



(Original Article)

Application Effect of Biochar, Activated Carbon and Microorganisms on Reducing the Phytotoxicity of Cd and Pb

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DOI: 10.21608/AJAS.2023.238008.1296

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Abstract

The objective of the current study was to isolate acid production bacteria (APB) from sewage wastewater-irrigated soil. The effect of Cd and Pb at levels of 0-1000 ppm with or without active carbon (AC) or biochar (BC) on the germination of corn seeds (*Zea mays* L.), and seedlings fresh and dry weight were also examined in germination experiment. In addition, a greenhouse pot experiment was conducted to study the effects of BC, AC, and bacillus (BA) on plant growth of *Zea mays* in heavy metal contaminated soil. The results showed that the solubilization index for bacterial isolates of APB-1, APB-2, APB-3 and APB-4 was 2.50, 1.62, 2.22, and 3.14, respectively. It was observed that the growth parameters of seedlings decreased with increasing doses of Cd or Pb, showing negative effects of these metals on seeds germination, seedling length, and fresh and dry matter of seedlings. The toxicity effect of Cd appeared to be greater than Pb on the seedling's growth. When applying AC or BC, however, the toxicity effect of toxic metals was reduced, which improved seedlings growth. In the greenhouse pot experiment, the application of AC, BC, or BA significantly increased the plant height and dry matter of maize shoots. It could be concluded that applying pyrolytic carbon-based materials or microbial inoculation enhanced plant growth and it may be due to improving soil quality and/or reducing heavy metals toxicity.

Keywords: Heavy metals, microbial inoculation, wastewater, biochar.

Introduction

Along with industrial practices, which are recognized to be the most widely utilized heavy metals and the most pervasive environmental contaminants, toxic heavy metal pollution is becoming a global problem (Rai *et al.*, 1981). People have been extracting lead metal and salts from the earth and using them for thousands of years, causing a continuous environmental accumulation and exposure of organisms and ecosystems (Ruden *et al.*, 2019). All developed countries in the globe that have industrialized rely heavily on lead (Pb). Through activities including extraction, smelting, engineering, waste dumping, and recovery, significant amounts are released into the environment. Leaded fuel burning has recently resulted in enormous amounts of lead and its compounds being emitted into the atmosphere (Byrd *et al.*, 1983).

The heavy metals cause the bacteria to respond in several ways, such as transport across the cell membrane, biosorption to the cell walls, trapping in extracellular capsules, precipitation, complexation, and oxidation-reduction reactions. In recent years, there has been a lot of interest in the use of microorganisms in the bioremediation of heavy metals. Organic acids are produced by a vast variety of microorganisms, including bacteria such *Arthrobacter paraffinens*, *Bacillus* sp., and *Corynebacterium* sp. (Lopez-Garcia, 2002). Citric, gluconic, itaconic, lactic, oxalic, fumaric, and malic acid are some of the organic acids that different microorganisms can create. The microbial fermentation of carbohydrates and related substrates results in the formation of these acids (Shaikh and Qureshi, 2013).

High cadmium concentrations caused heavy metal poisoning symptoms, including shorter roots, shoots, and leaves. Bleaching also changes the ultrastructure of chloroplasts and decreases photosynthetic performance. There were some symptoms at 100 μM Cd, but only 250 μM Cd was shown to have significant metal toxicity (Rascio *et al.*, 1993). Increased Cd levels prevent photosynthesis because they prevent nutrient intake, cell proliferation, plant growth, and seed germination, all of which result in slower growth and production. The accumulation of Cd disturbs the Calvin cycle enzymes, photosynthesis, and carbohydrate metabolism (Noor *et al.*, 2018).

