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Effects of Bacterial Inoculation to Immobilize Nickel in Wheat Grown on Ni-Contaminated Soil

Atta Rasool[a], Salar Ali[b], Waqar Ali[c], Atta Ur Rehman[d], and Said Muhammad[e]

[a]Department of Environmental Science, COMSATS University Islamabad (CUI), Vehari, Pakistan; [b]Department of Biological Sciences, University of Baltistan, Skardu, Pakistan; [c]State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, China; [d]School of life science Guizhou, Normal University Guiyang, Guiyang, China; [e]National Centre of Excellence in Geology, University of Peshawar, Peshawar, Pakistan

ABSTRACT

Plant growth stimulating bacteria are very effective in immobilization of metals and reducing their translocation in plants through precipitation, and adsorption. A pot experiment was conducted to investigate the effectiveness of chitosan- and hematite-modified biochar and bacterial inoculations on the immobilization of nickel (Ni) in polluted soil under wheat cultivation. Application of modified biochars and inoculation with *Pseudomonas putida* significantly increased both wheat root and shoot dry matter yields but decreased Ni phytoextraction efficiency. The Ni concentration, translocation factor and uptake in wheat shoot and root significantly decreased the application of either modified or unmodified biochars. Bacterial inoculation significantly decreased mean translocation factor and also root and shoot concentration and the uptake Ni in the shoot. Chitosan-modified biochar was the most influential treatment in decreasing Ni uptake by wheat followed by *P. putida* inoculation treatment. The results demonstrated positive effects of chitosan modified biochar and inoculation with *P. putida* in increasing dry matter yield and decreasing Ni uptake in wheat grown on Ni-contaminated soil. According to the results of present study, modified biochars application and bacterial inoculation are influential treatments which prevent Ni toxicity probably.

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Bacterial inoculation; immobilization; nickel; phytoextraction efficiency; translocation factor; wheat

Introduction

Climate changes have multifaceted effects on consequences of abiotic stress, threatening the sustainability and productivity of agricultural systems (Fahad, et al. 2014, 2017). However, changing climate, drought, and heat stress have become the most significant factors to crop productivity (Fahad and Bano 2012; Fahad, Hussain, Saud, Hassan, Chauhan, et al. 2016; Fahad, Hussain, Saud, Hassan, Ihsan, et al. 2016). Therefore, climate change is a major challenge for agricultural, food security, and the rural livelihoods of billions of people in the globe (Fahad and Bano 2012; Fahad et al. 2013; Fahad et al. 2014; Fahad et al. 2015; Fahad, Hussain, Saud, Hassan, Chauhan, et al. 2016; Fahad, Hussain, Saud, Hassan, Ihsan, et al. 2016; Fahad, Hussain, Saud, Hassan, Tanveer, et al. 2016 ; Fahad et al. 2017, 2018; Khan et al. 2017; Zahida et al. 2017; Adnan et al. 2018; Muhammad et al. 2019; Saud et al. 2020). Enhancement of toxic metals (HMs) in agricultural soils can reduce soil fertility as well as soil microbial activities and biodiversity (Cui et al. 2018; Jarrah et al. 2019). Nickel (Ni) is one of the most toxic metal which has a complex chemistry behavior due to its potential to easily convert from one oxidation state to another (Hu et al. 2014; Prado et al. 2016). Environmental contamination of Ni has gained substantial consideration worldwide due to its high levels in water and soil originating from both natural and anthropogenic activities such as smelting, mining, wastewater irrigation, agrochemical, manufacturing, industrial and vehicular emissions and weathering (Khan et al. 2010; Nawab et al. 2016). This metal absorbed from polluted soils through roots and translocated into aerial parts and finally enter the food chain, where it may pose serious health problem to human and animals (Ahmed et al. 2016; Rajendran et al. 2019). It is also a serious toxic element for plants growth and development (Adnan et al. 2018; Shahid et al. 2017). Plant exposure to Ni decrease or negatively affects growth by affecting photosynthesis and nutrient uptake of plant (Fahad et al. 2013; Khan et al. 2017; Sharma et al. 2016). Therefore, remediation techniques which reduce the bioavailability of Ni in contaminated soil are needed urgently.

