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EFFECT OF ENDOPHYTIC FUNGI ON CADMIUM TOLERANCE AND BIOACCUMULATION BY *FESTUCA ARUNDINACEA* AND *FESTUCA PRATENSIS*

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Endophytic fungi are a group of fungi that live asymptomatically inside plant tissue. These fungi may increase host plant tolerance to biotic and abiotic stresses. The effect of Neotyphodium endophytes in two grass species (*Festuca arundinacea *and* Festuca pratensis*) on cadmium (Cd) tolerance, accumulation and translocation has been our main objective. The plants were grown in a hydroponic system under different Cd concentrations (0, 5, 10, and 20 mg L^{-1}) for 6 weeks. They were also grown in soil spiked with different concentrations of Cd (0, 10, 20, and 40 mg kg^{-1}) for 2 months. The results from all Cd treatments showed higher biomass production (12–24%) and higher potential to accumulate Cd in roots (6–16%) and shoots (6–20%) of endophyte-infected plants than endophyte-free plants. Cadmium accumulation by plants indicated that the grasses were capable of Cd hyperaccumulation, a property that was augmented after endophyte infection. Maximum photochemical efficiency of photosystem II (F_v/F_m) revealed that Cd stress was significantly reduced in endophyte-infected plants compared to non-infected ones.

KEYWORDS: Phytoremediation, tall fescue, meadow fescue, *Neotyphodium*, heavy metals

INTRODUCTION

Current remediation methods applicable to soil and water contaminated with heavy metals such as excavation and physiochemical treatment *ex situ* are expensive, environmentally invasive, and labor intensive and therefore inappropriate for large polluted areas. Moreover, such treatments would inevitably dramatically affect structure, biota, and diverse functionality of the soil. Phytoremediation is considered an environmentally friendly and cost-effective alternative but it also has limitations (Pilon-Smits 2005). One key issue is that plants applied for phytoremediation must have ability to tolerate the pollutants. Therefore, toxicity level should allow plant growth. It seems that increasing plant tolerance to pollutant

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toxicity may enhance phytoremediation efficiency (Alkorta *et al*., 2004). Mycorrhizoremediation is a new approach for enhancing phytoremediation using arbuscular mycorrhizal fungi and plants symbiosis (Khan 2006; Göhre and Paszkowski 2006). *Neotyphodium* endophytes are another group of fungi that live their entire life cycle within the aerial portion of many grass species, forming nonpathogenic, systemic and usually intercellular associations (Bacon and De Battista 1991). Interactions between *Neotyphodium* endophytes and host plants vary from mutualistic to parasitic, depending on the resource environment in which the host–endophyte complex was formed and the conditions under which it is being grown (Malinowski and Belesky 2006). The most widely known *Neotyphodium* endophytes are *N. coenophialum*, *N. lolli* and *N. uncinatum* that colonize tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.) and meadow fescue (*Festuca pratensis* Huds.), respectively (Malinowski and Belesky 2000). *Neotyphodium* fungi have also been found in some *Festuca* species in Iran (Khayyam Nekouei 2001; Mohammadi and Mirlohi 2003). Similar to mycorrhiza, *Neotyphodium* endophytes may interact mutualistically with their host grasses and their role in protecting the hosts from metal toxicity has been reported regarding Al (Malinowski and Belesky 1999), Zn (Bonnet *et al*., 2000; Monnet *et al*., 2001) and Cu (Malinowski *et al*., 2004). *Neotyphodium* endophytes can affect mineral uptake (*i.e.*, Fe, Zn, Cu, Ca, and P) and their transport in tall fescue (Rahman and Saiga 2005; Malinowski *et al*., 2000). Ren *et al*. (2006) showed that endophyte-infected ryegrass accumulated more cadmium (Cd) than endophyte-free ones.

However, there is no information about the effect of endophytic fungi on Cd accumulation by *Festuca* species. In this investigation, we hypothesize that the presence of endophytes in *F. arundinacea* and *F. pratensis* may affect Cd accumulation and translocation in the plants, as well as reduce Cd stress. A hydroponic growth system was used to allow better characterization of contaminant uptake by plants because in this way potential interactions between the soil matrix and Cd are minimized. The high Cd concentration was used to test the ability of plants for Cd hyperaccumulation and to find the response of endophyte-infected plants to Cd stress. Plants were also grown in a Cd-spiked soil to evaluate their capability for Cd hyperaccumulation.

