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Uptake and Distribution of Zinc, Cadmium, Lead and Copper in *Brassica napus var. oleífera* and *Helianthus annus* Grown in Contaminated Soils

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ABSTRACT

Brassica napus var. oleifera and *Helianthus annus* were grown in artificially contaminated soils. Accumulation and translocation of the environmental pollutants zinc, cadmium, lead, and copper, was evaluated in different portions of the plants at two harvesting times. The distribution into the plants of these metal ions, as well as their capacity for contaminant phytoextraction and accumulation was assessed.

For this purpose, an analytical method utilizing focused ultrasound employed for extraction and stripping voltammetry for measurement has been optimized and validated for the simultaneous measurement of Zn, Cd, Pb, and Cu in plant extracts.

KEY WORDS: heavy metals, phytoremediation, *Brassica napus*, *Helianthus annus*, voltammetry.

I. INTRODUCTION

There is an enormous need for efficient, adaptable methodology to clean soil contaminated by hazardous substances such as heavy metals and PCBs. Heavy metals are naturally present in soils and elevated levels maybe a consequence of human activity. Soils are being contaminated due to direct deposition of pollutants from industries, waste disposal sites or dumps and agriculture, as well as by deposition via wastewater treatment and air pollution (Moffat, 1995; Alloway, 1995).

The prohibitively high cost of available soil remediation methods, which mainly involve soil removal and burial at costs of about $1 million per acre, has led to the

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development of phytoremediation of metals as a potentially cost-effective remediation solution for thousands of contaminated sites in the world (Salt *et al.*, 1998).

The value of metal-accumulating plants for environmental remediation has been recognized (Cunningham *et al.*, 1993: Wenzel *et al.*, 1993). Crop plants have been used to extract heavy metals from soil and sediments, followed by translocation of the contaminants to the harvestable stalks and leaves of plants (Raskin *et al.*, 1994). This application, called phytoextraction, may be used in the removal of heavy metals from soils, sludges, and sediments.

The largest challenge at any site comes in finding the right plant for the job. Most metal-accumulating plant species known today were discovered growing on soils containing high levels of heavy metals. These plants are often endemic to these types of soils, suggesting that metal accumulating is associated with heavy metal resistance (Baker and Brooks, 1989). The largest numbers of hyperaccumulating species belong to the Brassicaceae (Baker and Brooks, 1989). The optimum plant for phytoextraction would be able to both tolerate and accumulate high levels of heavy metals and also grow with a high biomass yield.

During phytoextraction, processes several sequential crops of hyperaccumulating plants may be used to reduce soil concentrations of heavy metals to environmentally acceptable levels. Dried, ashen, or composted plant residues, highly enriched in heavy metals may be isolated as hazardous waste or recycled as a source of metal ore (Ensley *et al.*, 1997).

The two plants selected in this work, *Brassica napus* var. oleifera and *Helianthus annus*, grow rapidly and are able to yield high biomass. Also, both plants are commonly grown in Europe and because they have high economic value, harvest processes are inexpensive. *Brassica napus* var. oleifera and *Helianthus annus* were grown in artificially contaminated soils. Phytoextraction, translocation, and the distribution of zinc, cadmium, lead and copper, all being important environmental pollutants, were evaluated in different portions of the plants.

The necessity of trace and ultratrace heavy metal measurement in different ecosystems has increased the application of electrochemical techniques. In its differential pulse mode, stripping voltammetry offers significant advantages for the determination of several important heavy metals. The potentialities of voltammetry arise from its high sensitivity, which permits the use of minute quantities of sample and simplicity of experimental procedures. The equipment is small, consumes little energy, and does not require special facilities such as cold or aeration, making the analytical cost low. Moreover, anodic stripping voltammetry (ASV) and catodic stripping voltammetry (CSV) permit simultaneous measurement of trace and ultratrace levels of several heavy metals in one solution (Zuhri, 1998). Therefore, this technique has been advantageously applied in relation to the most widely used methods for the determination of heavy metals in plant material, such as atomic absorption spectrometry (AAS) or inductively coupled plasma emission spectrometry (ICP-ES).

Queirolo *et al.* developed a differential pulse anodic stripping voltammetric method (DPASV) for the simultaneous determination of Cd, Pb, Cu, and Zn in cores of oak after wet digestion (Queirolo, 1987). DPASV was also employed for the measurement of the same elements in lignocellulose materials used as animal feeds (Lippolis, 1989) and in environmental samples (Ostapczuks, 1989; González Cuesta, 1989).

