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The application of phytoremediation technology in a soil contaminated by pyrite cinders

G. Fellet^{a,*}, L. Marchiol^a, D. Perosa^b, G. Zerbi^a

^a Department of Agriculture and Environmental Sciences, University of Udine, via delle Scienze 208, 33100 Udine, Italy

^b Caffaro srl, p.le Marinotti 1, 33050 Torviscosa, Italy

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ABSTRACT

A research project dealing with the phytoremediation of a soil polluted by pyrite cinders is currently running. The case study is represented by a polluted area located in an industrial site; since 2001, the site has been listed in the clean-up national priority list. Before the beginning of the *in situ* trials, two experiments in controlled conditions were carried out. The first pot experiments were performed growing *Glycine max*, *Sorghum bicolor*, *Zea mays* and *Helianthus annuus* on substrates polluted by different levels of pyrite cinders, containing As, Cd, Cu, Pb and Zn. In a second experiment, the possible influence of fertilization on the uptake of As and heavy metals by *S. bicolor* and *H. annuus* was observed.

The potential of phytoextraction of crops was evaluated considering the plant biomass, the concentration of As and heavy metals in the plant tissues and also the bioconcentration factor (BCF) and the translocation factor (TF).

In the most polluted substrate, *S. bicolor* appeared more efficient than *H. annuus* in the uptake of metals; the transport of the metals from roots towards stems and leaves was less efficient in *S. bicolor*.

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1. Introduction

The contamination of soils due to the presence of toxic metals can result in serious negative consequences, such as damage of ecosystems and of agricultural productivity, deterioration of food chain, contamination of water resources, economic damage and, finally, serious human and animal health problems (Raicevic et al., 2005). Therefore, the growing amounts of metals required in the world economy in terms of amount and extent of mining metals ores, of amount and diversity of finished products and by-products, and of amount and array of their disposal and containment methods cause new and increasing problems; this also because metals can affect environmental and human health in diverse settings—from the sites of mining to residential environments (Adriano et al., 2004).

Many approaches have been developed, assessed and performed to cope with the soil pollution. Current available soil clean-up technologies are often high-priced, energy consuming—therefore, concurring with the CO₂ emissions—and, most of the time, soil disturbing so that the soil itself can rarely be utilized after the treatment. For these reasons the importance of soft and low-cost technologies, such as those afforded in ecologically engineered systems, is rising (Mitsch and Jørgensen, 2003).

In these conditions, but overall for long-term projects, phytoremediation could be the cheaper and simplest option among the available soil clean-up strategies (Susarla et al., 2002). USEPA (2000) defines phytoremediation as “the use of plants for containment, degradation or extraction of xenobiotics from water or soil substrates”. It includes different technical approaches: phytoextraction of heavy metals by

* Corresponding author. Fax: +39 043 2558603.

E-mail address: guido.fellet@uniud.it (G. Fellet).

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growing crops and harvesting the above-ground material is a promising technology to remediate lightly or moderately contaminated soils (Bouwman et al., 2005).

Phytoremediation has demonstrated its efficiency in constructed wetlands treating effluents polluted by organics and heavy metals (Cheng et al., 2002; Davies et al., 2005; Bragato et al., 2006). On the other hand, it is widely recognized as potentially applicable in the clean-up of soils contaminated by several pollutants, but at this stage additional information and experience are needed to consolidate phytoremediation into a decision matrix and a cost model; therefore, it cannot be considered a mature technology for full scale applications.

The term phytoextraction refers to the process of using metal-tolerant or hyperaccumulating plants to remove metals and metalloids from soils. The plants sequester the metals in their shoots, which can then be harvested (Salt et al., 1998; Whiting et al., 2004).

Baker and Brooks (1989) defined the following values of metal concentration for hyperaccumulators: 100 ppm for Cd, 1000 ppm for Ni, Cu, Co, Pb, and 10000 ppm for Zn and Mn. These levels have been defined arbitrarily; however, they are at a concentration of one order of magnitude greater than those found in non-accumulator species (Salt and Kramer, 2000). Hyperaccumulators are found in 45 different families; the habitus of these plants varied from small annual herbs to perennial shrubs and trees; in temperate zones they are present mainly as herbs. The most studied hyperaccumulator of Zn and Cd is *Thlaspi caerulescens* (McGrath et al., 2006).

