

RECLAMATION OF POLLUTED SOIL: PHYTOREMEDIATION POTENTIAL OF CROP-RELATED *BRASSICA* SPECIES

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Abstract. Soils polluted by heavy metals can be reclaimed using a number of expensive tactics that either remove the contaminants or stabilize them within the soil. The value of metal accumulating plants for environmental remediation has recently been appreciated and promising results have been obtained. This paper reports a study on the behavior of *Brassica napus*, *Brassica juncea*, *Raphanus sativus* and *Brassica carinata* grown on a substrate contaminated by several heavy metals caused by the use of contaminated irrigation water. Data on carbon dioxide assimilation, biomass growth and the bioconcentration and translocation factor of each metal in each species were measured. The polluted substrate caused only a small variation in photosynthesis, however transpiration was more affected by the experimental substrate and in all three species of the genus *Brassica* the presence of metals in the substrate resulted in higher transpiration levels.

Two bioconcentration factors were calculated respectively for the roots (BCF) and the shoots (BCF^s); the BCF was >1 for all the species for Cd, Cu, Ni and Zn without significant differences among species. All the values of BCF^s were lower than 0.5; among the metals, all *Brassica* species demonstrated a similar performance for Cd and Zn, whereas for other elements the bioconcentration factor was very low.

Keywords: Bioconcentration factor, heavy metals, phytoremediation, soil pollution, translocation factor

1. Introduction

Soils polluted by heavy metals can be reclaimed using a number of expensive tactics that either remove the contaminants or stabilize them within the soil such as isolation, immobilization, physical separation and extraction (Mulligan, 2001).

The ability of certain plants to accumulate excess amounts of heavy metals in their tissues has been of interest to geobotanists and physiologists for many years. Only in the last 20 years the value of metal accumulating plants for environmental remediation purposes has been fully realized (Chaney, 1983).

Reeves and Brooks (1983) called plants that accumulate high concentrations of heavy metals in their tissues "hyperaccumulators". From an evolutionary point of



view these types of plants are the result of adaptation to extreme growth conditions (Baker *et al.*, 1994). Hyperaccumulators can concentrate high amounts of metals, often more than 50–100 times that of a non-hyperaccumulator. Up to now at least 45 plant families have been identified with metal-accumulating species, but others are still being sought worldwide (Guerinot and Salt, 2001).

Phytoremediation refers to the use of green plants to remove, contain or render the pollutants harmless (Chaney *et al.*, 1997), and phytoextraction refers to the use of pollutant-accumulating plants that can extract and translocate contaminants to the harvestable parts.

For phytoextraction to be practical we need a plant that should have the following traits: (i) ability to accumulate the metal(s) intended to be extracted, preferably in the aboveground parts; (ii) tolerance to high-metal concentrations in soils; (iii) fast growth and high accumulating biomass; (iv) easily grown as an agricultural crop and fully harvestable (Marchiol *et al.*, 2004). Unfortunately, no wild plants or crops have yet been identified that have these properties in common (Li *et al.*, 2003); moreover, hyperaccumulators are usually more selective for one particular metal (Baker and Brooks, 1989), while in soils contaminated by anthropogenic sources generally contain inorganic elements and organic compounds.

Two different approaches have been used to develop practical phytoextraction systems: the first using breeding and molecular techniques to engineer high-biomass plants (Nie *et al.*, 2002; Gisbert *et al.*, 2003) and the second focussing on identifying management practices to improve or maximize the phytoextraction efficiency of high biomass crops (Lasat, 2000; Klang-Westin and Perttu, 2002; Li *et al.*, 2003).

In phytoextraction, crop biomass yield is less important than the metal concentration in plant tissues (McGrath, 1998). It can therefore be concluded that no plants other than hyperaccumulators should be used for phytoextraction. However, it seems also reasonable to search for crop-related, non-hyperaccumulator, plants showing promising features for phytoextraction. Despite the knowledge gained in the last two decades, soil clean-up by means of growing plants is not yet a finished product either by hyperaccumulators or high biomass crops (McGrath *et al.*, 2001).

