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A preliminary study of the role of bacterial–fungal co-inoculation on heavy metal phytotoxicity in serpentine soil

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Abstract. This study was conducted to understand the role of bacterial–fungal interactions on heavy metal uptake by *Zea mays* plants. A pot experiment was conducted for 90 days with *Z. mays* in serpentine soil inoculated with a Gram-negative bacterium, fungus (*Aspergilllus* sp.) and both microbes to determine the effects of inoculation on nickel, manganese, chromium and cobalt concentrations in plant tissue and soil. Soil nutrients and soil enzyme activities were measured to determine the effect of inoculations on soil quality. Inoculation of microorganisms increased shoot and root biomass, and the maximum biomass was in the bacterial–fungal inoculation. This could be due to the solubilisation of phosphate and production of indole acetic acid. Although the combination treatment contributed to an increase in heavy metal uptake in *Z. mays* plants, the lowest translocation was observed in the combination treatment. Moreover, the soil available nitrogen, available phosphorous and total organic carbon content were increased with the microbial inoculation. Similarly, the soil dehydrogenase activity was higher as a result of microbial inoculation, whereas the highest dehydrogenase activity was reported in the combination inoculation. This study confirms the synergistic effect of bacterial–fungal inoculation as a soil-quality enhancer and as a plant-growth promoter in the presence of heavy metals.

Additional keywords: bioremediation, enzyme activity, heavy metal availability, soil quality, synergistic effect.

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Introduction

Weathering of ultramafic rocks produces soils and sediments that are non-anthropogenic sources of metal contamination. These soils are generally nutrient poor and contain high magnesium (Mg), iron (Fe), nickel (Ni), chromium (Cr) and cobalt (Co) and a high magnesium : calcium (Mg : Ca) ratio (Alexander 1988; Wenzel *et al*. 2003). In addition to the phytotoxic heavy metals, the high concentrations of Mg in soil can restrict Ca uptake, a limiting factor in plant tolerance to serpentine soils (Brady *et al*. 2005). Because of generally low organic matter and clay content (Proctor and Woodell 1975), serpentine soils often have a low water-holding capacity. Because of the high heavy metal concentrations, the microbial diversity is often low compared with non-serpentine soils (Panaccione *et al*. 2001; Southworth *et al*. 2014). Infertility, metal toxicity, and often sandy, rocky and shallow soils combined with low microbial diversity contribute to unique plant communities consisting of many rare and endemic species (Harrison and Rajakaruna 2011). Only well adapted species are able to tolerate the harsh chemical, physical and biological properties characteristic of serpentine soils (Anacker 2014).

The elevated metal concentrations associated with serpentinite rocks may cause ground-water pollution (Rajapaksha *et al*. 2012; Vithanage *et al*. 2014) and human and animal toxicities through plant uptake and food webs (Miranda *et al*. 2009). The presence of high concentrations of toxic metals can cause serious limitations to the use of areas overlaying serpentinites for agriculture and livestock farming (Shallari *et al*. 1998; Miranda *et al*. 2009). Geochemical studies from an agricultural area in Mouriki–Thiva in central Greece have revealed anomalous values of Ni (621–2639 mg kg^{-1}) and Cr (134–856 mg kg^{-1}), where Cr and Ni are primarily mobilised from chromite, olivine and serpentine minerals (Antibachi *et al*. 2012). Ni is substantially more labile, and, as a result, is readily available to plants in high concentrations (Antibachi *et al*. 2012; Vithanage *et al*. 2014). Ni is a known neurotoxin, reproductive toxin, nephrotoxin, hepatotoxin and a

carcinogenic agent (Denkhaus and Salnikow 2002). Cr is also a potent carcinogenic agent (Dayan and Paine 2001). Exposure to manganese (Mn) through drinking water can result in permanent neurological disorders and cardiac, liver, reproductive and fetal toxicities (Crossgrove and Zheng 2004).