For phytoextraction to work efficiently, plants need to grow vigorously and healthy, which is not so straightforward in polluted soils. Moreover, plants must have some other fundamental traits: (i) ability to accumulate the metal(s) intended to be extracted, preferably in the aboveground parts; (ii) high tolerance to the metal(s); (iii) fast growth and high biomass yield; (iv) cultivation as a crop and easily harvestable (Chaney et al., 2000). Unfortunately, a plant that combines all these features does not exist. Hyperaccumulators have an extraordinary efficiency in metal uptake and storage in their tissues, but the growth rate and the biomass yield of most of them are not satisfying in the perspective of full scale application. The use of high-yielding crop plants can overcome the low biomass of the hyperaccumulators, but they are less effective in the accumulation of metals.

Several groups are making many efforts in the field of biotechnologies (Eapen and D'Souza, 2005; LeDuc et al., 2006); in any case, the plants will have to be grown for a number of cycles on the polluted soil and, therefore, be managed in order to maximize both the biomass yield and the accumulation of the heavy metals. For these reasons the agronomic management of plants is an element of fundamental importance in the process of phytoremediation that needs investigation (McGrath et al., 2006).

For over a century the worldwide production of sulphuric acid used polymetallic pyrites galena (lead sulphide, PbS), pyrite (iron sulphide, FeS), chalcocite (copper sulphide Cu₂S) and sphalerite (zinc sulphide, ZnS) as a source of sulphur. The element was recovered by an industrial process of roasting; large amounts of cinders have been stockpiled or buried

without any system of containment. These wastes are of great environmental concern because of their content of As and several heavy metals that are very mobile and can be leached in the groundwater in acidic conditions (Ross, 1994).

A project financed by the Italian Ministry of Research and dealing with the *in situ* phytoextraction of heavy metals in soil polluted by pyrite cinders is currently running at Torviscosa (Udine, Italy).

Before starting the field trial we performed some pot experiments in controlled conditions in order to test the adaptation of crops that we had assumed to grow at the polluted site. This paper aims to report the results of these experiments performed using the polluted substrate collected at the experimental site of Torviscosa. As the substrate was contaminated by several heavy metals, it was essential to first test the plants *ex situ* to observe their behaviour when in contact with the pollutants. Besides, it is known that different species have different uptake vocation for different heavy metals. For these reasons, it is of great importance and interest in this scenario, to preliminarily investigate the species before proceeding with the field trials. The lab trials we performed also gave us insight on the crops' responses to the fertilization as an agronomical practice to be applied on assisted *in situ* phytoextraction experiments.

2. Materials and methods

The case-study is located in Torviscosa (45°49'N, 13°16'E, and 14 m altitude), Italy. The chemical plant of Torviscosa was established by SNIA in 1938. Initially, the main activity was the production of cellulose as primary component for synthetic fibers. During the following decades, the plants had been revamped in order to produce primary base and fine chemicals. The decree 468/2001 included this place and the surroundings in the national priority list of polluted sites. The site has been investigated in order to design a clean-up strategy for the whole area and to carry out emergency safety measures.

This is the case of the experimental site of Torviscosa: the soil collected in the profile 0–30 is polluted by As, Cd, Co, Pb and Zn (Marchiol and Fellet, 2004).

2.1. Soil characterization

Before starting the open field trials, some experiments were carried out in controlled conditions to observe the behaviour of crops grown on different concentrations of pyrite cinders and with different levels of mineral fertilization. In the spring of 2004, topsoil and pyrite cinders were collected from the experimental site of Torviscosa and analyzed.

The topsoil and the pyrite cinders collected at the experimental site were used to prepare different substrates for the experiments. Two topsoil–pyrite cinders mixtures were prepared and used to fill the pots. Topsoil and cinders were mixed respectively in 1:1 (v/v) ratio (P50%) and in 1:1.5 ratio (P66%). Physical and chemical characteristics of the topsoil and the mixtures are reported in Table 1. The contents of heavy metals and As of the pure pyrite cinders, the topsoil and the mixtures are reported in Table 2.

