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Effects of heavy metal resistant soil microbes inoculation and soil Cd concentration on growth and metal uptake of millet, couch grass and alfalfa

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Effects of cadmium (Cd) concentration and inoculation of heavy metal-resistant soil microbes on plant growth and Cd-accumulation by millet (*Pennisetum glaucum*), alfalfa (*Medicago sativa*) and couch grass (*Triticum repens*) were studied. A soil sample (Soil 1) was spiked with different (0 to 100 mg kg⁻¹) concentrations of Cd and incubated under periodic wetting-drying (WD) cycles for near seven months period. Another soil sample with a historical background of metal contamination (Soil 2), having heavy metals-resistant microbial communities, also was taken and used as inoculum. Considering the abundance of arbuscular mycorrhizal (AM) spores and rhizobacteria (RBs), 200 g of soil 2 was added to each 1 kg of Soil 1 as inoculum. For blank samples (samples without stress-adapted microbes), Soil 2 was sterilized prior to adding to Soil 1. This soil substrate was aged under WD cycles for further 3 months. The plants were grown in pots containing contaminated soils. At the end of growth period, plants shoots were harvested, washed, oven-dried, and grinded. Wet oxidation method was used for extraction of plant Cd. In order to test the phytotoxic effect of Cd and/or the effect of inclusion of stress-adopted microbes to soil on plant biomass production, the plants yield reductions were calculated. Bioconcentration factors (BCF) of soil Cd by plants were also calculated to estimate the potential uptake of Cd by the plants. According to the results, Cd showed considerable affinity for the studied soil. When no Cd was added to the soil, the introduction of the microbes caused a significant decline in plant biomass when compared to the non treated soil that could be attributed to the increased access of plants to the relatively immobile Cd existed in the soil and also to more metal contaminants absorption caused by the microbes. In most treatments, by increasing concentrations of Cd, there was obvious yield reduction. Also, the most tolerant and sensitive host plant against Cd contamination was couch grass and millet, respectively.

Key words: Cd contamination, phytotoxicity, plant metal uptake, metal-resistant microbes.

INTRODUCTION

In recent years, as a result of various industrial activities, the contamination of environmental components with chemical elements and compounds became a serious problem. Cadmium (Cd,) a heavy metal with an unknown

essential biological function, is one of the most toxic pollutants of the environment (Das et al., 1997; Oliver, 1997). Cd is a highly toxic and mobile element, easily absorbed by roots and transported to and uniformly distributed in shoots (Sekara et al., 2005). Consequently, the accumulation of Cd in soils has become a major concern as environmental viewpoint and food production.

Cd inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops

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and then enters the food chain with a significant potential to impair animal and human health (di Toppi and Gabrielli, 1999). Cd concentration in plants is related to the concentration of its forms available to plants (Das et al., 1997; Oliver, 1997). The bioavailability of Cd in soils is dependent on the partition of the metal between the solid and liquid phases. The sorption of the metal by surfaces of organic and inorganic soil constituents is largely responsible for the partition (Mullen et al., 1989; Kurek et al., 1996; Ledin et al., 1999). Plants provide valuable tools for reclamation of polluted soils through the so-called phytoremediation technology which enhances soil quality and improve recovery and re-establishment of biotic activities (Cunningham et al., 1997; Garbisu et al., 2002; Glick, 2003).

