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Minimizing Heavy Metal Accumulation in Edible Parts of Lettuce Plant

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THE experiment was conducted to reduce the buildup of heavy metals in lettuce plants grown in contaminated soil. Lettuce plants cv. Nader were cultivated on polluted soil in black polyethylene bags with or without the addition of biochar, rice straw, *Pseudomonas fluorescens* bacterial inoculation, biochar + rice straw, biochar + *P. fluorescens*, rice straw + *P. fluorescens*, and biochar + rice straw + *P. fluorescens*, as well as unpolluted soil as a control. The experiments were conducted in the seasons of 2019 and 2020 at the Vegetable Farm of the Faculty of Agriculture, Ain Shams University, Shubra El Kheima, Qalyubia Governorate, Egypt. The experimental treatments were distributed in a completely randomized design with three replicates. The attained results indicated that cultivating lettuce in polluted soil had negative effects on plant growth compared to those cultivated in unpolluted soil. Application of the experimental treatments improved the vegetative growth parameters, including plant length, leaf area, fresh and dry head weight, fresh and dry root weight, and head diameter. The results also showed that lettuce plants grown in heavy metal-polluted soil had lower values of vegetative growth parameters than those grown in polluted soil treated with biochar, rice straw, or *P. fluorescens*, and the interaction between them. When compared to bacterial inoculation treatment, the application of biochar and/or rice straw on lettuce plants grown in heavy metal-contaminated soil enhanced growth and leaf nutrient contents, while heavy metal contents decreased. The findings demonstrated that heavy metal-contaminated soil treated with biochar alone or in combination with rice straw and/or bacterial inoculation were effective in reducing the content of heavy metals in plant leaves and attenuating the harmful effects of heavy metals on grown lettuce plants.

Keywords: *Lactuca sativa,* Biochar, Rice straw, *P. fluorescens,* Heavy metals, Vegetative growth, Mineral contents, Nitrate content.

Introduction

The lettuce (*Lactuca sativa*) belongs to the Asteraceae family. Due to its succulent leaves, ancient Egyptians first cultivated lettuce as an oil-producing herb before transforming it into a leafy vegetable plant used in fresh salads (FAOSTAT, 2013). Owing to its high concentration of pro-vitamin A molecules and beta-carotene, lettuce provides the human body with about 20% of the daily recommended amount of vitamin A. It is also rich in folic acid and iron.

According to standard definitions, heavy metals are those with a specific density greater than 5 g cm^{-3}. Lead, cadmium, mercury, and arsenic are the main heavy metals that pose risks to human health. Continued exposure to heavy metal in some places around the world, is even rising despite the fact that the harmful health

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effects of heavy metals have long been known. There are many heavy metal-contaminated areas that are regrettably used for agricultural purposes in Egypt. Zorrig et al. (2013) reported that heavy metals are a major pollutant. Heavy metal contamination not only poses a threat to human health hazard but also has a significant detrimental effect on soil fertility and plant productivity (Arshad et al., 2017 and Iqbal et al., 2020). It can also cause changes in the composition and operation of an ecosystem due to its stability and toxicity (Mazhar et al., 2020). Fossil fuel combustion, mining, smelting, metal plating, Ni-Cd batteries, fungicides and pesticides, phosphate fertilizers, dyes, photographic processes, textile processes, stabilizers and alloy manufacturing all share and contribute to the release of toxic heavy metals into the environment (Boparai et al., 2011, Gutierrez-Segura et al., 2012 and Khan et al., 2017). According to the Agency for Toxic Substances and Disease Registry (ASTDR, 1999), the maximum concentration of Cd that can exist in the soil is 85 mg/kg, while the maximum concentration that can exist in plants is 0.02 mg/kg. Therefore, many documents found that when plants grow in heavy metal-contaminated soil, the heavy metal is absorbed, stored and accumulated in their edible tissues through the membrane transport routes used by Fe, Zn or Cu (Takahashi et al., 1999, Zorrig et al., 2011 and Zorrig et al., 2013) and thereby enters the food chain because it is easily soluble in water and has negative effects even at low concentrations. It increases the production of oxygen free radicals that may cause damage to DNA, RNA and proteins and it may eventually cause cell death, while lowering the levels of enzymatic and non-enzymatic antioxidants, which results in plant stunting, chlorosis, and leaf roll (Benavides et al., 2005 and Akoto et al., 2015). Reactive oxygen species (ROS) oxidize many proteins, including catalase, ascorbate peroxidase, and lipids, and are particularly toxic in higher concentrations (Ishibashi et al., 1990). The presence of heavy metals in plants also inhibits plant growth through stomatal closure, decreases nutrient uptake, and causes disruptions in photosynthesis and respiration as a result of mutations and changes in cell structure. In this regard, the lettuce plant makes a useful model for research into the processes that lead to the buildup of heavy metals in its tissues. Heavy metals can be removed from soil using a variety of methods, including physical, chemical, and biological ones. The physical and chemical methods are costly, labor-intensive, and alter the soil's characteristics permanently because they occasionally produce secondary pollutants. The use of biological methods, which is preferable in contrast, is both efficient, cost-effective and environmentally friendly (Itusha et al., 2019 and Zand et al., 2020). Therefore, bioremediation technology, which reduces the transfer of heavy metals to the soil by adding biosorbent materials like biochar and microorganisms, can be used to solve this issue. These biosorbent substances are influenced by factors like biomass, metals' physical and chemical properties, soil pH, and temperature.

According to Novak et al. (2009), biochar is charcoal that has been added to soil to enhance a variety of soil properties and boost productivity. It has a large surface area, has internally porous, highly efficient functional groups, and acts as a source of microorganism inoculum (Herrmann and Lesueur, 2013). Additionally, it has a high pH that immobilizes heavy metal cations in the soil (Beesley et al., 2010)**.** The addition of biochar to soil also enhances the availability of crucial macro- and micronutrients needed for sustaining plant growth (Major et al., 2010), as well as soil nutrient supply, microbial activity, and nutrient leaching (Lehmann et al., 2011 and Ventura et al., 2013). It also improves soil structure by increasing soil porosity and aeration (Lehmann et al., 2003), enhances nutrient storage in soil micropores (Lehmann and Joseph, 2015), and reduces the toxicity of heavy metals such as Zn, Cu, Cd, and Ni in many plant species through various mechanisms, including immobilization in the soil, changing pH, altering the redox state in the soil and improving of biological properties (Mazhar et al., 2020 and Mondal et al., 2020).

Microorganisms can improve the availability of heavy metals by solubilizing and mobilizing them in the soil solution by lowering soil pH (Iqbal et al., 2020 and Mazhar et al., 2020). In contrast, microorganisms can decrease metal availability through bioaccumulation (external and intracellular), immobilization, chelation, and active removal (Nadeem et al., 2017 and Iqbal et al., 2020). Pseudomonas is gram-negative aerobic bacteria that live in mine wastewater contaminated with various heavy metals (Singleton and Sainsbury, 1987 and Cho et al., 2001) and has been extensively studied because of its well-adapted metal-resistant properties (Singh et al., 2010 and Deb et al., 2013). Rice straw is a renewable, abundant, and inexpensive organic material as an agricultural residue (Zhong et al., 2003). Straw integration may improve soil porosity, water retention capacity, and nutrient and organic matter content (Bakht et al., 2009). Increased soil organic matter may reduce heavy metal availability in soils through adsorption or the formation of stable complexes (Guo et al., 2006).

Due to the increased demand for food, it has become necessary to remediate the heavy metal-contaminated soils and get them ready to sustainably produce safe food. The aim of this study was to find out whether biochar, rice straw and *Pseudomonas* bacterial inoculation could be used individually or in combination to lessen the negative effects of heavy metals on growth traits, physiological characteristics, and heavy metal accumulation in lettuce plants grown in black polyethylene bags using natural soil and heavy metal-contaminated soil.

