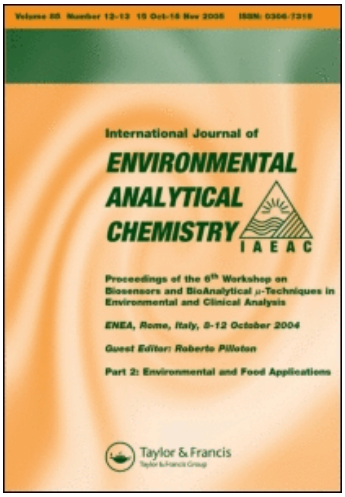

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[a] ICAM-Instituto de Ciências Agrárias Mediterrânicas, Universidade de Évora, Évora, Portugal [b] Centro de Química de Évora, Universidade de Évora, Évora, Portugal [c] Instituto Superior de Agronomia, UTL, Lisboa, Portugal [d] Instituto Superior Técnico, UTL, Lisboa, Portugal

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[a]*ICAM-Instituto de Ciências Agrárias Mediterrânicas, Universidade de Évora, Évora, Portugal;* [b]*Centro de Química de Évora, Universidade de Évora, Évora, Portugal;* [c]*Instituto Superior de Agronomia, UTL, Lisboa, Portugal;* [d]*Instituto Superior Técnico, UTL, Lisboa, Portugal*

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Remediation of sites contaminated with heavy metals using hyperaccumulators seems a promising alternative to engineering approaches. In this work, we compared cadmium (Cd) accumulation and tolerance (based on responses to oxidative stress) in three different species, *Brassica juncea* (L.) Czern, *Nicotiana tabacum* L. and *Solanum nigrum* L., described in the literature as very tolerant or even as hyperaccumulators. The plants were grown in soil spiked with different Cd concentrations ($0 - 35\,mg\,kg^{-1}$) over a period of 90 days.

The translocation factor (TF), used to measure the effectiveness to translocate Cd from roots to shoots, depended greatly on the species. *N. tabacum* was the plant which exhibited the highest TF values. It was the only plant under study that fulfilled the conditions of a hyperaccumulator for all levels of soil contamination. On the other hand, *S. nigrum* presented the highest Cd concentration in plant tissues, with $TF > 1$ in the presence of $5\,mg\,Cd\,kg^{-1}$ of soil. Although *B. juncea* had presented the lowest TF and Cd concentrations, it was the only plant with TF values increasing with the level of cadmium.

Oxidative stress in plants was evaluated by lipid peroxidation and activities of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD), both in roots and shoots. A significant enhancement (*versus* control) on lipid peroxidation and enzymatic activity of CAT and APX in shoots of *B. juncea*, *N. tabacum* and *S. nigrum* was observed for the highest levels of Cd in soil, 15 and/or $35\,mg\,Cd\,kg^{-1}$. *B. juncea* presented the most sensitive response of GPX, for all levels of Cd in soil. Lipid peroxidation and CAT activity were greater in shoots than in roots for all plants and soil Cd concentrations. SOD activity did not present consistent trends for any plant.

Keywords: antioxidative enzymes; cadmium; phytoremediation

1. Introduction

Rapid industrialisation and changed agricultural practices have enhanced the level of contaminants in the environment, with a consequent impact on human health. Cleaning up the environment by removal of hazardous contaminants is a crucial challenge, needing multi-faceted approaches to reach suitable solutions. Due to large costs associated with

*Corresponding author. Email: app@uevora.pt

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engineering approaches, attention has been focused on phytoremediation (use of plants to remediate polluted soils), which is considered an economic and environment-friendly technique [1]. Appropriate selection of plant species plays an important role in the development of methods for remediation (decontamination or stabilisation) of polluted soils [2].

Cadmium, which naturally occurs at low levels in the environment, tends to accumulate in soils due to agricultural practices such as the application of some phosphatic fertilisers, use of wastewater for irrigation, and application of biosolids and composts from urban wastes [3–4]. Maximum limits for Cd in arable soil vary between EU member states, but range between 0.4 and 3 mg kg^{-1} [5].

Cadmium accumulation by plants differs greatly between species and varies between organs of the same plant [6]. Considerable differences in Cd tolerance have been found among plant species and genotypes within a species [7].

Cadmium uptake by plants has been reported to impact a variety of cellular structures. It adversely affects membrane integrity [8–9]. Cadmium, with an oxidation state of +2, is a non redox-metal, and so is not able to generate reactive oxygen species (ROS) through Haber–Weiss reactions. However, overproduction of ROS in plants has been suggested as an indirect consequence of Cd toxicity [10].

ROS comprise radicals and other non-radical reactive species derived from oxygen. Among them, the superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) exert various deleterious effects on cell membranes [11], either directly or in cooperation with other molecules. For example, the oxidative damage of membrane integrity due to lipid peroxidation can result in the generation of highly cytotoxic compounds. Lipid peroxidation can be estimated by the contents of malondialdehyde (MDA) which is regarded as a reliable indicator of oxidative stress [12–13].

Numerous studies indicate an essential role of ROS in plant defence responses to abiotic stress, as they may act as a signal that activates defence mechanisms in plants [14]. ROS levels are strictly controlled by a complex and flexible network of antioxidant systems that maintains a balance between their negative and beneficial functions [15]. As the quantification of short-lived ROS in plants is difficult, their content is usually deduced from changes in the antioxidant system.

The major enzymatic ROS-scavenging components are catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD), as well as low molecular weight antioxidants like cysteine, non-protein thiol and ascorbic acid [13,16]. Changes in the antioxidant system may not reveal the direction and degree of ROS production, because the antioxidant system results from an adaptive response to reverse the effect of the ROS metabolism and that is affected by various factors [17]. This may partially explain why the antioxidant responses to Cd reported by different authors are somewhat inconsistent [18–19].

