Environ Sci Pollut Res (2015) 22:19408–19416
DOI 10.1007/s11356-015-4471-1

Phytoremediation using microbially mediated metal accumulation in *Sorghum bicolor*

René Phieler[1] · Dirk Merten[2] · Martin Roth[3] · Georg Büchel[2] · Erika Kothe[1]

Received: 22 December 2014 / Accepted: 30 March 2015 / Published online: 15 April 2015

Abstract Reclaiming land that has been anthropogenically contaminated with multiple heavy metal elements, e.g., during mining operations, is a growing challenge worldwide. The use of phytoremediation has been discussed with varying success. Here, we show that a careful examination of options of microbial determination of plant performance is a key element in providing a multielement remediation option for such landscapes. We used both (a) mycorrhiza with *Rhizophagus irregularis* and (b) bacterial amendments with *Streptomyces acidiscabies* E13 and *Streptomyces tendae* F4 to mediate plant-promoting and metal-accumulating properties to *Sorghum bicolor*. In pot experiments, the effects on plant growth and metal uptake were scored, and in a field trial at a former uranium leaching heap site near Ronneburg, Germany, we could show the efficacy under field conditions. Different metals could be extracted at the same time, with varying microbial inoculation and soil amendment scenarios possible when a certain metal is the focus of interest. Especially, manganese was extracted at very high levels which might be useful even for phytomining approaches.

Responsible editor: Robert Duran

✉ Erika Kothe
Erika.Kothe@uni-jena.de

[1] Microbial Communication, Institute of Microbiology, Friedrich Schiller University, Neugasse 25, 07743 Jena, Germany

[2] Applied Geology, Institute of Earth Science, Friedrich Schiller University, Burgweg 11, 07749 Jena, Germany

[3] Bio Pilot Plant, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll-Institute, Beutenbergstraße 11a, 07745 Jena, Germany

Keywords Phytoremediation · Heavy metals · *Sorghum bicolor* · Field trial · Pot experiments · *Rhizophagus* · *Streptomyces*

Introduction

Heavy metal and metalloid soil pollution through anthropogenic activities such as mining and smelting operations, burning of fossil fuels, applications of insecticides or fertilizers, and waste disposal are an increasing problem (Khan 2005; Yoon et al. 2006). High metal loads affect soil functions including soil structure and its productivity and may lead to contamination of ground- and surface waters (Ali et al. 2013; Vamerali et al. 2009). Decontamination of metal-polluted soils (Baker et al. 1994; Raskin et al. 1997) may be achieved by conventional remediation approaches like ex situ application of physicochemical methods; however, these techniques are both destructive and costly (Arthur et al. 2005; Saraswat and Rai 2009). Alternatively, bioremediation provides a sustainable and cost-efficient solution with phytoremediation aiming to apply metal accumulation in harvestable plant biomass (phytoextraction) with subsequent burning and ash deposition or to decrease metal mobility and toxicity (phytostabilization) (Brunetti et al. 2011; Dushenkov et al. 1997).

Phytoremediation strategies offer several benefits: they can be performed in situ and at low cost, prevent destroying soil structure and function, provide a vegetative ground cover abating erosion, and even are permissible for future land use and biorecovery of valuable metals (McGrath et al. 2001; Yang et al. 2005). Limitations that have been encountered are connected to soil properties, level of contamination, and bioavailability of pollutants (Pilon-Smits 2005). To overcome such limitations, organic or inorganic amendments have been

applied which, however, may result in nonpredictable results (Raskin et al. 1997). An alternative strategy is to modulate phytoremediation by addressing the microbial activities in the rhizosphere (Bolan et al. 2014; Sullivan et al. 2013).

Soil bacteria and mycorrhizal fungi can alter physicochemical properties in the rhizosphere and affect plant growth, thus changing metal uptake, e.g., by secretion of phytohormones (Zhuang et al. 2007b), production of chelators and siderophores (Dimkpa et al. 2009a; Raskin et al. 1997), acidification, and biomineralization (Abou-Shanab et al. 2008; Lasat 2002). It has been shown that specifically Gram-positive bacteria such as streptomycetes are ubiquitous in metalliferous soils where they thrive due to specific metal resistance traits aiding, in turn, plant growth (Abbas and Edwards 1989; Dimkpa et al. 2008, 2009a; Haferburg and Kothe 2007; Schmidt et al. 2005, 2009). These interactions between metal-tolerant soil microorganisms and plant roots play a significant role in remediation of heavy metals. Their beneficial effects on plant growth through nitrogen fixation, solubilization of phosphate, or acting as biocontrol agents (Ahemad and Kibret 2014) are well-studied features of plant-associated microorganisms with which they improve efficiency of phytoremediation. Already improved growth, increased metal bioavailability, and protection of plants against phytotoxic metal effects are among the desired characteristics of microbial bioinoculants for improved phytoremediation (Lasat 2002; Weyens et al. 2009).

The potential of heavy metal-resistant bacteria for enhancing the growth of host plants in contaminated soil has been reported (Nogueira et al. 2007; Sessitsch et al. 2013) For instance, *Streptomyces mirabilis* has been found to improve biomass productivity of *Sorghum bicolor* in metal-contaminated soil (Schütze et al. 2014). Fast growing crop plants, like *S. bicolor*, offer several advantages for phytoremediation processes because of its high biomass production, stress tolerance, and metal accumulation potential (Ciura et al. 2005; Epelde et al. 2009; Marchiol et al. 2007; Murillo et al. 1999; Zhuang et al. 2009). In the work presented here, we evaluated the application of two metal-resistant *Streptomyces* strains, isolated from a former uranium mining site and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* for microbially assisted phytoremediation approaches. The study investigated the impact of microbial amendment on plant performance and metal extraction by *S. bicolor* and examined metal mobility in contaminated soil in pot experiments and with field trials.

