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The reduction of chromium (VI) phytotoxicity and phytoavailability to wheat (*Triticum aestivum* L.) using biochar and bacteria

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ABSTRACT

Chromium (Cr) is considered a serious environmental pollutant due to its wide industrial use. Toxicity of Cr to plants depends on its valence state. Cr(VI) is highly toxic and mobile whereas Cr(III) is less toxic. Cr accumulation in plants causes high toxicity in terms of alterations in the germination process, reduction in the growth of roots, stems, and leaves, which may affect total dry matter production and yield. We performed a pot experiment to investigate chromium (50 mg kg^{-1}) induced phytotoxicity in wheat (*Triticum aestivum* L.) and to reduce its phytoavailability by amending the contaminated soil with chromium reducing bacteria (CRB) and 1% or 5% biochar.

For the phytotoxicity assay, wheat was grown at different concentrations of chromium (10, 20, 30, 40 and 50 mg L^{-1}). After 3 weeks a subsequent reduction in root and shoot length, fresh and dry biomass, percentage germination, total chlorophyll, and carbohydrates was observed.

Our results showed reduction in phytotoxic effects of Cr(VI) mainly due to a reduction of toxic Cr(VI) to Cr(III). Highest reductive transformation of Cr(VI) to Cr(III) was observed in T9 (5% biochar with bacterial consortia) in all three matrices i.e. soil (99%), root (98%) and shoots (97%). The highest (90%) Cr retention within soil was also observed in T9 with the addition of 5% biochar and bacterial consortia. Of the remaining 10% Cr retention (entering into the plant), 3 mg kg^{-1} and 1.3 mg kg^{-1} was found in roots and shoots (on dry weight basis), respectively. Soil inoculation with consortia showed 33% higher stabilization than individual strain application. Soil amendment with biochar and bacteria showed an improvement in plant height, biomass production, seed germination, chlorophyll, protein, and carbohydrate content ($p < 0.05$). Findings of this study may help to reduce food chain availability of potentially toxic Cr by employing cost-effective bioremediation amendments.

1. Introduction

Chromium (Cr) pollution through food chain circulation in agricultural soils has become an area of concern worldwide, due to its negative effects on crops and humans (Gangwar and Singh, 2011). With an average concentration of 122 mg kg^{-1} (Katz and Salem, 1994), Cr is the 7th most abundant element of the earth's crust (Kováčik et al., 2015). It is a redox sensitive polyvalent metal with seven oxidation states (−2 to +6) of which hexavalent and trivalent states are the most prevalent (Fendorf, 1995; Vernay et al., 2007). Cr(III) is 10–100 times less bioavailable, relatively stable and concurrently less toxic than Cr(V1) (Chattopadhyay et al., 2010). Cr (VI) is considered the most toxic form of Cr due to its strong oxidation potential, high solubility, mobility across the membranes and recently has been classified as a grade 'A' human carcinogen by the United States Environmental Protection Agency (U.S. EPA) (Maqbool et al., 2014). Unrestrained discharge of Cr(VI) containing industrial effluents has pernicious effects on crops at concentration of 5–100 mg kg^{-1} within soil (Turner and Rust, 1971). The phytotoxic effects of Cr(VI) include stunted growth, reduced biomass, chlorosis, impaired photosynthesis, and finally plant

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death (Sharma and Sharma, 1996; Vernay et al., 2008). Chromium (VI) is toxic to agronomic plants at concentration of 0.5 to 5.0 mg mL^{-1} in nutrient solution and 5–100 mg kg^{-1} in soil (Ali et al., 2013; Anderson, 2003; Chrysochoou et al., 2012; Khasim et al., 1989; Wilson and Pyatt, 2007). It would be beneficial to reduce Cr uptake by plants which would eventually limit the chromium food chain circulation up to higher living organisms. This aim can be achieved by manipulating Cr availabilty through (i) adsorption/immobilzation and (ii) transformation of more toxic form (Cr-VI) into a less toxic, stable state (Cr-III) (Kerger et al., 2009).

Chromium reducing microorganisms present an economic, safe and environmental friendly option for reduction of Cr(VI) to Cr(III) (Raspor et al., 2000). In bioremediation we utilize the capabilities of living organisms which are present in the environment and available free of cost. Thus, sophisticated equipments or expensive chemicals are not required to treat the Cr(VI) contaminated sites (Thakur, 2004). Chemical reduction techniques become ineffective or expensive when the concentrations of heavy metals are less than 100 mg L^{-1} (Ahluwalia and Goyal, 2007). Bioreduction of Cr (VI) can be a sustainable remediation technology to rectify and re-establish the natural condition of soil. Bioreduction is environmentally friendly and does not add chemicals or carbon sources into the environment. Microbes have the ability to adapt in contaminated environments and can carry out bioreduction for longer periods (Gadd, 2010).

In soil, bioavailability of Cr can further be controlled by organic amendments like biochar, which adsorbs the metals on its surface through a range of mechanisms e.g. complexation, precipitation, cation exchange and electrostatic interaction (Brassard et al., 2016; Doumer et al., 2016). Functional groups of biochar (that mainly consist of carboxylic, hydroxyl, phenolic etc.) provide excellent adsorption sites for binding of metals (Uchimiya et al., 2011). Cr is adsorbed on biochar through sorption, reduction or sorption coupled reduction (Saha and Orvig, 2010). Besides immobilization, biochar also plays a vital role in the reduction of hexavalent Cr into trivalent Cr. Fourier transform infrared spectroscopy (FTIR) analysis has confirmed that biochar contains several fused rings of polycyclic aromatic hydrocarbons (PAH's) which serve as ∏-electron donor for Cr(VI) reduction to Cr(III) (Brewer et al., 2009).