On the other hand, biochar from various biomass sources has drawn enormous research attention, mainly due to sequestering carbon (Sohi *et al.* 2010). Biochar is created from pyrolysis of organic wastes, and it can be as nutrient-rich fertilizer (Borges *et al.* 2020). According to recent studies (Enaime, and Lübken, 2021), biochars are efficient soil additives because they increase the agronomic qualities of soil. In addition, biochar has drawn a lot of interest as a viable and long-lasting soil amendment that can lessen the toxicity of heavy metals in the soil and enhance plant growth that is stressed by heavy metals (Khan *et al.*, 2022). Utilizing biochar improves soil quality, reduces pollutant movement, and increases carbon storage (Yuan *et al.*, 2019). Therefore, in the current study, it is important to reduce the toxicity of heavy metals especially Pb and Cd using immobilizing agents such as biochar. The use of an inexpensive and effective adsorption method based on biomass activated carbon and biochar to remediate heavy metal pollution is clearly desirable for economically disadvantaged developing countries (Hoang *et al.*, 2022). In this context, biochar and activated carbon might also function as a low-cost sorbent potentially immobilizing and reducing the toxicity of potentially toxic pollutants because of their porous nature and high specific surface area. Additionally, several mechanisms, including ionic exchange, complexation, electrostatic force attraction, and precipitation, could be by biochar in contaminated soil to lessen the toxicity of heavy metals (Inyang *et al.*, 2016; Guo *et al.*, 2023). Therefore, the objective of this study is to isolate and characterize acid production bacteria (APB), being resistant to Pb from sewage wastewater irrigated soil. Reducing the toxic effect of Cd and Pb using biochar, and active carbon was also investigated. In addition, a greenhouse pot experiment was

conducted to study the effects of biochar, activated carbon, and bacillus (BA) on plant growth of *Zea mays* in heavy metal contaminated soil.

Materials and Methods

Five Cd and Pb concentrations (0, 1, 10, 100 and 1000 ppm) with and without the application of active carbon (AC) or biochar (BC) were used in a germination experiment to test their effect on corn seeds germination and seedlings growth. Furthermore, four bacterial isolates were isolated from Arab El-Madabegh, Assiut, Egypt, where the soil was irrigated with sewage wastewater for more than 40 years. A pot experiment with contaminated soil was then carried out with treatments of biochar or activated carbon either alone or in combination with Bacillace (BA) for investigating the effect of these treatments on plant growth (plant height and dry matter) of *Zea mays*.

Germination Experiment

In the laboratory of soil and water department, at Assiut University, a germination experiment (in petri dishes) was conducted to examine the toxic effect of high Cd and Pb concentrations with and without biochar and activated carbon on the germination and seedlings growth of *Zea mays*. In this investigation, commercially manufactured biochar (BC) and activated carbon (AC) were used as adsorbents. Stock solutions of 1000 ppm Cd and Pb were prepared using Cd (NO₃)₂.4H₂O and Pb (NO₃)₂, respectively. Five concentrations of each element (0, 1, 10, 100 and 1000 ppm) were prepared to be used alone or in combination with 0.5 g active carbon (AC) or biochar (BC). After washing the seeds with distilled water, ten seeds were put in each petri dish filled with filter paper. Each treatment was replicated 2 times with a total of 30 dishes. The seeds of *Zea mays* were irrigated with a suitable amount of the prepared solution for each treatment with the consideration not to let the seed over dry or over irrigated. After 12 days, the germination percentage was calculated and seedlings length, fresh and dry weight.

Isolation of Acid Production Bacteria (APB) from Sewage Wastewater Irrigated Soil

Soil samples were collected from Arab El-Madabegh, Assiut, Egypt, where the soil was irrigated with sewage water for more than 40 years. The used soil samples had 77.2% sand, 6.0% silt, and 16.8% clay. The soil pH in 1:2.5 suspension, and EC in 1:2.5 extracts were 7.04 and 1.62 dS/m, respectively. For total content of heavy metals, soil Cd accounted for 3 mg/kg and soil Pb accounted for 68 mg/kg. Picovskaya's agar media (Rao and Sinha, 1963), was used for isolation of acid production bacteria (APB) from soil samples, had the following composition: containing of 2.5 mg MnSO₄.2H₂O; 5g Ca₃(PO₄)₂; 2.5 mg MgSO₄.7H₂O; 10 g glucose; 2.5 mg FeSO₄.7H₂O; 0.2 g NaCl; 20 g agar; 5 g yeast extract, diluted in 1 L distilled water, pH 7. After incubation for 7 days at 30 °C, colonies indicating halo zones with a large relative diameter were picked and purified on Picovskaya's agar medium for studying their characterizations. The medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 minutes. The medium was poured into sterile petri plates and 0.1 ml of the dilution sample