Material and methods

Site description and soil analysis

Pot and field experiments were carried out using contaminated soil material from the test site Gessenwiese installed by the University of Jena in 2004 on the basement of the former uranium leaching heap Gessenhalde near Ronneburg in Eastern Thuringia, Germany. Between 1952 and 1990, low-grade uranium ores were leached by irrigation with acid mine drainage (AMD) waters or diluted sulfuric acid (Büchel et al. 2005; Neagoe et al. 2005). After uranium mining operations were stopped in 1990, remediation started for restoration of this contaminated site. However, the drainage waters within this experimental site (Gessenwiese) still show high concentrations of heavy metals resulting in a spatially heterogeneous, but comparatively low, multimetal contamination (Schindler et al. 2012).

Soil samples were air-dried and sieved to a grain size up to 2 mm for determination of soil pH and total digestions and for sequential extractions. Soil pH was measured after shaking a 1:4 suspension for 1 h, left to settle for 24 h, and measured using pH330 (WTW). The same solution was used to determine electrical conductivity (EC; TetraCon 325 and LF320, WTW). Total heavy metal contents were determined using a pressure digestion system (DAS 30, PicoTrace). The bioavailable fraction of soil elements was determined following sequential extraction (Zeien and Brümmer 1989). The mobile fraction (F1) was extracted with 1 M NH_4NO_3 (p.a., Merck; compare Grawunder et al. 2009). Element contents were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; 725 ES, Varian) and inductively coupled plasma-mass spectrometry (ICP-MS, X-Series II, Thermo Fisher Scientific) in triplicates. The metal concentrations for total contents and bioavailable fractions are added as values before planting (t_0 at day 0) in the respective experiments, where t_0 was compared to soil concentrations after planting and inoculation. The sandy silt (53.93 % silt, 46.07 % sand) showed a cation exchange capacity of 9.07 mol/kg with a water content of 5.55 to 17.57 % and very low values for carbon, nitrogen, and sulfur as main nutrients (below detection limit for N and S, 1.01 to 1.17 % C).

Preparation of microbial inocula

The two multiresistant strains *Streptomyces acidiscabies* E13 and *Streptomyces tendae* F4 isolated from the former uranium mining site near Ronneburg, Germany (Amoroso et al. 2000), were used as bacterial inoculum. These strains are known to tolerate high concentrations of toxic metals and further for their plant growth promotion traits (Dimkpa et al. 2008; Schmidt et al. 2005). To prepare the bacterial inoculum for pot and field experiments, strains were cultivated in fermenters (7-L BIOSTAT B-DCUII, Sartorius Stedim Systems, or 300L Braun Biotech International). *S. acidiscabies* E13 was grown in medium 3 (glucose monohydrate, 5 g/l; soluble starch, 25 g/l; casein-peptone, 10 g/l; yeast extract, 5 g/l; $(NH_4)_2SO_4$, 1.5 g/l; KH_2PO_4, 1.5 g/l; trace element solution, 1 ml [$ZnCl_2$ 40 mg/l, $FeCl_3 \cdot 6\ H_2O$ 200 mg/l, $CuCl_3 \cdot 6\ H_2O$

10 mg/l, $MnCl_2 \cdot 4\ H_2O$ 10 mg/l, $Na_2B_4O_7$ 10 mg/l, $(NH_4)_2Mo_7O_{24} \cdot 6\ H_2O$ 10 mg/l, pH 7.0]) and *S. tendae* F4 was grown in medium 2 (replacing the C sources of medium 3 with glucose monohydrate, 30 g/l; casein-peptone, 10 g/l; cornsteep [Roquette]). Precultures for inoculating the fermenters were grown in the same media with additional 5 g/l $CaCO_3$. Fermentation conditions were 25 °C, 500 rpm, pO_2>20 %, aeration 2 slpm, and pH>6 controlled with 10 % NaOH (only for *S. tendae* F4). After 42 h of growth, mycelium was harvested by centrifugation (6000 rpm, 15 min, Avanti J-20 XP, Beckman) or separation (300–400 l/h, CSA8, Westfalia) and resuspended in tap water. Dead biomass was obtained by autoclaving. The arbuscular mycorrhizal inoculum was obtained as expanded clay containing spores of *R. irregularis* (Biofa AG, Münsingen, Germany) with 100 spores per gram.

Pot experiments

Pot experiments were carried out from May to October, 2012, on *S. bicolor* plants grown in a greenhouse (Thüringer Landesanstalt für Landwirtschaft, Jena). The setup consisted of 40 polyethylene pots (12×12×16 cm) filled with 2.5 kg contaminated soil from the test site. Each pot was sowed with 23 seeds of *S. bicolor*. After germination, seedlings were thinned to 12 plants per pot. The experimental design included four treatments: a negative control (unamended, C), amended with *Streptomyces* strains (S), amended with mycorrhizal fungus (M), and amended with a mixture of the two streptomycetes and the mycorrhizal fungus (MS). All treatments were carried out in five replicates.

Microbial inoculation was performed by mixing 20 ml of bacterial suspension and/or 4 g of *R. irregularis* granulate at the time of seeding. The pots were arranged in a randomized pattern and randomly rearranged every 4 days. Plants grew with natural day/night rhythm at ambient temperature between 15 and 30 °C. All plants were irrigated daily with distilled water. Aboveground biomass was harvested at 3 and 6 months after planting.