The efficiency and the type of metal uptake depend not only on the species of plant but also the action of rhizospheric organisms (Abou-Shanab *et al*. 2003; Ma *et al*. 2009). The rhizosphere is a complex and dynamic environment that involves many physical and chemical reactions (Jones and Darrah 1994; Anjum *et al*. 2012; Neilson and Rajakaruna 2012). In recent years, the role of rhizobacteria on plant heavy metal uptake has received some attention (Burd *et al*. 1998; Burd *et al*. 2000; Rajkumar and Freitas 2008). Many rhizospheric bacteria have the ability to promote plant growth through various mechanisms, including nitrogen (N) fixation, utilisation of 1-aminocyclopropane-1-carboxylic acid (ACC) and production of siderosphore and plant-growth regulators (Burd *et al*. 1998; Ma *et al*. 2009). These mechanisms increase the plant biomass and tolerance to heavy metal toxicity. Even though several studies have been conducted on the influence of bacteria in plant heavy metal uptake and immobilisation (Ma *et al*. 2009), there are no published reports of bacterial–fungal interactions on plant heavy metal uptake. Bacterial–fungal interactions are more apparent as biofertilisers in the form of biofilm (Seneviratne *et al*. 2009) and have shown their potential to be used in waste-water reactors for heavy metal remediation (Herath *et al*. 2013). Studies have also shown that their performance is higher than that of mono- or mixed cultures of bacterial biofilms (Herath *et al*. 2013). Thus, the present study was conducted to examine the role of bacterial–fungal inoculation on Ni, Mn, Cr and Co uptake on *Zea mays* plants grown in serpentine soils.

Materials and methods

Study site

Serpentine soil samples were collected from the Yudhaganawa serpentine site located within the Wasgamuwa National Park (7°71′67″N, 80°93′33″E) in the Matale and Polonnaruwa districts of north-central Sri Lanka (Vithanage *et al*. 2014), found in a transitional zone between the Highland and the Vijayan Complex. The climate is tropical with a dry period of 8–9 months. Rainfall is from the north-eastern monsoon from October to January and mean temperature is uniformly high at 32°C throughout the year. Mean annual rainfall ranges from 1750 mm to 2250 mm. The vegetation is mostly a dry mixed evergreen forest (57%) and a scrub jungle (27%).

Soil collection

Soil samples were collected within 10–15 cm from the surface after clearing the surface litter from five random locations. The samples were sealed in polythene bags and brought immediately to the laboratory and bulked and mixed together. The initial metal concentrations were 6567, 2609, 14 880 and 555 mg kg^{-1} of Ni, Mn, Cr and Co, respectively (Vithanage *et al*. 2014).

Preparation of microbial inoculums

Heavy metal-resistant bacteria were isolated in nutrient agar (NA) from serpentine soil collected from the serpentinite outcrop at Yudhaganawa. Dilution plating with serpentine soil from Yudhaganawa (10^{-1}–10^{-3}) was carried out to isolate the heavy metal-resistant bacteria present in serpentine soil. Fungal–bacterial biofilms were formed with an *Aspergillus* fungus (known for metal-tolerant strains; Ahmad *et al*. 2006; Anahid *et al*. 2011). The biofilms were subjected to a series of Ni concentrations (50–500 ppm) and the adsorption was determined. The biofilm with the highest adsorbing ability was used in the experiment.

The Gram-negative bacterium isolated from Ni-rich serpentine soil (currently, unidentified), a garden soil species of *Aspergillus*, and both bacteria and fungi were used as inoculums in the study. The bacterial cells were grown overnight in 250-mL Erlenmeyer flasks containing 100 mL of sterilised nutrient broth on a rotary shaker at 100 rpm at 30°C until late log phase. The fungus was cultured in 250-mL Erlenmeyer flasks containing 100 mL of Czapek dox broth in a rotary shaker at 100 rpm at 30°C for 48 h.

Glasshouse experiment

Serpentine soil was collected from Yudhaganawa outcrop and sieved to obtain the <2 mm fraction. The soils were inoculated with bacteria (B) (10 mL from the bradyrhizobium culture of 0.517 optical density at 600 nm), fungi (F) (10 mL of fungal broth culture containing 2 g of fungal mycelium) and bacteria and fungi together (BF), in triplicate (3 pots, 25 × 20 × 10 in size, per inoculum treatment). The control was filled with serpentine soil, without any microbial treatment. *Zea mays* was selected because it has the ability to tolerate heavy metal stress (Hall 2002; Nocito *et al*. 2006). Surface-sterilised *Z. mays* seeds were soaked in water overnight and allowed to germinate in a Petri dish lined with filter paper. After 1 week, three seedlings of equal height were planted in each pot. Plants were allowed to grow for 90 days in a glasshouse at 26−30°C and 70% relative humidity, with 12 h light/12 h dark conditions (natural light). Pots were watered periodically to keep the soils moist.