Phytoremediation method usually is time consuming process which is mostly as a result of low bioavailability of heavy metals in the soil environment and/or low biomass of hyperaccumulators (Cunningham et al., 1997; Khodaverdilo and Homae, 2008). It is well known that arbuscular mycorrhizal (AM) fungi and rhizobacteria (RBs) are ubiquitous in agricultural and natural ecosystems (Brundrett 1991, 2002) and that most plant species form symbiotic associations with these fungi (Newman and Reddell 1987) and bacteria (Belimove et al., 2004; Arshad et al., 2007). AM fungi forming obligatory mutualistic symbiotic associations with a range of plant species which enhance uptake of nutrient elements as well as water by host plants through their extraradical mycelial networks (Marschner and Dell, 1994). Numerous studies have also indicated that AM fungi and RBs can decrease the metal uptake of the host plants, thus protecting them against heavy metal toxicity (Leyval et al., 1997; Fein et al., 2001; Zaidi and Musarrat, 2004). The number of spores and subsequently root colonization of host plants are often reduced by soil disturbance (Waaland and Allen, 1987). However, AM fungal species and RBs which adapted to local soil conditions could be able to stimulate plant growth better than non-indigenous species. Indigenous AM fungal and RBs ecotypes result from long-term adaptation to soils with extreme properties (Sylvia and Williams, 1992; Bae et al., 2003). Therefore, inoculation of plants with indigenous and presumably stress-adopted AM fungi and RBs can be a potential biotechnological tool for successful restoration of degraded ecosystems (Dodd and Thompson, 1994; Mathur et al., 2007).

The aim of this study was to assess the effects of heavy metal resistant soil microbes inoculation and soil Cd concentration on growth and metal uptake of millet (*Pennisetum glaucum*), couch grass (*Triticum repens*) and alfalfa (*Medicago sativa*).

MATERIALS AND METHODS

Soils and their physicochemical and biological analyses

A soil sample belonging to Typic Calcixerepts subgroup according

to USDA Soil Taxonomy (Soil Survey Staff, 1999) was taken from Western Azerbaijan province, Iran (Soil 1).

To quantify the capacity of Soil 1 to sorb and immobilization of Cd, a 24 h batch equilibrium experiment was conducted using Cd solutions. For this purpose 2.5 g of soil samples were equilibrated with 25 ml of 0.01 M CaCl₂ solutions (as background electrolyte) including 0.5, 2, 6, and 10 mg Cd L⁻¹ (to provide Cd loading quantities of 5, 20, 60, and 100 mg kg⁻¹ soil, respectively) in 50-ml centrifuge tubes on a reciprocating shaker at 25°C temperature. The suspensions were centrifuged, then supernatants were filtered through Whatman No. 42 filter papers. Three replicates were used for collecting data. Amounts of Cd in supernatants were measured by flame atomic absorption spectrophotometry. Also, amount of absorbed Cd was calculated as the difference between the initial and final concentration.

To derive the Cd desorption isotherms, the soil retained after the centrifugation of Cd sorption were re-suspended in 30 ml of 0.01 M CaCl₂ solution and Cd was desorbed from the soil solid phase by the same procedure of shaking, centrifugation, filtration, and analyzing for Cd as described previously for the sorption stage. The amount of Cd retained (that is, remained sorbed after desorption test) was calculated as the difference between the Cd sorbed and the Cd desorbed into the solution in desorption stage.

The relative percentage change or sorption intensity (SI) was also used to compare Cd sorption capacity of the soil samples (Xiong et al., 2005). It can be calculated by taking the difference between the initial and equilibrium Cd concentrations, then dividing this difference by the initial Cd concentration, and finally expressing this as a percentage by multiplying by 100 for each initial concentration used (Sipos, 2009).

Percentage of Cd immobilized by soil samples (IP) was calculated by taking the difference between sorbed (C_s) and desorbed (C_{de}) amounts of Cd and dividing this difference by the sorbed Cd, and then expressing this as a percentage for each initial concentration used.

Samples of Soil 1 were passed through a 5 mm sieve before applying Cd treatments. The soils were then thoroughly mixed in plastic pots with Cd(NO₃)₂ in powder form. For each soil, Cd salt was ground and mixed well with a small portion of soil, and this metal/soil mixture was then thoroughly mixed with a large amount of soil in order to obtain total Cd concentrations of 5, 20, 60, and 100 mg kg⁻¹ soil. The spiked soils were subsequently packed into some plastic pots. The packed soils were incubated in a moisture regime entailing periodic wetting-drying (WD) cycles for near seven months period in room temperature. In each WD cycle, soils were saturated in pots (pot-saturation) and allowed to be air-dried to relatively constant moisture. To avoid leaching out of Cd, no drainage pathway was allowed. Each WD cycle lasted for 40 days. After each WD cycle, the soil was mixed thoroughly to ensure homogeneity of soil Cd.