Field experiment

The field experiment was carried out from May to September, 2013, on the test site Gessenwiese (50° 51′ 27″ N and 12° 08′ 82″ E) in the former uranium mining district Ronneburg, Germany (Büchel et al. 2005). *S. bicolor* was cultivated in two different plots of 12× 12 m each, one of which had been amended with 5 cm of calcareous topsoil in 2004 (topsoil plot), while the second plot was left unamended (control plot). *Sorghum* plants were subjected to three experimental treatments in three replicates at each plot: unamended control (C), inoculated with mycorrhizal *R. irregularis* (M), and inoculated with a mixture of mycorrhiza and streptomycetes (MS). For microbial inoculation, a volume of 20 l of bacterial suspension and/or granulate of *R. irregularis* as recommended were applied per subplot (Neagoe et al. 2014; Schindler et al. 2012). Harvest occurred after 17 weeks.

Plant analyses

After harvesting, plant shoots were thoroughly washed with deionized water and oven-dried at 40 °C until constant weight to determine shoot dry weight. Plants were then ground to a fine powder using an ultracentrifugal mill (ZM100, Retsch). Up to 200 mg of plant material was weighted and digested with 5 ml HNO_3 (65 %, supra, Merck) in a microwave pressure system (Mars 5 XPRESS, CEM, Germany). The digested samples were transferred into 25 ml flasks filled up with ultrapure water (PureLab Plus, USF Elga) and analyzed for heavy metals by ICP-OES (725 ES, Varian) and ICP-MS (X-Series II, Thermo Fisher Scientific) in triplicates. The precision and accuracy of the ICP-MS and ICP-OES measurements were proven by analyzing standard reference material SPS-SW2 (Spectrapure Standards AS) and NIST 1643e (NIST) and by measuring multielement standard solution (500 mg/l Ca, K, Mg, Bernd Kraft) each in dilution 1:5 (*v*/*v*) and comparison to the certified values. Typical precision for triplicate measurements was ≤2 % for ICP-MS and ≤5 % for ICP-OES.

Statistical analyses

All statistical analyses were performed with R 3.0.3. The data were analyzed for variance (ANOVA) with a confidence level of 95 %. Significant differences between treatment means were confirmed by Tukey's test or, for nonparametric data, by Kruskal-Wallis test ($P<0.05$). Means and standard deviations were calculated using Microsoft Excel 2007 (Microsoft Corporation) for Windows 7.

Results

Plant performance on contaminated substrate under glasshouse conditions

After 3 and 6 months of plant growth, the influence of microbial inoculation on biomass production of *S. bicolor* was evaluated by measuring shoot weight (Fig. 1). The biomass productivity of inoculated plants that were treated with both mycorrhiza and streptomycetes showed a slight, albeit statistically significant increase after 3 months.

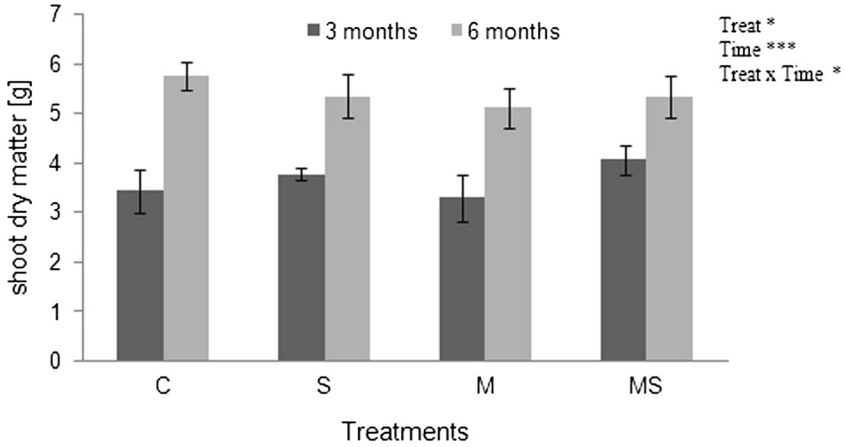

Fig. 1 Effects of treatments on shoot dry weight of *Sorghum bicolor* per pot after 3 and 6 months of growth in potting substrate. Values represent means±SD (n=5). Two-way ANOVA was performed to determine the effects of treatments and time. Significance levels of time, treatments (Treat), and the interaction treat×time are shown: *P<0.05; **P<0.01; ***P<0.001. *C* control treatment without inoculation, *S* amended with streptomycetes, *M* amended with mycorrhiza, *MS* amended with mycorrhiza and streptomycetes

The uptake of metals into shoots of *Sorghum* plants (Table 1) showed significant differences between treatments. Highest concentrations of Al, Co, and Ni were observed in the shoots of noninoculated *Sorghum* plants, while highest amounts of Cd, Mn, Sr, and Zn were found in plants with microbial amendment after 3 months.

After 6 months, *Sorghum* plants without microbial inoculation accumulated significantly higher levels of Al, Co, Mn, Ni, and Zn, while a significant contribution of bacterial and mycorrhizal inoculation could be observed for Cd and Sr uptake. The low bioavailability of U resulted in very low concentrations (0.01 without standard deviation) and was not further considered.

