

Potential of Organic and Inorganic Amendments for Stabilizing Nickel in Acidic Soil, and Improving the Nutritional Quality of Spinach

Umeed Ali

Huazhong Agriculture University

Muhammad Shaaban

College of resources and environment Huazhong agriculture university

Saqib Bashir

Key laboratory of arable land and conservation, College of resources and environment Huazhong Agriculture University,

Muhammad Afzal Chhajro

Sindh Madressatul Islam University, Karachi Pakistan

Li Qian

Huazhong Agricultural University College of Resources and Environment

Muhammad Shahid Rizwan

Cholistan Institute of Desert Studies, The Islamia University Bawalpur

Qingling Fu

Key Laboratory of arable land and conservation, college of resource and environment, Huazhong agriculture

Jun Zhu

Huazhong Agriculture University

Hongqing Hu (✉ hqhu@mail.hzau.edu.cn)

Key laboratory of arable land and conservation, College of resources and environment, Huazhong Agriculture University

Research Article

Keywords: Biochar. calcite. spinach. acidic Ni polluted soil

Posted Date: March 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-239959/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Contamination of soils by nickel (Ni) has become a serious environmental problem throughout the world, and this substance yields dangerous effects on the ecosystem and food chain. A pot experiment was conducted to examine the effect of rice straw (RS), rice straw biochar (BI) and calcite (CC) at 1% and 2% application rates in a Ni contaminated soil. The objective was to potentially stabilize Ni and reduce its bioavailability to spinach (*Spinacia Oleracea* L.). Spinach plants were grown in a Ni contaminated Ultisol (commonly known as a red clay soil). Physiological results indicated that a BI 2% application rate significantly increased the photosynthetic rate by 4-18.6 $\mu\text{mol m}^2 \text{S}^{-1}$ and transpiration rate by 1.7–8.9 $\text{mmol m}^2 \text{S}^{-1}$. Similarly, growth parameters for root and shoots dry biomass increased 1.7- and 6.3-fold, respectively, while essential nutrients were enhanced in the spinach plant compared to those in the untreated soil (CK). Moreover, adding amendments significantly decreased CaCl_2 extractable Ni by 62.5% 94.1%, and 87.2%, while the toxicity characteristics leaching procedure (TCLP) fell by 26.7%, 47.8%, and 41.7% when using RS, BI and CC, respectively, at 2% compared to CK. The Ni concentrations in the spinach roots declined by 51.6%, 73.3% and 68.9%, and in the shoots reduced by 54.1%, 76.7% and 70.8% for RS, BI and CC, at a 2% application rate, respectively. Bio-concentration factor (BCF) and translocation factor (TF) dropped significantly by as much as 72.7% and 20%, for BI 2% application rate. Results of the present study clearly indicated that biochar potential soil amendments for Ni stabilization, thereby reducing its bioavailability in the Ni contaminated soil. This process enhanced the safety of food to be consumed and mitigated security risks.

1 Introduction

Potential toxic elements (PTEs) that contaminate soil constitute one of the world's major problems regarding food security and human health (Xu et al. 2015; Azhar et al. 2019; Shaheen et al. 2019). Of all the PTEs, nickel (Ni) has been recognized as a serious threat to the environment and food safety due to increased globalization of economic systems, modern industrialization, household use, municipal effluents and too much application of pesticides and fertilizers (Ramzani et al. 2016; Shahzad et al. 2018). In south and central China, Ni has existed as a key pollutant in agriculture for some decades (MEEPRC 2018; Ali et al. 2020), where the mean concentration of Ni is 226 mg kg^{-1} and maximum 1000 mg kg^{-1} in the arable lands of Jinchang City. This municipality is also famously known as "Nickel City" in Gansu Province, which has the highest Ni concentration than permissible value, that is $> 40 \text{ mg kg}^{-1}$ (MEEPRC 2018).

Consequently, at low levels Ni plays a significant role in seedling growth and development (Rizwan et al. 2018; Shahzad et al. 2018). However, elevated Ni concentrations in soil constitute a major challenge because Ni typically has high availability, mobility, and toxicity (Shahbaz et al. 2018b; Shaheen et al. 2019), and is easily accumulated by leafy vegetables including spinach and cabbage (Salam et al. 2019; Bashir et al. 2020). Elevated Ni can in turn cause physiological disorders in plants and curtail crop growth (Mosa et al. 2016; Sehrish et al. 2019).

Spinach (*Spinacia Oleracea* L.) is one of the most popular leafy vegetables and it is grown throughout the world. In 2014, China became the world's largest spinach producer and in fact produced 22.1 million tonnes, representing 85% of the global supply (Amber Pariona 2017; Boostani et al. 2019). Compared to cereal crops, a leafy vegetable such as spinach exhibits a great potential to accumulate Ni from the soil, resulting in compromised growth, smaller yield, and poorer quality (Younis et al. 2015; Nawab et al. 2019). Khan et al. (2017) noted that larger amounts of essential nutrients and physiological characteristics of plant leaves are necessary to achieve proper spinach growth under conditions of Ni stress, during which amendments may have to be added. Therefore, minimizing or decreasing Ni toxicity from the soil while at the same time ensuring reduced Ni uptake by spinach are important processes for retaining a healthy environment and healthy people. Ensuring the nutritional quality of spinach requires the incorporation of various amendments in soil, and this can be achieved by minimizing Ni uptake in spinach and immobilizing/stabilizing Ni in contaminated soil.

Use of traditional techniques such as soil excavation, land filling, and soil washing for soil remediation have been developed but they are time-consuming, expensive and disturb the environment (Muhseen et al. 2018; Hamid et al. 2019). For these reasons, it is important to develop techniques that can immobilize/stabilize Ni *in situ* and the phytoremediation remediation technique are effective, cost-effective and eco-friendly (Shaaban et al. 2018; Azhar et al. 2019). Alternatively, using organic and organic amendments could be considered for the immobilization of Ni soils by curtailing Ni availability in polluted soils (Ahmad et al. 2015; El-Naggar et al. 2018).

In China the amount of annual residues of rice straw is estimated to be approximately 3.80 billion tonnes (Rizwan et al. 2016; Ali et al. 2020). Amendment of rice straw (RS) not only increases soil organic matter (SOM) but can also effectively reduce the availability of some PTEs including Ni in contaminated soils (Lee et al. 2004; Shaaban Rizwan et al. 2018). The pyrolyzed form (i.e., biochar) of rice straw application to soils has emerged as being better than rice straw (Khan et al. 2019). Biochar (BI) can serve as an effective organic amendment that possesses the ability to adsorb PTEs in soil by electrostatic interaction, complexation, ion exchange, precipitation and chemo-sorption (Shen et al. 2017; Ali et al. 2019). Different biochars have been widely incorporated in contaminated soils and they can effectively decrease the Ni availability and especially Ni uptake by spinach (Younis et al. 2015; Khan et al. 2019), significantly reduced root and shoot accumulation. Similarly, maize (Shahbaz et al. 2018b), wheat and Chinese cabbage (Rehman et al. 2017; Salam et al. 2019) significantly decreased the toxic metals uptake, enhanced growth and physiological parameters. More recently, Shaheen et al. (2019) reported that incorporation of BI in contaminated soils with PTEs: firstly, prominently enhances plants' tolerance of PTEs including Ni stress; and secondly, improves the soil's physicochemical properties.

As well as this, incorporation of inorganic amendments, i.e., calcite (CC) into contaminated soils can transform its mobile form to the most stable (residual) portion through a variety of processes such as adsorption, complexation and precipitation of PTEs in soil (Li et al. 2014; Mahar et al. 2017; Muhseen et al. 2018). According to Hamid et al. (2019) the application of CC as an inorganic amendment in polluted soil may have proved to be effective for PTEs mobility and bioavailability. This pot study was attempted

to investigate the soil pH and Ni availability to spinach by incorporation of organic and inorganic amendments in a Ni contaminated Ultisol and evaluate the potential amendments for Ni immobilization. Therefore, the aim of the current pot experiment was to assess the effectiveness of amendments on: (1) mitigation of Ni availability in Ultisol soil; (2) reducing Ni accumulation by Chinese spinach; and (3) enhancing the growth and nutritional quality of spinach.