MATERIALS AND METHODS

Plant Materials

Tall fescue (*F. arundinacea*) and meadow fescue (*F. pratensis*) were used as plant materials. Seeds of two plant species were originally collected from natural rangelands of Iran. These two plants were chosen because of high infection rates (almost 100%) of their seeds with *N. coenophialum* and *N. uncinatum,* respectively, which was confirmed by using direct staining method (Saha *et al*., 1988). Fifty seeds from each plant population were sown in plastic pots and grown in a greenhouse (temperature 27 $\pm$ 3C, relative humidity 45 $\pm$ 8%, 12 $\pm$ 0.5 h daylight). After 6 months, one single plant was selected from each population based on the fungus viability using microscopic detection and its hyphal density in leaf sheath tissue (Saha *et al*., 1988). Then all plants were clonally propagated by separation of secondary tillers from the main tillers. After 4 months, propagated plants were transferred to the field. Plants were allowed to grow in the field for three months and then half of the plants in each species were treated with a mixture of propiconazole and terbuconazole fungicides to eliminate endophytes according to a modified approach of Hill and Brown (2000). Plants were propagated in the field for 6 generations. Finally, the seeds

of endophyte-infected (E^+) and non-infected (E^-) plants were grown in the sand culture under controlled conditions (temperature 27 ± 3°C, relative humidity 45 ± 8%, 12 ± 0.5 h daylight). Plants were microscopically examined for the presence of endophytes before and after the experiment (Saha *et al*., 1988).

Hydroponics Experiments

Five 20-day-old seedlings of each grass/endophyte combination were transplanted to polyethylene pots containing 2 L of half Johnson's solution (Johnson *et al*., 1957). A buffer (0.5 mM MES (2- N-morpholino ethanosulfonic acid)) was used to stabilize the pH at 6.0. The nutrient solution was made to contain Cd^{2+} as $Cd(NO_3)_2{\cdot}4H_2O$ at different concentrations: 0, 5, 10, and 20 mg L^{-1} (Cd_0, Cd_5, Cd_{10}, and Cd_{20}). To develop the radical system, the plants were grown in the nutrient solution without Cd for the first 2 weeks, where after Cd at different concentrations was added to the nutrient solution for the next 6 weeks. Nutrient solutions were renewed each week and kept aerated by air-sparging. Total volume of nutrient solution with Cd was 4.5 L, corresponding to 0, 22.5, 45 and 90 mg of cumulative Cd added per unit to the respective experiments. After the 6 weeks with the Cd-enriched solutions, the plants were exposed to a 0.05 M $CaCl_2$ solution for 24 h to remove adsorbed Cd from water free spaces in the root (Utmazian *et al*., 2007). At the end of the experiment tiller number, root length and green leaves per pot were recorded. Plant samples were collected and washed with deionized water before being separated into shoots and roots. Then all samples were oven-dried (65°C) for 72 h to reach a constant weight and dry weight of shoots and roots were determined.

Chlorophyll fluorescence (maximum photochemical efficiency of the photosystem II =
F_v/F_m) and performance index (PI) of attached leaves were measured using a portable fluorescence spectrometer (PEA, Hansatech, UK) with the help of leaf clips one week before harvesting. Plants were dark-adapted for 20 min before measurement. All measurements were replicated with five different leaves of each plant. Due to close correlation of Fv/Fm with other measures of quantum efficiency of photochemistry, it has been widely used as a screening parameter for stress response (Bjorkman and Demmig 1987; Johnson *et al*., 1993; Stavrianakou *et al*., 2006). Performance index combines the density of reaction centers, the quantum yield of primary photochemistry and the ability to feed electrons into the electron chain between photosystem II and I, which are favorable to photosynthetic activity (Srivastava *et al*., 1999).

To determine plant translocation ability (from root to shoot) at different Cd concentrations, the transport index (TI) was calculated as proposed by Ghosh and Singh (2005):

$$\mathrm{TI}(\%) = \left[\frac{\mathrm{Cd\,Shoot\,(mg\,kg^{-1})}}{\mathrm{Cd\,Root\,(mg\,kg^{-1})}}\right] \times 100$$

Cd removal from each pot by plants was determined as:

$$\text{Cd Removal } (\%) = \left[\frac{\mathrm{Cd\,Shoot\,(mg\,kg^{-1})} \times \text{Shoot biomass (kg)} + \mathrm{Cd\,Root\,(mg\,kg^{-1})} \times \text{Root biomass (kg)}}{\text{Total added Cd per pot (mg)}}\right] \times 100$$

Soil Experiments

Soil samples collected from Lavark research station, Isfahan, Iran, were air-dried and passed through a 2 mm sieve. Soil (Typic Haplargid) texture was silty clay loam and soil pH was 7.8 ± 0.3 as determined in a 1:2.5 suspension of soil in 0.01 M $CaCl_2$. Soil calcium carbonate and organic carbon contents were $32 \pm 3.1\%$ and $0.500 \pm 0.007\%$ respectively. Cation exchange capacity (CEC) of the soil was 18.1 ± 2.0 cmol(+) kg^{-1} and total nitrogen and phosphorous contents of the soil were $0.10 \pm 0.003\%$ and 12 ± 2 mg kg^{-1}, respectively.