was spread over the agar plates. The inoculated plates were incubated at $28\pm 2^{\circ}\text{C}$ for 7 days. Colonies on plates which showed clear zone indicating the ability to secrete acids were selected. The diameter of clearance zone around the growing colony was measured, successively after 7 days. The solubilization index (SI) is the ratio of total diameter (colony + halo zone) and the colony diameter (mm) was determined, according to Edi- Premono *et al.* (1996).

Screening of Lead (Pb) Resistant Isolates Bacteria

All the isolates were checked for Pb tolerance. Minimum inhibitory concentration (MIC) was determined by the plate dilution method against respective heavy metals by gradually increasing the concentration of the heavy metals on nutrient agar plates until the strains failed to give colonies on the plate (Singh *et al.*, 2010). MIC is the lowest concentration of Pb which completely inhibits the growth of the isolate. In the preliminary test, each pure isolate after fresh growth for 24 h was streaked on nutrient agar (NA) plate supplemented with increasing concentration of Pb. The initial concentration used was 50 ppm and thereby gradual increasing the concentration each time on NA plates, as $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$. The plates were incubated at 30°C for 48 h and bacterial growth was observed. The growth of cultures on last concentration was transferred to the higher concentration by streaking on the plate. The lowest concentration that prevented bacterial growth was considered the MIC. The highest bacterial isolate for Pb resistant is preliminarily identified by the cell morphological, physiological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Whereas the isolate grown on nutrient agar media (24 hours and 7-day old cultures) were made for determinations of cell morphology, dimensions, Gram reaction, cell motility and colony characteristics. Besides, the isolates were tested for starch hydrolysis test and carbohydrate fermentation.

Greenhouse Pot Experiment

A greenhouse experiment was conducted in pots to study the effects of BC, AC, and BA on the growth of corn plants grown on Arab El-Madabegh contaminated soil irrigated with sewage wastewater. Three kilograms of soil were placed inside each pot. The soil samples were treated with the following treatments: T1: control soil (CK); T2: bacillus (BA); T3: activated carbon (AC); T4: biochar. The application rates of BC and AC were 1% w/w. In addition, 2 mL of bacterial culture of bacillus was added to each pot. Eight seeds of *Zea mays* L. were planted, and, after germination, they were thinned to three plants in each pot. The recommended doses of NPK fertilizers were applied and the plants were irrigated at the field capacity. After 50 days of germination, the plants were harvested. Plant height and the dry weight of the shoots were measured at the end of the experiment.

Statistical analysis

The collected data was statistically analyzed using XLSTAT. LSD (Least significant difference) test at 0.05 level was used to compare the treatment effects. Additionally, \pm SD (standard deviation) has also been reported.