A soil sample of alfalfa rhizosphere in vicinity of Pb, Zn-smelter from Zanjan province, Iran was also collected (Soil 2). This soil sample had a historical background of metal contamination. Also, it was supposed to have microbial communities with adaptability to heavy metals contamination stress and used as inoculum. AM fungal spores as well as RBs were isolated, identified, and abundant fungal species found in contaminated soil (Soil 2) were recorded. AM fungal spores were isolated using wet-sieving and centrifugation in sucrose solution (50%) technique (Gerdemann and Nicolson, 1963). Then fungal species were identified using synoptic keys of Raman and Mohankumar (Raman and Mohankumar, 1988), as well as Schenck and Perez (Schenck and Perez, 1987). The population of RBs including *Pseudomonas*, *Bacillus* and *Streptomyces* were 0.36 × 10³, 0.15 × 10³ and 0.23 × 10³ per 10 g of soil, respectively. Higher abundance of *Pseudomonas* bacteria (in particular fluorescent species) was found compared to *Bacillus* and *Streptomyces*. The dominant bacterial groups found in contaminated soil (Soil 2) were evaluated by nutrient media and

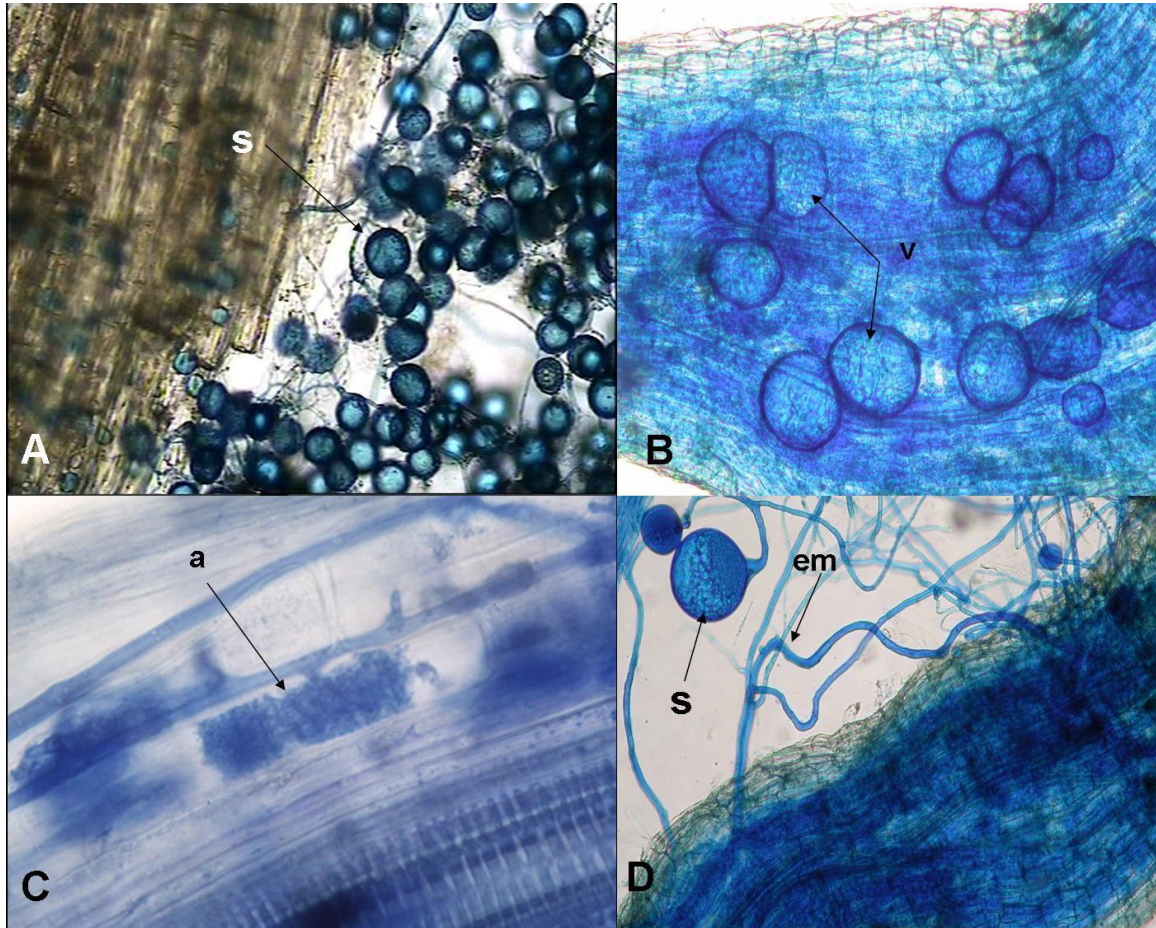


Figure 1. Colonized roots of alfalfa (A), (B), (C) and (D) by arbuscular mycorrhizal fungal after staining. Different fungal structures including vesicles (v), extraradical mycelia (em), arbuscules (a) and intraradical spores (s) could be observed.

analyzed for the occurrence of main RBs including *Pseudomonas*, *Bacillus* and *Streptomyces* species (Glick, 1995).

The root samples in Soil 2 were fixed in FAA [6 ml of formalin (40% formaldehyde), 1 ml of glacial acetic acid, 20 ml of ethanol (96%), and 40 ml of distilled water] once upon collection (Phillips and Hayman, 1970), cleared and stained for observation of fungal structures.

Arbuscular mycorrhizal fungal spores were observed in all collected soil samples from alfalfa fields. The most abundant hairy roots effect on most abundant fungal species since the fungal colonization occurs mostly around fine hairy roots. Also, the most number of fungal spores obtained in the end of growing season since complete growth of the host plant root system. There were 1117 fungal spores as average per 50 g soil sample in 3 replicates. After root clearing and staining in all studied plants, the most abundant observed fungal structures were vesicles as well as mycelia (Figure 1). The number of spores and arbuscules were low. However, these fungal structures could be observed intensively in alfalfa root samples due to its long lasting symbiosis time rather than other host plants. The results were in accordance with Muthukumar et al. (2004) findings.