Cadmium nitrate solution ($Cd(NO_3)_2.4H_2O$) was added to the air-dried soils at concentration of 0, 10, 20, and 40 mg kg^{-1} (Cd_0, Cd_{10}, Cd_{20}, and Cd_{40}). Treated soils were wetted for 2 weeks with deionized water at 60% soil water holding capacity, to enable the added Cd salt to reach a steady state. Soils were then dried at room temperature (25°C) for one week. The artificially contaminated soil was subjected to three wetting and drying cycles before using in pots (Blaylock *et al*., 1997), in which the Cd contents of the soil were 1.8 ± 0.1, 11.6 ± 0.3, 20.5 ± 1.2 and 42 ± 1.9 mg kg^{-1} for the Cd_0, Cd_{10}, Cd_{20} and Cd_{40} treatments. The plant available Cd contents (*i.e.*, AB-DTPA extractable Cd determined as described below) of the soil were 0.020 ± 0.003, 1.1 ± 0.3, 1.9 ± 0.2 and 2.5 ± 0.6 mg kg^{-1} for the Cd_0, Cd_{10}, Cd_{20} and Cd_{40} treatments. Each pot was filled with 1 kg of soil. Then five 20-day old seedlings of each plant (E^+ and E^-) were grown in pots for 2 months and watered based on 75% of soil field capacity, which was determined by weighing the pots every 3 days. Selected photosynthetic parameters (F_v/F_m and PI) of attached leaves were also measured one week before harvesting as described in the hydroponics experiments. After two months, the harvested plant samples were washed with deionized water and separated into roots and shoots. Finally, the samples were oven-dried (65°C) and their weights were determined.

Cadmium Analysis

Dried plant samples were ground in a metal free mill and 0.2 g was digested with 5 mL HNO_3 (65% w/w, Merck, Darmstadt, Germany) at 110°C for 2 h, cooled, added 1 mL of H_2O_2 (30% w/w, Merck, Darmstadt, Germany) and boiled for 1 h (Lim *et al*., 2004). The clear digests were diluted to 50 mL with triple deionized water. The Cd concentration was measured by flame atomic absorption spectrometry (Perkin Elmer, A-Analyst 200). For quality assurance, selected plant samples were sent to an accredited laboratory (Eurofins Lab., Copenhagen, Denmark) for determination of Cd (and other elements) by inductively coupled plasma mass spectroscopy (ICP-MS).

Plant available Cd content in the soil was determined using ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) method (Soil and Plant Analysis Council 1999). Twenty ml of AB-DTPA (pH = 7.6) was added to 10 g soil and shaken for 15 min in 180 cycles min^{-1}. Then the mixture was immediately filtered through a Whatman 42 filter paper. Total Cd content of the soil was determined using HNO_3 and H_2O_2 (Soil and Plant Analysis Council, 1999). The Cd concentration was measured by graphite furnace atomic absorption spectrometry (Perkin Elmer, Zeeman 5100).

Statistical Analysis

A completely randomized block design in a factorial scheme was implemented with two levels of endophytes (E^+, E^-) and 4 levels of Cd concentrations and three replications. Analysis of variance procedure (one way ANOVA) for all treatments was conducted using

the SAS program (Release 9.1). The difference between specific pairs of mean was identified using Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

Hydroponics Studies

Plants growth and photosynthetic parameters. The infection percentage of inoculated plants with endophytic fungi was 100%, while they were not detected in E^- plants before and at the end of the experiment. Shoot and root biomass of all plants significantly ($P < 0.05$) decreased under Cd stress (Cd_5-Cd_{20}) compared to the control (Cd_0) (Figures 1 and 2). However, plants infected with endophytic fungi had higher shoot, root and total biomass than non-infected counterparts (Figures 1 and 2, Table 1). Although, endophyte infection increased root biomass, it had no significant effect on root length (data not shown). Total biomass of *F. pratensis* (E^+) and *F. arundinacea* (E^+) was 8–14% and 9–26% larger than *F. pratensis* (E^-) and *F. arundinacea* (E^-), respectively. This effect is accentuated by data from tillering number and green leaves of plants (Table 2) where E^+ plants with more biomass had more green leaves and higher capability to tiller. These factors were highly influenced by Cd concentration in the solution. Regardless of endophyte status and Cd concentration in the solution, *Festuca pratensis* showed higher biomass in roots and shoots than *F. arundinacea*. This could possibly be related to difference in physiological characteristics of the two species. Kuldau and Bacon (2008) reported that increased plant growth is an effect observed in those endophyte-infected grasses such as tall fescue (*F. arundinacea*) and perennial ryegrass (*Lolium perenne*). An increase in the rate of growth and herbage yield may be due to physiological response of the grass from an increase in endogenous levels of plant hormones, which may be an additional effect of the fungal (De Battista *et al*., 1990).

