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PHYSIOLOGICAL STUDIES ON THE EFFECT OF SOME BENEFICIAL MICROORGANISMS ON LETTUCE PLANTS POLLUTED BY LEAD

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ABSTRACT

The present investigation was conducted during the two winter seasons of 2004/2005 and 2005/2006 to study the responses of growth characters, leaf water relations, chemical components, yield and its attributes to lead pollution treatments, the inoculation with different effective microorganisms and their interaction. Two pot experiments were carried out to investigate the effect of lead (Pb) pollution in different concentrations (0, 100, 250 and 500 mg/l) and the application of beneficial microorganisms [Halex 2 or EM (biofertilizers)] or lead bioremediator *Bacillus subtilis* strain in order to overcome the harmful effect of lead treatments on lettuce (*Lactuca sativa* L.) plants. The obtained results revealed that growth characters of lettuce plants expressed as plant height, root length, number of leaves, leaf length and width, leaf area as well as dry weight of roots and shoots were markedly decreased in response to lead pollution treatments. The highest significant reduction in these characters was more pronounced at higher lead rate (500 mg/l). RWC (%), photosynthetic pigments (chl. a, chl. b, chl. a+b and carotenoids), total soluble sugars, total carbohydrates concentrations and phenoloxidase activity were significantly decreased following the increase in lead levels in the growth medium. On the other hand, Pb treatments increased LWD (%), chl. a/b ratio, total free amino acids and proline accumulation in lettuce leaves compared with unpolluted plants. The concentration of minerals (N, P and K^+) was sharply reduced by Pb treatments, especially at higher lead levels. The concentration of lead in lettuce leaves and roots was positively correlated with Pb concentration in root medium. The increase in lead concentration was more pronounced in lettuce leaves than in roots. Yield and its components represented by average head weight, head length, head diameter, dry matter content % and TSS % were significantly decreased as a result of increasing Pb concentrations. The application of biofertilizers (Halex 2 or EM) or lead bioremediator *Bacillus subtilis* strain exhibited significant increases in most studied characters, but decreased LWD (%), chl. a/b ratio, proline accumulation in leaves and the concentration of lead in lettuce leaves and roots. The interaction between lead pollution treatments and the inoculation with different effective microorganisms mitigated the harmful effects exerted by lead pollution stress on lettuce plants and enhanced growth characters, leaf water relations, chemical parameters, yield and its components of lettuce plants.

Keywords: Lettuce plants, lead, Halex 2, EM, plant height, leaf area, photosynthetic pigments, minerals, yield, TSS %.

INTRODUCTION

Lettuce (*Lactuca sativa* L) is one of the most important vegetable crop grown in Egypt for local consumption and export. Lettuce is the word's most popular green salad. Its leaves are a good source of beta carotene, vitamin C, folic acid and mineral salts i.e. magnesium, iron and phosphorus.

Pollution caused by heavy metals is now a worldwide phenomenon. Soil, water and air pollution by heavy metals has caused serious environmental hazards recently, as a result of rapid industrialization. In association with environmental contamination, many health problems for human and animals were brought about, particularly with heavy metal pollution as they enter the food chain in their absorption and translocation (Wagner, 1993). These heavy metals were introduced to the Egyptian agroecosystem mainly through commercial fertilizers, pesticides, industrial activity, auto emission and wastewater used for irrigation (Aboulroos *et al*., 1996). Toxic effects of heavy metals include the blocking of functional groups of biologically important molecules such as enzymes, transport systems for essential nutrients and ions as well as inactivation of enzymes (Gadd and White, 1989).

Lead is the most widespread pollutant emitted from industry, stationary combustion plants and motor vehicles. Consequently, lead may be accumulated in the soil at high concentrations causing plant toxicity and vegetative damage (El-Ghinbihi, 2000).

Lead accumulation in the soil affects plants primarily through their root system, which rapidly responds to absorbed lead by changes in its growing rate (Breckle, 1991). The harmful effects of lead on the growth and the biochemical composition of the plant such as chlorophyll, sugars, protein, N, P and K^+ concentrations were observed by several authors (Burzynski, 1987; Poskuta *et al*., 1988 and El-Ghinbihi, 2000).

The role of beneficial microorganisms introduced into the rhizosphere in plant nutrition attracted the attention of many investigators such as Agwah and Shahaby (1993), Barakat and Gabr (1998) and Abd El-Fattah and Sorial (1998). Plant growth-promoting rhizobacteria (*Azospirillum, Azotobacter* and *Klebsiella*) may induce growth promotion and yield increases directly or indirectly. Direct influences include production of phytohormones (Noel *et al*., 1996), enhancement of the availability and acquisition of some minerals, liberation of phosphates and micronutrients (Ruiz-Lozano *et al*., 1995) and stimulation of disease resistance mechanisms, which all together may promote the vegetative growth. Indirect effects arise from altering the root environment and ecology, producing siderophores, which increase the uptake of nutrients from the soil. In addition, many *Azospirillum* spp. produced many plant hormones such as indole acetic acid, isobutyric acid and cytokinins (Omay *et al.*, 1993). These hormones were found to reverse the adverse effect of stress conditions (Strack and Karwowska, 1978). The positive effect of bifertilizer inoculation for increasing plant resistance to stress conditions were observed by Creus *et al*. (1997) and Hamdia and El-Komy (1998).

Bioremediation is an emerging new technology for cleaning up the environmental pollution using microorganisms such as bacteria, fungi and algae. Microorganisms can accumulate heavy metals from the external environment. The mechanism of bioremediation of heavy metals depends either on the inactivation or complexation of the metals or metals bind to the microbial cell wall or metal-siderophore interactions (Brierley, 1990).

Many soil bacteria can be applied in different forms to remediate heavy metal pollution. Ibeanusi *et al*. (1995) developed metal-tolerant microbial strains for increased different metal recovery. Cuero (1996) treated a sandy loam soil contaminated with heavy metal with *Bacillus subtilis* to determine its effect on heavy metal accumulation. Mahmoud and El-Beltagy (1998) isolated *Bacillus subtilis* strain from polluted soil, and used it for bioremediation process.

The technology of effective microorganisms (EM) was developed in Japan by Prof. Teruo Higa. EM is produced in about 15 countries and about 50 countries are using EM around the world (Higa and Parr, 1994). EM biofertilizer contains 5 groups of microorganisms i.e., lactic acid bacteria (*Lactobacillus plantarum*), photosynthetic bacteria (*Rhodopseudomonas palustris*), Yeasts (*Saccharomyces albus*), actinomycetes and fungi (*Aspergillus orgazae*). Thus the technology of effective microorganisms EM is safe, effective and environmentally friendly due to the fact that microbes are classified as biosafety, non-harmful or non-pathogenic which means that they are causing no diseases in a healthy human or not-genetically-engineered or modified (Zarb *et al*., 2001).

Effective microorganisms have been shown to promote germination, plant growth, flowering, fruiting, yield and quality of crops, moreover, enhance the photosynthetic capacity of plants (Higa and Wididana, (1991). EM biofertilizer is effective in promoting plant growth and productivity under stress conditions such as drought, heat, pollution, diseases, weed and insects (Higa and Parr, 1994). Application of effective microorganisms (EM) improved the physical, chemical and biological environments of cultivated soil by decreasing the soil electrical conductivity (EC), pH and increasing its organic matter content, polysaccharides, beneficial enzymes and organic acids that help build stable aggregate and soil structure (Sangakkara and Higa, 2000 and Salib *et al*., 2003)

The present study was undertaken to evaluate the effect of lead pollution treatments, the application of different beneficial microorganisms (Halex 2, EM and *Bacillus subtilis* strain) and their interaction on growth characters, leaf water relations, chemical composition, yield and its attributes of lettuce plants.

MATERIALS AND METHODS

Two pot experiments were performed at the Experimental Farm, Faculty of Agriculture, Minufiya University, Shibin El-Kom during the winter seasons of 2004/2005 and 2005/2006 in order to investigate the responses of growth characters, leaf water relations, chemical composition, yield and its components of lettuce plants to lead pollution treatments, the inoculation with different beneficial microorganisms and their interaction.

Seeds of lettuce (*Lactuca sativa* L.) variety Balady (Romaine) were obtained from the Horticulture Department, Faculty of Agriculture, Minufiya University and were sown in seed beds on October 15th and 17th in the first and second seasons, respectively. Forty five days later, 2 uniformed seedlings were transplanted in plastic pots 50 cm inner diameter and 40 cm depth, filled with 10 kg air dried soil.

Lead nitrate [$Pb(NO_3)_2$] was used as lead pollutant (Pb) at rates of 100, 250 and 500 mg/l besides tap water as control and was applied to irrigation water after 6 weeks from transplanting and were repeated 2 weeks later.