Plant tissue analysis

After 90 days, *Z. mays* plants were uprooted, washed with deionised water, and shoot and root samples were separated and dried at 50°C. Dried plant samples were weighed and digested with concentrated HNO_3 acid in a close-vessel temperature-controlled microwave digester system (Milestone ETHOS PLUS labstation with HRP-1000/10S high-pressure segmented rotor, Milestone Model START D, Italy). The digest was diluted to 100 mL with deionised water and Ni, Mn, Cr and Co concentrations were determined using an atomic adsorption spectrophotometer (GBC 933 M, Melbourne, Vic., Australia). Plant accumulation factor and translocation factor were calculated using the following equations:

$$\text{Plant accumulation factor} = \frac{\text{metal concentration in root}}{\text{metal concentration in soil}}, \text{ and} \quad (1)$$

$$\text{Translocation factor} = \frac{\text{metal concentration in shoot}}{\text{metal concentration in root}}. \quad (2)$$

Analysis of soil nutrients

Available phosphorus (P) was measured by the sodium bicarbonate extraction method. About 1.25 g of fresh soil was

shaken at 180 rpm with 25 mL of 0.5 M sodium bicarbonate for 15 min. The extract was filtered with Whatman No. 42 filter paper and 1 mL of it was used for analysis. To the filtrate, 4 mL of ascorbic acid and 3 mL of molybdate reagent were added and, after 1 h, absorbance was read at 880 nm (Watanabe and Olsen 1965). Available N was measured using the colourimetric method (Cataldo *et al*. 1975). A sample of 10 g of soil was shaken with 20 mL of K_2SO_4 for 30 min at 60 rpm. An aliquot of 0.5 mL of the extract was mixed with 1.0 mL of salicylic acid and mixed well with a vortex mixture. After 10 min, 10 mL of sodium hydroxide was added and mixed well. The mixture was incubated for 1 h for colour development and absorbance was read using a spectrophotometer (Shimadzu UV-2450, Japan) at 410 nm.

Measuring soil enzyme activities

To measure the polyphenol oxidase activity, ~5 g of soil was mixed with 10 mL H_2O, 6 mL 0.1% ascorbic acid and 10 mL 0.02 M catechol. It was incubated for 2 min in a water bath at 30°C and 3 mL of 10% phosphoric acid was added and finally the filtrate was titrated with 0.005 mol L^{-1} I_2. For the catalase-activity analysis, ~2 g of soil was mixed with 40 mL water and 5 mL 0.3% H_2O_2 for 20 min in a shaker at 150 rpm. The filtrate was titrated with 0.1 M $KMnO_4$ in the presence of H_2SO_4 (Achuba and Peretiemo-Clarke 2008). For dehydrogenase analysis, 20 g soil was mixed with 0.2 g $CaCO_3$ and 1 mL of 3% triphenyltetrazolium chloride (TTC) was added to 6 g of the mixture. It was incubated at 37°C for 24 h. A volume of 10 mL CH_3OH was added and shaken for 1 min. The absorbance was measured at 482 nm, with CH_3OH as the blank (Casida *et al*. 1964).

Fractionation of heavy metals

Sequential extraction of heavy metals was performed using the method used by Vithanage *et al*. (2014), as follows:

(i) Exchangeable: soil was reacted at room temperature for 1 h with 20 mL of magnesium chloride solution (1 M $MgCl_2$, pH 7.0) with continuous agitation.
(ii) Bound to carbonates: residue from (i) was leached at room temperature for 2 h with 20 mL of 1 M sodium acetate (NaOAc) adjusted to pH 5.0 with acetic acid (HOAc) and with continuous agitation.
(iii) Bound to Fe–Mn oxide: residue from (ii) was treated with 20 mL of 0.04 M hydroxylamine hydrochloride (NH_2OH – HCl) in 25% (v/v) HOAc heated at 90°C, with slow continuous agitation for 2 h.
(iv) Bound to organic matter: residue from (iii) was treated with 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 adjusted to pH 2 with HNO_3, heated to 85°C for 2 h with occasional agitation. Then 3 mL aliquot of 30% H_2O_2 (pH 2 with HNO_3) was added and the sample was heated again to 85°C for 3 h, with intermittent agitation. After cooling, 5 mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added and the sample was diluted to 20 mL and agitated continuously.

Statistical analysis

Data were analysed by ANOVA in SAS statistical package (version 9.1, Statistical Analysis System Institute Inc, NC, USA). Means were compared using Duncan's multiple-range test (DNMRT) at $P = 0.05$.

