Combined use of municipal solid waste biochar and bacterial biosorbent synergistically decreases Cd(II) and Pb(II) concentration in edible tissue of forage maize irrigated with heavy metal–spiked water

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ABSTRACT

A pot experiment was carried out to evaluate the effect of a municipal solid waste (MSW) biochar and a bacterial strain on the forage maize growth and the concentration of lead (Pb) and cadmium (Cd) in the edible tissue of maize irrigated with water contaminated with Cd (5 mg L⁻¹) and Pb (100 mg L⁻¹). Experimental treatments included (i) bacterial strain at two levels: no bacterial strain and Enterobacter cloacae R7; (ii) MSW biochar at three levels: 0, 1, and 3% (w/w); and (iii) irrigation water quality at five levels: plants irrigated with 100% freshwater (FW), plants irrigated with 75%FW + 25% contaminated water (CW), plants irrigated with 50%FW + 50% CW, plants irrigated with 25%FW + 75% CW, and plants irrigated with 100% CW. The effect of various treatments on maize growth indices and concentration of Pb(II) and Cd(II) in the plant was significant at 5% level. The concentration of these metals in the shoot of plants irrigated with 75 and 100% CW was higher than the permissible limits for Cd(II) and Pb(II) in livestock feed. However, the concentration of these metals in the shoot of the plants irrigated with 25 and 50% CW was lower than the permissible limit for this use. In this study, the combined application of 3%biochar and E. cloacae R7 had a significant effect on increased root dry weight (ranging from 29 to 33%), shoot dry weight (ranging from 32 to 43%) and bacterial root colonization (ranging from 33 to 53%) and on reduced concentration of Pb (ranging from 78 to 80%) and Cd (ranging from 72 to 76%) of the shoot of maize plant (edible tissues used by livestock), which was below the permissible limits for livestock feed, compared to corresponding controls. According to the results of this study, to reduce the concentration of the heavy metals in forage maize shoot (below the permissible limits for livestock feed), it is suggested using heavy metal–contaminated water either in combination with freshwater (50 or 75% FW) or in combination with biochar and bacterial biosorbent, averting human/animal health risk.

1. Introduction

Reuse of the untreated or treated wastewater (poorly treated wastewater) in agriculture is becoming more important every day because agriculture sector is one of the main consumers of water (more than 70% of water withdrawals (FAO, 2016)) in most of the countries located in arid and semi-arid areas (Niyonzima et al., 2013). The consequence of the use of such wastewater or constant use of treated wastewater in agriculture is the contamination of soils and groundwater and the accumulation of heavy metals in agricultural land (Abdu et al., 2011; Agari and Cornelis, 2015; Kiziloglu et al., 2008; Nzediegwu et al., 2019). Depending on the soil physical and chemical attributes (i.e., soil texture, soil CEC–cation exchange capacity, and soil pH) (Nzediegwu et al., 2019), various heavy metal species accumulated in the contaminated soils can be absorbed by food crops and be translocated to distinctive edible portions, where they may accumulate to toxic levels, and consequently they raise consequential threats to animal and human health and food security (Etesami, 2018; Singh et al., 2010a; Wang et al., 2018b). Multiple studies of heavy metal uptake by crop plants in soils irrigated with municipal and industrial wastewater have been reported (Chaoua et al., 2019; Hussain et al., 2013; Nzediegwu et al., 2019). Owing to their non–biodegradable and non–chemodegradable nature (Etesami, 2018; Muhammad et al., 2011), heavy metals critically restrict the advantageous use of wastewater for homebred or industrial usage (Petrus and Warchol, 2005).
Manipulating the bioavailability of heavy metals to plants by some amendments has become an achievable way to guarantee food security when irrigating plants with the wastewater (Hameeda et al., 2019; Nziediegwu et al., 2019; Tahir et al., 2018). There are many studies reported in the literature regarding the removal of diverse toxic metal ions including cadmium (Cd) and lead (Pb) using various composite materials (Awual, 2016, 2017, 2017a, b; Awual et al., 2020; Awual et al., 2018). Among various amendments, biochar, a carbon–rich solid residue produced by pyrolysis of biomass residues and an extensive range of organic wastes (Ding et al., 2017), has been greatly studied as an inexpensive biosorbent (Ahmad et al., 2014; Inyang et al., 2016; Wang et al., 2018b). Owing to their surface functional groups and adsorption sites, biochars have been also indicated to diminish the mobility and bio–availability of heavy metals (Lahori et al., 2017; O’Connor et al., 2018; Wang et al., 2018a) and prevent uptake and transfer of heavy metals to the food chain (Al-Wabel et al., 2015; Puga et al., 2015; Qiao et al., 2015) by many well–known mechanisms (Paz–Ferreiro et al., 2014; Uzoma et al., 2011).

Nowadays, a large quantity of municipal solid waste (MSW) (1.3 billion tonnes per year) is generated and appraised to augment up to 2.2 billion tonnes per year in 2025 with increasing global population, urbanization, industrialization, and a shift in consumption behavior, especially in developing countries (Hoornweg and Bhada-Tata, 2012; Lohri et al., 2017). The conversion of MSW to biochar has been proven to be an environmentally logical approach to alleviate negative impacts of pollutants from the MSW and manage waste instead of getting them evacuated in landfill site (Gnaraathne et al., 2019). Chemical analysis of MSW biochar has also shown that the concentration of potentially heavy metals (toxic elements) and polycyclic aromatic hydrocarbon in the material is minimal and no restrictions have been reported on their use (Gnaraathne et al., 2019; Jayawardhana et al., 2019; Taherymoosavi et al., 2017). The potential of MSW biochar to remove heavy metals from both soil and aqueous media and improve soil traits and plant growth has been previously investigated (Agrafioti et al., 2014; Gnaraathne et al., 2019; Jin et al., 2014; Taherymoosavi et al., 2017).

In addition to the heavy metal accumulation in plants, one of the disadvantages of using wastewater containing high concentrations of heavy metals for irrigation of plants (e.g., forage plants) is to diminish plant growth and yield (Etesami, 2018; Khan et al., 2015). Bacterial induced–palliation of heavy metal stress may have the potential to overcome the limitation (Etesami, 2018). It is well known that heavy metal resistant–plant growth promoting bacteria (PGPB) including some Enterobacter species (Banerjee et al., 2015; Paul and Mukherjee, 2016) can tolerate and absorb different types of heavy metals because of high adaptive nature and cellular mechanisms (Etesami, 2018). The PGBP can attenuate the heavy metal–induced stress (increase in immobility of heavy metals) and reduce the accumulation and translocation of these metals in the plants grown on soil contaminated to these metals and finally stimulate heavy metal–stressed plant growth by various mechanisms (Etesami, 2018).