Table 1 – Basic soil properties of the topsoil collected at the experimental site and of the substrates P50% and P66% used in the pot experiments

Parameter	Topsoil	P50%	P66%
Sand 2–0.2 mm (% w/w)	56	36	27
Sand 0.2–0.02 mm (% w/w)	21	37	41
Silt (% w/w)	0	5	12
Clay (% w/w)	23	22	20
pH (in water)	8.06	7.14	7.22
Electrical Conductivity (mS cm ⁻¹)	0.31	1.93	1.88
CEC (cmol kg ⁻¹)	5.82	3.32	2.33
Organic C (%)	1.09	0.68	0.58
Active CaCO ₃ (%)	4.22	1.74	0.99
Total N (%)	0.73	0.58	0.42
Available P (mg kg ⁻¹)	11.8	53.5	62.7
Exchangeable K (mg kg ⁻¹)	17.3	17.2	10.6
Total S (mg kg ⁻¹)	1.45	2.77	3.5
Exchangeable Ca (mg kg ⁻¹)	459	742	1314
Exchangeable Mg (mg kg ⁻¹)	20	23	28
Exchangeable Fe (mg kg ⁻¹)	13.1	19	20.3
Assimilable Mn (mg kg ⁻¹)	3.37	3.04	4.6
Soluble B (mg kg ⁻¹)	0.65	0.05	0.05

2.2. Plant growth experiments

Two plant growth experiments were carried out. The first experiment aimed to examine the response of four crops to the experimental substrates. Seeds of plants of *Glycine max* cv. Sapporo, *Sorghum bicolor* cv. Isadei, *Z. mays* cv. PR34F02 and *Helianthus annuus* cv. 289 × 978 were sown in 2 L pots containing topsoil as control and P50% and P66% mixtures as treatments. Plants were grown during 40 days on a laboratory bench lit by lamps which gave 500 μmol m⁻² s⁻¹ of photosynthetically active radiation (PAR) to the plant top with a 12 h:12 h photoperiod; the pots were rotated randomly and daily to equalize their light exposure. Ambient temperature was maintained at 25 ± 2 °C. Each pot was irrigated every 2 days with distilled water.

In the same growth conditions, a second experiment having a longer duration (50 days) was designed to identify the possible relationship between the nutritional state of plants and the phytoextraction of heavy metals and As.

Seeds of *S. bicolor* cv. Isadei and *H. annuus* cv. 289 × 978 were sown in 2 L pots containing the P66% mixture. The same species have been used by Madejón et al. (2003) and Murillo et al. (1999), in a soil polluted by pyrite cinders.

Two levels of fertilization were set: the controls did not receive any fertilization (No Fert), while the other plants were fertilized (+Fert). In particular, *S. bicolor* received 0.26 g of ammonium nitrate (equivalent to 125 kg ha⁻¹ N), 0.14 g of calcium phosphate (equivalent to 40 kg ha⁻¹ P₂O₅) and 0.11 g of potassium chloride (equivalent to 100 kg ha⁻¹ K₂O); *H. annuus* received 0.31 g NH₄NO₃ (equivalent to 150 kg ha⁻¹ N), 0.21 g CaHPO₄ (equivalent to 60 kg ha⁻¹ P₂O₅) and 0.33 g potassium chloride (equivalent to 290 kg ha⁻¹ K₂O).

At the end of both experiments, the plants were carefully harvested, washed with deionised water, divided into roots, shoots and leaves and oven dried at 105 °C for 24 h.

2.3. Determination of heavy metal content

Samples from each substrate were collected, air-dried, screened by means of a 2 mm sieve and finally oven dried (105 °C for 24 h). The samples were acid digested with a microwave oven (CEM, MARSXpress) according to the EPA method 3051 (USEPA, 1995a,b); after the mineralization the samples were filtered (0.45 μm PTFE) and diluted. Total content of As, Cd, Cr, Cu, Ni, Pb and Zn in topsoil and in the pyrite cinders were determined by an ICP-OES (Varian Inc., Vista MPX). The analysis for As were done separately with a continuous flow vapour generation system that provided improved detection limits for the element (VGA-77, Varian Inc.).

The plant samples were acid treated following the EPA method 3052 (USEPA, 1995a,b) and finally analyzed by an ICP-OES (Varian Inc., Vista MPX).

2.4. Experimental design and data analysis

Both experiments were arranged in a randomized block design. In order to evaluate the phytoextraction potential of the crops, the following parameters were calculated: (i) the plant average concentration of the plants fractions (mg kg⁻¹), (ii) the bioconcentration factor (BCF = C_{roots}/C_{soil}) and (iii) the translocation factor (TF = C_{shoots}/C_{roots}) where C represents the metal concentration.

The experimental data were subjected to a two-way analysis of variance (ANOVA); the comparisons between treatments used the Student–Newmann–Keuls's test ($p < 0.05$); each treatment consisted of eight replicates.

