Natural Resources / Environment (Group Hapi)

Studies on the Effect of Lead and some Agents for Remediation of Lead Ions on the Performance of Carrot Plant (*Daucus carrota L.*)

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Abstract

During the winter seasons of 1998-1999 and 1999-2000, pot trails were conducted at the Experimental Farm, Faculty of Agriculture, Shibin El-Kom, Egypt, with aim to study the performance of carrot plant cv. “Red Core Chantenay” grown in polluted soil with lead in forms of PCl_2 and $Pb(NO_3)_2$ at rates: 0, 1000, 2000 and 4000 μg Pb/g soil, without or with adding some agents for removal lead ions: Bacteria (*Bacillus subtilis, isolate No.13*) and phosphorus. Vegetative growth characters of roots and shoot were significantly inhibited with increasing the Pb concentration in soil. The inhibitory effect of Pb was more severely in the length and size of roots, root and shoot dry weights and root/shoot ratio than others characters, this effect was more pronounced in the presence of Pb $(NO_3)_2$ than in $PbCl_2$ application. Also, both Pb salt types had a deleterious effect on leaf chloroplast pigments, both chlorophyll a and b were more negative affected by Pb ions than that of carotenoids. Leaf water relations were significantly differed in responses to lead. Total water content (TWC), leaf water deficit (LWD) and transpiration were increased, whereas relative water content (RWC) was decreased as a result of Pb application. Pollution of soils with Pb significantly reduced the root, top and total yield of carrots more in the presence of Pb chloride than in that the presence of Pb nitrate. Total soluble solids (TSS) and total soluble sugars were increased, whereas carotene, vit. A, and vit. C were decreased in the roots of carrot plants treated with lead. Pb concentration in both root and top was significantly enhanced more in the presence of Pb chloride than that in the presence of Pb nitrate.
Adding Bacillus bacteria as a bioagent and phosphorus as a chemoagent to the Pb polluted soils not only led to overcome the deleterious effect of untolerable Pb levels (2000 and 4000 μg Pb/ g soil) on most above mentioned characters, but also stimulated the growth, increased the yield, regulated the plant water relation, protected the photosynthetic pigments and sharply reduced the Pb concentration in both root and top. Application of P was the best in this respect.

Key words*:* Carrot plant, lead, bacillus bacteria, phosphorus, growth, photosynthetic pigments, water relations, yield quantity and quality, lead accumulation.

Introduction

Lead is one of the heavy metals and is considered one of the dangerous environmental pollutants. It omitted from industries, motor vehicles, stationary fuel, road dust composition and traffic roads. Lead is not only a toxic element but also can be accumulated in plant organs and agricultural products (Burzynki, 1987; Mahmoud and El-Beltagy, 1998), consequently enter human food chain (Wagner, 1993). As a result of consumption of food, lead accumulates in human body and it may cause renal failer, brain and liver damage and it can attack the nervous system and cause failing of sickness (Lucky and Kenugopal, 1977; Ramade, 1987). Lead has a deleterious effect on crop plants. It was found that the high levels of lead inhibited the growth of higher plants such as wheat (Kletecka and Niklasova, 1986; Karataglis *et al*., 1991), vegetable crops (Ali, 1982; Xian, 1989; Moftah, 2000). Also, lead tended to have an inhibitory effect on some physiological processes, i.e. photosynthesis (Rebenchini and Harzly, 1974; Poskuta *et al*., 1987, Becerril *et al*., 1988), protein synthesis (Stibrova *et al*., 1986; Taiz and Zeiger, 1998) and amino acids (McCrea, 1984; Poskuta *et al*., 1988), carbohydrate and sugar content (Kandil, 1995), activity of some enzymes (Stibrova *et al*., 1986), chlorophylls (Prasa et al., 1989; Tomasevic et al., 1991) and some water relations (Burzynki, 1987; Ewais, 1997). In addition, some investigators found that lead in soil at higher rates decreased the yield of some crops whereas the lower one had an insignificant effect on it (Xian, 1989; Moftah, 2000). Nowadays, great efforts were made to remove or degrade and detoxify the heavy metal pollutants from water and soil using modern technology called bioremediation and phytoremediation. Many investigators used bacteria (Ibeanusi *et al*., 1995; Cuero, 1996; Margeay et al., 1997; Mahmoud and El-Beltagy, 1998) and fungi (Gadd, 1986) as bioremediants as well as algae (Oliguin, *et al*., 1994; Kaplan et al., 1998) and plants (Brown, 1995; Brook and Robinson, 1998) as phytoremediants. In this connection, Cuero (1996) studied the effect of the aminopolysaccrides chitosan and *Bacillus subtilis* each alone and in combination on metal accumulation in sandy loam soil contaminated with heavy metals. Mahmoud and El-Beltagy (1998) isolated and identified some lead tolerant bacteria strains from naturally lead polluted soils and tested them for lead reduction in rocket salad plant grown on polluted soils. They found that the reduction percentage of lead uptake by rocket salad plant using strains No.1 (*Streptomyces ambifaciens*), No.2 (*Streptomyces setonii*), No.13 (*Bacillus subtilis*), No. 15 (*Bacillus cereus*) and mixed were 72.6, 71.2, 96.4, 89.2 and 50.4, respectively. However, study the effect of such bioremediants on the growth, physiology and biochemistry as well as yield of plants were rare or not published. Also, application another agents as lead remediants such as nutrients or chemicals may be still under research. Moftah (2000), tested the responses of lead-polluted tomato and eggplant to the antioxidant ethylendiurea (EDU) and found that EDU treatment seemed to be useful in the counteracting the harmful effects exerted by Pb contamination on tomato and eggplant by regulating certain enzymes.