Albeit the potential of heavy metal resistant–PGPB and metal immobilizers such as biochar in reducing the absorption of heavy metals by various plants has been studied (Touceda-Gonzalez et al., 2015; Wang et al., 2018b), little is known about the alleviative and synergistic role of these bacteria and biochar, especially MSW–derived biochar, together in mitigating heavy metals stress and in reducing uptake of heavy metals by forage maize (Zea mays L.), as an energy–rich feed for ruminant livestock. It seems that heavy metal resistant–PGPB and MSW biochar can synergistically reduce uptake of heavy metal by forage maize plant. Moreover, no study has been carried on forage maize growth and edible tissue Cd and Pb concentration in the presence of heavy metal resistant–PGPB and MSW biochar. Heavy metal resistant–PGPB producing IAA (indole–3–acetic acid), siderophores, and ACC (1–aminocyclopropane–1–carboxylate) deaminase, and solubilizing insoluble phosphates stimulate plant growth and augment heavy metal resistance of the plants grown on heavy metal–contaminated soils (Etesami, 2018, 2020; Etesami and Maheshwari, 2018).

Although biochars have been reported to diminish soil available metal content via various mechanisms (e.g., adsorption, co–precipitation, and complexation), applying biochars or other immobilizers had a hazard on soil and water quality after a few years (Udewige et al., 2011; ur Rehman et al., 2018). Knowledge on the impacts of heavy metal resis–tant–PGPB, MSW biochar, and their combination on the plant growth and the accumulation of heavy metal in forage maize is a momentous prerequisite for the better grasp of their interactions with the forage maize for the extension of effective and safe production of forage maize irrigated with heavy metal–contaminated water (e.g., wastewater).

Given the merits of intimate quadruple interactions among biochars, plants, microorganisms, and heavy metals, the aim of the research was the feasibility of the joined and single application of MSW biochar, as a soil immobilizing agent (a promising adsorbent) for diminishing the availability of heavy metals and uptake by plants and as a suitable habitat provider for microorganisms, and heavy metal resistant–allochthonous bacterial strain Enterobacter cloacae R7, as an agent for heavy metal removal (a promising biosorbent) and plant growth promotion, to reduce the accumulation of heavy metals (within the permissible limit for live–stock feed) in forage maize plant irrigated with water spiked with Cd and Pb, which are mostly found as contaminants in wastewater and plant crops (Kachenko and Singh, 2006) and have been shown to have carcinogenic impacts (Awual et al., 2020; Jaishankar et al., 2014). We assumed that the combined use of MSW biochar (i.e., by providing a favourable habitat for bacterial proliferation and survival and heavy metal immobilization) and E. cloaceae R7 (i.e., by increased plant growth and plant resistance to heavy metal and bioaccumulation/biosorption) has the synergistic effects on increased forage maize biomass and decreased Cd and Pb concentration in the plant. Naturally, the results of this study can be an important and effective step in achieving a better method for reuse of wastewater for industrial and agricultural purposes. This study reports for the first time the potential of combined use of MSW biochar and a bacterial biosorbent for reducing the concentration of Pb and Cd, within the permissible limit for livestock feed, in forage maize irrigated with heavy metal contaminated water.

2. Materials and methods

2.1. Bacterial strain and preparation of inoculum

Enterobacter cloacae R7 (Accession number MF687205) was selected and used in this study. This strain was obtained from the rhizosphere soil of maize plants (Zea mays L. c.v., KSC–703) irrigated with industrial and municipal wastewater (Abedinzadeh et al., 2019). Some traits of this strain, measured in our previous study (Abedinzadeh et al., 2019), are shown in Table 1. For the preparation of bacterial inoculant for pot experiment, strain R7 was cultured in sterile NB (nutrient broth) (5.0% NaCl, 5.0% peptone, 1.5% beef extract, 1.5% yeast extract, and pH 7.4) in a rotary shaker (120 r/min) at 28 ± 2 °C for 24 h (exponential growth phase, OD600 = 0.5) in an incubator. The bacterial cells were collected by centrifugation at 7000 rpm at 4 °C for 10 min. The pellet was washed with a sterile saline solution (0.9% NaCl) twice and resuspended in sterile Milli–Q water to roughly 5 × 108 colony–forming unit (CFU) mL–1.

2.2. Production of MSW biochar and its characteristics

The MSW biochar used in this study was generated by pyrolysis (under the oxygen–limited conditions) of the manually segregated organic fraction of MSW, which was obtained from the landfill of Kahzarak, Tehran, Iran, according to the same methods described previously (Jayawardhana et al., 2019; Taherymoosavi et al., 2017). The pyrolysis process for conversion of MSW to biochar was executed at a temperature of 500 ± 10 °C with a temperature rise of 20 °C min–1 for 1 h in a muffle furnace (Linn High Therm). The temperature of 450–500 °C are the most suitable temperatures for biochar preparation for use in metal (i.e., Cd.
2.0 mg kg$^{-1}$ DTPA – Presence of functional groups of hydroxyl, carboxylic and phenolic

2.4. Experimental set up and treatments

To assay the single and combined effects of MSW biochar and bacterial biosorbent ($E.\text{cloacae}$ R7) on growth and concentration of Cd and Pb of forage maize irrigated with water contaminated with both heavy metals, a completely randomized design with factorial arrangement (a 2 × 3 × 5 factorial design) in three replications was carried out in a potted non–sterile soil. The experiment treatments included (i) bacterial strain factor at two levels: seeds non–inoculated with $E.\text{cloacae}$ R7 (B0) and seeds inoculated with $E.\text{cloacae}$ R7 (B1); (ii) MSW biochar factor at three levels: soil non–amended with MSW biochar (0%BC), soil amended with 1% MSW biochar (1%BC, w/w), and soil amended with 3% MSW biochar (3%BC, w/w); and (iii) irrigation water quality factor at five levels: plants irrigated with 100% freshwater (distilled water) (FW) (100%FW), plants irrigated with 75% FW and 25% water contaminated (CW) with 5 mg Cd L$^{-1}$ (in the form of analytical–grade salt of CdCl$_2$) and 100 mg Pb L$^{-1}$ (in the form of analytical–grade salt of Pb(NO$_3$)$_2$) (75%FW + 25%CW), plants irrigated with 50% FW and 50% water contaminated with 5 mg Cd L$^{-1}$ and 100 mg Pb L$^{-1}$ (50%FW + 50%CW), plants irrigated with 25% FW and 75% water contaminated with 5 mg Cd L$^{-1}$ and 100 mg Pb L$^{-1}$ (25%FW + 75%CW), and plants irrigated with 100% water contaminated with 5 mg Cd L$^{-1}$ and 100 mg Pb L$^{-1}$ (100%CW).