Table 2 – Total content of As and heavy metals in pyrite cinders, in the topsoil collected at the experimental site, in the substrated P50% and P66% utilized in the pot experiments and as a reference the thresholds fixed by DM 152/06 for residential and industrial sites

Element	Pyrite cinders	Topsoil	P50%	P66%	DM 152/06	
					(A)	(B)
As (mg kg ⁻¹)	964	19.9	586	718	20	50
Cd (mg kg ⁻¹)	9.84	0.83	6.43	7.53	2	15
Cu (mg kg ⁻¹)	3290	72.8	1589	1943	120	600
Pb (mg kg ⁻¹)	278	22.4	203	244	100	1000
Zn (mg kg ⁻¹)	1448	96.5	989	1322	150	1500

(A) residential sites; (B) industrial sites.

As far as the statistical analysis of the ICP data is concerned, for those samples where the element was not detected, its concentration was assumed to be one-half of the respective detection limit (Nadal et al., 2004). Detection limits were: $3 \mu\text{g L}^{-1}$ for As, $1.5 \mu\text{g L}^{-1}$ for Pb, $0.9 \mu\text{g L}^{-1}$ for Cu, $0.4 \mu\text{g L}^{-1}$ for Co and $0.2 \mu\text{g L}^{-1}$ for Cd and Zn.

3. Results

3.1. Characterization of experimental substrates

The results of the chemical–physical analysis of the topsoil collected at the experimental field site and the substrates used in the experiments are reported in Table 1.

All the substrates were very poor in organic C and the nutrient content of topsoil was rather low. However, the addition of pyrite cinders reduced to almost half the content of the organic C found in the topsoil; the substrates were low in CEC, total N and exchangeable K. Contents of exchangeable Ca, Fe and Mg increased in the mixtures due to the fraction of pyrite cinders.

Considering the Italian regulation, the decree 152/2006 fixes the thresholds for the heavy metals content and for a number of organic and inorganic contaminants in the soils of residential and industrial sites. When the concentrations of pollutants measured in a soil are above the thresholds, the soil is classified as polluted and the most appropriate clean-up method should be applied.

The concentrations of heavy metals measured in pure cinders were very high (Table 2); the mixtures resulted multicontaminated by heavy metals and As, as expected. In particular, taking as reference the thresholds of decree 152/2006, the concentration of As and Cu are higher than the limits for the industrial areas, while the values of Cd, Pb and Zn exceed the limits for residential areas but not the thresholds set for industrial soils.

3.2. Plant growth

In both experiments, there were no evidences of metal toxicity on the plants tissues; all the species tolerated the highest dose of pyrite cinders.

The total biomass of *G. max*, *S. bicolor*, *Z. mays*, and *H. annuus* measured at the end of the first experiment is showed in Fig. 1. As expected, ANOVA indicated a significant effect of the factor “species” ($p < 0.001$), but this was only due to the different ecological habitus of the plants. On the contrary, the factor “treatment” was statistically significant at $p = 0.038$. The plants of *Z. mays* grown in the substrate containing the highest rate of pyrite cinders produced, surprisingly, more biomass than others. No differences were recorded for the other species observed in the experiment.

In the second experiment, *S. bicolor* and *H. annuus* were studied; Fig. 2 reports the dry weight of the plant biomass of both control and fertilized plants. Apart the differences between the species, the fertilization did not result in an increase of the total plant biomass; in fact the dry matter accumulation in the plant tissues was lower in fertilized plants than in non-fertilized ones: the difference was statistically not significant.

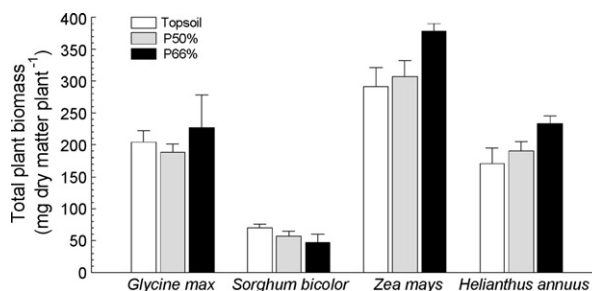


Fig. 1 – Total biomass (mg dry matter plant⁻¹) of *Glycine max*, *Sorghum bicolor*, *Zea mays* and *Helianthus annuus* grown on topsoil, P50%, and P66% substrates in experiment 1. Vertical bars represent the standard error for eight replicates.

3.3. As and heavy metals content in plants

The average concentration of As and the heavy metals found in the biomass of the plants observed in the experiment are the result of the process of element uptake and translocation (Fig. 3). Both experimental factors, “species” and “substrate”, affected significantly the plant growth for all the elements. It was observed that *G. max* and *S. bicolor* accumulated much more As and heavy metals than *Z. mays* and *H. annuus* (Fig. 3).