Therefore, the aim of this investigation was to study the effect of lead pollutant in forms $PbCl_2$ and $Pb(NO_3)_2$ at different rates, with or without application bacteria (*Bacillus subtilis*, isolate No. 13) and phosphorus in form calcium superphosphate, on growth, photosynthetic pigments, water relations and yield quantity and quality of carrot as well as lead accumulation in both root and top of carrot plant.

Material and Methods

Pot experiments were conducted at the Experimental Farm, Faculty Agriculture, Shibin El-Kom, Egypt. Sowing was carried out in plastic pots with 30 cm inner diameter, in October 5, 1998 and 1999 using carrot seeds cv. "Red Core Chantenay" obtained from the Horticulture Dept., Faculty of Agriculture, Minufiya University. Pots were filled with 8 kg clay loam soil taken from the Experimental Farm of Agric. Faculty of Agriculture (ECe=2.8 mmhos/cm; pH=7.9; Soluble salts=0.16%; Pb=68.6 ppm). Pots were divided into two sets: first was mixed with lead chloride and the second with lead nitrate at lead concentrations 0, 1000, 2000 and 4000 µg Pb/ g soil. Each set was divided into three groups: the first without any adding agents, the second inoculated with *Bacillus subtilis*, strain No. 13 grown in NPM and the third with adding phosphorus. *Bacillus subtilis* strain No. 13 as a Pb tolerant isolate was obtained from the Agricultural Microbiology Branch, Agric. Botany Dept., Faculty of Agriculture, Shibin El-Kom, Egypt. In P treatments, phosphorus was added as calcium superphosphate (15.5% P_2O_5) at rate 4 g/pot. Pots were irrigated with tap water whenever to keep the moisture in soil at about 65% of the total water holding capacity of the soil during the experimental period.

One week before the harvest time, a random sample of 10 plants was carefully taken from each treatment and the following measurements were done:

- Vegetative growth characters: Root length (cm), root and core diameter (cm), root size (cm^3), root, shoot and whole plant dry weights (g/plant), then the root/shoot ratio was calculated.
- Photosynthetic pigments were extracted from fresh leaves using acetone 80% and estimated according to Wettestein (1957), then calculated as mg/g dry weight.
- Leaf water relations: Total water content (TWC, %), relative water content (RWC, %), leaf water deficit (LWD, %) and transpiration rate (mg/ g fwt. h) according to Kalapos (1994) and Kreeb (1990).

At the harvest time, plants in each treatments were carefully taken and cleaned then the root, top and total yield/pot were estimated then calculated as kg/m^2. The harvest index (%) was measured using the formula (HI= (root yield/total yield)*100). Another random sample was taken and the total soluble solids (%) using an Abe hand refractometer, vit. A (IU/100 g fwt.), vit. C. (mg/100 g fwt.) and carotene (mg/ 100 g fwt.) in fresh root were estimated using the methods of A.O.A.C. (1970). Hundred grams from the roots and tops from each treatment were dried at 70°C, 0.2 gm from each dried ground organs was acid digested for estimation the total lead concentration (µg/ g dwt.) using Atomic Absorption Spectrophotometer at Faculty of Science, Minufiya University according to Allen (1974). Another 0.2 gm from dried ground root was used to determine the soluble sugars concentration (mg/g dwt.) according to Dubois *et al*., (1956).

A randomized complete block design with ten replicates was used. Data were statistically analyzed and the L.S.D. test at 5% level of probability was used to compare the means of the treatments (Waller and Duncan, 1969) with help the COSTAT C Statistical package (American Computer Program).

Results and Discussion

Vegetative growth

Root length: Data presented in Table (1) illustrate that lead at all levels significantly inhibited the root length and it was more severely at the high Pb level. Under the intolerable level (4000 µg Pb/g soil) the reduction in it of plants grown in soil polluted with $PbCl_2$ and $Pb(NO_3)_2$ reached about 13.1 and 25.5% (1st season) and 22.4 and 34.4% (2nd season) compared with the non treated plant, respectively, indicating that $Pb(NO_3)_2$ had more harmfully effect on root length. These results are in accordance with those obtained by Stiborova *et al*., (1986) and Obraucheva *et al.,* (1998).

In the unpolluted soil, adding bacillus bacteria caused a slight increase in it, whereas adding P significantly increased it. In the polluted lead soils at all Pb levels, application of both bacteria and P had a high significant effect in this respect. The increase in it resulted from adding bacillus bacteria to the polluted soil with $PbCl_2$ and $Pb(NO_3)_2$ at 4000 µgPb/g soil was about 15.8 and 19.3% (1st season), 30.7 and 33.3% (2nd season), whereas with adding P to both polluted soils, the increase was about 28.6 and 17.8% (1st season), 42.3 and 35.2% (2nd season), respectively. This indicates that application P to the polluted soil with $PbCl_2$ and $Pb(NO_3)_2$ was more useful for stimulating the growth of root.

Root diameter: The same trend of root length was observed in root diameter, but the percentages of reductions in root diameter of plants grown in soil polluted with 4000 µgPb/g soil were lower (-12.8 and -19.4% for $PbCl_2$; -16.3 and –26.8% for $Pb(NO_3)_2$). Also, inoculation the lead polluted soil with bacteria or adding P had a positive effect in this respect. Again, P was more effective in this respect.

