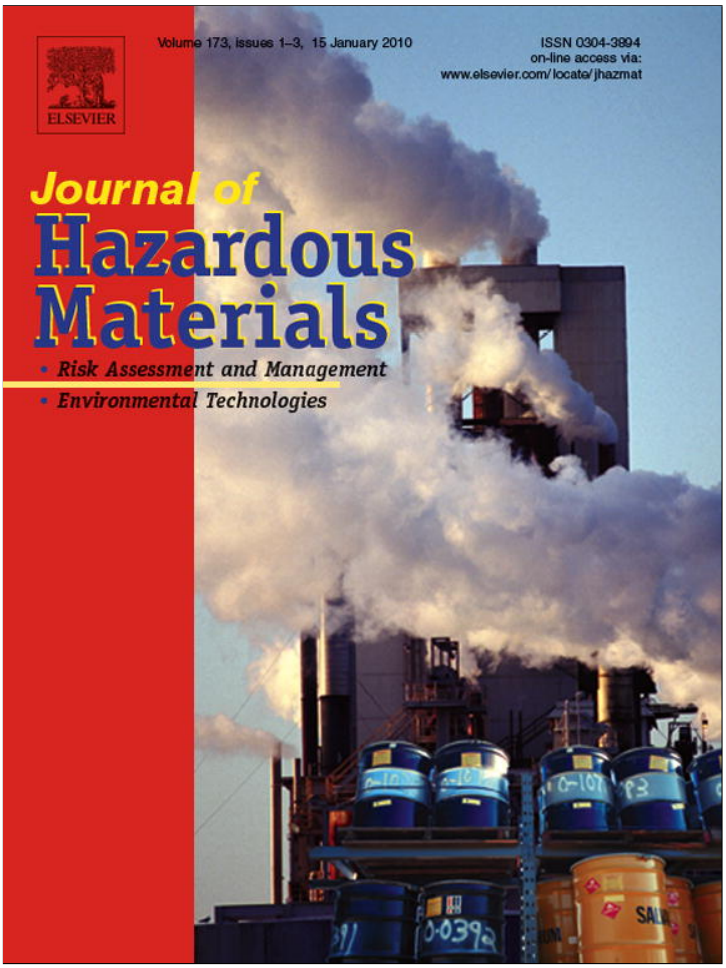

Journal of Hazardous Materials 173 (2010) 570–575

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research article

Improvement in soil and sorghum health following the application of polyacrylate polymers to a Cd-contaminated soil

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ARTICLE INFO

Article history:
Received 18 March 2009
Received in revised form 25 August 2009
Accepted 25 August 2009
Available online 31 August 2009

Keywords:
Cadmium
Enzymatic activities
Oxidative stress
Polyacrylate polymers
Sorghum

ABSTRACT

Contamination of soils with cadmium (Cd) is a serious global issue due to its high mobility and toxicity. We investigated the application of insoluble polyacrylate polymers to improve soil and plant health. Sorghum was grown in a Cd-contaminated sandy soil. Polyacrylate polymers at 0.2% (w/w) were added to half of the soil. Control soil without plants was also included in the experiment. Growth of sorghum was stimulated in the polymer-amended soil. The concentration of Cd in the shoots, and the activities of catalase and ascorbate peroxidase decreased in plants from polymer-amended soil compared with unamended control. The amount of $CaCl_2$-extractable Cd in the polymer-amended soil was 55% of that in the unamended soil. The Cd extracted in sorghum shoots was 0.19 mg per plant grown on soil without polymer and 0.41 mg per plant grown on polymer-amended soil. The total amount of Cd removed from each pot corresponded to 1.5 and more than 6% of soil $CaCl_2$-extractable Cd in unamended and polymer-amended soil, respectively. The activities of soil acid phosphatase, β-glucosidase, urease, protease and cellulase were greatest in polymer-amended soil with sorghum. In conclusion, the application of polyacrylate polymers to reduce the bioavailable Cd pool seems a promising method to enhance productivity and health of plants grown on Cd-contaminated soils.

1. Introduction

Soil contamination with cadmium (Cd) is a serious global issue due to its mobility and toxicity to plants and soil organisms. Furthermore, Cd may easily be transferred to the food chain and affect human health. The main sources of Cd are atmospheric deposition and some commercial phosphatic fertilizers, but other sources include municipal wastes and biosolids [1]. Inputs into soils exceed losses [2] and Cd content in soils is therefore likely to increase. The total content of Cd in soils varies between 0.01 and 1.1 mg Cd kg^{-1} DM, with higher values indicating anthropogenic contamination [3]. Cropped soils are considered safe if the total Cd concentration in the soil does not exceed 2 mg kg^{-1}, but above this level there are health risks for humans and animals [4]. Soil microbes are even more sensitive to Cd because they are in direct contact with the contaminated soil and can be damaged by levels of Cd above 0.08 mg kg^{-1} DM [4]. Soil microrganisms play an important role in maintaining soil fertility and health, as they are involved in biological cycles of important nutrients.