Results

Plant dry weight and height

Both shoot and root dry weights were higher in plants with microbial inoculation than in the control treatment (Table 1). Root dry weight showed a significant increase in B and BF treatments. The maximum root dry weight was recorded from BF treatment, which was 160% higher than that in the control. The shoot dry weight of microorganism-inoculated samples showed a significant increase over the control. The highest shoot dry weight was recorded for the F treatment. It was 73% higher than in the control. Both of the other microbial treatments (B and BF) showed a 63% increase of shoot dry weight over the control. There was a significant difference in shoot lengths among the treatments, with the highest shoot length reported in the F treatment, showing an increase of 29% compared with the control.

Even though not significant, the highest root length was observed in the BF treatment, with a 32% increase over the control.

Heavy metal accumulation in plant roots and shoots

Plant roots, irrespective of treatments, absorbed Ni in higher concentrations than other metals (Fig. 1). Shoot samples showed significantly higher concentrations of both Ni and Mn than Cr and Co, irrespective of the treatments. Plants treated with BF increased their uptake by 13% for Ni, 52% for Mn, 83% for Cr and 56% for Mn compared with control plants where no inoculum was added (Fig. 1). Even though it was not significant, the translocation factor (Table 2) was lowest in the BF treatment, for Ni and Mn, whereas it was second-lowest for Cr and not detected for Co. Among the heavy metals tested, the highest translocation factor was observed for Mn, suggesting higher accumulation in shoots. Although not significant, the plant accumulation factor was higher in the BF treatment than in the other treatments (Table 2). This indicated that the presence of microbes increased the heavy metal bioavailability and decreased translocation of Ni, Mn and Cr.

Table 1. Shoot and root lengths of *Zea mays* plants in different treatments
Values in parentheses represent standard deviation

Treatment	Root weight (g)	Shoot weight (g)	Root length (cm)	Shoot length (cm)
Control	0.05c ± (0.02)	0.19b ± (0.06)	14.6c ± (5.34)	9.6a ± (1.08)
Bacteria	0.09b ± (0.03)	0.31a ± (0.07)	18.8ab ± (7.91)	12.1a ± (1.24)
Fungi	0.06bc ± (0.02)	0.33a ± (0.08)	15.2a ± (4.54)	12.7a ± (0.97)
Bacterial–fungal inoculation	0.13a ± (0.03)	0.31a ± (0.07)	18.9b ± (3.29)	12.2a ± (1.47)

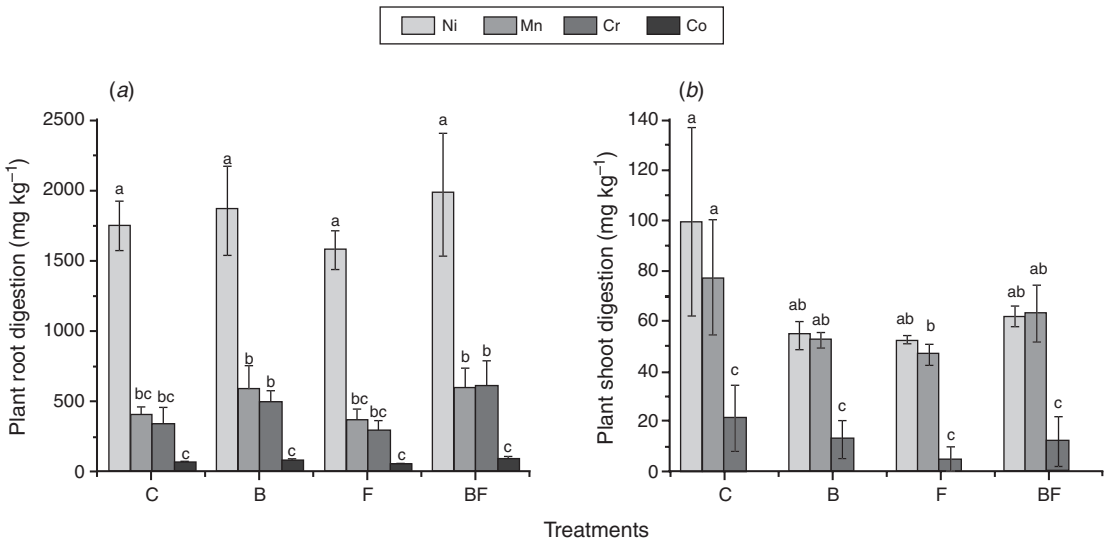

Fig. 1. Concentrations (mg kg^{-1}) of nickel (Ni), manganese (Mn), chromium (Cr) and cobalt (Co) in (*a*) roots and (*b*) shoots of *Zea mays* after 90 days of growth in serpentine soil inoculated with bacteria (B), fungi (F) and both bacteria and fungi (BF). Control (C) plants were provided with no inoculum. Different letters indicate significant differences ($P = 0.05$, d.f. = 8). Error bars represent the standard error of the mean.