The purpose of this study was to examine and compare the effects of different soil Cd concentrations on the growth, Cd uptake and oxidative stress of *Brassica juncea*, *Nicotiana tabacum* and *Solanum nigrum* seedlings, grown under similar experimental conditions. *B. juncea* has been identified as a plant that rapidly produces and has the capacity to take up and accumulate heavy metals such as Cd, Cu, Ni, Zn, Pb and Se [20]. This tolerance has practical applications in the bioremediation of polluted sites [21–22]. We have chosen this plant as a ''standard'' for comparing the performance of the two others, since the phytoextraction of this plant has been largely studied in literature.

N. tabacum is also a large biomass producing plant that can accumulate Cd (mainly in leaves), and has potential for use in phytoremediation studies [23–24]. *S. nigrum* has been identified very recently in the literature as a hyperaccumulator plant [25–26], but very few data are available.

Although the capacity of the three species to accumulate Cd has been emphasised in different studies, no systematic comparison of the three plants developed under the same experimental conditions has been undertaken, to the best of our knowledge. Information from such study should help identify critical values of Cd in soils that are phytotoxic, based on plant responses and biochemical parameters and also provide reference values for eco-toxicity assessment of Cd in soils.

2. Experimental

All chemicals were of analytical grade (p.a.) and the solutions prepared with Milli Q de-ionised and sterilised water.

2.1 *Experimental set up*

The pot experiment was conducted in a greenhouse (near the city of Évora, Portugal) with daily temperature ranging from 18 and 35°C. There was no supplemental lighting used for the growing period from August to November of 2006. The soil used was sandy with 95.4% sand, 2% silt and 2.6% clay. It contained 0.2% of organic matter and a total cadmium content of $0.4\,mg\,kg^{-1}$. Soil was air dried, passed through a 5-mm sieve and mixed with fertiliser and cadmium two months before the experiment. The fertilisers added (N (150), P (50), K (120), and Mg (10) mg per kg of soil) were supplied as ammonium nitrate, potassium dihydrogenophosphate, potassium sulphate and magnesium sulphate. Cadmium was added as Cd(II) nitrate solution to obtain a final concentration of 0 (control), 5, 15 and $35\,mg\,Cd\,kg^{-1}$ of soil.

Seeds of the three different plants – *Brassica juncea* L. Czern, *Nicotiana tabacum* L. (Cross Creek Seed Inc., var. K326) and *Solanum nigrum* L. – were sown in plastic containers filled with moist fine gravel previously irrigated with Milli-Q water. The content of water was controlled every day to maintain an adequate humidity. After germination, plants of each type with similar biomass were chosen to be transferred to the pots.

Each pot contained 2 kg of soil and received deionised water to achieve 70% of the maximum water holding capacity. Four plants of the same species were planted per pot. Pots were irrigated daily to maintain the water content near constant. Pots were completely randomised with three replicates of each plant species and Cd concentration.

Plants were harvested after 90 days' growth. Roots were then rinsed in distilled water to remove dust and soil mineral particles and were separated from shoots. Samples of fresh material (root and shoot) from each pot were immediately weighed and a sub-sample of 0.5–1.0 g was frozen in liquid nitrogen and kept at −80°C until used for enzymatic (or lipid peroxidation) determinations.

The remaining plant material was retained for biomass and Cd determination. It was dried at 60 ± 5°C for 2 days, the biomass evaluated, and finally ground to a fine powder (using a mortar) to pass a sieve of 100 μm.

Sub-samples of the soil were extracted with $CaCl_2$ 0.1 mol L^{-1} at the end of the experiment. A parallel investigation was conducted in 3 pots without plants, and sub-samples were extracted with $CaCl_2$ 0.1 mol L^{-1} after 2, 5 and 8 months.

2.2 *Cadmium analysis*

The plant material was re-dried at 60 ± 5°C until constant weight. Samples (~20 mg of plant material) were digested in 2 ml HNO_3 65% + 100 µL of H_2O_2 30%, using microwave acid digestion bombs from Parr® (Illinois, USA) and a Samsung MW81W (Samsung Electronics, South Korea). Cadmium was analysed in an atomic absorption flame spectrometer (Thermo Electron Corporation, type S4, from Thermounicam, Cambridge, UK).

2.3 *Activity of antioxidant enzymes*

All steps in the preparation of the enzyme extracts were carried out at 0–4°C. Frozen shoots samples (0.5 g) were ground to a fine powder in a cold glass mortar with 4.0 g of acid washed sand and homogenised in 2 ml of an extraction buffer with 100 mM Tris-HCl buffer solution (pH = 7.5), 1 mM EDTA, 3 mM 1,4-dithiothreitol and 2% (w/v) polyvinylpolypyrrolidone. Crude extracts for ascorbate peroxidase activity also contained ascorbate (10 mM). The homogenates were centrifuged at 12,000 *g* and 4°C for 30 minutes, and the resulting supernatants used for the determination of the enzymes activity (3 replicates were used for each determination). Absorbances were measured in a Hitachi U-2000 UV/Vis spectrophotometer. The enzyme activity was expressed as Unit g^{-1} fresh weight (FW) for catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and superoxide dismutase (SOD).

CAT (EC 1.11.1.6) activity was measured according to the method of Aebi [27]. One unit of CAT activity was defined as the amount of enzyme required to catalyze the dismutation of 1 µmol of H_2O_2 ($2H_2O_2 \rightarrow O_2 + 2H_2O$) per minute. The decrease of H_2O_2 was measured from the absorbance decay at 240 nm during 2 min ($\varepsilon_{H_2O_2} = 3.94 \times 10^{-2}$ mM^{-1} cm^{-1}). GPX (EC 1.11.1.7) activity was determined according to the method described by Tang *et al.* [28]. One unit of GPX activity is defined as the amount of enzyme required to catalyze the oxidation of 1 µmol of guaiacol per minute ($H_2O_2 + Guaiacol \rightarrow Tetraguaiacol + H_2O$). The increase of tetraguaiacol was measured from the absorbance at 420 nm during 2 minutes ($\varepsilon_{TG} = 26.6$ mM^{-1} cm^{-1}). APX (EC 1.11.1.11) activity was determined according to the method of Nakano and Asada [29]. One unit of APX activity was defined as the amount of enzyme required to catalyze the oxidation of 1 µmol of ascorbate per min ($Asc + H_2O_2 \rightarrow 2MDA + 2H_2O$). The decrease of ascorbate was measured from the absorbance decay at 290 nm during 2 minutes ($\varepsilon_{Asc} = 2.8$ mM^{-1} cm^{-1}). SOD (EC 1.15.1.1) activity was determined according to the method described in Prasad *et al.* [30]. One unit of SOD activity was defined as the amount of enzyme required to inhibit by 50% the reduction of Cytochrome C–Fe^{3+} to Fe^{2+}. Absorbances were measured at 550 nm over two minutes, in the absence and presence of SOD.