There is the need to select plants that can be grown in contaminated soils to retain metals and reduce losses via erosion or percolation through the soil profile [5]. Sorghum is relatively tolerant to Cd; in nutrient solution, biomass accumulation was even enhanced for low levels of Cd (up to 1 mg L^{-1} or 8.9 μM) and shoots had around 150 mg Cd kg^{-1} DM without any visual symptoms of toxicity [6]. Sorghum is the fifth most important cereal in the world, used as a main staple food or as animal feed, and the agronomic requirements for successful production are well established. It is a drought and heat tolerant plant [7] that can also be used as an energy crop for the production of bioethanol [8], which will be a major advantage if it were to be grown in contaminated soils.

Sorghum responds to Cd exposure with a significant increase of thiol groups inside the plant that can sequester the metal, but there is a maximum capacity for synthesis of these molecules in plant tissues [9]. Little is known about biochemical responses to Cd toxicity in sorghum, but metal toxicity in several species leads to an increased production of reactive oxygen species (ROS) [10]. ROS such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl

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doi:10.1016/j.jhazmat.2009.08.124

Table 1
Characteristics of the soil used in the experiment.

Texture	Sand
Organic C (g kg^{-1})	1.4
pH[a]	6.3
Total Cd (mg kg^{-1})	20.4
Extractable Cd (mg kg^{-1})[b]	9.8
Extractable P (mg kg^{-1})[c]	5.7
Extractable K (mg kg^{-1})[c]	25

[a] A water: soil ratio of 2.5 (v/m) was used.
[b] Extraction with 0.01 M $CaCl_2$.
[c] Extraction with 0.1 M ammonium lactate + 0.4 M acetic acid, pH 3.7.

radicals ($HO^{\bullet}$) and singlet oxygen ($^1O_2$), are produced in living cells and can be toxic at high concentrations because they can lead to lipid peroxidation, enzyme inactivation, membrane leakage and DNA damage [11]. Plants can activate several enzymatic defense pathways which include superoxide dismutase (SOD) to dissipate superoxide radicals, and catalase (CAT) and ascorbate peroxidase (APX), involved in the dissipation of H_2O_2 [10]. The balance between SOD, APX and CAT activities in cells is crucial to determine the steady-state level of superoxide radicals and hydrogen peroxide [12].

When soils are severely contaminated, amendments are needed to decrease the bioavailable pool and allow the establishment of plants [13]. Some of these amendments include additives used in agriculture such as lime and organic matter, but others are industrial products such as zeolites [14] or polyacrylate polymers. In artificially Cd-contaminated soils, polyacrylate polymers were able to remove at least 70% of the Cd in soil solution [15,16]. It is estimated that over 130 Gg of polyacrylates are used annually in diapers, paper towels and feminine products [17], and the lack of toxicity of these products is well established.

To test the effect of the application of insoluble polyacrylate polymers, it was necessary to choose indicators to monitor the improvement in soil and plant health. In the present work, we used plant growth, Cd concentration in the shoots and the activities of SOD, CAT and APX as indicators of plant health.

Biological indicators of soil health are numerous and include microbial activity and enzymatic activities related with nutrient cycling [18]. We chose to use soil dehydrogenase (microbial activity), soil enzymes related with C, N and P cycles, and bioavailable Cd to evaluate soil health.

The objectives of the present work were: (i) to test the use of polyacrylate polymer to decrease the Cd bioavailable pool in the soil, and enhance soil and plant health. (ii) To study the use of shorghum to extract Cd from the contaminated soil.

2. Materials and methods

2.1. Experimental set up

A Cd-contaminated soil (Haplic Podzol) from Pegões (38°24′N, 8°35′W), poor in organic matter and nutrients was used in the experiment (Table 1). This coarse-textured soil can be considered a long-term Cd-contaminated soil, as the extractable level of Cd had been stable over periods of months (data not shown).

The soil was passed through a 5-mm sieve and received a basal dressing of 125 mg P and 25 mg kg^{-1} of soil. The nutrients were supplied as calcium dihydrogen phosphate and magnesium sulphate, which were mixed thoroughly with the soil. Hydrophilic polyacrylate polymers $(-CH_2CHCOOH-)_n$ with K^+ (210 mg K g^{-1} of polymer) or NH_4^+ (100 mg N g^{-1} of polymer) as counter ions were added at 0.2% (0.1% of each polymer on a weight basis) to half of the soil at the time of the basal dressing. The polymers used were synthesized at our request by Marion-Roussel Ltd. They had linear chains with molecular weights of about 40 million, joined together by physical entanglements (i.e. without chemical cross-links).

The soil that received polymer was not supplied with additional K or N, while the other half received potassium sulphate and ammonium nitrate to compensate for the difference in K and N supply, so that all pots received 210 mg K and 100 mg N kg^{-1} DM of soil.