Materials and Methods

Experimental site and design, plant material, and cultivation

The main goal of this study was to find out whether biochar, rice straw, and *Pseudomonas* bacteria could be used individually or in combination to lessen the negative effects of heavy metals on the performance of lettuce through monitoring growth traits, SPAD readings, and heavy metal accumulation in lettuce plants grown in black polyethylene bags using non-contaminated soil as a control (Cont-s) and heavy metal-polluted soil (Poll-s) during 2019 and 2020 growing seasons. The experiment involved nine treatments distributed in a completely randomized design with three replicates. Each replicate consists of three bags with a capacity of one plant per bag. The experimental treatments were as follows: naturally polluted soil with heavy metals incorporated with biochar (BC), rice straw (RS), *Pseudomonas fluorescens* (PS), BC + RS, BC + PS, RS + PS, PS + RS + BC, untreated polluted soil (Poll-S) as well as non-polluted soil as a control (Cont-s). The black polyethylene bags had a diameter of 20 cm and a length of 30 cm, filled with 6 kg of soil per bag. The romaine-type lettuce, cv. Nader, was used as plant material in this study. The experiment was carried out at the Vegetable Farm of the Faculty of Agriculture, Ain Shams University, Shubra El Kheima, Qalyubia Governorate, Egypt (Latitude 30°06’45.3” N; Longitude 31°14’37.1” E).

Treatments

Soil types: The two soil types used were polluted and non-polluted soil, the polluted soil was collected from the El-Menaier area, Mashtoul El-Souqe, El-Sharqia Governorate. Naturally contaminated with heavy metals in this area due to factories wastewater in the Abo Zaabal industrial area. The collected, contaminated soil was transferred to the experimental site as indicated above. The non-polluted soil was brought from the Vegetable Farm of the Agriculture Faculty, where the experiment was conducted. The mechanical and chemical properties of the two soil types were analyzed at the Central Lab. of the Faculty

TABLE 1. Physical and chemical properties of the experimental soil, control soil (Cont-s) and polluted soil (Poll-s) before lettuce cultivation.

Parameters	Non-Polluted soil	Polluted soil	Parameters	Non-Polluted soil	Polluted soil
Texture	Sandy Clay loam	Sandy Clay loam	**Mg^{2+} (meq/l)**	2.68	7.02
Silt (%)	19.57	18.12	**K^{+} (meq/l)**	2.15	3.80
Clay (%)	23.58	20.22	**Na^{+} (meq/l)**	1.47	6.40
Sand (%)	56.85	61.46	**Cl^{-} (meq/l)**	1.30	8.25
pH	7.10	7.60	**HCO_3^{-}(meq/l)**	2.90	6.20
EC (dS/m)	1.63	2.77	**CO_3^{--} (meq/l)**	0.00	0.00
Ca^{2+} (meq/l)	2.58	5.75	**SO_4^{2-} (meq/l)**	2.99	7.05
Zn (ppm)	10.61	77.30	**Cu (ppm)**	2.81	11.31
Mn (ppm)	3.62	13.49	**Cd (ppm)**	0.11	7.02
Fe (ppm)	7.17	38.45	**Cr (ppm)**	0.21	17.90
Ni (ppm)	0.03	2.17	**Pb (ppm)**	0.40	15.20

TABLE 2. Chemical properties of the irrigation water used.

Soluble cations (meq/l)				Soluble anions (meq/l)				pH	EC (dS/m)
$C^{a2}+$	Mg^{2+}	K^+	Na^+	Cl^-	CO_3^{2-}	HCO^{3-}	$SO_4^{\ 2}$		
3.94	1.52	1.03	0.13	1.19	0.00	1.23	4.2	6.7	0.63

of Agriculture, Ain Shams University, Egypt. The irrigation water provided from the Nile River was also analyzed. The characteristics of the soil and water are shown in Tables 1 and 2.

Rice straw (RS): rice straw was brought from a farm that grows rice in the nearby area, Qalyubia Governorate. Before use, RS was threshed after being allowed to air dry. RS was added to the soil in each bag at a rate of 1unit RS per 3 units of soil, depending on the volume.

Biochar (BC): The used BC was purchased from a nearby place for commercial production of BC. The used BC has the following physical and chemical characteristics: an EC of 0.68 dSm^{-1}, a moisture content of 30 g kg^{-1}, and a pH of 7.10. The contents of N, P, K, and Cd were 27, 01, 8, 15, 12, and 0.00 g/kg, respectively. For each bag, BC was added at a rate of 50 g and thoroughly mixed into the soil.

Pseudomonas fluorescens (PS): PS was obtained from the Microbial Unit, Mircen, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University. The PS was in suspension form with a count of 10^6 bacterial cells per 1 ml, and 10 ml of bacterial suspension was added to each bag three times; 14, 28, and 42 after transplanting.

Data recorded

All lettuce plants were harvested after 70 days from all experimental plots by using a sharp knife to cut the heads from the soil surface. A plant sample of three plants from each replicate was randomly chosen and brought to the lab to be evaluated for vegetative growth, physiology, and chemical characteristics as follows:

Vegetative growth traits

Head length was measured. Head diameter was measured using a digital caliper. The harvested head sample was separated into leaves and roots and then the fresh weight per head was recorded. Afterward the samples were oven dried at 70°C to a constant weight, and the dry weights were recorded using a digital balance. Leaf area was determined according to Koller (1972) using the following equation:

$$\text{Leaf area} = \frac{\text{Disk area} \times \text{disks number} \times \text{leaves fresh weight}}{\text{Disks fresh weight}}$$

SPAD readings

SPAD readings were measured using a chlorophyll meter (Konica Minolta® SPAD 502). It was done on the fourth uncut leaf from the plant head outside at harvesting time (Neufeld et al., 2006).

Chemical analysis

To analyze the mineral content of lettuce plants, individual samples of the leaves and roots were randomly taken at harvesting. The leaves and roots were oven dried at 70°C to a constant weight, then dried samples were ground in a blender to a fine powder to pass through a 1-mm sieve and stored in glass vials for subsequent analyses. According to Thomas et al. (1967), 0.1 g of the dry leaf and root samples were wet digested with a solution of sulfuric acid (H_2SO_4 98%) and hydrogen peroxide (H_2O_2 30%). In this digestion extract, the elements under study were measured. Total nitrogen and nitrate were measured by the Kjeldahl technique, as described by Piper (1950). Macro- and microelements as well as heavy metals were measured using an inductively coupled plasma-optical emission spectrometry model (ICP-OES, Vista MPX, Varian Inc., California, USA) according to the procedure of Rodushkin et al. (1999).

Statistical analysis

In accordance with Waller and Duncan (1969), data from the two growing seasons were subjected to an analysis of variance using the CoStat package program for Windows (version 6.303, CoHort Software, CA, USA). After checking the generated data for error, homogeneity, and normality the combined analysis of variance for the data from the two seasons was performed. At a P≤0.05 probability level, Duncan's multiple range test was used to separate the significant differences among treatment means.

Results

Vegetative growth characteristics

The data presented in Figures 1 and 2 clearly demonstrate that using biochar, rice straw, and bacteria led to an increase in plant length, the fresh weight and dry weight of shoots, and the average leaf area of lettuce plants grown in black polyethylene bags under heavy metal contaminated soil conditions compared with Poll-s grown plants. The combination of biochar + rice straw + *Pseudomonas fluorescens* produced the highest significant values for plant length, head diameter, both the fresh and dry weight of the shoots and roots, average leaf area and SPAD readings (greenness) versus the contaminated soils in the control treatments during both growing seasons. These findings are consistent with earlier research that showed the application of rice straw, biochar, and *Pseudomonas fluorescens* significantly enhanced the vegetative and root growth parameters of various plants, as shown in Figures (1 and 2). Rice straw, biochar, bacteria, and their combinations had a significant impact on all lettuce plant growth data, including plant length, head diameter, plant fresh and dry weight, greenness, and leaf area. Treating contaminated soil with biochar, rice straw, and *Pseudomonas* bacterium individually or in combination had a significant impact on the above mentioned parameters compared to untreated contaminated soil. Treatment of contaminated soil with a combination of biochar + rice straw + bacteria conferred better values of these parameters than treatment with biochar and/or rice straw.