On the basis of typical wastewater concentrations of the heavy metals in developing countries, the concentration of Pb and Cd used in this assay was selected (Ahmad et al., 2011). MSW–biochar (on a dry weight basis) was blended completely into the soil (8 kg) and pots (80 cm in height and 21 cm in diameter) with drainage holes were filled with the soil according to corresponding treatments. Blending soil was also carried out in treatments non–amended with biochar (control). Before planting, pots including the biochar–soil mixture were moistened up to 60% moisture content of field capacity and pre–incubated for 30 days at temperature of

### Table 1. Some traits of bacterial strain Enterobacter cloacae R7 and municipal solid waste (MSW) biochar used in this assay.

| Traits | Value |
|--------|-------|
| MIC of Pb (mM) | 3 |
| MIC of Cd (mM) | 2.5 |
| MIC of Ni (mM) | 2.5 |
| MIC of Co (mM) | 0.5 |
| MIC of Cu (mM) | 2.5 |
| MIC of Zn (mM) | 3 |
| MIC of Cr (mM) | > 3.5 |
| Pb removal from contaminated liquid medium (%) | 88.95 |
| Cd removal from contaminated liquid medium (%) | 58 |
| Ability to colonize root surface and inside roots of maize in the presence and absence of Cd and Pb | + |
| IAA production (µg ml$^{-1}$) | 35.4 |
| Siderophore production | + |
| Insoluble phosphate solubilization | + |
| Mic, minimum inhibitory concentration; IAA, indole–3–acetic acid; ACC, 1–aminocyclopropane–1–carboxylate; +, the presence of activity, and EC, electrical conductivity. | |

And Pb) adsorption (Ding et al., 2016; Li et al., 2017a). The analysis of produced MSW biochar (2 mm–sieve) (Table 1) was performed on the basis of the experimental procedure characterized in previous studies (Ahmad et al., 2013; Taherymoosavi et al., 2017). The MSW biochar was stored in desiccator before use.

2.3. Soil sampling

In this study, a heavy metal–non–contaminated soil was selected and gathered from the surface soil layer (0–30 cm). Some chemical and physical characteristics of thoroughly mixed, sieved (a 2–mm sieve for chemical analysis) and air–dried soil were measured according to standard procedures. The soil had a sandy loam texture; pH, 7.78; EC, 1.10 dS m$^{-1}$; available P, 8.84 mg kg$^{-1}$; total N, 0.047%; available K, 325 mg kg$^{-1}$; Mg, 4.6 mg kg$^{-1}$; Ca, 18.40 mg kg$^{-1}$; Na, 6.0 mg kg$^{-1}$; available Fe, 2.0 mg kg$^{-1}$; saturation percentage (SP) of soil, 26; and organic C, 0.30%. Concentrations of available Cd and Pb were below the detection limits. This soil was also passed via a 4–mm stainless sieve prior to use in pots.

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room (25 °C) to stimulate the reaction and contact between soil particles and MSW–biochar (stabilization of chemical equilibria) and fixate the activity of microorganisms.

In this experiment, forage maize plant (*Zea mays* L. c.v., KSC–703) was used as a test plant. The seeds of this variety were taken from SPBRI. For seed bacterization, surface–disinfected seeds were imbibed in a bacterial cell suspension with a population density of $5 \times 10^8$ cells mL$^{-1}$ for 5 h. The control seeds were imbibed in the dead bacterial inoculum (autoclaved for 30 min at 121 °C). Six germinated healthful maize seeds were sown in each pot and then thinned to three equal seedlings in each pot seven days after planting. According to the soil test, urea fertilizer (at three times), triple superphosphate, and Hoagland solution (for three weeks with 100 mL of Hoagland solution) were used to meet N, P and micronutrients requirements of the plant. In the case of Pb, N added via its respective Pb(NO$_3$)$_2$ was taken into account when supplementing the recommended basal N dose. During the growth period, the plants were only irrigated with different water qualities according to the experimental design. The study was performed in a research greenhouse under 15000 Lux m$^{-2}$ with a 14 h photoperiod and controlled temperature (22–28 °C) and relative humidity (60%) located at Department of Soil

![Figure 1. The effect of bacterial strain *Enterobacter cloacae* R7 and municipal solid waste (MSW) biochar on the root dry weight (A) and the shoot dry weight (B) of forage maize plant irrigated with various irrigation water quality after 100 days of cultivation under greenhouse. B0, seeds non–inoculated with *E. cloacae* R7; B1, seeds inoculated with *E. cloacae* R7; 0%BC, soil non–amended with MSW biochar; 1%BC, soil amended with 1% MSW biochar; 3%BC, soil amended with 3% MSW biochar; 100%FW, plants irrigated with 100% freshwater (distilled water) (FW); 75%FW + 25%CW, plants irrigated with 75% FW and 25% water contaminated (CW) with 5 mg Cd L$^{-1}$ and 100 mg Pb L$^{-1}$; 50%FW + 50% CW, plants irrigated with 50% FW and 50% water contaminated with 5 mg Cd L$^{-1}$ and 100 mg Pb L$^{-1}$; 25%FW + 75%CW, plants irrigated with 25% FW and 75% water contaminated with 5 mg Cd L$^{-1}$ and 100 mg Pb L$^{-1}$; and 100%CW, plants irrigated with 100% water contaminated with 5 mg Cd L$^{-1}$ and 100 mg Pb L$^{-1}$. Data represent the mean ± SD ($n = 3$) and different letters indicate a significant difference ($P < 0.05$) using Duncan's test.
Biology and Biotechnology, University of Tehran, Iran. To eliminate the likely error of temperature, evaporation, light, transpiration, etc. to a possible extent to guarantee consistency of the circumstances, the place of the experimental treatments and their iterations were replaced in rotation. The duration of plant growth was 100 days in the greenhouse.

2.4.1. Measurements

The impacts of different treatments on root and shoot dry weight, oven-dried at 70 °C for 48 h, were estimated and recorded at the end of plant growth period. Moreover, the shoots and roots of the plant were cleaned with 0.01 M EDTA (Ethylenediaminetetraacetic acid) and distilled water to eliminate any non-specifically bound Cd and Pb, and then oven-dried (for 30 min at 105 °C, then at 65 °C, until they reached stable weights), ground, and were chemically analyzed. The content of Cd and Pb in these tissues was estimated by an inductively coupled plasma optical emission spectrometry (Jones and Case, 1990). All chemicals used in the research were of analytical grade. Root colonization by R7 strain (colony forming units, CFU g⁻¹) was also determined according to the same method explained by Abedinzadeh et al. (2019). Bacterial viability and CFU counts were calculated by plating serial dilutions on nutrient agar (NA) plates supplemented with 2 mM Cd and Pb. After comparing the content of Cd and Pb in the shoots with standards for

Figure 2. The effect of bacterial strain Enterobacter cloacae R7 and municipal solid waste (MSW) biochar on the root Pb concentration (A) and the shoot Pb concentration (B) of forage maize plant irrigated with various irrigation water quality after 100 days of cultivation under greenhouse. Data represent the mean ± SD (n = 3) and different letters indicate a significant difference (P < 0.05) using Duncan’s test. For description of treatments, see caption of Figure 1.
the contents of heavy metals (permissible limit of concentration of Cd and Pb in plants for animal feed), finally, the best treatment (or treatments) for irrigation of forage maize with heavy metal–contaminated water (wastewater) was introduced.