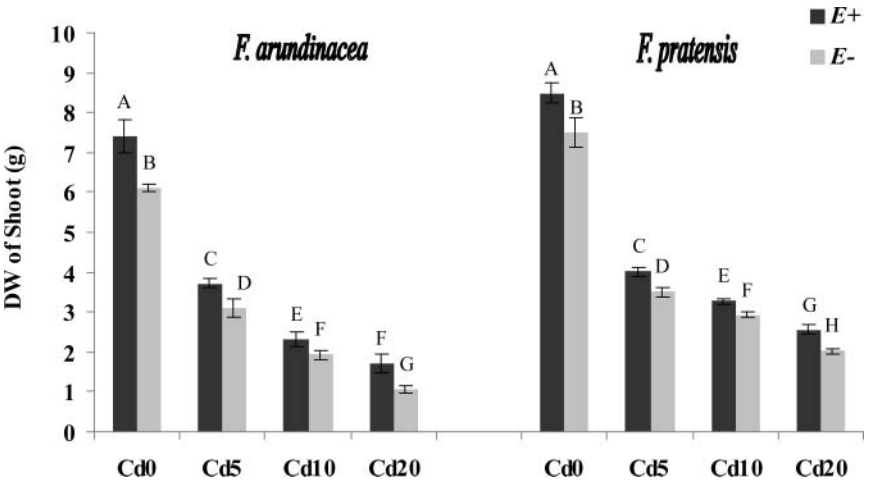

Figure 1 Shoot biomass (DW) of plants infected (E^+) and non-infected (E^-) with endophytic fungi in different Cd treatments (Cd_0, Cd_5, Cd_{10}, and Cd_{20} mg L^{-1}) in hydroponics. Values are means ± standard deviations. Different letters represent statistical differences ($P < 0.05$).

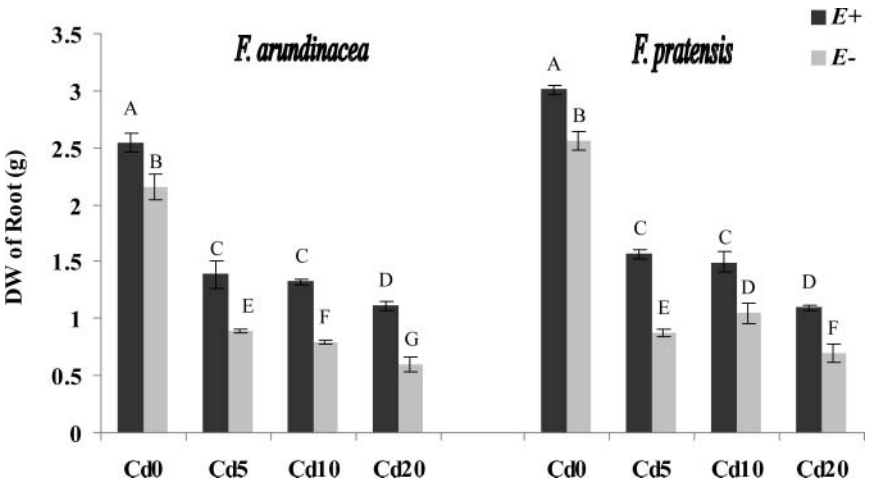

Figure 2 Root biomass (DW) of plants infected (E^+) and non-infected (E^-) with endophytic fungi in different Cd treatments (Cd_0, Cd_5, Cd_{10}, and Cd_{20} mg L^{-1}) in hydroponics. Values are means ± standard deviations. Different letters in each row represent statistical differences ($P < 0.05$).

Symptoms of Cd toxicity were noticed on plant leaves in the Cd_{20} treatment after six weeks, regardless of endophyte infection. These plants showed dryness and some brown spots on their leaves. The main reason for loss of leaves could be an increase of abscisic acid or ethylene due to Cd stress (Zhou and Qiu 2005). These symptoms were qualitatively more severe in endophyte-free plants than in the infected ones. Photochemical efficiency or quantum of yield potential of photosynthesis (F_v/F_m) revealed that E^+ plants suffered less Cd stress than E^- ones in the Cd_{10} and Cd_{20} treatments. The amount of F_v/F_m decreased with Cd increase in solution (Table 2). Therefore, F_v/F_m may be inhibited by Cd stress. In healthy leaves, optimal value of this parameter is always close to 0.83, not depending on plant species (Maxwell and Johnson 2000). A lower value indicates that a proportion of photosystem II reaction centers are damaged and the plant has reacted to stress (Maxwell and Johnson 2000). Endophytic fungi had a positive effect on the performance index (PI) in the host plants. *Festuca pratensis* showed higher PI than *F. arundinacea* in all treatments (Table 2). Since the biomass of plants (especially endophyte-infected ones) with higher PI was more than other ones with lower PI, it could be concluded that PI is closely related to photosynthetic activity. It also seems that the plants infected with endophytic fungi had higher photosynthetic activity. Monnet *et al*. (2001) showed that endophyte infected *Lolium perenne* had higher net photosynthetic rate and Fv/F_m than non-infected plants under Zn stress. In this study, increasing Cd concentration resulted in a PI decrease of 5–7 units (Table 2). This indicated that Cd-induced stress decreased plant photosynthetic activity and biomass production. This was similar to the results of Chugh and Sawhney (1999) for *Pisum sativum*.