The concentration of As recorded in *G. max* increased about 1.7 folds, passing from P50% to P66%. On the contrary, *S. bicolor* seemed to exclude As when the concentration rose towards the highest level of soil contamination. In fact, the plants grown in P66% had an As concentration more than 4.8 times lower than P50% ones (Fig. 3). The plants of *Z. mays* and *H. annuus* did not respond to the treatment having more or less the same As content.

A similar behaviour can be extended to Cu and Pb, even if some differences in the absolute values of concentration of the elements have been recorded, in particular for *S. bicolor* P66%. The plants of *G. max* did not respond positively to the increase of Cd and Zn concentration in the substrate; perhaps the plants reached their tolerance thresholds, ceasing the metal uptake process (Fig. 3).

In Fig. 4, the concentration of As and heavy metals found in plants of *S. bicolor* and *H. annuus* at the end of experiment 2 are shown. Both experimental factors, species and fertilization,

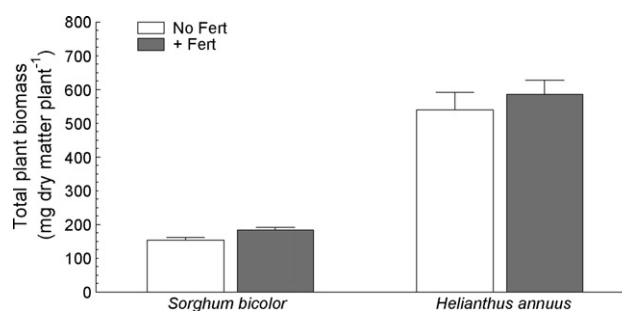


Fig. 2 – Total biomass (mg dry matter plant⁻¹) of *Sorghum bicolor* and *Helianthus annuus* grown on unfertilized and fertilized P66% substrate in experiment 2. Vertical bars represent the standard error for eight replicates.

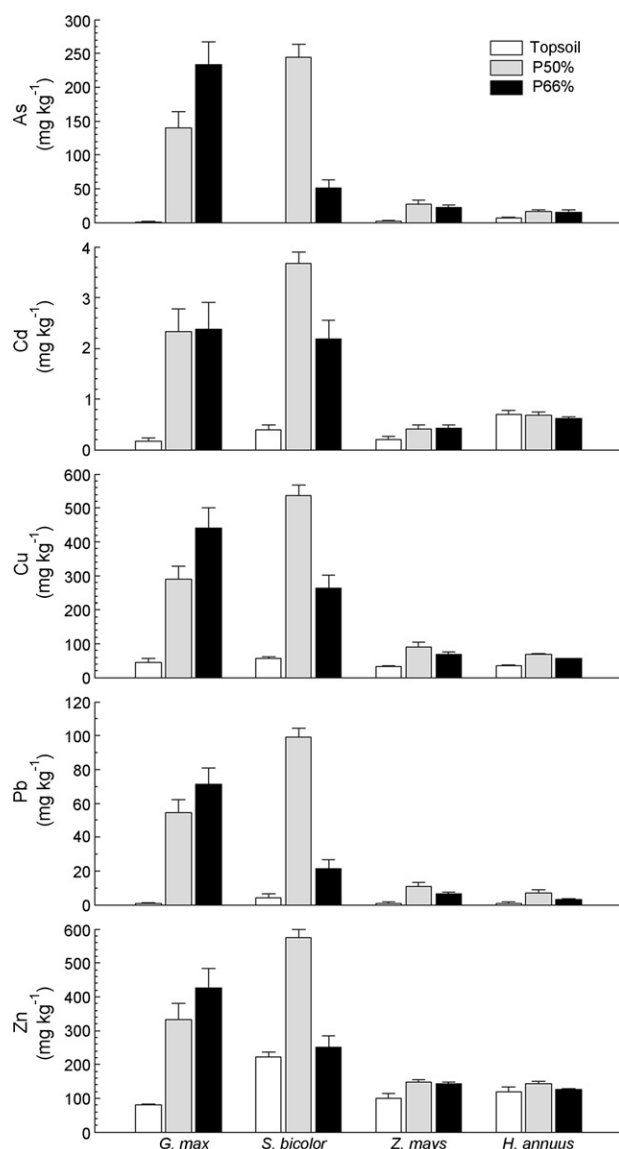


Fig. 3 – Average concentration (mg kg^{-1}) of As, Cd, Cu, Pb and Zn in plants of *Glycine max*, *Sorghum bicolor*, *Zea mays* and *Helianthus annuus* grown on topsoil, P50%, and P66% substrates in experiment 1. Vertical bars represent the standard error for eight replicates.