2.4 *Lipid peroxidation*

Frozen plant material was ground to a fine powder in a glass mortar with 2.5 ml of trichloroacetic acid (0.1% m/v). The homogenates were centrifuged at 10,000 *g* and 4°C

for 20 minutes. One mL of the resulting supernatants was collected in a tube (3 replicates for each determination). Four mL of thiobarbituric acid (0.5% m/v in trichloroacetic acid 20% m/v) were added to each tube, followed by their immersion in boiling water for 30 minutes, and cooling in ice for a further 5 minutes. The samples were centrifuged at 10,000 *g* for 15 minutes at room temperature. The lipid peroxidation was estimated by the contents of malondialdehyde (MDA), measured from the absorbance at 532 nm ($\varepsilon_{MDA} = 155\,mM^{-1}\,cm^{-1}$) and 600 nm for turbidity correction [12,25].

2.5 *Statistics*

Statistical analysis was carried out with one-way and two-way ANOVA using the statistical program SPSS® (version 14.0). A comparison of individual means was performed using the Tukey's test at $p \leq 0.05$ and $p \leq 0.01$ When necessary, data were log-transformed prior to analysis.

3. Results and discussion

In soil kept plant-free $80 \pm 5\%$ of the total Cd could be extracted by $CaCl_2$ 0.1 M after two months of Cd incubation (start of plant experiment in parallel pots), and $80 \pm 10\%$ could still be extracted after 5 and 8 months of incubation. These values are similar to those obtained from the planted pots when plants were harvested (i.e. 5 months after Cd incubation and 3 months after plantation).

The pH of soil samples from pots with and without plants was measured just before $CaCl_2$ extractions. The pH values varied in the range of 7.3 ± 0.3.

These results showed that soil pH and bioavailable Cd were stable over a period of 8 months in the absence of plants. A plant cover for a period of 90 days also did not influence these parameters.

3.1 *Effects of cadmium on plant growth*

Cadmium is a highly toxic element and its negative effects on plant growth have been described in many studies [10,31–32].

During the present 90-day experiment, all plants survived in the soil spiked with Cd. Based on biomass production, *Brassica juncea* was the species that best survived in soil containing 15 and 35 Cd kg^{-1} (Figure 1). Shoot biomass production of *Brassica juncea* and *Nicotiana tabacum* grown in 35 mg Cd kg^{-1} of soil was either not significantly different from controls or even larger. In contrast, the shoot biomass of *Solanum nigrum* was reduced in the treatments with Cd, due to Cd accumulation over 200 mg Cd kg^{-1} of shoots dry weight (DW) (Table 1). This is in agreement with Sun *et al.* [33], who showed a reduction in the growth of *S. nigrum* for Cd concentration above 125 mg Cd kg^{-1} of shoots DW. Based on the results of the present experiment, the concentration of 203 mg Cd kg^{-1} of shoots DW was obtained in a soil with only 5 mg Cd kg^{-1}, while those authors [33] used a soil with 25 mg Cd kg^{-1}. This can be explained by different bioavailability of Cd in the two soils. Ours was a slightly acidic sandy soil, very poor in organic matter, where part of the applied Cd remained in solution, while that used by Sun *et al.* [33] was a Haplic Luvisol with a pH of 6.6, where the bioavailable fraction should be smaller [4].

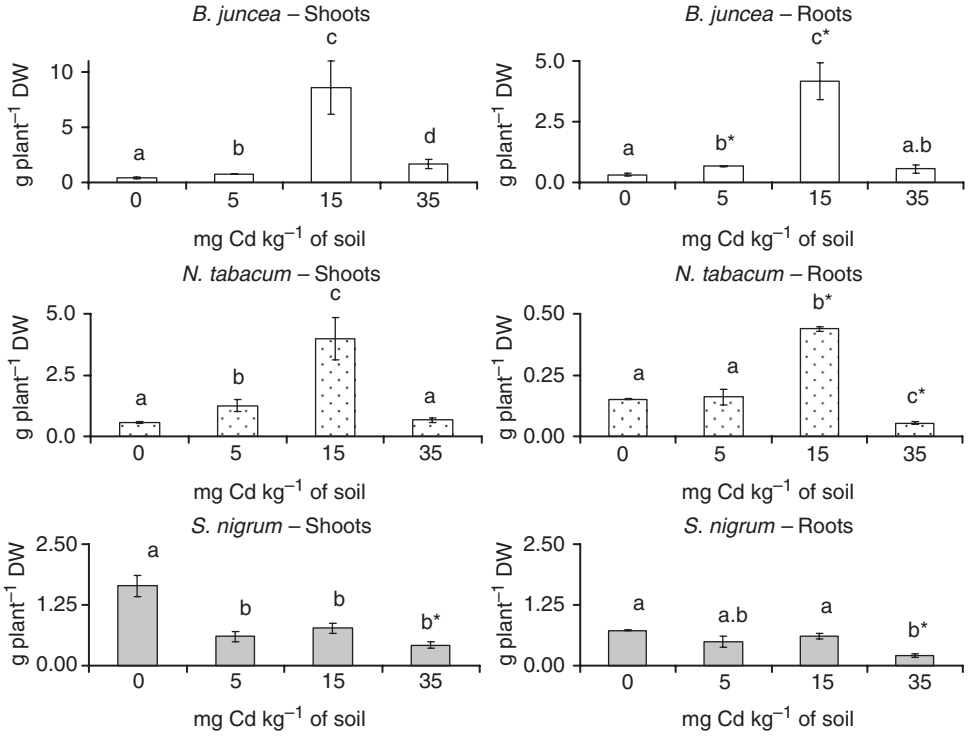

Figure 1. Effects of Cd treatment on shoot and root biomass (g plant^{-1} DW) of *B. juncea*, *N. tabacum* and *S. nigrum*.
Notes: DW = dry weight.
Vertical bars indicate the standard error of mean values ($n = 3$).
Values with the same letter are not significantly different at $P < 0.05$.
Values with * indicate significant differences from the control at $P < 0.01$.