Few environmental and biological samples can be analysed directly. Sample pretreatment depends on the type of sample, the analyte to be measured, and the analytical technique. Removal of organic matrix is a prerequisite of an accurate elemental analysis by voltammetry. Traces of dissolved organic substances may be adsorbed at the hanging mercury drop electrode (HMDE) and inhibit the electrode process causing suppression of the voltammetric signal. In the presence of dissolved organic matter containing catalytically active nitrogen compounds, the catalytic hydrogen ion reduction current may mask the signals of Cd and Zn and render their determination difficult or impossible (Nürnberg, 1960). The complete destruction of the organic matrix in plant material has been performed by ashing or by wet digestion with concentrated mineral acids (González Cuesta, 1989).

At the low concentration level of the metals to be determined, losses of metals by volatilization can deteriorate the analytical procedure.

Heavy metal extraction from plants has also been performed by mechanical shaking with HCl and HNO_3 (Sankam, 1992) but is time consuming. Ultrasonic extraction is emerging as an alternative method (Pérez-Cid, 1998) and applies intense, high-frequency sound to liquids, producing intimate mixing and powerful chemical and physical reactions. Again, organic compounds possibly extracted must be eliminated.

The aim of this work was the optimization and validation of an analytical method for the simultaneous measurement of Zn, Cd, Pb, and Cu using focused ultrasound for extraction and stripping volammetry for measurement. The method was then employed to assess the capacity of phytoextraction, accumulation and distribution in differents parts of the plants (roots, shoots, leaves, and flowers) of *Brassica napus var. oleifera* and *Helianthus annus* growing in two soils artificially contaminated with muds coming from a mining spill (Aznalcóllar, Spain) at two different levels of contamination. The study was carried out in two physiological stages, flowering and harvesting.

II. MATERIALS AND METHODS

A. Soil Contamination Process

Soil used was sampled at Montepríncipe (Madrid district, coordinates 40°25´N, 3°51´W), from a climax forest of holm oak (*Quercus ilex ssp. Ballota*). Soil was taken with a spade from the A horizon (approximately 0 to 10 cm) after careful removal of overlying layers. The soil was sieved at 2 mm. Physicochemical characteristics of the soil were pH 6.36, total nitrogen 1 $mg \cdot g^{-1}$, organic carbon 0.84%, N-ammonium 4.05 $\mu g \cdot g^{-1}$, *N*-nitrate 18.56 $\mu g \cdot g^{-1}$, phosphate 2.5 $\mu g \cdot g^{-1}$, texture: 75% sand, 15% silt and 10% clay and a cation exchange capacity of 16.48 meq 100 g^{-1} soil.

Six plastic pots (60×35×20 cm) were prepared for each plant species, two were controls containing "clean" soil, two contained material with a low level of contamination (200 $\mu g\ g^{-1}$ for Zn; 12 $\mu g\ g^{-1}$ for Cu; 125 $\mu g\ g^{-1}$ for Pb; and 0.055 $\mu g\ g^{-1}$ for Cd) and two contained a high level contamination (400 $\mu g\ g^{-1}$ for Zn; 25 $\mu g\ g^{-1}$ for Cu; 350 $\mu g\ g^{-1}$ for Pb; and 0.135 $\mu g\ g^{-1}$ for Cd). Contamination was artificially produced with contaminated muds coming from a mining spill (Aznalcóllar, Spain). Muds were poured on a control soil and maintained for 1 month, periodically being

watered to simulate rain. The two degrees of contamination were obtained through differential watering regimes, where the high level received twice the water as the low level. There was a pot of control soil at each watering level. The muds were subsequently removed and samples of control and polluted soils were analyzed for heavy metals. After the muds were removed, the soils were mixed prior to planting.

B. Plant Culture, Harvest and Biometrical Analysis

Thirty seeds of *Brassica napus var. oleifera* and *Helianthus annus* were germinated in each pot (control, low level of contamination and high level of contamination). Plants were periodically watered.

Four and a half months after planting (first sampling time), when plants had flowered, six plants were harvested from each pot. Roots were carefully washed to eliminate all the remaining soil and then plants were gently spread over filter paper with another layer of filter paper weighted over them. The filter paper was periodically changed until the plants were dried and then they were stored pending analysis. After 6 months (second sampling time), all remaining plants were harvested and treated in the same way. When plants were completely dried (45°C for 1 day), root, shoot, leaf, and flower weight was measured.

C. Heavy Metals Analysis in Soils

Heavy metal analysis in soils was performed as previously described (Marín *et al.*, 2001). The soluble fraction was determined to quantify available concentrations for plants. The conditions used in the focused ultrasound extraction were diethylentriaminopentacetic acid (DPTA) (Carlo Erba) 0.005 *M*; 0.2 g sample/25 ml of extractive agent; 10 min of sonication and 20 kHz of frequency.