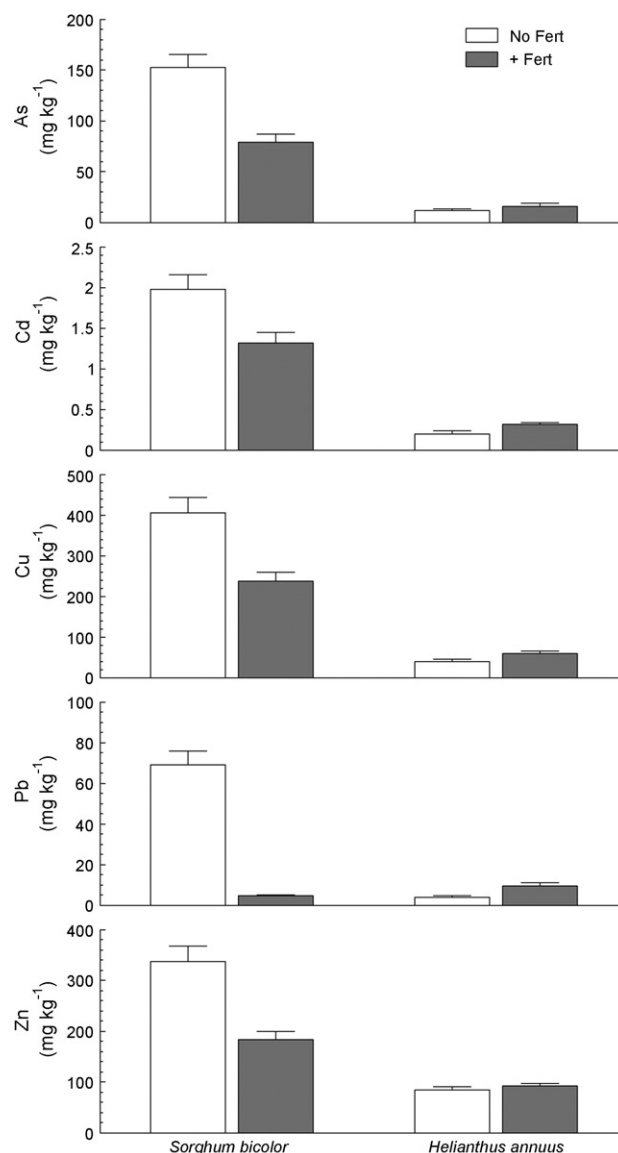


Fig. 4 – Average concentration (mg kg^{-1}) of As, Cd, Cu, Pb and Zn in plants of *Sorghum bicolor* and *Helianthus annuus* grown on unfertilized and fertilized P66% substrate in experiment 2. Vertical bars represent the standard error for eight replicates.

affected significantly the metal content in plants, while the interaction “species $\times$ fertilization” indicated that this species behaved differently in response to the treatment.

The amelioration of the substrate fertility did not result in an increase of the concentration of As and heavy metals in the plants of *S. bicolor* (Table 3); in particular the concentration of As, Cd, Cu and Zn in fertilized plants was respectively 2, 1.5, 1.7 and 1.8 times lower than the control plants.

On the contrary, the concentration of heavy metals in the plants of fertilized *H. annuus* was higher than unfertilized ones. The highest increase was recorded for Pb, which was 3 times higher than control; the concentration of the other elements was on average 1.3 times higher than controls.

3.4. Accumulation and translocation of As and heavy metals in plants

The mobility of the heavy metals from the polluted substrate into the roots of the plants and the ability to translocate the metals from roots to the harvestable aerial part were evaluated respectively by means of the bioconcentration factor (BCF) and the translocation factor (TF). BCF is defined as the ratio of metal concentration in the roots to that in soil; TF is the ratio of metal concentration in the shoots to the roots (Yoon et al., 2006).

Table 3 reports the BCF and TF calculated for the plants observed in experiment 1. The P50% substrate allowed plants to bioconcentrate much more As and heavy metals than P66%.

