

Physio-biochemical Behavior and Health Effects of Pepper Plants Subjected to Lead Stress and Their Responses to Remediating Agents as Microbial Activity and Phosphorus

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Keywords. *Bacillus* bacteria, bioconcentration factor, carcinogenic risk, daily intake of heavy metals, growth, health risk index, lead, lead accumulation, pepper plant, phosphorus, photosynthetic pigments, quality, tolerance index, water relations, yield quantity

Abstract. This work assessed the alleviating effects of bacteria (*Bacillus subtilis*) and phosphorus as environmentally friendly materials on the cultivation of pepper plants in polluted soil with lead (Pb) in forms of PbSO_4 and $\text{Pb}(\text{NO}_3)_2$ at rates of 0, 1000, 2000 and 3000 $\mu\text{g Pb/g}$ soil. Pot experiments were conducted to study the growth parameters, some physiological factors, biochemical constituents, and yield attributes, as well as the tolerance index (TI), translocation factor (TF), bioconcentration factor (BCF), and health effects [daily intake of heavy metals (DIM), health risk index (HRI), and carcinogenic risk (CR)]. Increasing the Pb concentration of all Pb salt used in soil severely affected the plant vegetative growth parameters. In comparison with other Pb salt forms, $\text{Pb}(\text{NO}_3)_2$ salt had a strong inhibitory impact. Additionally, the photosynthetic pigments in leaves were negatively impacted by all Pb salt forms. The application of Pb in all salt forms led to changes in the leaf water deficit (LWD), osmotic pressure, and membrane integrity and decreased the total water content, relative water content (RWC), transpiration rate, and leaf succulence. Pollution with Pb salts considerably decreased the yield constituents and various chemical properties of pepper, more so in the presence of Pb nitrate than in the presence of Pb sulfate type. A comparison of the concentration of Pb presence of Pb nitrate was greatly increased than the Pb sulfate in the whole plants. The safe limit of 0.3 mg/kg was exceeded by the Pb concentration in pepper fruits (6.3 and 4.3 mg/kg) cultivated in Pb-contaminated soil [with $\text{Pb}(\text{NO}_3)_2$ and PbSO_4 , respectively]. Additionally, Pb sulfate had a greater detrimental effect on Pb uptake in several plant organs than other Pb salt forms. The TI of pepper plants treated with salt types was >60% with PbSO_4 (75.6%), whereas it was <60% with $\text{Pb}(\text{NO}_3)_2$ (35.2%). The BCF values of pepper plants in the polluted Pb soils varied from 0.10 to 0.41, indicating a moderate accumulator plant. At every level of Pb contamination with all Pb salt types, the sequence of Pb TF values was as follows: roots (TFR) > shoots (TFsh) > fruits (TFf), with TF values < 1. When compared with TFR and TFsh, TFs for shoot to fruits (TFf) had the lowest values (range, 0.07–0.22). The DIM, HRI, and CR values of pepper plants revealed that the Pb of fruit of stressed pepper plants is within safe limits. In addition to reducing the detrimental effects of intolerable Pb levels (2000 and 3000 $\mu\text{g Pb/g}$ soil) on the majority of the aforementioned characters, adding *Bacillus* bacteria as a bio-agent and phosphorus as a chemo-agent to Pb-polluted soils also stimulated growth, increased yield, controlled plant water relations, protected photosynthetic pigments, and sharply decreased the Pb accumulation in plant organs. The *Bacillus* bacteria application resulted in some superior characteristics, such as root length, leaf number, leaf length, leaf area, leaf area index, fresh biomass, dry biomass, photosynthetic pigments, quantity yield attributes, reduction Pb accumulation in all plant organs, TI, TFR, TFf, BCF, in health effects trials, whereas phosphorus application improved plant height, leaf width, RWC, LWD, osmotic pressure, total soluble solids, acidity, total carbohydrates, total protein, and TFsh.

One of the most frequent metalloids contaminants in soil is lead (Pb). Additionally, Pb is one of the most dangerous environmental pollutants. The release of heavy metal pollutants into soils and water bodies is dangerous because of their nonbiodegradability (Thayaparan et al. 2013). Furthermore, Pb contributes to approximately 10% of all heavy metal contamination (Collin et al. 2022) resulting from road debris, motor vehicles, stationary fuel, and industry. Additionally, Pb is toxic and can accumulate in plant tissues and agricultural products (Ahmed et al. 2021) and subsequently enter the food chain (Wagner 1993). In the human body, Pb builds as a result of food consumption, and it can harm the kidneys, brain, liver, and nervous system as well as result in disease (Luckey and Venugopal 1977; Ramade and Hodgson 1987). Plant growth, photosynthetic activity, and metabolic processes are all impacted by Pb. Excessive Pb accumulation can impede root growth by as much as 42% (Collin et al. 2022). Additionally, Pb reduces the germination rate, germination index, root/shoot length, tolerance index, and dry mass of roots and shoots, inhibits seed germination, and slows seedling and plant growth (Cimrin et al. 2007; Mishra et al. 2006). Frequently, Pb also has detrimental effects on a number of physiological processes, including chemical characteristics, water interactions, and photosynthesis (Ewais 1997; Malar et al. 2016). The symptoms of Pb toxicity include rapid inhibition of root growth, stunted plant growth, blackening of the root system, and chlorosis. Moreover, Pb impairs the process of photosynthesis, function of enzymes, water balance, and mineral nutrition. Furthermore, Pb alters the hormonal status, disrupts mineral nutrition and water balance, and alters membrane shape and permeability (Ali and Nas 2018). Additionally, Pb can affect the amount and activity of a number of important enzymes, including those involved in the photosynthetic Calvin cycle, nitrogen metabolism, and sugar metabolism (Kumar and Dubey 1999; Stevens et al. 1997). Some researchers have reported that greater levels of Pb in the soil reduced the yield of the majority of crops (Opeolu et al. 2010).

The fruit of the sweet pepper plant, *Capsicum annuum* L., is an affiliate of the Solanaceae family and contains many crucial vitamins and minerals, including calcium, phosphorus (P), potassium, iron, sodium, zinc, and fluorine (Emmanuel-ikpeme et al. 2014). Green peppers are among the vegetables that are thought to protect against lung cancer. The root, shoot, and fruits of sweet peppers may become

contaminated with heavy metals, however. Consuming Pb-contaminated fruits can be harmful or fatal to humans (Akintan et al. 2019).

Because of the increasing heavy metal pollution of soils, it is necessary to search for remediating agents to alleviate the negative consequences of these pollutants and improve soil characteristics. Among metal-tolerant microorganisms, some bacteria, i.e., *Bacillus subtilis*, in particular, are capable of cleaning metal contamination, including Pb (Etesami 2018). This bacteria was found to improve nutrient uptake, facilitate the translocation of nutrients in various plant tissues, improve soil structure, and enhance the synthesis of essential biochemicals such as amino acids and carbohydrates, thereby enhancing plant growth through nitrogen fixation (Aslam et al. 2021; Etesami and Adl 2020). Moreover, *Bacillus* bacteria reduce the adverse effects of heavy metals on plants by modifying the activities of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, and catalase (Jung et al. 2019). *Bacillus* bacteria also help plants to reduce heavy metals by influencing phosphate solubilization, siderophore production, oxidative-reductive reactions, and nutrition uptake (Tiwari and Lata 2018). Additionally, *Bacillus* bacteria help plants produce more vitamins, enzymes, and phytohormones (Khanna and Kaur 2019).

Fouda (2005) concluded that treating the Pb-polluted soil with P, mycorrhizae, and yeast fungi improved plant development and eliminated the toxic effects of Pb in polluted soil. Unfortunately, there has been little information available about the effects of Pb and other remediates on the physiology, biochemistry, and growth of pepper plants, as well as the productivity and potential health risks. Therefore, this study investigated how Pb pollution in the forms of PbSO_4 and $\text{Pb}(\text{NO}_3)_2$ at various rates with or without the use of bacteria (*Bacillus subtilis*) and P in the form of calcium superphosphate affected pepper plant growth, photosynthetic pigments, water relations, yield quantity and quality, Pb tolerance, and Pb accumulation in pepper fruits, as well as its effects on human health.

Materials and Methods

Pot experiments were conducted during two growing seasons at the experimental farm of the Faculty of Agriculture, Shibin El-Kom, Menoufia University, Egypt, located at an altitude of 44 m above sea level (30.33°N latitude and 31.00°E longitude) during 2022 and 2023. This study aimed to evaluate physiological, biochemical, and yield characteristics, growth, tolerance index (TI), translocation factor (TF), bioconcentration factor (BCF), and health effects [daily intake of heavy metals (DIM), health risk index (HRI), and carcinogenic risk (CR)] of pepper plants grown in Pb-polluted soil at rates of 0, 1000, 2000, and 3000 μg Pb/g soil, with or without bacteria (*Bacillus subtilis*) and P for Pb ion removal.

Experimental design and approach. Three of the bottom drainage holes on polyethylene

pots (inner diameter, 30 cm; depth, 30 cm) were covered with sponge to restrict drainage. Each pot contained 8 kg of clay loam soil from the Faculty of Agriculture's experimental farm [electrical conductivity (EC) = $2.6 \text{ dS}\cdot\text{m}^{-1}$; pH = 7.8; soluble salts = 0.17%; Pb = 5.9 μg Pb/g soil]. Pots were divided into two groups, with Pb concentrations of 0, 1000, 2000, and 3000 μg Pb/g soil added to the first group, and Pb sulfate and Pb nitrate added to the second. Each set was split into three groups: one without any supplemental agents; one with *Bacillus subtilis* cultured in nutrient agar medium; and one with P added as calcium superphosphate 15.5% P_2O_5 . Green pepper (*Capsicum annuum* L. cv. California Wonder) was used during this study. In a greenhouse, the seeds were germinated on 5 Mar 2022 and 7 Mar 2023, respectively, using peatmoss media. In the previously mentioned plastic pots, the uniform seedlings of plants were transplanted on 5 Apr 2022 and 7 Apr 2023, with the same treatments. The pots were organized in a complete randomized block design with five replicates of each treatment. A Pb-tolerant isolate of *Bacillus subtilis* was obtained from the Agricultural Microbiology Branch, Agricultural Botany Department, Faculty of Agriculture, Shibin El-Kom, Egypt. For soil application, the bacteria were inoculated in nutrient broth medium for 3 d at 28 to 30 °C and applied to the treated pots surface. Then, P was added as calcium superphosphate (15.5% P_2O_5) at a rate of 4 g/pot as P treatments. Throughout the study period, pots were irrigated with tap water whenever necessary to maintain the soil moisture at approximately 65% of the total water holding capacity of the soil.

Experimental samples. The experiment involved 21 treatments with five replicates per treatment. Plant samples (three plant samples from each replicate) were randomly chosen and carefully obtained at 80 d after transplanting at 7:00 AM for all measurements.