A key role in the phytoremediation process is played by the ability of the plant to assimilate, concentrate and translocate metals from the root system to the aerial parts which must be harvested in order to remove the pollutants. To evaluate plants for phytoextraction purposes, the efficiency of a plant should be evaluated by means of both bioconcentration factor and translocation factor. The bioconcentration factor has been defined as the ratio of metal concentration in the plant biomass to metal concentration in the soil (McGrath and Zhao, 2003). The translocation factor is calculated taking into account the concentrations of heavy metals in the plant compartments and refers to the plant ability to translocate heavy metals from roots to the harvestable aerial part (Mattina *et al.*, 2003).

This paper contributes to the discussion on the phytoremediation potential of crop-related species. The experiment was designed to evaluate the behavior of four crop-related *Brassica* species in soil collected from a contaminated field. Carbon

dioxide assimilation, biomass growth, bioconcentration and translocation factors are reported.

2. Materials and Methods

The contaminated soil used in this experiment was collected in a farming area near Carpiano (northwest Italy). The soil pollution source on this site had been identified as the use of contaminated irrigation water for more than a century.

Preliminary tests on polluted soil indicated that plants performed poorly when sown in pots containing this soil. A milder substrate was therefore prepared by carefully mixing the soil with sand in a 1:3 (w/w) proportion. An uncontaminated agricultural soil (*Typic Udifluent*) taken from the experimental farm of Udine University was used as reference soil.

Table Ia and Ib reports, respectively physical and chemical parameters and the concentration of heavy metals in the reference soil and experimental substrate.

TABLE Ia
Physical and chemical characteristics of the reference soil and collected soil

Parameter		Reference	Collected soil
Particle size distribution (%)	Sand	36.8	78.0
	Silt	35.5	17.4
	Clay	27.6	4.6
pH		7.3	6.2
Organic C	g kg ⁻¹	17	40
Total N	g kg ⁻¹	30	3.7
Extr. P	P ₂ O ₅ Olsen (mg kg ⁻¹)	40	78
EC	dS m ⁻¹	0.13	0.48
CEC	cmol ₍₊₎ kg ⁻¹	17.2	14.6

TABLE Ib
Heavy metal concentration of reference soil and the experimental substrate

Element		Reference	Experimental substrate
Cd	mg kg ⁻¹	0.90	19
Cr	mg kg ⁻¹	18.2	85
Cu	mg kg ⁻¹	37.9	140
Ni	mg kg ⁻¹	19.6	24
Pb	mg kg ⁻¹	37.9	441
Zn	mg kg ⁻¹	66.1	3336

Previous experiments to screen the most tolerant varieties among different accessions of Brassica species indicated some cultivars as the most resistant to heavy metals among those tested, so these were used for the experiment. Seeds of canola – *Brassica napus* (cv. Kabel), Indian mustard – *Brassica juncea* (cv. Vitasso), wild radish – *Raphanus sativus* (cv. Rimbo) – and Abyssinian cabbage – *Brassica carinata* (cv. BRK13) – were sown in two L plastic pots containing the control soil or soil – sand mixture. The trial was arranged in a randomized design with 2 treatments (control and treated) and four pots per treatment, each pot containing 10 plants.

The plants were grown for 60 days on a laboratory bench lit by lamps providing $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR) at the plant top with a 12:12 h photoperiod. The pots were rotated randomly each day to equalize their light exposure. Ambient temperature was maintained at $22 \pm 2^\circ\text{C}$. Each pot was watered twice a week during the first month and then every 2 days, alternating distilled water and a modified diluted (1:5) Hoagland solution (Brown *et al.*, 1995) to supply sufficient mineral nutrition.

At the end of the growing period gas exchange measurements were done on both treated and control plants, sampling a leaf area of 6 cm^2 on five leaves per treatment. Carbon exchange rate (CER) and transpiration (T) were measured using a photosynthesis analyzer (LiCor-6400, LiCor, Nebraska). During the measurements leaf temperature was maintained at $22 \pm 0.2^\circ\text{C}$, dew point temperature in the cuvette at $12 \pm 0.5^\circ\text{C}$ and the influx air into the cuvette had a CO_2 content of 360 ppm. Data were logged continuously and recorded when a steady state of readings was reached (coefficient of variability 1%).