Biofertilizer (Halex 2), contains a mixture of growth promoting N-fixing bacteria of genera *Azospirillum, Azotobacter* and *Klebsiella*, which kindly supplied by Prof. Dr. M. G. Hassouna, Biofertilizer Unit, Plant Pathology Dept., Alexandria University. It was used at a rate of 1.5 g/pot in irrigated water 7 weeks after transplanting and this was repeated 2 weeks later.

Lead-tolerant *Bacillus subtilis* strain (Bs) was obtained from the Agric. Botany Department, Faculty of Agric., Minufiya University. This strain was identified by the PCR and DNA sequencing techniques (Sequence Analysis, Applied Biosystem, Japan) according to Mahmoud and El-Beltagy (1998). To study the effect of the lead tolerant strain as bioremediator. The strain was inoculated in a nutrient broth medium (2 liter) and incubated at 28-30^0 for 2 days, diluted to 20 liter with irrigation water and applied to the pots. The pots then irrigated at a rate of 1 liter/pot 7 weeks after transplanting and this was repeated 2 weeks later.

EM a biofertilizer, that contains different microorganisms i.e., photosynthetic bacteria (*Rhodopseudomonas palustris*), lactic acid bacteria (*Lactobacillus plantarum*), yeasts (*Saccharomyces albus*), actinomycetes and fungi (*Aspergillus orgzae*). The EM stock solution used in this study has been produced and made available by the Ministry of Agriculture, Egypt. It was used at a rate of 2 cm/pot in irrigated water 7 weeks after transplanting this was repeated 2 weeks later.

Each experiment included 16 treatments, which were all the possible combinations of the four lead pollution treatments (0, 100, 250 or 500 mg/l) with four effective microorganism applications [control, biofertilizer (Halex 2), *Bacillus subtilis* strain or biofertilizer (EM)]. The experimental design was split-plot in randomized complete blocks, with five replications for each treatment. The main plots were allocated for lead pollution levels, whereas, the sub-plots were occupied by microorganism treatments. The sub-plots were randomly assigned within each main plot.

The experimental soil was clay loam, the texture and some physical and chemical properties are presented in Table (1), according to *Page et al*. (1982). Moreover, the soil was analyzed for lead by atomic absorption spectrophotometer in an ammonium acetate extract following the method described by Jackson (1956), the obtained data are presented in Table (1).

All pots were fertilized with NPK^+. Each pot received 2.4 g P_2O_5 as calcium supperphosphate (15.5% P_2O_5), before transplanting, N and K^+ were applied in the form of ammonium nitrate (33% N) and potassium sulphate (48% K_2O) at the levels of 1.94 g N/pot and 1.16 g K_2O/pot, respectively. They were added in two equal doses during the growth season. Pots were irrigated with tap water whenever to keep the moisture in the soil at about 65% of the total water holding capacity of the soil during the experimental period. The other agricultural practices were done according to the recommended methods for lettuce crop.

Table (1): Physical and chemical properties of the experimental soil.

Property	Value
Physical analysis	
Sand (%)	34.5%
Silt (%)	21.3%
Clay (%)	44.2%
Soil texture	clay
Chemical analysis	
EC (dsm^{-1})	1.11
pH	7.72
Soluble cations (meq/l)	
Ca^{2+}	3.78
Mg^{2+}	3.17
Na^{+}	4.26
K^{+}	0.99
Pb^{2+} (mg/l)	0.044
Soluble anions (meq/l)	
HCO^{3-}	2.61
Cl^{-}	5.73

After 75 days from transplanting four plants were randomly taken from each treatment and the following data were recorded:

1- Growth Characters

In each plant sample, plant height (cm), root length (cm), number of leaves/plant, leaf length and width (cm), leaf area (cm^2/plant) using the dry weight method according to Aase (1978), as well as dry weight of roots and shoots (g/plant), (dried at 70ºC for 72 hrs.) were measured.

2- Leaf Water Relations:

Relative water content RWC (%) and leaf water deficit LWD (%) were determined according to Kalapos (1994).

3- Chemical Analysis:

Photosynthetic pigments were extracted from fresh leaves by acetone 85% and estimated according to Wettestein (1957), then calculated as mg/g dry weight. Total soluble sugars and total carbohydrates in dried leaves were determined colorimetrically by the phenol sulfuric acid method described by Dubois *et al*. (1956). Total free amino acids in dry leaves, were measured using the method of Rosen (1957). Free proline in fresh leaf samples was extracted by the method described by Bates *et al*. (1973). Phenoloxidase activity in O. D./g fresh weight after 45 min. was estimated in fresh leaves by the method of Broesh (1954). Total nitrogen concentration in dry leaves and roots was determined using micro-kjeldahl method according to Ling (1963). Phosphorus and potassium were estimated in dried leaves and roots following the method of Chapman and Pratt (1961). Lead was measured in dried leaves and roots using atomic absorption spectrophotometer according to Cottenie *et al*. (1982).

4- Yield and its Components

At harvest time, average lettuce head weight (g), head length (cm), head diameter (cm), dry matter content % in lettuce leaves were recorded. Five lettuce heads from each treatment were taken randomly to estimate the content of total soluble solids (TSS %) using hand refractometer according to A.O.A.C. (1985).

The collected data of both seasons were statistically analyzed using Costat Software program (1985). Treatment means were compared with the revised L. S. D. test at 0.05, level (Snedecor and Cochran, 1981).

RESULTS AND DISCUSSION

1- Growth Characters

Data presented in Tables (2 and 3) show that increasing lead concentrations in root medium up to 500 mg/l markedly decreased plant growth characters represented by plant height, root length, number of leaves, leaf length and width, leaf area as well as dry weight of roots and shoots, in both seasons. The highest significant reduction was observed under higher Pb concentration (500 mg/l). No significant differences were detected between the control and low Pb concentration (100 mg/l) in plant height, in both seasons and number of leaves and leaf width in the second season only. The inhibition of root growth may be due to the inhibitory effect of lead on cell division and elongation. The inhibitory effect of lead on cell division was through the reduction of meristem size and decreasing the number of mature cells (Obroucheva *et al*., 1998). Similar results were reported by Begonia *et al*. (1998) who found that *Brassica juncea* leaf area and root dry matter were reduced by lead treatments. Furthermore, Moftah (2000) revealed that lead concentration up to 300 mg/l significantly decreased plant height as well as roots and shoots dry weight of tomato and eggplant. Petersen *et al*. (2002) indicated that lettuce plants were poorly developed in Pb polluted soil and the dry weight of plants was decreased by increasing Pb concentrations. This decrease could be due to toxic effects of lead.

Results in the same Tables indicate that the inoculation of lettuce plants with different biofertilizers (Halex 2 or EM) or lead tolerant bacteria (*Bacillus subtilis* strain) significantly increased plant growth characters compared with untreated plants. In this regard the usage of *Bacillus subtilis* strain gave the maximum increases in plant height, leaf width, leaf area, root and shoot dry weight, meanwhile, the application of biofertilizer EM led to the highest significant increment in root and leaf length compared with the control plants. Furthermore, the inoculation with Halex 2 caused the greatest significant increase in number of leaves. No significant differences were observed between *Bacillus subtilis* strain and Halex 2 in dry weight of roots and shoots as well as between *Bacillus subtilis* strain and EM in plant height in both seasons. Similar results were observed by Idriss *et al.* (2002) who reported that several *Bacillus subtilis* strains process plant growth promoting activity and these strains stimulated the growth of maize seedlings. Furthermore, Bai *et al*. (2003) found that *Bacillus subtilis* strains increased weight of shoots and roots as well as total biomass of soybean plants under greenhouse and field conditions. The positive effect of boifertilizer (Halex 2 or *Azospirillum*) on growth characters was observed by Agwah and Shahaby (1993) and Abd El-Fattah and Sorial (1998) on lettuce plants.

T2

T3

This positive effect of Halex 2 may be attributed to the production of phytohormones, improving the availability and acquisition of nutrients, stimulation of disease resistance mechanisms, which all together may promote the growth characters (Noel *et al*., 1996 and Ruiz-Lozano *et al*., 1995). The significant increases in growth characters which were observed in this investigation by applying EM confirmed those obtained by Zaki and Salama (2006) on cucumber and El-Manawahly (2007) on pepper who mentioned that EM improved the plant growth characters. This effect may be due to the fact that EM increase the microorganisms in the soil, which convert the ability of mobilizing the unavailable forms of nutrient elements to available forms, moreover, these microorganisms produce growth promoting substances such as IAA and cytokinins which increase cell elongation and enhance plant growth characters (Higa and Parr, 1994 and Wididana and Higa, 1995).