For each treatment (with or without polymer), eight plastic pots were filled with 10 kg of soil. Sorghum (*Sorghum bicolor* (L.) Moench) was sown on May 2007 in half of the pots for each treatment, and plant number was adjusted to 8 per pot two weeks later. The pots were kept in an outside area protected with a net (minimum temperature: 13 °C; maximum temperature: 30 °C). Pots were weighed every other day and water added so that water content varied between 70 and 80% of the maximum water retention capacity.

Plant shoots (above-ground parts) were harvested two months after sowing, weighed, and cut into 5-cm fragments. Part of the plant material was immediately frozen in liquid nitrogen and kept at −75 °C until analyzed for enzymatic activity. The remaining plant material was weighed, dried at 65 °C, and weighed again.

The soil was removed from each pot, mixed, and a sample taken for analysis.

2.2. Plant analysis

Dried shoots were ground and 0.5 g samples were digested with 5 mL of concentrated nitric acid in a microwave oven (CEM MDS-2000) for 45 min. The solutions were then analyzed for Cd by electrothermal atomic absorption spectrophotometry (Unicam Solaar M).

Frozen shoots samples (0.5 g) were quickly macerated with a pestle and mortar in the cold (below 4 °C) with 1 mL of 100 mM Tris–HCl buffer (pH 7.8) containing 3 mM dithiothreithol and 1 mM EDTA, and in the presence of 2% (w/w) insoluble polyvinylpolypyrollidone. Crude extracts for ascorbate peroxidase activity also contained 10 mM ascorbate. The homogenate was centrifuged (10,000 × g for 30 min) and the supernatant filtered (0.20 μm). Two independent samples were analyzed from the frozen material corresponding to each pot (a total of eight analyses per treatment).

Catalase activity (EC 1.11.1.6) was determined according to Aebi [19] by measuring the decrease in absorbance at 240 nm for 2 min, in a solution containing 10 mM of H_2O_2 in 50 mM phosphate buffer (pH 7.0). One unit (U) of enzymatic activity was defined as the consumption of 1 μmol H_2O_2 per min and per cm^3 using a coefficient of absorbance of 39.4 mM^{-1} cm^{-1}.

Superoxide dismutase activity (EC 1.15.1.1) was determined by measuring the increase in absorbance at 550 nm for 2 min in a solution containing 0.5 mM xanthine, 0.05 mM ferricytochrome c, 0.1 mM EDTA and xanthine oxidase in 100 mM potassium phosphate buffer (pH 7.6) according to Rubio et al. [20]. One unit (U) of enzymatic activity was defined as the quantity of enzyme needed to inhibit the reduction of ferricytochrome-C by 50% per minute.

Ascorbate peroxidase (EC 1.11.1.11) activity was determined according to Ali et al. [21] in a reaction mixture contained 0.25 mM ascorbic acid and 0.3 mM hydrogen peroxide in 50 mM phosphate buffer (pH 7.0), following the decrease in absorbance at 290 nm. One unit (U) of enzymatic activity was defined as the consumption of 1 μmol ascorbate per min and per cm^3 using a coefficient of absorbance of 2.8 mM^{-1} cm^{-1}.

All absorbance determinations were measured using a Hitachi U-2000 UV/Vis Spectrophotometer (Tokyo, Japan). The enzymatic activities of catalase, superoxide dismutase and ascorbate peroxidase were reported as units (U) per g of fresh weight.

2.3. Soil analysis

All soil samples were sieved (<2 mm) before analysis. Fresh soil samples were immediately analyzed for dehydrogenase activity according to Tabatabai [22]. Soils were incubated for 16 h, at 25 °C, with 0.1% (w/v) triphenyltetrazolium chloride in Tris-buffer (0.1 M, pH 7.6), and the triphenylformazan formed was quantified spectrophotometrically, at 546 nm. Dehydrogenase activity corresponds to the overall activity of intracellular enzymes that catalyze oxidoreduction reactions of organic compounds.

Air-dried samples were used to measure pH in water (water:soil ratio of 2.5, v/w) and extractable Cd. Five grams of soil were shaken with 50 mL of 0.01 M $CaCl_2$ for 2 h and the suspension was filtered and analyzed for Cd by electrothermal atomic absorption spectrophotometry (Unicam Solaar M). Two independent replicates were performed for each sample and blanks were measured in parallel. $CaCl_2$-extractable Cd is considered as the mobile fraction, sometimes referred to as the "effective bioavailable metal fraction" [23].

Other soil samples were frozen at −20 °C until analyzed for several enzymatic activities. Cellulases were determined according to Hope and Burns [24]. In the context of this work, the term refers to the combined action of endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase and β-D-glucosidase on Avicel (Fluka), a purified depolymerised alpha cellulose, for 16 h at 40 °C, in a 0.1 M acetate buffer (pH 5.5, 0.2% NaN_3). The released reducing sugars were determined spectrophotometrically at 520 nm, after the addition of copper (II) and molybdo-arsenate.