2.5. Statistical analysis

The data were subjected to an analysis of variance (three–way ANOVA) using SAS software (version 9.2, SAS, Chicago, USA). Duncan’s Multiple Range Test was applied to analyze the significance of treatment means ± SD (standard deviations) at p < 0.05.

3. Results

3.1. Effect of MSW–biochar and bacterial strain on root and shoot dry weight

Root and shoot dry weight decreased with increasing the level of contamination in irrigation water (Figure 1A and B). The highest root (10.7 g pot⁻¹) and shoot (57.8 g pot⁻¹) dry weights were observed in inoculated plants and irrigated with freshwater (100%FW) without biochar application (B1 x 0%BC). In plants irrigated with freshwater (100%FW) and plants irrigated with 25% contaminated water (75%FW + 25%CFW), the root and shoot dry weights decreased significantly.

Figure 3. The effect of bacterial strain Enterobacter cloacae R7 and municipal solid waste (MSW) biochar on the root Cd concentration (A) and the shoot Cd concentration (B) of forage maize plant irrigated with various irrigation water quality after 100 days of cultivation under greenhouse. Data represent the mean ± SD (n = 3) and different letters indicate a significant difference (P < 0.05) using Duncan’s test. For description of treatments, see caption of Figure 1.
CW), the root and shoot dry weight decreased with augmenting levels of biochar both in the presence and absence of bacterial strain. But, the combined treatments of biochar and bacterial strain showed the greatest effect on root and shoot dry weight with increasing water contamination level (from 50%FW + 50%CW to 100%CW). Overall, based on the results shown in Figure 1, it can be concluded that when the maize plants were subjected to heavy metal stress (from 50%FW + 50%CW to 100%CW), the biochar or biochar with bacterial strain had greater effect on improving plant growth compared to under non-stressed conditions (100%FW) or under less stressed conditions (75%FW + 25%CW).

3.2. Effect of MSW–biochar and bacterial strain on plant Pb and Cd concentration

According to Figures 2 and 3, with increasing levels of contamination (from 100%FW to 100%CW) in irrigation water, the content of Pb and Cd in the plant root and shoot increased. Both *E. cloacae* R7 and biochar treatment led to a significant decrement in Pb and Cd concentration of root and shoot compared to corresponding controls (B0 × 0%BC). At all levels of irrigation water quality (from 100%FW to 100%CW), the lowest Pb and Cd concentrations in root and shoot were recorded at 3% biochar and bacterial strain treatments (B1 × 3%BC), and the highest Pb and Cd contents in root and shoot were registered in non–biochar and non–bacterial treatments (B0 × 0%BC). In the presence and absence of *E. cloacae* R7, the concentration of Pb and Cd of the root and shoot decreased with increasing biochar levels. The percentage of decrease in the Pb and Cd content in root and shoot plants inoculated with *E. cloacae* R7 without biochar (B1 × 0%BC) was lower than those grown on soil amended with biochar without *E. cloacae* R7 (B0 × 1%BC and B0 × 3%BC). In general, the combined application of *E. cloacae* R7 and biochar (1 and 3%) especially at its highest application rate, in comparison with the bacterial strain and biochar alone or controls caused a significant decrement in the Pb concentration of root (32–84% decrease) and shoot (18–78% decrease) and in the Cd concentration of root (21–81% decrease) and shoot (16–71% decrease) of the maize plant, indicating the key role of bacterial biosorbent and biochar in reducing Cd and Pb uptake by forage maize plants.

3.3. Effect of biochar on root colonization by bacterial strain

In order to acknowledge the colonization of the roots of forage maize plant irrigated with various levels of irrigation water quality (from 100%FW to 100%CW) by *E. cloacae* R7, CFU of the strain (bacterial viability) was counted. The population density range of this strain was reported in different treatments between 10^7 and 10^9 CFU g⁻¹. According to Figure 4, the population density of this bacterium increased in the rhizosphere soil as biochar application levels increased. In addition, with increasing concentration of heavy metals in irrigation water (from 100%FW to 100%CW), the bacterial population density in rhizosphere soil decreased. In fact, it can be stated that *E. cloacae* R7 had the favorable ability to colonize the root of maize plants irrigated with different irrigation water quality during plant growth period. In control treatments, no bacterial strain was also able to grow in NA medium containing 2 mM Pb and 2 mM Cd.

Figure 4. The effect of municipal solid waste (MSW) biochar on colony–forming unit (CFU) counts of Enterobacter cloacae R7 in rhizosphere soil of forage maize plant irrigated with various irrigation water quality after 100 days of cultivation under greenhouse. Data represent the mean ± SD (n = 3) and different letters indicate a significant difference (P < 0.05) using Duncan’s test. For description of treatments, see caption of Figure 1.
Cd and Pb concentration of forage maize as well as bacterial root colonization (as a mechanism involved) were characterized. Our results showed that E. cloacae R7, MSW biochar, and their combined use significantly augmented the maize plant root and shoot dry weight and decreased Cd and Pb concentration of the root and shoot of the forage maize plant. In addition, the combined use of MSW biochar and E. cloacae R7 reduced the concentration of Cd and Pb in edible tissue of forage maize irrigated with 75 and 100% contaminated water (CW), which was within the permissible limit for livestock feed. Since previous studies have fully focused on sorption of heavy metals on biochar, impact of biochar on soil traits (i.e., soil pH, and the availability of heavy metals in soil), and mechanisms by which biochars immobilized heavy metal in soil, much of this discussion is on biochar, bacterial strain, and heavy metal interactions, which has received less attention in previous research.

4.1. Effect of MSW–biochar and bacterial strain on plant biomass

In this study, the maize plants irrigated with water with the higher levels of contamination (75 and 100% contaminated water) showed a significant decrease in their growth compared to corresponding controls. In the study of Alaboudi et al. (2019), when the concentration of Pb, Cd and Cr ions augmented in the soil without biochar applications, a severe decrement in root length, plant height, and the dry weights of plant shoot and root were also observed. Based on the result of a previous study (Kabata-Pendias, 2000), diminished shoot dry weight with augmenting content of heavy metals (Cd and Pb) in the presence or absence of biochar in soil is possibly due to the impediment of metabolic activity in plants, resulting in restriction of growth of heavy metal–stressed plant.