Table 2. Plant accumulation factor and translocation factor of *Zea mays* plants in different treatments
Note: Values in parentheses represent standard deviation

Treatment	Plant accumulation factor				Translocation factor			
	Ni	Mn	Cr	Co	Ni	Mn	Cr	Co
Fungi	0.24a ± (0.02)	0.14a ± (0.03)	0.02a ± (0.01)	0.08a ± (0.04)	0.03a ± 0.01	0.13a ± 0.03	0.02a ± 0.02	n.d.
Bacteria	0.24a ± (0.05)	0.18ab ± (0.07)	0.03a ± (0.01)	0.11a ± (0.03)	0.03a ± 0.01	0.11a ± 0.02	0.02a ± 0.02	n.d.
Bacterial–fungal inoculation	0.30a ± (0.07)	0.23b ± (0.05)	0.04a ± (0.01)	0.16b ± (0.04)	0.03a ± 0.01	0.10a ± 0.04	0.02a ± 0.02	n.d.
Control	0.26a ± (0.02)	0.15a ± (0.02)	0.02a ± (0.01)	0.10a ± (0.01)	0.05a ± 0.02	0.20a ± 0.05	0.06a ± 0.02	n.d.

Soil nutrients

The total organic carbon (TOC) content was 2.3% in the serpentine soil from Yudhaganawa. After inoculation of microbes, F treatment showed a significant increase in TOC content, which showed a 15% increase over the control (Table 3). The B and BF treatments showed a significant reduction in TOC over the control by ~7.5% and 5%, respectively. Moreover, the inoculation of microbes led to an increase in both available N and P. Even though there was no significant difference in available N content among the treatments, the highest value was obtained in B treatment and the available P content was significantly higher in the BF treatment.

Soil enzyme activities

The dehydrogenase activity did not show a significant difference among the treatments. However, it was highest in the BF treatment. The polyphenol oxidase activity was significantly higher in the BF treatment, whereas, the catalase activity was significantly lower in the control (Fig. 2).

Fractionation of heavy metals

The sequential extraction results revealed that the inoculation of microbes into soil does not show significant differences of fractionation in Ni, Mn, Cr and Co among the treatments.

Table 3. Total organic carbon, available nitrogen (N) and available phosphorus (P) content in soil treated with bacteria, fungi, both bacteria and fungi and control without inoculation
Values in parentheses represent standard deviation

Treatment	%Organic carbon	Available N	Available P
Fungi	2.2a ± (0.65)	23.39a ± (5.63)	0.41a ± (0.15)
Bacteria	1.85b ± (0.07)	30.46a ± (4.92)	2.66a ± (0.20)
Bacterial–fungal inoculation	1.9b ± (0.07)	25.43a ± (7.54)	2.93a ± (0.66)
Control	2.0ab ± (0.12)	17.46a ± (5.23)	0.16a ± (0.22)

However, exchangeable fractions of Ni and Mn were higher (132 and 47 mg g^{-1}, respectively) in the B treatment than in the other treatments. Carbonate-bound fraction was higher in the BF treatment for Ni, Mn and Co than in the other treatments. Similarly, both Fe–Mn-bound and organic matter-bound fractions were higher in the BF treatment (Fig. 3).

Discussion

Even though heavy metals are toxic to living cells, the microorganisms that inhabit heavy metal-contaminated areas are resistant to metal toxicity (Haferburg and Kothe 2007; Gadd 2010). Therefore, several studies have focussed on the application of these microbes in bioremediation (Burd *et al.* 1998;