Growth parameters. Plant height (cm) was measured as the distance from the surface of the soil to the top of the plant, root length (cm), internode number per plant, leaf number per plant, leaf length and maximum width (cm), fresh and dry (dried in an electric oven at 70 °C for 72 h) weights of the root, stem, and leaves (g/plant), total leaf area (cm^2 /plant) measured using the disc method of Bremner and Taha (1966), specific leaf area and leaf area ratio (m^2/kg), leaf weight ratio (g/g), and root/shoot ratio. According to Simane et al. (1993), the relative growth rate (mg/g/week) and net assimilation rate ($\text{g/cm}^2/\text{week}$) during the course of 60 to 80 d and the leaf area index were estimated.

Photosynthetic pigments. According to von Wettstein (1957), photosynthetic pigments were extracted using 80% acetone from middle fresh leaves, assessed using a spectrophotometer, and quantified as mg/g dry weight (DW).

Water relations. Calculations were performed using the formula of Kalapos (1994) for total water content (%), relative water content (%), leaf water deficit (%), and sclerophylly degree.

Received for publication 31 Jan 2024. Accepted for publication 20 Feb 2024.

Published online 10 Apr 2024.

Prince Sattam bin Abdulaziz University project number (PSAU/2024R/1445).

The authors declare no conflict of interest.

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Total soluble solids. Using the Abbe refractometer (Portable Hand-Held Refractometer, WINCOM Company LTD, China), the total soluble solids of the cell sap from the fourth upper leaf test plants were determined, and the osmotic pressure values (bar) were computed using specific tables in accordance with the procedure outlined by Gosev (1960). The transpiration rate was determined using the weight method described by Kreeb (1990).

Membrane integrity. For each treatment, 20 pieces of fresh leaves (diameter, 1 cm) were placed in test tubes with distilled water. An CD-4301 Electronic Conductivity Meter (Lutron, Taiwan) was used to test the EC (C1) of the solution after the tubes were maintained at 30 °C for 3 h. The EC of the samples (C2) was measured again after 2 min of boiling. According to the methodology of Yan et al. (1996), the % of electrolyte leakage was estimated using the following formula: membrane integrity% = (C1/C2) × 100. The following formula proposed by Holbrook and Putz (1996) was used to determine the leaf succulence: degree of succulence = FW/DW; where FW is the fresh weight and DW is the dry weight.

Yield components. The following pepper yield characteristics and flower measurements were noted: number of blooms, number of fruits per plant, fruit yield (g/plant), and fruit weight (g). According to the AOAC (1990), the percentage of titratable acidity, total soluble solids, and vitamin C (mg/100 g FW) of fresh pepper fruits were measured. According to the work of Sadasivam and Manickam (1992), the total nitrogen in fruits was assessed, and then the total protein (%) and total carbohydrates (%) were determined.

Total Pb concentration (μg/g DW). Known weights from the root, shoot (stem plus leaves), and fresh fruits from each Pb treatment were dried at 70 °C, and 0.2 g from each dried ground organ was acid-digested using an atomic absorption spectrophotometer according to Allen et al. (1974).

The Pb uptake was determined using the following formula:

$$\text{Pb uptake}(\mu\text{g/plant}) = \text{Pb accumulation}(\mu\text{g/g}) \times \text{dry weight}(\text{g/plant}).$$

Tolerance index. The TI was calculated according to Tablang et al. (2021) as follows:

$$\text{TI}(\%) = (\text{Dry weight of plant in Pb treatment}(\text{g}) / \text{Dry weight of plant in control treatment}(\text{g})) \times 100$$

Bioconcentration factor. The ability of a plant to accumulate a particular metal in relation to its concentration in the soil is determined by the BCF, which is an index. It is an important predictor of the ability of a substance to bio-accumulate. According to Wilson and Pyatt (2007), it was calculated using the following equation:

$$\text{BCF} = \text{Pb concentration in whole plant} / \text{Pb concentration in soil}$$

Translocation factor. The TF was calculated to find the migration of metalloids from

soil to various plant components, as well as from the root to shoot, shoot to leaves, and leaves to fruit. The following formula, which was suggested by Padmavathamma and Li (2007), was used to calculate this factor:

$$\text{TF} = \text{concentration of heavy metals in the shoot} / \text{total heavy metal concentrations in root}$$

Daily intake of heavy metals. The DIM was determined by multiplying the heavy metal concentration in vegetables with conversion factors and the daily intake of vegetables. Then, the average body weight was divided by this value. The DIM value was determined according to the following equation Khan et al. (2009):

$$\text{DIM} = C_{\text{metal}} \times C_{\text{factor}} \times F_{\text{food intake}} / B_{\text{average weight}}$$

where C_{metal} is the concentration of heavy metals in vegetables (mg/kg), C_{factor} is the conversion factor of 0.085, $F_{\text{food intake}}$ is the daily intake of vegetables (kg/d), and $B_{\text{average weight}}$ is the average body weight (kg = 60 kg). The estimated value of the daily intake of pepper is 15 g/d/person.

Health risk index. The HRI was divided by the oral reference dose R_{fD} values to evaluate the HRI for the ingestion of pepper contaminated with Pb as follows:

$$\text{HRI} = \text{DIM} / R_{\text{fD}}$$

The R_{fD} is the maximum oral dose of a deadly drug that is permitted by the USEPA (2016). For the purposes of this investigation, the oral reference dose level for Pb (0.0035 mg/kg/d) was taken from the World Health Organization (WHO) (1993). For the exposed population, the safe HRI is not more than 1.0 (Tsafte et al. 2012).

Carcinogenic risk. The CR is an index that assesses the likelihood of developing cancer. The following equation, which was adapted from Shaheen et al. (2016), was used to determine the CR:

$$\text{CR} = \text{DIM} / \text{CSFo}$$

where CSFo is the oral carcinogenic slope factor, which is 0.0085 mg/kg/d for Pb (USEPA 2016).

Statistical analysis. An analysis of variance of the data was performed using SAS software version 9.2 (SAS 2008). Duncan's new multiple range test was used to compare individual treatment mean differences between groups at a 5% significance level.

To enable better visualization of the obtained results and a comprehensive view of the whole experiment, a group of the following most important studied traits was selected to be plotted in a heatmap figure: plant height, leaf area, DW of the whole plant, total photosynthetic pigments, total water content, transpiration rate, osmotic pressure, membrane integrity, fruit yield, vitamin C content in fruits, total protein, Pb concentration of the whole plant, Pb% remediation fruits, BCF, and HRI. Then, R 4.3.2 software was used to create the heatmap by utilizing the pheatmap package. Using a color scale and standardized

data, the data of the heatmap were created. For each trait, the average was subtracted from each value, and the resulting value was divided by the SD of the trait to standardize the data, which were measured in different units. Red color cells on the heatmap indicated high values of the trait, whereas blue color cells indicated low values of the trait.

Results and Discussions

Vegetative growth characteristics. The data in Tables 1 and 2 demonstrate that the pepper plant growth measurements decreased significantly ($P < 0.05$) with all Pb salt concentrations [PbSO₄ and Pb(NO₃)₂]. Compared with the untreated treatment with the high Pb level (3000 μg Pb/g soil), the height of the plants polluted with PbSO₄ and Pb(NO₃)₂ was reduced by 38% and 61%, respectively. The dry biomass values of root, stem, leaves, and whole plant were sharply decreased by approximately 56%, 46%, 58%, and 53% with PbSO₄, and by approximately 97%, 71%, 725, and 77% with Pb(NO₃)₂, respectively. The Pb salts might be grouped in the following order because of their detrimental impact on growth traits: Pb(NO₃)₂ > PbSO₄. When P and *Bacillus* bacteria were added to both unpolluted soil and polluted soil contaminated with Pb salts, the growth traits improved. The increases in plant height, leaf area (Fig. 1), dry matter of the whole plant (Fig. 2, Table 2), and relative growth rate were 23%, 106%, 57%, and 135%, respectively, with Pb(NO₃)₂ 3000 μg Pb/g soil with *Bacillus* bacteria, whereas the increases was 31%, 72%, 52%, and 15% with PbSO₄ 3000 μg Pb/g soil with P. This shows that increasing P with PbSO₄ as well as applying *Bacillus* bacteria with Pb(NO₃)₂ was more effective for promoting plant height under Pb stress conditions. These data were in agreement with those of Collin et al. (2022), who found that an excess of Pb accumulation caused up to a 42% reduction in the growth of the roots. The root exhibits a stronger inhibition, which may be attributable to its higher Pb content (Table 6). Additionally, Mishra et al. (2006) found that Pb inhibits seedling growth and decreases the root/shoot height, tolerance index, and dry biomass of roots and shoots. Gadallah (1995) stated that the reduction in plant height and increase in dry mass are symptoms of heavy metal toxicity.

At intolerable levels (2000 and 3000 μg Pb/g soil), Pb may have a detrimental effect on pepper plant growth because they delay cell division and differentiation, thus preventing their extension through a reduction in the meristem size and the number of mature cells, thus inhibiting plant growth (Obroucheva et al. 1998). The significantly reduced plant height, leaf count, and leaf area, as well as the energy required to manage the increased Pb concentration in their tissues (Karimi et al. 2012) and altered metabolic activities caused by decreased uptake of vital nutrients (Gopal and Rizvi 2008) are all possible explanations for the low biomass. The ability of *Bacillus* bacteria to bioremediate Pb from contaminated soil and inhibit its toxic effect (Ibeanusi et al.

Table 1. Effects of lead (Pb) salt forms and some remediating agents on vegetative growth characteristics of pepper plants after 80 d from transplanting.