Root size: It can be seen from the same Table that the root size was sharply decreased with increasing the Pb level in soils polluted with $PbCl_2$ and $Pb(NO_3)_2$ recording the smallest size at the level of 4000 µgPb/g soil with reduction reached about –33.9 and –47.8% (1st season), -50 and -64.8% (2nd season) less than the untreated plants. Similar results were achieved by Stiborova *et al*. (1986) and Obraucheva *et al*. (1998).

Treating the lead chloride and nitrate polluted soils with bacillus led to a great increase in root size reached to about 54.6 and 76.9% (1st season), 71.1 and 96.4% (2nd season), whereas its treating with P increased it more and reached about 79.3 and75.6% (1st season), 123.7 and 150.1% (2nd season) over the plants treated with 4000 µg Pb/g soil of two salt types.

Core diameter: A slight change in core diameter as a result of Pb treating was observed in the 1st season but a marked decrease was recorded in the 2nd one. Using bacillus bacteria in the lead polluted soils tended to be more effective in increasing the core diameter than using phosphorus (Table 1).

Plant height: A significant decrease in plant height was observed in the plants soils treated with 2000 (-20.9, -14.2, 1st season, and –23.01, -15.4%, 2nd season, for Pb chloride and nitrate respectively) and 4000 (-31.7, -35.3%, 1st season and –30.2, -28.2%, 2nd season) µg Pb/g soil of both salt types, whereas the low level (1000 µgPb/g soil) tended to increase it. The obtained results are in agreement with those reported by Gadallah (1995) who found that the heavy-metal toxicity appears in the reduction of plant height and dry mass accumulation.

The results obtained in Table (1) indicate that application of bacillus bacteria to the lead polluted soils by $PbCl_2$ and $Pb(NO_3)_2$ did not show a clear trend in this respect, whereas application of P overcame the deleterious effect of Pb and increased the plant height by about 22.5 and 21.8% in the 1^{st} season, 28.5 and 21.7% in the 2^{nd} season, respectively.

Dry weights of root, shoot and whole plant: Data presented in the same Table show that the dry weights of root and shoot as well as whole plant were significantly decreased with increasing the Pb level in soil. At the highest level of Pb (4000 µg Pb/g soil), the reduction in the dry weights of root, shoot and whole plant grown in polluted soils with $PbCl_2$ reached about –59.3, -43.5 and -54.2%, respectively, (1^{st} season); -32.9, -7.8 and –23.7% (2^{nd} season), but in the polluted soils with $Pb(NO_3)_2$ were about -68.9, -41.6 and –60.2% (1^{st} season); -35.1, -5.9 and 24.5% (2^{nd} season). These results indicate that Pb in form $Pb(NO_3)_2$ was more harmfully than in form $PbCl_2$. Similar results were obtained by Carlson et al. (1975) on maize plant, Ali (1982) on pepper and jews mellow plant; Gadallah (1995) on and Begonia *et al*., (1998) on *Brassica juncea* plant.

Inoculating the lead chloride polluted soils with bacillus bacteria as well as adding P was not only more useful in overcame the inhibitory effect of Pb on root dry matter but also a great increase in it (97.8 and 129.3% for bacteria; 57.6 and 154.4% for P) was recorded. Shoot dry matter as well as whole plant tended to be more affected by bacillus bacteria (35.2 and 101.1% for shoot; 73.1 and 116.9% for whole plant) than that by P (16.5 and 26.6% for shoot; 41.3 and 98.1% for whole plant). Regarding the polluted soils with lead nitrate, it was found that adding both of bacillus and P showed a positive effect on root dry matter but shoot and whole dry matters tended to be more affected by P than bacillus.

Root/Shoot ratio: Data in the same Table show that a significant reduction in R/S ratio in all Pb treatments was observed. In this connection, Mishra and Choudhuri (1998) found that Pb decreased shoot/root ratio of two cultivars rice differing in their tolerance to heavy metal stress. Using both bacteria and P in the untreated and polluted soils led to a great increase in it. Bacillus was more effective in the lead nitrate polluted soil whereas P was more useful in case of the lead chloride polluted soils.

The deleterious effect of lead at the intolerable levels (2000 and 4000 µg Pb/ g soil) on growth of carrot plant may be due to lead retarded cell division and differentiation thus inhibited their elongation and that lead to a reduction in plant growth (Kastori et al., 1998). Moreover, the inhibition in root growth may be ascribed to the toxic effect of lead on the meristimatic region of root, thus retarded their growth and distribution (Stiborova *et al.*, 1986) or/and that may be due to its inhibition effect on both cell division and elongation through the reduction of meristem size and decreasing the number of mature cells (Obroucheva *et al*., 1998).

The stimulating effect on growth parameters of carrot plant as a result of adding bacillus bacteria to the lead polluted soil may be attributed to action of bacteria for bioremediation of lead from contaminated soil thus inhibited its toxic effect (Ibeanusi et al., 1995), or may be due to their beneficial effect on improving nutritional status, producing growth regulators i.e. IAA, GA and cytokinins (Lazarovites, 1995; Arshad and Frankenberger, 1991) or to their ability to produce anti-bacterial and anti-fungal compounds that reduce diseases (Pandy and Kumar, 1989). As for the favourable effect of P on the growth may be due to its effect as a growth limiting factor or due to enhancing the absorption of other nutrients (Marschner, 1995), beside its inhibitory effect on lead by precipitation of lead ions.