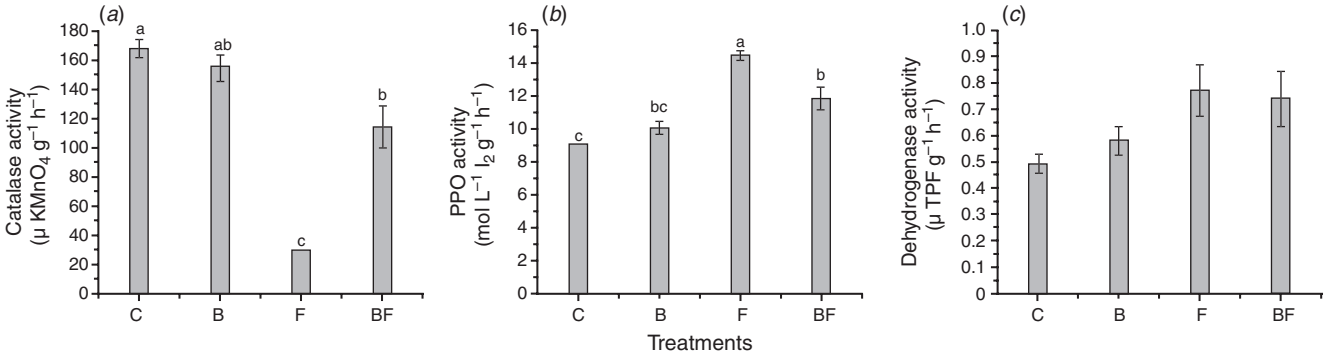

Fig. 2. (*a*) Catalase, (*b*) polyphenol oxidase and (*c*) dehydrogenase activity of serpentine soil inoculated with bacteria (B), fungi (F) and both bacteria and fungi (BF). Control soil (C) was provided with no inoculum. Different letters indicate significant differences ($P = 0.05$, d.f. = 8). Error bars represent the standard error of the mean.

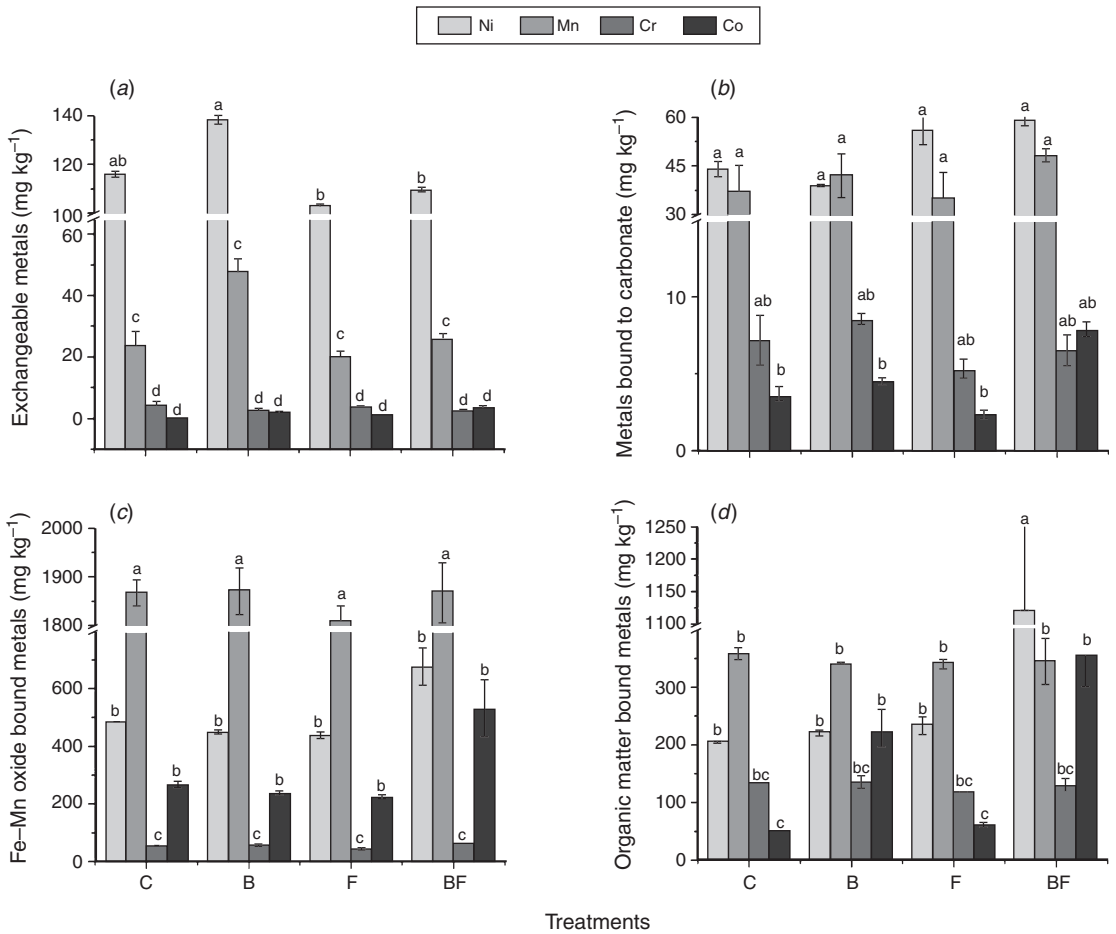

Fig. 3. (*a*) Exchangeable fraction, (*b*) carbonate bound fraction, iron (Fe)–manganese (Mn)-bound fraction of nickel (Ni), manganese (Mn), chromium (Cr) and cobalt (Co) in serpentine soil inoculated with bacteria (B), fungi (F) and both bacteria and fungi (BF). Control soil (C) provided with no inoculum. Different letters indicate significant differences ($P = 0.05$, d.f. = 8). Error bars represent the standard error of the mean.