Table 3 – Results of the Student–Newman–Keuls test ($p = 0.05$) for the bioconcentration factor (BCF) and the translocation factor (TF) calculated for plants of *Glycine max*, *Sorghum bicolor*, *Zea mays* and *Helianthus annuus* grown during experiment I on the substrates P50%, and P66%. $BCF = C_{\text{roots}}/C_{\text{soil}}$; $TF = C_{\text{shoots}}/C_{\text{roots}}$, where C represents the metal concentration in roots, shoots and in the experimental substrate

Factors	BCF					TF				
	As	Cd	Cu	Pb	Zn	As	Cd	Cu	Pb	Zn
Treatment										
P50%	0.552 a	0.780 a	0.467 a	0.620 a	0.704 a	0.039 a	0.110 b	0.061 a	0.983 a	0.122 a
P66%	0.369 b	0.466 b	0.345 b	0.346 b	0.442 b	0.020 a	0.215 a	0.057 a	0.784 a	0.119 a
Species										
<i>G. max</i>	0.809 a	0.967 a	0.570 a	0.805 a	0.810 a	0.019 a	0.047 b	0.039 bc	0.378 b	0.044 c
<i>S. bicolor</i>	0.583 b	0.979 a	0.631 a	0.692 a	0.832 a	0.041 a	0.147 b	0.026 c	0.699 b	0.062 c
<i>Z. mays</i>	0.225 c	0.306 b	0.230 b	0.235 b	0.419 b	0.014 a	0.044 b	0.047 b	0.751 b	0.108 b
<i>H. annuus</i>	0.223 c	0.239 b	0.192 b	0.201 b	0.231 c	0.042 a	0.412 a	0.124 a	1.70 a	0.269 a
ANOVA (p values)										
Sp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.2353	0.000	0.0000	0.0010	0.0000
Tre.	0.0002	0.0004	0.0001	0.0000	0.0000	0.1185	0.0025	0.5235	0.3507	0.8062
Sp. $\times$ Tre.	0.0000	0.0294	0.0007	0.0000	0.0001	0.0361	0.1181	0.0000	0.7669	0.0000

Table 4 – Results of the Student–Newman–Keuls test ($p = 0.05$) for the bioconcentration factor (BCF) and the translocation factor (TF) calculated for unfertilized (No Fert) and fertilized (+Fert) plants of *Sorghum bicolor* and *Helianthus annuus* observed in experiment II. $BCF = C_{\text{roots}}/C_{\text{soil}}$; $TF = C_{\text{shoots}}/C_{\text{roots}}$, where C represents the metal concentration in roots, shoots and in the experimental substrate

Factors	BCF					TF				
	As	Cd	Cu	Pb	Zn	As	Cd	Cu	Pb	Zn
Treatment										
No Fert	0.336 a	0.395 a	0.318 a	0.408 a	0.369 a	0.023 a	0.104 a	0.075 a	0.034 b	0.312 a
+Fert	0.270 b	0.365 a	0.302 a	0.174 b	0.311 a	0.017 a	0.087 a	0.031 b	0.211 a	0.163 b
Species										
<i>S. bicolor</i>	0.438 a	0.577 a	0.467 a	0.367 a	0.514 a	0.023 a	0.056 b	0.01 b	0.208 a	0.049 b
<i>H. annuus</i>	0.168 b	0.183 b	0.153 b	0.214 a	0.165 b	0.017 a	0.135 a	0.096 a	0.037 b	0.426 a
ANOVA (p values)										
Sp.	0.0000	0.0000	0.0000	0.0003	0.0000	0.1930	0.0487	0.0000	0.0000	0.0000
Tre.	0.016	0.3312	0.5483	0.0000	0.0596	0.2059	0.6586	0.0172	0.0000	0.0340
Sp. $\times$ Tre.	0.0001	0.0036	0.0007	0.0000	0.0001	0.083	0.2622	0.0176	0.0000	0.0180

In fact, for each element the BCF of P50% plants was significantly higher ($p < 0.001$) than others (Table 3). The species behaved differently; *G. max* and *S. bicolor* had the highest BCF of As and Cd respectively, while the BCF of all the elements were < 0.5 for *Z. mays* and *H. annuus* (Table 3). These species, however, having the highest TF for all the metals, resulted as the most efficient in the translocation of such elements; apart from the case of Cd, this process did not appear influenced by the level of pollution of the substrate (Table 3).

Table 4 reports the BCF and TF calculated for the plants of the experiment 2. The BCF values are all < 1 , indicating that the element concentrations never exceed the respective soil level. However, some differences have been observed among the species and comparing the treated and untreated plants. The average value of BCF of *S. bicolor* is always and significantly higher than *H. annuus*. BFC calculated on our experimental data are coherent with those presented by Mattina et al. (2003).