Results and Discussion

Germination Experiment

In comparison to the control (without the addition of Cd or Pb), the applied Pb or Cd had a harmful effect on the seedlings growth parameters (germination rate, and seedlings length, fresh weight, and dry weight). For example, at 1000 ppm Pb, the germination rate decreased from 85% (at zero Pb level) to 75% (at 1000 ppm Pb). In addition, the seedlings length, fresh weight, and dry weight decreased from 16.1 cm, 0.32 g/seedling, and 0.059 g/seedling (at zero level of Pb or Cd) to 2.23 cm, 0.09 g/seedling and 0.025 g/seedling (at 1000 ppm Pb), respectively. But compared to Pb, cadmium revealed more harmful consequences overall most added metal concentrations. At 100 ppm Cd, the germination rate decreased from 85% to 60%. Additionally, the seedlings length, fresh weight and dry weight decreased from 16.1 cm, 0.32 g/seedling, and 0.059 g/seedling (at zero level) to 4.0 cm, 0.12 g/seedling and 0.026 g/seedling (at 100 ppm Cd), respectively. At 1000 ppm, however, Cd exhibited the greatest toxicity and no seeds germinated or growth characteristics were observed. In this context, it has been reported that inhibiting plant growth is mainly due to higher toxicity of heavy metals (Hermans *et al.*, 2011; Gallego *et al.*, 1996; Haider *et al.*, 2021a). According to Gallego *et al.* (2012), cadmium poisoning has an impact on plants by preventing carbon fixation, lowering chlorophyll concentration, and decreasing photosynthetic activity, resulting in plant physiological damage. Furthermore, Pb has a considerable negative impact on plants' metabolic processes, resulting in decreased photosynthetic rates, seedling growth, germination percentage, root/shoot length, and dry mass of plants (Collin *et al.*, 2022).

It was generally observed that the seedlings growth parameters decreased with increasing doses of Cd or Pb applied, showing negative effects of these metals on seedlings fresh and dry matter. The toxicity effect of Cd seemed to be higher than Pb on the plant growth parameters. When applying pyrolytic carbon-based amendments, AC and BC reduced the toxicity impacts of added heavy metals, which improved plant growth characteristics. The use of carbon adsorbents (BC and AC) in contaminated soils has been reported to immobilize available fraction of soil metal, reducing heavy metal accumulation in plant tissues, and reducing the toxic effects of heavy metals (Burachevskaya *et al.*, 2021). The use of biochar or activated carbon in heavy metal-contaminated soil is considered an effective technique for controlling the phytoavailability and phytotoxicity of heavy metals due to its ability to metal immobilization, mainly due to its surface structure, functional groups, specific surface area, microporosity, and metal adsorption (Park *et al.*, 2011; Arabi *et al.*, 2021; Guo *et al.*, 2023). A study by Park *et al.* (2011) showed that biochar application significantly reduced the available concentrations

of Cd, Cu, and Pb in soil, indicating that these metals were immobilized. They also found that biochar application increased the plant dry biomass of shoots and roots by 353% and 572%, respectively. This may be due to decreased metal toxicity and increased availability of nutrients such as phosphorus and potassium.

Table 1. Effect of active carbon and biochar on seeds germination (G), seedling length (SL), fresh (FW) and dry weight (DW) of corn seedlings under spiked Cd and Pb (\pm SD)