2 Materials And Methods

2.1 Soil and amendments collection, preparation and characteristics

The soil used in this experiment is a brown-red type (Ultisol). The soil sampling site is located in Jiangxia district, Wuhan City, Hubei Province, China (30° 17.804' N, 114° 19.246). The region's climate is humid subtropical monsoon with an average temperature of 15.8–17.5 °C and 1269 mm annual rainfall, of which > 60% occurs from June-August (Yang et al. 2016; Ali et al. 2019). The soil sample was transferred to the laboratory, dried at room temperature and ground to pass through a 2 mm mesh sieve. Its physicochemical properties were analyzed (Table 1). The soil's pH and electrical conductivity (EC) were determined in 1:2.5 and 1:5 soil: water (m/V) ratio, respectively (Rizwan et al. 2016), while the wet oxidation method served for analyzing the soil organic matter (SOM) (Lu, 1999). Particle size distribution was determined by the pipette method (Gee et al. 1986), whereas soil cation exchange capacity (CEC) was determined by the NH_4OAC (pH 7.0) method.

Three amendments - RS, BI and CC - were employed in the present study's experiment. Rice straw was collected from an agriculture field at Huazhong Agriculture University, Wuhan, China. RS was cleaned with distilled water to remove impurities, and then the air-dried RS was ground into pieces smaller than 1.5 mm. Pyrolysis of collected rice straw was done in a high temperature furnace with a temperature control program. To ensure the pyrolysis worked successfully, the crucible filled with a precursor was sealed with tin foil beforehand. Following this the pyrolysis was done by raising the temperature to 500°C at a rate of 20°C min^{-1} and maintained for 2 h (Gao et al. 2020). Meanwhile, CC was purchased from Sinopharm Chemical Reagent Co. Ltd, Shanghai, China. Total concentration of Ni in soil and amendments were determined after digesting the samples with aqua regia ($\text{HCl-HNO}_3\text{-HClO}_4$). This was done by adopting the open Pyrex flask digestion procedure as recommended by Lu, (1999) and further measured on flame atomic absorption spectrophotometry (FAAS) (Varian AA240FS). Table 2 summarizes the physicochemical characteristics of the amendments. The amendments pH and EC were determined in 1:10 and 1:5 solid: water (m/V) ratio using a Mettler- Toledo FE20 pH meter and EC meter, respectively (Gao et al. 2020).

2.2. Pot experiment

A pot experiment was carried out in the warehouse of Huazhong Agricultural University, employing a completely randomized design (CRD) with replications done in triplicate and seven treatments as follows:

(1) untreated soil (CK), (2) RS 1%, (3) RS 2%, (4) BI 1%, (5) BI 2%, (6) CC 1%, and (7) CC 2%. In total, 2 kg air-dried soil sieved (2 mm) were put into plastic pots (14 cm height x 18 cm diameter). Soil was spiked with a nickel sulphate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) salt to obtain $100 \text{ mg Ni kg}^{-1}$ concentration in soil. Soil samples were incubated at $25 \pm 1 \text{ }^\circ\text{C}$ and constant humidity (70% of water holding capacity) for 60 days. Each pot was lined with a plastic bag in order to prevent metals and nutrients leaching. The Ni contaminated soil was thoroughly mixed with RS, BI and CC at the application rate of 1% and 2% (w/w) based on previous studies (Houben et al. 2013; Ali et al. 2019) and no amendment in the controlled soil and again incubated in a dark room at 25°C for more than 60 days.

Spinach seeds were disinfected by immersing them in H_2O_2 (2%, V/V) for a period of time, and then rinsed with distilled water (DI) 3–4 times. After seed drying eight seeds were cultivated in 21 pots including the control one. All pots were kept in a warehouse with a transparent roof and nylon net walls. The warehouse's average temperature was retained at a maximum 15°C and minimum 2°C , while humidity ranged between 76–78% throughout the experiment. After 14 days of seed germination, only four similar plants were maintained in each pot. During the experiment, pots were regularly irrigated with deionized water three times a week to avoid drought stress to plants and regularly changing the pots' position until harvesting. Moreover, N: P: K fertilizer at the rate of 0.19: 0.11: 0.13 g kg^{-1} was applied in urea, superphosphate and potassium sulfate form, respectively.

2.3. Physiological and growth parameters of spinach

Prior to harvesting (60 days), the physiological parameters such as photosynthetic rate (A) and transpiration rate (E) were observed in the youngest, fully-grown two healthy leaves using a Portable Infra-Red Gas Analyzer (IRGA) (Li-6400 Li-COR, Lincoln, NE, USA). These parameters were measured according to Hafeez et al. (2019). Briefly, gas exchange measurements were taken on a clear sunny day between 10:00–11:00 h a.m. with the following conditions: photosynthetic photon flux density of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, $380 \mu\text{mol mol}^{-1} \text{CO}_2$ concentration; 1.2–1.5 kPa vapor pressure gradient (VPD); relative humidity 50–65%; and leaf temperature $26\text{--}32^\circ\text{C}$.

Fully matured spinach was harvested and divided into roots and shoots. The fresh biomass of each treatment was recorded with a digital weighing balance. Roots and shoots were washed carefully with tap and distilled water, after which they were oven-dried to at 70°C to achieve a consistent weight. Next, the oven-dried root and shoot samples were ground separately using a stainless-steel grinder for further analysis.

2.4. Assessment of Ni and nutrients concentration in plant tissues and post-harvest soil analysis

The ground plant samples (root and shoot) were digested with a mixture of HNO_3 and HClO_4 at a ratio of (3:1 V/V) (Houben et al. 2013). Briefly, shoot and root samples were separately taken in a 100 mL conical flask and 10 mL of di-acid mixture was added and kept overnight. Next day, the digestion was done using a hot plate at $150\text{--}180^\circ\text{C}$ until 2–3 mL suspension remained, then suspension was diluted to 25 mL with

DI. Then, diluted supernatants were filtrated and stored at 4°C and their concentrations of Ni were determined using FAAS. The Ni concentration of the spinach roots and shoots were used to calculate several factors as described by Bashir et al. (2018b), as follows:

Bioconcentration factor (BCF) = Total Ni concentration_{Harvested plant tissues}/Total Ni concentration_{soil}

Translocation factor (TF) = Ni concentration_{shoot}/Ni concentration_{root}

The total concentration of phosphorus (P) and potassium (K) in plant and soil digest samples were measured on inductively coupled plasma mass spectrometer (ICP-MS-7890A), while the total nitrogen (N), concentrations were determined using a flow injection system (Auto-analyzer, Seal, Germany) (Lu, 1999). After plants were harvested, the leachable Ni was assessed in the rhizosphere soil that existed in each treatment through the toxicity characteristic leaching procedure (TCLP). This has been recently described in the paper by Bashir et al. (2020). Briefly, 1 g of soil was weighed into 50 mL centrifuges tube, and 20 mL of glacial acetic acid solution (pH 2.88) was added. The mixtures were put into a shaker for 18 h. Similarly, bioavailable Ni was examined by extracting the mixture with 0.01M CaCl₂ (2:20 *m/V*) suspensions as recommended by Houben et al. (2013). The mixtures were then shaken in a thermostatic reciprocating shaker for 2 h. Once the mixtures were equilibrated, liquids were separated by centrifuge at 3900 rpm for 20 min and then each solution was filtered and determined for Ni concentration (mg kg⁻¹) using FAAS.

2.5. Statistical analysis

The one-way analysis of variance (ANOVA) of three replicates was employed on data obtained from the experiment employing Statistic 8.1 (Analytical Software, USA). Tukey's Honestly Significant Difference (HSD) test was used to highlight significant differences between the mean of treatment at $P < 0.05$.

3 Results

3.1. Physicochemical properties of soil and amendments

The basic physicochemical properties of soil, organic and inorganic amendments are summarized in Tables 1 and 2. Soil presented a silty clay loam texture, acidic nature (pH 5.3) and a small amount of organic matter (14.79 g kg⁻¹). Moreover, all the amendments were alkaline in nature. Biochar had the highest C content 470.7 (g kg⁻¹) followed by RS 364.0 (g kg⁻¹) and CC 121.2 (g kg⁻¹) (Tables 1 and 2).

3.2. Effect of amendments on soil's post-harvest chemical properties

The current pot study confirmed that the incorporation of rice straw (RS), biochar (BI) and (CC) significantly ($P < 0.05$) improved the chemical properties of post-harvest contaminated soil. Changes in post-harvest, i.e. soil pH, total N, P and K contents differed among used amendment types and their application rates (Fig. 1 and Table 3). The highest soil pH value was observed in CC at 2%. The pH of the

original soil was 5.8 and it rose to 6.04, 6.49 and 6.94 with 1% RS, BI and CC addition, respectively. However, the soil pH increased to 6.47, 6.52 and 7.1 with 2% RS, BI and CC addition, respectively.