Cadmium accumulation in shoot. All of the plants in different treatments accumulated high amounts of Cd (704–2366 mg kg^{-1}) in their shoots without significant difference between the two plant species (Figure 3). The effect of endophyte infection on Cd accumulation by plant shoots in all treatments was significant as well. It was similar to the result of Ren *et al*. (2006) for endophyte infected ryegrass. Although it has been suggested that synthesis of some chelators such as phytochelatins and formation of stable

Table 1 Selected growth and photosynthetic parameters of plants (infected (E^+) and non-infected (E^-) with endophytic fungi) in different Cd treatments (Cd_0, Cd_5, Cd_{10}, and Cd_{20} mg L^{-1}) in hydroponics

	E^+				E^-			
	Cd_0	Cd_5	Cd_{10}	Cd_{20}	Cd_0	Cd_5	Cd_{10}	Cd_{20}
F. arundinacea								
Tiller num.	42.5 ± 3.5^{a}	33.0 ± 3.0^{b}	24.5 ± 0.7^{c}	18.7 ± 1.5^{d}	32.5 ± 3.5^{b}	30.5 ± 0.7^{b}	20.0 ± 0.9^{cd}	15.5 ± 0.7^{d}
Green leaves num.	190.5 ± 6.4^{a}	166.3 ± 11.2^{b}	66.5 ± 7.8^{d}	26.5 ± 2.1^{f}	173.5 ± 6.4^{b}	137.0 ± 4.2^{c}	50.0 ± 8.5^{e}	$23.0. \pm 2.8^{f}$
Fv/Fm	0.825 ± 0.003^{a}	0.816 ± 0.003^{ab}	0.795 ± 0.006^{b}	0.778 ± 0.001^{c}	0.830 ± 0.005^{a}	0.814 ± 0.001^{ab}	0.762 ± 0.059^{c}	0.700 ± 0.027^{d}
P Index	7.350 ± 0.306^{a}	6.187 ± 0.089^{c}	3.636 ± 0.165^{d}	1.445 ± 0.123^{f}	6.968 ± 0.047^{b}	3.523 ± 0.125^{d}	2.634 ± 0.292^{e}	0.478 ± 0.241^{g}
F. pratensis								
Tiller num.	49.0 ± 4.6^{a}	40.0 ± 1.4^{b}	25.0 ± 1.4^{d}	16.0 ± 2.8^{e}	41.5 ± 2.1^{b}	34.0 ± 1.4^{c}	19.5 ± 0.7^{de}	16.3 ± 1.2^{e}
Green leaves num.	244.0 ± 12.0^{a}	193.0 ± 5.7^{c}	83.0 ± 8.5^{d}	33.5 ± 6.4^{e}	217.0 ± 12.8^{b}	178.0 ± 2.9^{c}	68.5 ± 6.4^{d}	$33.7. \pm 3.1^{e}$
Fv/Fm	0.830 ± 0.002^{a}	0.806 ± 0.002^{ab}	0.793 ± 0.002^{b}	0.708 ± 0.009^{d}	0.828 ± 0.007^{a}	0.814 ± 0.004^{ab}	0.772 ± 0.007^{c}	0.676 ± 0.015^{e}
P Index	10.450 ± 0.185^{a}	8.032 ± 0.060^{c}	5.596 ± 0.284^{e}	4.099 ± 0.077^{f}	9.452 ± 0.599^{b}	6.251 ± 0.197^{d}	5.773 ± 0.173^{e}	4.562 ± 0.255^{f}

Values are means ± standard deviations (S.D.). Different letters in each row represent statistical differences ($P < 0.05$).