Agents	Treatments		Plant height (cm)	Root length (cm)	Internodes, no. per plant	Leaves, no. per plant	Leaf length (cm)	Leaf width (cm)	Leaf area (cm ² /plant)	Specific leaf area (m ² /kg)	Leaf area ratio (m ² /kg)	Leaf weight ratio (g/g)	Leaf area index
	Pb salts	µg Pb/g soil											
Control	PbSO ₄	0	51.00 bc	17.60 abcde	10.50 bcdef	35.25 cde	16.50 bcd	6.40 abc	215.52 h	1.64 efgh	6.17 efg	3.77 cde	1.22 ef
		1000	48.00 bcd	14.60 cdefg	12.00 a-d	41.25 ab	13.13 def	5.53 abc	230.52 g	1.54 efgh	5.95 bcde	3.86 cde	1.31 e
		2000	33.50 hg	12.00 fghi	9.00 defg	18.75 hi	18.27 bcd	6.67 abc	182.37 i	2.45 abc	8.61 bcde	3.52 cde	1.29 e
		3000	31.50 hi	7.80 hij	8.00 efg	15.00 i	15.93 cde	4.47 bc	91.50 o	1.25 gh	4.69 g	3.76 cde	0.65 h
<i>Bacillus</i>	Pb(NO ₃) ₂	1000	53.50 b	16.50 bcdef	11.00 abcde	33.75 de	15.87 cde	7.07 abc	110.19 m	1.76 defgh	7.44 cdef	4.22 bcd	0.78 fgh
		2000	39.00 efg	12.00 fghi	8.50 defg	19.50 hi	17.47 bcd	6.40 abc	106.08 mn	1.46 fgh	6.49 defg	4.45 bcd	0.75 h
		3000	19.80 j	5.00 j	7.00 fg	18.75 hi	10.30 f	3.57 c	60.15 p	2.21 bcde	13.24 a	5.99 a	0.43 efg
		0	52.00 bc	17.30 abcde	14.00 a	40.50 abc	17.70 bcd	7.20 ac	389.88 a	1.76 defgh	6.40 efg	3.64 cde	2.76 ab
Phosphorus	PbSO ₄	1000	54.10 b	22.00 abcde	10.50 bcdef	43.00 ab	21.37 ab	7.47 ab	288.75 d	1.18 defgh	4.76 g	4.04 cde	2.04 bcd
		2000	38.50 fg	17.80 abcd	9.00 defg	33.50 de	18.37 bcd	6.80 abc	178.77 i	1.47 fgh	4.84 g	3.28 de	1.27 e
		3000	37.50 fgh	16.20 bcdef	8.50 efg	30.00 ef	17.83 bcdd	7.00 abc	108.15 mn	1.49 fgh	4.58 g	3.07 de	0.77 gh
		1000	53.00 bc	18.20 abcd	12.50 abc	44.50 a	24.80 a	8.53 a	258.21 f	1.86 defgh	9.41 bc	5.07 abc	1.83 d
Phosphorus	Pb(NO ₃) ₂	2000	42.00 def	12.50 efg	10.75 bcdef	33.25 de	17.13 bcd	6.03 abc	135.87 k	1.75 cdefg	9.75 bc	5.57 bcd	0.96 gh
		3000	24.50 ij	7.20 ij	7.75 g	25.75 fg	11.00 ef	4.67 bc	123.84 l	2.02 ab	8.90 bcd	4.40 bcd	0.88 h
		0	55.30 b	20.10 ab	13.00 a	39.00 abcd	16.47 bcd	7.80 ab	310.23 c	1.87 cdefg	5.68 fg	3.03 ed	2.20 bc
		1000	69.50 a	15.00 bcdefg	15.00 bcdef	38.75 abcd	14.33 cdef	4.87 abc	373.41 b	1.98 cdef	6.32 efg	3.19 de	2.64 a
Phosphorus	Pb(NO ₃) ₂	2000	52.50 bc	13.90 cdefg	11.50 defg	24.00 gh	19.00 bc	7.00 abc	99.69 no	1.84 cdefgh	4.77 g	2.60 e	0.71 gh
		3000	41.00 def	19.00 abc	9.00 defg	18.00 i	18.50 bcd	7.20 abc	157.55 j	2.94 a	8.51 bcde	2.89 de	1.12 efg
		1000	48.00 bcd	17.00 abcdef	13.00 ab	38.25 bcd	19.40 bc	7.40 ab	270.63 e	1.92 cdefg	5.76 fg	3.00 de	1.92 cd
		2000	45.80 cde	13.50 defg	11.50 abcde	34.50 de	18.60 bc	6.13 abc	99.36 no	1.86 cdefg	5.66 fg	3.04 de	0.70 h
		3000	38.00 fgh	10.50 ghi	9.50 cdef	33.75 de	14.47 cdef	5.87 abc	96.45 o	2.36 abcd	10.32 b	4.36 bcd	0.68 gh

Means of the same treatments and those in the same column followed by the same uppercase or lowercase letter are not significantly different according to the Duncan multiple range test at $P \leq 0.05$.

1995) as well as the positive effects of *Bacillus* bacteria on the nutritional status and production of growth regulators like indoleacetic acid, gibberellic acid, and cytokinins (Lazarovits 1995) may be responsible for the stimulating effect on pepper plant growth parameters when these bacteria are added to Pb-polluted soil. Regarding the beneficial impact of P on growth, P acts as a growth stimulant, improves the uptake of other nutrients, and causes Pb ions to precipitate (Marschner 1995).

Photosynthetic pigments. Table 3 shows that chlorophyll a and chlorophyll b, total chlorophyll, carotenoids, and total pigments were all significantly reduced ($P < 0.05$) and degraded by Pb in any form. Compared with the control, decreases in the total pigments of 51% and 62% occurred at high levels of Pb in soil polluted with PbSO₄ and Pb(NO₃)₂. Similar outcomes were reported by Amin et al. (2018), who discovered that chlorophyll levels decreased significantly ($P < 0.05$) with a gradual increase in the Pb concentration from 0 to 1000 mg/kg.

Furthermore, Pb may have a negative impact on chloroplast pigments because it inhibits the production of aminolevulinic acid, a precursor to chlorophyll (Seregin and Kozhevnikova 2005), stimulates the activity of chlorophyllase and chlorophyll degradation (Drazkiewicz 1994), and causes chloroplast membranes to peroxide as a result of increased reactive oxygen species generation (Malar et al. 2016). Additionally, Pb may prevent the biosynthesis of chlorophyll by preventing the intake of crucial pigment-forming components, such as magnesium, potassium, calcium, and iron, which were disrupted when Pb replaced divalent cations during the process of photosynthesis (Gopal and Rizvi 2008).

In addition to reducing the inhibitory action of Pb forms, treating Pb-polluted soils with *Bacillus* and P also boosted the total pigments content in the plants cultivated in these soils. *Bacillus*-polluted soil with PbSO₄ and Pb(NO₃)₂ at 3000 µg Pb/g soil increased total pigments by 28% and 289%, respectively, compared to control soil and by 112% and 154%, respectively, compared to the untreated plants. In the Pb-contaminated plants, P was more efficient than *Bacillus* for all photosynthetic pigments. According to our results (Tables 6 and 7), bacteria and P can reduce the concentration of Pb ions in the soil, which may explain their capability to stimulate the growth of photosynthetic pigments both under normal conditions and in Pb-polluted soils. Additionally, they may provide plants with compounds like ATP and NADPH, which are essential for the biosynthesis of pigments such as chlorophyll and other photosynthetic pigments (Marschner 1995).

Leaf water relations. The leaf water deficit, sclerophyll degree, leaf osmotic pressure, and membrane integrity were increased by 64%, 5%, 67%, and 23%, respectively, compared to the untreated plants, whereas the leaf total water content, relative water content, transpiration rate, and leaf succulence tended to decrease in the plants at all levels of Pb, especially at high levels of Pb in the

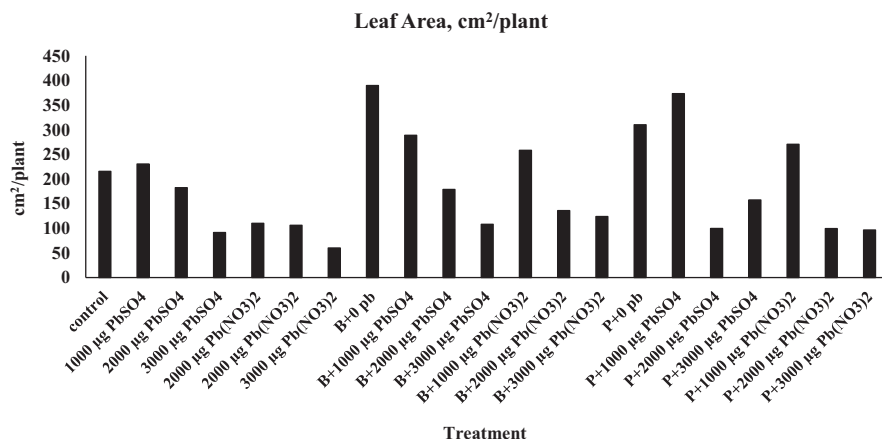


Fig. 1. Leaf area (cm²/plant) of pepper plants after 80 d from transplanting affected by some agents under different forms of lead (Pb) salts.

form of Pb(NO₃)₂ by 12%, 16%, 43%, and 8%, respectively (Table 4). Applications of P and *Bacillus* bacteria appeared to somewhat improve the values of water relations in the unpolluted soils. *Bacillus* and P treatments in the Pb-polluted soils enhanced every aspect of the water relationship. The data clearly showed that PbSO₄ treatments were superior to those comprising the other Pb salts for the treatment of *Bacillus* bacteria and P. With 3000 µg Pb/g soil in the form of PbSO₄ and *Bacillus* bacteria, the relative water content, leaf water deficit, transpiration rate, osmotic pressure, and membrane integrity improved in comparison with those of untreated plants by 12%, 31%, 67%, 8%, and 25%, respectively, whereas the increases with the P application were 10%, 26%, 9%, 24%, and 6%, respectively. In comparison with that of the P application, the impact of *Bacillus* bacteria was more obvious. Kastori et al. (1992) noted that excess Pb had damaged plant roots and decreased their transpiration strength, which resulted in decreased water intake and an insufficient supply of water to the aboveground parts of the plants. Additionally, Burzyński (1987) found that placing 2-week-old bean, wheat, and cucumber plants in a solution of Pb chloride resulted in a marked reduction of

transpiration and water uptake. Moreover, Pb disrupts the structure of the lipid membrane and protein fraction, allowing it to enter cells (Kastori et al. 1992). At extremely high concentrations, Pb causes cell membrane phytotoxicity. A few possible unexpected processes include modifications of the permeability of the plant cell membrane, interactions between sulphhydryl groups and cations, a possible propensity for reacting with phosphate groups, and active groups of ADP and ATP (Chugh and Sawhney 1999).

Yield components

Yield quantity. Table 5 demonstrates that yield quantity traits significantly decreased ($P < 0.05$) in comparison with those of the control plants with all studied Pb salt forms and rates. In comparison with other Pb salt types, the reduction in Pb(NO₃)₂-polluted soils was significantly greater. At 3000 µg Pb/g soil in the form of Pb(NO₃)₂, the largest reductions in the flower number, fruit number, weight, and yield per plant (g) were 74%, 90%, 61%, and 96%, respectively. However, at 3000 µg Pb/g soil in the form of Pb(NO₃)₂, there was a noticeable increase in the overall shedding percentage of approximately 80% when compared with the control. Parallel

results were observed by Opeolu et al. (2010), who reported that plants produced fewer flowers and less yield under high concentrations of Pb.

In comparison with untreated plants, *Bacillus* bacteria treatment of Pb-polluted soils increased the flower number, fruit number, fruit weight, and fruit yield (Fig. 3, Table 5) by approximately 46%, 25%, 98%, and 99% for PbSO₄, and by approximately 309%, 500%, 196%, and 1251% for Pb(NO₃)₂. With *Bacillus*, at a high concentration of Pb in the forms of PbSO₄ and Pb(NO₃)₂, respectively, the total shedding percentage was reduced by 6% and 14%, respectively. This indicated that *Bacillus* bacteria were not only helpful for removing Pb ions in nitrate form but also improved all yield attributes by three-times to 12-times. Using P as a Pb remediation agent not only eliminated the negative effects of Pb but also increased the flower number, fruit number, fruit weight, and fruit yield per plant (g), especially at 3000 µg Pb/g soil in form PbSO₄, by approximately 109%, 114%, 211%, and 483%, respectively, whereas the percentage of overall shedding decreased by approximately 8%. Although the P treatment was preferable, both *Bacillus* and P treatments successfully removed Pb ions in the nitrate form and increased fruit yield. Similarly, Mofath (2000) revealed that as the Pb concentration increased up to 300 mg/L, the tomato and eggplant yields significantly decreased.