Similarly, the concentrations of total N, P and K were in the following respective ranges: 0.22 to 0.44, 0.36 to 0.46 and 8.016 to 9.02 g kg⁻¹ (Table 3). Interestingly, the concentrations of total N, P and K in post-harvest were significantly ($P < 0.05$) increased by adding RS and BI application rates. Soil N rose by 68% and 100% when RS and BI were applied at the 2% application rate, respectively. Similarly, P rose by 5.55%, 27.77% and 16.66%, and K by 7.36%, 12.60% and 9.23% with the application of RS, BI and CC at 2%, respectively, relative to CK.

3.3. Effect of amendments on Ni bioavailability

After harvesting spinach plants, the bioavailable (CaCl₂ extractable) Ni concentration in the soil was determined in the ranges from 18 to 1.05 mg kg⁻¹ soil, respectively (Fig. 2). The CaCl₂ extractable Ni significantly decreased with the addition of RS, BI and CC at 1% and 2% rates. In the presence of RS, BI and CC at an application rate of 2% the reductions reached 62.5%, 94.1% and 87.2%, respectively, compared to CK. Overall, the maximum reduction in CaCl₂ extractable Ni concentration was noted in 2% BI application.

Likewise, TCLP extractable Ni concentration trend was similar to CaCl₂ extractable Ni. The TCLP Ni concentration in spinach in post-harvested soil varied from 28 to 14.6 mg kg⁻¹ soil, respectively (Fig. 3). The TCLP extractable Ni also significantly decreased after the incorporation of RS, BI and CC at 1% and 2% application rates. In the presence of RS, BI and CC 2%, the reductions reached 26.7%, 47.8% and 41.7%, respectively, compared to the CK. Overall, the maximum reduction of TCLP extractable Ni concentration was observed in 2% BI application.

3.4. Effect of amendments on spinach leaves' physiological and growth parameters

Soil amended with RS, BI and CC at the application rates of 1% and 2% and their effects on the physiological and growth parameters of spinach are illustrated in Fig. 4A and B and 5.A, B, C and D. All the amendments significantly ($P < 0.05$) enhanced physiological parameters, i.e., photosynthetic rate (A) and transpiration rate (E) in spinach leaves when compared with (CK) (Fig. 4A and B). The recorded ranges of A and E were 4-18.6 $\mu\text{mol m}^2 \text{s}^{-1}$ and 1.7–8.9 $\text{mmol m}^2 \text{s}^{-1}$, respectively. The BI 2% application to soil caused the highest increase (4.6-fold) in photosynthetic rate of spinach followed by CC 2% (3.24-fold) and RS 2% (2.21-fold), respectively (Fig. 4A).

Similarly, the maximum E was recorded with BI application rates, while the minimum was documented for CK. The BI 2% followed by CC 2% and RS 2% enhanced E of spinach relative to CK, i.e., 2, 5- and 3.2-fold, respectively (see Fig. 5A and B). Generally, in all amendments the values of A and E improved in the following order: BI > CC > RS > CK.

Plant growth parameters, i.e., biomass (shoot and root fresh and dry weight) and total yield of spinach were increased significantly ($P < 0.05$) through the application of organic and inorganic amendments relative to the control plants (Fig. 5A, B, C and D; Table 4). The fresh biomass of shoots and roots ranged from 9.3-31.19, and 1.87–3.05 g pot⁻¹, while the dry biomass of shoot and roots ranged from 2.4–4.2 and 0.32–1.90 g pot⁻¹, respectively, in current experiment (Fig. 6A, B, C and D). These parameters were observed to reach their maximum in biochar-treated soil. Relative to CK, fresh shoot biomass was enhanced by 2.4-fold, 3.4-fold, and 2.5-fold, and fresh root biomass by 1.4-fold, 1.6-fold, and 1.4-fold with RS, BI and CC at 2% application rate over the CK, respectively. Conversely, shoot and root dry biomass of spinach increased by 1.4-fold, 1.8-fold, 1.5-fold, and 2.2-fold, 5.9-fold, and 2.8-fold with RS, BI and CC at 2% application rate over the CK, respectively (Fig. 5A, B, C and D). As well, total dry biomass (TDM) of spinach enhanced by 43.9%, 136.3% and 66.05% in the presence of RS 2%, BI 2% and CC 2% application rate, respectively, when compared to CK (Table 4). In all the amendments the values of growth parameters were enhanced in the following order: BI > CC > RS > CK.

3.5. Effect of amendments on essential nutrient accumulation by plants

Incorporation of amendments in Ni acidic contaminated soil significantly ($P < 0.05$) increased N, P and K concentrations in spinach in comparison to CK (Table 3). The highest N, P and K concentrations were observed in BI 2% application rate. Spinach N content was increased from 4.1 to 11.9, 10.1, and 11.3 g kg⁻¹ DW, respectively, when BI, RS and CC were applied at the 2% application rate. Contrasted to this, spinach P and K concentrations were enhanced from 1.38 to 3.42, 1.94 and 2.86 g kg⁻¹ DW, and 25.4 to 56.05, 28.37 and 42.86 g kg⁻¹ DW, with the incorporation of BI, RS and CC were applied at the 2% application rate, respectively. Overall, the BI applied at the 2% rate was remarkable and on par with other amendments with reference to increasing the concentrations of N, P and K in spinach.

3.6. Effects of amendments on Ni concentrations in roots and shoots, bioconcentration factor and translocation factor

Nickel concentrations in the roots and shoots of spinach had the lowest values in amended soil when compared to CK (Fig. 6). The maximum Ni concentration in spinach was found in plants growing in untreated soil. However, the highest Ni reduction in roots (73.3%) and shoots (76.7%) were noted in BI 2% application rate compared to other treatments and CK. Incorporation of the amendments in the contaminated soil decreased the Ni concentration in roots by 51.6%, 73.3% and 68.9% with RS, BI and CC at 2%, respectively, over the untreated soil. Overall, spinach roots' Ni content decreased in the following order: BI 2% > CC 2% > BI 1% > RS 2% > CC 1% > RS 1% > CK.

Similarly, the maximum decreases in shoot Ni concentration of spinach were recorded with BI 2% application rate, and the minimum spinach Ni content was measured in the untreated soil (Fig. 7). Application of RS, BI and CC at 2% decreased spinach shoots' Ni content by 54.1%, 76.7% and 70.8%, respectively, over the untreated soil. The potential efficiency of incorporated amendments to reduce

spinach shoots' Ni concentration was recorded in the following order: BI 2% > CC 2%, BI 1%, RS 2%, CC 1%, RS 1% > CK.

The bioconcentration factor (BCF) and translocation factor (TF) represents the contents of Ni in spinach root and shoot to that applied in the contaminated soil. The BI 2% application to soil caused the highest reduction (72.7%) in BCF-shoot of spinach followed by CC 2% (63.6%) and RS 2% (45.4%), respectively, relative to CK (Table 4). Compared to CK, BCF-root was reduced by 44.8%, 65.5% and 62% in RS 2%, BI 2% and CC 2% treated spinach, respectively, whereas the maximum TF value fell (20%) in BI 2% application rate when compared to CK (Table 4). Our findings demonstrated the minimum bioavailability of Ni in polluted soil when amendments were added. Our study suggests that currently, all organic and inorganic biochar amendments enhance the reduction and adsorption of Ni in a leafy vegetable (spinach).

4 Discussion

The incorporation of organic and inorganic as soil amendments are promising solution for PTEs including Ni immobilization/stabilization within soils (Shahbaz et al. 2018; Shaheen et al. 2019). The main purpose of the current study was to assess the efficiency of rice straw, its derived biochar and calcite, in order to quantify their effectiveness in stabilizing Ni in soil, reducing Ni concentration by spinach, and improving its nutritional quality and yields.

A remarkable change occurred in soil pH, nutrients, extractable Ni, spinach growth and Ni concentration in Ni contaminated soil after these amendments were applied. After plant harvest, the soil pH significantly ($P < 0.05$) grew with increasing rates of amendments due to their alkaline nature (Table 2). Previous studies reported that the soil pH increased with the application of 2–5% of rice straw, biochar and calcite in Ni contaminated soils (Lee et al. 2004; Houben et al. 2013; Ahmad et al. 2015; Rehman et al. 2017). The increment in the soil pH when BI and CC were incorporated may have been due to the alkalinity and release of base cations into the soil (El-Naggar et al. 2018). The dissolution of these alkaline amendments into oxides, hydroxides and excessive calcium ions may increase soil pH. The present study's findings are consistent with what other analyses documented, for example Shahbaz et al. (2018a) and Salam et al. (2019). They revealed that high mineral ash content, presence of many surface functional groups and alkaline substances, like hydroxides and carbonates of amendments especially biochar, might increase soil pH. Similarly, calcite has a low surface area but high pH and sufficient amounts of calcium which could increase soil pH and promote metal carbonate through precipitation (Lahori et al. 2017). Ali et al. (2019) and Shaheen et al. (2019) concluded that adding different amendments in Ni contaminated soil could enhance soil pH compared with untreated soil. Several studies have discovered that BI is a competent amendment for increasing soil pH and inducing a liming effect on contaminated acidic soil (Rizwan et al. 2016; Bashir et al. 2020). Similarly, Houben et al. (2013) tested CC 5% addition in contaminated soil by growing rapeseed in post-harvested soil and the pH levels increased remarkably compared to biochar (10% application rate) and untreated soil.