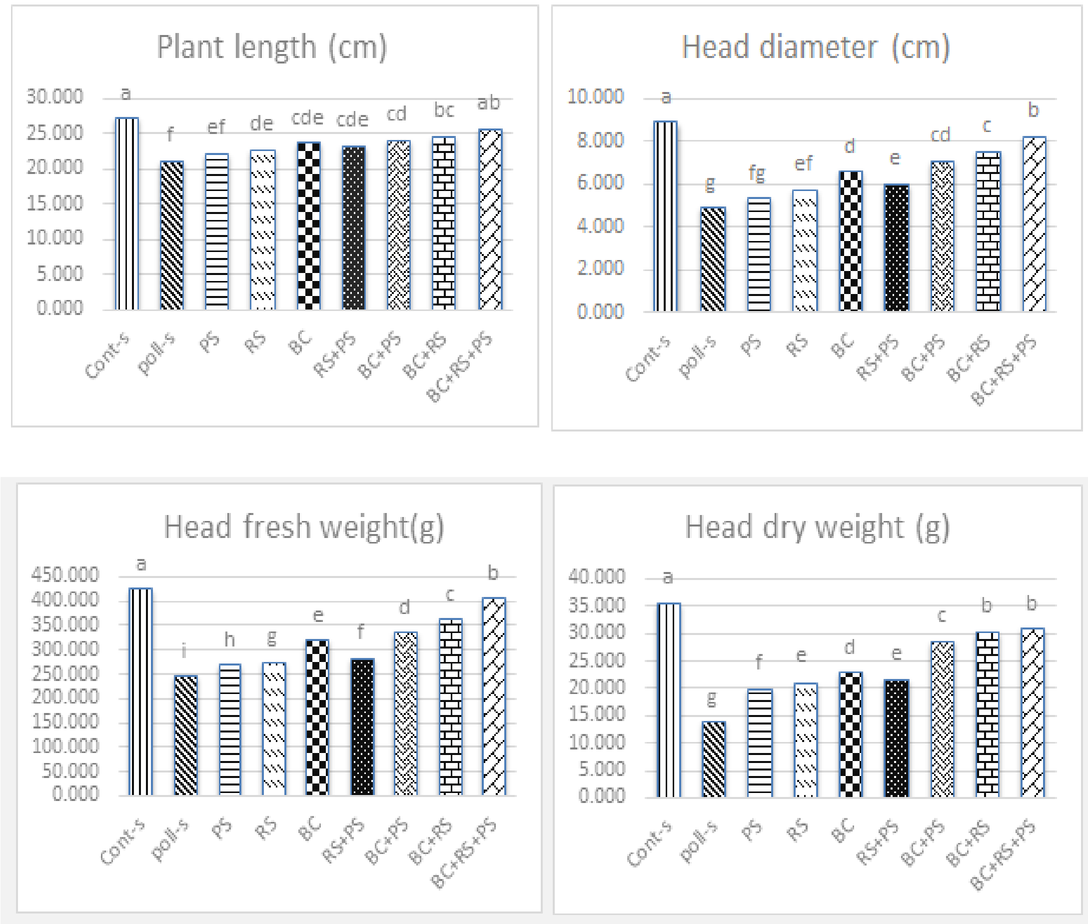

Fig. 1. Effect of soil incorporation with rice straw, biochar, *P. fluorescens* and their interactions on plant length, head diameter and head fresh and dry weight of lettuce plants cv. Nader (combined analysis of both seasons data).

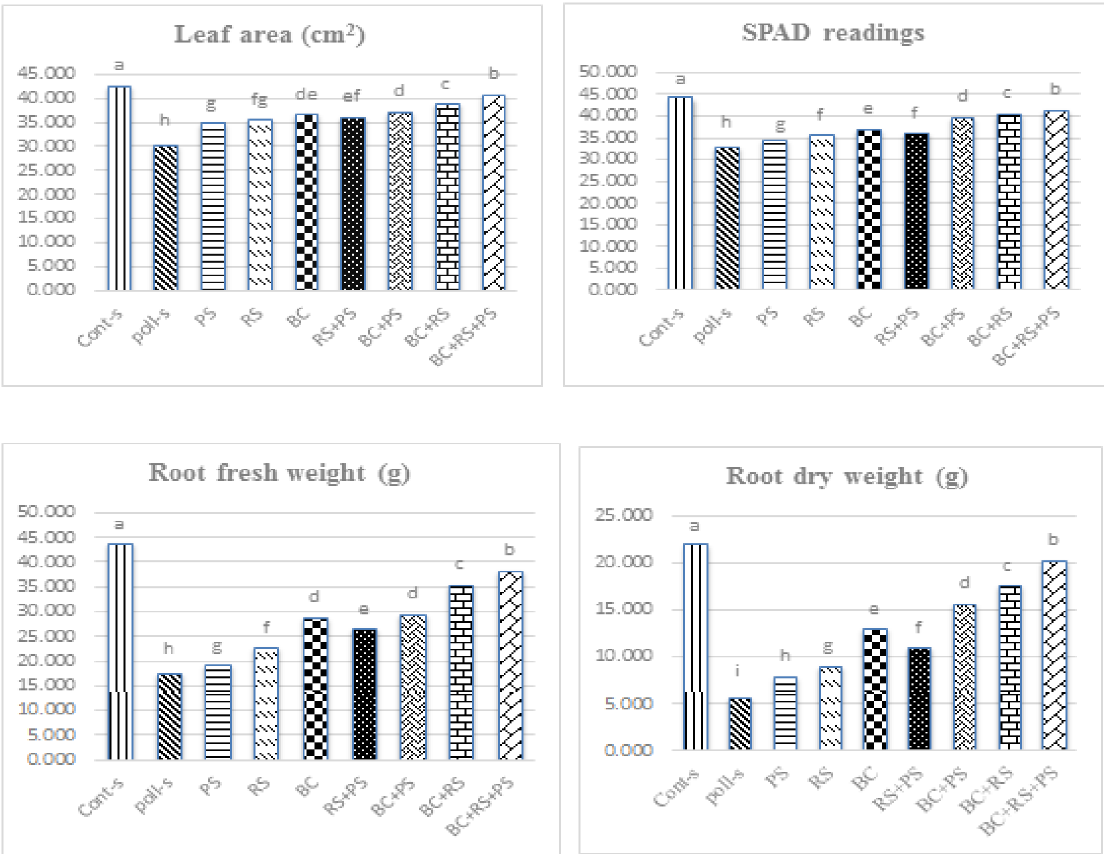

Fig. 2. Effect of soil incorporation with rice straw, biochar, P. fluorescens and their interactions on average leaf area, SPAD readings and roots fresh and dry weight of lettuce plants cv. Nader (combined analysis of both seasons data).

Mineral analysis of lettuce leaves and roots
Macroelements

The results shown in Figures (3, 4, 5 and 6) indicate that, in both growing seasons, the treatments with rice straw, biochar, *P. fluorescens*, and their combination significantly increased the percentage of N, P, K, Mg content in the roots and leaves of lettuce when compared to the contaminated soils (Poll-s). The level of nitrate (NO_3) content in the root and leaves was also decreased by these treatments.

Heavy metals and microelement contents of lettuce roots and leaves

Figures (7 and 8), clearly demonstrate the effects of the experimental treatments on the levels of Cu, Fe, B, Mn, Mo, and Zn in the lettuce roots and leaves. These treatments included rice straw, biochar, *P. fluorescens*, and their combinations. The combination of rice straw + biochar + P *fluorescens* was the best treatment for reducing the concentration of heavy elements when compared to the contaminated soils in the control treatment. The combination of the treatments performed better than each treatment alone.