Our findings show that Pb has a harmful effect on plant growth and inhibits the uptake and translocation of several major and trace elements in plant roots (Larcher 1980). The activity of some enzymes as well as biosynthesis of photosynthetic pigments that have an impact on fruit yield may be the cause of the inhibitory effect of Pb on the aforementioned yield attributes of pepper plants. It is possible that the removal of the harmful effects of Pb and the enhancement of strong root and shoot structure (Mohandas 1987), as well as the high bacterial production of phytohormones and improved nutrition (Lazarovits 1995), caused the noticeable increase of the yield components with the addition of bacteria or P.

Yield quality. Table 5 demonstrates that with all rates, there was a modest increase in the titratable acidity (%) of the fruits of plants subjected to Pb salt, which reached a maximum of 29% more than that of the control at a Pb concentration of 3000 µg/g soil. However, the amounts of vitamin C, total soluble solids, total carbohydrates, and total protein in pepper fruits were marginally reduced when Pb salts were applied at all concentrations, with reductions of 11%, 6%, 8%, and 17%, respectively, with 3000 µg Pb/g soil in the form of PbSO₄. The vitamin C, total soluble solids, total carbohydrates, and total protein in pepper fruits increased significantly when *Bacillus* bacteria were added to nonpolluted and Pb-polluted soils, with increases of 20%, 17%, 3%, and 12%, respectively, with high Pb in the form of PbSO₄/g soil. In contrast, with high Pb in the form of PbSO₄/g soil, the P treatment reduced these traits by approximately

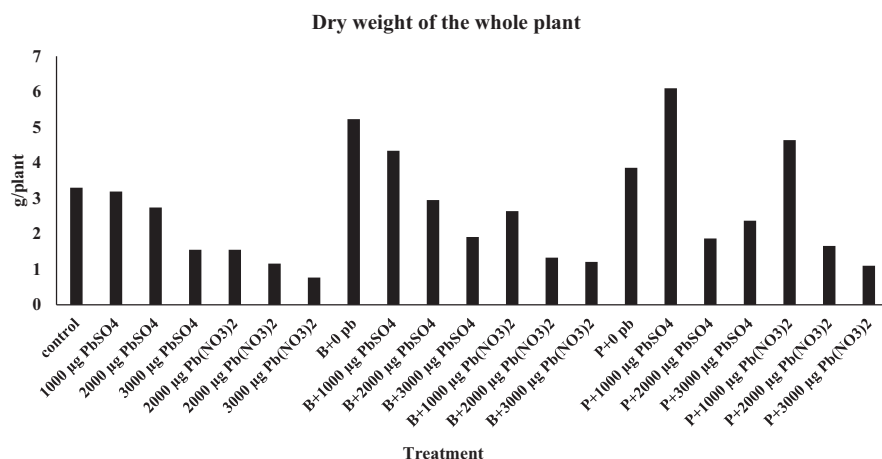


Fig. 2. Dry weight of the whole pepper plants (g/plant) after 80 d from transplanting affected by some agents under different forms of lead (Pb) salts.

Table 2. Effects of lead (Pb) salt forms and some remediating agents on fresh and dry weights of pepper plants after 80 d from transplanting.

Treatments			Fresh weight (g/plant)				Dry weight (g/plant)				Shoot/root ratio	RGR mg/g/week	NAR mg/cm ² /week
Agents	Pb salts	µg Pb/g soil	Root	Stem	Leaves	Whole plant	Root	Stem	Leaves	Whole plant			
Control	PbSO ₄	0	1.34 de	8.71 b	12.41 de	22.47 c	0.64 bcd	1.22 d	1.44 def	3.30 de	4.16 hi	1.22 cd	0.67 hi
		1000	3.04 b	6.44 c	12.88 c-e	22.35 c	0.61 c-e	1.04 e	1.54 c-e	3.19 de	4.23 hi	1.19 d	0.98 ef
		2000	2.19 c	5.93 cd	8.99 f	17.11 d	0.47 ef	1.06 e	1.22 e-g	2.74 ef	4.85 hi	1.12 d	0.98 ef
	Pb(NO ₃) ₂	3000	0.81 fg	3.17 efg	4.75 j	9.79 f	0.28 gh	0.66 hij	0.61 hi	1.55 hij	4.54 hi	0.60 efg	0.56 j
		1000	1.14 ef	3.97 defg	6.54 gh	11.64 ef	0.18 hij	0.63 ij	0.73 hi	1.55 hij	7.56 f	0.63 efg	0.63 ij
		2000	0.31 h	2.92 fg	6.46 ghi	9.68 f	0.08 ij	0.37 k	0.71 hi	1.16 ij	13.50 d	0.43 fgh	0.62 ij
<i>Bacillus</i>	PbSO ₄	3000	0.20 h	2.17 g	3.38 k	5.76 g	0.02 j	0.35 k	0.40 i	0.77 j	37.50 a	0.17 i	0.37 k
		0	1.74 d	10.06 b	19.02 a	30.82 b	1.08 a	1.55 c	2.60 a	5.23 ab	3.84 i	1.73 a	1.12 c
		1000	3.04 b	12.41 a	15.57 b	31.02 b	0.76 bc	1.65 c	1.93 bc	4.34 bc	4.71 hi	1.61 ab	1.51 a
	Pb(NO ₃) ₂	2000	2.85 b	5.89 cd	8.99 f	17.73 d	0.62 cde	0.94 ef	1.49 de	2.95 de	3.92 i	1.02 d	1.09 cd
		3000	1.22 c	3.81 defg	5.31 hij	10.34 ef	0.37 fg	0.82 fgh	0.72 hi	1.91 fghi	4.16 hi	0.67 ef	1.56 a
		1000	0.87 fg	4.67 cdef	13.35 cd	18.89 d	0.18 hij	0.74 ghi	1.72 bcd	2.64 efg	13.67 d	0.99 d	0.93 fg
Phosphorus	PbSO ₄	2000	0.87 fg	4.09 defg	5.91 ghij	10.87 ef	0.12 hij	0.50 jk	0.71 hi	1.33 ij	10.08 e	0.40 ghi	0.92 fg
		3000	0.37 h	2.75 fg	7.24 g	10.36 ef	0.04 j	0.45 k	0.83 ghi	1.21 ij	29.50 b	0.53 fgh	1.04 cde
		0	1.17 ef	8.37 b	11.71 e	21.25 c	0.51 def	1.29 d	2.07 b	3.86 cd	6.59 fg	1.45 bc	1.32 b
	Pb(NO ₃) ₂	1000	4.87 a	14.21 a	18.79 a	37.86 a	0.78 b	1.98 b	2.49 a	6.10 a	5.73 gh	1.7 bc	1.28 b
		2000	0.96 ef	5.91 cd	4.86 j	11.73 ef	0.22 ghi	0.98 ef	0.66 hi	1.87 fghi	7.45 f	0.72 e	1.00 def
		3000	0.84 fg	5.28 cde	6.40 ghi	12.52 e	0.37 gh	0.85 fg	1.05 fgh	2.37 efgh	5.14 ghi	0.69 ef	0.86 g

Means of the same treatments and those in the same column followed by the same uppercase or lowercase letter are not significantly different according to the Duncan multiple range test at $P \leq 0.05$.

NAR = net assimilation rate; RGR = relative growth rate.

12%, 25%, 5%, and 18%, respectively, in comparison with the untreated plants. In this regard, the bio-agent treatment outperformed the chemo-agent treatment. Kandil (1995) noted that Pb reduced the total, soluble, and nonsoluble carbohydrates of wheat grains. Sengar and Pandey (1996) reported that Pb treatment resulted in a decrease in photosynthetic pigments and ribulose diphosphate carboxylase, which caused a decrease in all sugar fractions (Stiborova et al. 1986). These decreases in photosynthetic pigments and ribulose diphosphate carboxylase are thought to cause the decreased carbohydrate concentration.

These results demonstrated that adding *Bacillus* bacteria or P to soils with or without Pb significantly improved the chemical characteristics of pepper fruits. This improvement may be attributable to the important roles of bacteria in the physiological and biochemical processes in plants as well as their promotion of growth and yield. Ali and Selim (1996) noted that tomato plants that had been inoculated with *Azotobacter* had higher fruit sugar and vitamin C contents. Using increased N, P, and potassium applications, Bagal et al. (1989) found that the concentrations of protein, carbohydrates, ascorbic acid, and minerals were greatly enhanced. Because P is a

component of RNA and DNA, as reported by Marschner (1995), it is reasonable to assume that the availability of P will have a significant impact on the biosynthesis of a variety of substances, including carbohydrates, proteins, and hormones. Additionally, Bender et al. (1986) revealed that plants with an optimal P supply have much higher levels of photosynthetic CO₂ fixation and assimilate translocation than plants with low P levels.

Pb concentration and uptake. Table 6 demonstrates that Pb accumulated approximately four- to five-times more in the root than in the shoot. The Pb concentration was drastically increased in the root, shoot, and

Table 3. Effects of lead (Pb) salt forms and some remediating agents on photosynthetic pigments of pepper plants after 80 d from transplanting.

Treatments			Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	Total photosynthetic pigments	Chlorophyll a/ chlorophyll b	Total chlorophyll/carotenoids
Agents	Pb salts	µg Pb/g soil	mg/g DW						
Control	PbSO ₄	0	2.66 h	0.98 fg	3.64 gh	1.10 fg	4.74 h-j	2.73 ef	3.30 cde
		1000	2.07 ij	1.02 f	3.08 hi	1.05 fg	4.04 jk	2.03 h	2.93 defg
		2000	1.89 jk	0.84 gh	2.72 hi	1.07 fg	5.09 ghij	2.26 h	2.54 fghi
	Pb(NO ₃) ₂	3000	1.39 m	0.31 l	1.70 j	0.54 h	2.24 l	4.52 ab	3.14 cde
		1000	2.46 hi	0.84 gh	3.40 gh	1.06 fg	4.46 ij	2.92 def	3.22 cde
		2000	1.33 lm	0.70 hi	2.03 ij	0.85 gh	2.89 kl	1.90 h	2.39 hi
<i>Bacillus</i>	PbSO ₄	3000	0.93 m	0.43 kl	1.35 j	0.45 h	1.80 l	2.18 h	3.03 def
		0	3.76 ef	1.41 de	5.17 ef	1.35 f	6.52 efg	2.67 fg	3.83 b
		1000	8.07 a	2.46 a	10.52 a	2.37 b	12.89 a	3.28 d	4.44 a
	Pb(NO ₃) ₂	2000	2.91 gh	0.95 fg	3.46 gh	1.42 ef	4.87 hij	3.08 def	2.44 ghi
		3000	1.55 kl	0.47 jk	2.03 ij	0.85 gh	2.87 kl	3.28 d	2.39 i
		1000	5.68 b	1.92 b	7.60 b	2.30 bc	9.90 b	2.95 def	3.31 cde
Phosphorus	PbSO ₄	2000	4.94 c	1.75 c	6.69 bc	2.04 bcd	8.74 bc	2.83 ef	3.28 cde
		3000	3.99 de	0.94 fg	4.92 ef	2.08 bcd	7.01 def	4.26 b	2.36 hi
		0	3.32 fg	1.02 f	4.33 fg	1.46 ef	5.79 fghi	3.27 d	2.96 def
	Pb(NO ₃) ₂	1000	5.68 b	1.54 d	7.21 bc	2.84 a	10.05 b	3.70 c	2.54 fghi
		2000	4.10 de	1.31 e	5.41 de	1.88 cd	7.29 de	3.12 ed	2.87 efgh
		3000	2.75 h	0.56 ijk	3.30 h	1.44 ef	4.74 hij	4.94 a	2.29 li
	Pb(NO ₃) ₂	1000	4.28 d	1.98 b	6.2 cd	1.80 de	8.06 cd	2.16 h	3.47 b-d
		2000	3.41 f	1.48 d	4.89 ef	1.36 f	6.24 efgh	2.31 gh	3.60 efgh
		3000	2.84 gh	0.60 ij	3.44 gh	1.13 fg	4.57 j	4.77 a	3.04 def

Means of the same treatments and those in the same column followed by the same uppercase or lowercase letter are not significantly different according to the Duncan multiple range test at $P \leq 0.05$. DW = dry weight.