The interaction between the pollution with different Pb rates and the application of different beneficial microorganisms reveal that under all Pb treatments the inoculation with these microorganisms overcame the negative effect of Pb pollutant and significantly increased plant growth characters, particularly under higher lead levels. Under Pb at a rate of 500 mg/l the inoculation with Halex 2 increased number of leaves by 71%, meanwhile, the inoculation with *Bacillus subtilis* strain increased plant height by about 43% and leaf width by 62.6%. On the other hand, the application of EM increased root length by 22% and leaf length by 45% during the first season compared with untreated plants. The effect of Bs strain on lettuce growth under Pb treatments may be due to the importance of this strain on detoxifying and/or bioleaching lead and render it to unavailable form to be absorbed by plant and poison it (Mahmoud and El-Beltagy, 1998). The positive effect of Halex 2 or *Azospirillum* on the growth characters under stress conditions was observed by Creus *et al*. (1997) and Hamdia and El-Komy (1998). Furthermore, Higa and Parr (1994) reported that under stress conditions EM promotes plant growth and productivity.

2- Leaf Water Relations

Results in Table (3) demonstrate that lead pollution treatments decreased RWC (%) and increased LWD (%) in lettuce leaves compared with untreated plants. The application of higher Pb concentration (500 mg/l) led to the highest significant reduction in RWC (%) and the greatest significant increment in LWD (%) compared with unpolluted plants. This inhibitory effect of lead on leaf water relations may be due to the effect of lead on transpiration and water content as a consequence of the negative effect on the transpiration system and the stomatal structure (Burzynski, 1987).

Data in the same Table mention that treating lettuce plants with different effective microorganisms (Halex 2, Bs strain or EM) significantly enhanced water status in lettuce leaves compared with untreated plants. In this regard, the inoculation with Bs strain gave the maximum increase in RWC (%) and the highest reduction in LWD (%) followed by EM and Halex 2.

Concerning the interaction between lead treatments and the application of biofertilizers (Halex 2 or EM) or lead tolerant Bs strain, results in Table (3) reveal that the application of these microorganisms significantly improved leaf water content of lettuce plants and alleviated the negative effect of lead pollution, especially under higher lead concentration.

3- Chemical Analysis

a- Photosynthetic Pigments

As seen in Table (4) chl. a, chl. b, total chlorophyll (chl. a+b) and carotenoids were sharply decreased in Pb-treated lettuce leaves compared to untreated plants. Increasing lead levels up to 500 mg/l significantly reduced photosynthetic pigments. The % reduction in chl. a, chl. b, chl. a+b and carotenoids at higher lead rate (500 mg/l) reached about 26%, 60%, 38% and 37% during the first season compared with unpolluted plants. Photosynthetic pigments have often been shown as one of the main sites of toxic lead. The inhibitory effect of lead on Fe uptake and transport to plant leaves may result in reducing chlorophyll synthesis and cause chlorosis (Foder *et al*., 1998). Lead may accumulate in chloroplast and causes the disorganization of their ultrastructure or the decrease of chloroplasts biosynthesis (Burzynski, 1985). Similar results were obtained by Romanowska *et al.* (1998) who reported that Pb treatments completely inhibited photosynthesis in pea and maize leaves. Furthermore, Legrady and Lang (1998) on maize and Moftah (2000) on tomato and eggplant mentioned that photosynthetic pigments were decreased in Pb treated leaves.

Data presented in Table (4) show that chl. a/b ratio was significantly increased by increasing Pb concentrations. In this regard, the highest Pb rate showed the highest chl. a/b ratio in both seasons.

Presented data in the same Table reveal that the inoculation of lettuce plants with different beneficial microorganisms (Halex 2, *Bacillus subtilis* strain or EM) significantly increased chl. a, chl. b, chl. a+b and carotenoids compared with untreated plants, meanwhile it decreased chl. a/b ratio. Similar results were obtained by Agwah and Shahaby (1993); Abd El-Fattah and Sorial (1998) on lettuce and Barakat and Gabr (1998) on tomato who recorded that inoculation with *Azospirillum* or Halex 2 significantly increased leaf chlorophyll concentration. Furthermore, Zaki and Salama (2006) mentioned that application of EM significantly increased total chlorophyll concentration in cucumber leaves. This increase may be due to the fact that EM contains photosynthetic bacteria (*Rhodopseudomonas* sp.), which enhanced the plant photosynthetic rate (Xu *et al*., 2001).

The highest mean values of chl. a, chl. b, chl. a+b were obtained from the application of Bs strain, meanwhile, the highest mean values of carotenoids were observed under EM treatment. No significant differences were detected between EM, Bs strain and Hatex 2 in chl. a, chl. b, chl. a+b and chl. a/b ratio.

The interaction between Pb treatments and the application of Hatex 2, Bs strain or EM significantly enhanced photosynthetic pigments and alleviated the negative effect of lead hazards on photosynthetic pigments. Under the higher pb level (500 mg/l) the inoculation with Bs gave the highest significant increases in photosynthetic pigments. This increment reached about 45% for chl. a, 88% for chl. b and 54% for chl. a+b during the first season. The positive effect of Bs strain may be due to its effect as bioremediator of lead from the polluted soil.

T4

b- Total Soluble Sugars and Total Carbohydrates

Analysis of variance show that increasing lead concentration significantly decreased the concentration of total soluble sugars and total carbohydrates in lettuce leaves compared with unpolluted plants (Table, 5). Under higher lead concentration (500 mg/l) the reduction in total soluble sugars reached about 30% and 27% and in total carbohydrates reached about 34% and 35% in the first and second seasons, respectively, compared with the control plants.

No significant differences were observed between control plants and low lead concentration (100 mg/l) in total soluble sugars in both seasons. The deleterious effect of higher lead levels on total soluble sugars and total carbohydrates may be due to its negative influence on photosynthesis and other physiological processes such as transpiration in lead treated plants (Burzynski, 1987). The obtained results confirmed those obtained by El-Ghinbihi (2000) who found that lead in different concentrations decreased total carbohydrates in common bean leaves.

Data recorded in Table (5) indicate that the application of different beneficial microorganisms significantly increased total soluble sugars and total carbohydrates compared with untreated plants. The higher increment in these respects was observed by the inoculation of lettuce plants with Bs strain which increased total soluble sugars by 68% and 69% and total carbohydrates by 79% and 82% in the first and second seasons, respectively, followed by EM. The addition of these microorganisms under lead pollutant condition significantly increased total soluble sugars and total carbohydrates and mitigated the negative effect of lead treatments, especially under higher lead concentration (500 mg/l) compared with untreated plants. In this concern, El-Manawahly (2007) reported that the inoculation with EM significantly increased total carbohydrates in pepper seedlings. The promoting effect of EM on total carbohydrates could be attributed to the fact that EM enhances nutrient availability and stimulates plant growth as well as photosynthetic pigments (Xu *et al*., 2001). Moreover, Abd El-Fattah and Sorial (1998) indicated that Halex 2 significantly increased total carbohydrates in lettuce leaves.

c- Total Free Amino Acids and Proline

Results recorded in Table (5) mention that treating lettuce plants with different lead levels significantly increased the concentration of total free amino acids and proline accumulation in leaves. The highest significant increment in total free amino acids and proline was observed under higher lead concentration and reached about 170% and 183% for total free amino acids and 92% and 93% for proline in the first and second seasons, respectively, compared with untreated plants.

T5

Data in the same Table show that the inoculation of lettuce plants with different beneficial microorganisms (Halex 2, *Bacillus subtilis* strain or EM) significantly increased total free amino acids and decreased the accumulation of proline in lettuce leaves compared with uninoculated plants. Best results were observed by the application of Bs strain followed by EM or Halex 2.

The interactive effect of lead pollution treatments and the inoculation with Halex 2, Bs strain or EM significantly increased total free amino acids concentration and decreased the accumulation of proline in lettuce leaves, especially under higher lead concentration. The inoculation with Bs strain led to the best results in this concern compared with the other treatments and the control plants.

d- Phenoloxidase Activity

As seen in Table (5) phenoloxidase activity was decreased significantly as a result of increasing lead concentration in root medium compared with untreated plants. The highest reduction in phenoloxidase activity was observed under the higher lead concentration (500 mg/l) and reached about 28% and 26% in the first and second seasons, respectively, compared with unpolluted plants. No significant differences were observed between low lead concentration (100 mg/l) and the control plants in both seasons. The obtained results were in line with those observed by Romanowska *et al*. (1998) who mentioned that phosphoenolpyruvate carboxylase and ribulose-bisphosphate carboxylase activities in pea and maize leaves decreased in response to Pb treatments.