Photosynthetic pigments

Data presented in Table (2) reveal that a sharply decrease and degradation in both chlorophyll a and b as well as total chlorophyll and carotenoids. In the lead chloride polluted soil, the percentage reduction in the above-mentioned pigments were about –90.9, -89.1, -90.2 and -44.5%, respectively, in the 1st season, and about –95.9, -90.7, -94 and -36.4 % in the 2nd one. Meanwhile in the lead NO_3 polluted soil, they were about –88.8, -53.9, -75.3 and –80.7% (1st season); -80.5, -90.7, -84.3 and –63.1% (2nd season), respectively. This indicates that Pb in any form had a severely harmful effect on all photosynthetic pigments. Similar results were reported by Burzynski (1985); Stbirova *et al*., (1986); Sengar and Pandey (1996) and Fodor *et al*. (1998). The deleterious effect of lead on chloroplast pigments may be due to that Pb inhibits the biosynthesis of aminolevulinic acid (ALA) a precursor of chlorophyll (Thomas and Singh, 1996), and/or stimulates the activity of chlorophyllase and chlorophyll degradation (Abdel Basset *et al.,* 1995), and/or it can alter chlorophyll biosynthesis by inhibiting protochlorophllide reductase through interfering the sulfhydryl site on the enzyme (Lagriffoul *et al*., 1998), and/or it decreases the carotenoids that prevent chlorophyll photodestruction, or/and it inhibits Fe uptake and transport to plant leaves (Fodor *et al*., 1998).

Treating the lead polluted soils with bacillus and P not only led to counteracting the inhibitory effect exerted by Pb but also increased their concentrations from seven to ten times for chl. a, one to five times for chl. b, one to seven times for total chlorophyll, and less or more than unit for carotenoids. P was more effective than bacillus in this respect.

The role of bacteria in enhancing the photosynthetic pigments may be attributed to its indirect effect by reducing the concentration of lead ions in root medium to extent to become non toxic. The promoting effect of P on photosynthetic pigments under the normal conditions as well as the lead polluted soils may be due to its effect on reducing the concentration of lead ions as shown our results in the same work and/or due to its providing the plants with ATP and NADPH and other compounds that play a vital role in biosynthesis of chlorophylls and other pigments (Marschner, 1995).

Leaf water relations

Data recorded in Table (3) indicate that the leaf total water content (TWC) tended to be decreased in the plants grown in the lead chloride polluted soils (-3.44% and –1.635% at the rate of 4000 µg Pb/g soil), whereas it tended to be increased in the lead nitrate polluted soil (+8.11 and +7.4% at the same Pb rate). In the unpolluted soils, application bacillus bacteria seemed to have not a clear effect on TWC (increased in the 1st season but decreased in the 2nd one), whereas adding P led to decrease it. In the lead-polluted soils, bacillus treatments caused a decrease in TWC ranged from 2 to 7%, whereas P treatments increased it.

Concerning the relative water content (RWC) and leaf water deficit (LWD) in relation to Pb treatments, agents treatments as well as their interactions, it was found that RWC sharply decreased in the Pb polluted soils with $PbCl_2$ (-14.52 and –11%) and $P(NO_3)_2$ (-15.5 and –12.62%) at rate 4000 µg Pb/g soil, whereas LWD was dramatically increased by 66.9 and 49.4% (chloride) and 71.4 and 56.8% (nitrate) at the same Pb rate. Treating the polluted soils with bacillus bacteria relatively improved RWC and increased it by about 7.2 and 6.6 (chloride); 11.1 and 12.3% (nitrate), whereas treating with P increased it more (15.4 and 16%, chloride; 22.6 and 19.9%, nitrate). LWD was decreased by about –16.9 and -17.7% (chloride); -25.3 and –31% (nitrate) in the polluted soils treated with bacillus, meanwhile the decrease in it was higher as a result of treating with P and reached about –36.3 and -42.9% (chloride); -51.3 and –49.8% (nitrate). Regarding the rate of leaf transpiration (TR), data in the same Table show that TR was significantly increased under lead contamination conditions reached about 55 and 25.9% (chloride); 30.2 and 58.2% (nitrate) at rate of 4000 μg Pb/g soil. Both bacillus bacteria and P regulated the loss of water from leaves and decreased its rate. P treatment was more effective than bacillus in this respect. The obtained results agreed with those obtained by Burzysnki (1987) who found that the placement of two week old bean, wheat and cucumber plants in lead chloride solution caused a significant decrease in transpiration and water uptake.

Yield attributes

Root, top and total yield: The results obtained in Table (4), show that the root and top as well as total yield of carrot were negatively affected by Pb treatments and were severely in the polluted soils with Pb chloride and nitrate at rates of 2000 and 4000 μg Pb/g soil. The highest reduction was recorded in root yield (-56.3 and –57.14, (Pb chloride); -45.8 and -58.1% (Pb nitrate)) followed by total yield (-51.8 and –49.8%, (Pb chloride), -34.8 and –45.8% (Pb-nitrate). A higher reduction in top yield was observed in the plants grown in contaminated soils with $PbCl_2$ (-41.5 and –33.8%) whereas a lower reduction in it (-10.1 and -19.2%) was recorded in soils with $Pb(NO_3)_2$ indicating that lead chloride was more harmful than nitrate. Similar results were reported by McCrea (1984) and Xian (1989) on kidney beans and Moftah (2000) on tomato and eggplant.

