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The use of vetiver for remediation of heavy metal soil contamination

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Abstract The use of *Vetiveria zizanioides* (vetiver) was
studied to evaluate its efficiency for the remediation of soils
contaminated by heavy metals. Vetiver plants were tested
for Cr, Cu, Pb and Zn. Phytoextraction and bioremediation
experiments were carried out by irrigating the vetiver plants
and the dry plants with solutions containing suitable
amounts of Cr, Cu, Pd and Zn. The concentrations of the
heavy metals were determined in both experiments in shoot
and root parts of vetiver plants using inductively coupled
plasma atomic emission spectroscopy after a mineralization
step. Phytoextraction experiments showed a poor efficiency
of vetiver for Cr and Cu uptake (both less than 0.1% in
shoots and roots after 30 days), but a quite high capability
of Pb and Zn uptake (0.4% in shoots and 1% in roots for Pb
and 1% both in shoots and in roots for Zn, after 30 days).
For these reasons the vetiver plant can be considered a quite
good "hyperaccumulator" only for Pb and Zn. As for
bioremediation experiments, the vetiver plant showed
heavy metal uptake values significantly lower than those
obtained with other biological substrates.

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Introduction

Soils are generally contaminated with heavy metals as the
result of numerous anthropogenic activities, primarily
associated with industrial processes, manufacturing and
disposal of industrial and domestic refuse and waste
materials [1]. Soils and water contaminated with heavy
metals represent a serious environmental and human health
problem—owing to the possible transfer to the food chain—
that needs an effective technological solution. Most current
methods to remediate heavy metal contaminated soils
consist of labor-intensive and costly excavation, landfilling
and leaching technologies [2]. These methods are generally
very expensive and cannot always be applied.

Phytoremediation is considered an innovative, econom-
ical and environmentally compatible method for heavy
metal remediation [2, 3]. Phytoremediation is a general
term which refers to the use of plants for environmental
cleanup. It can be divided into (1) phytoextraction, in which
heavy metal accumulating plants are used to transfer and
concentrate metals from the soil into the roots and the
above shoots [4]; (2) rhizofiltration, in which plant roots
absorb and concentrate heavy metals from the soil [5]; (3)
phytostabilization, in which plants stabilize the pollutants in
soils, reducing their mobility into the soil and therefore the
risk of leaching into ground water. Phytoextraction also
includes bioremediation, in which plant roots together with
their rhizospheric microorganisms are used to remediate
contaminated soils with organics and the air-purifying uses
of some plants [6]. The phytoremediation technique has a
lot of advantages: it is a natural, in situ, low-cost and

nondestructive technique which totally preserves the natural state of the environment [7–12].

Vetiver grass (*Vetiveria zizanioides*) is a perennial grass with strong ecological adaptability and large biomass. It is easy to manage and it grows in different soil conditions. Vetiver, owing to these unique morphological and physiological characteristics, is commonly known for its effectiveness in erosion and sediment control [13] and for its tolerance to extreme soil conditions, including heavy metal contamination [14]. Because of these characteristics, vetiver grass has, in principle, great potential in phytoremediation of heavy metal contaminated soils [15, 16].

The present study investigates the efficiency of vetiver grass in phytoextraction of Cr, Cu, Pb and Zn. The aim is to establish whether this plant can be considered a good hyperaccumulator of the above mentioned heavy metals and whether it can be successfully applied to the remediation of heavy metal contaminated soils.

Experimental

This study was performed as a laboratory experiment. Four vetiver plants were used to investigate the uptake of Cr, Cu, Pb and Zn.

$CrCl_3$, $CuCl_2$, $PbCl_2$ and $ZnCl_2$ were obtained from Carlo Erba (Milan, Italy). A proper amount of each salt was dissolved in distilled water to obtain the following heavy metal solutions: 623 ppm Cr, 190 ppm Cu, 621 ppm Pb and 653 ppm Zn. These concentrations were choosen because they are reported in the literature as the lowest toxic levels in soils [13]. Higher concentrations of heavy metals could cause toxicity symptoms, including death of the vetiver plants during experiments.

All solutions were prepared with high-purity water produced by a Milli-Q system.

Phytoextraction experiments

Vetiver plants in pots were irrigated daily with 250 ml of the heavy metal solutions for 30 days. The shoot and root parts of the vetiver plants were harvested every day during the first week (short-term experiment) and every 5 days up to 30 days (long-term experiment). Shoot and root samples (1 g each) were carefully rinsed with distilled water and successively cut in small pieces and dried in an oven at 70 °C for 2 h to obtain a stable dry weight. All dried parts were dissolved in 8 ml distilled water and digested with 2 ml HNO_3 and 2 ml H_2O_2 using a microwave mineralizator (CEM-MARS 5). The digested liquid was filtered with a 0.5-μm nitrocellulose membrane filter and the filtrate was analyzed for heavy metals by inductively coupled plasma (ICP) spectrometry (Varian Vista-MPX CCD simultaneous

ICP optical emission spectrometer). For the analytical determinations of the heavy metals the following wavelengths were used: 267 nm for Cr, 213 nm for Cu, 220 nm for Pb and 260 nm for Zn. Reference solutions were prepared by diluting the 1,000 mg/l standard solutions with 2% HNO_3.