Data in the same Table show that the inoculation of lettuce plants with Halex 2, Bs strain or EM significantly increased phenoloxidase activity, especially under lead stress treatments. The highest increase was observed by the application of Halex 2 followed by EM. The obtained results were in agreement with those observed by Abd El-Fattah and sorial (1998) who found that phenoloxidase activity in lettuce leaves was increase by the inoculation with Halex 2.

e- Mineral Concentration

Data presented in Tables (6 and 7) indicate that N, P and K^+ concentrations were higher in lettuce leaves than in roots. The usage of the lower lead concentration (100 mg/l) caused a significant decrease in the N concentration in roots and K^+ concentration in leaves and roots. No significant decrease in the N concentration in the leaves and the P concentration in leaves and roots of plants was observed. Increasing lead levels significantly decreased N, P and K^+ concentrations in leaves and roots. The highest negative effect of lead treatments on the concentration of N, P and K^+ was observed under the high lead level (500 mg/l). The maximum reduction in N concentration reached 20% for leaves and 40% for roots, meanwhile, the highest reduction of P concentration reached 27% for leaves and 25% for roots, the maximum reduction in K^+ concentration reached about 21% for leaves and 22% for roots during the first season compared with untreated plants.

T6

T7

Similar results were observed by El-Ghinbihi (2000) who mentioned that treating common bean plants with lead significantly decreased N, P and K^+ concentrations in leaves and roots. This inhibitory effect of lead on N concentration may be due to its effect on N absorption from the growth culture. The inhibitory effect of lead on P concentration may be due to the action of lead on the uptake and translocation of P element within plant roots (Larcher, 1980).

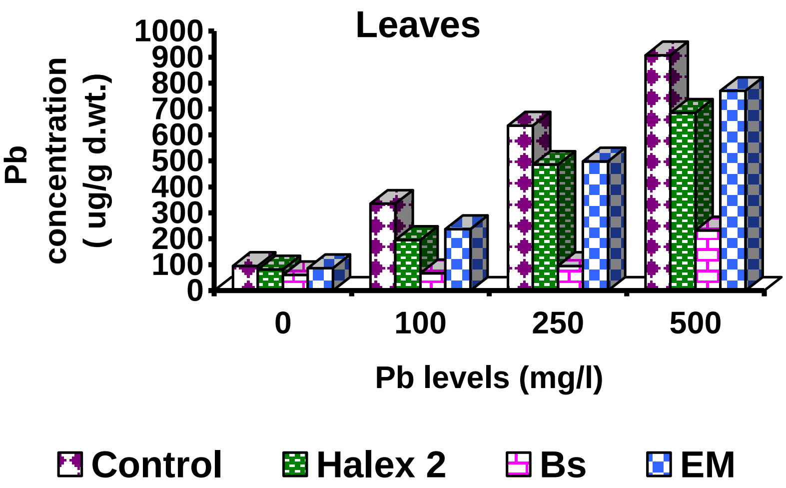
Results in Tables (6 and 7) reveal that the application of beneficial microorganisms significantly increased N, P and K^+ concentrations in leaves and roots compared with untreated plants. In this regard, the application of EM gave the greatest significant increment in the N concentration in roots followed by Halex 2. On the other hand Hatex 2 led to the highest significant increase in N concentration in leaves. The inoculation with Bs strain caused maximum increases in P and K^+ in leaves and roots compared with uninoculated plants. In this connection, Bai *et al*. (2003) indicated that *Bacillus subtilis* strains increased total N concentration in soybean plants. The same author reported that increased root development means increased nutrient uptake and increase N supply capability and some beneficial bacteria are known to exert their plant growth promoting effect via stimulating root growth through production of IAA. Furthermore, Zaki and Salama (2006) on cucumber and El-Manawahly (2007) on pepper found that the inoculation with EM significantly increased the concentration of N, P and K^+ compared with the control. This effect may be attributed to the fact that applying EM may promotes the microorganisms in the soil, which release of nutrients from organic matter to mineral form and enhances utility values of organic matter (Sangakkara and Weerasekera, 2001). On the other hand, Abd El-Fattah and Sorial (1998) on lettuce and Barakat and Gabr (1998) on tomato recorded that the inoculation with Halex 2 increased N, P and K^+ concentrations in leaves. These results can be related to the role of non-symbiotic N_2-fixing bacteria in improving the availability and acquisition of nutrients.

The interaction between lead treatments and the application of beneficial microorganisms indicate that these microorganisms mitigated the harmful effect of lead pollution treatments and significantly enhanced N, P and K^+ concentrations in lettuce leaves and roots.

f- Lead Concentration

Data recorded in Table (7) and Fig. (1), indicate that in all lead treated plants the concentration of lead was higher in leaves than in roots. In this regard, a significant amount of lead was transported to the shoots of lettuce plants. In this concern, Baker (1987) reported that many plant species are resistant to or can tolerate different amounts of heavy metals. Metal-tolerant plants accumulate higher amounts in roots as compared to non-tolerant ones, which accumulate higher heavy metal amounts in shoots.

Increasing Pb concentration in root medium significantly increased its accumulation in the leaves and roots of lettuce plants. The application of the higher pb concentration (500 mg/l) led to maximum significant increases in pb concentration in leaves and roots compared to untreated plants.



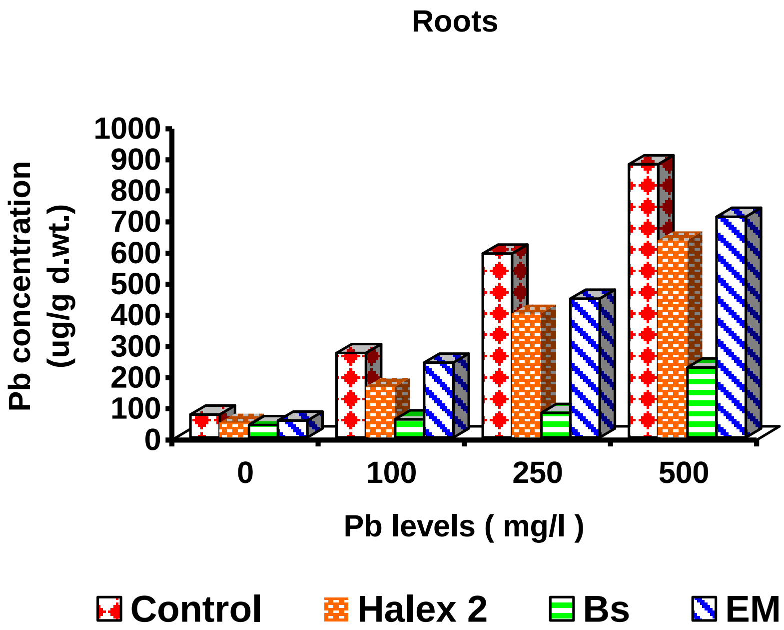


Fig. (1): Effect of lead levels, application of Halex 2, Bs strain and EM on Pb concentration in lettuce leaves and roots during 2004/2005 season.

The obtained results confirmed those obtained by Begonia *et al*. (1998) who demonstrated that shoot and root Pb concentration in *Brassica juncea* was increased with increasing Pb levels. Moreover, Moftah (2000) revealed that the concentration of lead in leaves and roots of tomato and eggplant increased significantly with increasing its applied rate to soil. Petersen *et al*. (2002) indicated that increasing Pb concentration in soil increased its accumulation in lettuce plants.

The interaction between the lead treatments and the application of Hatex 2, *Bacillus subtilis* strain or EM show that under lead treatments the use of these microorganisms significantly decreased the accumulation of Pb in lettuce leaves and roots compared with the control plants (Table, 7). Under higher lead concentration (500 mg/l) the use of Bs strain reduced Pb accumulation by about 74% and 75% in leaves and roots in the first and second seasons, respectively, compared with their controls.

The obtained results were in full agreement with those observed by Mahmoud and El-Beltagy (1998) who found that under different lead concentrations (100, 400 and 800 mg/l) the inoculation with *Bacillus subtilis* strain significantly decreased lead concentration in rocket salad. This reduction reached about 93% at 400 mg/l Pb. These results demonstrate the positive role of the lead tolerant strain on reducing lead levels in plant tissues compared with control plants. The mechanisms of bioremediation of lead from contaminated soil by microorganisms may be due to precipitation of metal ions, adsorption at bacterial sites and reduction by change of oxidation states (Ibeanusi *et al*., 1995).

4- Yield and its Attributes

Data recorded in Table (8) indicate that yield and its attributes represented by average head weight, head length, head diameter, dry matter content % and TSS % were negatively affected by lead stress conditions. Yield and its components were decreased with increasing Pb concentrations in growth media. In this respect the most reduction was recorded at 500 mg/l of Pb at which the average head weight was decreased by 30%, head length by 25%, head diameter by 29%, dry matter content by 25% and TSS% by 16% compared with untreated plants, during the first season compared with unpolluted plants.