Table 2 Cadmium removal by total biomass of plants (infected (E^+) and non-infected (E^-) with endophytic fungi) after 6 weeks in different Cd treatments (Cd_0, Cd_5, Cd_{10}, and Cd_{20} mg L^{-1}) in hydroponics

	E^+				E^-			
	Cd_0	Cd_5	Cd_{10}	Cd_{20}	Cd_0	Cd_5	Cd_{10}	Cd_{20}
F. arundinacea								
Cd removal (%)	—	24.2 ± 1.0^a	15.1 ± 0.9^b	7.8 ± 0.0^c	—	14.2 ± 0.4^b	8.8 ± 0.4^c	4.1 ± 0.0^d
Total biomass (g)	9.9 ± 0.4^a	5.1 ± 0.3^c	3.6 ± 0.2^e	2.8 ± 0.3^f	8.3 ± 0.2^b	4.0 ± 0.3^d	2.7 ± 0.1^f	1.6 ± 0.1^g
F. pratensis								
Cd removal (%)	—	31.2 ± 1.2^a	21.0 ± 0.9^b	11.4 ± 0.2^e	—	16.3 ± 0.9^c	13.5 ± 0.3^d	6.7 ± 0.1^f
Total biomass (g)	11.5 ± 0.2^a	5.5 ± 0.1^c	4.8 ± 0.2^d	3.6 ± 0.1^f	9.1 ± 0.3^b	4.4 ± 0.1^d	4.0 ± 0.1^e	2.8 ± 0.1^g

Values are means ± standard deviations. Different letters in each row represent statistical differences ($P < 0.05$).

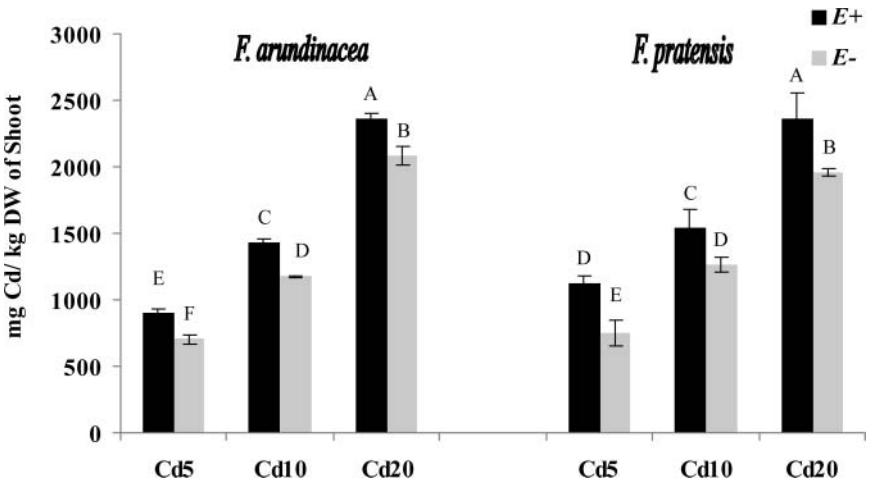

Figure 3 Shoot Cd concentrations (mg Cd kg^{-1} DW) of plants which were infected (E^+) and non-infected (E^-) with endophytic fungi in different Cd treatments (Cd_5, Cd_{10}, Cd_{20} mg L^{-1}) in hydroponics. Values are means ± standard deviations. Different letters represent statistical differences ($P < 0.05$).

complexes are important parameters for Cd tolerance and accumulation by plants (Sun *et al*., 2007; Pilon-Smits 2005), the mechanism of Cd accumulation by endophyte infected plants has not been recognized yet.

Cadmium accumulation in root. Cadmium content in roots ranged from 1407 to 3725 mg kg^{-1} in different treatments (Figure 4). Cd accumulation in the roots of *F. pratensis* was significantly higher than in the roots of *F. arundinacea* for all the treatments. Plants infected by endophyte showed higher amount (up to 6–16%) of Cd in their roots than in non-infected ones. So far, it is not clear how endophytes affect Cd root accumulation.

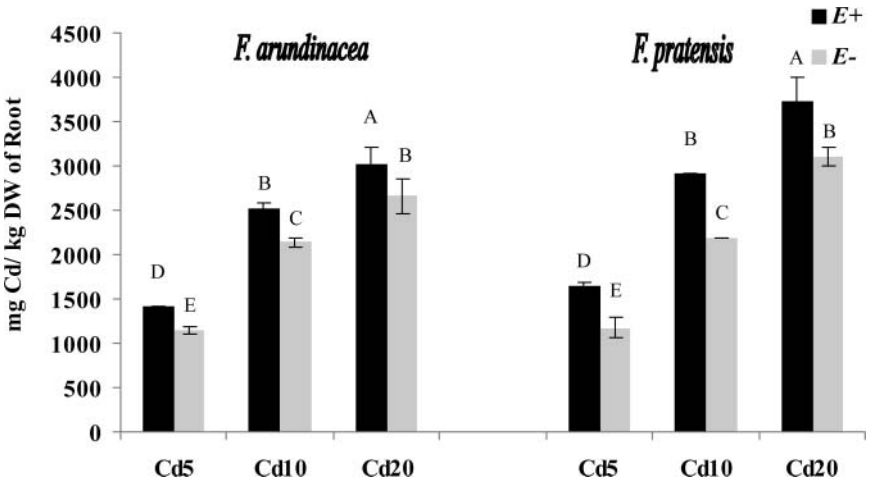

Figure 4 Root Cd concentrations (mg Cd kg^{-1} DW) of plants which were infected (E^+) and non-infected (E^-) with endophytic fungi in different Cd treatments (Cd_5, Cd_{10}, Cd_{20} mg L^{-1}) in hydroponics. Values are means ± standard deviations. Different letters represent statistical differences ($P < 0.05$).