Rajkumar and Freitas 2008). Belimov *et al*. (2005) reported that the inoculation of the cadmium (Cd)-resistant bacterial strains isolated from the rooting zone of Indian mustard (*Brassica juncea*) grown in Cd-contaminated areas enhances its growth under toxic heavy metal concentrations. Similarly, Jiang *et al*. (2008) reported that the inoculation of *Burkholderia* sp. isolated from a lead (Pb)- and Cd-contaminated field enhanced the growth of tomato and maize under Pd and Cd stress. Serpentine soil is a naturally metal-contaminated soil and, therefore, the microbes that live in this habitat are likely to be more resistant to heavy

metals than those in more recently contaminated areas. (Ma *et al*. 2014) reported that *Psychrobacter* sp. and *Pseudomonas* sp. isolated from serpentine soil improved the growth of *B. juncea* and *Ricinus communis* grown in serpentine soil.

In the present study, the bacterial strains were isolated from serpentine soils with an objective to assess the effects of the metal-resistant and plant growth-promoting bacteria (PGPB) on plant growth and uptake of Ni, Mn, Cr and Co by *Z. mays*. The study showed that the inoculation of microorganisms increased the growth of *Z. mays* and was also effective in protecting plants from growth inhibition caused by heavy metals. Both shoot and root dry weight and shoot length and root length were higher in microorganism-inoculated samples than in the control. Similarly, a significant increase in shoot and root was observed with the inoculation of *Methylobacterium oryzae* strain and *Burkholderia* sp. into tomato plants grown under Ni and Cd stress (Madhaiyan *et al*. 2007). In our study, the highest root weight was observed in the bacterial–fungal treatment (BF), which was 2.6 times higher than in the control. This may be due to the secretion of plant growth hormones (IAA) by the synergistic effect of the bacterial–fungal interaction (Glick 2012).

Heavy metal-tolerant bacteria in the rhizosphere play an import role in growth promotion by possessing many different mechanisms, such as siderophore production, utilisation of ACC and by the production of growth-promoting substances (Sheng *et al*. 2008). Most of the plant growth-promoting bacteria contain the enzyme ACC deaminase, which hydrolyses ACC and thereby decreases ACC within the plant, including a reduction of plant ethylene (Grichko and Glick 2001). The lowering of ACC levels within the plant results in a reduction of plant ethylene and decreases the extent of ethylene inhibition of seedling root elongation (Burd *et al*. 1998). Similarly, the present study showed that microorganism-inoculated treatments induce root elongation of plants. The B and BF treatments showed a significant increase in root elongation, which implies growth promotion and stress reduction by microorganisms. The metal-resistant bacteria belonging to different genera such as *Pseudomonas*, *Mycobacterium*, *Agrobacterium* and *Arthrobacter* have been found to have plant growth-promoting features that can potentially promote plant growth and reduce stress in plants (Dell'Amico *et al*. 2005; Rajkumar *et al*. 2005). The heavy metal uptake and accumulation depends on availability of heavy metals in soil, metal speciation, plant species and rhizospheric activity (Gupta and Sinha 2006). The rhizospheric microbes can affect mobility and availability of trace metals to the plant, by producing siderophores for ensuring iron availability, reducing soil pH, or by solubilising phosphates (Smith and Read 1996; Sheng and Xia 2006; Zaidi *et al*. 2006).

In the present study, inoculation by both fungi and bacteria together influenced the quantity of accumulation of Ni, Cr, Mn and Co in the root system. This may be due to the mobilisation of heavy metals and increasing their availability to plants by lowering the soil pH. The addition of Cd-resistant bacterial strains to *Brassica napus* grown in metal-contaminated soil significantly increased the plant Cd uptake when compared with non-inoculated controls as a result of pH reduction (Gadd and Sayer 2000; Sheng and Xia 2006). Similar results were obtained for the inoculation of *Psychrobacter* to *B. juncea*, resulting in higher Ni accumulation in both the shoots and roots of *B. juncea* with the inoculation of *Psychrobacter* (Ma *et al*. 2009). However, contradictory results were obtained with the inoculation of both bacteria and fungi separately. Many studies have reported that rhizobacteria are able to reduce the heavy metal uptake in plants in the presence of siderophores (Burd *et al*. 1998, 2000). Even though there are several studies on bacterial influence on heavy metal uptake in soil, very few have focussed on fungi. However, studies have reported the effect of mycorrhizal fungi on heavy metal uptake; uptake by mycorrhizal fungi depends on plant growth conditions, the fungal partner, heavy metal and amount of metal present in soil (Weissenhorn *et al*. 1995; Southworth *et al*. 2014).