Totally, 10 fungal species from two genera including *Glomus* (Glomeraceae, Glomerales) and *Acaulospora* (Acaulosporaceae, Diversisporales) were identified in alfalfa rhizosphere soil from Zanjan region. Among them, 9 species were of *Glomus* including *G.*

fasciculatum, *G. mosseae*, *G. intraradices*, *G. caledonium*, *G. geosporum*, *G. constrictum*, *G. versiforme*, *G. etunicatum* and *G. ambisporum*. One species belonged to *Acaulospora* (*A. mellea*). The most and least abundant species were *G. fasciculatum* and *A. mellea*, respectively.

According to the results obtained from analyzing Soil 2 for abundance of AM spores and RBs, 200 g of Soil 2 was added to each 1 kg of Soil 1 as inoculums. For blank samples (samples without stress-adapted microbes), Soil 2 was sterilized prior to adding to Soil 1. This soil substrate was aged under a WD moisture regime for a further 3 months as described above. This soil substrate was packed in some 5 kg plastic pots in nine replicates for each treatment (each three replicates were used for each plants as discussed below).

Subsamples of the prepared soil substrate were air dried and ground to pass through a 2-mm sieve before analysing. Soil pH was determined in 1:5 soil to 0.01 M CaCl_2 suspension with a glass electrode. Particle size was also determined by hydrometer method (Gee and Boudier, 1986). The total carbonates in the soil expressed as the calcium carbonate equivalent (CCE) was also determined by a rapid titration method (Rayment and Higginson, 1992). Organic matter was determined by dichromate oxidation (Walkley and Black, 1947). Soil EC was determined in a saturated paste extract. The cation exchange capacity (CEC) was measured using sodium acetate (1 M NaOAc) at pH 8.2 (Chapman, 1965). The total

amounts of some inherent metals (Zn, Cu, Mn, Fe, Pb, and Cd) in the prepared soil substrate were extracted by adding 10 ml of HNO₃ (1:1) to 2.0 g dried soil and heating for 15 min at 95°C on a heating block followed by 2 ml deionized water and 3 ml of 30% H₂O₂ (Soon and Abboud, 1993; USEPA, 1986; Gupta, 2000).