Table 4. Effects of lead (Pb) salt forms and some remediated agents on water relations of pepper plants after 80 d from transplanting.

Treatments			Total water content (%)	Relative water content (%)	Leaf water deficit (%)	Sclerophylly degree (%)	Transpiration rate mg H ₂ O ₂ /cm ² /h	Leaf succulence	Osmotic pressure mmHg	MI (%)	
Agents	Pb salts	μg Pb/g soil									
Control	PbSO ₄	0	90.70 a	79.60 abcde	20.40 ef	7.59 cdef	8.63 bcd	10.75 bc	2.86 e	16.34 e	
		1000	92.39 a	76.76 bcdf	23.24 d	6.67 ef	7.18 defg	13.15 a	3.94 cd	20.54 bcd	
		2000	89.09 a	74.04 efgh	25.96 c	8.32 bcd	6.82 efg	9.16 bcde	4.63 b	21.63 abc	
	Pb(NO ₃) ₂	3000	82.16 b	71.44 f-i	28.56 b	8.76 bc	5.77 ghi	8.45 de	5.19 a	21.84 ab	
		1000	90.74 a	67.12 hi	32.88 a	6.41 f	7.60 cdef	10.80 bc	4.08 cd	21.00 bc	
		2000	89.87 a	71.50 fgh	28.50 b	7.46 cdef	6.68 efg	9.87 bcde	4.08 cd	22.95 a	
		3000	79.87 b	66.58 i	33.42 a	7.95 cde	4.92 i	9.87 bcde	4.77 ab	21.60 abc	
	Bacillus	PbSO ₄	0	92.75 a	81.01 abcde	19.00 efg	8.26 bcd	7.97 cde	8.89 de	3.54 d	15.49 e
			1000	90.31 a	82.84 abc	17.16 hij	8.17 bcde	6.86 efg	10.32 bcd	4.77 ab	16.25 e
			2000	89.38 a	79.23 abcde	20.77 ef	8.61 bcd	10.22 a	9.41 bcde	4.77 ab	16.71 e
Pb(NO ₃) ₂		3000	88.21 a	80.35 abcde	19.65 efg	10.54 a	9.67 ab	7.82 e	4.77 ab	16.38 e	
		1000	88.80 a	82.32 abc	17.68 ghi	8.36 bcd	5.28 hi	8.93 bcde	4.63 b	19.00 d	
		2000	89.86 a	67.79 ghi	32.22 a	7.11 def	8.26 bcde	9.86 be	3.81 cd	20.95 bc	
		3000	89.10 a	70.70 fghi	29.30 b	7.97 cde	9.05 abc	9.17 bcde	4.77 ab	20.60 bcd	
Phosphorus	PbSO ₄	0	88.93 a	81.58 abcd	18.42 fgh	7.12 def	6.87 efg	9.04 bcde	4.22 bc	16.95 e	
		1000	90.92 a	84.91 a	15.10 j	7.82 cdef	8.49 bcd	11.02 b	4.22 bc	19.10 d	
		2000	88.84 a	74.36 defg	25.64 c	8.54 bcd	8.07 cde	8.96 bcde	3.94 cd	20.93 bc	
	Pb(NO ₃) ₂	3000	88.19 a	78.74 abcde	21.26 d	9.536 ab	6.31 fgfi	8.47 de	3.95 cd	20.54 bcd	
		1000	88.06 a	83.95 ab	16.05 ij	10.23 a	7.32 defg	8.37 de	3.56 d	20.00 cd	
		2000	90.55 a	79.78 abcde	20.22 ef	7.69 cdef	7.24 defg	10.59 bc	4.28 bc	21.95 ab	
		3000	90.35 a	76.60 cde	23.40 d	7.57 cdef	8.97 abc	10.36 bd	3.94 cd	21.60 abc	

Means of the same treatments and those in the same column followed by the same uppercase or lowercase letter are not significantly different according to the Duncan multiple range test at $P \leq 0.05$.

MI = membrane integrity.

fruit as well as in the entire pepper plant with increased rates of Pb salts in the soil. In comparison with the control plants, at the highest Pb concentration in the forms of PbSO₄ and Pb(NO₃)₂, the increases in the Pb concentration in the entire plant were approximately 1963% and 2841%, respectively. This demonstrated that pepper plants prefer to absorb Pb in the salt form of Pb(NO₃)₂ rather than PbSO₄. These findings may help to explain why Pb nitrate had more severe negative effects on most characteristics as exhibited by the root, shoot, and fruit production. These results are somewhat consistent with those of Mofath (2000) and Ahmed et al. (2021). When bio-remediate (*Bacillus* bacteria) for Pb ions was added to soils polluted with PbSO₄ and Pb(NO₃)₂ at 3000 μg Pb/g soil, the Pb concentration in the whole plant decreased significantly by approximately 30% and 24%, respectively, and that in the fruits decreased significantly by approximately 76% and 87%, respectively (Fig. 4, Table 6).

The introduction of P as a chemical agent to soils contaminated with PbSO₄ and Pb(NO₃)₂ at 3000 μg Pb/g soil, reduced the Pb concentration by ~21% and 16% in entire plant, respectively, and by ~47% and 73% in pepper fruits, respectively. These findings demonstrated that using *Bacillus* bacteria as a bio-remediation agent was more effective at removing Pb from soil and, as a result, at reducing the toxicity of Pb to pepper plants. Mahmoud and El-Beltagy (1998) reported similar results and showed that the use of *Bacillus* bacteria strains in naturally Pb-polluted soils reduced the Pb uptake by the rocket salad plant by 96.4% compared with a reduction of 73.49% in the soil that contained 400 ppm Pb. Bacteria acting as bio-remediate can reduce Pb buildup through a variety of mechanisms, including metal ion precipitation, bacterial

adsorption, and reduction through oxidation state changes (Ibeanusi et al. 1995). Regarding the P-Pb interaction, Gaweda (1997) reported that applying fertilizers, such as P (800 mg P/kg DW), calcium (1500 mg calcium/kg DW), and magnesium (240 mg/kg DW), or liming the pH increase of the soil from 5.1 to 6.2 significantly reduced the accumulation of Pb in carrot roots.

Pb tolerance index, Pb bioaccumulation factor, Pb translocation factor

Pb tolerance index. Table 7 illustrates the capacity of green pepper plants to tolerate large accumulations of Pb metal within plant tissue based on the dry biomass and Pb TI (%). Pepper plants exposed to Pb at doses of 2000 and 3000 μg Pb/g soil had less dry matter than untreated plants (Table 2). Plants exposed to PbSO₄ and Pb(NO₃)₂ at 3000 μg Pb/g soil showed TIs of 47% and 23%, respectively, which are extremely low levels. Additionally, PbSO₄ and Pb(NO₃)₂ had TI values of 83% and 35%, respectively, at 2000 μg Pb/g soil. The average TIs of Pb-stressed pepper plants were 76% with PbSO₄ and 35% with Pb(NO₃)₂. According to Herlina et al. (2020), plants with TI values more than 100% showed net gains in biomass and tolerance acquisition, whereas plants with TI values less than 100% showed net decreases in biomass and stressed plant states. However, Zhivotovsky et al. (2011) and Wang et al. (2014) claimed that a TI threshold value of 60% would represent the ability of plants to tolerate metalloids.

The TI values of pepper plants treated with various Pb salt types were less than 100%, more than 60% for PbSO₄, and less than 60% for Pb(NO₃)₂. This indicated that the plants underwent stress during the Pb(NO₃)₂ treatments, as evidenced by the slowed growth of plants. *Bacillus* treatment increased the TI

values of PbSO₄ and Pb(NO₃)₂-polluted soils by 58% and 37%, respectively, whereas P treatment increased the TI values of plants treated with 3000 μg Pb/g soil for all Pb salt types to approximately 72% and 33%, respectively. Accordingly, the average TIs of Pb-stressed pepper plants inoculated with *Bacillus* were 93% with PbSO₄ and 52% with Pb(NO₃)₂; however, with P, the average TIs were 104% with PbSO₄ and 75% with Pb(NO₃)₂. These findings suggest that *Bacillus* and P treatments are beneficial to plants cultivated with PbSO₄.

Various plant species use distinct defense mechanisms to endure increased metal concentrations (Syuhaida et al. 2014). To prohibit heavy metal ions from diffusing into plant cells, a polysaccharide similar to callose (β-1, 3 glucan) must be created and deposited on the outside of the cell membrane. During soil tests and in situ treatments, plant roots released exudates into the soil matrix to chelate metals and prevent their uptake into cells (Furini 2012; Jutsz and Gnida 2015).

Bioconcentration factor. The BCF can be used to quantify the capacity of a plant to take-up heavy metals from soils and store them inside the plant. This is defined as the ratio of the metal concentration in the entire plant to that in the soil. Ghosh and Singh (2005) reported that BCF values ranging from 1.0 to 10 signify hyperaccumulator plants, those ranging from 0.1 to 1.0 signify moderate-accumulator plants, those ranging from 0.01 to 0.1 signify low-accumulator plants, and those with values less than 0.01 signify nonaccumulator plants.

The BCF values of pepper plants increased when the Pb levels of all Pb salt forms increased (Table 7). The BCF values of pepper plants in the polluted Pb soils varied from 0.10 to 0.41, indicating moderate-

Table 5. Effects of lead (Pb) salt forms and some remediating agents on pepper yield and some chemical characteristics of pepper fruits.