Treatment effects on metal availability and contents in the potting substrate

Both total metal contents and bioavailability were examined in order to evaluate the potential of microbially assisted phytoremediation under controlled conditions. The substrate showed multimetal contamination with high Al bioavailability at pH 4.4 to 4.6 and an electrical conductivity of 439± 12 μS cm^{-1}. Soil bacteria and mycorrhizal fungi can change soil pH and, hence, alter bioavailability. Additional metal tolerance mechanisms including chelator or siderophore production may lead to changes in metal transfer from soil into plant biomass. Thus, the changes in bioavailable metal contents

Table 1 Metal concentrations in shoots of *Sorghum bicolor* grown in greenhouse pots

Growth time	Treatments	Metal concentration in shoots [mg kg^{-1}]							
		Al	Cd	Co	Mn	Ni	Sr	U	Zn
3 months	Control	99.6±31.9	1.19±0.13	0.98±0.17	278±39	43.9±7.9	8.0±0.6	0.01±0.00	12.0±0.9
	Streptomyces	75.5±24.9	1.19±0.20	0.81±0.26	289±22	31.7±14.2	7.7±0.6	0.01±0.00	12.1±1.5
	Mycorrhiza	86.9±18.6	1.34±0.15	0.68±0.07	320±36	24.5±1.6	9.4±0.8	0.01±0.00	10.7±2.1
	Mycorrhiza+*Streptomyces*	58.9±11.1	1.16±0.11	0.56±0.04	281±14	21.4±2.7	8.1±0.9	0.01±0.00	10.6±1.2
6 months	Control	41.9±9.2	0.57±0.06	0.59±0.20	271±27	25.0±7.8	7.5±0.5	0.01±0.00	17.6±3.3
	Streptomyces	25.7±5.7	0.55±0.08	0.44±0.20	252±19	20.1±7.4	7.1±0.8	0.01±0.00	14.2±2.9
	Mycorrhiza	25.5±9.4	0.54±0.13	0.42±0.05	264±18	15.5±2.4	7.4±0.5	0.01±0.00	14.6±2.6
	Mycorrhiza+*Streptomyces*	25.2±4.3	0.62±0.12	0.40±0.14	263±24	16.2±2.4	7.8±0.3	0.01±0.00	13.3±1.7
ANOVA	Treatment	**	n.s.	**	n.s.	***	*	*	n.s.
	Time	***	***	***	**	***	***	*	***
	Treatment×time	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.

n.s. nonsignificant at the P<0.05 level

*P<0.05; **P<0.01; ***P<0.001

within the soil after the plant growth season were checked with respect to the different microbial treatments. For most metals, a decrease in mobile fraction was seen after the pot experiment, most prominent for Al, while a slight mobilization of Co and Mn had occurred for both inoculated and noninoculated pots after 6 months. In contrast, Cd showed very low mobilization for all treatments (Table 2). The inoculation did show a statistically significant effect on reduction of mobile Al and Ni contents.

Biomass production and metal uptake in the field trial

S. bicolor was grown in both field substrates which featured pH 5.2 to 5.4 and conductivity of 276±60 μS cm^{-1} for the topsoil field site and pH 4.4 to 5.2 and 249±99 μS cm^{-1} for the control soil plot. The microbial amendments of either mycorrhiza or a mixture of mycorrhiza and streptomycetes were applied to evaluate microbial impact on plant growth. This was following the results of the pot experiments in which either mycorrhiza alone or, in most parameters, combined streptomycete and mycorrhiza application had induced changes in metal bioavailability.

As the field site had been amended in 2004 with 5 to 10 cm topsoil to allow for better plant performance, this effect was evaluated in addition to microbial inoculation. Generally, an effect on biomass production by adding low amounts of topsoil in 2004 was not observed. In addition, for plants grown on the topsoil plot, microbial inoculation did not enhance biomass production (Fig. 2). In contrast, in the nonamended control soil, aboveground biomass was significantly increased by combined inoculation with mycorrhiza and streptomycetes. Thus, an effect of topsoil addition was seen, albeit only with the help of microbial amendments. While microbial addition could help plant growth on the unamended control soil, the topsoil addition had been sufficient—potentially even by adding the soil microbial community—to support plant growth in a way that made additional microbial inoculation superfluous.

In line with the lack of a measurable effect of inoculation on the topsoil-treated field site, microbial inoculation had no significant effect ($P<0.05$) on general metal accumulation in *S. bicolor* shoots (Table 3). This was found for both substrates. In a more detailed analysis, the topsoil field-grown plants showed higher levels of U and Zn in inoculated subplots. However, the combined application of mycorrhiza and streptomycetes decreased the uptake of Ni into shoots. On the unamended control soil, Co and Mn were accumulated in high amounts into shoot biomass of inoculated *Sorghum*, while the concentration of Ni was lowest in the shoots of plants treated with mycorrhiza.

To test the effects of planting and inoculation on the respective substrate, metal mobility was scored by sequential extraction before and after planting (Table 4). In the topsoil plot, neither plant growth nor inoculation induced visible changes in metal mobility recorded at the end of the growing season for most metals. However, there was a significant increase in Sr in the mobile fraction, while U availability was slightly reduced. Only few significant changes in bioavailable metal concentrations were detected for the control soil, with increases in Al and U and decreases in Co and Mn after plant growth.