Treatments	Growth parameters			
	G, %	SL, cm	FW, g/seedling	DW, g/seedling
zero level of Cd or Pb (CK)	85 \pm 5	16.1 \pm 1.56	0.32 \pm 0.0	0.059 \pm 0.02
<i>With Pb addition (with and without addition of AC or BC)</i>				
1 ppm Pb	85 \pm 15	11.1 \pm 0.63	0.14 \pm 0.01	0.043 \pm 0.01
10 ppm Pb	85 \pm 15	6.75 \pm 0.75	0.15 \pm 0.01	0.054 \pm 0.01
100 ppm Pb	80 \pm 20	3.75 \pm 0.13	0.10 \pm 0.01	0.031 \pm 0.0
1000 ppm Pb	75 \pm 5	2.23 \pm 0.10	0.09 \pm 0.01	0.025 \pm 0.0
1 ppm Pb + AC	80 \pm 20	15.6 \pm 1.13	0.38 \pm 0.07	0.071 \pm 0.01
10 ppm Pb + AC	90 \pm 10	16.6 \pm 0.38	0.34 \pm 0.03	0.085 \pm 0.01
100 ppm Pb + AC	100 \pm 0.0	11.5 \pm 2.75	0.15 \pm 0.01	0.068 \pm 0.01
1000 ppm Pb + AC	80 \pm 20	3.25 \pm 1.08	0.08 \pm 0.02	0.024 \pm 0.01
1 ppm Pb +BC	100 \pm 0.0	10.0 \pm 3.25	0.42 \pm 0.03	0.088 \pm 0.01
10 ppm Pb + BC	90 \pm 10	12.6 \pm 2.88	0.17 \pm 0.02	0.067 \pm 0.01
100 ppm Pb +BC	95 \pm 5	4.63 \pm 0.38	0.08 \pm 0.0	0.037 \pm 0.0
1000 ppm Pb +BC	95 \pm 5	3.75 \pm 0.75	0.09 \pm 0.01	0.039 \pm 0.01
<i>With Cd addition (with and without addition of AC or BC)</i>				
1 ppm Cd	95 \pm 5	11.4 \pm 1.6	0.12 \pm 0.01	0.045 \pm 0.0
10 ppm Cd	100 \pm 0.0	8.75 \pm 0.0	0.19 \pm 0.01	0.059 \pm 0.01
100 ppm Cd	60 \pm 10	4.00 \pm 0.70	0.12 \pm 0.0	0.026 \pm 0.0
1000 ppm Cd	0 \pm 0.0	0 \pm 0.0	0 \pm 0.0	0 \pm 0.0
1 ppm Cd + AC	90 \pm 5	16.5 \pm 0.0	0.30 \pm 0.03	0.081 \pm 0.0
10 ppm Cd + AC	95 \pm 5	7.00 \pm 0.8	0.23 \pm 0.01	0.056 \pm 0.01
100 ppm Cd + AC	80 \pm 0.0	5.63 \pm 0.6	0.21 \pm 0.03	0.046 \pm 0.0
1000 ppm Cd + AC	25 \pm 5	1.63 \pm 0.1	0.10 \pm 0.0	0.018 \pm 0.0
1 ppm Cd +BC	85 \pm 15	16.8 \pm 1.8	0.15 \pm 0.03	0.045 \pm 0.01
10 ppm Cd + BC	65 \pm 5	11.0 \pm 2.3	0.48 \pm 0.02	0.087 \pm 0.0
100 ppm Cd +BC	85 \pm 5	5.88 \pm 0.1	0.20 \pm 0.02	0.043 \pm 0.01
1000 ppm Cd +BC	15 \pm 5	3.00 \pm 0.0	0.18 \pm 0.03	0.011 \pm 0.0

Isolation and Screening of Acid Production Bacteria

Four bacterial isolates were isolated from contaminated soil. They were selected for formation of clear zone around their colonies on Picovskaya[™] s agar media, which indicating the ability to secrete acids (Fig. 1). Table (2) shows the label of the isolated bacterial strains and the solubilization index (SI). The solubilization index of bacterial isolates APB-1, APB-2, APB-3 and APB-4 was 2.50, 1.62, 2.22, and 3.14, respectively. The highest solubilization index was recorded by the strain APB-4 and the lowest by the strain APB-2.

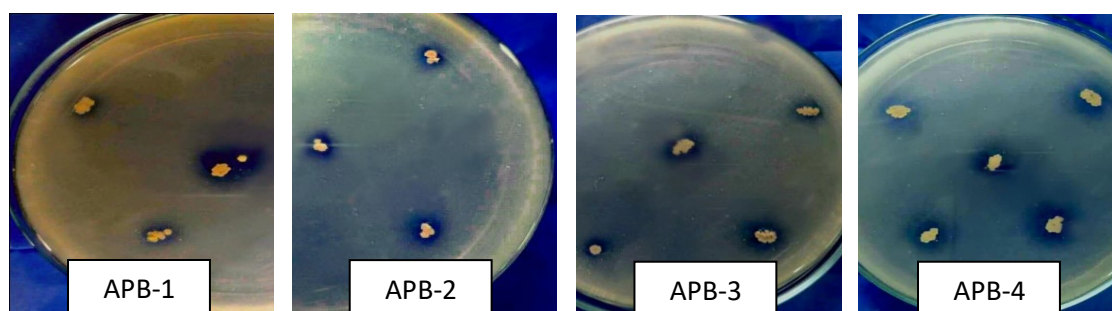


Figure 1. Colonies of isolated bacterial strains grown for 7- days on Pikovskaya's medium showing the clear zone.