Many strategies have been applied to remediate contaminated soil, which can be divided into *ex situ* and *in situ* strategies. *In situ* immobilization of contamination is greatly more cost effective and environmentally friendly in comparison with *ex situ* soil excavation, removal, and dumping elsewhere (Wang et al. 2019). Effectiveness of *in situ* techniques can be increased through the application of biological treatments such as biochars and some microorganisms. Biochar, a carbon-rich solid product of the pyrolysis of biomass under anaerobic condition, has gained increasing attention

CONTACT Atta Rasool drattarasool@gmail.com Department of Environmental Sciences, COMSATS University Islamabad (CUI), Vehari 61100, Pakistan

owing to its multifunctionality including carbon sequestration, improvement of soil fertility and environmental cleanup (Manolikaki and Diamadopoulos 2019; Patra et al. 2017). Biochar has been used as a high-efficient adsorbent for in situ remediation of organic and inorganic pollutants, and also as a soil passivator to immobilize metals, resulting in reducing their bioavailability (Lu et al. 2018; Sigmund et al. 2018; Yu et al. 2018). It has been shown that numerous modification approaches, can improve biochar application in environmental cleanup (Zhang et al. 2012; Zhou et al. 2013). Such modification may increase the surface functional groups which might be the dominant control on sorption of HMs ions by biochar (Qiu et al. 2008; Uchimiya et al. 2011). In this perspective environment, chitosan-modified biochars (CMBs) would combine the advantages of relatively large surface area and porous network of biochars with chitosan's high chemical affinity to metals (Zhou et al. 2013). Biochar characteristics also could be modified by incorporating hematite as one of the most abundant natural iron oxide minerals for enhance the Ni reduction reaction (Fahad, Hussain, Saud, Hassan, Tanveer, et al. 2016).

Microbial reduction of Ni is also important for bioremediation point which can be considered as an additional Ni immobilization mechanism (Cervantes et al. 2001; Fahad et al. 2015). Hence, more attention should be paid to the application of modified biochars and microorganisms on the immobilization of Ni in polluted soils. The main objective of present study was, therefore, to evaluate the effectiveness of chitosan modified- and hematite modified-biochars and microbial inoculation in the remediation of Ni-contaminated soils.

Material and methods

Soil collection and characterization

The soil samples were collected from different ecological sites in Normal University, Guiyang at surface layer (0–30 cm). The geology of the study was documented in different karst landforms: limestone soil, paddy soil, and yellow soil. The collected soils were homogeneously mixed, air-dried, and sieved (<2 mm). The major physico-chemical characteristics of the soil were measured using standard methods and are presented in Table 1. Particle size distribution and organic matter content (OM) were determined using the hydrometer procedure (Bouyoucos 1962) and Walkley–Black method, respectively. Cation exchange capacity (CEC) and calcium carbonate equivalent (CCE) were determined by replacing cations with NaOAc (Jackson 1958) and neutralization with HCl (Allison and Moodie 1965), respectively.

Table 1. Chemical physical properties of studies soil.

Sand	Silt (%)	Clay	pH	EC (dsm^{-1})	CEC ($Cmol + kg^{-1}$)	CCE (%)	OM (%)
30	34	36	7.3	1.2	26	32	1.1

Chitosan and biochar preparation

Chitosan was prepared from shrimp shells through four main steps including demineralization, deproteination, dehydration, and deacetylation (Hataf et al. 2018; Heidari et al. 2018). Aborementional steps was performed through the application of diluted HCl (7% *w/w*), diluted NaOH (10% *w/w*), 96 and 100% ethanol and 30% *w/w* of NaOH. Biochar was prepared following the method proposed by Agrafioti et al. (2014) through pyrolysis of rice husk powder in a muffle furnace at 600 °C with a temperature increase rate of 15 °C min^{-1}.