Adding bacillus bacteria to the lead polluted soils led to counteract the deleterious effect lead and increased the root, top and total yields by about 30.1, 35.9 and 32.3% (1^{st} season); 72.3, 20 and 42.9%(2^{nd} season), respectively, in the soils polluted with $PbCl_2$, meanwhile by about 13.5, 12.7 and 13.1% (1^{st} season); 94.9, 3.3 and 51.9% (2^{nd} season) in the soils polluted with $Pb(NO_3)_2$. Using P as a remediant for lead did not only remove the harmful effect of lead but also gave higher increases in root yield (106.4 and 65.3%), top yield (88 and 25%) and total yield (99.6 and 48.6%) in case of $PbCl_2$. The same trend was observed in the soil polluted soils but the percentage increases in yield were lower.

Harvest index: Data in the same Table indicate that the Harvest index (HI) was significantly decreased at all Pb levels and recorded the lowest values at the rates of 2000 and 4000 μg Pb/g soil. These reductions ranged from about 9 to 25%. Adding bacillus bacteria to the polluted soils had no significant effect on it in the 1^{st} season but a clear increase (21.1 and 28.3%) was observed in the 2^{nd} one. A slight increase in HI (3.4 and 4.6%) in the 1^{st} season but a relatively increase (11.2 and 13.1%) in the 2^{nd} one was recorded with the P treatments.

The inhibitory effect of lead on the above mentioned yield attributes of carrot may be due to its toxic effect on growth as shown our results in the same work and its inhibitory effect on the uptake and translocation of some major and micro elements within plant roots (Larcher, 1980); activity of some enzymes as well as biosynthesis of photosynthetic pigments which reflect an carrot yield. Adding bacteria or P led to a marked increase in yield components and that may be not only due to its role in removing the toxic effect of lead but also its role that play in promotion of strong root and shoot (Mohandas, 1987), the high bacterial production of phytohormones (Lazarovites, 1995), improving nutrition (Lazarovites, 1995) as well as increasing the root weight (Selim, 1999).

Chemical properties of roots

Total soluble solids (TSS): Data given in Table (5) reveal that TSS was positively affected by Pb application more in the presence of lead nitrate (28.9 and 26.8%) than in lead chloride (3.8 and 5%). TSS tended to be decreased in the polluted soils and treated with bacillus bacteria but increased in the roots of plants grown in non polluted soils. P significantly increased it in the non-polluted and polluted soils with $PbCl_2$, however a higher decrease (-24.1 and -21.2%) was observed by $Pb(NO_3)_2$.

Carotene: A significant decrease in carotene content was found in the roots of plants grown in contaminated soils with lead chloride and nitrate at rates of 2000 and 4000 µg Pb/g soil but a slight increase in it was found at rate 1000 µg Pb/ g soil (Table 5). Treating the polluted soils with bacillus bacteria gave a great increase in root carotene content arrived to 56.5 and 110.14% in the presence of $PbCl_2$, and to 101.2 and 48.1% in the presence of $Pb(NO_3)_2$. P treatments gave higher increases in the presence of $PbCl_2$ (85 and 122%) but lower increases in the presence of $Pb(NO_3)_2$ (48.8 and 11%). Similar results were observed by Moftah (2000) on tomato and eggplant.

Vitamin A: It was found that Vit. A was significantly decreased with increasing the Pb concentrations in the lead polluted soils, reached the lowest content at the highest rate of Pb (4000 µg Pb/g soil), (Table 5). The deleterious effect of lead was more pronounced in the presence of $PbCl_2$ (-25.3 and –38%) than in the presence of $Pb(NO_3)_2$ (-17.6 and -21.3%). Adding bacillus bacteria to the non polluted soils caused a significant increase in it whereas in the polluted soils did not show a clear trend. Using P in both the non-polluted as well as lead chloride polluted soils resulted in increasing Vit. A, meanwhile a slight increase in the presence of lead nitrate.

Vitamin C: Data given in the same Table demonstrate that Vit. C in root was negatively affected by Pb at all concentration and was more severely at the rate of 4000 µg Pb/g soil in both salt types. The reduction in it reached about –27.4 and –30.1% in the presence of lead chloride and –49.1 and –25.6% in the presence of lead nitrate if compared with the control. Inoculation the non-polluted as well as the polluted soils by lead nitrate with bacillus bacteria led to a significant increase but not in presence of lead chloride. Adding P to the non and the polluted soils with lead (two types) overcome the deleterious effect and increased it by about +15 and +36.1 (Chloride) and +27.3 and +2.3% (Nitrate).

Soluble sugars: Data illustrated in Fig (1) showed that the total soluble, reducing and non-reducing sugars were markedly decreased under all levels of lead application. Under the lead stress conditions, application of both bacillus and P led to marked increases in the concentrations of total soluble and non-reducing sugars but the reducing sugars were decreased. In this respect, Kandil (1995) revealed that lead decreased total, soluble and non-soluble carbohydrates of wheat grains. Also, Ali (1982) found that 100-1000 ppm of lead as foliar application or soil treatment decreased the non-reducing sugars in some vegetable crops. The reduction in carbohydrate concentration as a result of lead treatment may be attributed to Pb causes a decrease in the photosynthetic pigments (Sengar and Pandey, 1996) and ribulose diphosphate carboxylase (the key enzyme for carbohydrates synthesis) which in turn in decreasing in all sugar fractions (Stibrova *et al*., 1986).