Table 2. The solubilization index (SI)* of isolated bacterial strains grown for 7- days on Pikovskaya's medium

Isolate label	Diameter of	Diameter of	Solubilization
APB-1	15	6	2.50
APB-2	13	8	1.62
APB-3	20	9	2.22
APB-4	22	7	3.14

*SI: is the ratio of total diameter (colony + halo zone) and the colony diameter (mm).

Many microorganisms including bacteria such as *Arthrobacter paraffinens*, *Bacillus* sp, *Corynebacterium* sp., produce organic acids (Lopez-Garcia, 2002). The organic acids produced by various microbes are citric, gluconic, itaconic, lactic, oxalic, fumaric, malic acid. These acids are formed through the microbial fermentation of carbohydrates and related substrates (Shaikh and Qureshi, 2013).

Screening of Lead (Pb) Resistant Isolates Bacteria

Four heavy metal resistant isolates were from contaminated soil irrigated with sewage wastewater. The minimum inhibitory concentration (MIC) of bacterial isolates APB-1, APB-2, APB-3 and APB-4 was 250, 100, 200, and 300 ppm of Pb, respectively. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for lead (50 ppm). The isolate APB-4 showed the highest tolerance to Pb (300 ppm), while the isolate APB-2 showed the lowest tolerance (100 ppm).

The microbial resistance to heavy metal is attributed to a variety of detoxifying mechanisms developed by resistant microorganisms such as complexation by xopolysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another (Silver, 1996). Filali *et al.* (1999) studied wastewater bacteria isolates *Psuedomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus* resistant to heavy metals. Similarly, Sharma *et al.* (2000) isolated highly cadmium resistant *Klebsiella* that was found to precipitate significant amount of Cd.

Characterization of Acid Production Isolates

The isolate APB-4 showed highest tolerance to Pb (300µg/ml), is identified by the cell morphological, physiological and biochemical characteristics. Results of the tested morphological, cultural and physiological characteristics of the isolate are shown in Table (3). Colonies and vegetative cells of acid production isolate APB-4 are shown in Figs. 2 and 3. Cell shape of the isolate is rod, ranging from 0.5 to 1 µm in diameter and from 2 to 3 µm in length. On nutrient agar medium, the isolate produced smooth, irregular, convex colonies 1-15 mm in diameter. The isolate APB-4 was able to ferment glucose, sucrose, mannitol and maltose but unable to ferment lactose and fructose sugars. The isolate APB-4 can hydrolyze starch and gelatin. According to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994), considering all the identifying characteristics selected isolate was identified as *Bacillus* sp.



Fig 2. Colonies of acid production isolate APB-4 on nutrient agar medium after 3 days of incubation.



Fig 3. Vegetative cells of the acid production isolate APB-4 from 48 hrs old cultures on nutrient agar medium. (X 4000)

Table 3. Morphology, physiology and cultural characteristics of acid production isolate APB-4

Test	APB-4	Biochemical properties	
Gram reaction	-	Starch hydrolysis	+
Motility	+	Gelatin hydrolysis	+
Pellicle formation	-	Carbon source	
Colony type	Round	Glucose	+
Colony Size mm	1-4 mm	Sucrose	+
Colony shape	Convex	Mannitol	+
Colony edge	Irregular	Lactose	-
Cell morphology	Rod	Fructose	-
Spore formation	+	Maltose	+