Discussion

Phytoextraction of heavy metals by using crop species with high biomass production is a promising approach to remediate

Table 2 Alteration of soil metal bioavailability in pots after plant growth

Soil metal content [mg kg^{-1}]		Al	Cd	Co	Mn	Ni	Sr	Zn
Before planting	Total soil content	57820±300	0.52±0.03	14.7±0.1	477±7	54±1.4	96.6±2.3	75.4±2.1
	Bioavailable	51.3±0.3	0.09±0.00	0.41±0.02	46.6±0.8	5.4±0.1	3.3±0.0	1.91±0.03
3 months	Control	46.1±1.8	0.09±0.01	0.28±0.03	39±2	5.5±0.2	3.5±0.1	1.7±0.1
	Streptomyces	47.4±1.1	0.08±0.01	0.30±0.00	39±2	5.5±0.2	3.4±0.0	1.7±0.1
	Mycorrhiza	47.5±2.4	0.08±0.00	0.26±0.02	36±1	5.6±0.2	3.5±0.1	1.7±0.0
	Mycorrhiza+*Streptomyces*	48.5±1.2	0.08±0.00	0.29±0.02	37±1	5.5±0.3	3.4±0.1	1.6±0.1
6 months	Control	35.6±1.3	0.08±0.01	0.58±0.16	49±8	4.9±0.3	3.2±0.2	1.5±0.0
	Streptomyces	34.9±0.2	0.08±0.01	0.54±0.02	50±1	4.9±0.0	3.2±0.0	1.7±0.0
	Mycorrhiza	34.3±0.4	0.08±0.00	0.54±0.13	49±8	4.8±0.1	3.3±0.2	1.5±0.1
	Mycorrhiza+*Streptomyces*	30.1±2.7	0.09±0.01	0.54±0.03	49±3	4.3±0.2	3.0±0.2	1.5±0.1
Statistical significance	Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Time	***	n.s.	***	***	***	***	***
	Treatment×time	**	n.s.	n.s.	n.s.	*	n.s.	n.s.

n.s. nonsignificant at the $P<0.05$ level

*$P<0.05$; **$P<0.01$; ***$P<0.001$

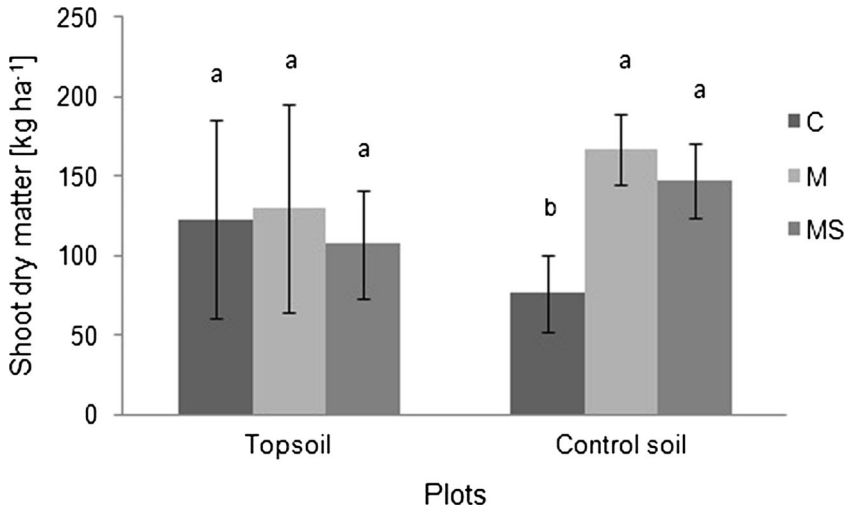

Fig. 2 Effects of treatments on shoot dry weight of *Sorghum bicolor* grown in two different field substrates. Values represent means±SD (n=9). One-way ANOVA was performed for each field substrate. *Means with different letters* are significantly different (P<0.05) according to Tukey's test. *C* control treatment without inoculation, *S* amended with streptomycetes, *M* amended with mycorrhiza, *MS* amended with mycorrhiza and streptomycetes

low to moderately contaminated soils (Ernst 2005). In our study, we evaluated the role of microbial inoculation on metal extraction and uptake by *S. bicolor* grown on multimetal-contaminated soil. Besides metal accumulation capacity, plant biomass production was measured to define a phytoextraction potential for this particular plant species known to tolerate heavy metals including Zn, Cu, Cd, Ni, and Pb (Hernández-Allica et al. 2008; Zhao et al. 2003; Zhuang et al. 2007a). We did not consider root concentrations which are often included in phytoextraction calculations. The crop harvestable biomass exclusively consists of the aerial part, and thus, roots may not be considered for phytoextraction or phytomining as long as the root is not harvested (which would be the case for, e.g., potato or beets).

The diffusive metal translocation in soil and root apoplast reflects the bioavailability of a given metal, making it essential to analyze the bioavailable fraction. The impact of soil microbes on bioavailability is an essential part influencing plant uptake. Soil bacteria and mycorrhizal fungi facilitate an increase in soil metal mobility (Zhuang et al. 2007b) and can significantly promote heavy metal uptake by plants (Rojas-Tapias et al. 2012; Usman and Mohamed 2009).

Metal-resistant streptomycetes were applied to enhance plant performance. These Gram-positive, aerobic soil bacteria have been found to promote plant growth on metalliferrous soils (Dimkpa et al. 2008, 2009b). The effects of streptomycetes on plant development can be triggered by various mechanisms including phosphate solubilization, production of phytohormones, and siderophore excretion (Dimkpa et al. 2009a; Langella et al. 2014). Additionally, metal-resistant arbuscular mycorrhizal fungi have been extensively investigated for application in soil remediation (Griffioen 1994; Khan 2005; Khan et al. 2000; Turnau et al. 2001). They can support growth of host plants in metal-contaminated environments by enhanced uptake of nutrients and water and by modification of metal toxicity via complexation or precipitation (Ernst 2005; Gaur and Adholeya 2004; Wang et al. 2007). In mycorrhizal plants, toxic elements were found to be either more highly concentrated or reduced through fungal metal-binding processes within the rhizosphere (Toler et al. 2005; Usman and Mohamed 2009).