Treatments		Fruit										Vitamin C		Total soluble		Total		Total	
Agents	Pb salts	µg Pb/g soil	Flowers no./plant	Fruits no./plant	Shedding (%)	Fruit weight (g)	Fruit yield/plant (g)	Fruit yield/m ² (kg)	Fruit yield (ton/feddan.)	Titrate acidity (%)	(mg ascorbic acid/100 g FW fruit)	Total solids (%)	carbohydrates (%)	protein (%)					
Control	PbSO ₄	0	13.67 ijk	7.67 fg	43.96 e	10.95 ef	83.42 j	2.36 hi	9.92 hi	0.56 a	150.39 b-c	5.90 a	31.66 abcde	19.79 abcd					
		1000	14.33 ij	5.17 i	63.89 bcd	6.51 g	34.11 n	0.97 k	4.06 k	0.60 a	145.05 cd	5.93 a	29.97 cde	18.96 abcde					
		2000	15.00 hi	5.50 hi	63.19 bcd	6.58 g	36.54 mn	1.03 k	4.34 k	0.61 a	140.39 def	6.10 a	29.61 cde	18.13 abcde					
		3000	11.67 k	4.67 i	63.64 bcd	7.33 g	34.25 n	0.97 k	4.07 k	0.64 a	133.72 f	6.27 a	29.00 e	16.46 f					
<i>Bacillus</i>	Pb(NO ₃) ₂	1000	27.67 c	6.89 gh	74.87 a	10.50 f	73.22 k	2.07 ij	8.71 ij	0.60 a	145.05 cde	6.00 a	32.99 a	20.46 ab					
		2000	12.33 jk	2.67 j	78.42 a	6.17 gh	16.58 o	0.47 l	1.97 l	0.62 a	137.05 ef	6.33 a	31.38 abcde	18.79 bcde					
		3000	3.50 l	0.75 k	78.9 a	4.33 h	3.17 p	0.09 i	0.38 i	0.64 a	130.39 f	6.67 a	29.94 cde	17.79 ef					
		0	22.00 e	13.67 a	36.11 g	23.17 a	221.00 c	6.26 c	26.28 c	0.51 a	163.72 a	6.12 a	32.75 ab	20.79 a					
<i>Phosphorus</i>	PbSO ₄	1000	33.67 a	12.50 ab	62.18 bcd	22.83 a	337.50 a	9.55 a	40.13 a	0.60 a	153.72 abc	6.33 a	31.66 a-e	20.13 abc					
		2000	18.67 fg	6.67 gh	64.48 bcd	20.33 b	91.67 i	2.59 h	10.90 gh	0.60 a	157.05 ab	6.67 a	30.24 bcde	19.13 abcde					
		3000	17.00 gh	5.83 hi	59.82 d	14.50 cd	68.17 k	1.93 j	8.10 ij	0.56 a	160.39 ab	7.33 a	29.97 cde	18.46 cde					
		1000	29.67 b	11.50 bc	62.44 bcd	23.32 a	305.83 b	8.66 b	36.36 b	0.54 a	160.39 ab	6.33 a	30.58 a-e	20.46 ab					
<i>Phosphorus</i>	Pb(NO ₃) ₂	2000	17.00 gh	5.50 hi	67.69 b	15.67 c	60.83 l	1.72 j	7.23 j	0.55 a	157.05 ab	6.60 a	29.63 cde	19.25 abcde					
		3000	14.33 ij	4.50 i	68.29 b	12.83 de	42.83 m	1.21 k	5.09 k	0.56 a	155.72 a-c	7.17 a	29.28 de	18.83 bcde					
		0	14.67 i	10.33 cd	29.52 f	23.17 a	107.00 h	3.03 g	12.72 g	0.56 a	160.39 ab	7.00 a	32.03 abc	20.00 abc					
		1000	22.33 e	8.50 ef	62.37 bcd	14.50 cd	150.83 e	4.27 e	17.93 e	0.54 a	157.05 ab	7.20 a	31.75 abc	20.21 abc					
<i>Pb(NO₃)₂</i>	Pb(NO ₃) ₂	2000	24.67 d	9.83 de	60.08 cd	20.33 b	193.50 d	5.48 d	23.01 d	0.55 a	155.72 abc	7.63 a	31.66 abcde	19.54 abcde					
		3000	24.33 d	10.00 d	58.89 d	22.83 a	200.00 d	5.66 d	23.78 d	0.57 a	150.39 bcd	7.83 a	30.42 abcde	19.50 abcde					
		1000	24.67 d	9.67 de	60.59 cd	23.32 a	195.67 d	5.54 d	23.26 d	0.58 a	160.39 ab	7.50 a	32.28 abc	20.29 abc					
		2000	20.67 ef	7.83 fg	62.10 bcd	15.67 c	122.83 g	3.48 f	14.60 f	0.59 a	153.72 abc	7.77 a	31.36 abcde	20.21 abc					
3000	20.67 ef	7.50 fg	66.35 bc	12.83 de	142.83 f	4.04 e	16.98 e	0.60 a	153.72 abc	7.93 a	31.00 abcde	19.50 abcde							

Means of the same treatments and those in the same column followed by the same uppercase or lowercase letter are not significantly different according to the Duncan multiple range test at $P \leq 0.05$.

accumulator plants. The BCF of Pb of several plants ranged from 0.0001 to 0.0648 (Chang et al. 2014).

The BCF of Pb in pepper plants varied depending on the Pb salts used (Table 7). The BCF of Pb ranged from 0.22 to 0.29 with PbSO₄ and from 0.20 to 0.41 with Pb(NO₃)₂. The average BCFs of Pb metal in pepper plants were as follows: 0.323 with Pb(NO₃)₂, 0.252 with PbSO₄, and 0.241 with Pb(NO₃)₂ at 3000 µg Pb/g soil (the highest BCF value).

These results are agreement with those observed by Ahmed et al. (2021), Chang et al. (2014), and Ghosh and Singh (2005). Adding both *Bacillus* and P to the soils contaminated with all Pb salt types caused the average values of BCF to change to 0.17 and 0.185 with PbSO₄, respectively, and to 0.234 and 0.273 with Pb(NO₃)₂, respectively (Table 7). These findings demonstrated the value of using *Bacillus* bacteria as a bio-remediated agent to reduce the Pb BCF of pepper plants contaminated with PbSO₄ and Pb(NO₃)₂.

Translocation factor. The calculated TFs are shown in Table 7 as the mobilization ratios of Pb metal from the soil to roots, shoots, and fruits. With increasing Pb levels in the Pb-contaminated soils, the TFs of roots (TFR), shoots (TFsh), and fruits (TFf) increased. With all Pb salt types, the TFs of Pb were as follows: TFR > TFsh > TFf. However, Pb(NO₃)₂ was the most successful at transporting Pb ions from the soil to roots, as evidenced by the greatest TFR, which was 0.77 at 3000 µg Pb/g soil, and the PbSO₄ type had the greatest TFsh value (0.253) and effectively transported Pb ions from the root to the shoot. At 3000 µg Pb/g soil, the TFf values for PbSO₄ and Pb(NO₃)₂ were 0.08 and 0.1, respectively. It is interesting that the *Bacillus* bacteria with 3000 µg Pb/g soil in the form of Pb(NO₃)₂ significantly reduced the TFR, TFsh, and TFf values of pepper plants by 15%, 26%, and 80%, respectively, compared with those of untreated plants; however, P with 3000 µg Pb/g soil in the form of Pb(NO₃)₂ resulted in decreases of 4%, 43%, and 50%, respectively. Napoli et al. (2019) reported that a high value of TF (TF > 1) indicates the potential of a plant to move metalloids from roots to aerial tissues. In contrast, a low number (TF < 1) denotes the restricted ability of a plant to translocate the metal to aerial tissues. Pepper plants in this study had TF values of 1.0. As a result, the plants may be categorized as excluders of Pb because of the low capacity to absorb large amounts of Pb. According to the results of previous studies, which were comparable to ours in this regard, the majority of plants had a TF value of 1.0. Additionally, Ahmed et al. (2021) observed that fruits produced in high Pb-contaminated soil exceeded the recommended limit of 0.3 mg/kg in chili plants. At every level of pollution, the TF for Pb was discovered as follows: leaves > shoots > roots > fruits. *Brassica juncea* efficiently transferred Pb from roots to leaves, which is necessary for phytoextraction of Pb (Mohanty et al. 2010). Additionally, low levels of Pb translocation showed that plants may have

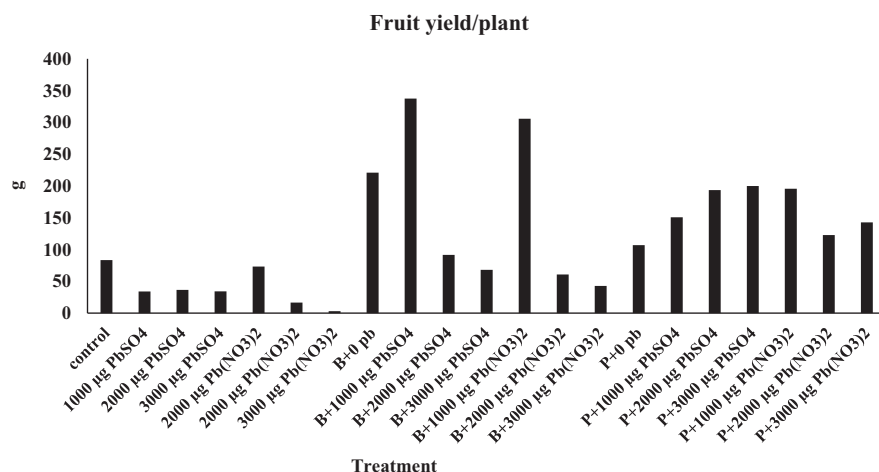


Fig. 3. Fruit yield/plant (g) after 80 d from transplanting affected by some agents under different forms of lead (Pb) salts.

been reluctant to move metal from their roots to their shoots because of Pb toxicity. Moreover, Pb can be harmful to enzymes involved in the synthesis of chlorophyll, photosynthetic activity, and antioxidants (Kim et al. 2003). High root-to-shoot translocation of heavy metals indicated that these plants have essential traits to be exploited during phytoextraction of these metals (Ghosh and Singh 2005). Plant species with $TF > 1$ can move metals from roots to shoots more readily than species with roots that are restricted by those metals. Dan et al. (2002) proposed that lignification of the cell wall and the development of metal-lignin complexes may be one of the main mechanisms underlying the Pb tolerance in the roots of fragrant *Geranium* plants. The mobility of Pb and its competition with other metals inside plants may have an impact on the uptake and translocation of Pb.

Based on the aforementioned explanation, pepper plants accumulate Pb to a modest extent.