According to the current study findings, amendments specially the addition of biochar to polluted soil could enhance soil and plant nutrients, which might be due to the dissolution of biochar liable carbon and excessive elements in the soil solution (Li et al. 2014; Ramzani et al. 2016). Ding et al. (2016) revealed that BI has potential nutrients source and can release considerable amounts of N, P, and K. Therefore, our results concluded that BI can potentially increase total N, P and K content in soil and plant when compared to other amendments (Table 3). Similar results were observed in the work by Bashir et al. (2018a), who indicated that incorporation of rice straw biochar in contaminated soil significantly increased the total amounts of N, P, and K by 75.7%, 79.5% and 40.4%, respectively, relative to the CK. Our results concur with the findings reported by Khan et al. (2017) and Khan et al. (2019), where spinach and tomato growth and nutrient contents rose in the presence of straw and biochar application. Overall, the application of BI to Ni acidic contaminated soil has great potential for improving soil fertility and promoting spinach growth in soils with high concentrations of Ni. This is due to BI having a huge specific surface area (SSA), excessive amounts of elemental minerals and plenty of liming capacity (Table 2). The present study used biochar properties that are almost similar to those employed by Kamran et al. (2020) and Gao et al. (2020).

The remarkable changes in post-harvest soil Ni in CaCl_2 and TCLP extract following the amendments' incorporation may also influence Ni immobilization/stabilization. We detected a significant decrease in CaCl_2 and TCLP-extractable Ni concentration when RS, BI and CC were applied at the 1% and 2% application rates (Figs. 2 and 3). The significant increase in Ni stabilization with amendments application could be associated with an increase in soil pH due to alkaline amendments (Table. 2) being added in acidic polluted soil (Xu et al. 2015; Shaheen et al. 2019). Specifically, BI at 2% significantly curtailed the extractability of Ni by discouraging Ni metal toxicity via immobilization in the soil. Incorporation of BI into a contaminated soil can improve its fertility which leads a substantial improvement in soil's physicochemical properties. Similar studies have documented that PTEs stabilization increased with different rates of BI and CC through different mechanism such as adsorption and precipitation due to its porous structure, greater SSA and functional groups (Shen et al. 2017; Hamid et al. 2019; Ali et al. 2019). Bashir et al. (2018b) noted a 73% and 38% reduction in CaCl_2 and TCLP extractable soil concentration, respectively, when adding rice straw biochar in contaminated soil and comparing this to the untreated soil. Previous studies stated that application of different organic and inorganic amendments (agriculture wastes, biochar and lime materials) has the potential to decrease PTEs bioavailability and leachability in polluted soil, due to the their physicochemical characteristics (Ramzani et al. 2016; Mahar et al. 2017; Rehman et al. 2017). Conversely, our findings concur with those of Houben et al. (2013) who in their rapeseed study, suggested that incorporating BI and CC at various rates in contaminated soil: firstly, significantly decreased Cd, Pb, and Zn availability; and secondly, improved plant growth and biomass. Recently, Salam et al. (2019) found that the incorporation of organic amendments, such as rice straw biochar in naturally acidic contaminated soil improved leafy vegetable growth as well as significantly decreased TCLP and CaCl_2 extractable Pb and Cu.

Physiological and growth parameters are considered to be key indicators that can help evaluate the damage caused by Ni toxicity to plants (Xu et al. 2015; Shahzad et al. 2018; Azhar et al. 2019). We discovered a remarkable increment physiological and growth parameter in amended pot. Likewise, the most significant results were noted in BI 2% treated plants (Fig. 4A and B and Fig. 5.A, B C and D; Table 4). This increase in above parameters can be attributed to Ni stabilization and enhanced nutritional quality after added RS, BI and CC. This finding agrees with previous analyses where Ni stress caused a decline in physiological parameters in red clover plant (Shahbaz et al. 2018a). Similar results regarding Ni-induced reductions in physiological and growth parameters in different plants have been reported by Rehman et al. (2016) and Sehrish et al. (2019). The decline in biomass and physiological parameters might be due to Ni induced reduction in nutrient uptake and ultra-structural changes in plants species (Rizwan et al. 2018; Shahzad et al. 2018). These results agreed with Boostani et al. (2019), who reported the adding BI at 2.5% increased the shoots' dry weight of spinach by about 46%, compared to the respective Ni contaminated soil. Increase in spinach biomass was a key factor when judging the effects of Ni on plant growth. In our findings, we observed that all factors play vital role in enhancing plant dry biomass, which was consistent with Younis et al. (2015), who investigated that incorporation of cotton sticks biochar could increase biomass of *Spinacia oleracea* fresh and dry biomass shoot and root by 78% and 58%, respectively, compared to the control. The incorporation of RS and CC elevated soil pH, and reduction in Ni solubility in soil solution which could increase plant growth and soil health and our finding were accordance documented by Houben et al. (2013), who suggested that CC amendments have sufficient amounts of calcium ions, which offered more benefits by: firstly, increasing soil pH and better rapeseed growth; and secondly, decreasing toxic metal mobility. The possible mechanism for the improvement in above parameters of spinach as a leafy vegetable, through the application of organic and inorganic amendments, could be due to physiological parameters being enhanced via chlorophyll content, facilitation of electron transport and better water mobility in spinach leaves via the xylem process (Xu et al. 2015; Rizwan et al. 2018).

The content of Ni in spinach shoots and roots was significantly ($P < 0.05$) different among all amended pots. Applying amendments reduced Ni content by spinach, which must indicate the poorer bioavailability of Ni in contaminated soil with increasing rates of amendments and especially BI rates (Shen et al. 2016; Shaheen et al. 2019). Rice straw biochar may have the highest absorption capacity because it is alkaline in nature and has a larger surface area as compared to CC and RS in Table 2. These characteristics suggest that application of BI could enhance Ni absorption on BI amended soil, which might be one of the mechanism/reasons for minimizing Ni availability to spinach relative to CK. The minimum absorption of Ni by spinach tissues is due to the lowest concentration values of Ni mobility and leachability in soil, which was directly related to the increment of soil pH in biochar amended soil. Our findings agree with the work conducted by Rehman et al. (2016), who stated that Ni decreased significantly when the rate of adding biochar increased from 1–2%. Therefore, the incorporation of BI significantly reduced the Ni absorbed by plant tissues, and this proved to be consistent with Khan et al. (2017), who observed that application of miscanthus derived biochar significantly reduced Ni content in spinach by up to 58%, relative to CK. Similar findings were observed by Salam et al. (2019), who concluded that the application

of biochar remarkably reduced toxic metal content by up to as much as 37.81% when employing *Brassica chinensis*. Similarly, the addition of CC decreased Ni content in spinach tissue. Inorganic amendment especially CC is reported to raise soil pH in acidic soil (Hamid et al. 2019). This elevated soil pH with CC liming amendment may be attributed to reduced bioavailability and increased metal immobilization in post-harvest contaminated soil (Houben et al. 2013). The possible mechanisms for this resultant concentration of Ni in spinach root and shoot are the formation of stable BI complexes with Ni either by surface sorption onto its different functional groups. This could be due to the exchange of Ni with various cations associated with BI and CC (Shen et al. 2016; Lahori et al. 2017).

The BCF refers to the ratio of metal in a plant compared to its content in the soil, whereas TF is another important factor that can assess the ability of spinach plants to transfer Ni from roots to shoots (Mosa et al. 2016; Boostani et al. 2019). In the current experiment the BCF and TF values were noted to be highest in roots compared to shoots (Table 4). Meanwhile, the extent of BCF in shoots and roots were recorded in CK. The uptake of Ni in shoots is possible due to there being more Ni in the roots. Nawab et al. (2019) and Salam et al. (2019) investigated the distribution ratio of metals' concentrations in the total amount of metals in leafy vegetable (spinach) and Chinese cabbage in root. They noted that this may play an important role in the Ni concentration in plant shoots. The BCF and TF values calculated in the present study were lower than the unity in all amended soil samples. The reduced BCF and TF values which were less than unity in spinach under amended soil conditions, confirming that BI and CC could reduce Ni uptake by spinach. Our findings are virtually the same as those of other studies by Herath et al. (2015) and Bashir et al. (2018a), who noted the BCF and TF values after CC and BI application may have resulted from their great ability to minimize plants' uptake of metal substances. In addition, the BCF and TF factors were the chief components that made it possible to assess the ability of plants to survive in contaminated soils.