Heavy metal content of roots and leaves:

Tables (3 and 4) demonstrate the impact of the designate treatments, biochar, rice straw, *P. fluorescens*, and their combinations on the levels of Cd, Pb, and Cr in the lettuce the leaves and roots. The combination of biochar + rice straw + *P. fluorescens* was the best treatment for reducing the concentration of these elements in both leaves and roots of lettuce plant when compared to the contaminated soils (Poll-s) in the control treatment. The combination of the treatments performed better than each treatment alone.

Discussion

The negative effects of heavy metal stress were lessened by the soil incorporation of biochar,

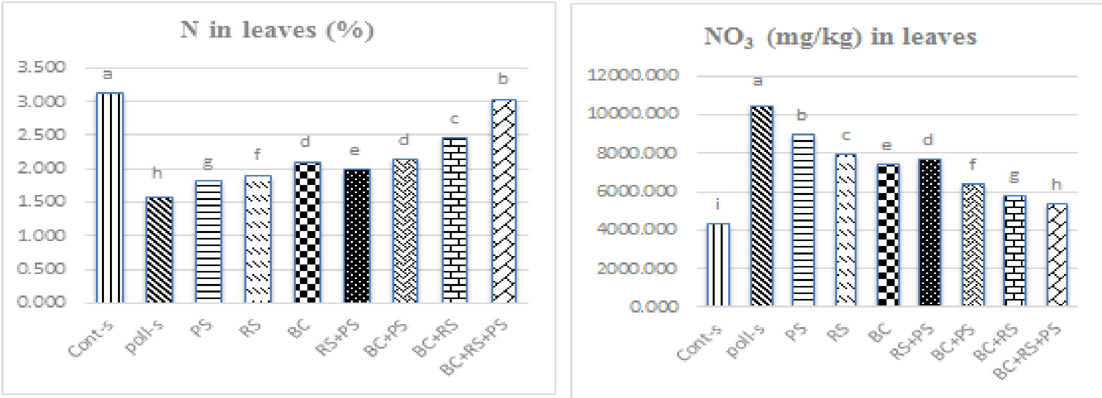

Fig. 3. Effect of soil incorporation with rice straw, biochar, *P. fluorescens* and their interactions on leaf N and NO_3 of lettuce plants cv. Nader (combined analysis of both seasons data).

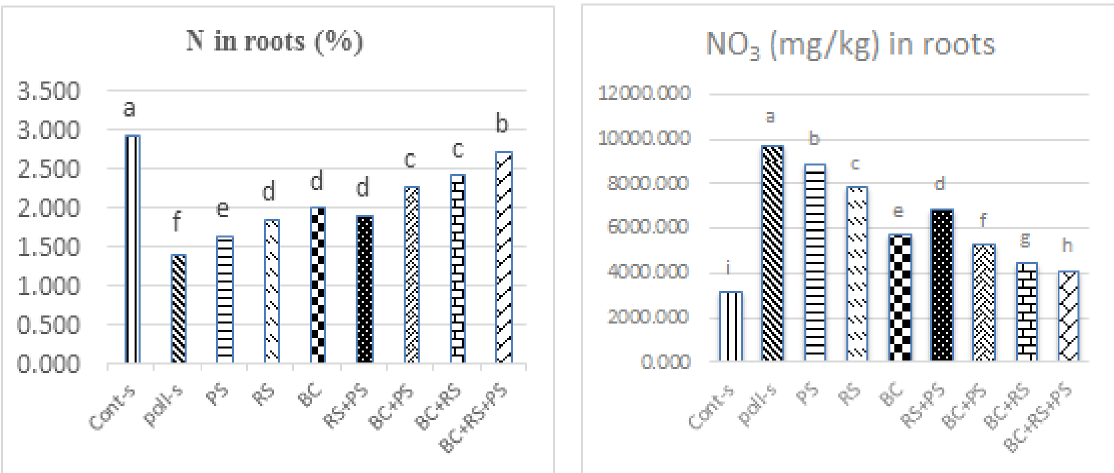

Fig. 4. Effect of soil incorporation with rice straw, biochar, *P. fluorescens* and their interactions on root N and NO_3 of lettuce plants cv. Nader (combined analysis of both seasons data).

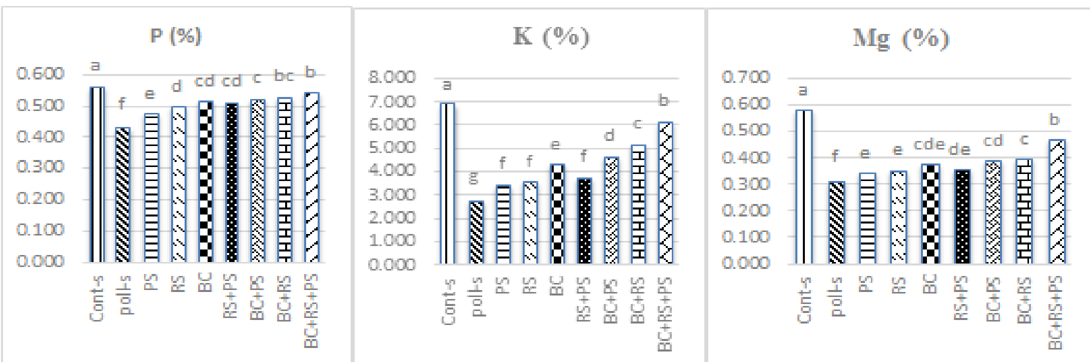

Fig. 5. Effect of soil incorporation with rice straw, biochar, *P. fluorescens* and their interactions on leaf P, K and Mg percentages of lettuce plants cv. Nader (combined analysis of both seasons data).

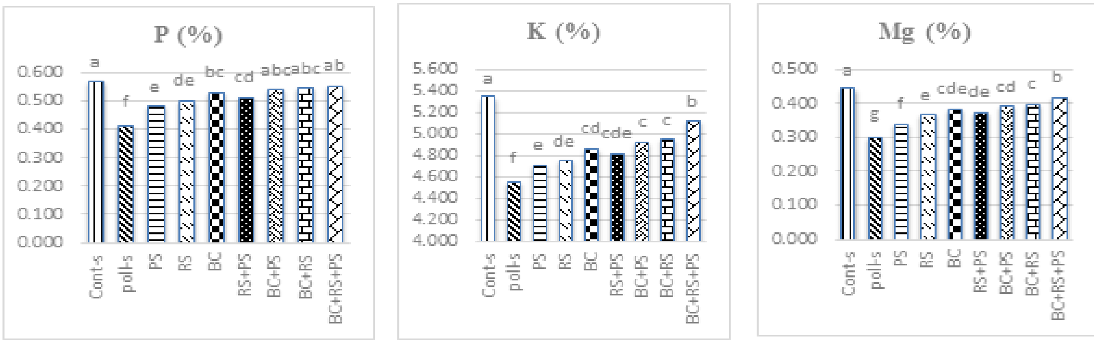

Fig. 6. Effect of soil incorporation with rice straw, biochar, *P. fluorescens* and their interactions on root P, K and Mg percentages of lettuce plants cv. Nader (combined analysis of both seasons data).