Modification of biochar using chitosan and hematite

Chitosan was dissolved in acetic acid (2%) and biochar was added to the solution. After stirring the combination for 30 min, the homogenous suspension was added dropwise into a NaOH (1.2%) solution and kept for 12 h. Then the mixture was rinsed with deionized water to remove the excess of NaOH and oven-dried for 24 h at 70 °C (Zhou et al. 2013). Hematite-modified biochar was prepared following the method proposed by Wang et al. (2015). Hematite powder was added to deionized water and the mixture was sonicated for 30 min with Ultrasonic Homogenizer (SONOPULS HD-4200). In hematite suspension, rice husk was added and stirred for 1 h; solid phase was then separated from the mixture and oven dried at 80 °C. Finally, hematite-treated rice husk powder was pyrolyzed at 600 °C for 1 h.

Bacterial inoculum preparation

Two bacteria *Pseudomonas putida* (PTCC No. 1694) and *Bacillus megaterium* (PTCC no. 1656) purchased from Normal University research center, Guiyang. Pure bacterial culture was grown on nutrient broth (NB) medium in a shaker incubator, at 28 °C for 36 h. The bacterial population was uniformized by McFarland method. The bacterial suspension had a population of 108 colony forming units (CFU) mL^{-1}.

Treatments and experimental design

A factorial 4×4 greenhouse pot experiment was conducted according to a completely randomized design with three replicates. Soil samples placed in plastic bags and Ni was added at the rate of 250 mg kg^{-1}. Treatments consisted of amendments in 4 levels (control, or 1% of each unmodified biochar (B), CMB, and natural hematite-modified biochar (HMB)) and tree levels of bacteria (control, *B. megaterium* and *P. putida*). Biochars were added to each soil sample individually, at 1% *w/w* level, and soil samples were mixed carefully. Treated soils samples were incubated for one month at 25 ± 2 °C under field capacity.

Table 2. Effects of modified biochars and bacterial inoculation on wheat dry weight.

	Treatments				
Microbial inoculation	C	B	HMB	CMB	Mean
Root dry weight (g pot^{-1})					
N	0.38e	0.85d	1.13c	1.45b	0.93B
BM	0.34e	0.77d	1.40Bb	1.22C	0.93B
PP	0.33e	1.19c	1.55b	1.81a	1.19A
Mean	0.35D	0.94C	1.36B	1.49A	
Shoot dry weight (g pot^{-1})					
N	0.7f	2.59de	3.99b	4.22b	2.87AB
BM	0.72f	2.24e	4.17b	3.56bc	2.67B
PP	0.6f	3.14cd	4.06b	5.19a	3.25A
Mean	0.67C	2.66B	4.07A	4.32A	

Note: Means with the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). C: control; B: unmodified biochar; HMB: hematite-modified biochar; CMB: chitosan-modified biochar; N: non-inoculated; BM: inoculated with *Bacillus megaterium*; PP: inoculated with *Pseudomonas putida*.

Plant harvesting and analyses

Pots were filled with 2.5 kg of soil and uniformly fertilized according to the results of soil testing. Six seeds of wheat were planted and thinned to three uniform stands 1 week after emergence. Soil moisture was kept near field capacity during the growth period. Eight weeks after emergence, shoots were harvested and roots were separated from soil carefully. Both parts were rinsed with distilled water and dried at 65 °C for 72 h, weighed, ground, and dry-ashed at 550 °C for 4 h and dissolved in 2 M hydrochloric acid (Jarrah et al. 2014). The concentration of Ni was determined by using an atomic absorption spectrophotometer (Shimadzu AA 670 G, Tokyo, Japan). In addition, phytoextraction efficiency and translocation factor (TF) of Ni were calculated as follows (Asilian et al. 2018):

$$\text{Translocation factor} = \text{Cr concentration in the shoot}/\text{Cr concentration in the root}$$

$$\text{Phyto} - \text{extraction efficiency} = \text{Shoot Cr uptake}/\text{Root dry weight}$$