The determination of Zn, Cu, Pb and Cd was carried out by using an Atomic Absorption Spectrophotometer (Perkin-Elmer model 2380) with an acetylene-air flame (Zn and Cu) and graphite furnace (Pb and Cd). Hollow cathode lamps (Perkin-Elmer) were used as a radiation source for all the studied elements. The instrumental parameters employed were a spectral bandwidth of 0.7 nm, a 30-mA lamp intensity and the following wavelengths: 213.9, 324.8, 283.3, and 228.8 nm for Zn, Cu, Pb, and Cd, respectively.

D. Heavy Metals Extraction and Analysis in Plants

The metal extraction procedure is described in Figure 1. Four mL of the extract containing the heavy metals were passed through a C_{18} solid phase extraction cartridge (Bond Elut JR., Scharlau), previously activated as described in the instructions. The first two mililiters were discarded and the remaining were used for measurement.

Zn, Cd, Pb, and Cu stock reference standards 1000 mg L^{-1} were from Merck. Working standard solutions were a mixture of Zn, Cd, Pb, and Cu (26.1 μg mL^{-1}, 2.2 μg mL^{-1}, 4.4 μg mL^{-1}, and 17.3 μg mL^{-1}, respectively) were prepared daily.

A 1% HNO_3 solution was used as extracting agent. A pH 4.6 buffer solution containing 2 *M* acetic acid and 1 *M* ammonia was used as supporting electrolyte for

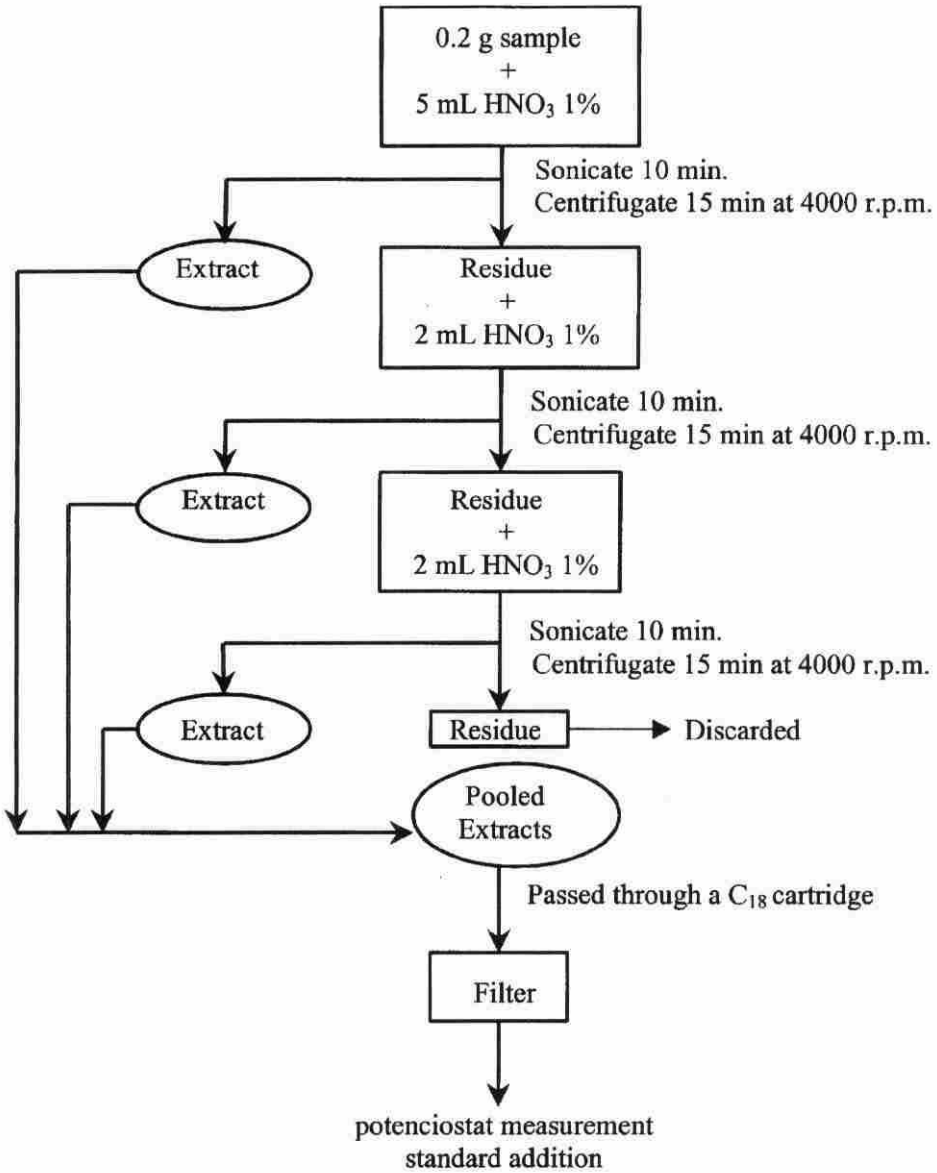

FIGURE 1. Sample treatment.

the electrochemical detection. All reagents were Suprapur® grade (Merck) and water was deionized from a Milli-Q® system.