Although a number of studies have investigated microbial reduction of Cr(VI) to Cr(III), (Bolan et al., 2013; Camargo et al., 2003; Chrysochoou et al., 2013; Srivastava and Thakur, 2006) or chromium adsorption by biochar (Agrafioti et al., 2014; Choppala et al., 2012; Mohan et al., 2011), none of the studies focused on the synergistic effects of bacterial inoculation and biochar application on Cr uptake, speciation, fractionation and growth of the wheat plant. Therefore, the objective of the present study was to investigate the influence of co-application of biochar amendment and bacterial inoculation on reduced phytotoxicity and phytoavailability of Cr(VI) through reductive biotransformation and adsorption in Cr-spiked soil. Wheat (*Triticum aestivum* L.) was selected as model plant for this study according to the Organization for Economic Cooperation and Development (OECD) guideline 208 for testing of chemicals. Followed by coarse grain and rice, wheat is the third most important and extensively cultivated crop (650 million tons/year) globally (Alburquerque et al., 2013). In Pakistan, wheat is a staple food, grown across some 8,069,000 ha. According to the Pakistan Agricultural Research Council (PARC), per capita wheat consumption of the country is 120 kg per year – among the highest in the world (Hassan et al., 2013). This crop is in danger of Cr(VI) contamination as approximately 547–814 m^3 of tannery effluents/day containing 592.20 mg L^{-1} of Cr(VI) is discharged from 72 tanning units from Sialkot city only, which exceeds the average allowable Cr(VI) concentration (1 mg L^{-1}) that might be used for irrigation of agricultural soils (Ali et al., 2013). This study will also address the resolution of Pakistan's sustainable agriculture by alleviating Cr(VI) stress through biochar amendment and microbial remediation in polluted croplands.

2. Materials and methods

2.1. Seed collection and sterilization

Seeds of the wheat variety Pak-13, were collected from the Institute of Field Crop (wheat section), National Agricultural Research Centre (NARC), Islamabad. In order to avoid any microbial contamination, seeds were surface sterilized by dipping in 95% ethanol for 60 s and then in 70% sodium hypochlorite (NaOHCl) for ten minutes and washed six times with sterilized water. Prior to sowing, seeds were soaked in distilled water for two hours to enhance the process of seed germination (Abdul-Baki and Anderson, 1973).

2.2. Identification of bacterial strains

Two bacterial strains, Cr122 (*Pseudomonas japonica*) and S2C4 (*Bacillus cereus*) used in this study were isolated from a Cr contaminated site. Chromium resistant bacteria were isolated with a serial dilution method, whereby serial dilutions up to 10^{-7} were spread on Nutrient agar containing 50 mg kg^{-1} filter-sterilized chromium. The distinct colonies of bacteria were streaked three times on Nutrient agar containing 50 mg L^{-1} Cr followed by incubation at 30 °C in order to obtain pure cultures. Minimum inhibitory concentration assays (MIC) were performed to identify the most efficient Cr resistant strains. Glycerol stock of selected strains was prepared by mixing equal amounts of bacterial culture and 80% glycerol (giving a final 40%) stored at −20 °C.

The isolates were sequenced by the Genome Analysis Department Macrogen Inc. Korea. Sequences obtained were analyzed using BLAST search from National Center for Biotechnology Information (NCBI) databases revealing up to 99 or 100% similarity to different bacterial species. Alignments of resultant sequences with related sequences at the NCBI database were carried out using CLUSTALW. 16S rRNA sequences of Chromium resistant organisms were submitted to GenBank and were assigned accession numbers KT758725 (*Pseudomonas japonica*) and KT758726 (*Bacillus cereus*).

2.3. Biochar properties

Ready to use biochar was obtained from the AIT Austrian Institute of Technology. Biochar was prepared by slow pyrolysis (525 °C) of mixed wood chips (2 × 2 dimension) for 60 min in a rotary furnace. Some basic physicochemical properties of biochar were; specific surface area 26.4 $m^2 g^{-1}$, ash content 15.2%, pH 8.9 (in $CaCl_2$), EC 1.6 mS cm^{-1} and Cation Exchange Capacity (CEC) 93.0 $mmol_c kg^{-1}$. Biochar characterization was as described in Kloss et al. (2014). Two different concentrations (1% and 5% v/v of soil) of biochar were used in this study to evaluate the effect of biochar amendment on reduced uptake of Cr by wheat (*Triticum aestivum* L.).

2.4. Germination experiment

Prior to pot experiments, a germination experiment was performed to evaluate the toxic effects of Cr on growth of wheat. Healthy and uniform sized surface sterilized seeds (16 seeds/plate) were sown in autoclaved Petri plates lined with double layer of Whatman filter paper No.1. For control treatments, the filter paper was moistened by adding 8 mL of distilled water. For the Cr treatments, the same quantity of solutions containing Cr i.e.