Daily intake of heavy metals, health risk index, and carcinogenic risk. Table 7 shows the predicted daily Pb intake (DIM), HRI, and CR for pepper plants grown in soils that were artificially contaminated with Pb using various Pb salt forms. The DIM values at high Pb levels in the pepper plants were grouped in the following order according to the different forms of Pb salts: $Pb(NO_3)_2$ ($\approx 25.6 \times 10^{-5}$) > $PbSO_4$ ($\approx 16.2 \times 10^{-5}$) > control (untreated plants; $\approx 0.9 \times 10^{-5}$). The maximum DIM value was observed with $Pb(NO_3)_2$ ($\approx 25.6 \times 10^{-5}$) at 3000 µg Pb/g soil, whereas the lowest value was observed with $PbSO_4$ ($\approx 3.40 \times 10^{-5}$) at 1000 µg Pb/g soil. It is noteworthy that the DIM values for all Pb salts were not higher than 0.214 with any level, as recommended by the WHO/

Food and Agriculture Organization (FAO). Regarding the impact of *Bacillus* and P on the DIM values of pepper plants, it was found that the DIM significantly decreased with 3000 µg Pb/g soil compared with that of untreated plants by approximately 76% and 48% with $PbSO_4$, respectively, and 87% and 73% with $Pb(NO_3)_2$, respectively. These findings demonstrated that it was more beneficial to reduce the Pb DIM values of pepper plants that had been polluted with Pb salts by using *Bacillus* bacteria as a bioremediate agent. However, according to Likuku and Obuseng (2015), chili pepper plants cultivated in heavily contaminated soil had higher DIM values than that recommended by the WHO/FAO. Furthermore, the accumulations of chromium, Pb, and zinc in green peppers (*Capsicum annum* L.) were significantly higher than the safe limits recommended by the joint WHO/FAO Food Standards Program Code Alimentarius Commission. Although pepper is a necessary part of the daily diet in Egypt and is often consumed in tiny amounts as a spice and food additive, previous research showed that the DIM values for Pb were low at all levels of Pb contamination. This could explain why the green pepper plants in our study had lower DIM levels.

Regarding the HRI values of Pb in pepper plant fruits, it should be noted that all Pb levels across all Pb salt types in our study were less than 1, indicating that it was safe to consume Pb-polluted pepper plant fruits. The HRI values for $PbSO_4$ and $Pb(NO_3)_2$ varied from 0.01 to 0.05 and 0.01 to 0.07, respectively. The Pb-stressed pepper plants treated with *Bacillus* and P resulted in noticeably lower HRI values (Table 7).

According to the USEPA (2016), CR values exceeding 1×10^{-4} cause significant cancer risks. The CR values of the present study

Table 6. Effects of lead (Pb) salt forms and some remediating agents on the Pb concentration and uptake in different pepper organs as well as the Pb remediation percentages of the whole plant and fruit.

Treatments			Root		Shoot		Fruits		Whole plant		Pb% remediation whole plant	Pb% remediation fruits
Agents	Pb salts	µg Pb/g soil	Conc. µg/g DW	Uptake µg/plant	Conc. µg/g DW	Uptake µg/plant	Conc. µg/g DW	Uptake µg/plant	Conc. µg/g DW	Uptake µg/plant		
Control	$PbSO_4$	0	17.56 lm	11.24 mn	4.25 op	11.31 m	0.40 jkl	2.34 mn	11.10 o	36.64 m		
		1000	70.22 k	42.83 g	17.01 k	43.88 ij	1.60 hi	3.83 lm	44.41 l	141.68 j		
		2000	152.84 i	71.83 e	41.20 f	93.94 d	3.61 de	9.23 gh	98.82 j	270.78 d		
	$Pb(NO_3)_2$	3000	360.96 d	101.07 c	89.62 b	113.81 a	7.60 b	18.22 e	229.09 d	355.09 b		
		1000	70.44 k	12.68 m	7.40 n	10.07 mn	1.62 hi	8.29 hi	39.73 l	61.58 l		
		2000	232.44 fg	18.60 l	56.05 e	60.54 g	5.23 c	6.07 jk	146.86 g	170.36 h		
		3000	520.90 a	10.42 n	120.10 a	90.08 e	12.03 a	2.67 mn	326.52 a	251.42 e		
	$PbSO_4$	0	6.63 m	7.16 o	1.89 q	7.84 n	0.12 l	1.86 n	4.32 p	22.61 n	61.07 a	69.96 c
		1000	44.23 kl	33.61 i	12.60 m	45.12 i	0.80 jk	18.95 e	28.82 mn	125.06 k	35.12 cd	49.94 f
		2000	100.48 j	62.30 f	28.05 i	68.16 f	1.62 hi	10.37 g	65.07 k	191.96 g	34.15 cd	55.21 e
<i>Bacillus</i>	$Pb(NO_3)_2$	3000	257.12 f	95.13 d	64.10 d	98.71 c	1.84 h	8.78 gh	161.53 f	308.52 c	29.49 def	75.79 b
		1000	42.01 kl	7.56°	4.60°	11.32 m	0.61 jkl	12.97 f	23.61 n	62.33 l	40.58 c	62.55 d
		2000	182.00 h	21.84 k	34.00 g	41.15 j	1.62 hi	6.88 ij	108.81 i	144.72 j	25.91 e-g	69.11 c
	$PbSO_4$	3000	424.02 c	16.96 l	73.61 c	94.22 d	1.62 hi	4.84 kl	249.62 c	302.04 c	23.55 fg	86.57 a
		0	8.05 m	4.11 p	2.56 pq	8.59 mn	1.31 n	1.31 n	5.39 p	20.81 n	51.45 b	56.17 e
		1000	46.00 kl	35.88 h	14.60 l	65.27 f	1.00 ij	10.59 g	30.80 m	187.90 g	30.64 de	37.39 g
		2000	104.01 j	22.88 k	32.00 h	52.49 h	2.60 g	35.23 b	69.31 k	129.61 k	29.87 def	27.90 h
		3000	304.04 e	112.49 a	56.08 e	106.56 b	4.02 d	56.22 a	182.07 e	431.50 a	20.52 gh	47.17 f
	$Pb(NO_3)_2$	1000	62.01 k	33.49 i	4.04 op	16.56 l	0.70 jkl	9.59 gh	33.38 m	154.86 i	16.00 h	56.74 e
Phosphorus	$PbSO_4$	2000	220.02 g	110.01 b	24.02 j	27.62 k	2.80 fg	24.11 d	123.42 h	204.88 f	15.96 h	46.41 f
		3000	480.08 b	28.80 j	64.06 d	65.98 f	3.22 ef	32.15 c	273.68 b	301.04 c	16.18 h	73.27 bc

Means of the same treatments and those in the same column followed by the same uppercase or lowercase letter are not significantly different according to the Duncan multiple range test at $P \leq 0.05$.

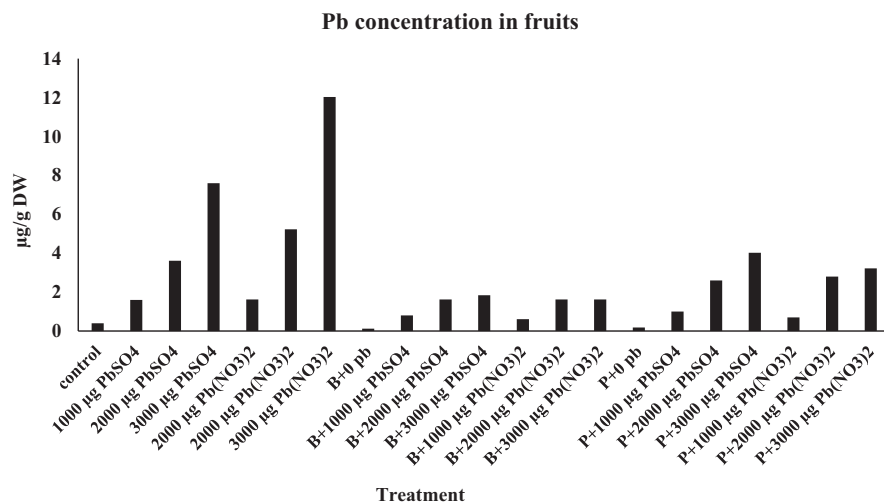


Fig. 4. Lead (Pb) concentration in fruits [$\mu\text{g/g}$ dry weight (DW)] after 80 d from transplanting affected by some agents under different forms of Pb salts.

(Table 7) indicated that Pb contamination of pepper plants can be regarded as safe in terms of the CR. The average CR values in our study were 1×10^{-6} for the untreated plants and ranged from 4.1×10^{-6} to 19×10^{-6} for PbSO_4 and from 4×10^{-6} to 30×10^{-6} for $\text{Pb}(\text{NO}_3)_2$. When stressed plants were treated with P and *Bacillus*, CR values were noticeably reduced until they were within the permissible limit. Ruchuwarak et al. (2019) noted that *Centella asiatica* and other various species of vegetable plants showed increased CRs with Pb. All levels of contamination according to the DIM for chromium and Pb were inadequate, and Pb had a higher DIM than chromium (Zewail et al. 2020).

Heatmap analysis. Figure 5 shows the heatmap of the associations between the treatments and measured traits (plant height,

leaf area, DW of the whole plant, total photosynthetic pigments, total water content, transpiration rate, osmotic pressure, membrane integrity, fruit yield, vitamin C content in fruits, total protein, Pb concentration of the whole plant, Pb concentration of remediation fruits, BCF, and HRI). The heatmap in Fig. 5 shows high values of fruit yield, total photosynthetic pigments, DW of the whole plant, leaf area, total protein, plant height, total water content, Pb concentration of remediation fruits, and vitamin C content in fruits (orange and red colors) with treatment (T) 8 (*Bacillus* + 0 Pb), T9 (*Bacillus* + 1000 μg PbSO_4), T12 [*Bacillus* + 1000 μg $\text{Pb}(\text{NO}_3)_2$], T15 (P+0 Pb), T16 (P+1000 μg PbSO_4), and T19 [P+1000 μg $\text{Pb}(\text{NO}_3)_2$]. Low values were found with T4 (3000 μg PbSO_4) and T7 [3000 μg $\text{Pb}(\text{NO}_3)_2$]. Additionally, with

T4 and T7, the BCF, Pb concentration of the whole plant, HRI, membrane integrity, and osmotic pressure were highest, indicating undesired effects of high levels of PbSO_4 and $\text{Pb}(\text{NO}_3)_2$. A comparison of $\text{Pb}(\text{NO}_3)_2$ and PbSO_4 indicated that the most harmful effects were observed with $\text{Pb}(\text{NO}_3)_2$. *Bacillus* bacteria had more beneficial effects that alleviated the adverse effects of Pb pollution, especially those associated with PbSO_4 salt.

Conclusion

Increasing the Pb concentration of both PbSO_4 and $\text{Pb}(\text{NO}_3)_2$ resulted in considerable inhibition of growth characteristics of pepper plants. The inhibitory effect of Pb was more pronounced in the presence of $\text{Pb}(\text{NO}_3)_2$ than in the presence of PbSO_4 , with the leaf area, dry biomass, and relative growth rate being more negatively affected. The photosynthetic pigments in plant leaves and their water status were adversely affected by both forms of Pb salts. The effect of $\text{Pb}(\text{NO}_3)_2$ was more harmful than that of PbSO_4 on yield quantity and quality. The Pb levels of pepper fruits cultivated with both Pb salts were higher than the recommended limit of 0.3 mg/kg. The TI of pepper plants treated with PbSO_4 was >60%, but it was 60% in plants treated with $\text{Pb}(\text{NO}_3)_2$. The bio-concentration factor values of pepper plants polluted with Pb salts varied from 0.10 to 0.41, indicating that pepper is a moderate-accumulator plant. With all Pb levels of the salt forms, the TF values were ranked as follows: $\text{TFR} > \text{TFsh} > \text{TFf}$. With TF values <1.0, the DIM, HRI, and CR of pepper fruits of plants cultivated in the polluted soils were within safe limits. In addition to helping to mitigate the negative effects of excessive Pb levels, *Bacillus* bacteria as a bio-agent and P as a chemo-agent in Pb-polluted

Table 7. Effects of lead (Pb) salt forms and some remediating agents on the Pb tolerance index (TI), bioconcentration factor (BCF), and translocation factor (TF), as well as the daily intake of heavy metals (DIM), health risk index (HRI), and carcinogenic risk (CR).