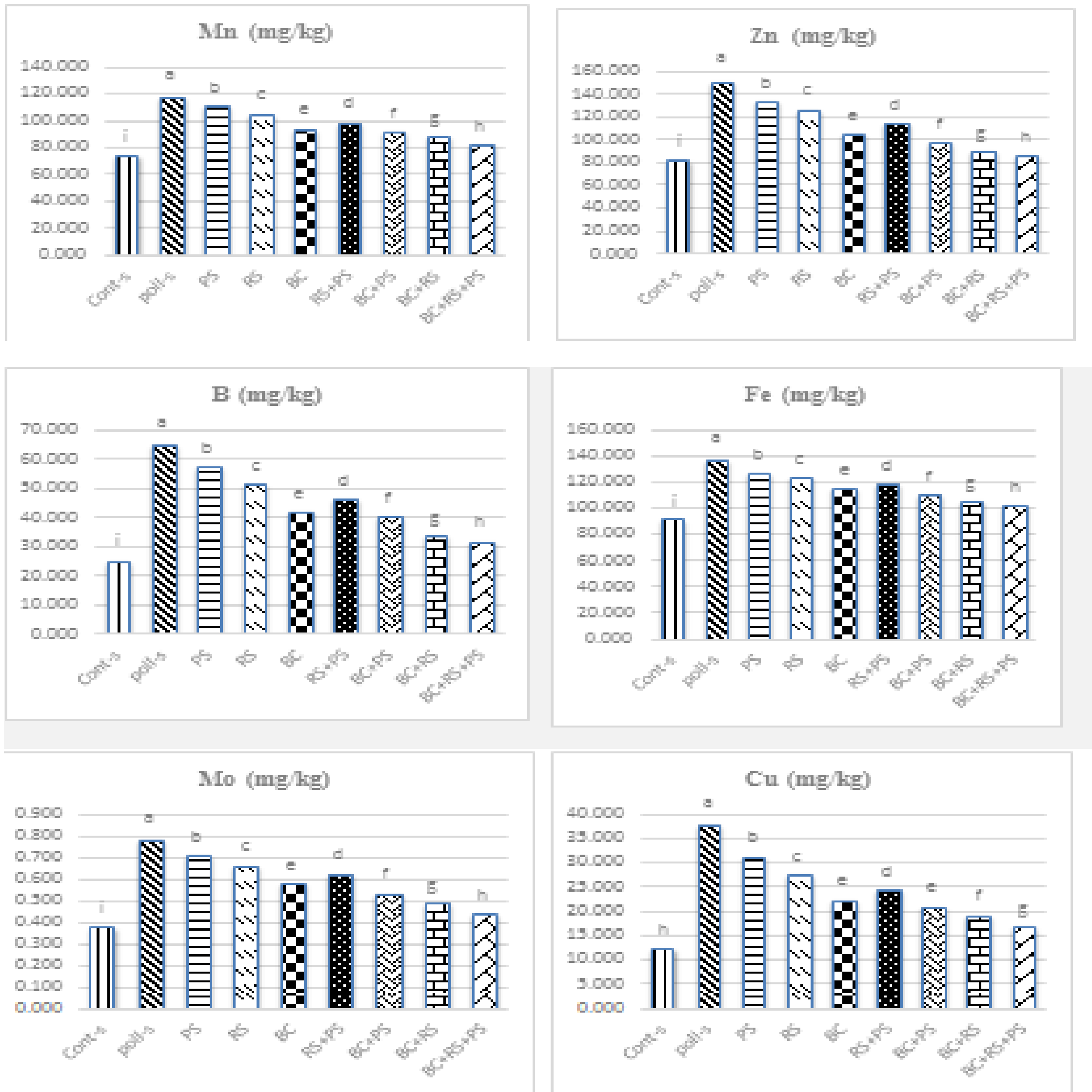

Fig. 7. Effect of soil incorporation with rice straw, biochar, *P. fluorescens* and their interactions on leaf Mn, Zn, B, Fe, Mo and Cu of lettuce plants cv. Nader (combined analysis of both seasons data).

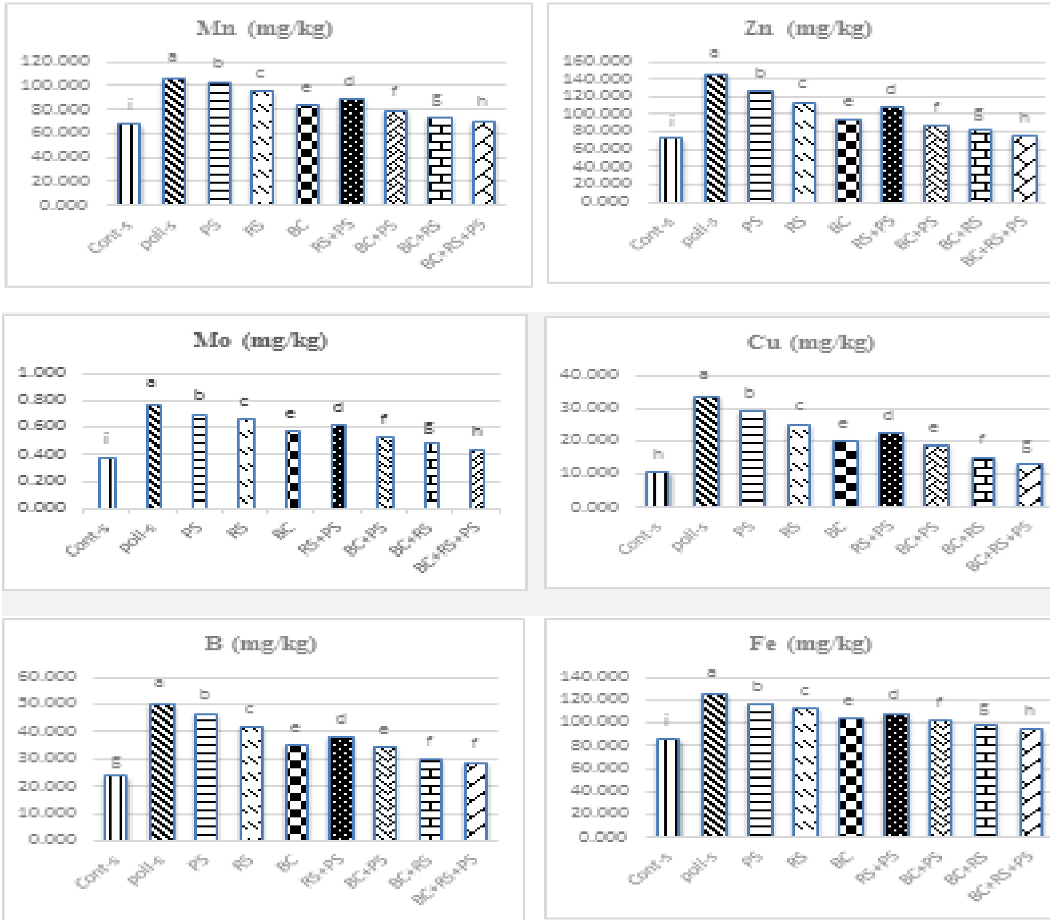

Fig. 8. Effect of soil incorporation with rice straw, biochar, P. fluorescens and their interactions on root Mn, Zn, Mo, Cu, B and Fe of lettuce plants cv. Nader (combined analysis of both seasons data).

TABLE 3. Effect of soil incorporation with rice straw, biochar, *P. fluorescens* and their interactions on leaf Cd, Pb and Cr of lettuce plants cv. Nader (combined analysis of both seasons data).

Treatment	Cd (mg/kg)	Pb (mg/kg)	Cr (mg/kg)
Cont-s	0.307 f	0.133 i	0.017 g
Poll-s	0.863 a	0.830 a	0.123 a
PS	0.627 b	0.627 b	0.090 b
RS	0.500 c	0.583 c	0.077 c
BC	0.403 d	0.433 e	0.050 d
RS+PS	0.480 c	0.530 d	0.073 c
BC+PS	0.397 d	0.353 f	0.040 e
BC+RS	0.377 de	0.307 g	0.040 e
BC+RS+PS	0.363 e	0.247 h	0.030 f

Cont-s = control soil, Poll-s = polluted soil. PS = *P. fluorescens*, RS = Rice straw, BC = Biochar RS+PS = Rice straw + *P. fluorescens*, BC+PS = Biochar + *P. fluorescens*. BC+RS = Biochar + Rice straw and BC+RS+PS = Biochar + Rice straw + *P. fluorescens*.

TABLE 4. Effect of soil incorporation with rice straw, biochar, P. *fluorescens* and their interactions on root Cd, Pb and Cr of lettuce plants cv. Nader (combined analysis of both seasons data).