Host plants used in this study were millet (*Pennisetum glaucum*), couch grass (*Triticum repens*) and alfalfa (*Medicago sativa*). Seeds of plants were cultured in pots containing 5 kg of Cd contaminated soils in greenhouse condition. After four weeks, the emerged seedlings were thinned for keeping 14 and 10 strongest seedlings for millet and couch grass and alfalfa per pot, respectively.

No fertilizer was applied, except that the increasing inputs of nitrogen resulting from different concentrations of Cd(NO₃)₂ application was taken into account and adjusted by adding appropriate amounts of urea.

The plants were harvested by cutting the shoots at the soil surface at the end of growth period. After harvesting, subsamples of pot soil (2 mm) were analysed for extractable soil Cd concentration. Plant shoots were carefully washed with tap water to remove any adhering soil particles and rinsed twice with distilled water followed by drying at 75°C for 72 h and dry weights were recorded. For plant shoots Cd analysis, 2.0 g aliquots of ground shoots were digested in 30 ml of HNO₃, HClO₄, and H₂SO₄ mixture (40:4:1) followed by 20 ml of deionized water (Gupta, 2000). The Cd concentrations in soil and plant extracts were measured by atomic absorption spectroscopy (AAS, Shimadzu 6300).

In order to simultaneously test the phytotoxic effect of Cd and/or the inclusion effect of stress-adopted microbes to soil on plant biomass production, the plants yield reductions were calculated as the relative percentage of dry biomass of a given plant at each treatment (Y_c) to its dry biomass at control treatment (the treatment with no added Cd and no stress-adopted microbial community) (Y₀):

$$RY = \frac{Y_c}{Y_0} \times 100 \quad (1)$$

With this parameter, simultaneous evaluation of Cd contamination and inclusion of stress-adopted microbes was done, so that, the relative yield of the control treatment was regarded as 100% and the changes in relative yield as a result of Cd contamination and/or microbial inoculation were calculated when compared with the control treatment.

Bioconcentration factors (BCF) of soil Cd by plants were also calculated to estimate the potential uptake of Cd by the plants as follow:

$$BCF = \frac{\text{totalPbin plantdrymatter}(mg\ kg^{-1})}{\text{totalPbin soil}(mg\ kg^{-1})} \quad (2)$$

RESULTS AND DISCUSSION

Soil 1 was a non saline (EC = 1 dS/m), loam texture soil with 10.1% calcium carbonate equivalent. As we used this soil as the main substrate to grow the plants, the sorption/desorption of Cd by the soil was studied. This helped us to discuss the results obtained for Cd phytotoxicity and Cd accumulation by the studied plants.

Sorption, desorption and immobilization of the added Cd by Soil 1

The results of the sorption/desorption experiments are

Table 1. sorption, desorption and immobilization of Cd by Soil 1.

C _i (mg/l)	C _e (mg/l)	C _s (mg/kg)	C _{des} (mg/kg)	IP (%)	SI (%)
0.5	0.09	4.1	0.6	85.0	82.0
2	0.29	17.1	1.9	88.9	85.3
6	0.55	54.5	7.2	86.8	90.8
10	0.73	92.7	11.0	88.1	92.7

C_i and C_e: initial and equilibrium concentration of Cd in solution, respectively; C_s and C_{des}: Cd sorbed and desorbed by soil, respectively; IP: percent of Cd immobilized by soil; SI: sorption intensity.

summarized in Table (1). With increase in the initial Cd loading quantities (C_i), there was a slight increase in the amounts of Cd remained solution after equilibration (C_e). A linear sorption of Cd was recorded for the soil, indicating the C-type isotherm with a constant affinity of Cd sorption for the range of applied concentrations. The maximum metal sorption capacity of the soil was considerable as they were able to sorb and immobilize most of the applied Cd. A small amount of sorbed Cd was desorbed by soils, indicating the strong and irreversible binding of Cd in the studied soil (Table 1).