Table 1. Amount of cadmium in shoots per plant, cadmium concentration in shoots and roots, translocation factor (TF, ratio of metal ion concentration between shoots and roots) and bioconcentration factor (BF, ratio of metal ion concentration between shoots and soil) of *B. juncea*, *N. tabacum* and *S. nigrum* grown for 90 days in a Cd-contaminated soil. Average ±SE.

Plant	Cd in soil (mg Cd kg^{-1})	Cd in shoots (mg Cd per plant)	Cd in shoots (mg Cd kg^{-1})	Cd in roots (mg Cd kg^{-1})	TF	BF
B. juncea	**5**	0.019 ± 0.005	25 ± 7	103 ± 23	0.21 ± 0.02	5.0 ± 0.8
	15	0.50 ± 0.10	63 ± 2	127 ± 4	0.49 ± 0.01	4.20 ± 0.06
	35	0.31 ± 0.09	183 ± 15	255 ± 13	0.73 ± 0.08	5.2 ± 0.3
N. tabacum	**5**	0.17 ± 0.03	135 ± 2	23 ± 7	7.6 ± 0.8	27.0 ± 0.3
	15	0.60 ± 0.10	161 ± 8	71 ± 25	2.4 ± 0.7	11.0 ± 0.4
	35	0.31 ± 0.06	461 ± 68	440 ± 77	1.0 ± 0.1	13 ± 1
S. nigrum	**5**	0.12 ± 0.02	203 ± 8	80 ± 2	2.50 ± 0.05	41 ± 1
	15	0.30 ± 0.07	383 ± 66	540 ± 24	0.64 ± 0.06	26 ± 2
	35	0.23 ± 0.05	539 ± 60	921 ± 153	0.64 ± 0.01	15 ± 1

Note: DW = dry weight.

A stimulatory effect on plant growth was exhibited by *N. tabacum* and *B. juncea*, with significant increases in both biomass of shoot and root at a soil Cd concentration of 15 mg kg^{-1}, compared to the control (Figure 1). This effect was observed on both dry and fresh weight.

A positive effect on plant growth at low Cd concentration has been reported by other authors, in hydroponic experiments with plants such as rice [34–35], soybean [36], barley [7], miscanthus [37] and sorghum [38]. This effect depends on plant species and experimental conditions such as metal concentration and stress duration [7,32]. The stimulatory effect of Cd on plant growth is normally related to a so-called hormetic effect that may represent an 'overcompensation' response to a disruption in the homeostasis of the organism [35]. Stimulatory effects on growth may derive from enhanced cell division, which has been observed in both animal and plant cells culture [36,39]. According to Sobkowiak and Deckert [36], the stimulation of cell proliferation by low doses of Cd could be related to the capacity of Cd^{2+} to substitute Zn^{2+}, a component of key enzymes involved in replication and translation [32].

In this work, other factors could have contributed for the large increase in *N. tabacum* and *B. juncea* biomass at a soil Cd concentration of 15 mg kg^{-1}, directly or by accelerating the hormetic effect. Temperature could have been one factor, since the biomass increase was not so apparent in similar experiments carried out during the winter (data not shown).

3.2 *Cd accumulation and distribution*

Mechanisms for plant tolerance to Cd have been reported previously and they include two strategies: exclusion and accumulation [40]. Plants with a strategy of accumulation translocate Cd to the shoots, with only a small amount of Cd being stored in the roots.

Ideally, plants to be used in phytoextraction should produce a relatively large shoot biomass, and have large bioconcentration factor (BF, ratio of metal ion concentration between shoots and soil) and translocation factor (TF, ratio of metal ion concentration between shoots and roots), i.e. they should have an accumulation mechanism.

In this work a large proportion of Cd was accumulated in *B. juncea* roots (*versus* shoots) for all Cd levels in the soil (Table 1), which may be a natural protective response of plants to defend the above-ground parts from Cd toxicity. In parallel, the TF increased with Cd soil contamination (Table 1), which indicated a better performance of this plant as phytoextractor for high Cd concentrations. Nouairi *et al.* [20] observed a TF of 0.3 for *B. juncea* exposed to 11 mg Cd L^{-1} during 15 days in hydroponic solution, inbetween the TF values obtained in this work for *B. juncea* exposed to 5 and 15 mg Cd kg^{-1} in soil.

In contrast to *B. juncea*, *N. tabacum* had greater Cd concentrations in shoots than in roots (Table 1). The concentration of Cd in *N. tabacum* shoots was greater than that of *B. juncea*, for all levels of Cd concentration in the soil, which also represents an important advantage in terms of phytoextraction. However, the TF decreased from 8 to 1 in *N. tabacum*, for Cd concentration in soil ranging from 5 to 35 mg Cd kg^{-1} (Table 1), suggesting a limited mechanism of metal ion tolerance.

The accumulation of Cd in *S. nigrum* roots and shoots increased with increasing Cd concentrations in the soil, as for *B. juncea* or *N. tabacum*. Greater Cd concentrations in shoots relative to roots only occurred at a concentration of 5 mg $kg^{-1}$of soil, with TF = 2.5. Increasing Cd concentration in soil promoted Cd accumulation in roots, leading to TF values below 1. Sun *et al.* [4] also found a decrease in TF values with the increase

of Cd, which indicates that Cd concentration in tissues strongly affects the capability of plants to transport Cd from roots to shoots.

S. nigrum was the plant with the highest Cd concentration in shoots. However, the amount of Cd extracted per plant and accumulated in shoots did not follow this behaviour (Table 1). The amount of Cd in shoots is one of the most important parameters for phytoremediation, as pollutants are removed from a contaminated site by plant harvesting.