Treatment	Cd (mg/kg)	Pb (mg/kg)	Cr (mg/kg)
Cont-s	0.313 h	0.140 g	0.017 f
Poll-s	0.953 a	1.153 a	0.123 a
PS	0.670 b	0.590 b	0.073 b
RS	0.620 c	0.467 c	0.057 c
BC	0.517 e	0.337 d	0.050 cd
RS+PS	0.573 d	0.433 c	0.053 cd
BC+PS	0.467 f	0.280 de	0.047 cd
BC+RS	0.417 g	0.240 ef	0.040 de
BC+RS+PS	0.347 h	0.180 fg	0.030 e

Cont-s = control soil**,** Poll-s = polluted soil**.** PS = *P. fluorescens***,** RS = Rice straw**,** BC = Biochar RS+PS = Rice straw + *P. fluorescens***,** BC+PS = Biochar + *P. fluorescens***.** BC+RS = Biochar + Rice straw and BC+RS+PS = Biochar + Rice straw + *P. fluorescens***.**

inoculation with *P. fluorescens*, rice straw, and their combinations, which also improved the vegetative growth parameters such as plant length, leaf area, and head diameter of lettuce plants grown under heavy metal stress. These changes in lettuce's growth parameters are brought on by a decrease in the mobility of heavy metals in the soil following the use of bioadsorbents compared to other physical and chemical methods, the use of microbial metabolic capacity to absorb or remove environmental pollutants offers a cost-effective and secure alternative. According to Desoky et al. (2020), Pb^{2+} or Cd^{2+} stress greatly decreased the fresh and dry weight of plants as well as the leaf chlorophyll content of spinach plants. The reason for the occurrence of significant increases in Cd or Pb content in spinach roots and leaves, suggests that exposure to Cd and/or Pb in the culture medium leads to disruption of the cell's antioxidant defense system compared with the control (without heavy metals), where plant cells are unable to preserve lower levels of Cd or Pb through effective detoxification mechanisms, and as a result, oxidative damage occurs to various cellular components that reduce plant growth, as shown in this study. Application of heavy metals-remediation bacteria has confirmed plant cells to preserve a decrease in the level of Cd or Pb ions, resulting in healthy growth of seedlings under heavy metals. For non-essential elements such as Cd or Pb, there are no specific transport channels in plants. These elements are transported to plants through transporters (Clemens et al., 1998). Plant growth-promoting rhizobacteria can contribute to plant growth improvement through various direct and indirect mechanisms. For example, some reports have been recognized the specific processes of PGPR that stimulate plant growth in the presence of heavy metals, such as the production of IAA, ACC deaminase, and siderophores. IAA-producing bacteria can stimulate plant growth. ACC deaminase inhibits ethylene production (Glick et al., 2007), and siderophores help plants obtain sufficient iron under heavy metal stress (Bruins et al., 2000). On the other hand, biochar is added to soil to improve soil quality and crop productivity; it has a large surface area, a high number of functional groups, and generally a high pH, which makes it able to immobilize heavy metal cations in soils (Beesley et al., 2010). Similarly, biochar application can reduce metal leaching through its effect on metal redox reactions (Choppala et al., 2012). Previous studies showed that the addition of 1% biochar (sawdust fly ash, bagasse fly ash, and rice husk ash), with 2% microorganisms (*P. aeruginosa*, *Bacillus subtilis*, and *Beauveria bassiana*), led to enhanced growth parameters for rice grown in Cd-contaminated soil (Mondal et al., 2020) showed that biochar application (5.5 Mg ha^{-1}) and PGPR (*Rhizobium*, *Bacillus* sp., *Azotobacter*, and *Azospirillum*) enhanced the biomass (roots and shoots) of common bean plants (*Phaseolus vulgaris* L. cv. Falguni) treated with different levels of Cadmium. A similar trend was observed by Mazhar et al. (2020) who found that PGPR strains (*Bacillus*) inoculated along with 1% biochar led to an ameliorated stress effect of Chromium and the ability to improve the plant growth attributes of wheat. The application of rice straw altered the amount of humic compounds in the soil as well as their Cu and Cd levels. Humic compounds can bind with metals to produce organic complexes and chelates because they include a variety of functional groups like carboxyls and different hydroxyls (Clemente and Bernal, 2006). The obtained data herein shown in Figure 2 illustrates how physiological traits and photosynthetic pigments are crucial in enhancing leaf health and crop performance. The lettuce plants were susceptible to the harmful effects of heavy metal stress on plant systems, such as the emergence of leaf chlorosis and deteriorated physiological characteristics as a result of a deficiency in chlorophyll biosynthesis. This might be the result of Cd^{2+} taking the place of Mg^{2+} in the chlorophyll molecule (Wierzbicka et al., 2007). Oxidative stress in plants is facilitated by stress-induced excess ROS (Cheeseman, 2007). Chlorophyll is degraded, membrane functions are changed, and DNA, RNA and proteins are harmed by the elevated ROS levels. Furthermore, heavy metal toxicity results in ion imbalance, osmotic stress, and oxidative stress in plant tissues (Zhu, 2001), which inhibit pigment synthesis and photosynthesis process (Sheng et al., 2008), and stimulates an excessive reduction of reaction centers especially in PS-II, which worsens the photosynthesis mechanism in plants that are unable to use up excess energy (Baker, 2008). The reduction in chlorophyll content, gas exchange, and hydration status of plants brought on by Cd^{2+} and/or Pb^{2+} toxicity is lessened by the use of heavy metal remediation bacteria. It was found that bacteria are critical for binding heavy metals to cell walls and preventing their transfer to plants. In addition to preventing premature leaf senescence and increasing the area of leaves with higher pigment concentrations for photosynthesis, this lessens the negative effects of heavy metal stress on the plant (Cervantes et al., 2001). However, inoculation with *P. fluorescens* and rice straw (interaction between the two) and application of biochar to the soil significantly improved spate in heavy metal-contaminated soil. This is directly related to the leaf's nitrogen content because nitrogen is a macronutrient required for plant growth and chlorophyll synthesis. When rice straw, biochar, and *P. fluorescens* were specifically combined in this study, the results can explain the increase in leaf greenness that was observed and is reflected in healthy plants.

Conclusion

This study shows that soil amendments (biochar, rice straw, and inoculation with *P. fluorescens* and their combinations) improved vegetative growth parameters, and the percentage of N, P, K and Mg in leaves and roots, and reduced Cd, Pb and Cr content in leaves. This study recommends the application of biochar + rice straw + *P. fluorescens* on lettuce cv. Nader plants, which developed the highest values of vegetative growth parameters, improved the accumulation of elements in the human diet and reduced the content of heavy minerals in lettuce leaves under the conditions of Qalyubia Governorate, Egypt.

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Conflicts of interest

The authors declare that they have no competing interests.

References

Akoto, O., Addo D., Baidoo E., Agyapong E.A., Apau J. and Fei-Baffoe B. (2015) Heavy metal accumulation in untreated wastewater-irrigated soil and lettuce (*Lactuca sativa*). *Environ. Earth Sci.*, **74**, (7), 6193–6198. DOI:10.1007/s12665-015-4640-z

Arshad, M., Khan A.H., Hussain I., Anees M., Iqbal M., Soja G., Linde C. and Yousaf S. (2017) The reduction of chromium (VI) phytotoxicity and phyto availability to wheat (*Triticum aestivum* L.) using biochar and bacteria. *Appl. Soil Ecol.*, **114**, (1), 90–98. https://doi.org/10.1016/j.apsoil.2017.02.021

ASTDR, (1999) Toxicological profile for cadmium; Prepared for US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ASTDR); Atlanta, GA, USA.