E. Apparatus

Voltammetric measurements were performed in a potenciostat-galvanostat Autolab PGSTAT10 (Ecochemie) equipped with a polarographic stand VA663 (Metrohm). The anodic stripping voltammograms were obtained using the GPES 4.4 electrochemical software. The electrodes employed were a Metrohm 6.1246.020 mercury electrode in the static drop mode, a Metrohm 6.0728.000 Ag/AgCl reference electrode, and a Metrohm 6.1247.000 glassy-carbon rod counter electrode.

F. Voltammetric Determination

Zn, Cd, Pb, and Cu were determined by differential pulse anodic stripping voltammetry (DPASV), using the method described in the DIN standard 38406, part 16 (1990).

Blank solutions were prepared by transferring 10 mL of supporting electrolyte buffer solution into a 50-ml volumetric flask, and making up to 50 mL with Milli-Q® water. These blank solutions were transferred into the electrochemical cell and deaerated by passing through a pure nitrogen stream for 15 min. An accumulation potential of –1.20 V was applied to a fresh drop of mercury while the solution was stirred at 500 rpm throughout an accumulation period of 60 s. When accumulation time was completed, the stirring was stopped and, after a 30 s rest period, a differential pulse scan, with a scan rate of 10 mV s^{-1} and a 50 mV pulse amplitude, was initiated towards more positive potential values to an end potential of +0.10 V.

After recording the background voltammogram, a 250 µL aliquot of plant sample extract was added, and the accumulation-stripping cycle was repeated. During stripping, well-separated peaks of the four metals were recorded at –1.00, –0.56, –0.40, and 0.00 V for Zn, Cd, Pb, and Cu, respectively, which enabled their simultaneous determination from one sample. The quantitative determination was done by applying the standard addition method, which involved successive 50 µL additions of the four elements working standard solution.

G. Method Validation

Validation was performed to demonstrate the suitability of the analytical method for the intended purpose and therefore the reliability of the results.

Linearity, accuracy, and precision for standards and samples were tested employing two certified reference materials: Tea leaves GBW 07605 (State Bureau of Technical Supervision, The People's Republic of China) and spinach leaves 1570 a (NIST). The certified contents were 26.3 ± 0.9 µg g^{-1} for Zn, 4.4 ± 0.2 µg g^{-1} for Pb, and 17.3 ± 1.0 µg g^{-1} for Cu in tea and 2.89 ± 0.07 µg g^{-1} for Cd in spinach. Standards additions were performed from 3.26 to 22.82 ng mL^{-1} for Zn, from 0.28 to 1.96 ng mL^{-1} for Cd, from 0.56 to 3.92 for Pb, and from 2.16 to 15.12 for Cu.

The validation parameters are summarized in Table 1. These data show the good performance of the method for the intended purpose.

Table 1. Main validation parameters for Zn, Cd, Pb and Cu voltammetric measurement in plants.

VALIDATION PARAMETERS			Zn	Cd	Pb	Cu
Standards linearity		a ± L.C.	0.7 ± 0.4	0.01 ± 0.02	0.01 ± 0.07	0.11 ± 0.33
		b ± L.C.	1.37 ± 0.03	0.90 ± 0.03	0.50 ± 0.04	1.43 ± 0.05
		r	0.996	0.997	0.996	0.994
Samples linearity		a ± L.C.	3.7 ± 0.4	0.18 ± 0.03	0.26 ± 0.06	2.7 ± 0.1
		b ± L.C.	1.27 ± 0.04	0.73 ± 0.08	0.65 ± 0.08	1.78 ± 0.05
		r	0.999	0.998	0.998	0.999
Standards accuracy		Recovery %	100.6 ± 0.4	101.7 ± 0.6	100.6 ± 0.3	100.4 ± 0.3
		RSD %	1%	2%	1%	1%
Samples accuracy		Recovery %	99.6 ± 3	94 ± 3	92 ± 3	96 ± 2
		RSD %	6%	6%	7%	4%
Method precision	Intra-assay day 1	Mean µg/g	27 ± 1	2.6 ± 0.2	3.8 ± 0.1	16.8 ± 0.2
		RSD	5%	7%	3%	1%
	Intra-assay day 2	Mean µg/g	26 ± 1	2.8 ± 0.2	4.2 ± 0.3	16.4 ± 0.8
		RSD	5%	5%	7%	5%
	Intra-assay day 3	Mean µg/g	25 ± 1	2.8 ± 0.1	4.1 ± 0.3	16.5 ± 0.7
		RSD	5%	5%	7%	4%
	Intermediate	Mean µg/g	26.2 ± 0.8	2.72 ± 0.08	4.0 ± 0.1	16.5 ± 0.3
		RSD	6%	6%	7%	4%
Instrumental precision		Mean µg/g	26.5 ± 0.5	2.8 ± 0.3	3.9 ± 0.5	16.6 ± 0.8
		RSD	1%	3%	2%	1%
Limit of detection		Concentration	0.88 ng/g	0.21 ng/g	0.36 ng/g	0.40 ng/g
Limit of cuantification		Concentration	2.93 ng/g	0.71 ng/g	1.21 ng/g	1.34 ng/g