5 Conclusion

Pot experiment investigations clearly demonstrated that incorporation of rice straw, biochar and calcite can potentially alleviate Ni toxicity by reducing Ni bioavailability and leachability in Ni contaminated Ultisol. However, the incorporation of organic amendments biochar with high Ni-accumulating spinach was deemed to be an efficient method for remediating Ni contaminated soil, improving soil pH and ensuring food was nutritionally safe. Specifically, the addition of all these amendments to Ni contaminated soil significantly reduced CaCl_2 and TCLP extractable Ni. Maximum decreases Ni concentration and increases in nutrient contents in spinach as well as in the post-harvest soil were noted in BI 2% application rate compared to all treatments and untreated soil. Similarly, the lowest BCF and TF values for Ni in spinach were observed in BI 2%, compared to untreated soil, respectively. Meanwhile the highest soil pH values were observed in CC 1% and 2% application rates in comparison to all treatments. These findings suggest that the application of 2% biochar is very promising in reducing Ni uptake, and can reduce toxicity to plants, and reduce bioavailability and leachability in the soil. It is further suggested

that more studies must be conducted to elucidate the effects of various amendments on Ni immobilization under field conditions.

Declarations

Acknowledgements

This work received financial support from the National Sci-Tech Support Plan of China (2015BAD05B02).

References

1. Ahmad I, Akhtar MJ, Zahir ZA, Mitter B (2015) Organic amendments: effects on cereals growth and cadmium remediation. *Int J Environ Sci Technol* 12:2919–2928. doi.org/10.1007/s13762-014-0695-8
2. Ali U, Shaaban M, Bashir S, Fu Q, Zhu J, Shoffikul IM, Hu H (2020) Effect of rice straw, biochar and calcite on maize plant and Ni bio-availability in acidic Ni contaminated soil. *J Environ Manage* 259:109674
3. Ali U, Shaaban M, Bashir S, Gao R, Fu Q, Zhu J, Hu H (2019) Rice straw, biochar and calcite incorporation enhance nickel (Ni) immobilization in contaminated soil and Ni removal capacity. *Chemosphere* 244:125418
4. Azhar M, Zia Ur Rehman M, Ali S, Qayyum MF, Naeem A, Ayub MA, Anwar U. Haq M, Iqbal A, Rizwan M (2019) Comparative effectiveness of different biochars and conventional organic materials on growth, photosynthesis and cadmium accumulation in cereals. *Chemosphere* 227:72–81
5. Bashir S, Salam A, Chhajro MA, Fu Q, Khan MJ, Zhu J, Shaaban M, Kubar KA, Ali U, Hu H (2018a) Comparative efficiency of rice husk-derived biochar (RHB) and steel slag (SS) on cadmium (Cd) mobility and its uptake by Chinese cabbage in highly contaminated soil. *Int J Phytoremediation* 20(12):1221–1228. doi:10.1080/15226514.2018.1448364
6. Bashir S, Hussain Q, Shaaban M, Hu H (2018b) Efficiency and surface characterization of different plant derived biochar for cadmium (Cd) mobility, bioaccessibility and bioavailability to Chinese cabbage in highly contaminated soil. *Chemosphere* 211:632–639
7. Bashir S, Ali U, Shaaban M, Gulshan AB, Iqbal J, Khan S, Husain A, Niaz A, Mehmood S, Kamran M, Hu H (2020) Role of sepiolite for cadmium (Cd) polluted soil restoration and spinach growth in wastewater irrigated agricultural soil. *J Environ Manage* 258:110020
8. Boostani HR, Najafi-Ghiri M, Mirsoleimani A (2019) The effect of biochars application on reducing the toxic effects of nickel and growth indices of spinach (*Spinacia oleracea* L.) in a calcareous soil. *Environ Sci Pollut Res* 26::1751–1760
9. Ding Y, Liu YG, Liu SB, Li ZW, Tan XF, Huang XX, Zeng GM, Zhou L, Zheng BH (2016) Biochar to improve soil fertility. A review. *Agron sustain dev* 36:36:1–18

10. El-Naggar A, Raja PAU, Shaheen SM, Rinklebe J, Ok YS (2018) Potential of Biochar to Immobilize Nickel in Contaminated Soils. *Nickel in Soils and Plants*, pp. 293–318
11. Gao R, Hu H, Fu Q, Li Z, Xing Z, Ali U, Zhu J, Liu Y (2020) Remediation of Pb, Cd, and Cu contaminated soil by co-pyrolysis biochar derived from rape straw and orthophosphate: Speciation transformation, risk evaluation and mechanism inquiry. *Sci Total Environ* 730:139119
12. Hafeez A, Ali S, Ma XL, Tung SA, Shah AN, Ahmad S, Chattha MS, Souliyanonh B, Zhang Z, Yang GZ (2019) Photosynthetic characteristics of boll subtending leaves are substantially influenced by applied K to N ratio under the new planting model for cotton in the Yangtze River Valley. *Field Crops Res* 237:43–52
13. Hamid Y, Tang L, Lu M, Hussain B, Zehra A, Khan MB, He Z, Gurajala HK, Yang X (2019) Assessing the immobilization efficiency of organic and inorganic amendments for cadmium phytoavailability to wheat. *J Soils Sediments* 19:3708–3717
14. Herath I, Kumarathilaka P, Navaratne A, Rajakaruna N, Vithanage MJ (2015) Immobilization and phytotoxicity reduction of heavy metals in serpentine soil using biochar. *J Soils Sediments* 15:126–138
15. Houben D, Evrard L, Sonnet P (2013) Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (*Brassica napus* L.). *Biomass Bioenergy* 57:196–204
16. Kamran M, Malik Z, Parveen A, Huang L, Riaz M, Bashir S, Mustafa A, Abbasi GH, Xue B, Ali U (2020) Ameliorative effects of biochar on rapeseed (*Brassica napus* L.) growth and heavy metal immobilization in soil irrigated with untreated wastewater. *J Plant Growth Regul* 39:266–281
17. Khan MN, Lan Z, Sial TA, Zhao Y, Haseeb A, Jianguo Z, Zhang A, Hill RL (2019) Straw and biochar effects on soil properties and tomato seedling growth under different moisture levels. *Arch Agron Soil Sci* 65:12,1704–1719. doi:10.1080/03650340.2019.1575510
18. Khan WU, Ramzani PMA, Anjum S, Abbas F, Iqbal M, Yasar A, Ihsan MZ, Anwar MN, Baqar M, Tauqeer HM, Virk ZA, Khan SA (2017) Potential of miscanthus biochar to improve sandy soil health, in situ nickel immobilization in soil and nutritional quality of spinach. *Chemosphere* 185:1144–1156
19. Lahori AH, Zhang Z, Guo Z, Mahar A, Li R, Awasthi MK, Sial TA, Kumbhar F, Wang P, Shen F, Zhao J, Huang H (2017) Potential use of lime combined with additives on (im)mobilization and phytoavailability of heavy metals from Pb/Zn smelter contaminated soils. *Ecotoxicol Environ Saf* 145:313–323
20. Lee TM, Lai HY, Chen ZS (2004) Effect of chemical amendments on the concentration of cadmium and lead in long-term contaminated soils. *Chemosphere* 57:1459–1471
21. Li F, Cao X, Zhao L, Wang J, Ding Z (2014) Effects of mineral additives on biochar formation: carbon retention, stability, and properties. *Environ Sci Technol* 48:11211–11217
22. Mahar A, Wang P, Ali A, Lahori AH, Awasthi MK, Wang Z, Guo Z, Wang Q, Feng S, Li R, Zhang Z (2017) Im)mobilization of soil heavy metals using CaO, FA, sulfur, and Na₂S: a 1-year incubation study. *Int J Environ Sci Technol* 15:607–620