Baker, N.R. (2008) Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annu. Rev. Plant Bio.,* **59**, (1), 89–113. DOI: 10.1146/annurev.arplant.59.032607.092759

Bakht, J., Shafi M., Jan M.T. and Shah Z. (2009) Influence of crop residue management, cropping system and N fertilizer on soil N and C dynamics and sustainable wheat (*Triticum aestivum* L.) production. *Soil Tillage Res.*, **104**, (2), 233–240. DOI:10.1016/j.still.2009.02.006.

Beesley, L., Moreno-Jimenez E. and Gomez-Eyles J.L. (2010) Effect of biochar and green waste compost amendments on mobility, bioavailability, and toxicity of inorganic and organic contaminants in a multielement polluted soil. *Environ. Pollut.,* **158**, (6) 2282–2287. doi: 10.1016/j.envpol.2010.02.003.

Benavides, M.P., Gallego S.M. and Tomato M.L. (2005) Cadmium toxicity in plants. *Braz. J. Plant Physiol.,* **17**, (1), 21–34. https://doi.org/10.1590/S1677-04202005000100003

Boparai, H.K., Joseph M. and O'Carroll D.M. (2011) Kinetics and thermodynamics of cadmium ions removal by absorption onto nano zerovalent iron particles. *J. Hazard Mater.*, **186**, (1),458–465. DOI: 10.1016/j.jhazmat.2010.11.029.

Bruins, M.R., Kapil S. and Oehme F.W. (2000) Microbial resistance to metals in the environment. Ecotoxicol. Environ. Saf., **45**, (3), 198-207. doi: 10.1006/eesa.1999.1860.

Cervantes, C., Campos-García J., Devars S., Gutiérrez-Corona F., Loza-Tavera H., Torres-Guzmán J.C. and Moreno-Sánchez R. (2001) Interactions of chromium with microorganisms and plants. *FEMS Microbiol. Rev.,* **25**, (3) 335–347. https://doi.org/10.1111/j.1574-6976.2001.tb00581.x.

Cheeseman, J.M. (2007) Hydrogen peroxide and plant stress: a challenging relationship. *Plant Stress Global Science Books,* **1**, (1), 4–15.

Cho, J.S., Hur J.S., Kang B.H., Kim P.J., Sohn B.K., Lee H.J., Jung Y.K. and Heo J.S. (2001) Biosorption of copper by immobilized biomass of *Pseudomonas stutzer*. *J. Microbiol. Biotechnol.,* **11**, (6) 964–972.

Choppala, G.K., Bolan N.S., Megharaj M., Chen Z. and Naidu R. (2012) The influence of biochar and black carbon on reduction and bioavailability of chromate in soils. *J. Environ. Qual.,* **41**, (4), 1175-1184. DOI: 10.2134/jeq2011.0145.

Clemente, R. and Bernal M.P. (2006) Fractionation of heavy metals and distribution of organic carbon in two contaminated soils amended with humic acids. *Chemosphere*, **64**, (8), 1264–1273. doi: 10.1016/j.chemosphere.2005.12.058.

Clemens, S., Antosiewicz D.M., Ward J.M., Schachtman D.P. and Schroeder J.I. (1998) The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. *Proc. Natl. Acad. Sci. U.S.A*, **95**, (20), 12043–12048. https://doi.org/10.1073/pnas.95.20.12043.

Deb, S., Ahmed S.F. and Basu M. (2013) Metal accumulation in cell wall: A possible mechanism of cadmium resistance by *Pseudomonas stutzeri*. *Bull. Environ. Contamin. Toxicol.,* **90**, (3), 323–328. doi: 10.1007/s00128-012-0933-z.

Desoky, E.S.M., Merwad A.R.M., Semida W.M., Ibrahim S.A., El-Saadony M.T. and Rady M.M. (2020) Heavy metals-resistant bacteria (HM-RB): potential bioremediators of heavy metals-stressed *Spinacia oleracea* plant. *Ecotoxicol. Environ. Saf.,* 198, 110685. DOI: 10.1016/j.ecoenv.2020.110685.

FAOSTAT, (2013) "Production of Lettuce & Chicory by countries for 2013". Food and Agriculture Organization of the United Nations, Rome, Italy. Statistics Division.

Glick, B.R., Todorovic B., Czarny J., Cheng Z., Duan J. and McConkey B. (2007) Promotion of plant growth by bacterial ACC deaminase. *Crit. Rev. Plant Sci.,* **26**, (5-6), 227-242. https://doi.org/10.1080/07352680701572966.

Guo, G., Zhou Q. and Ma L.Q. (2006) Availability and assessment of fixing additives for the in-situ remediation of heavy metal contaminated soils: A review. *Environ. Monit. Assess.*, **116**, (1-3), 513–528, DOI: 10.1007/s10661-006-7668-4.

Gutierrez-Segura, E., Solache-Rio M., Colin-Cruz A. and Fall C. (2012) Adsorption of cadmium by Na and Fe modified zeolitic tuffs and carbonaceous material from pyrolyzed sewage sludge. *J. Env. Manag.,* **97**, (1), 6–13. DOI: 10.1016/j.jenvman.2011.11.010.

Herrmann, L. and Lesueur D. (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. *Appl. Microbiol. Biotechnol.,* **97**, (20), 8859–8873. DOI: 10.1007/s00253-013-5228-8.

Iqbal, A., Mushtaq M., Khan A., Nawaz I., Yousaf S. and Iqbal M. (2020) Influence of *Pseudomonas japonica* and organic amendments on the growth and metal tolerance of *Celosia argentea* L. *Environ. Sci. Pollut. Res.*, **27**, (20), 24671–24685. doi: 10.1007/s11356-019-06181-z.

Ishibashi, Y., Cervantes C. and Silver S. (1990) Chromium reduction in *Pseudomonas putida*.

Appl. Environ. Microbiol., **56**, (7), 2268–2270. https://doi.org/10.1128/aem.56.7.2268-2270.1990.

Itusha, A.W., Jabez O. and Mohanasrinivasan V. (2019) Enhanced uptake of Cd by biofilm forming Cd resistant plant growth promoting bacteria bioaugmented to the rhizosphere of *Vetiveria zizanioides*. *Int. J. Phytoremediat.*, **21**, (5) 487–495. doi: 0.1080/ 15226514. 2018.1537245.

Khan, M.A., Khan S., Khan A. and Alam M. (2017) Soil contamination with cadmium, consequences and remediation using organic amendments. *Sci. Total Environ.,* 601-602, 1591–1605. doi: 10.1016/j.scitotenv.2017.06.030.

Koller, H.R. (1972) Leaf area-leaf weight relationships in the soybean canopy. *Crop Sci.,* **12**, (2), 180-183. https://doi.org/10.2135/ cropsci1972. 0011183X 0012000 20007x.

Lehmann, J. and Joseph S. (2015) Biochar for environmental management: An introduction. In Biochar for Environmental Management: Science, Technology and Implementation, 2nd ed.; Lehmann, J. and Joseph, S., Eds.; Earthscan from Routledge: London, UK, pp. 1–1214.

Lehmann, J., da Silva J.P., Steiner C., Nehls T., Zech W. and Glaser B. (2003) Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. *Plant Soil*, **249**, (2) 343–357. DOI:10.1023/A:1022833116184.

Lehmann, J., Rillig M.C., Thies J., Masiello C.A., Hockaday W.C. and Crowley D. (2011) Biochar effects on soil biota–a review. *Soil Biol. Biochem.*, **43**, (9), 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022.

Major, J., Lehmann J., Rondon M. and Goodale C. (2010) Fate of soil-applied black carbon: Downward migration, leaching and soil respiration. *Glob. Chang. Biol.,* **16**, (4) 1366–1379. https://doi.org/10.1111/j.1365-2486.2009.02044.x.

Mazhar, R., Ilyas N., Arshad M., Khalid A. and Hussain M. (2020) Isolation of heavy metal tolerant PGPR strains and amelioration of chromium effect in wheat in combination with biochar. Iran. *J. Sci. Technol. Trans. A Sci.*, **44**, (6), 1–12. DOI:10.1007/s40995-019-00800-7.