Cadmium showed considerable affinity for the studied sample (Table 1) as most of the Cd present in the initial solutions was sorbed (the SI value was 82%) and immobilized (the IP values were more than 85%) by the soil. Fontes et al. (2000) found similar sorption behavior for metals in limed soil samples. Their results indicated that the presence of carbonates in the soil created new sorption sites and also favored the precipitation of metals. Sipos et al. (2008) found the precipitation of metals in a carbonatic soil using X-ray diffractometric analyses. It is likely that this process contributed to the high sorption and immobilization of Cd in this case as well. Metal precipitation in soils can occur at alkaline conditions, relatively high metal concentrations, and low metal solubility or also in the case of small number of specific adsorption sites (Brümmer, 1986). In these samples, the presence of carbonates resulted in an elevated pHs (pH = 7.5) which were in the weakly alkaline range. According to Brookins (1988) these conditions may favor the precipitation as Cd-carbonates (octavite).

Some physicochemical characteristics of the prepared soil used in this study are presented in Table 2. As indicated in Table (2) the experimental soil is a clay loam, non saline, weakly alkaline and calcareous soil with some degrees of inherent contamination with Cd. Concentration of other metals is far below their acceptable levels in soil.

Effects of soil contamination with Cd on mean relative yield of plants, as an index for phytotoxicity, is presented in Table 3. Decline in mean relative yield ranged from 58.6% (with 68 mg Cd kg⁻¹ soil) to 100% (with 8 mg Cd kg⁻¹ soil) for millet and from 35.1% (with 108 mg Cd kg⁻¹ soil) to 100% (with 8 mg Cd kg⁻¹ soil) for alfalfa and from 70% (with 28 mg Cd kg⁻¹ soil) to 100% (with 8 mg Cd kg⁻¹ soil).

Table 2. Some physicochemical properties of the pot soil used for this study.

Properties	Value
Clay (%)	28
Sand (%)	24
Silt (%)	48
Texture	clay loam
pH	7.5
OM (%)	2.6
EC (dSm ⁻¹)	1.0
CEC (cmol _c kg ⁻¹)	24.7
CCE (%)	10.1
Total Zn (mg kg ⁻¹)	12.7
Total Cu (mg kg ⁻¹)	30.6
Total Mn (mg kg ⁻¹)	422.9
Total Fe (mg kg ⁻¹)	377.9
Total Cd (mg kg ⁻¹)	8.0
Total Pb (mg kg ⁻¹)	22.6

OM: organic matter, EC: electrical conductivity, CEC: cation exchangeable capacity, CCE: calcium carbonate equivalent.

soil) for couch grass. These results showed that, at the same levels of soil contamination, the Cd-tolerance of couch grass was higher as compared to millet and alfalfa. As shown, when no Cd was added to the soil, the introduction of soil microbial inoculum caused a significant decline in plant biomass when compared to the non inoculated soil. However, this reduction could be observed in most of the treatments inoculated with stress-adapted microbes as well. These results are in disagreement with the results of Chen et al. (2004) and Biro and Takacs (2007).

As discussed previously, most of the Cd added to the main soil was immobilized and resulted in low soluble Cd in the soil. The pot soil also has similar physicochemical properties as Soil 1 and one may expect a similar retention of Cd in this case as well. Therefore, reduction of plant biomass as a result of stress -adapted microbes in treatments without adding any Cd, possibly, could be attributed to the increased access of plants to the relatively immobile inherent elements pre-existed in the prepared soil (Table 2) and in other treatments may be due to more metal contaminants absorption caused by soil microbial activity.

As shown in Table 3, soil Cd concentrations below 20 and 5 mg kg⁻¹, respectively for alfalfa and couch grass, not only had no phytotoxicity, but also had a statistically significant stimulating effect of Cd on the plant growth. This indicated favorable effect of soil Cd at relatively low concentrations (28 mgkg⁻¹ Cd) on plant yield. Such a stimulating effect of Cd on plant yield was previously found by several researchers (Bosiacki, 2008; John et al., 2009) but so far it has not been explained. Generally, in most of the treatments, increasing in concentrations of

Cd, resulted in reduction yield and inoculation of stress-adapted microbes further increased this reduction. Also, the most Cd-tolerant plant was couch grass with the least rate of yield reduction. However, the most sensitive host plants were alfalfa and millet, respectively.