23. MEEPRC (2018) (Ministry of Ecology and Environment of the People's Republic of China). Soil environmental quality Risk control standard for soil contamination of agricultural land. (GB 15618 – 2018) (in Chinese)
24. Mosa A, El-Banna MF, Gao B (2016) Biochar filters reduced the toxic effects of nickel on tomato (*Lycopersicon esculentum* L.) grown in nutrient film technique hydroponic system. *Chemosphere* 149:254–262
25. Nawab J, Khan N, Ahmed R, Khan S, Ghani J, Rahman Z, Khan F, Wang XP, Muhammad J, Sher H (2019) Influence of different organic geo-sorbents on *Spinacia oleracea* grown in chromite mine-degraded soil: a greenhouse study. *J Soils Sediments* 19:2417–2432
26. Pariona, Ameber, World leaders in spinach production." World atlas, Apr. 25, 2017, worldatlas.com/articles/world-leaders-in-spinach-production.html
27. Ramzani PMA, Iqbal M, Kausar S, Ali S, Rizwan M, Virk ZA (2016) Effect of different amendments on rice (*Oryza sativa* L.) growth, yield, nutrient uptake and grain quality in Ni-contaminated soil *Environ. Sci Pollut Res* 23:18585–18595
28. Rehman MZ, Rizwan M, Ali S, Fatima N, Yousaf B, Naeem A, Sabir M, Ahmad HR, Ok YS (2016) Contrasting effects of biochar, compost and farm manure on alleviation of nickel toxicity in maize (*Zea mays* L.) in relation to plant growth, photosynthesis and metal uptake. *Ecotoxicol Environ Saf* 133:218–225
29. Rehman MZU, Khalid H, Akmal F, Ali S, Rizwan M, Qayyum MF, Iqbal M, Khalid MU, Azhar M (2017) Effect of limestone, lignite and biochar applied alone and combined on cadmium uptake in wheat and rice under rotation in an effluent irrigated field. *Environ Pollut* 227:560–568
30. Rizwan M, Mostofa MG, Ahmad MZ, Imtiaz M, Mehmood S, Adeel M, Dai Z, Li Z, Aziz O, Zhang Y, Tu S (2018) Nitric oxide induces rice tolerance to excessive nickel by regulating nickel uptake, reactive oxygen species detoxification and defense-related gene expression. *Chemosphere* 19:23–35
31. Rizwan MS, Imtiaz M, Chhajro MA, Huang G, Fu Q, Zhu J, Aziz O, Hu H (2016) Influence of pyrolytic and non-pyrolytic rice and castor straws on the immobilization of Pb and Cu in contaminated soil. *Environ Technol* 37:2679–2686
32. Salam A, Bashir S, Khan I, Hu H (2019) Two years impacts of rapeseed residue and rice straw biochar on Pb and Cu immobilization and revegetation of naturally co-contaminated soil. *Appl Geochem* 105:97–104
33. Sehrish AK, Aziz R, Hussain MM, Rafiq MT, Rizwan M, Muhammad N, Rafiq MK, Sehar A, Din Ju, Al-Wabel MI, Ali S (2019) Effect of poultry litter biochar on chromium (Cr) bioavailability and accumulation in spinach (*Spinacia oleracea*) grown in Cr-polluted soil. *Arab j geosci* 12:12–57
34. Shaaban M, Van Zwieten L, Bashir S, Younas A, Nunez-Delgado A, Chhajro MA, Kubar KA, Ali U, Rana MS, Mehmood MA, Hu R (2018) A concise review of biochar application to agricultural soils to improve soil conditions and fight pollution. *J Environ Manage* 228:429–440
35. Shahbaz AK, Iqbal M, Jabbar A, Hussain S, Ibrahim M (2018a) Assessment of nickel bioavailability through chemical extractants and red clover (*Trifolium pratense* L.) in an amended soil: Related

- changes in various parameters of red clover. *Ecotoxicol Environ Saf* 149:116–127
36. Shahbaz AK, Lewińska K, Iqbal J, Ali Q, Iqbal M, Abbas F, Tauqeer HM, Ramzani PMA (2018b) 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. *J Environ Manage* 218:256–270
 37. Shahbaz AK, Ramzani PM, Saeed R, Turan V, Iqbal M, Lewinska K, Abbas F, Saqib M, Tauqeer HM, Iqbal M, Fatima M, Rahman MU (2019) Effects of biochar and zeolite soil amendments with foliar proline spray on nickel immobilization, nutritional quality and nickel concentrations in wheat. *Ecotoxicol Environ Saf* 173:182–191
 38. Shaheen SM, Mladenovic S, Wang SL, Niazi NK, Tsadilas C, Ok YS, Rinklebe J (2019) Nickel mobilization/immobilization and phytoavailability in soils as affected by organic and inorganic amendments. *Nickel in Soils and Plants*, pp. 265–292
 39. Shahzad B, Tanveer M, Rehman A, Cheema SA, Fahad S, Rehman S, Sharma A (2018) Nickel whether toxic or essential for plants and environment-A review. *Plant physiol bioch* 132:641–651
 40. Shen Z, Som AM, Wang F, Jin F, McMillan O, Al-Tabbaa A (2016) Long-term impact of biochar on the immobilisation of nickel (II) and zinc (II) and the revegetation of a contaminated site. *SciTotal Environ* 542:771–776
 41. Shen Z, Zhang Y, McMillan O, Jin F, Al-Tabbaa A (2017) Characteristics and mechanisms of nickel adsorption on biochars produced from wheat straw pellets and rice husk. *Environ Sci Pollut Res* 24:12809–12819
 42. Xu CY, Hosseini Bai S, Hao Y, Rachaputi RC, Wang H, Xu Z, Wallace H (2015) Effect of biochar amendment on yield and photosynthesis of peanut on two types of soils. *Environ Sci Pollut Res* 22:6112–6125
 43. Younis U, Athar M, Malik A, Shah MHR, Mahmood S (2015) Biochar impact on physiological and biochemical attributes of spinach *Spinacia oleracea* (L.) in nickel contaminated soil. *Glob J Environ Sci Manag* 1(3):245–254

Tables

Table 1. Soil physicochemical characteristics.

Parameter	Soil
pH	5.3
EC (mS cm ⁻¹)	0.2
Texture	Silty clay loam
Sand (g kg ⁻¹)	124.6
Silt (g kg ⁻¹)	507.2
Clay (g kg ⁻¹)	368.2
Organic matter (g kg ⁻¹)	14.79
CEC (cmolc kg ⁻¹)	12.75
Total Ni (mg kg ⁻¹)	35
Available phosphorus (mg kg ⁻¹)	8.74
Available potassium (mg kg ⁻¹)	150

Table 2. Physicochemical characteristics of organic and inorganic amendments used in this study.

Parameter	Rice straw	Biochar	Calcite
pH	6.8	10.29	8.6
EC (mS cm ⁻¹)	-	1.1	0.1
Total Ni (mg kg ⁻¹)	1.5	10.75	0.75
BET-SA (m ² g ⁻¹)	2.3	14.5	1.77
C (g kg ⁻¹)	364	470.7	121.2
N (g kg ⁻¹)	19.9	25.3	1.02
O (g kg ⁻¹)	401.9	175	327
H (g kg ⁻¹)	58.6	19.6	-
P (g kg ⁻¹)	1.15	1.63	-
K (g kg ⁻¹)	13.45	15.86	-
Ash (g kg ⁻¹)	-	653	-
Yield (g kg ⁻¹)	-	330	-

Table 3. Effect of amendments applied to the Ni contaminated soil on nutrients in the soil and plant after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means (n = 3)

Treatment	Soil			Plant		
	N g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹	N g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹
CK	0.22±0.19 d	0.36±0.048 e	8.016±0.308 c	4.1±0.10 e	1.38±0.05 g	25.42±0.41 f
RS 1%	0.28±0.21 c	0.37±0.095 de	8.417±1.791 b	6.68± 0.20 c	1.75±0.01 f	26.88±0.64 ef
RS 2%	0.37±0.14 b	0.38±0.066 de	8.603±0.794 b	8.18±0.21 c	1.94±0.08 e	28.37±0.20 e
BI 1%	0.34±0.18 b	0.41±0.041 bc	8.59±0.909 b	10.05±0.29 b	3.14±0.05 b	37.74±1.05 c
BI 2%	0.43±0.09 a	0.46±0.046 a	9.02±0.186 a	11.93±0.42 a	3.42±0.03 a	56.05±0.71 a
CC 1%	0.29±0.24 c	0.39±0.283 cd	8.49±0.988 b	8.646±0.41 c	2.31±0.05 d	34.30±0.99 d
CC 2%	0.31±0.01 c	0.42±0.278 b	8.75±0.648 ab	11.24±0.13 a	2.86±0.10 c	42.86±0.59 b

Different letters indicate significant differences among treatments at P < 0.05.

Table 4. Effect of amendments applied to the Ni contaminated soil on plant bioconcentration factor (BCF), translocation factors (TF) and total dry biomass (TDM) after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means (n = 3).