Mondal, S.C., Sarma B., Farooq M., Nath D.J. and Gogoi N. (2020) Cadmium bioavailability in acidic soils under bean cultivation: Role of soil additives. *Int. J. Environ. Sci. Technol.,* **17**, (12) 153–160. https://doi.org/10.1007/s13762-019-02263-0.

Nadeem, S.M., Imran M., Naveed M., Khan M.Y., Ahmad M., Zahir Z.A. and Crowley D.E. (2017) Synergistic use of biochar, compost, and plant growth-promoting rhizobacteria for enhancing cucumber growth under water deficit conditions. *J. Sci. Food Agric.,* **97**, (15), 5139–5145. DOI: 10.1002/jsfa.8393.

Neufeld, H.S., Chappelka A.H., Somers G.L., Burkey K.O., Davison A.W. and Finkelstein P.L. (2006) Visible foliar injury caused by ozone alters the relationship between SPAD meter readings and chlorophyll concentrations in cut leaf coneflower. *Photosynth. Res.,* **87**, (3), 281–286. DOI: 10.1007/s11120-005-9008-x.

Novak, J.M., Busscher W.J., Laird D.L., Ahmedna M., Watts D.W. and Niandou M.A.S. (2009) Impact of biochar amendment on fertility of a Southeastern Coastal Plain soil. *Soil Sci.,* **174**, (2), 105–112. DOI: 10.1097/SS.0b013e3181981d9a.

Piper, C.S. (1950) Soil and plant analysis .1st ed. Inter. *Science Publishers Inc*., New York, USA, pp.30-59.

Rodushkin, L., Ruth T. and Huhtasaari A. (1999) Comparison of two digestion methods for elemental determinations in plant material by ICP techniques. *Anal. Chim. Acta*, **378**, (1-3),191-200. DOI:10.1016/S0003-2670(98)00635-7.

Sheng, M., Tang M., Chen H., Yang B., Zhang F. and Huang Y. (2008) Influence of ar-buscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza*, **18**, (7-8), 287 -296. **DOI**: 10.1007/s00572-008-0180-7.

Singh, P.B., Hatton J.B., Singh B., Cowie L.A. and Kathuria A. (2010) Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *J. Environ. Qual,* **39**, (4), 1224–1235. DOI: 10.2134/jeq2009.0138.

Singleton, P. and Sainsbury D. (1987) Dictionary of microbiology and molecular biology. *J. Basic Microbiol. NY, USA,* **28**, (7), 470. https://doi.org/10.1002/jobm.3620280712.

Takahashi, R., Ishimaru Y., Nakanishi H. and Nishizawa N.K. (1999) Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. *Plant Signal. Behav.,* **6**, (11), 1813–1816. doi: 10.4161/psb.6.11.17587.

Thomas, R.L., Sheard R.W. and Moyer J.R. (1967) Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. *Agron. J.,* **59**, (3), 240-243. https://doi.org/10.2134/agronj1967.00021962005900030010x.

Ventura, M., Sorrenti G., Panzacchi P., George E. and Tonon G. (2013) Biochar reduces short-term nitrate leaching from a horizon in an apple orchard. *J. Environ. Qual.,* 42, (1), 76–82. DOI: 10.2134/jeq2012.0250.

Waller, R.A and Duncan D.B. (1969) A bayes rule for the symmetric multiple comparison problem. *J. Am. Stat. Assoc.,* **64**, (328), 1484-1503.

Wierzbicka, M.H., Przedpełska E., Ruzik R., Ouerdane L., Połeć-Pawlak K., Jarosz M., Szpunar J. and Szakiel A. (2007) Comparison of the toxicity and distribution of cadmium and lead in plant cells. *Protoplasma*, **231**, (1-2), 99-111. DOI: 10.1007/s00709-006-0227-6.

Zand, A.D., Alireza M.T. and Azar V.H. (2020) Application of titanium dioxide nanoparticles to promote phytoremediation of Cd-polluted soil: Contribution of PGPR inoculation. *Bioremediate. J.*, **24**, (2-3), 171–189. DOI: 10.1080/10889868.2020.1799929.

Zhong, H., Yue Y. and Fan J. (2003) Characteristics of crop straw resources in China its utilization. *Resour. Sci.*, **25**, 62–67.

Zhu, J.K. (2001) Plant salt tolerance. *Trends Plant Sci.,* **6**, (2),66-71. http://dx.doi.org/10.1016/S1360-1385(00)01838-0

Zorrig, W., Abdelly C. and Berthomieu P. (2011**)** The phylogenetic tree gathering the plant Zn/Cd/Pb/Co P1B-ATPases appears to be structured according to the botanical families. *Comptes Rendus Biol.,* **334**, (12), 863–871. https://doi.org/10.1016/j.crvi.2011.09.004.

Zorrig, W., El Khouni A., Ghnaya T., Davidian J.C., Abdelly C. and Berthomieu P. (2013) Lettuce (*Lactuca sativa*): A species with a high capacity for cadmium (Cd) accumulation and growth stimulation in the presence of low Cd concentrations. *J. Hortic. Sci. Biotechnol.*, **88**, (6),783–789. https://doi.org/10.1080/ 14620316. 2013. 11513039.

تقليل تراكم العناصر الثقيلة فى الاجزاء المستهلكة من الخس

ياسين محمد شيبه ، سلامه عبد الحميد عبد الهادي ، صبري موسى سليمان يوسف ومحمد زكي الشناوي
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أجريت هذه الدراسة بغرض الحد من تراكم العناصرالثقيلة في الأجزاء الصالحة للأكل من الخس المزروع في تربه ملوثة. لذلك تمت زراعة نبات الخس صنف نادر في تربة ملوثة وكانت المعاملات كالتالى فحم حيوي، قش الأرز، التلقيح ببكتريا سيدموناس فلورنسيس، الفحم + قش الأرز، الفحم + البكتريا، القش + البكتريا، الفحم + القش + البكتريا، بالإضافة إلى تربة ملوثة بدون أي إضافات والتربة غير الملوثة (كنترول) وأجريت التجارب في موسمي ٢٠١٩ و٢٠٢٠ في مزرعة الخضر بكلية الزراعة بجامعة عين شمس. أشارت النتائج المتحصل عليها إلى أن العناصر الثقيلة لها آثار سلبية على نمو نباتات الخس المزروعة في التربة الملوثة بالمقارنة بالنباتات المزروعة في التربة غير الملوثة. وأدى تطبيق هذه المعاملات إلى تحسين الصفات الخضرية (إرتفاع النبات، ومساحة الأوراق، ووزن الرأس الطازج والجاف، ووزن الجذر الطازج والجاف، وقطر الرأس). كما أظهرت النتائج أن نباتات الخس المزروعة في تربة ملوثة بالمعادن الثقيلة بدون أي إضافات قد سجلت أقل القيم معنوياً لجميع الصفات المدروسة وسجلت أعلى القيم معنوياً بالنسبة لمحتوى الاوراق والجذور من العناصر الصغرى والثقيلة. وبالتالى فإن استخدام قش الأرز أوالبكتريا أوالفحم أوالدمج بينهم حسن من نموالنباتات ومحتوى العناصر الغذائية في الأوراق وإن أظهرت النتائج أن الفحم الحيوى وحده أو بالأشتراك مع القش اوالبكتريا كان فعال فى تقليل تراكم العناصر الثقيلة فى الأوراق وتقليل الأثار الضارة للمعادن الثقيلة على نبات الخس ويمكن أن تكون التوصية للاستخدام اذا دعت الحاجة إلى زراعة نباتات الخس في مثل هــذه الأراضي وتحت هذه الظروف.

الكلمات الدالة:

نبات الخس ، الفحم النشاط، قش الأرز ، ببكتريا سيدموناس فلورنسيس ، العناصر الثقيلة ، النمو الخضرى ، المحتوى المعدني و محتوى النترات فى الأوراق والجذور.