Heavy metal accumulation in each part of the plant was determined as the weight (milligrams) of heavy metal per kilogram of dry plant (parts per million).

Five samples of shoots and roots were prepared and analyzed.

Bioremediation experiments

Vetiver plants were cleaned with water, cut and separated into roots and shoots. These parts were immersed in 250 ml of heavy metal solutions for 30 days. Shoot and root samples (1 g each) were taken and analyzed every day during the first week (short-term experiment) and every 5 days up to 30 days (long-term experiment).

The samples were rinsed with distilled water, cut in small pieces, dried in an oven at 70 °C for 2 h and successively dissolved in 8 ml distilled water and digested with 2 ml HNO_3 and 2 ml H_2O_2 and analyzed for their heavy metal content by ICP spectrometry, as previously mentioned.

Five samples of shoots and roots were prepared and analyzed.

Blank experiments were carried out for both phytoextraction and bioremediation studies. They consisted of the analysis of shoot and root samples of a vetiver plant not treated with the metal solution. Each measurement was repeated five times.

Desorption experiments

After the test at 30 days for the phytoextraction and bioremediation experiments, vetiver plants and the vetiver samples were rinsed with distilled water daily for 9 days and analyzed daily for heavy metals by ICP spectrometry.

Cyclic voltammetry experiments

The solutions of Cr, Cu, Pb and Zn in three different solvents, distilled water with 0.1 M sodium perchlorate as supporting electrolyte, extract of shoots and extract of roots, were analyzed by cyclic volatmmetry using a computer-controlled potentiostat (Amel 433, Milan, Italy), a graphite electrode as the working electrode (3-mm diameter), a platinum disk and a Ag/AgCl/KCl(sat) as counter and reference electrodes, respectively. The electrochemical cell was an one-compartment cell with an internal volume of 10 ml. All potentials are referred to the Ag/AgCl/KCl(sat) electrode. The cyclic voltammetry was

Fig. 1 Metal uptake in vetiver shoots (*triangles*) and roots (*circles*) during the short-term phytoextraction experiments: Cr (**a**); Cu (**b**); Pb (**c**); and Zn (**d**). *Error bars* represent 95% confidence intervals (*n*=5)
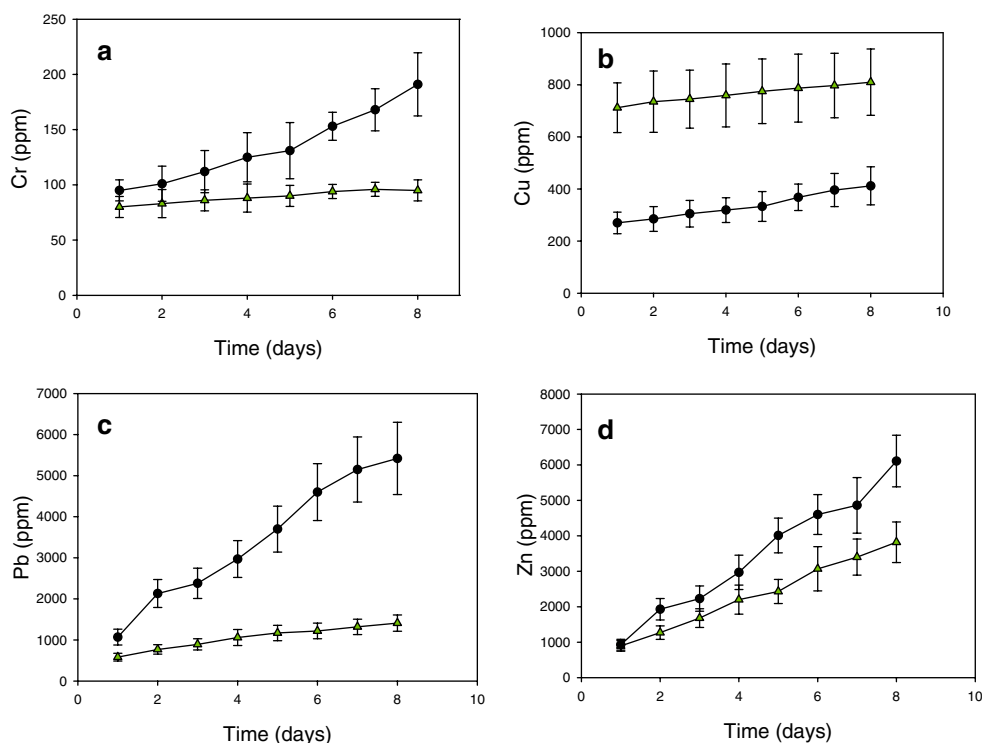
carried out by varying the potential between -0.5 and -1.5 V. The graphite surface was polished with 0.003 μm alumina powder, sonicated in water for 10 min and then washed with water. All measurements were carried out at 20.0±0.2 °C using a thermostatic bath. All solutions were carefully deaerated before analysis and maintained under a nitrogen atmosphere during the voltammetry experiments.