Acid phosphomonoesterase (EC 3.1.3.2) and β-glucosidase (EC 3.2.1.21) were measured by incubating the soil with a substrate containing a *p*-nitrophenyl moiety [25,26]. For acid phosphatase, the soil was incubated with *p*-nitrophenyl phosphate in modified universal buffer (pH 6.5) at 37 °C. After 1 h, 0.5 M $CaCl_2$ was added to stop the reaction and the *p*-nitrophenol released was extracted with 0.5 M NaOH and measured spectrophotometrically at 400 nm. For β-glucosidase the procedure was the same, except that the substrate used was *p*-nitrophenyl-β-D-glucopyranoside, and the *p*-nitrophenol released was extracted with 0.1 M THAM-NaOH, pH 12.0.

Acid phosphomonoesterase (acid phosphatase) catalyses the hydrolysis of organic P esters into inorganic P, and β-glucosidase catalyses the hydrolysis of carbohydrates with β-D-glucoside bonds like cellobiose, providing energy substrates for soil heterotrophic microrganisms.

Urease (EC 3.5.1.5) was determined according to Kandeler and Gerber [27], by measuring the NH_3 released after incubating the soil with a solution of urea for 2 h at 37 °C, in a borate buffer (pH 10). The ammonium content of the centrifugated extracts was determined spectrophotometrically, at 690 nm, after reaction with sodium dicloroisocyanide 0.1%. Urease catalyses the hydrolysis of urea to CO_2 and NH_3.

Protease activity was determined after the incubation of 1 g of soil with sodium caseynate (2%, w/v) in Tris-buffer pH 8.1, during 2 h at 50 °C [28]. The tyrosine formed reacted with Folin-phenol reagent to form a blue complex, which was determined spectrophotometricaly at 700 nm. The term protease includes several enzymes that catalyze the hydrolysis of proteins and oligopeptides to amino acids.

Table 2
Biomass accumulation, and Cd concentration and content in shoots of sorghum grown in a Cd-contaminated soil, with or without application of polyacrylate polymers.

Polymer	Dry weight (g per pot)	Cd concentration (mg kg^{-1} DW)	Cd present in shoots (mg per plant)
–	14.8 b	100.4 a	0.19 b
+	93.5 a	35.3 b	0.41 a

Values in a column followed by the same letter are not significantly different as estimated by the Newman–Keuls test at $p < 0.05$.

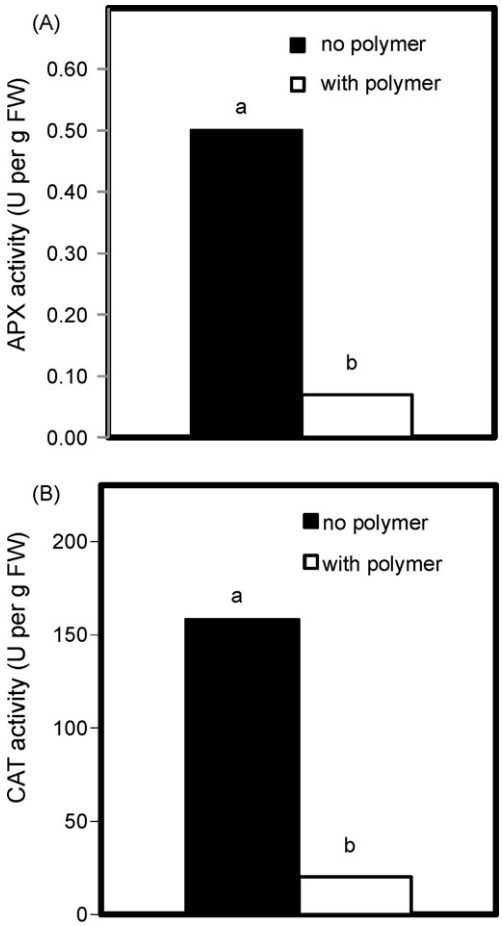

Fig. 1. Activities of plant enzymes related to oxidative stress defense mechanisms. (A) Catalase; (B) ascorbate peroxidase. In each figure, values followed by the same letter are not significantly different as estimated by the Newman–Keuls test at $p < 0.05$.

2.4. Statistics

All data were analyzed for variance by the General Linear Model (GLM) and mean separation was performed using the Newman–Keuls test at $p \leq 0.05$.

3. Results

Growth of sorghum was considerably greater in polymer-amended soil than in unamended control (Table 2). In contrast, Cd concentration was smaller in the shoots of plants from polymer-amended soil than in those from soil without polymer.

The activities of plant enzymes related to oxidative stress defense mechanisms responded differently to polymer application to soil. CAT and APX had greater activities in plants from unamended soil than in those from soil with polymer (Fig. 1). In contrast, SOD activity did not respond to treatment and had an average value of 0.05 U g^{-1} fresh weight (data not shown).