Treatment	BCF _(shoot)	BCF _(root)	TF	TDM (g pot ⁻¹)
CK	1.1±0.08 a	2.9±0.110 a	0.40±0.011a	2.71±0.37 d
RS 1%	0.7±0.06 b	2.3±0.043 b	0.34±0.031 b	3.7±0.505 bcd
RS 2%	0.6±0.05 b	1.6±0.060 c	0.32±0.09 b	3.9±0.2 bcd
BI 1%	0.6±0.03 b	1.6±0.040 cd	0.31±0.03 b	4.785±0.68 ab
BI 2%	0.3±0.006 c	1.0± 0.008 e	0.30±0.04 b	6.405±0.34 a
CC 1%	0.6±0.02 b	1.3±0.22 c	0.39±0.11 a	3.695±0.38 cd
CC 2%	0.4±0.002 c	1.1±0.043 de	0.35±0.01 b	4.5±0.08 bc

Different letters indicate significant differences among treatments at P < 0.05.

Figures

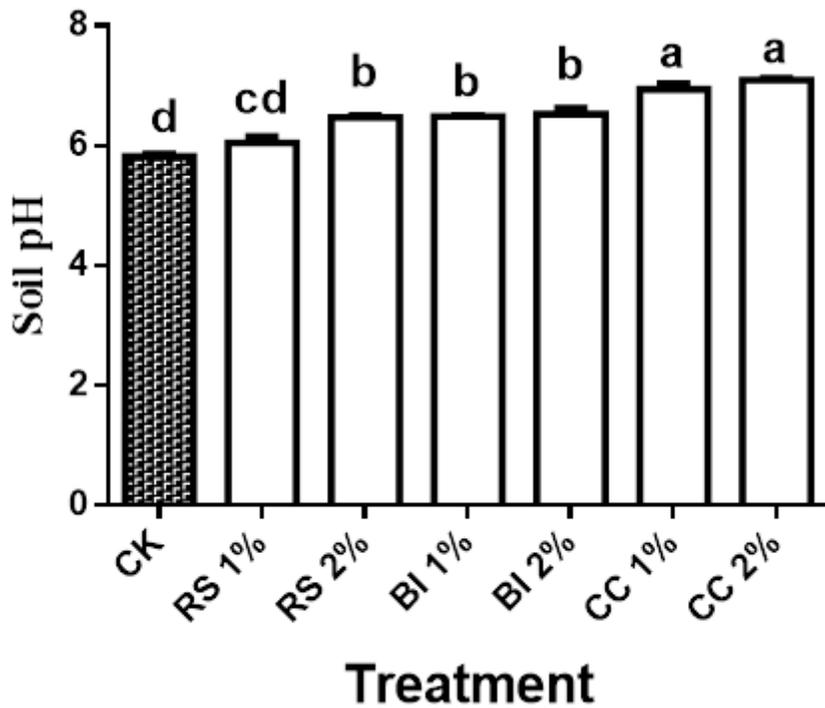


Figure 1

Effect of amendments applied to the Ni contaminated soil on soil pH after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means ($n = 3$). Different letters indicate significant differences among treatments at $P < 0.05$.

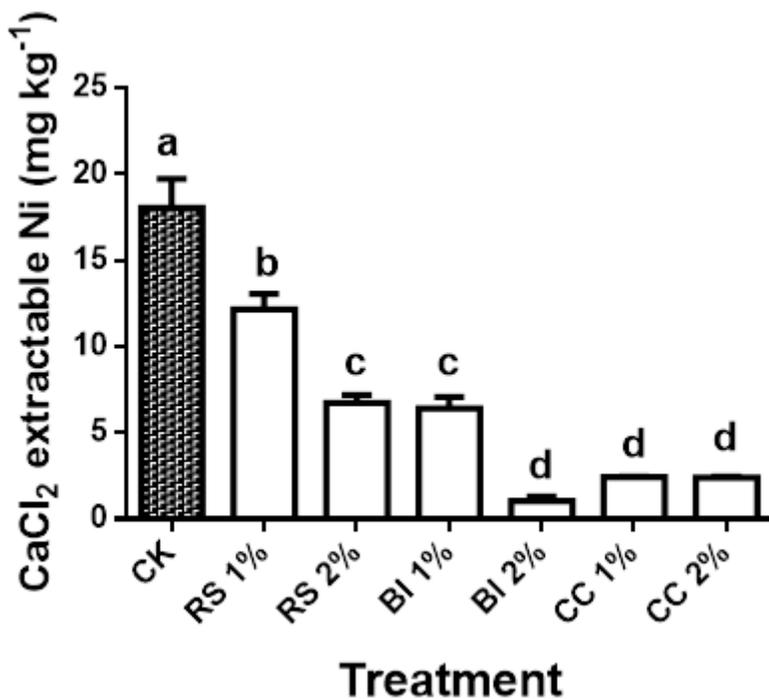


Figure 2

Effect of amendments applied to the Ni contaminated soil on CaCl₂ extractable Ni after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means (n = 3). Different letters indicate significant differences among treatments at P < 0.05.

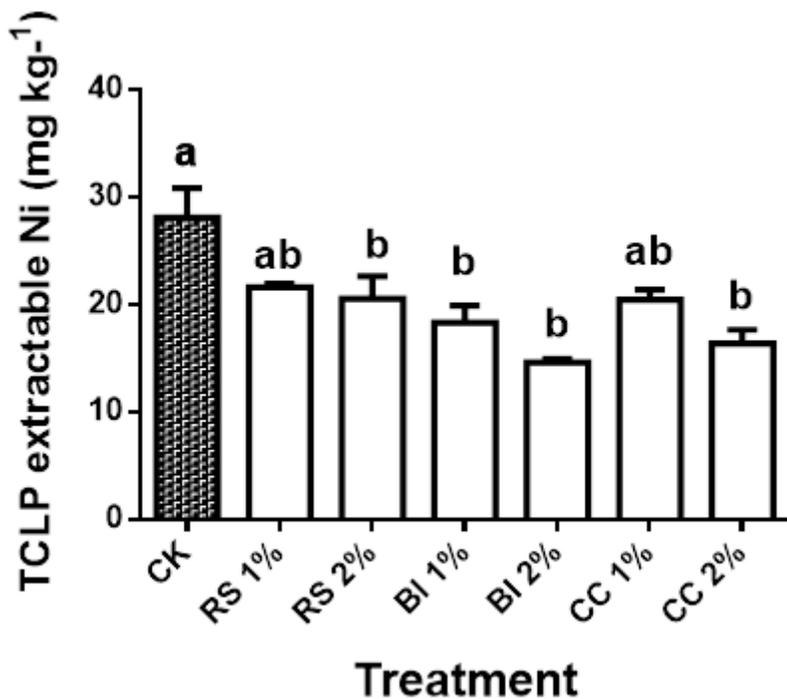


Figure 3

Effect of amendments applied to the Ni contaminated soil on TCLP extractable Ni after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means (n = 3). Different letters indicate significant differences among treatments at P < 0.05.

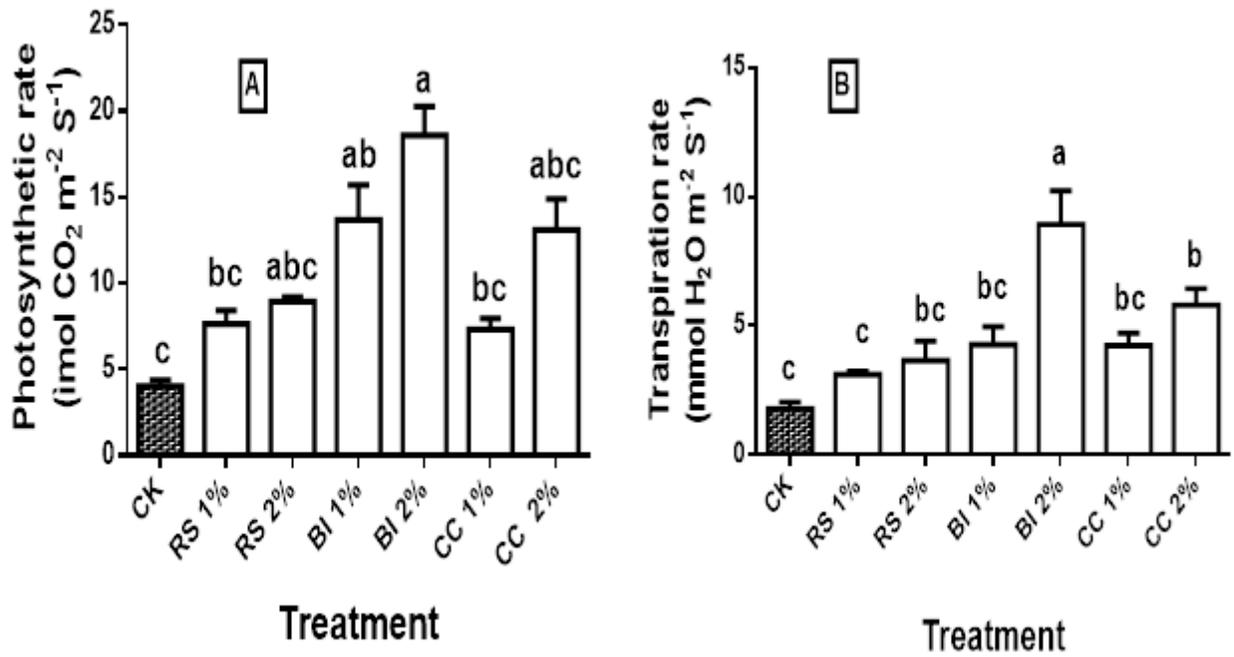


Figure 4

Effect of amendments applied to the Ni contaminated soil on photosynthetic rate (A) and transpiration rate (B) in the leaves after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means (n = 3). Different letters indicate significant differences among treatments at P < 0.05.