Results and discussion

Effect of modified biochars and bacterial inoculation on dry weight of wheat

The impacts of modified biochar and bacterial inoculation on dry weight of wheat are shown in Table 2. The results indicated that the addition of the amendments significantly increased mean root and shoot dry weight of wheat with the exception of BM. The shoot dry weight in HMB and CMB were 4.07 and 4.32 g pot^{-1}, respectively, 53 and 62.4% greater than unmodified biochar (2.66 g pot^{-1}). Maximum root and shoot dry weight was observed in the treatment received CMB and inoculated with *P. putida* (1.8 g root pot^{-1} and 5.19 g shoot pot^{-1}). Guan et al. (2009) found that chitosan treatment enhanced plant germination and seedling growth on wheat.

Effect of modified biochars and bacterial inoculation on Ni concentration and uptake in wheat

The results indicated that the addition of amendments significantly decreased Ni content in root and shoot compared with control treatment (C) (Table 3). In the evaluation, application of HMB and CMB resulted in an extreme decrease in Ni concentration in both wheat root and shoot. Compared to control, application of HMB and CMB treatments caused 81.12 and 85.08% decrease in root Ni concentration, respectively. And 86.34 and 91.58% decrease in shoot Ni concentration, respectively. Likewise, the inoculation of microorganism (BM and PP) caused decreases in Ni concentration in both wheat root and shoot. The decreases in Ni concentration of HMB and CMB (modified biochars) treatments were considerably greater than that in the unmodified biochar treatment (B), suggesting that modification of biochar effectively improved its ability in Ni immobilization. It appears that application of HMB and CMB can effectively decrease the accumulation of Ni in wheat, subsequently prevent phytotoxicity. Similarly, Lyu et al. (2018) reported that the addition of biochar supported carboxymethyl cellulose (CMC)-stabilized nanoscale iron sulfide (FeS) composite (CMC-FeS@biochar) greatly reduced the bioavailability of Ni to wheat.

Table 4 shows the effects of different modifications on Ni uptake in wheat root and shoot of wheat. As shown in Table 4, Ni uptake significantly decreased in shoot following the addition of modified biochars and inoculation with microorganisms. The lower content of Ni in wheat showing higher capability of the amendment in Ni-fixation (Khan et al. 2017). The highest effects were found for CMB, followed by PP. The overall effect of the amendments in Ni stabilization was in order of CMB > PP > HMB > BM > B. Nickel uptake decreased in shoot of wheat plant by 47.65, 29.54, 16.90, and 15.91%; following the use of CMB, PP, HMB, and BM, respectively. Higher efficiency of CMB in Ni stabilization in comparison with the other treatments is most likely because of the presence of chitosan with a large number of adsorption sites in its composition. The effect of modified biochar on Ni uptake by wheat root showed a similar trend with that of wheat shoot. However,

Table 3. Effects of modified biochars and bacterial inoculation on the concentration of nickel in wheat root and shoot.

	Treatments				
Microbial inoculation	C	B	HMB	CMB	Mean
Root nickel concentration (μg g^{-1})					
N	5001a	1570c	981e	741fg	2050A
BM	4367b	1581c	926ef	755fg	1908B
PP	4773a	1196d	748fg	604g	1829B
Mean	4713A	1449B	885C	700D	
Shoot nickel concentration (μg g^{-1})					
N	101.23b	33.20c	19.26e	11.85fg	41.28A
BM	99.2b	27.75d	16.30ef	11.67fgh	38.58B
PP	114.6a	20.78e	9.84gh	6.23h	37.89B
Mean	105A	27B	15C	30D	

Note: Means with the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). C: control; B: unmodified biochar; HMB: hematite-modified biochar; CMB: chitosan-modified biochar; N: non-inoculated; BM: inoculated with *Bacillus megaterium*; PP: inoculated with *Pseudomonas putida*.