A dual inoculation with arbuscular mycorrhizal fungi and rhizospheric bacteria, specifically streptomycetes, showed enhanced plant biomass productivity and increased levels of mycorrhization (Abdel-Fattah and Mohamedin 2000). In contrast, antagonistic interactions between AM fungi and actinomycetes have also been reported (Adriano-Anaya et al. 2006; Ames et al. 1984; Schreiner and Koide 1993), underlining the

Table 3 Metal concentrations in shoots of *Sorghum bicolor* grown in the field trial

Metal concentration [mg kg^{-1}]		Al	Cd	Co	Mn	Ni	Sr	U	Zn
Topsoil	Control	54.4±5.7	2.8±1.3	1.3±0.8	397±152	21.0±13	7.4±0.6	0.02±0.00	21.1±7.1
	Mycorrhiza	57.4±11.4	2.2±1.7	1.0±0.2	308±15	13.1±2.9	7.4±1.5	0.02±0.01	24.5±12.9
	Mycorrhiza+*Streptomyces*	53.1±16	2.6±1.6	1.4±0.2	385±125	12.3±2.2	7.6±0.6	0.03±0.00	30.1±15.8
Control soil	Control	164±32	3.8±1.3	1.6±0.6	494±126	28.2±3.1	7.6±1.1	0.10±0.02	35.1±16.1
	Mycorrhiza	126±4.6	2.0±0.1	0.8±0.3	303±92	18.3±1.8	8.2±0.8	0.07±0.01	13.3±0.8
	Mycorrhiza+*Streptomyces*	144±24.5	2.1±0.1	3.2±0.8	798±134	29.0±2.4	6.2±0.5	0.08±0.02	14.5±0.2

Table 4 Alteration of soil metal bioavailability in the field after plant growth

Metal concentration [mg kg^{-1}]		Al	Cd	Co	Mn	Ni	Sr	U	Zn
Topsoil	Total soil content	50178±632	0.8±0.1	15.7±1.1	664±63	53.6±2.8	106±3	5.8±0.2	79.4±2.1
	Bioavailable	6.5±3.3	0.17±0.02	0.8±0.55	119±27	8.9±2.3	6.1±0.5	0.004±0.001	3.6±0.9
	Control	17.8±8.8	0.25±0.08	0.33±0.12	92.1±15.5	12.9±5.1	6.3±0.1	0.003±0.00	4.6±1.2
	Mycorrhiza	9.4±5.3	0.19±0.04	0.69±0.42	92.6±24.6	12.0±6.2	6.8±0.3	0.004±0.00	4.3±1.9
	Mycorrhiza+*Streptomyces*	8.3±5.3	0.18±0.04	0.60±0.65	90.8±28.5	10.4±2.8	7.0±0.4	0.003±0.00	4.0±1.0
ANOVA	Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Time	n.s.	n.s.	n.s.	n.s.	n.s.	*	***	n.s.
	Treatment×time	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Control soil	Total soil content	54181±1508	0.72±0.07	17.0±2.0	663±179	53.7±5.3	106±2	6.5±0.2	70.4±1.2
	Bioavailable	33.3±17.2	0.19±0.05	0.87±0.35	87.6±14.9	9.8±2.3	2.9±0.2	0.023±0.001	2.4±0.9
	Control	47.6±4.8	0.20±0.01	0.24±0.10	57.8±4.0	10.6±1.4	3.2±0.3	0.04±0.01	2.2±0.5
	Mycorrhiza	58.1±14.2	0.21±0.06	0.26±0.12	60.5±17.1	10.7±4.3	3.1±0.2	0.04±0.02	2.6±1.2
	Mycorrhiza+*Streptomyces*	74.6±27.5	0.22±0.06	0.44±0.35	72.0±21.8	11.7±3.1	3.0±0.1	0.06±0.03	2.8±1.2
Statistical significance	Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Time	**	n.s.	**	**	n.s.	n.s.	*	n.s.
	Treatment×time	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. nonsignificant at the $P<0.05$ level

*$P<0.05$; **$P<0.01$; ***$P<0.001$

necessity to first score the microbial interactions in pot experiments.

The potential of metal removal of a given plant species is mainly influenced by its metal accumulation capacity and biomass productivity (Lasat 2002; Zhuang et al. 2007a). In our experiment, microbial inoculation could partially enhance shoot metal concentration of *Sorghum* plants under greenhouse conditions, while due to high variability, the effect was less clear under field conditions. These differences in metal uptake between small-scale and open environment, large-scale field trials may be caused by different physiological states of the plants and heterogeneous soil conditions.

The bioconcentration factor (BCF; metal concentration in harvested shoots/soil content in mobile fraction F1) is one of the most important variables for a successful phytoextraction process (McGrath and Zhao 2003). *Sorghum* plants showed relatively high BCF values for Cd and Zn (Table 5). Shoot metal concentrations, including BCF values, decreased during growth except for Zn (compare Epelde et al. 2009).

Since only a small fraction of heavy metals is bioavailable for plant uptake, it is necessary to follow metal mobility in soil (Violante et al. 2010). Besides physicochemical properties like soil pH, redox potential, or metal speciation, which strongly influence bioavailability of heavy metals, soil microorganisms can significantly promote metal solubility and mobilization in the soil through acidification or by producing chelators (Marques et al. 2013; Sheng et al. 2012). The microbial inoculation had a significant impact on reduction of bioavailable soil fractions of Al and Ni after 6 months of plant growth under controlled conditions (see also Schütze et al. 2014).