a: intercept (nA); b: slope (nA. L/ µg) ; L.C. : limits of confidence (p=95%)

III. RESULTS

Contaminating the soils with muds from a mining spill partially mimics the situation produced in Aznalcollar, Spain. In addition to being of interest for soil remediation, it is also worth knowing the degree of metal translocation in plants growing in the soils after mud removal as other biota can be affected (honeybees, birds, and other animals).

Dry weight in the different parts of the plant during flowering and harvest are shown in Figures 2 and 3, respectively. Measurements during flowering represents the mean of six plants, whereas after harvesting they are the mean of all the plants.

As can be seen, at flowering, sunflower growth was slightly depressed with increasing contamination whereas oil rape plants were unaffected at low contamina-

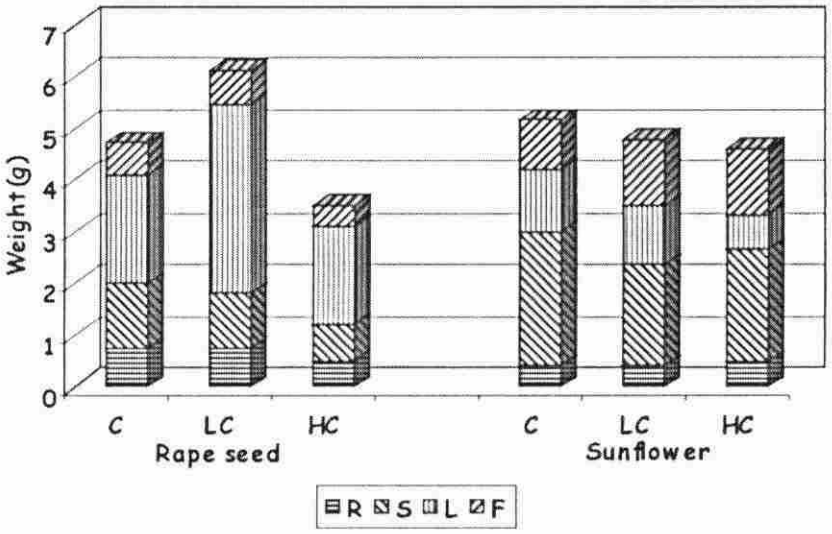

Figure 2. Weight of plants at flowering sampling. C: control; LC: low contamination. level; HC: high contamination level. R: roots; S: shoots; L: leaves; F: flowers.

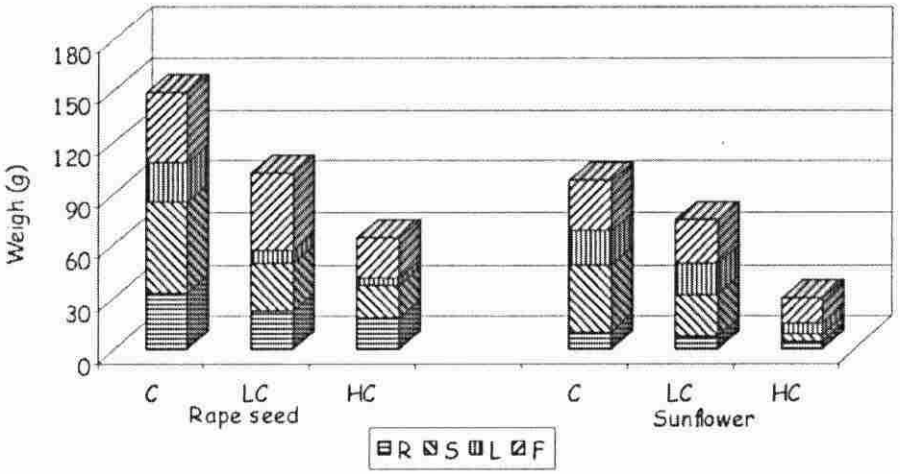

Figure 3. Weight of plants at harvesting sampling. C: control; LC: low contamination level; HC: high contamination level. R: roots; S: shoots; L: leaves; F: flowers.

tion level, but growth was decreased at high contamination level. In sunflower, the part of the plant with greatest biomass was the shoot (50% of total weight), whereas in oil rape the leaves had the highest weight (50% of total weight).

