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Research article

Improvement in productivity, nutritional quality, and antioxidative defense mechanisms of sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.) in nickel contaminated soil amended with different biochar and zeolite ratios

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Nickel (Ni) contaminated soils pose a potential ecological risk to the environment, soil health, and quality of food produced on them. We hypothesized that application of miscanthus biochar (BC) and cationic zeolite (ZE) at various proportions into a Ni contaminated soil can efficiently immobilize Ni and reduce its bioavailability to sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.). An electroplating effluent contaminated soil was amended with BC and ZE, as sole treatments (2% w/w) and their combinations of various ratios (BC, ZE, BC25%ZE75%, BC50%ZE50% and BC75%ZE25%) for immobilization of Ni in the soil. Furthermore, the associated effects of these treatments on residual and DTPA-extractable Ni from the soil; concentrations of Ni in shoots, roots, and grain; growth, physiology, biochemistry and the antioxidant defence mechanisms of sunflower and maize were investigated. Results revealed that BC50%ZE50% treatment efficiently reduced DTPA-extractable Ni in the soil, Ni concentrations in shoots, roots, and grain, while improved selective parameters of both plants. Interestingly, the BC75%ZE25% treatment significantly improved the biomass, grain yield, physiology, biochemistry and antioxidant defense machinery, while decreased Ni oxidative stress in both sunflower and maize, compared to rest of the treatments. The results demonstrate that the BC50%ZE50% treatment can efficiently reduce Ni concentrations in the roots, shoots and grain of both sunflower and maize whereas, an improvement in biomass, grain yield, physiological, biochemical, and antioxidant defense machinery of both crops can only be achieved with the application of BC75%ZE25% treatment in a Ni contaminated soil.
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1. Introduction

Nickel (Ni) in the soil environment has become a leading health concern worldwide (Jiang et al., 2017; Shaheen et al., 2017). The primary anthropogenic source of Ni contamination in the soil is the discharge of effluents from industries like electroplating (Harasim and Filipek, 2015; Lee et al., 2017), cadmium batteries (Chakankar et al., 2017), nickel steel and iron alloys (Harasim and Filipek, 2015).

The elevated Ni deposition in the soil adversely influences soil health and fertility (Ngole-Jeme and Fantke, 2017; Ramzani et al., 2016), plants (Ramzani et al., 2017; Soares et al., 2016), animals (Williams et al., 2017), humans (Goodson et al., 2015) and micro-

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organisms (Nie et al., 2015). Besides these facts, an excessive quantity of Ni^{2+} ions in the soil can also disturb metabolic reactions in sunflower and maize through the initiation of oxidative damage i.e. increasing the contents of reactive oxygen species (ROS) like malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) in leaves tissues (Hassanpour and Rezeyatmand, 2015; Najafi et al., 2011; Sharma and Dhiman, 2013; Wang et al., 2009; Wani et al., 2017) and can influence normal functions of cells (Parlak, 2016; Ramzani et al., 2017). Parallel to Ni oxidative stress, an antioxidant defense machinery in leaf tissues of plants might also be affected by high Ni stress through less production of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), ascorbic acid (AsA) and dehydroascorbate reductase (DHAR) (Ahmad et al., 2017; Kotapati et al., 2017) in sunflower (Gopal and Khurana, 2011; Hassanpour and Rezeyatmand, 2015; Sharma and Dhiman, 2013) and maize (Baccouch et al., 1998; Eriyamremu and Lolodi, 2010). Nickel also disturbs seed germination (Jadia and Fulekar, 2008) and biochemical attributes like soluble sugar, starch, protein, fiber, fat and carotenoids in sunflower (Gopal and Khurana, 2011; Hassanpour and Rezeyatmand, 2015) and maize (Nie et al., 2015). Moreover, Ni creates osmotic imbalance, disturbance in cell structure and low photosynthesis in plants (Ali et al., 2009; Sachan and Lal, 2017) which consequently leads to reduction of biomass in sunflower (Dhiman et al., 2017; Mohammadzadeh et al., 2014; Sharma and Dhiman, 2013) and maize (Nie et al., 2015; Torres et al., 2016).