The amount of $CaCl_2$-extractable Cd present in the unamended soil was about 1.8 times greater than that from soil amended with polymer (Fig. 2A). The presence of sorghum did not lead to any significant changes in $CaCl_2$-extractable Cd.

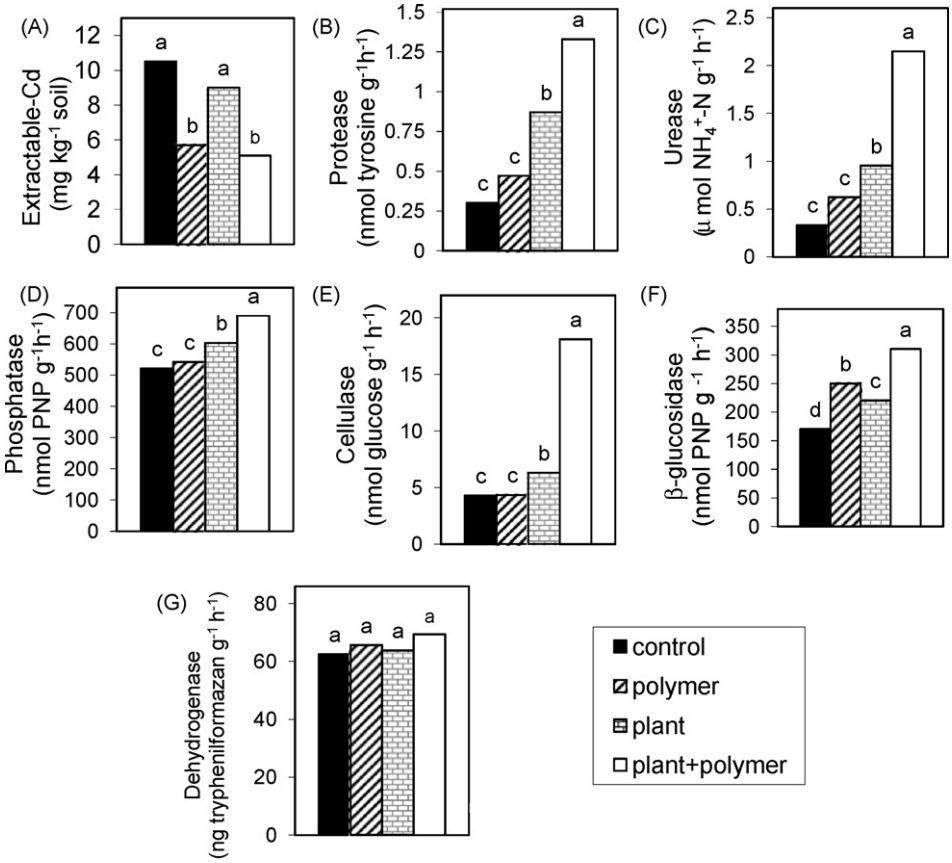

Fig. 2. Soil $CaCl_2$-extractable Cd and activity of hydrolytic enzymes expressed per hour and gram of dry soil. In each figure, values followed by the same letter are not significantly different as estimated by the Newman–Keuls test at $p < 0.05$.

Although the Cd bioavailable pool decreased following polymer application, more Cd was extracted by sorghum (0.41 mg per plant) from polymer-amended soil than from soil without polymer (0.19 mg per plant). This resulted from the greater biomass accumulation by sorghum in the polymer-amended soil. In terms of the amounts of Cd removed per kilogram of soil, these corresponded to 0.33 and 0.15 mg, respectively. In turn, the values represented 1.5% of the $CaCl_2$-extractable Cd in unamended soil, and more than 6% in the polymer-amended soil.

The dehydrogenase activity did not respond to treatments and had an overall average value of 65 ng triphenylformazan g^{-1} h^{-1} (Fig. 2G).

The activities of all other soil enzymes tested (protease, urease, acid phosphatase, β-glucosidase and cellulase) had the greatest values in polymer-amended soil with sorghum (Fig. 2). β-Glucosidase was stimulated by polymer application even in the absence of sorghum (Fig. 2F). The other enzymes were consistently stimulated by the presence of sorghum, even in unamended soil.

4. Discussion

The soil used in the experiment had a total concentration of Cd of about 20 mg kg^{-1} DM, of which approximately half was $CaCl_2$-extractable and could be considered as bioavailable (Table 1). Depending on soil properties, level of contamination, and the method used for Cd extraction, the bioavailable fraction of Cd in soils can vary from less than 10% to more than 80% [29,30,31]. A soil with 20 mg Cd kg^{-1} should not be used to grow edible crops, as it exceeds the maximum limits for Cd in arable soils that range between 0.4 and 3 mg kg^{-1} [32]. Soils severely contaminated have either to be set aside, be subjected to remediation, or be used for alternative crops such as those to be used for biofuel production.