The extracts of shoots and roots were prepared as follows: 1 g of shoots or roots was carefully cut in small pieces, diluted to 250 ml with distilled water with 0.1 M sodium perchlorate and left at 4 °C for 10 days. The solutions were then filtered and used for the preparation of the heavy metal solutions.

Results and discussion

Phytoextraction experiments

Short-term experiments

Figure 1 shows the heavy metal uptake of vetiver plants both in shoots and roots when irrigated daily with solutions of 623 ppm Cr (Fig. 1a), 190 ppm Cu (Fig. 1b), 621 ppm Pb (Fig. 1c) and 653 ppm Zn (Fig. 1d). The results show that the vetiver plant can take up large amounts of Pb and Zn (Fig. 1c,d) but only very low amounts of Cr and Cu (Fig. 1a,b), both in the shoot and in the root parts of the

Fig. 2 Comparison between the heavy metal accumulation in shoots and roots in the short-term phytoextraction experiments
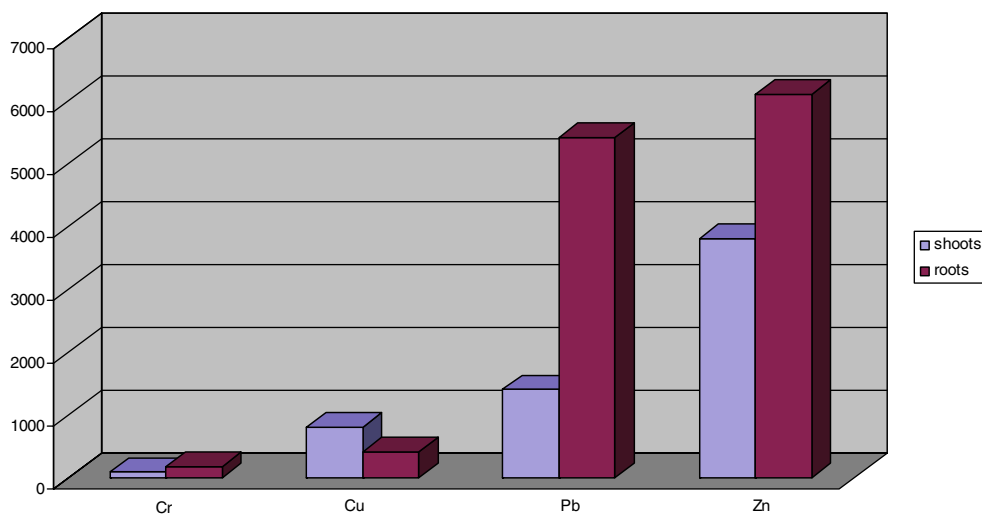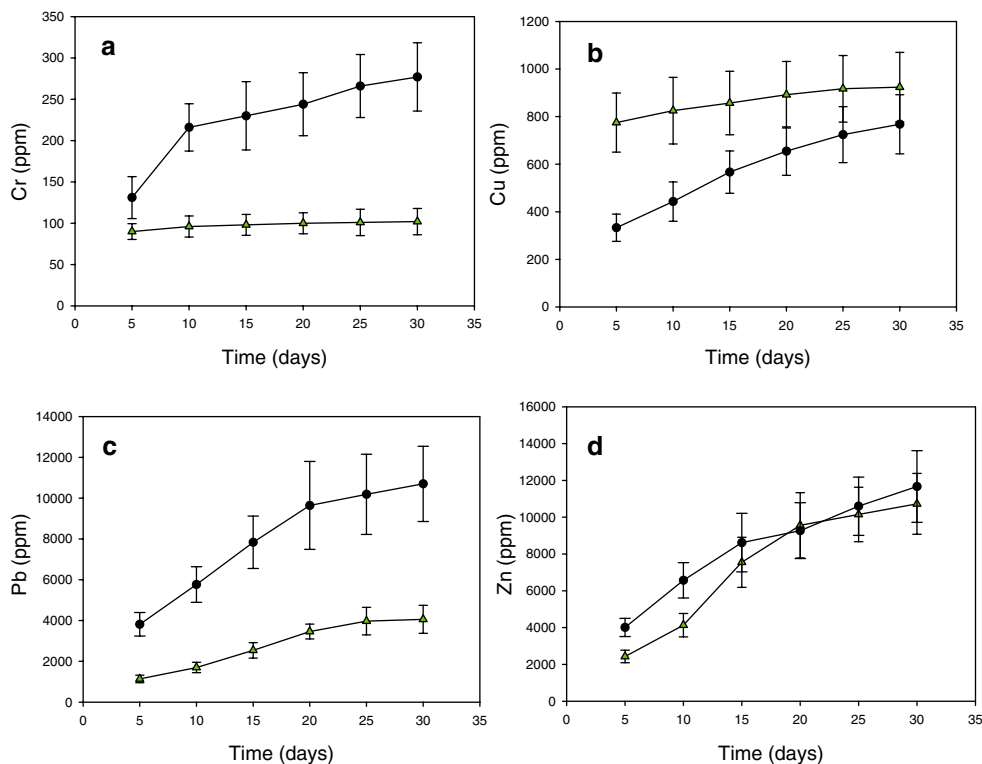
Fig. 3 Metal uptake in vetiver shoots (*triangles*) and roots (*circles*) during the long-term phytoextraction experiments: Cr (**a**); Cu (**b**); Pb (**c**); and Zn (**d**). *Error bars* represent 95% confidence intervals (*n*=5)