Treatments				TF							
Agents	Pb salts	μg Pb/g soil	TI (%)	BCF	Soil to roots (TF _r)	Root to shoot (TF _{sh})	Shoot to fruits (TF _f)	DIM	HRI	CR	
Control	PbSO ₄	0	100.00 f	0.22 a	0.55 bcde	0.24 abcd	0.09 def	0.851 × 10 ⁻⁵ jk	0.0024 hi	1.00 × 10 ⁻⁶ k	
		1000	96.67 f	0.22 fg	0.54 bcde	0.24 abcd	0.09 def	3.404 × 10 ⁻⁵ h	0.0097 fgh	4.01 × 10 ⁻⁶ h	
		2000	83.03 gh	0.25 efg	0.58 bc	0.27 abc	0.09 def	7.667 × 10 ⁻⁵ de	0.0219 de	9.02 × 10 ⁻⁶ e	
	Pb(NO ₃) ₂	3000	46.97 lm	0.29 cde	0.58 bc	0.25 abcd	0.08 def	16.153 × 10 ⁻⁵ b	0.0462 b	19.00 × 10 ⁻⁶ b	
		1000	46.97 lm	0.20 ghij	0.42 def	0.11 ef	0.22 a	3.438 × 10 ⁻⁵ h	0.0098 ef	4.05 × 10 ⁻⁶ h	
		2000	35.15 n	0.37 ab	0.74 a	0.24 abcd	0.09 def	11.118 × 10 ⁻⁵ c	0.0318 c	13.08 × 10 ⁻⁶ c	
Bacillus	Pb O ₄	3000	23.33 o	0.41 a	0.77 a	0.23 abcd	0.10 de	25.568 × 10 ⁻⁵ a	0.0731 a	30.08 × 10 ⁻⁶ a	
		0	158.48 b	0.14 jk	0.35 fg	0.28 ab	0.06 fgh	0.256 × 10 ⁻⁵ k	0.0007 i	0.30 × 10 ⁻⁶ l	
		1000	131.52 d	0.14 jk	0.32 fg	0.28 ab	0.06 fgh	1.704 × 10 ⁻⁵ ij	0.0049 ghi	2.01 × 10 ⁻⁶ ij	
	Pb(NO ₃) ₂	2000	89.39 g	0.16 ijk	0.33 fg	0.28 ab	0.06 fgh	3.434 × 10 ⁻⁵ h	0.0098 fgh	4.04 × 10 ⁻⁶ h	
		3000	57.88 j	0.20 ghij	0.40 efg	0.25 abcd	0.03 hi	3.910 × 10 ⁻⁵ h	0.0112 fg	4.60 × 10 ⁻⁶ h	
		1000	80.00 h	0.12 k	0.25 g	0.11 ef	0.13 c	1.288 × 10 ⁻⁵ k	0.0037 ghi	1.52 × 10 ⁻⁶ jk	
	Phosphorus	PbSO ₄	2000	40.30 mn	0.27 def	0.56 bcd	0.19 bcde	0.05 ghi	3.434 × 10 ⁻⁵ h	0.0098 cd	4.04 × 10 ⁻⁶ h
			3000	36.67 n	0.31 cd	0.65 ab	0.17 de	0.02 i	3.434 × 10 ⁻⁵ h	0.0098 fgh	4.04 × 10 ⁻⁶ h
			0	116.97 e	0.15 jk	0.33f g	0.32 a	0.07 efg	0.373 × 10 ⁻⁵ k	0.0011 hi	0.44 × 10 ⁻⁶ l
			1000	184.85 a	0.15 jk	0.43 cdef	0.32 a	0.07 efg	2.131 × 10 ⁻⁵ i	0.0061 ghi	2.51 × 10 ⁻⁶ i
Pb(NO ₃) ₂	2000	56.67 jk	0.17 hijk	0.31 fg	0.31 a	0.08 efg	5.528 × 10 ⁻⁵ g	0.0158 ef	6.50 × 10 ⁻⁶ g		
	3000	71.82 i	0.23 fgh	0.52 bcde	0.18 cde	0.07 efg	8.534 × 10 ⁻⁵ d	0.0244 cd	10.04 × 10 ⁻⁶ d		
	1000	140.61 c	0.17 hijk	0.51 bcde	0.07 f	0.17 b	1.488 × 10 ⁻⁵ j	0.0043 ghi	1.75 × 10 ⁻⁶ jk		
	2000	50.30 kl	0.31 cd	0.74 a	0.11 ef	0.12 cd	5.959 × 10 ⁻⁵ g	0.0170 def	7.01 × 10 ⁻⁶ g		
	3000	33.33 n	0.34 bc	0.74 a	0.13 ef	0.05 ghi	6.834 × 10 ⁻⁵ f	0.0195 de	8.04 × 10 ⁻⁶ f		

Means of the same treatments and those in the same column followed by the same uppercase or lowercase letter are not significantly different according to the Duncan multiple range test at $P \leq 0.05$.

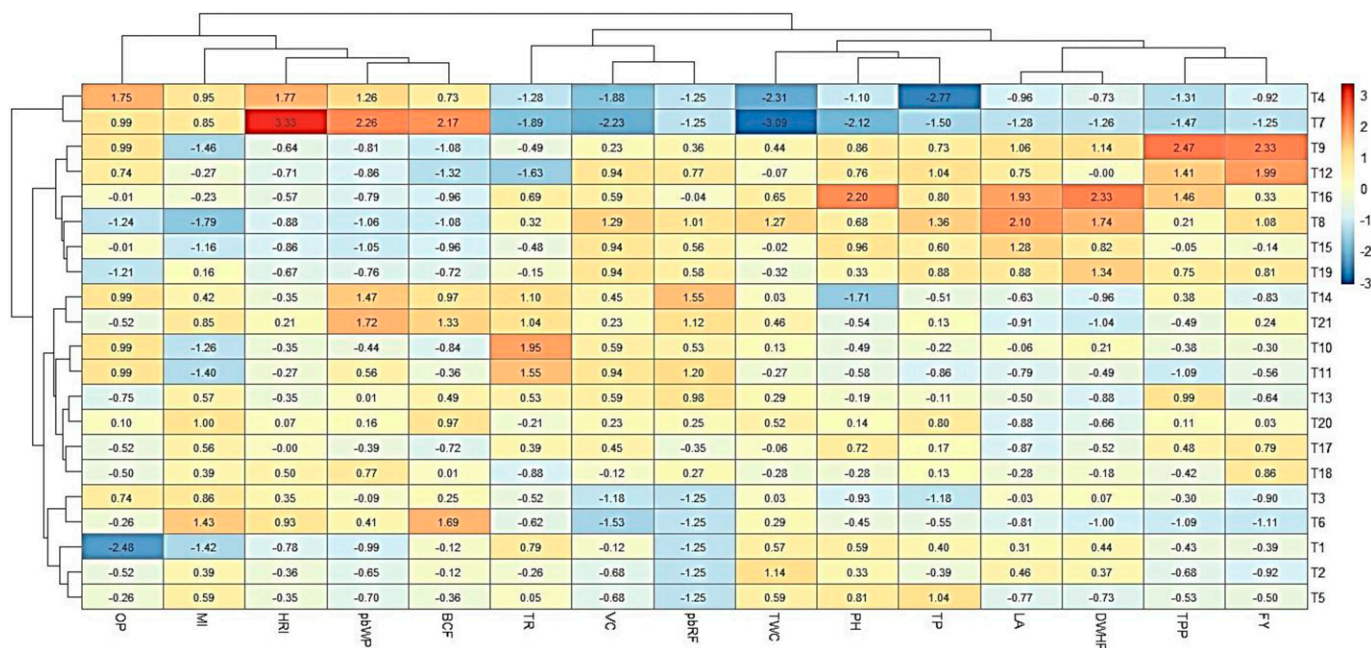


Fig. 5. Heatmap summarizing the relationship between the treatments and the measured traits. Plant height (PH), leaf area (LA), dry weight of the whole plant (DWHP), total photosynthetic pigments (TPPs), total water content (TWC), transpiration rate (TR), osmotic pressure (OP), membrane integrity (MI), fruit yield (FY), vitamin C content in fruits (VC), total protein (TP), Pb concentration of the whole plant (PbWP), Pb concentration of remediation fruits (PbRF), bioconcentration factor (BCF), and health risk index (HRI) of pepper plants after 80 d from transplanting. Treatment (T) 1 = control; T2 = 1000 $\mu\text{g PbSO}_4$; T3 = 2000 $\mu\text{g PbSO}_4$; T4 = 3000 $\mu\text{g PbSO}_4$; T5 = 1000 $\mu\text{g Pb(NO}_3)_2$; T6 = 2000 $\mu\text{g Pb(NO}_3)_2$; T7 = 3000 $\mu\text{g Pb(NO}_3)_2$; T8 = *Bacillus*+0 Pb; T9 = *Bacillus*+1000 $\mu\text{g PbSO}_4$; T10 = *Bacillus*+2000 $\mu\text{g PbSO}_4$; T11 = *Bacillus*+3000 $\mu\text{g PbSO}_4$; T12 = *Bacillus*+1000 $\mu\text{g Pb(NO}_3)_2$; T13 = *Bacillus*+2000 $\mu\text{g Pb(NO}_3)_2$; T14 = *Bacillus*+3000 $\mu\text{g Pb(NO}_3)_2$; T15 = phosphorus+0 Pb; T16 = phosphorus+1000 $\mu\text{g PbSO}_4$; T17 = phosphorus+2000 $\mu\text{g PbSO}_4$; T18 = phosphorus+3000 $\mu\text{g PbSO}_4$; T19 = phosphorus+1000 $\mu\text{g Pb(NO}_3)_2$; T20 = phosphorus+2000 $\mu\text{g Pb(NO}_3)_2$; and T21 = phosphorus+3000 $\mu\text{g Pb(NO}_3)_2$.

soils also stimulated growth, increased yield, controlled plant water relations, protected photosynthetic pigments, and significantly decreased Pb accumulation in plant organs. Moreover, the use of *Bacillus* bacteria as well as P had beneficial effects on the TI, TFR, TFF, and health traits of pepper plants. Compared to P application, *Bacillus* bacteria application more effectively enhanced most of the studied parameters.

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