At the end of the experiment, the total area of leaves in the control and treated plants was measured using a leaf area meter (LiCor-3000, LiCor). Leaves, stems and roots of five plants per treatment were randomly selected and collected, taking particular care with the root apparatus. The plant fractions (leaves, stems and roots) were fresh weighed and the oven-dried (105°C , 24 h) to determine the dry matter. An amount of plant tissue and soil sample (max 0.50 g) was digested following the US EPA Method 3051 (1994) on a CEM Mars 5 microwave sample preparation system; after cooling, the vessel contents are filtered with Whatman PuradiscTM 0.2 μm PTFE filters. Total Cd, Cr, Cu, Ni, Pb and Zn contents in the plant and soil samples were determined by an ICP-ES (Perkin Elmer 3000).

From the ICP data, the concentration of the heavy metals in the plant biomass were calculated as follows:

$$[\text{metal}]_{\text{plant}} = \frac{[(\text{dw}_{\text{leaves}}) \bullet (\text{ppm}_{\text{leaves}})] + [(\text{dw}_{\text{stems}}) \bullet (\text{ppm}_{\text{stems}})] + [(\text{dw}_{\text{roots}}) \bullet (\text{ppm}_{\text{roots}})]}{(\text{dw}_{\text{leaves}} + \text{dw}_{\text{stems}} + \text{dw}_{\text{roots}})}$$

To evaluate the phytoextraction potential, from the ICP data were calculated (i) two bioconcentration factors ($\text{BCF} = C_{\text{roots}}/C_{\text{soil}}$ and $\text{BCF}' = C_{\text{shoots}}/C_{\text{soil}}$, where C represents the metal concentration) (Mattina *et al.*, 2003) and (ii) the translocation factor $\text{TF} = [(C_{\text{aerial}}/C_{\text{plant}}) \times 100]$, where C_{aerial} represents the metal concentration in the aerial part of the plant (Ekvall and Greger, 2003).

2.1. DATA ANALYSIS

Analysis of variance and Duncan's test ($p < 0.05$) were used to compare treatment means. Data are presented as means with standard error. All statistics were computed using CoStat (CoHort Software, Monterey, CA).

3. Results

3.1. GAS EXCHANGES AND PLANT GROWTH

Data from the gas exchange analysis are reported in Table II. In general, *B. napus* and *B. juncea* showed significantly higher CO₂ fixation than *B. carinata* and *R. sativus*; the average of CER calculated for the plants that were growth in the reference soil and in the experimental substrate, were respectively 12, 7, 12.7, 6.21 and 6.60 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for *B. napus*, *B. juncea*, *B. carinata* and *R. sativus*. The CER was slightly but significantly affected by the presence of heavy metals; the CO₂ assimilation was reduced in plants of *B. juncea* and *R. sativus* (Table II). Transpiration (T) was affected more by the experimental substrate, and in all three *Brassica* species the presence of metals in the substrate resulted in higher T levels. Deriving water use efficiency (WUE) from the CER/ T ratio showed that most treated species had lower WUE than the controls (Table II).

Figure 1 reports the plant biomass data expressed on a dry matter basis. In *B. juncea*, *B. carinata* and *R. sativus* plant biomass production was reduced by soil pollution, but *B. napus* was not affected (Figure 1). *R. sativus* and *B. juncea* were particularly affected by the soil contamination and biomass production was less than half that of the plants growth in the reference soil with (a reduction of respectively -52% and -55%). *B. carinata* gave the highest yield in the reference soil (1.21 g of dry matter per plant) and was less affected than radish and indian mustard by the experimental substrate (-25%) (Figure 1). All plants grown in the polluted soil showed foliar symptoms of toxicity (chlorosis).

3.2. PLANT UPTAKE AND ALLOCATION OF HEAVY METALS

The uptake and translocation of heavy metals in the *Brassica* species observed in our experiment are reported in Figure 2 and Tables III and IV. Figure 2 shows the weight average of the concentration of heavy metals in the plant biomass. The ANOVA did not indicate any significant differences. High data variability recorded in particular for nickel and lead, had differences among the species observed in the experiment (Figure 2).