plant. The Pb uptake by shoots is higher than 0.1% (dry weight) from the fourth day and about 3 times (approximately 0.3%) higher by roots after the same time. The Zn uptake is even higher, about 0.38 and 0.61% after 8 days in shoots and roots, respectively. As the term "hyperaccumulator" is used for a plant capable of accumulating in the shoots concentrations of trace metals of at least 0.1% (dry weight) with the exception of Zn, for which the concentration must be about 1%, from the results obtained it is possible to consider the vetiver plant as a quite good hyperaccumulator only for Pb and Zn.

Figure 2 shows a comparison between the heavy metal uptake in shoots and roots. The results obtained clearly indicate that vetiver can take up heavy metals preferably in the roots, with the only exception being Cu.

Long-term experiments

Figure 3 show the heavy metal uptake of vetiver when irrigated daily with the same solutions used in the short-term experiments but harvested every 5 days for a period of 30 days.

Fig. 4 Comparison between the heavy metal accumulation in shoots and roots in the long term phytoextraction experiments
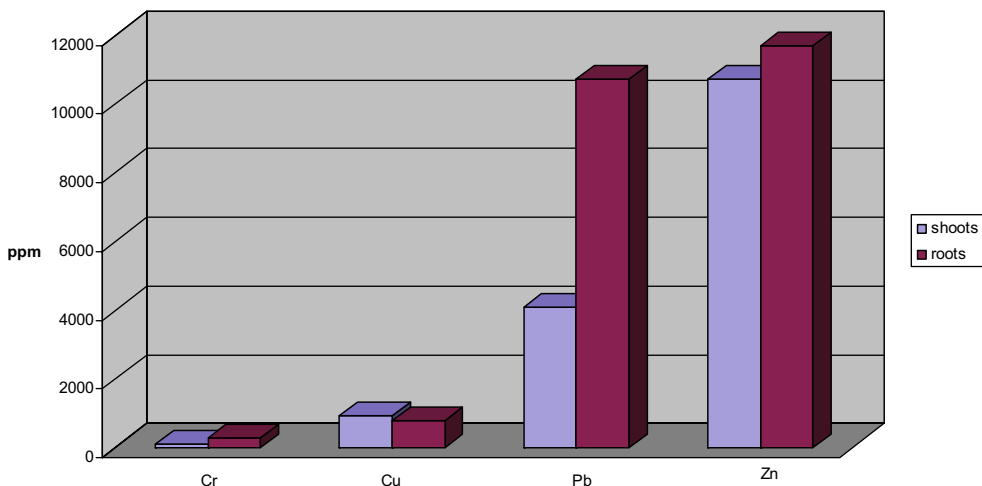
Table 1 Linear and logarithmic equations approximating the heavy metal content versus time plots in the short-term and long-term phytoextraction experiments