Metal hyperaccumulating plants have the ability to solubilise metal ions from the soil matrix, efficiently absorb them into the roots and translocate them to the shoots [41–42]. They are defined as plants with Cd concentration in shoots over $100\,mg\,kg^{-1}$ [43]; a bioaccumulation factor greater than 1.0, sometimes reaching 50–100 [44]; and a translocation factor greater than 1.0 [45].

N. tabacum was the only plant under study that fulfilled those conditions for all levels of soil contamination, although at $5\,mg\,Cd\,kg^{-1}$ *S. nigrum* had presented competitive parameters for phytoextraction. For *B. juncea*, considered a very tolerant plant, the TF was always lower than 1. It also presented a much lower value for the BF at each Cd concentration in the soil, compared with the other two plants.

In conclusion, *S. nigrum* would be the best choice for phytoremediation of soils with small levels of Cd ($\leq 5\,mg\,Cd\,kg^{-1}$), but for greater levels *N. tabacum* had a better performance. *B. juncea* may become competitive for soil concentrations greater than $35\,mg\,Cd\,kg^{-1}$, since the TF increased with soil contamination, and the amount of Cd accumulated in shoots was similar or slightly greater compared with the other two plants for that level of soil Cd.

3.3 *Effect of cadmium on lipid peroxidation*

The malondialdehyde (MDA) content in shoot tissues exposed to Cd was greater than in controls in *N. tabacum*, and especially in *B. juncea* (Figure 2). The enhancement in MDA was probably due to the Cd-induced oxidative damage of cell membranes. Previous studies also shown that Cd enhances lipid peroxidation in other plants such as *Phaseolus vulgaris* [46–47], *Helianthus annuus* [48], *Pisum sativum* [49–50], *Bacopa monnieri* L. [13], *Macrotyloma uniflorum* L. and *Cicer arietinum* L. [51].

The greatest MDA content in shoots was present in *B. juncea* and *N. tabacum* exposed to $15\,mg\,Cd\,kg^{-1}$, although lipid peroxidation was not deleterious for plant growth as the greatest biomass was obtained for that level of Cd. A similar result was reported for rooted cuttings of black poplar grown in a contaminated sandy soil, where a simultaneous increase of MDA (3 times) and shoot length was observed [52].

For *S. nigrum*, a significant increase in MDA shoot concentration was observed only for $35\,mg\,Cd\,kg^{-1}$ (Figure 2), although a reduction in plant growth was observed from $5\,mg\,Cd\,kg^{-1}$ treatment. The extent of lipid peroxidation in shoots of *S. nigrum* exposed to $5\,mg\,Cd\,kg^{-1}$, similar to control, together with the large amount of Cd accumulated in shoots (greater than in *B. juncea* or *N. tabacum* shoots), seems to indicate that *S. nigrum* possesses other strategies to cope with Cd in soils that are not severely contaminated. According to Sun *et al.* [33], organic acids in *S. nigrum* have an important role in binding Cd, so that it can be safely stored in vacuoles.

The three plants presented a greater concentration of MDA in shoots than in roots, for all levels of Cd in the soil (Figure 2). On the other hand, MDA concentration in roots

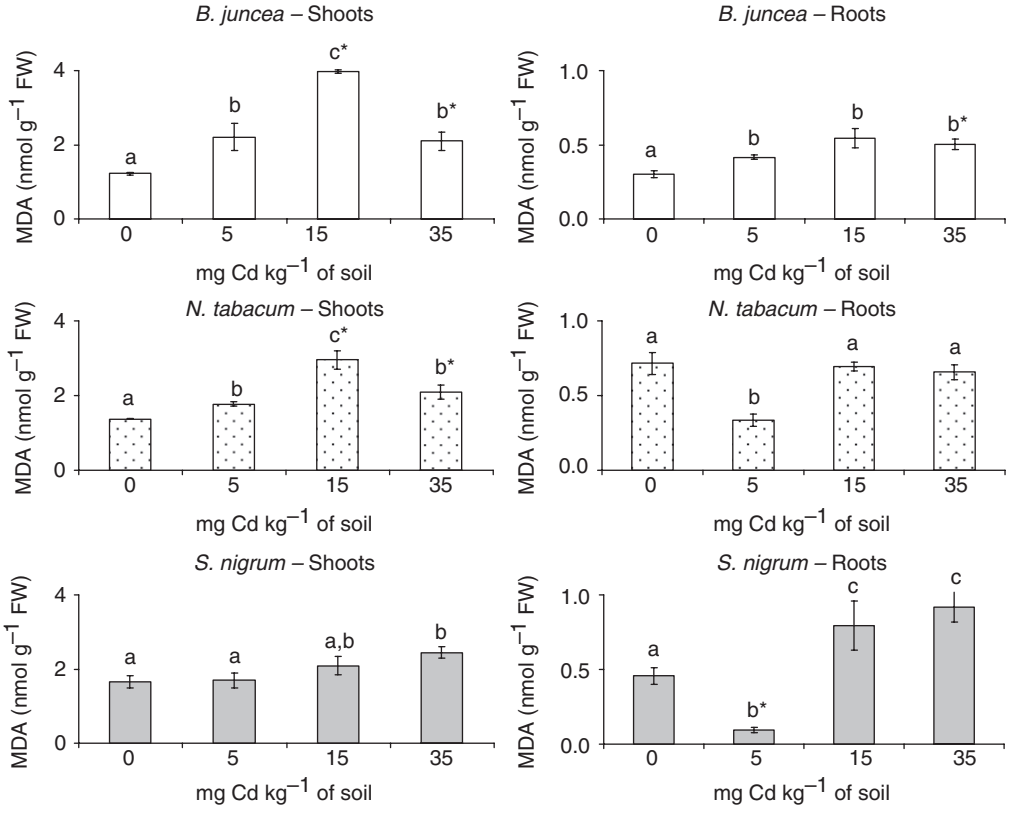

Figure 2. MDA content (nmol g^{-1} FW) in shoots, and in roots of *B. juncea*, *N. tabacum* and *S. nigrum* grown for 90 days with 5, 15 and 35 mg Cd kg^{-1} of soil.
Notes: MDA = malondialdehyde. FW = fresh weight.
Vertical bars indicate the standard error of mean values ($n = 3$).
Values with the same letter are not significantly different at $P < 0.05$.
Values with * indicate significant differences from the control at $P < 0.01$.

did not follow the trends observed in shoots, namely: (i) the increase of MDA content in the presence of Cd was no longer observed in *N. tabacum*; (ii) *N. tabacum* and *S. nigrum* contained surprisingly low values of root MDA concentration at 5 mg Cd kg^{-1} of soil. The small lipid peroxidation and enzymatic activity values at 5 mg Cd kg^{-1} for *N. tabacum* and *S. nigrum* roots seems to indicate that the detoxification mechanisms used are adequate in that compartment and for that level of soil Cd.