Table 5 Bioconcentration factors for metal accumulation into *Sorghum* shoots (shoot metal concentration/soil content in mobile fraction F1)

Treatments		Al	Cd	Co	Mn	Ni	Sr	U	Zn
Pot experiment	Control	1.9	13.4	2.4	6.0	8.1	2.4	0.8	6.3
	Streptomyces	1.5	13.5	2.0	6.2	5.9	2.3	0.7	6.4
	Mycorrhiza	1.7	15.2	1.7	6.9	4.5	2.8	0.8	5.6
	Mycorrhiza+*Streptomyces*	1.1	13.1	1.4	6.0	4.0	2.4	0.6	5.6
Field experiment on topsoil plot	Control	20.0	16.5	2.9	3.4	2.3	1.2	6.4	6.8
	Mycorrhiza	8.8	14.2	1.6	2.9	1.6	1.3	5.2	7.1
	Mycorrhiza+*Streptomyces*	8.9	15.8	2.3	3.0	1.4	1.2	5.8	7.9
Field experiment on control soil plot	Control	1082.1	27.5	3.6	6.8	3.3	2.4	18.0	26.7
	Mycorrhiza	4.5	11.9	0.8	3.6	2.3	2.8	3.3	7.1
	Mycorrhiza+*Streptomyces*	4.3	10.9	4.2	8.5	3.1	2.2	3.7	5.9

Thus, our study support the use of *Sorghum* for phytoextraction specifically for Cd and Co, while microbial inoculation can lead to higher plant survival by minimizing the toxic effects of other metals like Ni in the multimetal-contaminated substrate that is usually found at anthropogenically contaminated, metalliferous sites. In our experiments, we were able to extract, choosing the right conditions, approximately 0.5 g Co or Cd per hectare and 4.5 and 1.2 g Ni and Sr per hectare, respectively, and at the same time, 15 mg of highly detrimental U and 120 g Mn per hectare. This multielement remediation can provide a suitable method to stabilize contaminated land and provide future land use, potentially with alternating extraction and renewable energy plant production cycles.

Acknowledgments We would like to thank the Research Training Group (DFG-Gk1257) and the Excellence Graduate School for Microbial Communication (JSMC) for financial support. We are grateful to Dr. Hubert Schröter (TLL, Jena), Ulrike Buhler and Ines Kamp (Applied Geology, FSU Jena), Benjamin Funai (Microbial Communication, FSU Jena), and Dr. Christine Fischer (Ecohydrology, FSU Jena) for assistance and help with this interdisciplinary work.

Conflict of interest There are no potential conflicts of interest.

Compliance with ethical standards This research does not involve human participants or animals.

References

Abbas A, Edwards C (1989) Effects of metals on a range of *Streptomyces* species. Appl Env Microbiol 55:2030–2035

Abdel-Fattah GM, Mohamedin AH (2000) Interactions between a vesicular-arbuscular mycorrhizal fungus (*Glomus intraradices*) and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. Biol Fertil Soils 32:401–409

Abou-Shanab RA, Ghanem K, Ghanem N, Al-Kolaibe A (2008) The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World J Microbiol Biotechnol 24:253–262

Adriano-Anaya ML, Salvador-Figueroa M, Ocampo JA, Garcia-Romera I (2006) Hydrolytic enzyme activities in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) roots inoculated with *Gluconacetobacter diazotrophicus* and *Glomus intraradices*. Soil Biol Biochem 38: 879–886

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881

Ames RN, Reid CPP, Ingham ER (1984) Rhizosphere bacterial population responses to root colonization by a vesicular arbuscular mycorrhizal fungus. New Phytol 96:555–563

Amoroso MJ, Schubert D, Mitscherlich P, Schumann P, Kothe E (2000) Evidence for high affinity nickel transporter genes in heavy metal resistant *Streptomyces spec*. J Basic Microbiol 40:295–301

Arthur EL, Rice PJ, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR (2005) Phytoremediation—an overview. Crit Rev Plant Sci 24:109–122

Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Res Conserv Recycl 11:41–49

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal(-loid)s contaminated soils—to mobilize or to immobilize? J Hazard Mat 266:141–166

Brunetti G, Farrag K, Rovira PS, Nigro F, Senesi N (2011) Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, Southern Italy. Geoderma 160:517–523

Büchel G, Bergmann H, Ebenå G, Kothe E (2005) Geomicrobiology in remediation of mine waste. Chem Erde – Geochem 65:1–5

Ciura J, Poniedzialek M, Sekara A, Jedrszczyk E (2005) The possibility of using crops as metal phytoremediants. Pol J Env Stud 14:17–22

Dimkpa CO, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces* spp. Chemosphere 74:19–25

Dimkpa CO, Merten D, Svatos A, Buchel G, Kothe E (2009a) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696

Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009b) Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biol Biochem 41: 154–162

Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B (1997) Phytoremediation: a novel approach to an old problem. In: Wise DL (ed) Studies in environmental science. Elsevier, Amsterdam, pp 563–572

Epelde L, Mijangos I, Becerril JM, Garbisu C (2009) Soil microbial community as bioindicator of the recovery of soil functioning derived from metal phytoextraction with sorghum. Soil Biol Biochem 41:1788–1794

Ernst WHO (2005) Phytoextraction of mine wastes—options and impossibilities. Chem Erde – Geochem 65:29–42

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86: 528–534

Grawunder A, Lonschinski M, Merten D, Büchel G (2009) Distribution and bonding of residual contamination in glacial sediments at the former uranium mining leaching heap of Gessen/Thuringia, Germany. Chem Erde – Geochem 69S2:5–19

Griffioen WAJ (1994) Characterization of a heavy metal-tolerant endomycorrhizal fungus from the surroundings of a zinc refinery. Mycorrhiza 4:197–200

Haferburg G, Kothe E (2007) Microbes and metals: interactions in the environment. J Basic Microbiol 47:453–467