The bioconcentration factors – BCF and BCF', respectively for the roots (BCF) and the shoots (BCF') – calculated from the ICP-ES data are reported in Table IIIa

TABLE II
Carbon exchange rate (CER), transpiration (*T*) and water use efficiency (WUE) of plants of *B. napus* cv. Kabel (Bna), *B. juncea* cv. Vittasso (Bju), *B. carinata* cv. BRK13 (Bca) and *R. sativus* cv. Rimbo (Rsa) grown in the reference soil (Ref.) and in the experimental substrate (Exp. sub.)

	Bna			Bju			Bca			Rsa			ANOVA	
	Ref.	Exp. sub.	Ref.	Exp. sub.	Ref.	Exp. sub.	Ref.	Exp. sub.	Ref.	Exp. sub.	Ref.	Exp. sub.	Treatment	Species
CER ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	12.6 $\pm$ 0.1 [†]	12.8 $\pm$ 0.1	13.1 $\pm$ 0.1	12.4 $\pm$ 0.1	6.01 $\pm$ 0.04	6.42 $\pm$ 0.1	7.03 $\pm$ 0.1	6.17 $\pm$ 0.03	* [‡]	*				*
Transpiration (mmol H ₂ O m ⁻² s ⁻¹)	1.86 $\pm$ 0.02	3.13 $\pm$ 0.04	2.17 $\pm$ 0.02	3.21 $\pm$ 0.06	1.19 $\pm$ 0.01	1.80 $\pm$ 0.05	1.51 $\pm$ 0.05	1.57 $\pm$ 0.03	*	*				*
WUE (adimensional)	6.89 $\pm$ 0.07	4.18 $\pm$ 0.05	6.08 $\pm$ 0.06	4.01 $\pm$ 0.06	5.08 $\pm$ 0.04	4.10 $\pm$ 0.06	5.29 $\pm$ 0.14	4.13 $\pm$ 0.06	*	*				*

[†]Standard error; [‡]* $P < 0.05$

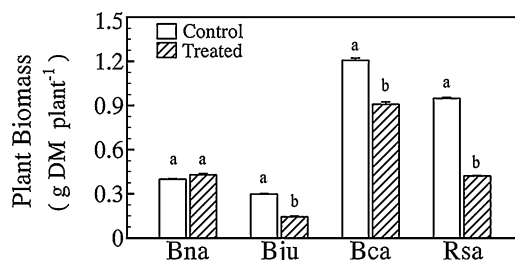


Figure 1. Plant biomass (dry weight) of plants of *B. napus* (Bna), *B. juncea* (Bju), *B. carinata* (Bca) and *R. sativus* (Rsa) growth in the reference soil and in the experimental substrate. Vertical bars represent the standard error for five replicates; letters above bars indicate significant differences between plants growth in the reference soil and in the experimental substrate.