Greenhouse Pot Experiment

The results of the greenhouse pot experiment showed that the application of AC, BC, or BA significantly increased the height and dry weight of maize plants (Table 4). The highest value of plant height was recorded with BA treatment (42 cm) followed by BC (36 cm), AC (33 cm), and CK (27 cm). The shoot dry matter significantly increased from 0.78 g/pot for control soil to 1.95 g/pot for BA, 1.77 g/pot for AC, and 2.13 g/pot for BC. This result indicates that the highest value of shoot dry weight was recorded with BC (2.13 g/pot) followed by BA (1.95 g/pot) and AC (1.77 g/pot). According to Bargaz *et al.* (2018), soil microbes are crucial to increasing soil fertility and hence promoting plant growth. As a result, the soil in the current study that had been inoculated with bacillus polymyxa increased the height and dry weights of maize plants. According to Chen *et al.* (2023), the application of biochar and inoculated microorganisms may increase the soil's fertility, which is beneficial to plant development and production. In heavy metal-polluted soils, application of biochar and microorganisms may improve the heavy metals immobilization, controlling heavy metals uptake by plants, and encourage the plant growth (Zhang *et al.*, 2019; Haider *et al.*, 2021b). The effect of applying biochar and microorganisms on stabilizing cadmium (Cd) and enhancing maize development in Cd-contaminated soil has been investigated by Haider *et al.* (2022). They found that the use of BC or microbes dramatically enhanced plant growth and decreased root and shoot Cd.

Table 4. Effect of active carbon, biochar, and bacillus on height and shoot dry weight of corn plants (pot experiment)

Parameters/treatments	CK	BA	AC	BC	LSD (p= 0.05)
Plant height (cm)	27	42	33	36	3.8
Dry weight (g/pot)	0.78	1.95	1.77	2.13	0.27

CK: control soil, BA: bacillus, AC: activated carbon, BC: biochar, least significant differences at p = 0.05.

Conclusion

All the identified characteristics isolates that were isolated from the sewage wastewater irrigated soil were identified as bacillus sp. The isolated APB-4 showed the highest tolerance to Pb (300 ppm), while the isolated APB-2 showed the lowest tolerance (100 ppm). Increasing Pb or Cd decreased the germination percentage, this toxic effect was lowered by the application of AC and BC. The application effect of BC, AC, or BA enhanced plant growth. For future studies, however, co-application effect of biochar and microorganisms on phytoremediation of heavy metals from contaminated soil should be investigated.

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تأثير استخدام الفحم الحيوي والكربون النشط والكائنات الحية الدقيقة في تقليل السمية النباتية للكادميوم والرصاص

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الملخص

كان الهدف من البحث الحالي هو عزل البكتيريا المنتجة للحامض (APB) من التربة المروية بمياه الصرف الصحي. كما تم دراسة تأثير الكادميوم والرصاص عند مستويات (0-1000 جزء في المليون) مع أو بدون الكربون النشط (AC) أو الفحم الحيوي (BC) على إنبات بذور الذرة والوزن الطازج والجاف للنبات في تجربة الإنبات. بالإضافة إلى ذلك، تم إجراء تجربة أصيص لدراسة تأثيرات BC أو AC بمفردها أو بالإضافة المشتركة مع بكتريا (*Bacillus*, BA) على نمو نبات الذرة في التربة المروية بمياه الصرف الصحي. أظهرت النتائج أن مؤشر الذوبان للعزلات البكتيرية (APB-1 و APB-2 و APB-3 و APB-4) كان 2.50، 1.62، 2.22، و 3.14، على التوالي. في تجربة الانبات، لوحظ بشكل عام أن مؤشرات نمو النبات تنخفض مع زيادة تركيزات الكادميوم أو الرصاص، مما يدل على التأثيرات السلبية لهذه العناصر على المادة الطازجة والجافة في النبات. يبدو أن التأثير السمي للكادميوم أكبر من الرصاص على مؤشرات نمو النبات. في تجربة الاصص، أدى تطبيق (BC أو AC أو BA) إلى زيادة طول النباتات والوزن الجاف لنباتات الذرة بشكل ملحوظ. من هذه الدراسة، يمكن استنتاج أن التلقيح الميكروبي وتطبيق المواد المعتمدة على الكربون والمنحلة حرارياً يعزز من نمو النبات وقد يكون سبب ذلك تقليل امتصاص العناصر الثقيلة وتحسين جودة التربة الملوثة.

الكلمات المفتاحية: العناصر الثقيلة؛ التلقيح الميكروبي؛ مياه الصرف الصحي؛ الفحم الحيوي.