At harvesting, sunflower and oil rape biomass decreased proportionally to the contamination level. The inhibition of growth was observed in all plant parts and was related to the degree of soil contamination. In all of the plants, the aerial vegetation was 80 to 90% of the total plant weight. It is necessary to highlight that, at harvesting, oil rape plants have higher biomass than the corresponding sunflower, in both control and contaminated soils. At harvest, leaves were the part of oil rape with lowest biomass but in sunflower, the roots were lowest.

Results on metal content in four parts of the plant are summarized in Table 2 (flowering) and in Table 3 (harvesting). The results indicate that zinc, lead, and copper, but no cadmium were found for the first sampling (flowering time) in some of the plant organs (roots, shoots, leaves, and flowers) in both tested plants (oil rape and sunflower).

Zn was the most abundant metal in all parts of control and contaminated plants as it was in soils. In both species, zinc in the organs increases significantly (ANOVA test p=95%) paralleled with the corresponding increase in soil concentration. In oil rape, higher levels of zinc were found in flowers for plants growing in control soils. Nevertheless, leaves were the organs with higher zinc content in contaminated soils. The increase in Zn content was significant when comparing plants from control soils with those growing in both levels of contamination. This indicates efficient translocation of the metal to the aerial part of the plant. Similar findings were observed with sunflower, but with the leaves being the organs with higher zinc accumulation in both control and contaminated soils.

With the exception of lead, which remains approximately constant with both levels of contamination, metal concentrations in plants are directly correlated to the soil.

Lead, the second most abundant element in contaminated plants, showed a different pattern. For this metal, the increase in oil rape was only significant in the root's of plants growing in contaminated soils, whereas there was a significant decrease in the aerial part (shoots and leaves) when comparing low and high contamination levels. For sunflower, the shoots accumulated significantly more lead when the contamination level in soils increased. The significant decrease in lead content of sunflower roots when comparing low and high soil contamination degree is noteworthy. Both oil rape and sunflower growing in control soils showed the maximum lead concentration in flowers, but as the soil contamination increased, the root was the organ with greatest increases of the metal, indicating poor translocation to the aerial vegetation.

Copper behavior is different in both plants. Maximum copper levels in oil rape grown in control soils were found in leaves and flowers. When contamination increased, root content increased while shoots content significantly decreased, again indicating poor translocation. In sunflowers growing in control soil, the maximum copper concentration was found in flowers. When the contamination increased, a significantly higher content of copper was found only in roots and leaves. Roots were

Table 2. Results at flowering

		Concentration (μg/g)							
		Oil rape				Sunflower			
Heavy metal	Contamination level	Root	Shoot	Leaf	Flowers	Root	Shoot	Leaf	Flowers
Zn	Control	x 54±4 **a**	x 37±5 **b**	x 41±4 **b**	x 69±6 **c**	x 24±3 **a**	x 20.7±0.8 **b**	x 87±2 **c**	x 54±2 **d**
	Low	y 740±35 **a**	y 384±21 **b**	y 1014±23 **c**	y 278±23 **d**	y 225±9 **a**	y 132±3 **b**	y 239±16 **c**	y 114±3 **d**
	High	z 1480±83 **a**	z 929±34 **b**	z 2046±61 **c**	z 764±44 **d**	z 769±27 **a**	z 469±4 **b**	z 991±24 **c**	z 139±7 **d**
Cd	Control	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Low	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	High	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Pb	Control	x 3.3±0.5 **a**	x 0.07±0.02 **b**	x 0.46±0.09 **c**	x 4.09±0.08 **d**	x 2.9±0.4 **a**	x 0.62±0.02 **b**	x 4.3±0.5 **c**	x 14±1 **d**
	Low	y 143±6 **a**	y 10±1 **b**	y 18±2 **c**	y 29±4 **d**	y 167±2 **a**	y 24±3 **b**	y 31±1 **c**	y 31±3 **c**
	High	z 161±7 **a**	z 8±2 **b**	z 13±2 **c**	y 27±4 **d**	z 108±6 **a**	z 37±4 **b**	y 32±2 **c**	y 29±2 **d**
Cu	Control	x 8.3±0.5 **a**	x 3.2±0.3 **b**	x 11.0±0.7 **c**	x 10.9±0.8 **c**	x 5.2±0.8 **a**	x 5.0±0.2 **a**	x 0.56±0.04 **b**	x 14±3 **c**
	Low	y 40±4 **a**	y 36±5 **a**	y 30±4 **b**	y 26±2 **b**	y 27±3 **a**	y 13±2 **b**	y 4.5±0.4 **c**	y 18±3 **d**
	High	z 75±6 **a**	z 22±1 **b**	y 35±2 **c**	y 30±1 **d**	z 42±1 **a**	y 14±1 **b**	z 15±2 **b**	y 19±2 **c**

Letters a, b and c show the significant differences between root, shoot, leaf and flowers in each plant. Different letters indicate significant statistical ($p<0.05$).