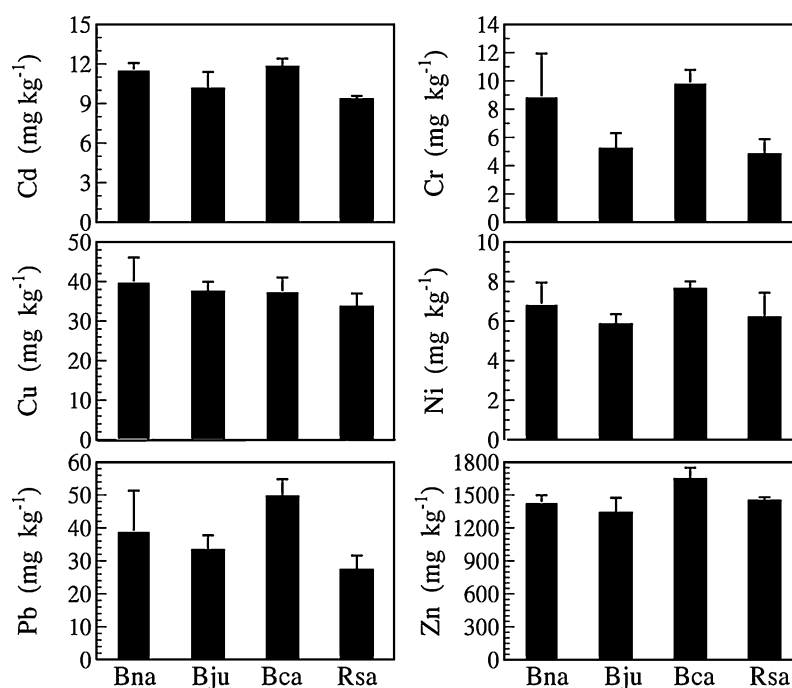


Figure 2. Weight average concentration of cadmium, chromium, copper, nickel, lead and zinc in the biomass of plants of *B. napus* (Bna), *B. juncea* (Bju), *B. carinata* (Bca) and *R. sativus* (Rsa) growth in the reference soil and in the experimental substrate. Vertical bars represent the standard error for five replicates.

and IIIb. The BCF was >1 in all the species for Cd, Cu, Ni and Zn without any significant difference among species. All species demonstrated good tolerance to a high-root concentration of heavy metals (Table IIIa).

BCF^r refers to the most important plant feature in phytoremediation: the uptake of metals, their mobilization into plant tissues and storage in the aerial plant biomass.

TABLE IIIa

Bioconcentration factor ($BCF = C_{\text{roots}}/C_{\text{soil}}$) calculated for plants of *B. napus* cv. Kabel (Bna), *B. juncea* cv. Vittasso (Bju), *B. carinata* cv. BRK13 (Bca) and *R. sativus* cv. Rimbo (Rsa) grown in the experimental substrate

Element	BCF			
	Bna	Bju	Bca	Rsa
Cd	1.46 a†	1.40 a	1.58 a	1.91 a
Cr	0.70 a	0.50 a	0.58 a	0.45 a
Cu	1.22 a	1.17 a	1.10 a	1.25 a
Ni	1.20 a	1.41 a	1.05 a	1.24 a
Pb	0.62 a	0.55 a	0.62 a	0.45 a
Zn	1 a	1.01 a	1 a	1.08 a

†Means with the same letter within a row are not significantly different ($p < 0.05$).

TABLE IIIb

Bioconcentration factor ($BCF' = C_{\text{shoots}}/C_{\text{soil}}$) calculated for plants of *B. napus* cv. Kabel (Bna), *B. juncea* cv. Vittasso (Bju), *B. carinata* cv. BRK13 (Bca) and *R. sativus* cv. Rimbo (Rsa) grow in the experimental substrate

Element	BCF'			
	Bna	Bju	Bca	Rsa
Cd	0.49 a	0.45 a	0.44 ab	0.32 b
Cr	0.02 a	0.02 a	0.02 a	0.01 a
Cu	0.15 ab	0.18 a	0.10 c	0.12 bc
Ni	0.16 a	0.12 a	0.17 a	0.14 a
Pb	0.01 b	0.03 a	0.01 b	0.01 b
Zn	0.35 a	0.34 a	0.40 a	0.36 a

†Means with the same letter within a row are not significantly different ($p < 0.05$).

The BCF' values were lower than 0.5 among the metals. All *Brassica* species demonstrated a similar performance for Cd and Zn, while the BCF' was much lower for the other variables (Table IIIb).

The allocation of metals in plant tissues is estimated by the translocation factor (TF) reported in Table IV. Cadmium and zinc gave the highest TF values (65 and 71%, respectively, averaging the species). Within species, the experimental data had some variability, but ANOVA was significant at $p = 0.056$ for Cd, indicating that the species behave differently. In particular, *B. napus* and *B. juncea* performed better than the other two species.

On average, about 45% of absorbed copper and nickel was mobilized from the roots to the aerial biomass (Table IV). *B. juncea* was the most efficient species in the experiment.