T8

These results were in agreement with those obtained by Xian (1989) who demonstrated that high levels of lead significantly decreased the yield of kidney bean. Moreover, Moftah (2000) recorded that yield of tomato and eggplant was decreased with increasing Pb concentrations up to 300 mg/l. In addition, Petersen *et al*. (2002) found that increasing Pb concentrations in soil decreased lettuce yield.

The application of beneficial microorganisms significantly enhanced yield and its composition, furthermore, they alleviated the inhibitory effect of lead treatments and significantly increased yield and its characteristics compared with untreated plants (Table, 8). Under the high lead treatment (500 mg/l) the inoculation with Bs strain increased average head weight by 88% and 30% as well as head length by 18% and 12%, the application of Halex 2 increased head diameter by 49% and 53% as well as dry matter content % by 47% and 60%, the usage of EM increased TSS % by 61% and 52% in the first and second seasons, respectively, compared with untreated plants. The enhancing effect of microorganisms on yield and its composition which were observed in this study may be related to their role in increasing plant growth characters (Tables 2 and 3) and chemical parameters (Tables, 4, 5, 6 and 7) which were previously discussed. Similar results were observed by Bai *et al*. (2003) who revealed that *Bacillus subtilis* strains increased the grain yield of soybean plants. The promoting effect of *Bacillus subtilis* strain on lettuce yield and its components which was obtained in this work may be related to its lead bioremediation efficiency and/or attributed to the fact that *Bacillus subtilis* strains produce a wide variety of antibacterial and antibiotics like subtilin, which reduces plant diseases and antagonize fungal pathogens by stimulating the defensive capacities of the plant and made the plants more healthy and vigorous and increased the yield (Leclere *et al*., 2005). The enhancing effect of EM on yield was observed by Zaki and Salama (2006) working on cucumber and El-Manawahly (2007) working on pepper who found that EM application led to high increases of the yield. Furthermore, the positive effect of *Azospirillum* or Halex 2 on yield was observed by Agwah and Shahaby (1993) and Abd El-Fattah and Sorial (1998) on lettuce as well as Barakat and Gabr (1998) on tomato who gained yield increases by the inoculation with Halex 2. The improving effect of Halex 2 on yield may be due to N fixation capacity, production of growth phytohormones, antibacterial and antifungal compounds and siderophores (Omay, *et al*., 1993 and Noel *et a*l., 1996).

It could be concluded that treating lettuce plants with different lead concentrations up to 500 mg/l markedly decreased growth characters, RWC (%), most of chemical composition, yield and its characteristics, but it increased LWD (%), total free amino acids, proline concentration in leaves and the accumulation of lead in leaves and roots compared with unpolluted plants. The inoculation of lettuce plants with different beneficial microorganisms (Halex 2, *Bacillus subtilis* strain or EM) significantly improved growth characters, leaf water relations, chemical parameters, yield and its components. The interaction between lead treatments and the application of Halex 2, lead tolerant *Bacillus subtilis* strain or EM indicate that the usage of these microorganisms alleviated the deleterious effects of lead pollution treatments and significantly enhanced growth characters, leaf water relations, chemical composition, yield and its attributes. Therefore, it can be recommended that the application of different beneficial microorganisms such as lead bioremediator bacteria (*Bacillus subtilis* strain) or biofertilizers (Halex 2 or EM) be used to remediate lead from polluted soils and to alleviate the harmful effect of lead pollution of lettuce plants via improving soil properties and producing healthy plants.

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دراسات فسيولوجية على تأثير بعض الكائنات الحية الدقيقه النافعة على نباتات الخس الملوثة بالرصاص

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أجريت هذه الدراسة فى تجربتى أصص بالمزرعة التجريبية لكلية الزراعة - جامعة المنوفية خلال الموسمين الزراعين ٢٠٠٤/٢٠٠٥ و٢٠٠٥/٢٠٠٦ وذلك لدراسة استجابة صفات النمو الخضرى والعلاقات المائية والتركيب الكيماوى للأوراق والمحصول لنباتات الخس صنف بلدى (رومانى) للتلوث بالرصاص وكذلك دراسة تأثير استخدام أنواع مختلفة من الكائنات الحية الدقيقة النافعة (السماد الحيوى هالكس ٢ أو السماد الحيوى EM أو السلالة البكتيرية باسيلس ساتليس ذات القدرة على أزالة الرصاص من التربة الملوثة به) وأيضا دراسة التفاعل بين هذه المعاملات المختلفة وذلك كمحاولة للتقليل الآثار الضارة الناتجة عن تلوث نباتات الخس بالرصاص.

وقد عرضت نباتات الخس للتلوث بالرصاص باستخدام نترات الرصاص بتركيزات (صفر, ١٠٠، ٢٥٠، ٥٠٠ ملليجرام/لتر). وقد أوضحت النتائج المتحصل عليها ما يلى:

* أدى تعرض نباتات الخس للتلوث باستخدام تركيزات مختلفة من الرصاص الى حدوث نقص معنوى فى صفات النمو الخضرى المتمثلة فى أرتفاع النبات وطول الجذر وعدد الأوراق وطولها وعرضها ومساحتها وكذلك الوزن الجاف للجذور والمجموع الخضرى.
* أظهرت النتائج أن تعرض نباتات الخس للتلوث بالرصاص قد أدى الى حدوث نقص معنوى فى المحتوى النسبى للماء فى الأوراق وكذلك تركيز صبغات البناء الضوئى (كلوروفيل أ ، كلوروفيل ب ، كلوروفيل أ+ب ، الكاروتينيدات) والسكريات الذائبة الكلية والكربوهيدرات الكلية فى أوراق الخس مقارنه بالنباتات الغير معاملة.
* أدت زيادة تركيز الرصاص فى بيئة النمو الى نقص معدل النشاط الأنزيمى لأنزيم الفينول أوكسيديز معنويا.
* أوضحت الدراسة أن معاملة نباتات الخس بتركيزات مختلفة من الرصاص أدت الى حدوث زيادة معنوية فى النسبة المئوية لمعدل نقص الماء فى الأوراق وأيضا نسبة كلوروفيل أ/ب وكذلك تركيز الأحماض الأمينية الحرة الكلية وتراكم البرولين فى أوراق الخس مقارنة بالنباتات الغير معاملة.
* أشارت الدراسة الى حدوث نقص معنوى فى تركيز كل من النتروجين والفوسفور والبوتاسيوم فى أوراق وجذور نباتات الخس المعاملة بالرصاص خاصة عند استخدام التركيزات العالية.
* أدت المعاملة بالرصاص الى حدوث زيادة معنويه فى تركيز الرصاص فى كل من أوراق وجذور النباتات المعاملة وكان تركيز الرصاص فى الأوراق أعلى من تركيزه فى الجذور.
* أدت معاملة نباتات الخس بتركيزات مختلفة من الرصاص الى حدوث نقص معنوى فى محصول الخس المتمثل فى متوسط وزن الرأس وطول الرأس وقطرها والنسبه المئويه لكلا من المادة الجافة والمواد الصلبة الذائبة.
* أظهرت النتائج أن استخدام أنواع مختلفة من الكائنات الحية الدقيقه النافعة أدى الى حدوث زيادة معنوية فى صفات النمو الخضرى والمحتوى النسبى للماء فى الأوراق والمكونات الكيماوية وكذلك المحصول ومكوناته لنباتات الخس.
* أدى استخدام هذه الكائنات الحية الدقيقة الى تقليل البرولين المتراكم فى الأوراق وتركيز الرصاص فى أوراق وجذور النباتات.
* أشارت النتائج أن التفاعل بين المعاملة بتركيزات مختلفة من الرصاص والتلقيح باستخدام الأسمدة الحيويه (هالكس ٢ أو EM) أو استخدام السلالة البكتيرية باسيلس ستليس ذات القدرة على أزالة الرصاص من التربة الملوثه به أدى الى الاقلال من التأثيرات الضارة الناتجة عن تلوث نباتات الخس بالرصاص وكذلك أدى الى زيادة صفات النمو الخضرى وتحسين العلاقات المائيه والتركيب الكيماوى للأوراق وكذلك المحصول لنباتات الخس.
* ومن نتائج هذه الدراسة يمكن التوصيه باستخدام هذه الكائنات الحية الدقيقه لتقليل التأثيرات الضارة للتلوث بالرصاص.

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Table (2): Effect of lead levels, application of different microorganisms [Halex 2, lead torrent *Bacillus subtilis* strain (Bs) or EM] and their interaction on some growth characters of lettuce plants during 2004/2005 and 2005/2006 seasons.