As shown from the above mentioned results, adding bacillus bacteria or phosphorus as calcium superphoshate to the polluted and non polluted soils with lead significantly improved the chemical properties of carrot roots and that may be due to its promotion effect on growth and yield as well as vital roles in physiological and biochemical pro??cesses in plant. In this connection, Antipchuk *et al*. (1982) and Ali and Selim (1996) observed that inoculation of tomato plants with Azotobacter resulted in a rise in fruit sugars and vitamin C contents. Bagal *et al*. (1989) found that protein, sugars, ascorbic acid and mineral contents were significantly increased by increasing the rates of N, P and K application. Marschner (1995) stated that P is a component of RNA and DNA, therefore, it might be expected that P supply would have important effect on biosynthesis of many compounds e.g. sugars, proteins and hormones. Moreover, Bender *et al.* (1986) revealed that photosynthetic CO_2 fixation and assimilates translocation were considerably increased in plant with the optimum P supply in comparison with the low P level.

Lead concentration

Data illustrated in Fig. (2&3) that Pb accumulated more in shoot than in root and reached about five times. In both shoot and root Pb concentration was dramatically increased with increasing the Pb rates in soils. At the highest level of Pb, the increase percentage in Pb accumulation in shoot reached about 900 and 985.4% in the presence of $PbCl_2$, about 433.3 and 448.9% in the presence of $Pb(NO_3)_2$, whereas in root reached about 85.7 and 115.5% in the presence of $PbCl_2$, while about 57.2 and 84.6% in the presence of $Pb(NO_3)_2$, if compared with its concentration in the control plants. This indicates that carrot plants prefer to Pb uptake in salt form $PbCl_2$ more than in form $Pb(NO_3)_2$. These results may be explained why the deleterious effect of lead chloride on most characters measured in shoots was more extremely. The obtained results are in agreement with some those obtained by Gaweda (1995 and 1997), Hooda *et al.* (1997) and Moftah (2000).

In the polluted lead soils with $PbCl_2$ at rates of 1000, 2000 and 4000 µg Pb/g soil, adding bacillus bacteria as bioremediant for lead ions led to a reduction in its concentration by about 50.1, 43.4 and 51.6% in shoot, and by about 44.6, 51.3 and 53.2% in root, respectively, whereas in the presence $Pb(NO_3)_2$ were about 32.3, 32.2 and 65.1% in shoot, 44.1, 42.4 and 49.8% in root, (Fig. 4). Regarding the effect of using P as a chemical agents for lead remediation, it was found that adding P to the contaminated soils with $PbCl_2$ at the same above mentioned rates reduced Pb concentration by about 62, 36.6 and 59.3% in shoot, about 48, 60.4 and 72.1% in root, whereas in the presence of $Pb(NO_3)_2$ the reductions were about 38.4, 53.8 and 55.8% in shoot, about 58.7, 47.9 and 54.2% in root (Fig. 4). These results pointed out that using the P was more useful for lead remediation and consequently overcome the toxicity effect of lead on carrot plant. The obtained results of bacillus-lead interaction are in accordance with those reported by Mahmoud and El-Beltagy (1998), who found that using bacillus bacteria strain No. 15 in the naturally lead-polluted soils reduced the lead uptake by 96.4% in the rocket salad plant, whereas the reduction was 73.49% in the soil polluted with 400 ppm lead. The reduction in lead accumulation as a result of using bacteria as bioremediants may be attributed to precipitation of metal ions, adsorption at bacterial sites and reduction by change of oxidation states (Ibeanusi *et al*., 1995)

As for the P-lead interaction, Gaweda (1997) indicated that using phosphorurs (800 mg P/kg DW), calcium (1500 mg Ca/kg DW) and magnesium (240 mg/kg DW) as fertilizer or liming to increase soil pH from 5.1 to 6.2 considerably limited the accumulation of Pb in carrot roots.

Figures 1 - 4[4]

References:

Abdel-Basset, R.; A.A. Issa and M.S. Adam (1995): Chlorophyllase activity: Effects of heavy metals and calcium. Photosynthetica 31: 421-425.
Ali, E.A. (1982): Physiological studies on the contamination and toxicity of some plants by certain heavy metals. Ph.D. Thesis, Faculty of Agric. Cairo University.
Ali, F.A. and A-F. H. Selim (1996): Response of tomato (*Lycopersicon esculentum* L. Castle Rock) to inoculation with *Azotobacter* and different levels of phosphorus and potassium fertilization. Menofiya J. Agric. Res., 21(4): 795-817.
Allen, S.E. (1974): Chemical analysis of ecological materials. Bluckuel Scientific Publications. Goney Mead., Oxford, 563 pp.
Antipchuk,A.F.; E.V. Tantsyurenko and R.M. Mantselyruk (1982): Effect of bacteria on tomato yield and quality. In Tekhnologiya Pr-Vai Effektinost Primeneiya Bakteri-Lnnkh Udobrenii, Moscow, USSR: 98-103.
A.O.A.C. (1970): Association of Official Agricultural Chemists. Methods of Analysis. 11th Ed. The Association, Washington, D.C.
Arshad, M. and W.T.Jr. Frankenberger (1991): Microbial production of plant hormones. In: Rhizosphere and Plant Growth (D.L. Keister and P.B. Cregan, Eds), Kluwer, Dortrecht, pp 327-334.
Bagal, S.D.; G.A. Shaikh and R.N. Adsule (1989): Influence of different N, P and K fertilizers on the protein, ascorbic acid, sugars and mineral contents of tomato. J. Maharashta Agric. Univ., 1989, 14(2): 153-155 (C.F. Hort. Abstr., 1992, Vol. 62).
Becerril, J.M.; A. Munoz-Rueda; P. Aparicio-Tejo and C. Gonzallez-Murua (1988): The effects of cadmium and lead on photosynthetic electron transport in clover and lucerne. Plant Physiol. Biochem., 26: 357-363.
Begonia, G.B.; C.D. Davis; M.F.T. Begonia and C.N. Gray (1998): Growth responses of indian mustard (*Brassica juncea*, L.) and its phytoextraction of lead from a contaminated soil. Bulletin Environ. Contam. and Toxic, 61(1): 38-43.
Bender, L.; B. Steibeling and K.H Neumann (1986): Investigations on photosynthetic and assimilates translocation in *Daucus carrota* L as influenced by a varied phosphorus supply and changes in the endogenous hormonal system following GA_3 treatments. Zeitschrift für Pflanzenernährung und Bodenkunde, 149 (5): 533-540.
Brooks, R.R. and B.H. Robinson (1998): Aquatic phytoremediation by accumulator plants. Plants that hyper accumulate-heavy metals: their role in phytoremediation, microbiolgy, archaeology, meniral exploration and phytomining, 203-226, CAB International, Walling Ford; UK.
Brown (1995): The green clean. Bio., 45: 579-582.
Burzynki, M. (1987): The uptake and transpiration of water and the accumulation of lead by plant growing on lead chloride solutions. Acta Soc. Bot., 56: 271-280.
Cuero, R.G. (1996): Enhanced heavy metal immobilization by a bacterial-chitosan complex in soil. Biotechnology Letters, 18(5): 511-514.
Dubois, M.; K.A. Gilles; J.K. Hamilton; P.A. Robers and F. Smith (1956): Colorimeteric methods for determination of sugars and related substances. Anals Chem., 20: 350-356.
Ewais, E.A. (1997): Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. Biol. Plant., 39: 403-410.
Fodor, F.; E. Cseh; A. Varga and G. Zaray (1998): Lead uptake, distribution and remobilization in cucumber. J. Plant Nutr., 21: 1363-1373.
Gadallah, M.A.A. (1995): Effects of cadmium and kinetin on chlorophyll content, sacchrides and dry matter accumulation in sunflower plants. Biol. Plant., 37: 233-240.
Gadd, G.M. (1986): The uptake of heavy metals by fungi and yeasts: The chemistry and physiology of the process and applications for biotecnology. In: Immobilization of ions by biosorption. Eccles H. and S. Hut (eds.). Ellis Horwood, Chchester, U.K., 135-147.
Gaweda, M. (1995): The effect of organic matter in soil on the lead level in edible parts of lettuce and carrot. Acta Hort., 379: 221-228.

[4] Figures 1-4 and the tables 1 to 5 are only available in the print copy (Beiheft zu Der Tropenlandwirt Nr. 71).