3.4 *Effect of cadmium on antioxidant enzymes*

Cd-induced changes in activities of reactive oxygen species (ROS)-scavenging enzymes have been detected in several plants [32,48–49,53–54]. The response of antioxidant enzymes to Cd, and in general to metal ions, can vary among species, among different tissues, and depends on the level of free radicals resulting from the balance between generation and scavenging [53–54].

Catalase (CAT), localised in peroxisomes, is an indispensable enzyme that scavenges ROS, particularly H_2O_2 in plant cells. It participates in the main defence system against

accumulation and toxicity of hydrogen peroxide and can play a role in controlling H_2O_2 level in cells, which is of utmost importance in cellular metabolism [55–56].

In this work, increases in CAT activity of shoots and roots exposed to 15 and 35 mg Cd kg^{-1} (Figure 3), indicated that ROS induced by Cd were metabolised by CAT in peroxisomes. It has been reported that CAT is indispensable for oxidative stress tolerance in tobacco plants [57]. For all plants CAT activity was much greater in shoots than in roots (Figure 3), and *S. nigrum* had a considerably greater CAT activity in shoots compared with the other two plants. For *N. tabacum* less root CAT activity was observed when plants were exposed to 5 mg Cd kg^{-1}, compared to the control, following the trend already observed for MDA.

Ascorbate peroxidase (APX), in contrast to CAT, is present in almost all cellular compartments such as chloroplasts, cytosol and microbodies [58–59]. For all plants, APX activity was much smaller compared to CAT (Figures 3–4), following the trend usually observed for other species. The different activity range of APX and CAT suggests that they belong to different classes of H_2O_2-scavenging enzymes: APX might be responsible

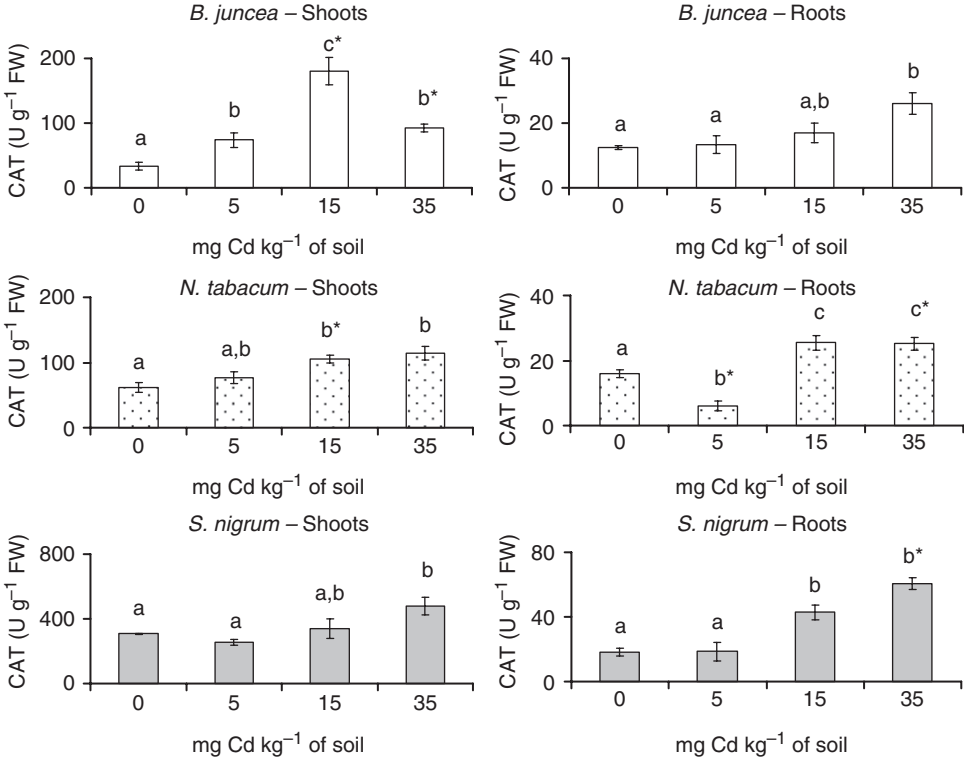

Figure 3. Effects of Cd treatment on the activities of CAT (U g^{-1} FW), in shoots and roots of *B. juncea*, *N. tabacum* and *S. nigrum*.
Notes: CAT = catalase. FW = fresh weight.
Vertical bars indicate the standard error of mean values ($n = 3$).
Values with the same letter are not significantly different at $P < 0.05$.
Values with * indicate significant differences from the control at $P < 0.01$.

for the fine modulation of ROS for signalling, whereas CAT might be responsible for the removal of ROS excess during stress [14,60–61].

In the present study, the greatest values of APX activity were found in shoots and roots of *N. tabacum* (Figure 4). A significant enhancement was generally observed in plants exposed to 15 and/or 35 mg Cd kg^{-1}, as observed for CAT. Similar or smaller APX activity was observed in plants exposed to 5 mg Cd kg^{-1}, compared to the control, trend also presented by CAT in *N. tabacum* and *S. nigrum*. APX activity in shoots of *B. juncea* was greater at 15 mg Cd kg^{-1}, as observed for CAT. For *S. nigrum*, APX activity was much greater in shoots than in roots (trend followed by CAT, for all plants). This result is in discrepancy with Dinakar *et al.* [62], who reported a greater APX activity of *S. nigrum* in roots than in leaves in hydroponic experiments of 10–25 days, with 0.1 mmol Cd L^{-1}.