The results show that sorghum can be grown in a Cd-contaminated soil, although growth was impaired when plants were grown in unamended soil compared with plants from soil receiving polymer (Table 2). In fact, polymer application to the soil led to a six-fold increase in biomass accumulation by sorghum. Therefore, plant growth was greatly stimulated when the soil Cd bioavailable pool was depleted by application of polyacrylate polymers. Polyacrylate polymers contain many carboxyl groups capable of forming chelates with metals. In fact, applying polymers to the soil decreased the $CaCl_2$-extractable Cd by more than 40% (Fig. 2A). Considering it is a long-term contaminated soil, this result compares favourably with the depletion of 70% of the Cd in soil solution in artificially Cd-contaminated soils reported previously [15,16].

The concentration of Cd in the shoots of sorghum grown in polymer-amended soil was 35 mg kg^{-1} compared with 100 mg kg^{-1} in plants from unamended soil (Table 2). Together with the changes in biomass, these results show that application of polyacrylate polymers protects plants from excessive levels of Cd.

Although there is no definite understanding of the biochemical responses of plants to Cd, it is now generally accepted that Cd toxicity is associated with an increase in ROS [33,34]. These species are constantly produced due to leakages from the electron transport chain, but plants usually keep ROS at acceptable levels with antioxidative enzymatic responses. Few studies on Cd toxicity have been carried out in soil-grown plants and no information is available for sorghum. It is known, however, that chromium at toxic levels enhances the levels of SOD, CAT and APX in sorghum grown for 10 days in a nutrient solution [35].

In the present experiment, the activity of SOD was not affected by treatment. In wheat an increase in SOD activity in leaves after two weeks of growth was only observed for a level of Cd of 33 mg kg^{-1} [33]. This might indicate that there was no excessive accumulation of superoxide anions in sorghum or that after two months of plant growth these had been dissipated. Alternatively, the decrease in the Cd concentration in the shoots in plants from polymer-amended soil was not sufficient to cause a change in this enzymatic activity.

In contrast, the activities of CAT and APX were affected by treatment (Fig. 1). Both these enzymes are involved in intracellular H_2O_2 removal. CAT is exclusively present in peroxisomes while APX is present in almost all cellular compartments [12]. CAT and APX activities increased in several plant species exposed to Cd [10,33,36] and CAT transcripts were induced in pea plants exposed to Cd [34].

In the present work, the increase of both CAT and APX activities seemed to be important to prevent oxidative damage caused by excess H_2O_2. The lower concentration of Cd in plants grown in polymer-amended soil seemed to decrease the need for CAT and APX in sorghum cells. In fact, in sorghum from unamended soil the activities of these enzymes were about eight times greater than in plants from polymer-amended soil (Fig. 1). In *Arabidopsis thaliana* the major cause of oxidative stress was the accumulation of hydrogen peroxide, and APX seemed to have a crucial role in Cd tolerance [37].

Cadmium is considered particular toxic towards soil microrganisms, although severe adverse effects have generally only been detected for high Cd concentrations. For example, Moreno et al. [38] reported that dehydrogenase activity only decreased for Cd concentrations greater than 800 mg kg^{-1} of soil. In the present experiment, the treatment (application of polyacrylate polymers and a plant cover) had no effect on the overall activity of the microbial population, as there were no significant differences in dehydrogenase activity (Fig. 2G). Chronic exposure to trace elements may change microbial communities with an increase in metal-resistant species [39]. Alternatively, physiological adaptations may take place, such as physical exclusion or intracellular sequestration [40]. Either the microrganisms present were well adapted to high levels of Cd, or the microbial community changed as a result of the treatment but maintained a similar overall activity.

Although the microbial activity was unchanged by treatment, activities of soil enzymes related to C, N and P cycles were increased by polymer application and the presence of sorghum (Fig. 2B–F). There are two possible explanations for this increase. First, plant exudates could contribute to the labile organic matter pool, providing new sources of nutrients to soil microrganisms. The increase in all enzymes when sorghum was present (both in unamended and polymer-amended soil) supports this hypothesis. However, although sorghum exudates are capable of chelating Cd this seemed to result in enhanced bioavailability of Cd, and not the opposite [9]. Second, Cd has a particular affinity for sulfhydryl groups and also reacts with hydroxyl groups and nitrogen-containing ligands. Consequently, the metal ion can inactivate enzymes such as acid phosphatases [41]. The decrease in bioavailable Cd due to polymer application could thus result in a greater activity of hydrolytic soil enzymes, although only β-glucosidase was stimulated by polymer application in the absence of sorghum.

Sorghum grown in unamended soil had a Cd concentration in shoots of 100 mg kg^{-1} (Table 2), the threshold value for hyperaccumulators indicated by Baker et al. [42]. However, plant biomass was greatly impaired, and the amount of Cd extracted from the soil and accumulated in the shoots was only 0.15 mg kg^{-1} of soil, corresponding to just 1.5% of the bioavailable fraction (extracted with $CaCl_2$).