According to the results obtained from this study (Figure 1), the effect of MSW biochar and bacterial biosorbent (E. cloacae R7) on root and shoot dry weight varied depending on the quality of irrigation water (from 100%FW to 100% contaminated water). In general, irrigation of forage maize plants with contaminated water (CW) significantly decreased the root and shoot dry weights from 9 to 49% depending on the quality of the used irrigation water. For example, in maize plants irrigated with freshwater (100%FW) and maize plants irrigated with 25% contaminated water (75%FW + 25%CW), MSW biochar had a negative effect on root and shoot dry weight compared to corresponding controls and also showed a positive effect on these growth indices in plants irrigated with 50, 75, and 100% contaminated water over the corresponding controls; although in some treatments this effect (negative or positive) was not significant compared to the corresponding controls (Figure 1A and B). Previous studies have also reported positive and negative impacts of biochar on soil fertility and plant yield (Lehmann and Joseph, 2015; Rees et al., 2015; Sohi et al., 2010; Xu et al., 2016). In a previous study (Al-Wabel et al., 2015), application of various rates of Concarpus biochar (0.0, 1.0, 3.0, and 5.0% w/w) augmented shoot dry biomass of maize plants by 54.5–266%. The addition of biochar (2%, w/w) and crop straws remarkably diminished shoot dry weight in ryegrass grown on a soil contaminated with Cd and Pb (Xu et al., 2016). Following the use of biochar, Zang et al. (2012) not only did not observe an increase in plant yield, but some of the used biochars also reduced plant yield. In a greenhouse study, Rajkovich et al. (2012) showed that high levels of biochar application (7%) decreased maize plant yield, while lower levels of biochar addition (2%) increased the yield of this plant. In the present study, the effect of 1% biochar on the root and shoot dry weight was more than that of 3% biochar in plants irrigated with freshwater (FW) and 25% contaminated water. Novak et al. (2009) reported that biochar application in soil can increase crop yield by 60% or decrease crop yield up to 30% and this increase or decrease of crop depends on soil type. In general, the impact of biochar on plant growth varies depending on the nature of its feedstock, the temperature of pyrolysis, the toxic and volatile compounds produced during pyrolysis, the type of plant and soil, and the duration of application to the soil (Lehmann and Joseph, 2015).

Based on previous studies, the reason for the decrease in plant growth at various biochar levels was attributed to the presence of toxic compounds in biochar, the adsorption of essential nutrients by the biochar, and an increment in pH, which results in a decrement in the accessibility of essential nutrients such as P and micronutrients (Guo et al., 2016; Lehmann and Joseph, 2015). The presence of toxic compounds in the biochar used in this study may not be a reason for decreased biomass of maize plant irrigated with 100%FW and 75%FW + 25%CW compared to the relative controls because the MSW biochar used in this study could increase the biomass of the maize plant irrigated with 50, 75, and 100% contaminated water (CW) compared to corresponding controls.

Competing ions (metal ion selectivity) are one of the most important factors influencing adsorbent efficiency (Awual, 2016; Awual et al., 2018). In previous studies, it has been well proven that the sorption sequence of trace elements on biochar is as Pb > Cu > Cd > Zn (Namgaya et al., 2010). This indicates that when Pb or Cd is not present or is in low concentrations (e.g., plants irrigated with 100%FW and 25% contaminated water), the adsorption sites of biochar are occupied by the essential nutrients for the plant such as Cu and Zn, but in other treatments (i.e., plants irrigated with 50, 75, and 100% contaminated water), Pb and Cd are first adsorbed and prevent the adsorption of essential plant nutrients (i.e., prevention of forming less soluble Zn/Ca–biochar complexes unavailable to the plants). This may be the reason for the positive impact of biochar on root and shoot dry weight in maize plants irrigated with 50, 75, and 100% contaminated water. Based on previous laboratory studies (Jiang et al., 2012; Trakal et al., 2011; Xu et al., 2013), Pb consistently also outcompetes Zn for sites on biochar in both single and poly–metal systems.

In plants irrigated with 50, 75, and 100% contaminated water (CW), the MSW biochar treatments with bacterial strain resulted in greater root and shoot dry weight than biochar treatments without bacterial strain. It has been reported that biochar addition boosts tolerance of plants to various stresses including metal toxicity through adsorption of heavy metals and decrease in uptake of these metals by the plant (Kammann et al., 2015; Schmidt et al., 2015; Wang et al., 2018b). In a previous study, improved heavy metal–stressed plant growth by biochar was reported (Seneviratne et al., 2017). It is known that MSW biochar serves as an advantage for the soils that affirmed both chemically and biologically (Gunarathne et al., 2019; Jayawardhana et al., 2018). Ayiania et al. (2019) and Randolph et al. (2017) attributed an increase in crop yield caused by soil added MSW biochar to improved pH of the soil and the CEC and to increased micro and–macro nutrients and ability of the biochar to act as a carbon sink through maintaining the carbon content in the soil.

In some treatments, although the MSW biochar increased maize plant root and shoot dry weight in comparison to corresponding controls; this effect was not statistically significant (Figure 1). Similar observation was reported by Namgaya et al. (2010). These researchers did also not observe any considerable impact on the dry biomass of maize plants grown on soil polluted with heavy metals (As, Cd, Cu, Pb, and Zn) and amended with biochar (0, 5, and 15 g/kg), even at the highest biochar application rate. In the present study, the absence of significant impact of biochar on the dry biomass of maize plant is also consistent with the findings of a study by Hartley et al. (2009), where biochar application rate did not indicate any significant impact on the dry biomass of Silvgrass (Miscanthus).

Since the deficiencies of the nutrients of soil used in this study received sufficient contents of essential nutrients by basal N and P fertilizers in all treatments and probably the maize plant irrigated with the lower percentages of contaminated water (25 and 50%CW) was not under heavy metal (Cd and Pb) stress, a significant positive response in root and shoot dry weight via biochar application compared with the treatments non–amended with biochar (control) was not expected. In addition, there is a possibility that higher levels of MSW biochar application may affect plant dry matter yield. For instance, in the study of Rondon et al. (2007) and Alaboudi et al. (2019), biochar application rates of 6, 5, 9, and 10% could increase the dry matter yield of maize plant.
grown on soil contaminated with Pb, Cd, and Cr. These researchers attributed this effect to augmented CEC and water-holding capacity. But, in the present study, levels of MSW biochar application were 1 and 3%.

In this study, the maize plants just inoculated with *E. cloacae* R7 had greater root and shoot dry weight than un inoculated plants but grown on the soil treated with MSW biochar. In previous studies, the reason for increased growth of heavy metal-stressed plants inoculated with heavy metal-resistant bacteria has been attributed to the ability of these bacteria to reduce the toxic effects of metals (Etesami, 2018; Sessitsch et al., 2013). It has been reported that rhizosphere bacteria can enhance plant growth through (i) producing plant hormones such as auxin (e.g., IAA increases protection to plants from external stress), (ii) increasing nutrient availability, (iii) facilitating nutrient uptake, and (iv) reducing heavy metal toxicity in heavy metal-stressed plants. In this study, bacterial strain alone had a positive effect on maize growth indices. In fact, under heavy metal stress, heavy metal-resistant rhizosphere bacteria can reduce plant heavy metal uptake, reduce “stress ethylene” levels due to heavy metal toxicity, promote better plant establishment, enhance plant root system growth, and help increase plant growth (Etesami, 2018; Ma et al., 2016c; Sessitsch et al., 2013).