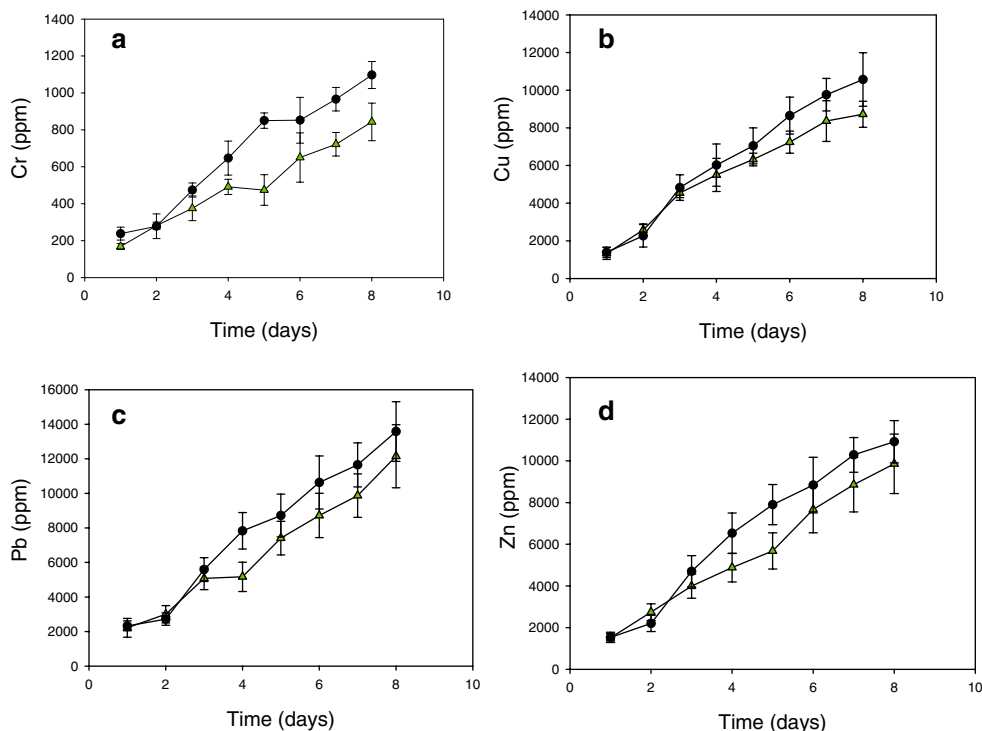|	Short-term experiment		Long-term experiment	
	Linear regression equation[a]		Logarithmic regression equation[b]	
	Shoots	Roots	Shoots	Roots
Chromium	$c = (2.3 \pm 0.1)t + (78.5 \pm 0.9)$ $R^2=0.9608$ $R_p^2 = 0.9065$ MEP=1.7	$c = (13 \pm 1)t + (74 \pm 5)$ $R^2=0.9665$ $R_p^2 = 0.9259$ MEP=8.6	$c = (6.6 \pm 0.4)\ln t + (80 \pm 1)$ $R^2=0.9860$ $R_p^2 = 0.9347$ MEP=1.0	$y = 77.16\ln x + 18.54$ $R^2=0.9572$ $R_p^2 = 0.8192$ MEP=20.3
Copper	$c = (13.5 \pm 0.5)t + (704 \pm 2)$ $R^2=0.9911$ $R_p^2 = 0.9791$ MEP=4.5	$c = (21 \pm 1)t + (242 \pm 6)$ $R^2=0.9814$ $R_p^2 = 0.9685$ MEP=0.6	$c = (87 \pm 4)\ln t + (630 \pm 1)$ $R^2=0.9892$ $R_p^2 = 0.9655$ MEP=9.8	$y = 251.61\ln x - 99.15$ $R^2=0.9812$ $R_p^2 = 0.9117$ MEP=45.6
Lead	$c = (537 \pm 39)t + (115 \pm 8)$ $R^2=0.9734$ $R_p^2 = 0.9449$ MEP=62.5	$c = (591 \pm 159)t + (630 \pm 31)$ $R^2=0.9851$ $R_p^2 = 0.9699$ MEP=252.5	$c = (1{,}790 \pm 210)\ln t - (2{,}033 \pm 582)$ $R^2=0.9479$ $R_p^2 = 0.7907$ MEP=508.9	$c = (4{,}083 \pm 282)\ln t - (3{,}059 \pm 783)$ $R^2=0.9812$ $R_p^2 = 0.9424$ MEP=598.5
Zinc	$c = (443 \pm 56)t + (423 \pm 11)$ $R^2=0.9959$ $R_p^2 = 0.9942$ MEP=74.2	$c = (291 \pm 179)t + (703 \pm 35)$ $R^2=0.9850$ $R_p^2 = 0.9718$ MEP=272.7	$c = (5{,}064 \pm 523)\ln t - (6{,}279 \pm 145)$ $R^2=0.9591$ $R_p^2 = 0.8735$ MEP=1,112.6	$c = (4{,}193 \pm 195)\ln t - (2{,}890 \pm 542)$ $R^2=0.9914$ $R_p^2 = 0.9776$ MEP=381.5

The prediction determination coefficient $\left(R_p^2\right)$ and the root mean square error of prediction (*MEP*) were evaluated by cross-validation.

R^2 coefficient of determination

[a] $c(ppm) = (a \pm SD_a)t(days) + (b \pm SD_b)$, where a is the slope, b is the intercept and SD_a and SD_b are the standard deviations of the slope and the intercept, respectively

[b] $c(ppm) = (a \pm SD_a)\ln t(days) + (b \pm SD_b)$, where a and b are the parameters of the logarithmic equation and SD_a and SD_b are the standard deviations of the two parameters, respectively

Fig. 5 Metal uptake in vetiver shoots (*triangles*) and roots (*circles*) during the short-term bioremediation experiments: Cr (**a**); Cu (**b**); Pb (**c**); and Zn (**d**). *Error bars* represent 95% confidence intervals (*n*=5)

As already observed in the short-term experiments, the vetiver plant is able to take up only very low concentrations of Cr and Cu but very high concentrations of Pb and Zn are accumulated both in shoots and in roots. The Cr uptake is much lower than 0.1% both in shoots and in roots after 30 days (Fig. 3a). Similarly, the Cu uptake is slightly higher but is always lower than 0.1% after 30 days both in shoots and in roots of the Vetiver plant (Fig. 3b). The other heavy metals studied showed the opposite behavior: the results indicate a very large Pb and Zn uptake, with values higher than 1% from day 25 in the roots for Pb (Fig. 3c) and both in the shoots and in the roots for Zn (Fig. 3d).