While considering the limitations of various conventional remediation techniques used for heavy metals polluted soils (Edao, 2017; Sruthy and Jayalekshmi, 2014), the in-situ remediation techniques especially immobilization of Ni in the soil using various organic and inorganic immobilizing agents is gaining perspective attentions of researchers in recent years (Friesl-Hanl et al., 2017; Khan et al., 2017; Radziemska and Mazur, 2016). Biochar (BC) is prepared with organic feedstock under pyrolysis condition (Khan et al., 2017; Oustriere et al., 2017) and exhibits exclusive attributes like a large surface area, porosity and cation exchange capacity (CEC) (Ding et al., 2016; Rajapaksha et al., 2016) which as a result, increase soil pH, CEC, water holding capacity (WHC) and sorption of Ni on the surfaces of BC (Khan et al., 2017; Shen et al., 2017). Furthermore, BC also improves soil health and fertility through the provision of essential nutrients (Bandara et al., 2017; Herath et al., 2015) which can improve growth, yield and quality of sunflower (Alburquerque et al., 2014; Paneque et al., 2016; Tatarková et al., 2013; Turan et al., 2017) and maize (Rehman et al., 2016). Similarly, zeolite (ZE) is a type of aluminium silicates characterized by three-dimensional frameworks of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedral linkage which contains channels and cavities for the exchange of cations (Khatamian et al., 2017; Oste et al., 2002). Zeolite also exhibits a large surface area, porous structure and high CEC which as a result, works as an efficient molecular sieve (Apreutesei et al., 2008; Jiménez-Castañeda and Medina, 2017) for Ni immobilization in the soil (Radziemska and Mazur, 2016; Tahervand and Jalali, 2017) and can reduce its bioavailability to different plants (Sachan and Lal, 2017) including sunflower (Gheshlaghi et al., 2008) and maize (Radziemska et al., 2014).

Till yet, sunflower and maize have been dynamically used in the phytoremediation of heavy metals from polluted soil. However, a limited research has been carried out regarding the role of BC and ZE for Ni immobilization in contaminated soils and consequently, their effects on various parameters like growth, physiology and biochemical compounds of sunflower and maize. Therefore, the objectives of this study were (i) to test the efficiency of BC and ZE (as sole treatments and their combinations of various ratios) on immobilization of Ni in a Ni contaminated soil, (ii) observing the profitable effects of Ni immobilization on the quality and productivity of sunflower and maize, (iii) and to determine the extent of Ni uptake and its effect on the physiology, biochemistry, oxidative stress and antioxidant defense machinery of sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.).

2. Materials and methods

2.1. Collection and characterization of experimental soil

Nickel polluted soil [top layer (0–15 cm)] was collected from an area receiving effluents from the electroplating industry named Al-Badar Engineering Co Pvt Ltd., Sheikhupura road, Qila Sattar Shah, Ferozwala, near Lahore, Pakistan (31°40′18.5″N 74°06′31.3″E). This soil has also been used in our previous experiment (Shahbaz et al., 2018). Prior to use in this pot experiment, the soil was air-dried and passed through a sieve (2-mm size mesh). Several physicochemical characteristics of the soil were measured by adopting standard procedures and methods. To determine soil texture, the hydrometer method proposed by Gee and Bauder (1986) was used. The soil pH was measured in a saturated soil paste by using a calibrated pH meter (model WTW 7110, Weilheim, Germany) after subsequent shaking of a soil-water suspension for 1 h. For the determination of CEC of soil, the method suggested by Rhoades (1982) was adopted. Similarly, the contents of soil organic matter (SOM) were calculated by Walkley-Black method (Jackson, 1962). The soil was analyzed to estimate total phosphorus and exchangeable potassium by adopting the methods recommended by Watanabe and Olsen (1965) and Richards (1954), respectively. The contents of $CaCO_3$ in the soil were calculated by using procedure proposed by Allison and Moodie (1965). The bioavailable Ni in the soil was measured on atomic absorption spectrophotometer [AAS (280FS, Agilent Technologies, Santa Clara, CA, USA)] after extracting with 0.005 M DTPA extractant (Lindsay and Norvell, 1978). Similarly, total Ni in the soil was estimated after digesting the soil with aqua regia (HCl: HNO_3, 3:1 v/v) by adopting the open flask digestion procedure as proposed by Chen and Ma (2001) and further measured on AAS. All physicochemical properties of the experimental soil are illustrated in Table 1.

2.2. Selection of immobilizing agents

Biochar (BC) used in this pot experiment was prepared by using miscanthus as a feedstock (*Miscanthus nepalensis*) which has already been used in our previous experiments (Khan et al., 2017; Shahbaz et al., 2018). The feedstock of miscanthus was passed through the pyrolysis process at 350 °C in a closed reactor. Prior to its experimental usage, the prepared BC was air-dried, thoroughly

Table 1
Physicochemical properties of experimental soil. The origin of these data is Shahbaz et al. (2018).

Parameters	Units	Values
Sand	%	16
Silt	%	41
Clay	%	43
pH	–	8.2
Organic matter	%	1.1
$CaCO_3$	%	3.1
HCO_3	%	0.04
EC	dSm^{-1}	1.9
CEC	$cmol_c\ kg^{-1}$	16.7
Nitrogen	$mg\ kg^{-1}$	139
Phosphorus	$mg\ kg^{-1}$	6.9
Potassium	$mg\ kg^{-1}$	138
DTPA-Ni	$mg\ kg^{-1}$	3.94
Total-Ni	$mg\ kg^{-1}$	77

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