The *E. cloacae* R7 used in this study possesses multiple plant growth-promoting (PGP) traits such as insoluble phosphate dissolution and the ability to generate siderophore, IAA, and ACC deaminase, which may be responsible for the protection of the *E. cloacae* R7-inoculated forage maize plants against Cd and Pb toxicity and the promotion of growth of heavy metal-stressed plant. In the study of Wang et al. (2018b), a heavy metal heavy resistant bacterial strain with multiple PGP traits, *Neurospora crassa* R7, increased growth of maize plants in the presence or absence of Cd augmented root length of *Brassica juncea* L. Czern (Indian mustard) seedlings in a Cd-contaminated soil. In another study, promoted plant growth, decreased Ni and Cd accessibility in soil, and reduced their concentration in roots and shoots of *Lycopersicon esculentum* L. inoculated with plant growth-promoting bacterial strains *Burkholderia* sp. CBMB40 and *Magnaporthe oryzae* CBMB20 were observed (Madhaiyan et al., 2007).

In this study, except for the maize plants irrigated with 100%FW and 75%FW + 25%CW, combined use of *E. cloacae* R7 and MSW biochar at higher levels of contamination of irrigation water (50, 75, and 100%CW) considerably augmented root and shoot dry weight of forage maize plant compared to corresponding controls (Figure 1). This increase in growth indices was attributed to the biochar effect on soil microbial activity (*E. cloacae* R7). Analogous observations were reported by Hale et al. (2015), where use of biochar and *E. cloacae* strain UWS considerably augmented root and shoot dry weight and root system development of plant. The results of various studies show that biochar application, depending on the biochar properties, causes changes in soil microbial community (Egamberdieva et al., 2018; Palansooriya et al., 2019b). Recently, promoted plant growth and nutrient uptake by biochar-based inoculants have been reported (Egamberdieva et al., 2017; Gódowska et al., 2017; Tripti et al., 2017). Watzinger et al. (2014) stated that biochar addition increased microbial biomass and changed microbial community. Increased microbial biomass in their research was attributed to increased available P and K concentrations, increased soil water retention capacity, boosted soil CEC, improved soil nutritional and physical conditions, and increased ability of bacteria to adapt to environmental changes. In another study, the plants inoculated with *Bacillus* sp. and grown on soil amended with biochar indicated an increment in the root and shoot biomass compared to control plants. In addition, the bacterial population was higher in the rhizosphere of these plants (Saxena et al., 2013). Augmented plant growth and yield and uptake of nutrients (i.e., N, P, and K) of maize plants by combined application of biochar and *Azospirillum* inoculants were also reported by Saranya et al. (2011). The study of Sun et al. (2016) also reported increased population of *Pseudomonas putida*, which effectively colonized the rhizosphere of plants and increased plant biomass, following application of biochar produced at 600 °C.

### 4.2. Effect of MSW-biochar and bacterial strain on plant Pb and Cd concentration

Irrigating forage maize plants with Pb and Cd contaminated water also significantly resulted in augmented content of these heavy metals in plant root and shoot compared to corresponding controls. In this study, the absorption of Pb and Cd by maize plants was different. In the study of the impact of industrial wastewater irrigation on the amount of heavy metals in maize and rapsseeded plants, Khurana and Aulakh (2010) found that the amount of Cd accumulated in maize and rapsseeded irrigated with effluent was twice as high as that of control plants. In this study, the content of Pb and Cd in maize plant roots was also higher than that of these metals in maize shoots. Similar observations were also reported by Alaboudi et al. (2019) and Xu et al. (2016) in maize plants grown on soil contaminated with Pb, Cd, and Cr. In this assay, only the concentration of Pb and Cd in shoot of forage maize plants irrigated with freshwater and 25 and 50% contaminated water was below the permissible limits for livestock feed (Figures 2B and 3B), 10 mg kg⁻¹ and 1 mg kg⁻¹ for Pb and Cd metals, respectively (European Commission Directive, 2002/32/EC), both in the presence and absence of *E. cloacae* R7 and MSW biochar. The results showed that application of bacterial strain *E. cloacae* R7 and MSW biochar at different levels of irrigation water quality (0, 25, 50, 75% and 100% contaminated water) had a significant impact on reducing heavy metal absorption. In this study, the exogenous introduction of *E. cloacae* R7 and MSW biochar (the combined application of bacterial strain and biochar) was observed to induce growth of forage maize plant significantly (*P < 0.05*) while diminishing Pb and Cd concentration in root and shoot of the forage maize plant and inhibiting translocation of these heavy metals in the plant root and shoot compared to respective controls. The biochar and bacteria-induced deterrence of Cd and Pb translocation in forage maize plant was acknowledged from the translocation factor of the corresponding heavy metals (data not shown). The concentration of Pb and Cd of the shoot of forage maize plants irrigated with 75% and 100% contaminated water in the treatments without bacterial inoculation and biochar exceeded the permissible limits for livestock feed (Figures 2B and 3B). But, combined use of *E. cloacae* R7 and MSW biochar could reduce the concentration of these two heavy metals, which was below the permissible limits for livestock feed.

In this study, in the absence of bacterial biosorbent, applying MSW biochar significantly also reduced root and shoot Pb concentration (18–84% decrease) and root and shoot Cd concentration (16–81% decrease) in forage maize plants in response to augmenting application rates (0, 1, and 3% w/w). In a antecedent study (Al-Wabel et al., 2015), biochar addition (at rates of 0.0, 1.0, 3.0, and 5.0% w/w) also diminished the content of Cd in the maize plants grown on soil contaminated with heavy metals by up to 47.2–53.2%. In another previous study, biochar produced from agriculture residues diminished Pb and Cd toxicity via immobilization of them into more fixed forms and decreased heavy metals absorption by maize plant growing on soils contaminated with Pb, Cd, and Cr (Alaboudi et al., 2019). Bian et al. (2013) reported a decrement in Cd concentration in rice grains ranging from 20 to 90 percent owing to the use of biochar to a soil contaminated with Cd. In another study, the content of Cd in maize shoots was also diminished by 50.9% with the bamboo biochar (Xu et al., 2016). In the study of Namgay et al. (2010), biochar addition could decrease the content of As, Cd, and Cu (0, 10, and 50 mg/kg) in maize shoots, specifically at the highest application rate of heavy metals.

According to an antecedent report (Al-Wabel et al., 2015), a decrease in metal availability in soil by multiple mechanisms like sorption, ion exchange, precipitation, complex formation, and co-precipitation, and an increment in soil pH are some of the major reasons for biochar-induced decrease in metal concentration in the plants grown on...
heavy metal–polluted soil. In the present study, the application of MSW–biochar to soil augmented soil pH by 0.18–0.92 unit compared with pH 7.78 of the non–amended soil and significantly reduced ammonium acetate–or ammonium bicarbonate–DTPA extractable Cd and Pb concentrations of soils, indicating metal immobility (data not shown), which caused a decrease in concentration of root and shoot Pb and Cd. Due to its low organic matter content and as a result low buffering capacity, the used soil could not reduce the liming performance of the supplemented biochar. In previous studies, at the low pH, due to the presence of H⁺ ions competing with the metal ions for the adsorption sites of the adsorbents, the adsorption of heavy metals (e.g., Cd and Pb) was also low (Awwal, 2016, 2019a; Awwal et al., 2018). For example, with boosting soil pH, formation of insoluble Cd species such as Cd(OH)₂ and Cd (OH)⁺ increases (Awwal et al., 2018). In a previous study (Awwal et al., 2018), when augmenting pH, the protonation reaction becomes weak and the Cd²⁺ ions interplay with the functional groups on the surface of the nanocomposite adsorbent becomes dominant, and then the Cd(II) ions adsorption was boosted.