Hernández-Allica J, Becerril JM, Garbisu C (2008) Assessment of the phytoextraction potential of high biomass crop plants. Env Poll 152: 32–40

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Langella F, Grawunder A, Stark R, Weist A, Merten D, Haferburg G, Büchel G, Kothe E (2014) Microbially assisted phytoremediation approaches for two multi-element contaminated sites. Env Sci Poll Res 21:6845–6858

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Env Qual 31:109–120

Marchiol L, Fellet G, Perosa D, Zerbi G (2007) Removal of trace metals by *Sorghum bicolor* and *Helianthus annuus* in a site polluted by industrial wastes: a field experience. Plant Physiol Biochem 45: 379–387

Marques APGC, Moreira H, Franco AR, Rangel AOSS, Castro PML (2013) Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria—effects on phytoremediation strategies. Chemosphere 92:74–83

McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282

McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207–214

Murillo JM, Maranon T, Cabrera F, Lopez R (1999) Accumulation of heavy metals in sunflower and sorghum plants affected by the Guadiamar spill. Sci Total Env 242:281–292

Neagoe A, Ebena G, Carlsson E (2005) The effect of soil amendments on plant performance in an area affected by acid mine drainage. Chem Erde – Geochem 65:115–129

Neagoe A, Stancu P, Onete M, Bodescu F, Gheorghe R, Iordache V (2014) Effects of arbuscular mycorrhizal fungi on *Agrostis capillaris* grown on amended mine tailing substrate at pot, lysimeter, and field plot scales. Environ Sci Pollut Res 21:6859–6876

Nogueira MA, Nehls U, Hampp R, Poralla K, Cardoso E (2007) Mycorrhiza and soil bacteria influence extractable iron and manganese in soil and uptake by soybean. Plant Soil 298:273–284

Pilon-Smits E (2005) Phytoremediation. Ann Rev Plant Biol 56:15–39

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226

Rojas-Tapias D, Bonilla R, Dussán J (2012) Effect of inoculation with plant growth-promoting bacteria on growth and copper uptake by sunflowers. Water Air Soil Pollut 223:643–654

Saraswat S, Rai JPN (2009) Phytoextraction potential of six plant species grown in multimetal contaminated soil. Chem Ecol 25:1–11

Schindler F, Gube M, Kothe E (2012) Bioremediation and heavy metal uptake: microbial applications at field scale. In: Kothe E, Varma A (eds) Bio-geo interactions in metal contaminated soils. Springer, Heidelberg, pp 365–384

Schmidt A, Haferburg G, Sineriz M, Merten D, Büchel G, Kothe E (2005) Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils. Chem Erde – Geochem 65: 131–144

Schmidt A, Haferburg G, Schmidt A, Lischke U, Merten D, Ghergel F, Büchel G, Kothe E (2009) Heavy metal resistance to the extreme: *Streptomyces* strains from a former uranium mining area. Chem Erde – Geochem 69:35–44

Schreiner RP, Koide RT (1993) Streptomycin reduces plant response to mycorrhizal infection. Soil Biol Biochem 25:1131–1133

Schütze E, Klose M, Merten D, Nietzsche S, Senftleben D, Roth M, Kothe E (2014) Growth of streptomycetes in soil and their impact on bioremediation. J Hazard Mater 267:128–135

Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, Puschenreiter M (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem 60:182–194

Sheng X, Sun L, Huang Z, He L, Zhang W, Chen Z (2012) Promotion of growth and Cu accumulation of bio-energy crop (*Zea mays*) by bacteria: implications for energy plant biomass production and phytoremediation. J Env Manag 103:58–64

Sullivan TS, McBride MB, Thies JE (2013) Rhizosphere microbial community and Zn uptake by willow (*Salix purpurea* L.) depend on soil sulfur concentrations in metalliferous peat soils. Appl Soil Ecol 67: 53–60

Toler HD, Morton JB, Cumming JR (2005) Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. Water Air Soil Poll 164:155–172

Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D (2001) Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. Mycorrhiza 10: 169–174

Usman ARA, Mohamed HM (2009) Effect of microbial inoculation and EDTA on the uptake and translocation of heavy metal by corn and sunflower. Chemosphere 76:893–899

Vamerali T, Bandiera M, Mosca G (2009) Field crops for phytoremediation of metal-contaminated land. Env Chem Lett 8: 1–17

Violante A, Cozzolino V, Perelomov L, Caporale AG, Pigna M (2010) Mobility and bioavailability of heavy metals and metalloids in soil environments. J Soil Sci Plant Nutr 10:268–292

Wang FY, Lin XG, Yin R (2007) Inoculation with arbuscular mycorrhizal fungus *Acaulospora mellea* decreases Cu phytoextraction by maize from Cu-contaminated soil. Pedobiologia 51:99–109

Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27:591–598

Yang X, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353

Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Env 368:456–464

Zeien H, Brümmer G (1989) Chemische Extraktionen zur Bestimmung von Schwermetallbindungsformen in Böden. Mitt Dtsch Bodenkundl Ges 59:505–510

Zhao FJ, Lombi E, McGrath SP (2003) Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil 249:37–43

Zhuang P, Yang QW, Wang HB, Shu WS (2007a) Phytoextraction of heavy metals by eight plant species in the field. Water Air Soil Poll 184:235–242

Zhuang X, Chen J, Shim H, Bai Z (2007b) New advances in plant growth-promoting rhizobacteria for bioremediation. Env Int 33: 406–413

Zhuang P, Shu W, Li Z, Liao B, Li J, Shao J (2009) Removal of metals by sorghum plants from contaminated land. J Env Sci 21:1432–1437