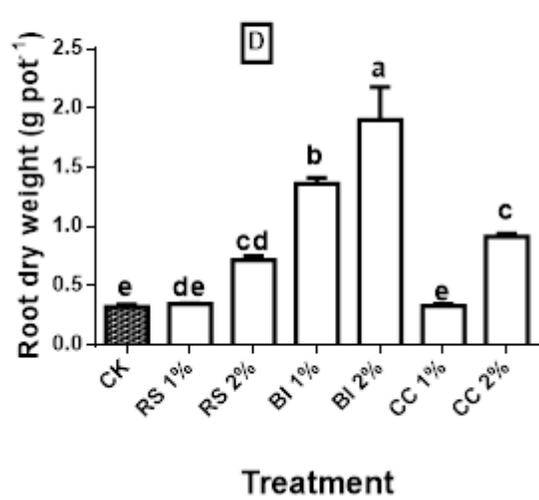
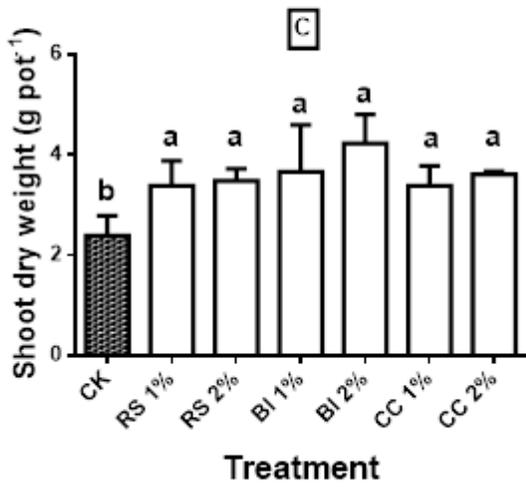
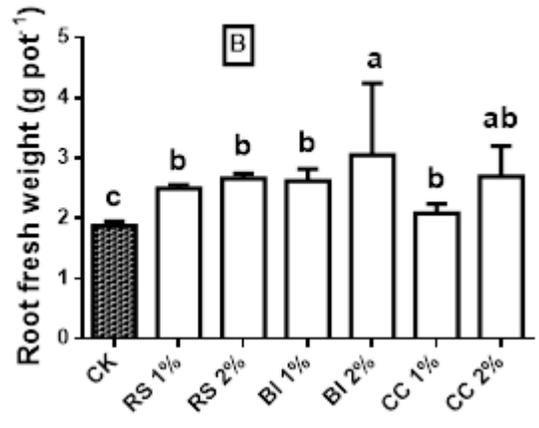
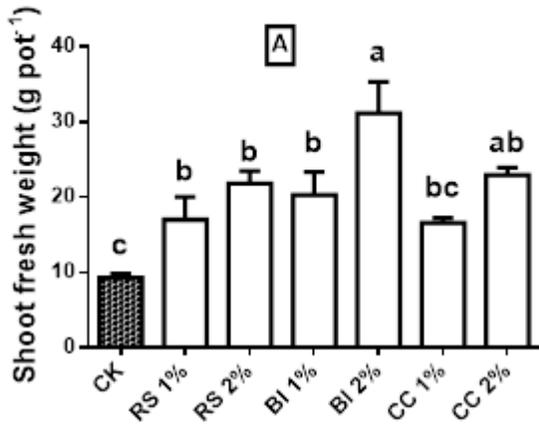


Figure 5

Effect of amendments applied to the Ni contaminated soil on shoot fresh weight (A), root fresh weight (B), shoot dry weight (C) and root dry weight (D) after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means (n = 3). Different letters indicate significant differences among treatments at P < 0.05.

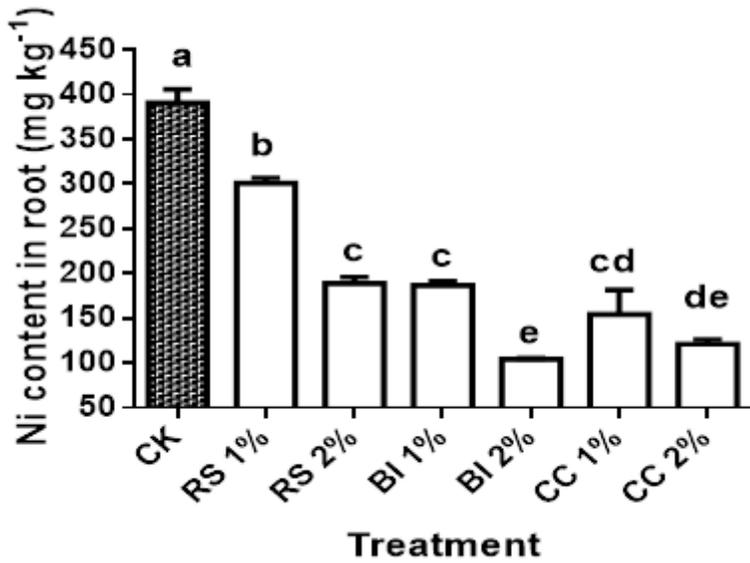


Figure 6

Effect of amendments applied to the Ni contaminated soil on Ni concentration in roots after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict standard deviations (SDs) of the means (n = 3). Different letters indicate significant differences among treatments at P < 0.05.

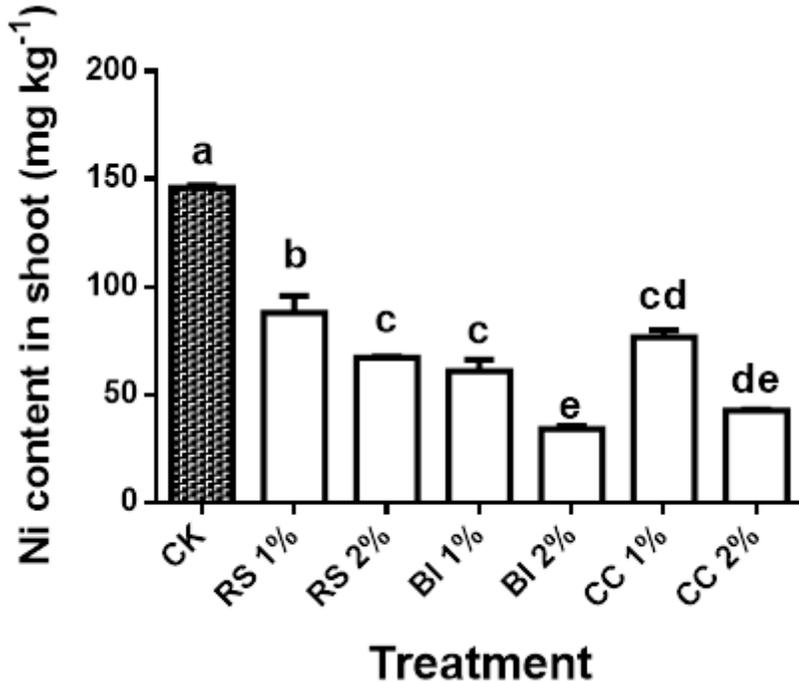


Figure 7

Effect of amendments applied to the Ni contaminated soil on Ni concentration in shoots after spinach was grown. Treatments: untreated soil (CK), rice straw (RS), biochar (BI) and calcite (CC). Error bars depict

standard deviations (SDs) of the means ($n = 3$). Different letters indicate significant differences among treatments at $P < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalabstractSpinach20201102.tif](#)