Some plant root exudates such as phenolic compounds may be important in this mechanism. Malinowski *et al*. (2004) showed that endophyte infection increased Cu binding activity of extracellular root exudates of tall fescue in response to P-deficiency in nutrient solution. Chelating effect of released phenolic compounds has been proposed for Al sequestration on root surface of E^+ tall fescue (Malinowski and Belesky 1999). Lavid *et al*. (2001) suggested that the main mechanism for Cd accumulation in water plants is based on trapping of Cd by polymerized phenols in specialized epidermal structures as indicated by peroxidase and polyphenol oxidase activities. Therefore, plants that have greater peroxidase activities and hence higher polyphenol production will be more resistant to Cd.

Cadmium removal and transfer index (TI). There was a significant difference in Cd removal between E^+ and E^- plants. Endophyte infected plants with highest biomass production and Cd content in root and shoot showed higher removal efficiency (Table 1). Cadmium removal by plants was in the range of 4.4–31%, while E^+ plants showed 3–15% higher removal efficiency than E^- plants (Table 1). Despite of higher Cd content in plant roots and shoots in Cd_{20} and Cd_{10} treatments, removal efficiency was the highest in Cd_5 treatment because of higher biomass production and lower Cd concentration in the growth environment. *Festuca pratensis* had higher potential for Cd removal than *F. arundinacea* (Table 1).

All plants could translocate more than 50% of Cd from roots to shoots but we did not see a significant difference between infected and non-infected plants in this respect (data not shown). The highest TI was in Cd_{20} treatment (78%), where Cd toxicity symptoms were seen on plant leaves. Mechanisms of Cd translocation in plants could be complexation of Cd with low molecular weight organic ligands, such as citrate and histidine, and movement due to transpiration from the leaves, or chelating of Cd by phytochelatins (Gong *et al*., 2003; Salt *et al*., 1995).

Soil Studies

Plants growth and photosynthetic parameters. Results showed that there was no significant difference between root and shoot biomass of plants grown in all treatments (Control, Cd_{10}, Cd_{20}, and Cd_{40}). Furthermore, as shown in Table 3, there was no difference in the produced biomass of infected and non-infected plants. This may be due to generally less growth of plants in soil compared with the growth in the hydroponic system where all essential nutrients for optimum growth were available. The differences between the plants biomass may be seen in longer growth period of plants in soil. Photochemical efficiency (F_v/F_m) revealed that there was no sign of Cd stress in plants after two months growth in the Cd spiked soil. Regardless of the endophyte infection, F_v/F_m was 0.79–0.82, which revealed no stress in the plants. The lower Cd bioavailability of the soil with high pH and calcium carbonate content in comparison to the hydroponic system and therefore lower Cd uptake by plants could be the main reason for absence of Cd stress in soil rather than in the hydroponic system. Overall, no significant differences were seen between *F. pratensis* vs. *F. arundinacea* and endophyte infected vs. non-infected plants according to shoot and root biomass, F_v/F_m and PI parameters in the soil experiments.

Cd accumulation in shoot and root. The results showed that *F. pratensis* and *F. arundinacea* had the potential to accumulate Cd in their roots and shoots when planted in contaminated soil (Table 4). Although Cd accumulation in root and shoot of plants grown in contaminated soil was noticeable but it was lower than the accumulation obtained in the hydroponic system. Regardless of endophyte infection, the observed Cd accumulation was

Table 3 Cadmium removal by total biomass of plants (infected (E^+) and non-infected (E^-) with endophytic fungi) after 2 months in different Cd treatments (Cd_0, Cd_{10}, Cd_{20}, and Cd_{40} mg kg^{-1}) in soil

	E^+				E^-			
	Cd_0	Cd_{10}	Cd_{20}	Cd_{40}	Cd_0	Cd_{10}	Cd_{20}	Cd_{40}
F. arundinacea								
Cd removal (%)	—	1.7 ± 0.2^{ab}	1.9 ± 0.1^{a}	1.4 ± 0.2^{bc}	—	1.2 ± 0.1^{c}	1.5 ± 0.2^{bc}	1.5 ± 0.2^{bc}
Total biomass (g)	4.7 ± 0.5^{a}	4.4 ± 0.4^{a}	4.7 ± 0.9^{a}	3.6 ± 0.3^{a}	4.9 ± 0.2^{a}	4.9 ± 0.8^{a}	4.1 ± 1.0^{a}	4.1 ± 0.5^{a}
F. pratensis								
Cd removal (%)	—	2.3 ± 0.1^{a}	2.4 ± 0.2^{a}	1.7 ± 0.1^{b}	—	1.9 ± 0.1^{b}	1.9 ± 0.1^{b}	1.7 ± 0.2^{b}
Total biomass (g)	4.4 ± 1.0^{a}	4.4 ± 0.2^{a}	4.8 ± 0.6^{a}	3.5 ± 0.2^{a}	4.9 ± 0.1^{a}	4.5 ± 0.1^{a}	3.9 ± 1.0^{a}	$5.0. \pm 0.4^{a}$