Cd concentration in shoot dry weight of alfalfa, millet and couch grass at different concentrations of soil Cd and presence or absence of stress-adapted microbes is presented in Table 4. The maximum shoot Cd concentrations for couch grass, alfalfa, and millet, were 23.6, 13.4, and 15.1 mg kg⁻¹ dry weight respectively. All these maximum levels were observed at soil total Cd of 108 mg kg⁻¹ (Table 4). Results in Table 4 indicate that couch grass is tending to take up more Cd than alfalfa and millet. The shoot cadmium (Cd) content in inoculated as well as in non-inoculated plants increased with increasing soil Cd level. In general, at lower soil Cd contents (28, 8, and 68 mg Cd per kg soil for millet, alfalfa, and couch grass, respectively) a significant increase of Cd uptake was observed as a result of inoculation with stress-

adapted microbes. However, at more soil Cd concentrations, there was no significant difference or a lower shoot Cd concentration was recorded in inoculated treatments (Table 4). More Cd uptake in inoculated treatments could be attributed to more access of plants to soil Cd through microbial assistance, for example, mycorrhizal hyphae. Other researchers (Gildon and Tinker, 1983; Takacs and Voros, 2003; Biro and Takacs, 2007) also found a decrease of shoot Cd content by inoculation of microbes at high soil Cd concentrations.

As shown in Table (4), alfalfa, millet and couch grass maintained low and constant metal concentration over a broad range of Cd concentration in soil. In this study the ability of alfalfa, millet and couch grass to Cd uptake were low. As a severe phytotoxicity of Cd was not recorded (Table 3), the low shoot Cd contents, probably, could be attributed to low bioavailability of Cd in the soil used as discussed previously (Table 1). Furthermore, as the plants are relatively Cd-tolerant, their low shoot Cd contents may suggest that roots of plants are non-efficient barriers to Cd uptake and/or translocation to the above ground plant parts as well. The uptake of metals by plant is coupled to a chemiosmotic process across the membrane of intact root cells (Mitchell, 1979). Tolerance and root to shoot metal transport are often negatively correlated, and tolerance is often associated with enhanced metal retention in roots (Harmens et al., 1993). Zhu et al.(1999) also observed higher accumulation of Cd into the roots of *B. juncea* as compared to above ground parts by over expressing gamma-glutamylcysteine synthetase.

BCF values of Cd for alfalfa, millet and couch grass at different concentrations of soil Cd, is presented in Table 5. For all three plants, values of BCF for Cd were less than unity with an exception of alfalfa when grown on soil with 8 mg Cd kg⁻¹ (Table 5). The same trend as shoot Cd content (Table 4) was recorded for the effect of inoculation of microbes on BCF. The results indicated

Table 3. Relative yield (Y_c/Y_0)[#] of millet, alfalfa and couch grass grown in a soil with different levels of Cd contamination with and without introducing stress-adapted microbes.

Calculated soil total Cd (mg kg ⁻¹)	Relative yield of plants (%)					
	Millet		Alfalfa		Couch grass	
	Non inoculated [§]	Inoculated [§]	Non inoculated	Inoculated	Non inoculated	Inoculated
8	100.0	75.4	100.0	68.9	100.0	73.0
13	74.0	76.3	107.4	92.1	103.8	84.0
28	73.8	69.7	115.4	93.6	74.5	70.0
68	58.6	67.7	76.9	56.7	98.8	84.7
108	66.0	58.8	74.2	35.1	84.8	86.3

#: the plants yield reductions were calculated as the relative percentage of dry biomass of a given plant at each treatment (Y_c) to its dry biomass at control treatment of control treatment (the treatment with no added Cd and no stress-adopted microbial community) (Y_0). §: non inoculated and inoculated denotes for the treatments without and with inoculation of stress-adapted microbes, respectively.

Table 4. Cd concentration in shoot dry weight of alfalfa, millet and couch grass at different concentrations of soil Cd contamination with and without inoculation of stress-adapted microbes.