Gaweda, M. (1997): The control of lead accumulation in carrot (*Daucus carrota* L.) plants by some components of the substrate. J. Appl. Genet., 38A: 206-213.
Hooda, P.S.; D. McNulty; B.J. Alloway and M.N. Aitken (1997): Plant availability of heavy metals in soils previously amended with heavy applications of sewage sludge. J. Sci. Food and Agric., 73(4): 446-454.
Ibeanusi, V.M.; E.A. Archibold and B.S. Schepart (1995): Mechanisms of heavy metal uptake in a mixed microbial ecosystem. C.F. Bioremediation of pollutants in soil and water.
Kalapos, T. (1995): Leaf water potential, leaf water deficit relationship for ten species of a semi arid grassland community. Plant and Soil, 160: 105-112.
Kandil, O.S. (1995): Effect of some sources of pollution on wheat plants. M.Sc. Thesis, Faculty of Agric. , menufiya Univ.
Karataglis, S.; M. Moustakus and L. Symeonidis (1991): Effect of heavy metals on isoperoxidase of wheat. Biol. Plant., 33: 3-9.
Kaplan, D.; N.T. Prakash and A. Abeliovich (1998): Glutathion-induced recovery in Chlorella cells from metal toxicity. Fresenius-Environ. Bulletin, 7: 153-159.
Kastori, R.; M. Plesnicar; Z. Sakac; D. Pankovic and I. Arsenijevic (1998): Effects of excess lead on sunflower growth and photosynthesis. J. Plant Nutr., 21: 75-85.
Kletecka, J. and Z. Niklasova (1986): Evaluation of the effect of lead in soil of some cereals, grasses and legumes. Rostinna-Vyroba, 32: 229-237.
Kreeb, K.H. (1990): Methoden zur Pflanzenokologie und Bioidikationen. Gustav Fisher, Jena, 327 pp.
Lagriffoul, A.; B. Mocquot; M. Mensh and J. Vangronsveld (1998): Cadmium toxicity effects on growth, mineral and chlorophyll contents and activities of stressed enzymes in young maiz plants (*Zea mays*, L.). (cited after Moftah, 2000).
Larcher, W. (1980): The utilization and cycling of mineral elements. In Physiological Plant Ecology, pp. 158-205. Spring Veralg, New York.
Lazarovites, G. (1995): Application of growth promoting rhizobacteria to transplant plug and seed. Agriculture & Agrifood Canada, Pest Management Research Center, London, Ontario,Canada, N5M 4T3 (C.F. Hort. Sci., 3(3), 1995).
Lucky, T.D. and B. Venugopal (1977): Metal toxicity in mammals. Plenum Press NewYork and London.
Mahmoud, W.H and A. El-Beltagy (1998): Isolation, identification and potential use of lead reduction from heavy metal polluted soils. Menufiya J. Agric. Res., 23(6): 1461-1473.
Margeay, M.; J.R. Wild; S.D. Varfolomeyev and A. Scozzafava (1997): Microbial resources for bioremediation of sites polluted by heavy metals. Perspectives in bioremediation; technologies for environmental improvement. Proceedings of s Workshop, Lviv, Ukraine, 65-73. Kluwer Academic Publishers; Dordercht; Netherlands.
Marschner, H. (1995): Mineral nutrition of higher plants, 2nd edition. Academic press, New York, Tokio, London, pp. 889**.**
McCrea, P.R. (1984): An assessment of the effects of road dust on agricultural production system. Research Report in Agricultural Economics, Newzealand, 156: 10-18.
Mishra, A. and M.A. Choudhuri (1998): Amelioration of lead and mercury effects on germination of rice seedling growth by antioxidants. Biol. Plant., 41:469-473.
Mohandas, S. (1987): Field response of tomato (*Lycopersicon esculentum* Mill, “Pusa Ruby”) to inoculation wit a VA mycorrhiza fungus Glomus fasiculatum and Azotobacter vinelandii. Plant and Soil., 98: 295-297.
Moftah, A.E. (2000): Physiological responses of lead-polluted tomato and eggplant to the antioxidant ethylendiurea. Menufiya Agric. Res., 25(4): 933-955.
Obraucheva, N.V.; E.I. Bystrova; V.B. Ivanov; O.V. Antipova and I.V. Seregin (1998): Root growth response to lead in young maize seedlings. Plant and Soil, 200: 55-61.
Oliguin, E.J.; L. Gonzalez; F. Sanchez and J.J. Pena-Cabriales (1994): Bioremediation potential ion. Mexico. 15th World Congress of Soil Science, Acapulco, Mexico, 10-16, 1994.
Pandy, A. and S. Kumar (1989): Potential of Azotobacters and Azospirilla as biofertilizer for upland agriculture. A review. J. Scientific and Industerial Research, 48(3): 134-144.
Poskuta, J.W.; E. Parys and E. Romanoska (1987): The effect of lead on the gaseous exchange and photosynthetic carbon metabolism of pea seedlings. Acta. Soc. Bot. Poll., 56: 127-137.
Poskuta, J.W.; E.Parys; E. Romanoska; M. Gagdis and B. Worblewska (1988): The effect of lead on photosynthesis C^{14} distribution among photoassimilates and transpiration of maize seedling. In: Quantitative organic microanalysis, edt. J. and A. Churchill, London.
Prasad, D.D.K.; G. Sathi and A.R.K. Prasad (1989): Regulation of prophrin biosynthesis by lead and mercury in mug bean (*P. vulgaris*) seedlings. Biochem. Inst., 19: 1403-1417.
Ramade, F. (1987): Ecotoxicity. John Wiley and Sons, Chichester, 119-135.

Rebenchini, H.M. and I. Harzly (1974): Lead induced ultrastructural changes in the chloroplast of hydrophyte *Ceratophylum demersun*. Z. Pflanzen Physiol., 73: 377-386.
Selim, A-F. H. (1999): Root characters, leaf area index and duration, yield and water use efficiency of sugar beet plants under several nutrient regimes. The International Conference on “Water Resources Management, Use and Policy in Dry Areas”, 1-3 December 1999, Amman, Jordan.
Sengar, R.S. and M. Pandey (1996): Inhibition of chlorophyll biosynthesis by lead in greening *Pisum sativum* leaf segments. Biol. Plant., 38(3): 459-462.
Stibrova, M. ; M. Doubravova; A. Brezlnova and A. Fridrich (1986): Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley. Photosynthetica, 20: 416-425.
Taiz, L. and E. Zeiger (1998): Heavy metal stess and homeostasis. In: Plant Physiology. Sinauer Assoc., Mas., USA, p 116.
Tomas, R.M. and V.P. Singh (1996): Reduction of cadmium-induced inhibition of chlorophyll and carotenoids accumulation in Cucumis sativus L. by uniconazole (S.3307). Photosynthetica, 32: 145-148.
Tomasevic, M.; M. Bogdanovic and D. Stoiunovic (1991): Influence of lead on some physiological characteristics of bean and barley. Perlodicum Biologorum, 93: 337-338.
Wagner, G.J. (1993): Accumulation of heavy metals in crop plants and its consequences to human health. Adv. Agron., 51: 173-177.
Waller, P.A. and D.B. Duncan (1969): A bays rule for symmetric multiple comparison problem. Amer. Sta. Assoc. J., pp. 1485-1503.
Wettestein, D.V. (1957): Chlorophyll-Letal und submikroskopische Formelwechsel der Plastiden. Exptl. Cell Res., 12: 427-433.
Xian, X. (1989): Responses of kidney bean to concentration and chemical form of cadmium, zinc and lead in polluted soil. Environ. Poll., 57:127-137.