Values are means ± standard deviation (S.D.). Different letters in each row represent statistical differences ($P < 0.05$).

Table 4 Cadmium accumulation in shoot and root (mg kg^{-1} DW) of plants (infected (E^+) and non-infected (E^-) with endophytic fungi) after 2 months in different Cd treatments (Cd_0, Cd_{10}, Cd_{20}, and Cd_{40} mg kg^{-1}) in soil

	E^+				E^-			
	Cd_0	Cd_{10}	Cd_{20}	Cd_{40}	Cd_0	Cd_{10}	Cd_{20}	Cd_{40}
F. arundinacea								
Shoot	ND[†]	32.9 ± 2.6^{e}	66.5 ± 5.0^{c}	98.1 ± 3.6^{a}	ND	25.2 ± 1.3^{f}	49.8 ± 8.1^{d}	80.3 ± 11.6^{b}
Root	ND	60.9 ± 2.3^{e}	137.4 ± 22.8^{c}	244.7 ± 32.0^{a}	ND	43.2 ± 6.8^{f}	110.3 ± 9.1^{d}	172.0 ± 32.8^{b}
F. pratensis								
Shoot	ND	38.9 ± 5.7^{e}	86.2 ± 9.9^{c}	130.1 ± 25.2^{a}	ND	29.1 ± 4.6^{f}	63.3 ± 7.5^{d}	100.2 ± 7.5^{b}
Root	ND	95.0 ± 11.8^{e}	182.7 ± 9.7^{c}	302.1 ± 13.9^{a}	ND	87.1 ± 6.6^{f}	150.0 ± 12.3^{d}	$230.4. \pm 24.2^{b}$

[†]Not Detected. Values are mean ± standard deviations (S.D.). Different letters in each row represent statistical differences ($P < 0.05$).

higher in the roots compared to the shoots in all treatments (Table 4). Plants containing endophytic fungi had more capability to accumulate Cd in their roots and shoots than E^- plants. In fact, as these grasses could accumulate more than 100 mg Cd kg^{-1} dry weight, which is considered the criteria for hyperaccumulators (Baker *et al*., 2000), they could have the capability of Cd hyperaccumulation, although they are less effective than *Thlaspi caerulescens* that can accumulate up to 10,000 mg Cd kg^{-1} in the shoot under hydroponic conditions (Lombi *et al*., 2000).

Cadmium removal and transfer index (TI). Cadmium removal by plants showed that endophyte infected plants had a higher potential to remove Cd from the soil than non-infected ones in Cd_{10} and Cd_{20} treatments (Table 3) and they showed higher Cd removal efficiency than Cd_{40} treatment. The main reason for decreasing Cd removal efficiency in soil studies in comparison to hydroponics studies was the lower Cd accumulation by plants grown in the soil.

Regardless of endophyte infection, TI of plants was 40 to 58% with no significant difference in all treatments. However, the highest TI was 78% in hydroponics studies showing that plants grown in hydroponics could translocate more Cd from root to shoot. However, there was no significant difference between endophyte infected and non-infected plants in both soil and hydroponics studies that revealed that endophyte could not affect Cd translocation from root to shoot of *F. pratensis* and *F. arundinacea.*

This study showed that *F. pratensis* and *F. arundinacea* had a noticeable potential for Cd accumulation. Infecting the plants with endophytic fungi may increase Cd phytoremediation efficiency via higher biomass production and more Cd accumulation. Because of more Cd tolerance of endophyte infected plants in the presence of very high Cd concentration, it seems that using these plants could be efficient for remediation of strongly Cd contaminated soils such as those near smelters (Buchauer 1973). But it should be considered that there might be a major difference in phytoremediation efficiency between using Cd contaminated soils of industrial sites and Cd-spiked soils.

CONCLUSION

Endophyte infection of *F. pratensis* and *F. arundinacea* increases the plants' ability to accumulate more Cd in roots and shoots as well as to decrease plant stress. Furthermore, endophyte infected plants showed more biomass production than non-infected plants in the hydroponic system. Accordingly, the grasses showed a high potential for removal of Cd from both aqueous solution and soil, which was significantly augmented when they were infected by endophyte.

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