Table 4. Effect of modified biochars and bacterial inoculation on the uptake of Ni by wheat root and shoot.

	Treatments				
Microbial inoculation	C	B	HMB	CMB	Mean
Root nickel uptake (μg pot^{-1})					
N	1755a	1332bcd	1085def	1060de	1306A
BM	1398bc	1198cde	1283be	912e	1198A
PP	1488b	1291b – e	1147c–f	1088def	1254A
Mean	1547A	1273B	1172B	1020C	
Shoot nickel uptake(μg pot-1)					
N	70ab	88a 73ab	73ab	50cd	69A
BM	72ab	60bdc	65b	36de	58B
PP	68b	63bc	36de	28e	49C
Mean	70A	70A	63B	38C	

Note: Means with the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). C: control; B: unmodified biochar; HMB: hematite-modified biochar; CMB: chitosan-modified biochar; N: non-inoculated; BM: inoculated with *Bacillus megaterium*; PP: inoculated with *Pseudomonas putida*.

Table 5. Effect of modified biochars and bacterial inoculation on the translocation factor (TF) and Phytoextraction efficiency of Ni in wheat plant.

	Treatments				
Microbial inoculation	C	B	HMB	CMB	Mean
Translocation factor					
N	0.04bc	0.023abc	0.021cd	0.015ef	0.020A
BM	0.024ab	0.019de	0.018de	0.015fg	0.018B
PP	0.026a	0.018de	0.013g	0.009h	0.016C
Mean	0.03A	0.02B	0.017C	0.013D	
Phyto-extraction efficiency					
N	195b	102c 66de	73ab	33fg	99A
BM	222a	78d	47ef	30fg	94A
PP	216a	58de	24g	17g	78B
Mean	211A	79B	48C	27D	

Note: Means with the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). C: control; B: unmodified biochar; HMB: hematite-modified biochar; CMB: chitosan-modified biochar; N: non-inoculated; BM: inoculated with *Bacillus megaterium*; PP: inoculated with *Pseudomonas putida*.

microorganisms had no significant effect on Ni uptake by wheat root.

Effect of modified biochars and bacterial inoculation on the translocation factor (TF) and

Phytoextraction efficiency (PE) of wheat

Internal partitioning of Ni between aerial and underground parts of wheat plant was evaluated using the translocation factor (TF), which shows the ratio of Ni concentration in root to the shoot (Asilian et al. 2018). Results indicated that the addition of the amendments significantly decreased TF of Ni in wheat plant (Table 5). Generally, among HMs, Ni is reported to be the least mobile element in the plant roots (Shukla et al. 2007). As shown in Table 3, the concentration of Ni in roots is sometimes 100 times higher than the shoots (Shanker et al. 2005). Ni transfer from plant roots to aerial tissues is very low and dependent on its chemical form inside the tissue.

The values of Ni phytoextraction efficiency (PE) which indicate the ability of the root to transport Ni to shoot are shown in Table 5. The results indicated that the application of the amendments with the exception of BM significantly decreased the values of PE. Compared to control, application of B, HMB, and CMB treatments caused 62.49, 78.62, and 87.82% decrease in PE, respectively. Drastic decreases in PE values subsequent the application of the amendments well designate high capability of the amendments in diminishing Ni phytotoxicity in wheat.

Conclusion

Results of the present study revealed that although biochar was relatively effective in Ni immobilization, modification with hematite and chitosan significantly improved its capability in Ni immobilization. Application of CMB had the highest impact on Ni immobilization followed by inoculation with *P. putida*. These treatments effectively prevented Ni phytotoxicity in wheat probably through the transformation of Ni from mobile and mobilizable fractions to unavailable forms.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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