TABLE IV

Translocation factor (TF = $[(C_{\text{aerial}}/C_{\text{plant}}) \times 100]$) calculated for plants of *B. napus* cv. Kabel (Bna), *B. juncea* cv. Vittasso (Bju), *B. carinata* cv. BRK13 (Bca) and *R. sativus* cv. Rimbo (Rsa) grown in the experimental substrate

Element	TF			
	Bna (%)	Bju (%)	Bca (%)	Rsa (%)
Cd	71.0 ab†	75.2 a	57.2 b	56.9 b
Cr	20.2 a	27.9 a	14.3 a	13.7 a
Cu	49.9 ab	60.1 a	30.0 b	42.1 ab
Ni	50.6 a	43.1 a	44.7 a	44.0 a
Pb	15.7 b	33.4 a	9.27 b	21.0 ab
Zn	71.4 a	76.2 a	65.4 a	72.3 a

†Means with the same letter within a row are not significantly different ($p < 0.05$).

4. Discussion

One of the problems applying phytoremediation is that soil pollution is always a combination of inorganic elements and/or organic compounds (Mattina *et al.*, 2003), but there are very few hyperaccumulator plants with the capacity for multiple metal bioaccumulation (Baker, 2000). What about tolerant but less specialized plants? Also for this reason and to widen the field of application for phytoremediation, the potential of crop-related species should be considered. In particular, metal uptake efficiency and translocation rate from the soil to the harvestable plant biomass must be evaluated, as these were inherited from some *Brassica* ancestors growing in metal-rich soils (Bañuelos *et al.*, 1999; Zaman and Zereen, 1998), but as yet not verified in polluted soils.

Our results showed that *Brassica* species grown in the polluted soil tolerated the presence of heavy metals even if there were signs of physiological imbalance and biomass growth reduction. Despite the contemporary presence of several metals, *B. napus* and *B. carinata* showed good tolerance in terms of biomass production.

The higher transpiration rate in treated plants contrasted with observations made on soybean (Marchiol *et al.*, 1996), barley (Vassilev *et al.*, 1998) and *B. juncea* (Haag-Kerwer *et al.*, 1999) grown in hydroponic conditions in the presence of Cd, however the different experimental conditions confound any direct comparison. Angle *et al.* (2003) studying the effects of soil moisture on phytoextraction of Ni and Zn by some hyperaccumulators, observed greater metal uptake at higher soil moisture values and also that plants produce greater biomass at higher soil moisture levels that further enhances the amount of metals extracted from soil. From this point of view it could be interesting to study the relationships between the gas-exchanges parameters and the metal translocation via the root–shoot pathway in the perspective of phytoextraction.

As stated by Zhao *et al.* (2003), plant biomass, bioconcentration factor and soil mass are the three variables that define the phytoremediation potential of a given plant species. In our experiment, the low calculated BCF' values indicate to a great extent, that plants had difficulties in mobilizing all the elements. On the other hand, metal uptake and translocation of an element from roots to shoots is basically linked to the element speciation, soil pH and soil organic matter (Kabata-Pendias and Pendias, 1992).

Mattina *et al.* (2003) demonstrated the simultaneous bioconcentration of some heavy metals and an organic pollutant by some non-hyperaccumulator, non-*Brassica* species such as *Lactuca sativa*, *Cucurbita pepo*, *Cucumis sativus*, *Lycopersicon esculentum*, *Cirsium arvense*, *Lupinus albus* and *Spinacia oleracea*. Our BFC data are coherent with those presented by Mattina *et al.* (2003) but the same does not apply to the data on BCF'.

The amounts of heavy metals extracted from the polluted soil in our experiments are not *per se* satisfying for phytoremediation and, from this viewpoint, it appears crucial to gain more knowledge on techniques that can enhance the efficiency of heavy metal removal. A critical aspect is the condition of multi-contamination that could affect the plant performances. Improvements to induced-phytoextraction could be obtained by implementing specific agronomic practices that have a positive influence on the efficiency of this process, such as mineral nutrition (Ekvall and Greger, 2003; Weih and Nordh, 2002), water supply, soil amendments and corrections (Jauert *et al.*, 2002; Madrid *et al.*, 2003).

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