The plant accumulation factor was highest with Ni, followed by Mn, showing the favourability of Ni and Mn uptake over other metals (Table 3). The plant accumulation factor was lowest with Cr and this could be due to the toxic nature of Cr. It is reported that Mn is a readily translocatable metal, whereas Ni is intermediate and Cr is categorised as the least translocatable metal (Alloway 1995). The amount of heavy metal translocation is a critical consideration for both phytoremediation and vegetative consumption. Higher translocation is favourable in phytoremediation processes, whereas it is less desirable in edible plants used for consumption. In the present study, the translocation of Ni, Mn, Cr and Co was lowest in the BF treatment, showing the lowest accumulation in shoots. Translocation factor, the ratio of shoot to root for metals, indicates internal metal transportation (Kabata-Pendias 2010). It is mainly dependent on heavy metal mobility and toxicity. We report the maximum translocation for Mn, a micronutrient, likely explaining the higher translocation we observed. Ni, which also showed considerable translocation, is also reported as a plant micronutrient important for growth and metabolism (Mishra and Kar 1974; Brown *et al*. 1987; Barker and Pilbeam 2014). Our results indicated that metals accumulated by *Z. maize* were largely retained in roots, as shown by values of translocation factor of <1.

The F-treatment was more effective than the other treatments with increasing TOC. The secretion of mucilage/polysaccharides by inoculated fungi could be the reason for the increase in the TOC concentration (Srivastava *et al*. 2012). The content of available N did not show a significant increase or decrease with the introduction of microbes. However, the P availability was higher in the B and BF treatments. Soil microorganisms produce a range of phosphatases, which have the capacity to utilise P from various forms of organic P that occur in soil. Enhanced phytase activity in the rhizosphere is responsible for P deficiency across a wide range of plant species and is commonly reported to be higher in P-deficient soils (Richardson and Simpson 2011). A wide range of microorganisms able to solubilise inorganic P have been cultured from soil, including bacteria (e.g. *Actinomycetes*, *Pseudomonas* and *Bacillus* spp.) and fungi (e.g. *Aspergillus* and *Penicillium* spp.) (Richardson and Simpson 2011).

Soil enzyme activities are directly related to soil physiochemical characteristics, soil microbial diversity, and soil nutrients (Caldwell 2005). Among the different soil enzyme activities, dehydrogenase activity is an indicator for potential non-specific intracellular enzyme activity of the total microbial biomass (Ladd 1978; Chu *et al*. 2007). In the present study, the microorganism-innoculated samples showed a higher

dehydrogenase activity than did the control, which reflects the increase of microbial activity as a result of inoculation.

The results obtained in the present study indicated that the inoculation of microbes seemed to be very effective in growth promotion of plants under heavy metal stress. The BF treatment showed the highest growth promotion and the highest heavy metal accumulation in the roots. Interestingly, the lowest translocation factor was also recorded in the BF treatment.

Conclusions

Although the Gram-negative bacterial strain we have extracted and used in the present study is unidentified (to be identified via DNA sequencing), our study demonstrated that the inoculation of heavy metal-resistant and serpentine-associated bacterial strains, in association with a common and metal-tolerant soil fungus (*Aspergillus* sp.), seemed to be very effective in protecting plants from growth inhibition caused by Ni, Mn Cr and Co. The BF treatment also increased the root biomass and enhanced root architecture. An increase in plant growth promotion was also observed with microbial treatments compared with the control. Even though the highest plant accumulation factor was recorded for the BF treatment, the translocation factor was lowest in the BF treatment. This treatment led to a reduction of heavy metal accumulation in plant shoot, a beneficial feature for crop plants grown for consumption. We also observed that the BF treatment increased soil nutrients, such as available N and P. Moreover, the microbial inoculation increased dehydrogenase activity, reflecting the increase of soil microbial activity. Current research is aimed at confirming the taxonomic status of the bacterial strain we have isolated as well as testing the efficacy of the biofilm against a range of other heavy metals commonly found in metal-contaminated soils in Sri Lanka.

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