In polymer-amended soil, biomass accumulation was greatly enhanced, so that over a short period of just two months sorghum extracted 0.33 mg Cd kg^{-1} of soil, corresponding to more than 6% of the bioavailable pool. Although the Cd concentration in sorghum shoots was smaller than in plants from unamended soil (Table 2), it was still well over the threshold value of 10 mg kg^{-1} needed for a phytoextraction process to be considered feasible [43].

5. Conclusions

Growth and health of sorghum was greatly improved following the application of insoluble polyacrylate polymers to a Cd-contaminated soil. The concentration of Cd in the shoots and the activities of catalase and ascorbate peroxidase were smaller in plants from amended soil. The presence of a plant cover and the decrease in soil bioavailable Cd following polymer application resulted in improved soil health as shown by a greater activity of soil hydrolytic enzymes related to C, N and P cycles. Sorghum could be used to extract Cd from the soil as the bioavailable soil pool was depleted by more than 6% over a period of just two months.

Acknowledgements

This study was funded by projects PPCDT/AMB/57586/2004 and PPCDT/AMB/55312/2004 from the Portuguese Foundation for Science and Technology (FCT). Qu Guiwei and Ana Isabel Cardoso are grateful for the grants from FCT.

References

[1] A. Keller, R. Schulin, Modelling regional-scale mass balances of phosphorus, cadmium and zinc fluxes on arable and dairy farms, Euro. J. Agron. 20 (2003) 181–198.
[2] S.W. Moolenaar, P. Beltrami, Heavy metal balances of an Italian soil as affected by sewage sludge and Bordeaux mixture application, J. Environ. Qual. 27 (1998) 828–835.
[3] P.C. Srivastava, U.C. Gupta, Trace Elements in Crop Production, Science Publishers Inc., Lebanon, USA, 1996.
[4] R.W. Scholz, U. Schnabel, Decision making under uncertainty in case of soil remediation, J. Environ. Mag. 80 (2006) 132–147.
[5] I.D. Pulford, C. Watson, Phytoremediation of heavy metal-contaminated land by trees—a review, Environ. Int. 29 (2003) 529–540.
[6] A.P. Pinto, A.M. Mota, A. de Varennes, F.C. Pinto, Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants, Sci. Total Environ. 326 (2004) 239–247.
[7] G. Tuck, M.J. Glendining, P. Smith, J.I. House, M. Wattenbach, The potential distribution of bioenergy crops in Europe under present and future climate, Biomass Energy 30 (2006) 183–197.
[8] L. Barbanti, S. Grandi, A. Vecchi, G. Venturi, Sweet and fibre sorghum (*Sorghum bicolor* (L.) Moench), energy crops in the frame of environmental protection from excessive nitrogen loads, Euro. J. Agron. 25 (2006) 30–39.
[9] A.P. Pinto, A. de Varennes, M.L.S. Gonçalves, A.M. Mota, Sorghum detoxification mechanisms, J. Plant Nutr. 29 (2006) 1229–1242.
[10] P.L. Gratão, A. Polle, P.J. Lea, R.A. Azevedo, Making the life of heavy metal stressed plants a little easier, Funct. Plant Biol. 32 (2005) 481–494.
[11] I.M. Møller, P.E. Jensen, A. Hansson, Oxidative modifications to cellular components in plants, Annu. Rev. Plant. Biol. 58 (2007) 459–481.
[12] R. Mittler, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 7 (2002) 405–410.
[13] D.C. Adriano, W.W. Wenzel, J. Vangronsveld, N.S. Bolan, Role of assisted natural remediation in environmental cleanup, Geoderma 122 (2004) 121–142.
[14] W. Friesl, E. Lombi, O. Horak, W. Wenzel, Immobilization of heavy metals in soils using inorganic amendments in a greenhouse study, J. Plant Nutr. Soil Sci. 166 (2003) 191–196.
[15] C. Lindim, A. de Varennes, M.O. Torres, A.M. Mota, Remediation of a sandy soil artificially contaminated with cadmium using a polyacrylate polymer, Commun. Soil Sci. Plant Anal. 32 (2001) 1567–1574.
[16] A. de Varennes, M.J. Goss, M. Mourato, Remediation of a sandy soil contaminated with cadmium, nickel and zinc using an insoluble polyacrylate polymer, Commun. Soil Sci. Plant Anal. 37 (2006) 1639–1649.
[17] E. Martin, Environmental impact studies of the disposal of polyacrylate polymers used in consumer products, Sci. Total Environ. 19 (1996) 225–234.

