous species, although we believe that more profitable progress could be achieved with an integrated approach involving genetics, biology, physiology and especially agronomy, to maximise plant adaptation and growth. In any case, identification of a pool of plants to be cultivated in association or in rotation is necessary, in order to cover the soil permanently and reduce possible damage by parasites. Assisted phytoextraction seems difficult to manage as regards timing and dosages of the compounds used and frequently reduce biomass yield and metal removal.

The phytomanagement of sites polluted by pyrite waste may simply involve the establishment of a vegetation cover with cultivated plants left to reproduce themselves or with biomass harvesting and annual sowing. However, besides phytoextraction, long-term stabilisation of metals in plant roots is an important issue to consider, in view of the high metal retention at root level, and recent evaluation at the University of Padova showed that 6 % of tap root biomass in rapeseed was recalcitrant to degradation after about 18 months from shoot harvest.

References

Anderson CWN, Brooks RR, Chiarucci A, LaCoste CJ, Leblanc M, Robinson BH, Simcock R, Stewart RB (1999) Phytomining for nickel, thallium and gold. J Geochem Explor 67:407–415

Bandiera M, Mosca G, Vamerali T (2009) Humic acids affect root characteristics of fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.) in metal-polluted wastes. Desalination 247:79–92

Bandiera M, Mosca G, Vamerali T (2010) Phytotoxicity and metal leaching in EDDS-assisted phytoextraction from pyrite wastes with Ethiopian mustard and fodder radish. Plant Biosyst 144:490–498

Brooks RR, Lee J, Reeves RD, Jaffre T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–57

Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309–315

Clemente R, Almela C, Pilar Bernal M (2006) A remediation strategy based on active phytoremediation followed by natural attenuation in a soil contaminated by pyrite waste. Environ Pollut 143:397–406

Coletto L, Vamerali T, Mosca G (2006) Characterisation of a site polluted by pyrite cinders and heavy metals for setting up profitable phytoremediation strategies. In: Fotyma M, Kaminska B (eds) Proceedings of the IX ESA congress, vol. 11/06 (2), Warsaw, Poland

Delfine S, Tognetti R, Desiderio E, Alvino A (2005) Effect of foliar application of N and humic acids on growth and yield of durum wheat. Agron Sustain Dev 25:183–191

Dickinson NM, Pulford ID (2005) Cadmium phytoextraction using short rotation coppice *Salix*: the evidence trail. Environ Int 31:609–613

Evangelou MWH, Dagan H, Schaeffer A (2004) The influence of humic acids on the phytoextraction of cadmium from soil. Chemosphere 57:207–213

Fellet G, Marchiol L, Perosa D, Zerbi G (2007) The application of phytoremediation technology in a soil contaminated by pyrite cinders. Ecol Eng 31:207–214

Goins GD, Russelle MP (1996) Fine root demography in alfalfa (*Medicago sativa* L). Plant Soil 185:281–291

Heim A, Luster J, Brunner I, Frey B, Frossard E (1999) Effects of aluminium treatment on Norway spruce roots, aluminium binding forms, element distribution, and release of organic acids. Plant Soil 216:103–116

Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534

Laureysens I, Blust R, De Temmerman L, Lemmens C, Ceulemans R (2004a) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. Environ Pollut 131:485–494

Laureysens I, Bogaert J, Blust R, Ceulemans R (2004b) Biomass production of 17 poplar clones in a short-rotation coppice culture on a waste disposal site and its relation to soil characteristics. For Ecol Manag 187:295–309

Liphadzi MS, Kirkham MB, Paulsen GM (2006) Auxin-enhanced root growth for phytoremediation of sewage-sludge amended soil. Environ Technol 276:695–704

Lopez ML, Peralta-Videa JR, Benitez T, Gardea-Torresdey JL (2005) Enhancement of lead uptake by alfalfa (*Medicago sativa*) using EDTA and a plant growth promoter. Chemosphere 61: 595–598

Marchiol L, Fellet G, Perosa D, Zerbi G (2007) Removal of trace metals by *Sorghum bicolor* and *Helianthus annuus* in a site polluted by industrial wastes: a field experience. Plant Physiol Biochem 45:379–387

Marmiroli M, Antonioli G, Maestri E, Marmiroli N (2005) Evidence of the involvement of plant ligno-cellulosic structure in the sequestration of Pb: an X-ray spectroscopy-based analysis. Environ Pollut 134:217–227

Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3:153–162

Mosca G, Vamerali T, Ganis A, Coletto L, Bona S (2004) Miglioramento dell'efficienza agronomica della fitodecontaminazione di metalli pesanti. In: Zerbi G, Marchiol L (eds) Fitoestrazione di metalli pesanti–Contenimento del rischio ambientale e relazioni suolo-microrganismi-pianta. Forum Editrice, Udine (In Italian)

Pietrini F, Zacchini M, Iori V, Pietrosanti L, Bianconi D, Massacci A (2010) Screening of poplar clones for cadmium phytoremediation using photosynthesis, biomass and cadmium content analyses. Int J Phytoremediation 12:105–120

Pignatti S, Mengarda F (1962) Un nuovo procedimento per l'elaborazione delle tabelle fitosociologiche. Rendiconti della classe di Scienze fisiche, matematiche, naturali, s. 8, 32:215–222 (In Italian)

Pulford ID, Dickinson NM (2005) Phytoremediation technologies using trees. In: Prassad MNV, Naidu R (eds) Trace elements in the environment. Taylor and Francis, CRC, New York

Pulford ID, Watson C (2003) Phytoremediation of heavy metal contaminated land by trees – a review. Environ Int 29:529–540

Quartacci MF, Irtelli B, Baker AJM, Navari-Izzo F (2007) The use of NTA and EDDS for enhanced phytoextraction of metals from a multiply contaminated soil by *Brassica carinata*. Chemosphere 68:1920–1928

Rascio N, Dalla Vecchia F, La Rocca N, Barbato R, Pagliano C, Raviolo M, Gonnelli C, Gabbrielli R (2008) Metal accumulation and damage in rice (cv. *Vialone nano*) seedlings exposed to cadmium. Environ Exp Bot 62:267–278

Raskin I, Nanda-Kumar PBA, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290

Robinson B, Schulin R, Nowack B, Roulier S, Menon M, Clothier B, Green S, Mills T (2006) Phytoremediation for the management of metal flux in contaminated sites. For Snow Landsc Res 80:221–234

Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal-contaminated soil. Plant Soil 256:265–272

Saison C, Schwartz C, Morel JL (2004) Hyperaccumulation of metals by *Thlaspi caerulescens* as affected by root development and Cd-Zn/Ca-Mg interactions. Int J Phytoremediation 6:49–61

Salt DE, Blaylock M, Nanda-Kumar PBA, Dushenkov V, Ensly BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic elements from the environment using plants. Biotechnology 13:468–475

Sheldon AR, Menzies NW (2005) The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. Plant Soil 278: 341–349

Shen H, Christie P, Li XL (2006) Uptake of zinc, cadmium and phosphorus by arbuscular mycorrhizal maize (*Zea mays* L.) from a low available phosphorus calcareous soil spiked with zinc and cadmium. Environ Geochem Health 28:111–119

Tandy S, Bossart K, Mueller R, Ritschel J, Hauser L, Schulin R, Nowack B (2004) Extraction of heavy metals from soils using biodegradable chelating agents. Environ Sci Technol 38: 937–944

Ubi W, Osodeke VE (2007) Toxicity of aluminium to pineapple (*Ananas comosus*) grown on acid sands of Cross River State, Nigeria. Global J Environ Sci 6:15–20

Vamerali T, Bandiera M, Coletto L, Zanetti F, Dickinson NM, Mosca G (2009) Phytoremediation trials on metal- and arsenic-contaminated pyrite wastes (Torviscosa, Italy). Environ Pollut 157: 887–894

Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8:1–17

Vamerali T, Bandiera M, Hartley W, Mosca G (2011a) Assisted phytoremediation of mixed metal (loid)-polluted pyrite waste: effects of foliar and substrate IBA application on fodder radish. Chemosphere 84:213–219

Vamerali T, Bandiera M, Mosca G (2011b) *In situ* phytoremediation of arsenic- and metal-polluted pyrite waste with field crops: effects of soil management. Chemosphere 83: 1241–1248

Vidal M, López-Sánchez JF, Sastre J, Jiménez G, Dagnac T, Rubio R, Rauret G (1999) Prediction of the impact of the Aznalcóllar toxic spill on the trace element contamination of agricultural soils. Sci Tot Environ 242:131–148

Wang FY, Lin XG, Yin R (2007) Effect of Arbuscular mycorrhizal fungal inoculation on heavy metal accumulation of maize grown in a naturally contaminated soil. Int J Phytoremediation 9:345–353

Wiegleb G, Felinks B (2001) Primary succession in post-mining landscapes of lower Lusatia – chance or necessity. Ecol Eng 17:199–217

Yang XE, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353

Yoon JK, Cao XD, Zhou QX, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368:456–464