Fig. 6 Metal uptake in vetiver shoots (*triangles*) and roots (*circles*) during the long-term bioremediation experiments: Cr (**a**); Cu (**b**); Pb (**c**); and Zn (**d**). *Error bars* represent 95% confidence intervals (*n*=5)
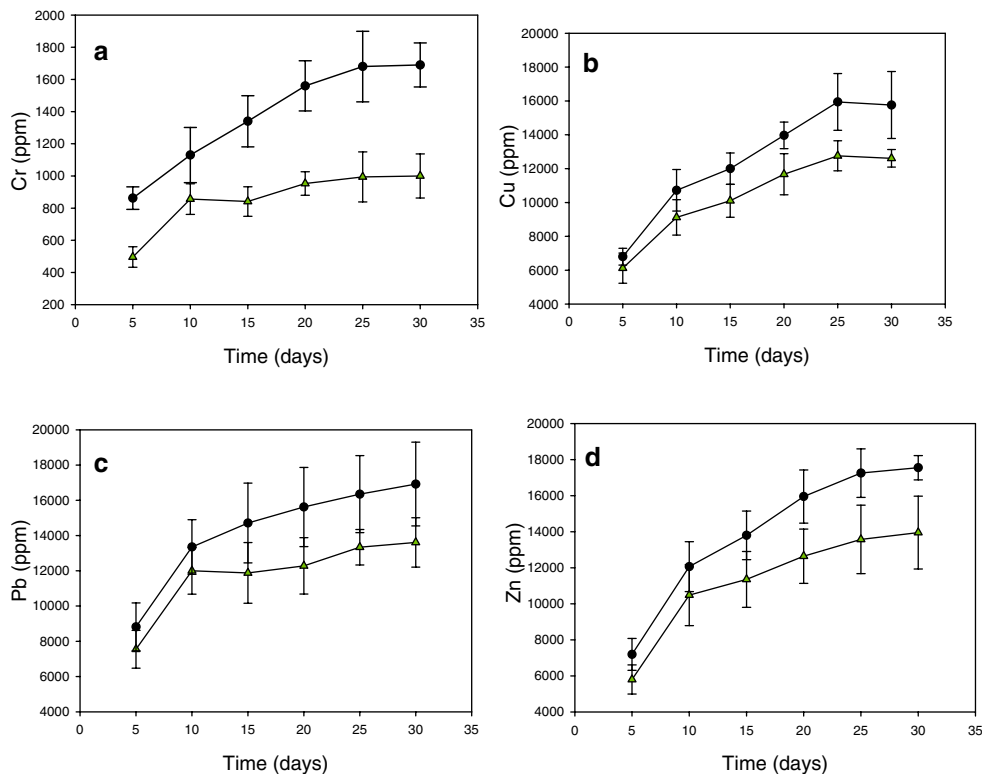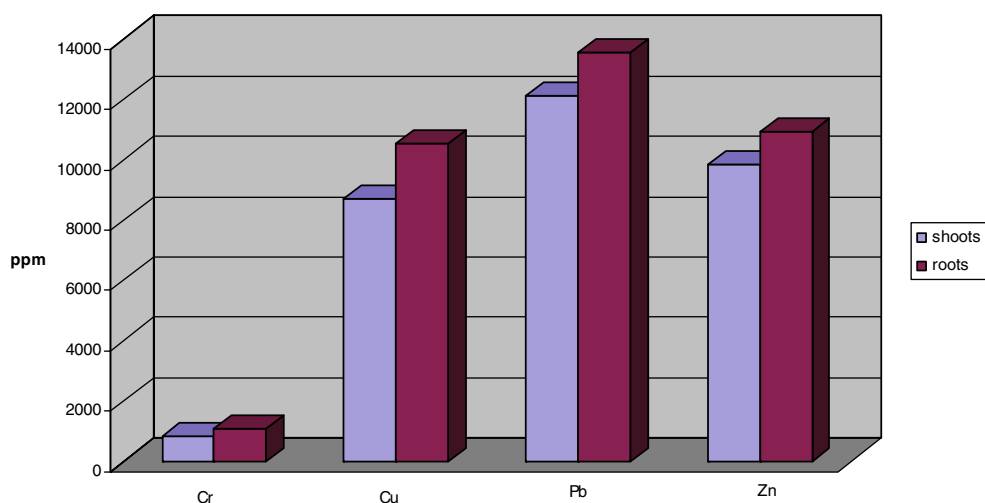
Fig. 7 Comparison between the heavy metal accumulation in shoots and roots in the short-term bioremediation experiments

These results seem to confirm the efficiency of vetiver as a hyperaccumulator for Pb and Zn and not for Cr and Cu, as already reported for the short-term experiments.

Once again, the results showed that the total amount of heavy metals accumulated in roots is much higher then in shoots for all the heavy metals tested, with the exception of Cu. Figure 4 shows clearly the comparison between shoot and root uptake.

It is interesting to note that the plots of the heavy metal content versus time show a linear increase up to 8–10 days. After this period the increase of the heavy metal content both in shoots and in roots becomes progressively lower. This behavior is particularly evident starting from day 20.