[18] F. Gil-Sotres, C. Trasar-Cepeda, M.C. Leirós, S. Seoane, Different approaches to evaluating soil quality using biochemical properties, Soil Biol. Biochem. 37 (2005) 877–887.
[19] H.E. Aebi, Catalase, in: U.S. Bergmeyer (Ed.), Methods of Enzymatic Analysis, vol III: Oxidoreductases, Transferases, Verlag Chemie, Weinheim, 1983, pp. 273–277.
[20] M.C. Rubio, E.M. Gonzalez, F.R. Minchin, K.J. Webb, C. Arrese-Igor, J. Ramos, M. Becana, Effects of water stress on antioxidant enzymes of leaves and nodules of transgenic alfalfa overexpressing superoxide dismutases, Physiol. Plant. 115 (2002) 531–540.
[21] M.B. Ali, N. Singh, A.M. Shohael, E.J. Hahn, K.Y. Paek, Phenolics metabolism and lignin synthesis in root suspension cultures of Panax ginseng in response to copper stress, Plant Sci. 171 (2006) 147–154.
[22] M.A. Tabatabai, Soil enzymes, in: S.H. Mickelson, J.M. Bigham (Eds.), Methods of Soil Analysis, Part 2, Soil Science Society of America, Madison, WI, USA, 1994, pp. 775–833.
[23] M. Pueyo, J.F. López-Sanchez, G. Raurest, Assessment of $CaCl_2$, $NaNO_3$ and NH_4NO_3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils, Anal. Chim. Acta 504 (2004) 217–226.
[24] C.F.A. Hope, R.G. Burns, Activity, origins and location of cellulase in a silt loam soil, Biol. Fertil. Soils 5 (1987) 164–170.
[25] F. Eivazi, M.A. Tabatabai, Phosphatases in soils, Soil Biol. Biochem. 9 (1977) 167–172.
[26] F. Eivazi, M.A. Tabatabai, Glucosidases and galactosidases in soils, Soil Biol. Biochem. 20 (1988) 601–606.
[27] E. Kandeler, H. Gerber, Short-term assay of soil urease activity using colorimetric determination of ammonium, Biol. Fertil. Soils 6 (1988) 68–72.
[28] J.N. Ladd, J.H.A. Butler, Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates, Soil Biol. Biochem. 4 (1972) 19–30.
[29] S.B. Pandeya, A.K. Singh, P. Jha, Labile pools of cadmium in sludge-treated soils, Plant Soil 203 (1998) 1–13.
[30] E. Smolders, K. Brans, A. Foldi, R. Merckx, Cadmium fixation in soils measured by isotopic dilution, Soil Sci. Soc. Am. J. 63 (1999) 78–85.
[31] A.L. Nolan, M.J. McLaughlin, S.D. Mason, Chemical speciation of Zn, Cd, Cu and Pb in pore waters of agricultural and contaminated soils using Donnan dialysis, Environ. Sci. Technol. 37 (2003) 90–98.
[32] COM, Report from the Commission to the Council and the European Parliament on the Implementation of Community Waste Legislation for the Period 1998–2000, Brussels, 2003, 11.7.2003, 250 final/3.
[33] R. Lin, X. Wang, Y. Luo, W. Du, H. Guo, D. Yin, Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.), Chemosphere 69 (2007) 89–98.
[34] M.C. Romero-Puertas, F.C. Corpas, M. Rodríguez-Serrano, M. Gómez, L.A. del Rio, L.M. Sandalio, Differential expresión and regulation of antioxidative enzymes by cadmium in pea plants, J. Plant Physiol. 164 (2007) 1346–1357.
[35] A. Shanker, G. Pathmanabhan, Speciation dependant antioxidative response in roots and leaves of sorghum (*Sorghum bicolor* (L.) Moench cv CO 27) under Cr (III) and Cr (VI) stress, Plant Soil 265 (2004) 141–151.
[36] P. Kumar, R.K. Tewari, P.N. Sharma, Cadmium enhances generation of hydrogen peroxide and amplifies activities of catalase, peroxidases and superoxide dismutase in maize, J. Agron. Crop Sci. 194 (2008) 72–80.
[37] U.-H. Cho, N.-H. Seo, Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation, Plant Sci. 168 (2005) 113–120.
[38] J.L. Moreno, T. Hernandez, C. Garcia, Effects of cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil, Biol. Fertil. Soils 28 (1999) 230–237.
[39] M. Mergeay, Bacteria adapted to industrial biotopes: metal-resistant Ralstonia, in: G. Storz, R. Hengge-Aronis (Eds.), Bacterial Stress Responses, ASM Press, 2000, pp. 403–414.
[40] G.M. Gadd, Interactions of fungi with toxic metals, New Phytol. 124 (1993) 25–60.
[41] N.G. Juma, M.A. Tabatabai, Distribution of phosphomonoesterases in soils, Soil Sci. 126 (1978) 101–108.
[42] A.J.M. Baker, S.O. McGrath, R.D. Reeves, J.A.C. Smith, Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils, in: N. Terry, C. Bañuelos (Eds.), Phytoremediation of Contaminated Soil and Water, Lewis Publisher, Boca Raton, USA, 2000, pp. 85–107.
[43] S.P. McGrath, E. Lombi, C.W. Gray, N. Caille, S.J. Dunham, F.J. Zhao, Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*, Environ. Pol. 141 (2006) 115–125.