Letters x, y and z show the significant differences between control, low and high contamination level in each heavy metal. Different letters indicate significant statistical ($p<0.05$).

Table 3. Results at harvesting

		Concentration (μg/g)							
		Oil rape				Sunflower			
Heavy metal	Conta. level	Root	Shoot	Leaf	Flowers	Root	Shoot	Leaf	Flowers
Zn	Control	x 33±2 a	x 32±2 a	x 182±10 b	x 56±6 c	x 31±2 a	x 37±7 ab	x 59±2 c	x 38±3 b
	Low	y 669±39 a	y 575±30 b	y 1110±32 c	y 519±53 b	y 273±35 a	y 262±13 a	y 445±17 b	y 171±1 c
	High	z 3452±225 a	z 4333±183 b	z 6041±311 c	z 3332±150 a	z 1805±88 a	z 1419±85 b	z 3601±284 c	z 897±38 d
Cd	Control	x 0.8±0.2 a	x 1.4±0.2 b	x 0.30±0.02 c	x 0.23±0.03 c	x 0.8±0.1 a	x 0.29±0.05 b	x 0.11±0.01 c	x n.d d
	Low	y 10.4±0.4 a	y 4.7±0.2 b	y 4.3±0.2 c	y 4.6±0.6 cb	y 8.7±0.4 a	y 6.9±0.1 b	y 7.5±0.6 c	y 6.3±0.8 b
	High	z 14±1 a	z 11±2 b	z13±1 ac	z 12.3±0.5 c	y 9±1 a	y 7.6±0.4 b	y 7.8±0.5 b	z 8±1 b
Pb	Control	x 3.8±0.4 a	x 3.2±0.3 a	x 3.5±0.5 a	x 15±1 b	x 5.4±0.6 a	x 1.97±0.07 b	x 3.8±0.4 c	x 5.2±0.2 a
	Low	y 136±16 a	y 32±4 b	y 72±5 c	y 70±6 c	y 211±13 a	y 21±3 b	y 58±2 c	y 18±1 b
	High	z 303±7 a	y 29±2 b	z 244±14 c	y 82±7 d	z 135±9 a	z 17±2 b	z 49±5 c	z 21±2 d
Cu	Control	x 4.5±0.8 a	x 8.2±0.4 b	x 15±1 c	x 5±0 d	x 3.4±0.3 a	x 5.2±0.5 b	x 4.3±0.4 c	x 4.5±0.5 c
	Low	y 62±5 a	y 13±2 b	y 36±5 c	y 30±5 c	y 24±1 a	y 15±1 b	y 12.7±0.6 c	y 8.4±0.8 d
	High	z 98±8 a	z 66±6 b	z 92±5 a	z 87±4 a	z 135±9 a	z 21±2 b	z 22±3 b	z 15±3 c

Letters a, b and c show the significant differences between root, shoot, leaf and flowers in each plant. Different letters indicate significant statistical ($p<0.05$).

Letters x, y and z show the significant differences between control, low and high contamination level in each heavy metal. Different letters indicate significant statistical ($p<0.05$).

the sunflower organ with higher copper accumulation when the soil presented higher levels of contamination.

At harvest (second sampling, Table 3), the four metals (Zn, Pb, Cu, and Cd) were found in the four organs tested (roots, shoots, leaves, and flowers). Cadmium was under detection limits at flowering, but at harvest levels were over the limits prescribed by legislation.

In both plants (oil rape and sunflower), zinc accumulation follows the same pattern. In control plants, the higher zinc content was found in leaves. When contamination increased, zinc content increased in the four organs, but increases were greatest in the leaves. As in the first sampling, these findings indicate efficient translocation of this metal to the aerial tissues.

For cadmium, the two plant species show a different behavior. For oil rape grown in control soil, the higher level of cadmium was found in shoots, but in the contaminated soils, cadmium levels increased significantly in the four vegetative organs. At lower contamination, roots were the organs with higher cadmium content, but at higher contamination, roots and leaves became highest. Meanwhile, sunflower roots were the organ with higher cadmium levels in control, low and high contamination. These findings indicate a poor capacity to translocate the metal to the aerial tissues.

The amount of lead in oil rape roots and leaves at harvest significantly increased as related to the soil contamination level. Flowers are the organs with higher lead content in oil rape grown in control soil, whereas roots are the accumulators in soils at both levels of contamination. Sunflower followed a different pattern. There was a significant decrease of lead content in root, shoot, and leaves of plants grown in soils with a higher contamination level when compared with the low level and a significant increase in flowers. In all cases, the organ with a higher lead level was the root, confirming the results from the first sampling suggesting poor translocation of this metal to the aerial vegetation.