Calculated soil total Cd (mg kg ⁻¹)	Cd concentration in shoot dry weight of plants (mg kg ⁻¹)					
	Millet		Alfalfa		Couch grass	
	Non inoculated [§]	Inoculated [§]	Non inoculated	Inoculated	Non inoculated	Inoculated
8	1.8	1.9	10.2	12.0	6.4	6.2
13	4.3	5.0	12.4	12.4	7.4	8.6
28	5.9	6.4	12.4	12.5	10.6	11.3
68	12.5	12.3	13.1	13.2	12.3	13.3
108	15.1	14.5	13.4	13.3	23.6	20.2

§: non inoculated and inoculated denotes for the treatments without and with inoculation of stress-adapted microbes, respectively.

Table 5. BCF values of Cd for alfalfa, millet and couch grass at different concentrations of soil Cd contamination with and without inoculation of stress-adapted microbes.

Calculated soil total Cd (mg kg ⁻¹)	BCF (-)					
	Millet		Alfalfa		Couch grass	
	non inoculated [§]	inoculated [§]	non inoculated	inoculated	non inoculated	inoculated
8	0.22	0.24	1.27	1.50	0.80	0.73
13	0.33	0.38	0.95	0.95	0.57	0.66
28	0.21	0.22	0.44	0.44	0.37	0.40
68	0.18	0.18	0.19	0.19	0.18	0.21
108	0.13	0.13	0.12	0.12	0.18	0.19

§: non inoculated and inoculated denotes for the treatments without and with inoculation of stress-adapted microbes, respectively.

that the BCF values of all three plants decreased with increasing Cd contamination in soil (Table 5). There were two possibilities for the interpretation why BCF values decreased with increasing soil contamination. One possible reason was that the self-adjusting of plants plays an important role on sequestering the metals in their roots. Only small amounts of metals are translocated to the up-ground parts of plants. Another possible reason is that plants grow unfavorably on the contaminated soils

(Wang et al., 2004). It is especially true on the heavily contaminated soil where plants had weak growth. In this case, because of the adverse living condition, the metal uptake ability by plants was weakened and the metal content in plant shoots was decreased. On the contrary, the corresponding metal content of soils was quite high, so the BCF values of plants in heavily contaminated soils were significantly decreased. Similar results were also found by Wang et al. (2004).

Conclusions

Generally, in most of the treatments, increasing Cd concentrations resulted in reduction of yield, and inoculation of stress-adapted microbes further increased this reduction. This could be attributed to the increased access of plants to the relatively immobile Cd existing in the soil as well as to more metal contaminants absorption caused by soil microbial activity. Similar to other researchers (Bosiacki, 2008; John et al., 2009), some favorable effect of soil Cd at relatively low concentrations (28 mg kg^{-1} Cd) was recorded on plant yield. Also, the most Cd-tolerant plant was couch grass with the least rate of yield reduction. However, the most sensitive host plants were alfalfa and millet, respectively. The shoot Cd content in inoculated as well as in non inoculated plants increased with increasing soil Cd level. In general, at lower soil Cd contents a significant increase of Cd uptake was observed as a result of inoculation with stress-adapted microbes.

However, at more soil Cd concentrations, there was no significant difference or a lower shoot Cd concentration was recorded in inoculated treatments. More Cd uptake in inoculated treatments could be attributed to more access of plants to soil Cd through microbial assistance, for example, mycorrhizal hyphae. Some studies have demonstrated that microbes such as bacterial isolates significantly increased the bioavailability of heavy metals in soil (Chen et al., 2005; Abou-Shanab et al., 2006; Sheng and Xia, 2006). As a severe phytotoxicity of Cd was not recorded, the low shoot Cd contents, probably, could be attributed to low bioavailability of Cd in the calcareous soil used. Furthermore, as the plants are relatively Cd-tolerant, their low shoot Cd contents may suggest that roots of plants are non-efficient barriers to Cd uptake and/or translocation to the above ground plant parts as well. Low bioavailability of heavy metals in soils and low biomass production of the plants may limit the efficiency of phytoremediation.

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