Compared to the control plants, the BCF in treated plants of *S. bicolor* decreased a percentage comprised between 19 and 34%. Conversely, in *H. annuus* the BCF always responded positively to the fertilization, even at a lower absolute value (Table 4).

The TF values indicate the rate of translocation of the metal absorbed by the root system to the aboveground biomass. In general, TF did not follow the same trend observed for BCF. On average the TF is greater in *H. annuus* than *S. bicolor*; higher translocation was observed in *H. annuus* for Cd, Cu and Zn overall, while lower values have been calculated for *S. bicolor*. In the case of TF, the interaction between the experimental factors resulted significant, too. With regard to As and Cd, the fertilization slightly increased the translocation factor of *S. bicolor*. The same treatment caused the decrease of the translocation root–shoot of As (–76%), Cu (–56%) and Zn (–47%) in *H. annuus* (Table 4).

4. Discussion

This paper reported data about the adaptation of some crops which have been grown during a pot experiment on a multicontaminated soil. The basic soil properties and the total content of As and heavy metals have been reported. Surprisingly, pH data measured in all the substrates are above the neutrality; considering the chemical properties of the pyrite

cinders, this evidence indicates that these wastes lost most of their acidic potential. From an environmental point of view this means a lower mobility of the pollutants along the soil profile. On the other hand, this suggested that the bioavailable fraction of As and the metals could not be very high.

Relevant and interesting differences in the average concentration of metals founded in the tissues of the plants have been detected. In the perspective of phytoremediation, this is not “per se” very conclusive since it gave an indication about the capability of our plants to uptake the pollutants present in the soil.

For the future implications of our field experiment, the most important evidences were those regarding the BCF and TF; on the other hand these parameters are considered the basic test that can be used to evaluate the potential of plants for phytoremediation.

In general, the BCF's values <1 the crops are not as efficient as hyperaccumulators in the uptake of As and heavy metals. The BCF values ranged from 0.192 to 0.979; these values are coherent with those reported by Yoon et al. (2006) on native plants collected in pollutes areas.

We observed an interesting behaviour by *G. max*, in terms of both BCF and biomass growth. Considering the BCF it comes out that the species has a similar behaviour to *S. bicolor* with the exception for As. Taking into account the capacity of this plant to translocate the pollutants, together with its BCFs, it appears that soybean can preferentially uptake and accumulate Pb to the aboveground biomass rather than the other pollutants. Unfortunately no other similar experiments on soybean have been reported in literature; therefore, we can not estimate our data. Andrade et al. (2004) provided data about the amelioration of Pb uptake by *G. max* that was recorded after the establishment of a double symbiosis with an arbuscular mycorrhizal fungus and *Bradyrhizobium*. We did not used any inoculation, so we can suppose that in our conditions *G. max* could perform better.

Data from the second experiment showed that *S. bicolor* was more efficient than *H. annuus* in the translocation of As, Cd, Cu and Pb; Zn appeared to be more translocated towards the shoots in *H. annuus*.

Lasat (2000) and more recently Li et al. (2003) pointed out the role of soil fertility on the increase of metal phytoextraction; in our pot experiment we did not clearly verify the same result having had some unexpected result from fertilized plants. However, the early results of the field trial that is running at the experimental site of Torviscosa (Marchiol et al., 2007) strongly confirmed the data provided by Lasat (2000) and Li et al. (2003): fertilization, allowing plants to increase the biomass yield promote a more efficient removal of pollutants. The highest efficiency of this process can be reached by using a high biomass yielding plant having a bioconcentration factor >1.

Although phytoremediation is still a new technology, in the last few years a lot of basic research has been carried out in an attempt to understand how plants take up large quantities of metals, together with the mechanisms of metal translocation from roots to shoots, storage and detoxification. However, more applied projects in the field are needed to clarify the real potential of this technology, since the knowledge needed for proper cultivation of plants is still lacking.

One of the general principles of phytoremediation is to match the proper plant species to the contaminated site. This means that consideration must be given to soil condition, micro-climate, pests and diseases as well as the contaminant to be cleaned up (Licht and Isebrands, 2005). Agronomists will have to further provide solutions to applied aspects of crop management practices that have the potential to maximize the clean-up efficiency of remediative plants. Planting practices, seedbed conditions, irrigation, crop rotations, crop cycle duration, and, finally, methods and scheduling of the harvest should be investigated. To date, for all these aspects there is a lack of experience in field conditions particularly for multicontaminated soils. Several questions need appropriate clarification and research.

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