Treatments		Plant height (cm)		Root length (cm)		Number of leaves/plant		Leaf length (cm)		Leaf width (cm)	
Pb levels (mg/l)	Microorg.	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season
0		30.93 a	31.87 a	9.57 a	14.00 a	20.84 a	22.00 a	28.39 a	29.12 a	11.02 a	11.65 a
100		29.71 a	30.77 a	8.24 b	12.93 b	18.83 b	21.00 a	27.04 b	26.79 b	10.07 b	10.99 a
250		27.66 b	28.65 b	7.57 c	11.11 c	16.92 c	18.67 b	24.63 c	24.93 c	9.55 b	9.89 b
500		25.75 b	26.39 c	6.70 d	9.26 d	15.25 d	16.75 c	21.29 d	21.57 d	7.85 c	8.87 c
	Control	24.40 c	25.93 c	7.19 c	9.28 d	13.17 c	14.83 d	21.20 d	21.61 d	7.93 c	8.72 c
	Halex 2	28.75 b	29.46 b	7.84 b	11.66 c	21.84 a	23.34 a	25.07 c	25.11 c	10.53 a	11.20 a
	Bs	30.97 a	31.68 a	7.96 b	12.01 b	19.17 b	20.84 a	26.08 b	26.31 b	10.83 a	11.50 a
	EM	29.93 ab	30.60 ab	9.10 a	14.35 a	17.67 b	19.42 c	29.00 a	29.38 a	9.20 b	9.98 b
0	Control	28.43 bcdef	30.00 bcde	8.54 c	12.40 d	17.67 de	19.33 efg	25.07 ef	26.7 e	9.68 cde	10.03 def
	Halex 2	32.20 ab	33.10 ab	10.27 ab	14.10 c	24.67 a	25.67 a	30.37 b	29.33 c	13.33 a	13.96 a
	Bs	28.93 bcdef	30.33 bcde	8.77 c	12.50 d	18.33 cde	19.67 defg	25.57 ef	26.89 e	9.85 bcde	10.52 cde
	EM	34.17 a	34.03 a	10.70 a	17.00 a	22.67 ab	23.33 abc	32.53 a	33.57 a	11.23 bc	12.10 bc
100	Control	25.03 fg	27.21 efg	7.43 ef	9.63 f	13.00 fgh	17.00 gh	22.10 i	22.00 j	8.23 def	9.70 efg
	Halex 2	29.83 abcde	30.07 bcde	7.60 de	12.50 d	22.67 abc	24.33 ab	26.90 d	26.20 f	10.42 bcd	11.15 bcde
	Bs	33.60 a	34.15 a	8.23 cd	14.10 c	21.33 bcd	22.67 bcd	28.83 c	28.55 d	11.93 ab	12.77 ab
	EM	30.37 abcd	31.63 abcd	9.70 b	15.47 b	18.33 cde	20.00 def	30.32 b	30.42 b	9.70 cde	10.32 cde
250	Control	23.20 gh	24.36 gh	6.77 fg	8.21 h	11.33 gh	12.67 i	20.44 j	20.01 k	7.72 efg	8.00 gh
	Halex 2	27.40 cdefg	28.70 def	7.10 ef	11.10 e	21.67 abc	22.67 bcd	23.62 h	24.62 h	10.10 bcd	10.37 cde
	Bs	31.32 abc	32.42 abc	7.72 de	11.61 e	19.33 bcd	21.33 cde	26.14 de	26.70 e	11.67 abc	11.98 bcd
	EM	28.70 bcdef	29.10 de	8.67 c	13.50 c	15.33 ef	18.00 fgh	28.31 c	28.40 d	8.70 def	9.22 efg
500	Control	20.93 h	22.13 h	6.00 h	6.87 i	10.67 h	10.33 i	17.18 k	17.72 l	6.07 g	7.15 h
	Halex 2	25.57 efg	25.97 fg	6.37 gh	8.92 g	18.33 cde	20.67 cdef	19.37 j	20.30 k	8.28 def	9.32 efg
	Bs	30.03 abcd	29.83 cde	7.10 ef	9.84 f	17.67 de	19.67 defg	23.77 gh	23.11 i	9.87 bcde	10.73 cde
	EM	26.47 defg	27.64 ef	7.32 ef	11.42 e	14.33 fg	16.33 h	24.83 fg	25.14 g	7.17 fg	8.28 fgh

Values marked with same alphabetical letter(s), within a comparable group of means, do not significantly differ using revised L.S.D. test at 0.05 level.

Table (3): Leaf area, dry weight of roots and shoots and leaf water relations of lettuce plants as affected by lead levels, the inoculation with different microorganisms (Halex 2, Bs or EM) and their interaction during 2004/2005 and 2005/2006 seasons.

Pb levels (mg/l)	Microorg.	Leaf area (cm^2 /plant) First Season	Leaf area (cm^2 /plant) Second Season	Root dry Weight (g/plant) First Season	Root dry Weight (g/plant) Second Season	Shoot dry weight (g/plant) First Season	Shoot dry weight (g/plant) Second Season	RWC (%) First Season	RWC (%) Second Season	LWC (%) First Season	LWC (%) Second Season
0		2236.5 a	2185.25 b	3.41 a	3.12 a	7.36 a	6.93 a	82.37 a	78.99 a	17.64 c	21.01 c
100		1994.5 b	2228 a	3.15 a	2.91 b	7.05 b	6.28 b	81.42 a	77.54 b	18.58 c	22.46 c
250		1798 c	2022.5 c	2.65 b	2.44 c	5.95 c	5.44 c	78.60 b	74.61 c	21.40 b	25.39 b
500		1385.75 d	1418 d	1.97 c	1.85 d	4.56 d	4.61 d	75.38 c	71.22 d	24.62 a	28.78 a
	Control	1229.25 d	1366.5 d	1.79 c	1.71 d	3.73 c	3.46 c	73.26 d	69.70 d	26.74 a	30.30 a
	Halex 2	1983.25 c	1992.75 c	2.68 b	2.55 c	6.59 b	5.67 b	79.52 c	75.80 c	20.48 b	24.20 b
	Bs	2183 a	2360.5 a	3.46 a	3.06 a	7.43 a	7.17 a	83.21 a	79.49 a	16.79 d	20.51 d
	EM	2019.25 b	2134.0 b	3.25 a	2.99 b	7.17 a	6.96 a	81.78 b	77.36 b	18.22 c	22.64 c
0	Control	1715 a	1510 g	2.88 cdef	2.63 f	6.12 d	5.74 ef	79.25 ef	76.89 de	20.75 c	23.11 def
	Halex 2	2688 b	2551 b	3.56 abc	3.39 b	8.21 b	7.00 d	83.87 bc	80.45 bc	16.13 fg	19.55 efgh
	Bs	1638 i	1954 f	2.93 cdef	2.72 ef	6.11 d	5.77 ef	80.38 def	76.09 ef	19.62 cd	23.91 cdef
	EM	2905 a	2726 a	4.28 a	3.72 a	9.01 a	9.21 a	85.96 ab	82.52 ab	14.04 gh	17.48 gh
100	Control	1302 j	1483 g	2.01 efg	2.06 h	3.46 f	3.13 i	74.00 g	70.22 hi	26.00 b	29.78 b
	Halex 2	2018 f	2348 d	2.87 cdef	2.77 e	7.90 b	6.21 e	81.25 de	77.38 de	18.75 cdef	22.62 def
	Bs	2617 c	2753 a	4.10 ab	3.42 b	8.82 a	7.93 b	87.87 a	83.56 a	12.13 h	16.44 h
	EM	2041 f	2328 d	3.63 abc	3.39 b	8.00 b	7.83 bc	82.56 cd	78.98 cd	17.44 def	21.02 efg
250	Control	896 n	1527 g	1.45 gh	1.38 j	2.84 g	2.61 ij	71.36 h	67.84 i	28.64 b	32.16 b
	Halex 2	2093 e	1910 f	2.49 cdefg	2.41 g	6.00 d	5.48 f	78.95 ef	74.10 fg	21.05 c	25.90 bcd
	Bs	2256 d	2431 c	3.65 abc	3.19 c	7.94 b	7.74 bc	83.76 bc	80.63 bc	16.24 efg	19.37 fgh
	EM	1947 g	2222 e	3.00 bcde	2.77 e	7.01 c	5.92 ef	80.32 def	75.85 ef	19.68 cd	24.15 cde
500	Control	1004 m	946 j	0.82 h	0.77 k	2.50 g	2.36 j	68.43 i	63.85 j	31.57 a	36.15 a
	Halex 2	1134 l	1162 i	1.79 fgh	1.64 i	4.23 e	4.00 h	74.01 g	71.26 h	25.99 b	28.74 b
	Bs	2221 d	2304 d	3.17 abcd	2.91 d	6.85 c	7.23 cd	80.82 de	77.69 de	19.18 cde	22.31 def
	EM	1184 k	1260 h	2.10 defg	2.09 h	4.65 e	4.86 g	78.27 f	72.09 gh	21.73 c	27.91 bc

Values marked with same alphabetical letter(s), within a comparable group of means, do not significantly differ using revised L.S.D. test at 0.05 level.