Copper content in the four organs of oil rape significantly increased with the soil contamination level. Plants grown in control soils showed the higher content in leaves, but with increasing contamination, roots are the main accumulators, again indicating poor translocation. In sunflower, copper content significantly increases in all organs when soil contamination level increases and the roots are the best accumulators.

Generally, an increase was observed for the four metals from flowering to harvest, and this was positively correlated with contamination level in the soil. The exception to this trend was lead in sunflower, with these plants showing a slight increase in concentration with the lower contamination level of lead.

IV. DISCUSSION

The aim of this study was to assess the capacity of two plants (oil rape and sunflower) to remediate soils polluted with two different levels of four heavy metals. Both plants were able to survive at high and low levels of contamination, indicating these plants have mechanisms to tolerate the presence of heavy metals in the substrate where they grow. Salt *et al.* (1998) indicated that plants have efficient mechanisms

for the detoxification of the accumulated metals, including chelation, compartmentalization, biotransformation, and celullar repair mechanisms.

In this type of study, it is also very important to know the distribution within the plant of the metals being accumulated. Zinc is the most abundant metal in both sampling times, in both plants and in all organs studied. Also, the concentration in the plants increases with an increase of zinc in the substrate. This effect has been observed for several plants such as wheat and Betula (Santa María and Cogliatti, 1998; Denny and Wilkins, 1987). This metal is necessary as a minor nutrient and it is known that plants have special zinc transporters to absorb this metal (Guerinot, 1997). However, an excessive accumulation of this element in living tissues leads to toxicity symptoms (Marschner, 1985). In our experiment, leaf is the major organ of zinc accumulation. Brune *et al.* (1994), showed that the acumulation of this metal in leaf can be a mechanism to avoid toxic effects of Zn in shoot.

Cadmium was only detected in the vegetation at the second sampling time, and the observed concentration was the lowest with regard to other metals. The reason could be the interactions between Cd and other metals and the low Cd level in the muds. Synergistic and antagonistic effects on metal uptake have been reported; for example, Cd uptake can be decreased by additional Zn supply to soil (Cataldo *et al.*, 1983).

In oil rape, root and leaf are the major organs of cadmium accumulation, and in sunflower it is the root. Studies done in tobacco showed that the compartmentation of Cd in vacuoles of root is a physiological mechanism of Cd tolerance (Vögeli-Lange and Wagner, 1990). Although the genetics of Cd tolerance are still unclear (Schat and Vooijs, 1997), it has been demonstrated that the physiological mechanisms of Cd tolerance are not based on an enhanced synthesis of phytochelatins (De Knecht *et al.*, 1995). De Knecht *et al.* (1992) found that Cd-tolerant plants exhibited a higher Cd root:shoot ratio than sensitive plants. In our study, oil rape showed a root:shoot ratio higher than sunflower with both contamination levels, indicating that oil rape is more tolerant than sunflower to this metal. The accumulation of Cd in leaf at the high level of soil contamination is noteworthy; other work with a plant of the same family showed that leaf thichomes also appear to provide a site for the sequestration of Cd (Salt *et al.*, 1995).

At both sampling times, in both plants, and with both contamination levels, the maximum concentration of lead and copper was found in root. This was also observed by Poschenrieder *et al.* (2001). They studied 32 plants and only two accumulated more copper in shoot than in root, with only one specie able to resist high concentrations of copper. Under these conditions, the amount of copper accumulated in shoot was higher than in root. Copper is an essential micronutrient for plant life but it is toxic at high concentration (Burzynski and Buczek, 1997).

It is noteworthy that lead concentration decreased at the first sampling time in shoot, leaf, and flower in oil rape and in the flower of sunflower when the soil contamination level increased. In the second sampling time, this effect also occurred in shoot and flower in oil rape and in root, shoot, and leaf in sunflower. This effect indicates that this metal does not move easily out of the root. These results indicate that these plants should not be used to remediate this metal, because it is very important that the maximum accumulation is in the aerial, easily harvestable plant organs (Saxena *et al.*, 1999).

In general, oil rape accumulates a higher amount of metals than sunflower and it could be considered as a better phytoextractive plant. However, other parameters such as biomass production must be considered. For example, the dry weight of rape oil in soil at the high contamination level decreased at the first sampling time, but dry weight of sunflower was unaffected. Finally, another very important aspect in this type of studies is the interactions between metals. Keltjens and van Beusichem (1998) indicated that the competition between metals at common root absorption sites could result in a low overall metal toxicity, despite high metal levels present in the rooting medium. Also, these interactions can cause differences in absorption and translocation capacities of the plant used.

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