For this reason the plots of the heavy metal uptake versus time were approximated by linear equations for the short-term experiments and by nonlinear equations, in particular logarithmic curves, for the long-term experiments. The equations are reported in Table 1. It is possible to observe that these approximations are quite good, as almost all R^2 values were higher than 0.98. Moreover, the values of the intercept of the linear curves were in very

good agreement with the blank values calculated before heavy metal irrigation for both shoots and roots. The difference between the intercept values and the blank values was always less than 6%.
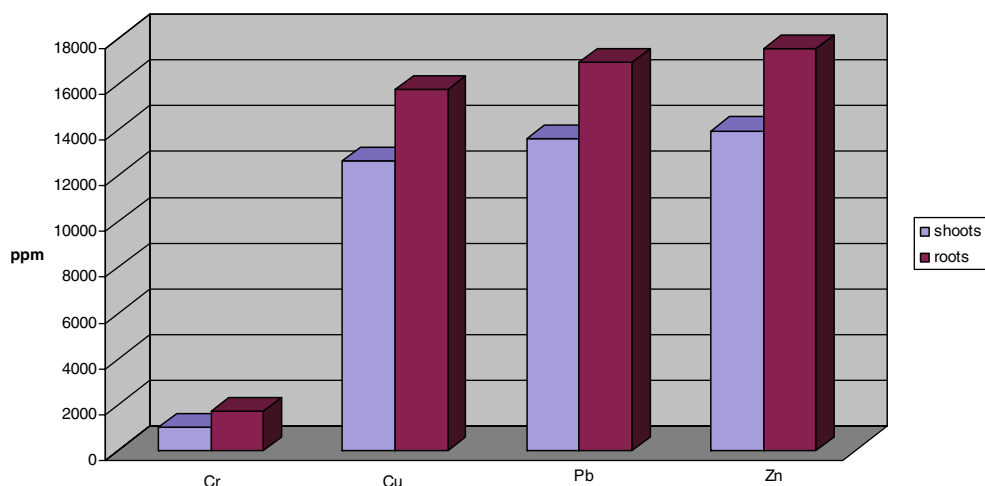
Bioremediation experiments

Short-term and long-term experiments

Figures 5 and 6 show the heavy metal uptake of vetiver shoot and root samples immersed in the heavy metal solutions and analyzed daily within the first 8 days (short-term experiment, Fig. 5) and every 5 days up to day 30 (long-term experiment, Fig. 6). Very low amounts of heavy metals were taken up both by the shoots and by the roots within the whole period studied. It is important to note that these concentrations are much lower than those accumulated by other substrates, such as bacteria, fungi and algae [17].

As already observed for the phytoextraction experiments, it seems that vetiver takes up heavy metals preferably in roots compared with shoots for all the heavy

Fig. 8 Comparison between the heavy metal accumulation in shoots and roots in the long-term bioremediation experiments

metals tested. Figures 7 and 8 show these results for the short-term and long-term experiments, respectively.

The trend of the heavy metal uptake in the bioremediation experiments is similar to that already reported for the phytoextraction experiments. The heavy metal uptake increases almost linearly within the first 8–10 days. Then the increase becomes progressively less and reaches a steady state between day 25 and day 30.

Table 2 shows the equations of the linear and logarithmic curves which better approximate the experimental curves. R^2 values are quite good, and almost all are between 0.97 and 0.99.

Desorption experiments

Table 3 shows the heavy metal desorption in vetiver shoots and roots rinsed daily with distilled water for 9 days. A fast desorption is observed for Cr, Cu and Pb, with desorption values after 9 days between 40 and 59% in shoots and between 44 and 56% in roots. The rapidity of the desorption seems to confirm the hypothesis of an uptake process based on a physical mechanism.

Different behavior is registered for Zn, with a desorption value of 42% in shoots and of only 32% in roots. This result seems to indicate a possible chemical nature of the metal uptake.

Cyclic voltammetry experiments

In order to better understand the nature of the heavy metal uptake, cyclic voltammograms of solutions containing the same amount of heavy metals in three different solvents, distilled water, extract of shoots and extract of roots, were recorded.

For each of the first three heavy metals studied, Cr, Cu and Pb, the cyclic voltammograms obtained in the three different solvents were absolutely identical, showing cathodic peaks at the same potentials and with the same current intensities, independently of the used solvent. This result seems to confirm the physical nature of the Cr, Cu and Pb adsorption.

In contrast, the voltammograms obtained with Zn showed different behavior, depending on the solvent employed. In distilled water the cyclic voltammogram showed an irreversible voltammogram with a cathodic peak with E_p=-1.34 V versus Ag/AgCl and an intensity $i_p \approx$ 35 μA. No anodic peak was observed in the reverse scan. Both in shoot and in root extracts, the shapes of the voltammograms are similar but the cathodic peaks appeared with a slight cathodic shift (E_p=-1.36 V vs. Ag/AgCl) and much lower intensities ($i_p \approx$ 13 μA).