Table (4): Photosynthetic pigments concentration (mg/g d. wt.) in lettuce leaves as affected by different lead treatments, the application of different microorganisms (Halex 2, Bs or EM) and their interaction in 2004/2005 and 2005/2006 seasons.

Treatments		Chlorophyll a		Chlorophyll b		Chlorophyll a+b		Carotenoids		Chlorophyll a/b ratio	
Pb levels (mg/l)	Microorg.	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season
0		3.95 a	4.04 a	2.14 a	2.35 a	6.09 a	6.39 a	3.66 a	3.85 a	1.85 d	1.77 c
100		3.65 ab	3.78 b	1.78 b	2.32 a	5.42 b	6.10 a	3.35 a	3.66 a	2.09 c	1.72 c
250		3.36 bc	3.40 c	1.29 c	1.64 b	4.65 c	5.04 b	2.78 b	3.13 b	2.67 b	2.15 b
500		2.93 c	2.94 d	0.84 d	1.09 c	3.77 d	4.03 c	2.30 c	2.36 c	3.59 a	2.96 a
	Control	2.95 b	3.07 c	1.13 b	1.22 b	4.08 b	4.28 c	1.92 c	2.09 c	2.94 a	2.80 a
	Halex 2	3.51 a	3.48 b	1.54 a	1.89 a	5.05 a	5.37 b	3.15 b	3.40 b	2.55 b	2.05 b
	Bs	3.77 a	3.85 a	1.71 a	2.20 a	5.48 a	6.05 a	3.23 b	3.44 b	2.35 b	1.79 b
	EM	3.67 a	3.77 a	1.67 a	2.10 a	5.33 a	5.87 a	3.79 a	4.07 a	2.37 b	1.96 b
0	Control	3.36 bcdef	3.41 defg	1.72 bcdef	1.74 cde	5.08 cde	5.15 e	2.73 ef	2.91 de	1.95 def	1.96 de
	Halex 2	4.17 ab	4.26 b	2.39 ab	2.79 a	6.56 ab	7.05 abc	4.09 ab	4.60 ab	1.74 f	1.53 e
	Bs	3.55 bcde	3.66 de	1.95 abcd	1.86 bcd	5.50 bcd	5.52 de	2.88 ef	2.99 de	1.82 f	1.97 de
	EM	4.73 a	4.84 a	2.50 a	3.01 a	7.23 a	7.85 a	4.92 a	4.90 a	1.89 ef	1.61 e
100	Control	3.14 cdef	3.20 fgh	1.35 defg	1.38 def	4.49 de	4.58 efg	2.20 fg	2.49 ef	2.33 cde	2.32 bcd
	Halex 2	3.66 bcde	3.79 cd	1.72 bcdef	2.32 abc	5.38 bcd	6.11 cd	3.38 bcde	3.69 bcd	2.13 def	1.63 de
	Bs	4.08 abc	4.36 b	2.18 abc	2.99 a	6.26 abc	7.35 ab	3.93 bc	4.09 abc	1.87 ef	1.46 e
	EM	3.71 bcde	3.78 cd	1.85 abcde	2.58 ab	5.56 bcd	6.36 cd	3.89 bcd	4.35 ab	2.01 def	1.47 e
250	Control	2.86 ef	2.97 ghi	0.89 gh	1.10 def	3.75 ef	4.07 fgh	1.79 gh	1.97 f	3.21 b	2.70 bc
	Halex 2	3.31 bcdef	3.06 fghi	1.24 efgh	1.48 de	4.55 de	4.54 efg	2.84 ef	3.02 de	2.67 c	2.07 cde
	Bs	3.92 abcd	4.13 bc	1.63 cdef	2.29 abc	5.55 bcd	6.42 bcd	3.17 bcdef	3.73 bcd	2.40 cd	1.80 de
	EM	3.35 bcdef	3.44 def	1.41 defg	1.70 cde	4.76 de	5.14 e	3.33 bcde	3.81 bcd	2.38 cd	2.02 de
500	Control	2.42 f	2.69 i	0.57 h	0.64 f	2.99 f	3.33 h	0.96 h	0.99 g	4.25 a	4.20 a
	Halex 2	2.91 def	2.81 hi	0.80 gh	0.95 ef	3.71 ef	3.76 gh	2.30 fg	2.29 ef	3.64 b	2.96 b
	Bs	3.52 bcde	3.24 efgh	1.07 fgh	1.67 cde	4.59 de	4.91 ef	2.93 def	2.94 de	3.29 b	1.94 de
	EM	2.87 ef	3.02 fghi	0.90 gh	1.11 def	3.77 ef	4.13 fgh	3.01 cdef	3.22 cde	3.19 b	2.72 bc

Values marked with same alphabetical letter(s), within a comparable group of means, do not significantly differ using revised L.S.D. test at 0.05 level.

Table (5): Effect of lead levels, application of different microorganisms and their interaction on total soluble sugars, total carbohydrates, total free amino acids concentrations (mg/g d. wt.), proline concentration (ug/g d. wt.) and phenoloxidase activity (O. D./g fresh wt.) in lettuce leaves in 2004/2005 and 2005/2006 seasons.

Treatments		Total soluble sugars		Total carbohydrates		Total free amino acids		Proline		Phenoloxidase activity	
Pb levels (mg/l)	Microorg.	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season
0		27.91 a	29.09 a	305.70 a	296.36 a	65.56 d	60.55 d	247.88 d	242.37 d	0.32 a	0.34 a
100		27.87 a	28.89 a	302.49 b	291.99 b	115.97 c	112.35 c	276.65 c	268.76 c	0.30 a	0.33 a
250		24.43 b	25.94 b	232.75 c	219.27 c	157.67 b	148.29 b	354.38 b	367.19 b	0.26 b	0.28 b
500		19.51 c	21.25 c	201.43 d	193.17 d	176.73 a	171.56 a	475.22 a	468.64 a	0.23 c	0.25 c
	Control	17.46 c	18.55 d	174.32 d	165.35 d	67.68 d	62.55 d	417.95 a	412.13 a	0.20 d	0.22 d
	Halex 2	23.69 b	24.83 c	248.00 c	239.35 c	119.22 c	115.17 c	308.01 c	299.82 c	0.34 a	0.36 a
	Bs	29.40 a	31.29 a	312.35 a	301.12 a	168.97 a	160.65 a	252.91 d	265.94 d	0.27 c	0.30 c
	EM	29.17 a	30.50 b	307.70 b	294.96 b	160.06 b	154.37 b	375.25 b	369.06 b	0.30 b	0.34 b
0	Control	23.56 e	24.69 fg	267.81 h	259.33 h	45.90 j	42.33 m	273.54 i	270.13 j	0.25 fgh	0.27 gh
	Halex 2	28.72 c	29.33 d	305.63 e	298.14 d	62.09 i	57.72 l	210.11 m	203.71 o	0.40 a	0.43 a
	Bs	23.19 e	25.00 f	273.44 g	264.12 g	73.25 h	65.13 k	260.58 j	254.24 l	0.27 defgh	0.29 efg
	EM	36.17 a	37.32 a	375.90 b	363.84 b	81.00 g	77.02 h	247.27 k	241.39 m	0.34 bc	0.38 b
100	Control	18.45 g	18.72 i	176.88 l	172.73 l	47.25 j	43.94 m	309.77 g	299.97	0.22 hi	0.24 hi
	Halex 2	25.66 d	27.03 e	280.75 f	269.75 f	71.04 h	68.31 j	272.63 i	265.41 k	0.36 ab	0.37 bc
	Bs	35.11 a	36.84 a	393.38 a	382.53 a	182.25 d	178.75 c	235.09 l	230.14 n	0.30 cdef	0.34 cd
	EM	32.26 b	32.97 c	358.94 c	342.94 c	163.35 e	158.38 f	289.11 h	279.52 h	0.32 bcd	0.36 bc
250	Control	15.70 h	17.13 j	137.44 m	122.61 m	82.25 g	74.19 i	458.10 c	451.73 c	0.19 i	0.21 i
	Halex 2	22.72 ef	23.43 gh	212.81 j	201.93 j	161.16 e	158.34 f	313.63 f	302.83 f	0.31 bcde	0.32 de
	Bs	32.47 b	34.37 b	307.82 d	294.20 e	204.69 c	189.26 b	204.67 n	277.37 i	0.26 efgh	0.28 fg
	EM	26.83 d	28.82 d	272.91 g	258.32 h	182.59 d	171.36 e	441.10 d	436.81 d	0.29 cdefg	0.31 def
500	Control	12.14 i	13.64 k	115.13 n	106.74 n	95.33 f	89.75 g	630.40 a	626.70 a	0.12 j	0.15 j
	Halex 2	17.66 g	19.53 i	192.82 k	187.58 k	182.59 d	176.31 d	435.67 e	427.34 e	0.28 defg	0.30 efg
	Bs	26.83 d	28.94 d	274.75 g	263.62 g	215.69 a	209.44 a	311.28 g	302.00 g	0.24 gh	0.27. gh
	EM	21.42 f	22.87 h	223.00 i	214.72 i	213.30 b	210.72 a	523.52 b	518.52 b	0.26 efgh	0.29 efg

Values marked with same alphabetical letter(s), within a comparable group of means, do not significantly differ using revised L.S.D. test at 0.05 level.