According to antecedent studies, the biochar application–mediated increment of soil pH is owing to the pyrolysis temperature of biochar, ash increment, solubilization of hydroxides and carbonates present in biochar, the liberation of basic cations into the soil, the alkaline nature of biochar, and biochar surface basicity (Beheshti et al., 2017; Lehmann and Joseph, 2015; Singh et al., 2010b). In this context, the MSW–biochar used in this study is classified as alkaline biochar (pH of 9.04).

Decreased content of heavy metals in forage maize shoots in biochar–treated soil can be ascribed to the immobilized accessible metals and dilution impact as a result of augmenting plant biomass (Al-Wabel et al., 2015; Park et al., 2011). In addition, it has been found that a significant amount of Cd and Pb in soils treated with biochar can be bound to organic matter/biochar via non–exchangeable specific adsorption (the adsorption and complex formation of Cd and Pb on biochar), specifically at high pH, which is neither extractable by DTPA nor available to heavy metal–stressed plants (Alaboudi et al., 2019; Liu et al., 2009; Namgaya et al., 2010). The previous results indicated that augmented rate of added biochar significantly diminished the bioaccessible forms of Pb and Cd (Alaboudi et al., 2019). The reduction in Cd and Pb uptake with MSW–biochar application at various irrigation water quality suggests that the Cd and Pb entered soil was sorbed by MSW–biochar and thus was not accessible to plants. The functional groups of the MSW–biochar used in this study were mainly hydroxyl, carboxylic and phenolic (Table 1). Previous studies also have showed that biochars including these functional groups have been identified as the best material for the adsorption of heavy metals (Ahmad et al., 2014; Hayyat et al., 2016).

In this study, concentration of Pb and Cd was lower in plants inoculated with E. cloaca R7 and grown on biochar non–amended soil than that in non–inoculated and biochar–untreated plants. The bacterial strain E. cloaca R7 used in this study was capable of not only diminishing the content of Cd and Pb in root and shoot forage maize plants but also significantly augmenting the root and shoot dry weight of this plant in the presence or absence of those heavy metals. Similar observation was reported by Mondal et al. (2019). In our antecedent study (Abedinzadeh et al., 2019), E. cloaca R7 was able to remove 88.95% Pb and 58% Cd from the liquid medium contaminated to these metals. In another study, Banerjee et al. (2015) also reported the bioaccumulation capacity of the bacterial strain E. cloaca B1 to be 64.17% and 95.25 % for Cd and Pb, respectively.

Augmented immobilization of heavy metals in soil (Bolan et al., 2014; Fauzia et al., 2017), decreased translocation of the heavy metals from soil to plant, and therewith reduced phyto–accumulation of the heavy metals by some bacteria have been reported in several antecedent studies (Biswas et al., 2018; Etsami, 2018; Han et al., 2018). For example, heavy metal–resistant Bacillus sp. KUM2 diminished the content of As, Cd, Cu, and Ni in lentil plant (Lens culinaris) by immobilizing these metals in the rhizosphere soils (Mondal et al., 2019). Cadmium–resistant B. megaterium H3 boosted immobilization of Cd and diminished metal bioaccessibility, absorption and translocation in rice plant, therewith attenuating metal toxicity (Li et al., 2017b), whereas B. thuringiensis X30 diminished Cd and Pb concentration in radish by immobilizing Cd and Pb in the rhizosphere soils (Han et al., 2018). In the study of Wang et al. (2018b), Neorhizobium huautlense strain T1–17 significantly decreased the concentration of Cd (22.2%) and Pb (59%) in the shoot of Brassica rapa, subspecies pekinesis (Chinese cabbages) compared with relative controls. In a recent study, heavy metal–resistant bacteria–mediated immobilization has been attributed to generate extracellular polymeric substances which can impressively chelate the ions of heavy metals (Biswas et al., 2018). Heavy metal–accumulating bacteria, due to producing metabolites having adhesives traits (e.g., organic acids, siderophores, humic and fulvic acids, alcohols, and polysaccharides) and anionic groups of peptidoglycan component of the bacterial cell and some functional groups (e.g., salto- nate, sulphydryl, carboxylyl, hydroxyle, amine and amide groups) binding with ions of heavy metals, can entrap heavy metals and their sulphinides and oxides (Wu et al., 2010) and can also diminish metal toxicity and hinder heavy metal content by plants (Ma et al., 2011).

The content of Cd and Pb in the forage maize plants inoculated with E. cloaca R7 and grown on MSW biochar non–amended soil was higher than that in the forage maize plants non–inoculated with E. cloaca R7 and grown on MSW biochar–amended soil. In addition to diminishing heavy metal uptake by plants, some bacteria also increase the uptake of metals by the plants. For example, Rahamanian et al. (2011), by examining the growth of some plants in Cd–contaminated soils and in the presence of resistant microorganisms, showed that these microorganisms increased absorption of Cd by the plants by increasing available forms of Cd for the plant. Inoculation with Neorhizobium huautlense strain T1–17 also significantly augmented the root Cd content (29%) of Chinese cabbages (Wang et al., 2018b).

4.3. Effect of MSW–biochar on bacterial colonization of forage maize plant

In this study, the lowest concentration of Pb and Cd was observed in root and shoot of forage maize plants inoculated with bacterial strain and grown on soil amended with MSW biochar, showing the synergistic effects of E. cloaca R7 and MSW–biochar on increased maize plant biomass and diminished attainable Cd and Pb content in the soil (data not shown) and the absorption of these metals by forage maize. In the study of Wang et al. (2018b), in combined treatment of biochar + Neorhizobium huautlense strain T1–17, the Cd concentration of the vegetables edible tissues was considerably reduced (ranging from 46% to 80% for Brassica rapa subsp. pekinesis and from 55% to 64% for Raphanus sativus) in comparison to the plants non–inoculated with bacterial strain (controls).

Environments under various stresses such as heavy metals stress force bacteria to be adopted many different types of metabolic strategies and diverse degree of resistance/tolerance (Gillian et al., 2015). The bacterial strain (E. cloaca R7) used in this study successfully colonized the rhizoplane/rhizosphere of forage maize plants grown under either control (plants irrigated with freshwater) or heavy metal–contaminated soils (plants irrigated with Cd and Pb contaminated water), reflecting the potential of E. cloaca R7s as an efficient bio–inoculant in bioremediation. In our previous study, E. cloaca R7 also indicated the highest population size on the root of forage maize plants under Pb and Cd stressed and non–stressed conditions (Abedinzadeh et al., 2019). Effective root colonization by this bacterial genus in the plants grown on heavy metal–polluted soil has been also stated (Sayed et al., 2015). In a former study (Mondal et al., 2019), an allochthonous wastewater bacterium (Bacillus sp. KUM2) could also successfully colonize the rhizosphere of plants (~5–6 log CFU g⁻¹ soil) grown on soil polluted with heavy metals (i.e., As, Cd, Cu, and Ni).