A possible explanation of the results obtained is that the Zn uptake by vetiver shoots and roots is based also on a

Table 2 Linear and logarithmic equations approximating the heavy metal content versus time plots in the short-term and long-term bioremediation experiments

	Short-term experiment		Long-term experiment	
	Linear regression equation[a]		Logarithmic regression equation[a]	
	Shoots	Roots	Shoots	Roots
Chromium	$c = (86 \pm 28)t + (92 \pm 5)$ R^2=0.9791 $R^2_p = 0.9697$ MEP=37.1	$c = (97 \pm 47)t + (128 \pm 9)$ R^2=0.9699 $R^2_p = 0.9526$ MEP=65.0	$c = (270 \pm 45)\ln t + (125 \pm 13)$ R^2=0.8969 $R^2_p = 0.5699$ MEP=113.3	$c = (497 \pm 33)\ln t + (33 \pm 9)$ R^2=0.9572 $R^2_p = 0.9522$ MEP=66.3
Copper	$c = (776 \pm 364)t + (1,066 \pm 72)$ R^2=0.9733 $R^2_p = 0.9423$ MEP=594.8	$c = (199 \pm 35)t + (1,359 \pm 70)$ R^2=0.9841 $R^2_p = 0.9692$ MEP=551.2	$c = (3,791 \pm 251)\ln t + (137 \pm 70)$ R^2=0.9827 $R^2_p = 0.9599$ MEP=463.0	$c = (5,203 \pm 351)\ln t - (1,549 - 973)$ R^2=0.9821 $R^2_p = 0.9660$ MEP=585.9
Lead	$c = (433 \pm 396)t + (1,393 \pm 78)$ R^2=0.9813 $R^2_p = 0.9645$ MEP=607.4	$c = (412 \pm 44)t + (1,659 \pm 88)$ R^2=0.9833 $R^2_p = 0.9710$ MEP=652.7	$c = (3,082 \pm 597)\ln t + (3,436 \pm 1,654)$ R^2=0.8697 $R^2_p = 0.4567$ MEP=1473.6	$c = (4,395 \pm 414)\ln t + (2,403 \pm 1,149)$ R^2=0.9657 $R^2_p = 0.8343$ MEP=1101.1
Zinc	$c = (244 \pm 220)t + (1,201 \pm 43)$ R^2=0.9922 $R^2_p = 0.9889$ MEP=291.6	$c = (187 \pm 45)t + (1,428 \pm 89)$ R^2=0.9773 $R^2_p = 0.9561$ MEP=693.5	$c = (5,898 \pm 294)\ln t - 1,994 \pm 816)$ R^2=0.9901 $R^2_p = 0.8451$ MEP=1076.7	$c = (4,444 \pm 418)\ln t - (727 \pm 116)$ R^2=0.9658 $R^2_p = 0.9668$ MEP=653.8

[a] As in footnotes a and b to Table 1

Table 3 Heavy metal desorption values in vetiver shoots and roots

	Shoots			Roots		
Time (days)	Cr (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)	Cr (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)
Chromium						
Uptake day 30	102	±15.9		277	±41.3	
Desorption day 3	80	±19.1	22	224	±41.3	19
Desorption day 6	45	±6.4	56	148	±25.4	46
Desorption day 9	42	±26.4	59	132	±19.1	53
Copper	Cu (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)	Cu (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)
Time (days)						
Uptake day 30	925	±146.3		769	±124.0	
Desorption day 3	756	±127.2	21	561	±104.9	26
Desorption day 6	552	±89.0	40	449	±73.1	41
Desorption day 9	470	±63.6	49	429	±104.9	44
Lead	Pb (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)	Pb (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)
Time (days)						
Uptake day 30	4,068	±683.7		10,700	±1,844.4	
Desorption day 3	3,579	±496.1	12	8,540	±1,240.2	21
Desorption day 6	2,549	±432.45	37	5,172	±753.7	52
Desorption day 9	2,458	±397.5	40	4,811	±740.9	56
Zinc	Zn (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)	Zn (ppm)	95% confidence interval (ppm) ($n=5$)	Desorption (%)
Time (days)						
Uptake day 30	10,727	±1,653.6		11,671	±1,949.3	
Desorption day 3	8,954	±1,275.2	16	10,083	±1,520.0	14
Desorption day 6	7,350	±1,376.8	32	8,853	±1,806.2	24
Desorption day 9	6,304	±1,087.6	42	8,011	±1,039.9	32

chemical mechanism involving the formation of a complex between the metal and some chelating molecules within the plants. This hypothesis is largely confirmed by the literature where is reported that metal-chelating molecules (phytosiderophores) can be secreted into the rhizosphere to chelate and solubilize the "soil-bound" metals, in particular Fe and Zn [2].

Conclusions

The vetiver plant has been tested to evaluate the possibility of its use for heavy metal soil remediation.

Phytoextraction experiments showed that vetiver can be considered a hyperaccumulator of Pb and Zn, but quite low concentrations of Cr and Cu were accumulated both in the shoot and in root parts of the plant.

In contrast, bioremediation experiments showed a very low efficiency of vetiver for accumulating all the heavy metals tested both in shoots and in roots.

In both experiments the heavy metal accumulation in roots was always higher than that observed in shoots.

From the results obtained, it is possible to conclude that vetiver could, in principle, be successfully used for remediation of Pb- and Zn-contaminated soils. However, further research should be done in order to enhance the uptake rate of these metals and to study the possibility of improving the uptake of other metals by utilizing, for example, chelating agents.

However, it is important to note that the phytoremediation process can show, in principle, a decrease of the biomass production because of the strong inhibition of numerous enzymes by heavy metals with a consequent negative effect on some important physiological processes of the plants, such as chlorophyllous photosynthesis.

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