Guaiacol peroxidase (GPX) is mainly a cell wall bound enzyme, although it is also found in other compartments such as cytosol, vacuole and extra-cellular spaces [63]. It can be considered a stress marker, having a broad specificity for phenolic substrates, and a greater affinity for H_2O_2 than CAT [14].

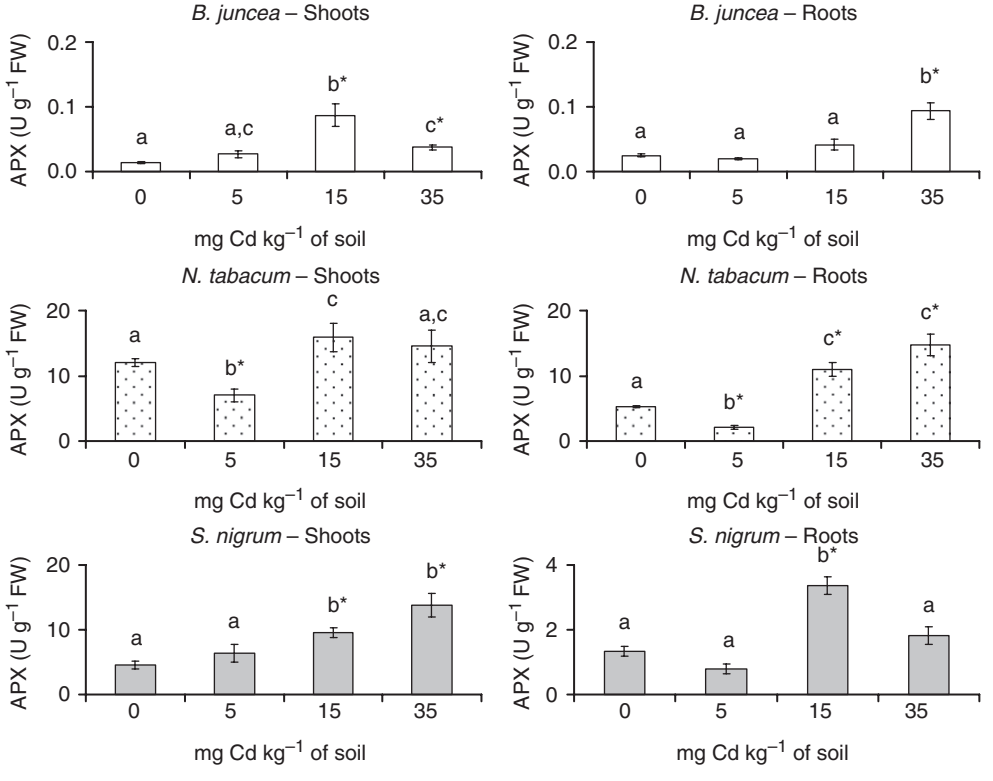

Figure 4. Effects of Cd treatments on the activities of APX (U g^{-1} FW), in shoots and roots of *B. juncea*, *N. tabacum* and *S. nigrum*.
Notes: APX= ascorbate peroxidase. FW = fresh weight.
Vertical bars indicate the standard error of mean values ($n = 3$).
Values with the same letter are not significantly different at $P < 0.05$.
Values with * indicate significant differences from the control at $P < 0.01$.

For *B. juncea*, GPX activity in shoots and roots was greater than in the respective controls at all soil Cd concentrations (Figure 5). A notable enhancement (6 times) of GPX activity in shoots was observed in the presence of Cd (*versus* control). Thus, GPX seems to play a lead role in shoots of *B. juncea*, since the increment observed was much greater than those of APX and CAT. In *S. nigrum* the GPX activity in shoots also increased with Cd content, although to a less extent than in *B. juncea*. GPX activity of *N. tabacum* was not enhanced by Cd compared with the control, indicating that GPX is not as important as CAT or APX in ROS detoxification in this plant. Only for *S. nigrum* was GPX activity in shoots greater than in roots (trend followed by CAT in all plants).

A decrease of enzymatic activity in roots of *N. tabacum* and *S. nigrum* exposed to 5 mg Cd kg^{-1} was observed. The significant decrease of lipid peroxidation as well as CAT, APX and GPX activities, generally observed in the roots of *N. tabacum* and *S. nigrum* exposed to that Cd concentration, may have been due to an adjustment of the plant antioxidative system, within a period of adaptation to the Cd stress, which may even overcompensate the stress, resulting in a decrease of free radicals.