The population density of this bacterial strain (CFU g⁻¹) in the rhizosphere soil increased with augmenting MSW biochar application levels and decreased with boosting heavy metal content in irrigation water (from 100%FW to 100%CW). It is well known that abiotic stresses including heavy metal toxicity have a considerable adverse impact on
bacterial survival and proliferation and their interplay with plants (Etesami, 2018; Etesami and Maheshwari, 2018; Romdhane et al., 2009). The population density of this bacterial strain in the rhizosphere of the maize plants grown on soil treated with MSW biochar was higher than that in the rhizosphere of the corresponding control maize plants. It is well known that biochar addition to soils influences plant performance and soil quality by changing soil physicochemical traits and stimulating the activities of soil microorganisms (Lehmann et al., 2011). Depending on the physicochemical attributes of biochars (e.g., pH, nutrient content, solubilized organic C content, specific surface area, C:N (carbon–to–nitrogen) ratio, water-holding capacity, pore size, pore volume, etc.), they can provide appropriate habitats for microorganisms (Egamberdieva et al., 2018; Palansooriya et al., 2019b) and also improve plant–microbe interactions. It has been reported that the highly porous nature of biochars and their high surface area are conducive habitats for soil bacteria and support proliferation of the bacteria via boosted aeration and desired micro/macronutrient (e.g., N, P, K, Zn, Cu, Fe, and Mn) supply for growth, as well as guarding against disparate biotic and abiotic stresses (e.g., heavy metal, adverse pH, high salinity, drought, pathogens, etc.), and provide a greater access for better colonization of microorganisms (Egamberdieva et al., 2018; Jaafar et al., 2015; Luo et al., 2017; Ye et al., 2017). Bacteria living inside pores may get better preserved from external stressful factors like heavy metals toxicity in contaminated soil (Chen et al., 2013). Due to having the properties that support microbial life, biochars have been suggested as a carrier for microbial inoculants including bacteria that supported a high survival rate of inoculated bacteria and obviously augmented colonization of the rhizosphere/rhizoplane of the inoculated plant even in hostile environments (Egamberdieva et al., 2018).

The survival of introduced bacteria in soil and the rhizosphere/rhizoplane and their ability to colonize roots are critical for their advantageous impact on plant development and are also an essential condition for PGPB to be considered as a true plant growth–boosting bacterium (Egamberdieva and Kucharrova, 2009; Silva et al., 2003). According to Figure 4, it appears that the properties of the MSW–biochar used in this study were suitable for the growth and activity of E. cloacae R7 (e.g., increase in CFU g⁻¹ and decrease in heavy metal concentration in maize plants) and could support a high proliferation and survival rate of introduced E. cloacae R7.

In the study of Hale et al. (2015), pinewood biochar also increased the survival of plant growth–boosting E. cloacae strain UW5 and sustained higher population density of the bacterial strain. In foregone studies (Lehmann et al., 2011; Palansooriya et al., 2019a), the highly porous structure of biochar makes provision habitats for bacteria to colonize, allowing their growth in the environment of stressed soil. It has been stated that biochar can also improve the biological activity in contaminated soils by altering soil pH and reducing the heavy–metal bioaccessibility (Palansooriya et al., 2019b; Prayogo et al., 2014). For instance, in a study (Nie et al., 2018), use of biochar at a rate of three tones per hectare augmented the population size of bacteria of soil contaminated with Cu, Cd, and Pb by 2.8 times in comparison to the control.

In general, the mode of action of biochars resulting in stimulating bacterial activities and population density is complex (e.g., direct/indirect interplays among biological and non–biological factors and processes in the soil) and is affected by the nature of biochar used (e.g., biochar feedstock, pyrolysis conditions, and modification technique), biochar application rates, soil type, and experimental conditions (Palansooriya et al., 2019b; Zhu et al., 2017). In addition, the amount of heavy metal accumulating bacterial strains depend on several factors, including physical, chemical, and biological characteristics of contaminated soil, bacterial assemblage, the plant species, nature and concentration of heavy metals, and rhizospheric microenvironment (Mallick et al., 2014; Ndeddy Aka and Babalola, 2016). According to results of this study, the inoculation of forage maize plants irrigated with Cd and Pb–contaminated water containing Cd and Pb as high as in the natural wastewater with exogenous introduction of allochthonous E. cloacae R7 along with MSW biochar was proved to be effective to diminish the concentration of Cd and Pb in the shoot (edible part) of the forage maize plants, which was within the permissible limits for livestock feed, which guarantees food safety from animal health viewpoint.

5. Conclusions

According to the results of the research, the biomass of forage maize plants irrigated with Cd and Pb–contaminated water containing Cd and Pb as high as in the natural wastewater was significantly reduced. However, with the application of municipal solid waste (MSW) biochar to soil, especially in 75 and 100% contaminated water (CW), the biomass of the maize plant inoculated and non–inoculated with E. cloacae R7 was increased. Irrigating forage maize plant with Pb and Cd contaminated water (75 and 100%CW) also resulted in increased concentration of these heavy metals in plant shoots, which was above the permissible limits for livestock feed. But the concentrations of these metals in the shoots of maize plants irrigated with 25 and 50% water contaminated with Pb and Cd were below the permitted limits for livestock feed. In this study, combined application of biochar (3%) and bacterial strain E. cloacae R7, as a viable, inexpensive, and feasible technique, had a synergetic effect on diminishing the heavy metal uptake by forage maize plant, averting human/animal health risk.

Since the use of industrial and municipal wastewater in agriculture due to water scarcity and drought has become common in recent years, it is suggested using heavy metal–contaminated water either in combination with freshwater (50 or 75%freshwater) or in combination with biochar and bacterial biosorbent. The potential of the MSW biochar and E. cloacae R7 in reducing heavy metal accumulation in waste–water–irrigated forage maize plants should be assayed in field–scale experiments in the future. A further understanding of the interaction between allochthonous E. cloacae R7 and MSW biochar is indispensable for the development of efficacious and safe production of forage plants such as forage maize plant. This may therefore provide new potent heavy metal–accumulating bacterial strains plus immobilizers for increasing the biomass production (edible tissues) and diminishing the absorption of heavy metals by forage plants irrigated with wastewater.

Declarations

**Author contribution statement**

M. Abedinzadeh: Performed the experiments.
H. Etesami: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
H. A. Alikhan: Analyzed and interpreted the data.
S. Shafiei: Contributed reagents, materials, analysis tools or data.

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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