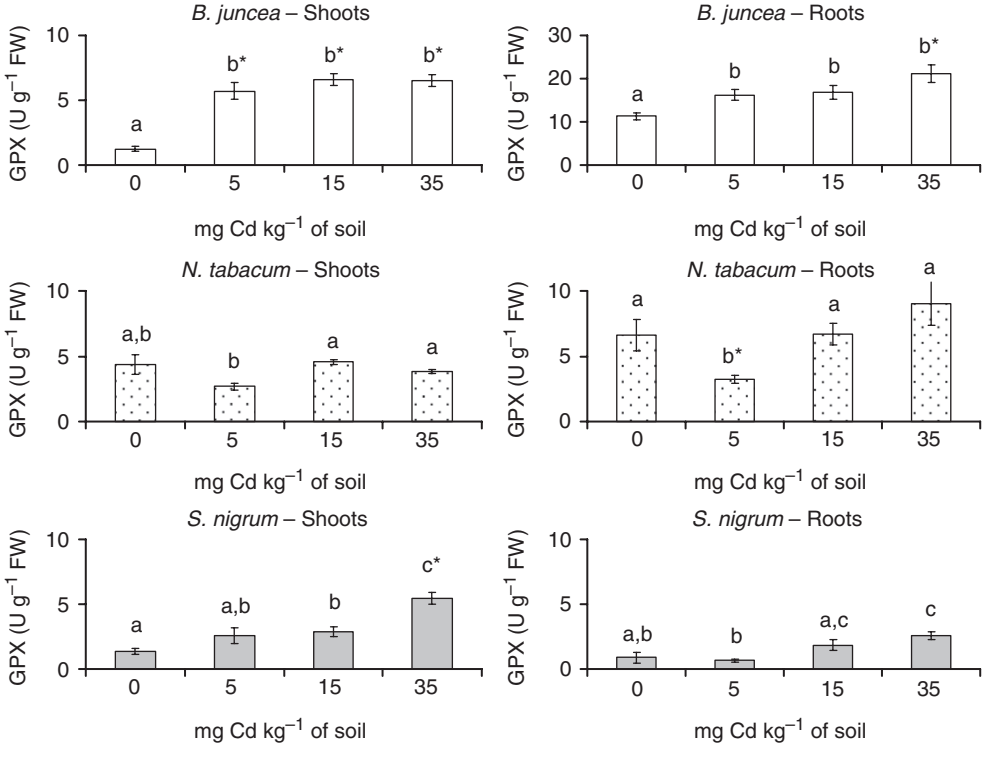

Figure 5. Effects of Cd treatments on the activities of GPX (U g^{-1} FW), in shoots and roots of *B. juncea*, *N. tabacum* and *S. nigrum*.
Notes: GPX = guaiacol peroxidase. FW = fresh weight.
Vertical bars indicate the standard error of mean values ($n = 3$).
Values with the same letter are not significantly different at $P < 0.05$.
Values with * indicate significant differences from the control at $P < 0.01$.

Superoxide dismutase (SOD) activity protects the cell against oxidative stress. SOD dismutes $O_2^{\bullet -}$ to H_2O_2, an excess of which may be subsequently detoxified by CAT, GPX or APX [63]. The balance between the activities of these enzymes in cells is crucial to determine the steady-state level of superoxide radicals and hydrogen peroxide [14]. An enhancement of SOD activity in plants grown in contaminated media has been detected in several studies. Shanker and Pathmanabhan [64] observed that chromium toxicity enhanced the levels of SOD, CAT and APX in sorghum grown for 10 days in nutrient solution. Lin *et al.* [32] observed an enhancement on SOD activity in leaves of wheat grown during 2 weeks in a soil with 33 mg Cd kg^{-1}. However, in soil experiments lasting 50 [65] and 120 days [52], the contamination by trace metals did not affect, or even decreased, the activity of SOD, with a simultaneous increase of APX and CAT due to the intensive reaction of plants against H_2O_2. In the present work, SOD activity at the end of the experiment (90 days) also did not increase significantly in the shoots or roots of any of the three plants exposed to Cd (data not shown). These results suggest that the period of the experiment plays an important role in SOD performance. The activity level detected in shoots and roots of the three plants, between 20 and 36 U g^{-1} of fresh weight (FW), indicates that SOD is still active on dismutation of superoxide and can be responsible for the formation of H_2O_2 used as substrate by CAT.

4. Conclusions

No reduction in plant root and shoot dry biomass of *Nicotiana tabacum* and *Brassica juncea* was noted when the plants were grown at cadmium concentration up to 15 mg Cd kg^{-1}. Comparing these two plants, *N. tabacum* presented a higher amount of Cd extracted per plant, bioconcentration factor (BF) and translocation factor (TF). With TF and BF being >1.0, *N. tabacum* plant can be classified as Cd-hyperaccumulator. On the other hand, the TF favoured *B. juncea* as a phytoextractant plant at very high levels of Cd, since TF increased with soil Cd contamination. The corresponding BF was always >1, showing an efficient accumulation of Cd.

It was also observed that *Solanum nigrum* showed the highest TF and BF for Cd concentration in soil of 5 mg Cd kg^{-1}. Higher soil Cd concentration promoted a higher Cd accumulation in roots, but restricted translocation into the shoots, resulting in a decrease of TF and BF. Although *S. nigrum* presented the highest control biomass, *B. juncea* and *N. tabacum* growth was less affected at concentrations of 35 mg Cd kg^{-1} in the soil.

The results obtained clearly showed the activation of important components of the enzymatic antioxidant defence mechanisms in the three species, especially in treatments with 15 and 35 mg Cd kg^{-1} of soil, as an adaptive response to reverse the effect of reactive oxygen species. However, this response was not enough to prevent oxidative damage, leading to an increase of lipid peroxidation. Different enzymes and activity levels in roots and shoots suggested that different antioxidant mechanisms were active in the various plant compartments.

Generally a significant enhancement was observed on lipid peroxidation and enzymatic activities of catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in shoots and roots of *B. juncea* and *S. nigrum* for the highest levels of Cd soil contamination, 15 and/or 35 mg Cd kg^{-1}. In *N. tabacum* shoots, the same trend was observed for CAT, APX and lipid peroxidation. CAT activity was significantly greater in shoots than in roots for all the three plants and all soil Cd concentrations, probably caused

by photosynthetic and photorespiration activity in leaves, leading to an enhanced H_2O_2 content. GPX seems to play a lead role in shoots of *B. juncea*, since the increment observed in the presence of Cd was much greater than those of APX and CAT.

Plant growth and enzymatic responses can be used to assess the ecotoxicity of soil contaminated by heavy metals such as cadmium. The growth of *S. nigrum* shoots was very sensitive to Cd, with significant reductions from $5\,mg\,Cd\,kg^{-1}$ of soil, due to the large values of Cd concentration found in shoots ($\geq 200\,mg\,Cd\,kg^{-1}$ of shoots DW). Compared to the other plants, *B. juncea* had the most sensitive response of GPX, with significant increases in this enzymatic activity in roots and shoots, for all Cd levels in the soil. *N. tabacum* was the plant that presented significant increases of CAT activity, in both shoots and roots.

Although *N. tabacum* and *S. nigrum* belong to the Solanaceae family (*B. juncea* belongs to the Cruciferae family), they did not present a common trend in enzymatic behaviour. It is well known that different species within the same family, or even different varieties within the species, can present very different responses of the anti-oxidant defence system.

The duration of the experiment seemed to play an important role for SOD performance. A dynamic experiment to monitor SOD activity changes with time would be helpful and it is suggested as future work.

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