Table (6): N and P concentrations (mg/g d. wt.) in lettuce leaves and roots as affected by lead treatments and the application of different microorganisms (Halex 2, Bs or EM) and their interaction during 2004/2005 and 2005/2006 seasons.

Treatments		N concentration				P concentration			
		Leaves		Roots		Leaves		Roots	
Pb levels (mg/l)	Microorg.	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season
0		28.85 a	27.05 a	18.69 a	17.37 a	3.39 a	3.56 a	2.06 a	2.21 a
100		27.63 a	25.70 a	16.39 b	14.88 b	3.25 a	3.42 a	2.00 a	2.17 a
250		25.48 b	23.36 b	14.70 c	13.29 c	2.84 ab	2.97 ab	1.81 ab	1.96 b
500		22.80 c	21.50 c	11.16 d	10.28 d	2.48 b	2.65 b	1.54 b	1.67 c
	Control	20.55 d	19.51 d	11.41 d	10.50 c	2.11 b	2.29 b	1.42 c	1.55 c
	Halex 2	31.04 a	28.80 a	17.22 b	15.90 b	3.55 a	3.71 a	2.09 ab	2.21 a
	Bs	27.58 b	25.54 b	13.09 c	11.58 c	3.65 a	3.79 a	2.18 a	2.32 a
	EM	25.59	23.75 c	19.22 a	17.85 a	2.64 b	2.81 b	1.73 bc	1.91 b
0	Control	24.50 de	23.72 def	14.32 defg	13.72 ef	2.87 bcdefg	2.94 bcde	1.81 abcd	1.96 cdef
	Halex 2	35.50 a	32.50 a	21.75 ab	20.21 ab	4.36 a	4.62 a	2.36 a	2.48 ab
	Bs	26.00 cd	24.33 cde	15.72 de	13.92 ef	2.98 bcdef	3.14 bcde	1.96 abcd	2.11 bcde
	EM	29.40 bc	27.63 bc	22.97 a	21.64 a	3.34 abcde	3.52 abcd	2.12 abc	2.27 abc
100	Control	22.10 ef	20.78 fgh	12.92 fgh	11.34 gh	2.23 efg	2.47 cde	1.47 abcd	1.62 fgh
	Halex 2	31.46 b	29.69ab	18.50 c	16.96 d	3.75 abc	3.91 abc	2.20 ab	2.32 abc
	Bs	29.50 bc	27.13 bcd	13.90 efg	12.42 fg	4.15 ab	4.33 ab	2.37 a	2.54 a
	EM	27.44 cd	25.19 cde	20.23 bc	18.78 bc	2.86 bcdefg	2.97 bcde	1.96 abcd	2.21 abcd
250	Control	19.50 f	18.31 h	11.40 hi	10.12 hi	1.76 fg	1.92 e	1.32 bcd	1.48 ghi
	Halex 2	29.57 bc	26.99 bcd	16.12 d	14.67 e	3.36 abcde	3.44 abcd	2.05 abc	2.15 bcde
	Bs	28.33 bc	25.83 cd	12.25 ghi	10.84 gh	3.89 abc	3.93 abc	2.27 ab	2.41 ab
	EM	24.50 de	22.32 efg	19.04 c	17.52 cd	2.34 defg	2.57 cde	1.61 abcd	1.78 efg
500	Control	16.10 g	15.21 i	7.00 j	6.82 j	1.58 g	1.81 e	1.07 d	1.15 i
	Halex 2	27.63 cd	26.03 cd	12.50 ghi	11.74 gh	2.74 cdefg	2.87 bcde	1.76 abcd	1.88 def
	Bs	26.48 cd	24.88 cde	10.50 i	9.12 i	3.57 abcd	3.74 abc	2.11 abc	2.23 abcd
	EM	21.00 f	19.87 gh	14.63 def	13.44 ef	2.01 fg	2.18 de	1.22 cd	1.39 hi

Values marked with same alphabetical letter(s), within a comparable group of means, do not significantly differ using revised L.S.D. test at 0.05 level.

Table (7): Effect of lead levels, the application of different microorganisms and their interaction on K^+ concentration (mg/g d. wt.) and Pb concentration (ug/g d. wt.) in lettuce leaves and roots during 2004/2005 and 2005/2006 seasons.

Treatments		K^+ concentration				Pb concentration			
		Leaves		Roots		Leaves		Roots	
Pb levels (mg/l)	Microorg.	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season
0		35.18 a	33.65 a	31.98 a	30.76 a	81.38 d	79.13 d	54.50 d	52.00 d
100		33.43 b	31.98 b	30.84 b	29.37 b	208.63 c	203.06 c	183.88 c	181.06 c
250		30.50 c	28.85 c	27.54 c	28.37 c	428.75 b	424.38 b	379.19 b	375.75 b
500		27.65 d	26.29 d	25.05 d	23.44 d	648.44 b	640.25 a	611.81 a	607.75 a
	Control	26.94 d	25.72 d	24.15 c	22.80 d	493.31 a	486.44 a	454.06 a	451.13 a
	Halex 2	30.62 c	29.32 c	30.73 a	29.01 b	362.00 c	357.50 c	310.75 c	307.81 c
	Bs	36.25 a	34.71 a	32.27 a	30.97 a	113.88 d	109.94 d	101.44 d	98.19 d
	EM	32.96 b	31.02 b	28.25 b	29.14 c	398.00 b	392.94 b	363.13 b	359.44 b
0	Control	31.03 f	30.00 e	28.20 fg	26.74 e	96.0 j	93.25 k	74.50 l	71.50 k
	Halex 2	37.33 b	35.67 b	36.12 a	34.51 a	81.75 k	80.25 m	48.00 o	46.00 m
	Bs	32.94 de	31.72 d	29.25 ef	28.94 d	60.50 l	58.00 o	41.00 p	38.75 n
	EM	39.41 a	37.21 ab	34.33 ab	32.84 b	87.25 jk	85.00 l	54.50 n	51.75 lm
100	Control	28.11 g	27.42 fg	25.03 hi	24.17 f	335.00 g	327.00 g	272.00 g	270.00 g
	zalex 2	31.60 ef	30.01 e	32.13 cd	30.43 c	195.75 i	192.25 j	163.00 g	160.25 j
	Bs	39.48 a	37.84 a	35.67 ab	33.14 b	66.75 l	62.00 n	59.50 m	56.00 l
	EM	34.53 cd	32.65 cd	30.51 de	29.72 c	237.00 h	231.00 h	241.00 h	238.00 h
250	Control	25.67 h	24.13 i	23.14 ij	22.04 g	635.75 d	629.25 d	591.75 d	587.00 d
	Halex 2	28.43 g	26.72 gh	28.19 fg	26.74 e	485.25 f	481.50 f	398.25 f	395.00 f
	Bs	37.63 b	35.87 b	33.64 bc	32.05 b	96.00 j	93.25 k	79.75 k	77.00 k
	EM	30.28 f	28.69 ef	25.20 h	32.64 f	498.00 e	493.50 e	447.00 e	444.00 e
500	Control	22.94 i	21.33 j	20.24 k	18.26 h	906.5 a	896.25 a	878.00 a	876.00 a
	Halex 2	25.11 h	24.88 i	26.46 gh	24.37 f	685.25 c	676.00 c	633.75 c	630.00 c
	Bs	34.94 c	33.42 c	30.53 de	29.74 c	232.25 h	226.50 i	225.50 i	221.00 i
	EM	27.62 g	25.54 hi	22.96 j	21.37 g	769.75 b	762.25 b	710.00 b	704.00 b

Values marked with same alphabetical letter(s), within a comparable group of means, do not significantly differ using revised L.S.D. test at 0.05 level.

Table (8): Effect of lead levels, the inoculation with different microorganisms (Halex 2, Bs or EM) and their interaction on yield and its attributes of lettuce plants during 2004/2005 and 2005/2006 seasons.

Treatments		**Average head weigh (g)**		**Head length (cm)**		**Head diameter (cm)**		**Dry matter content %**		**TSS %**	
Pb levels (mg/l)	**Microorg.**	**First Season**	**Second Season**	**First Season**	**Second Season**	**First Season**	**Second Season**	**First Season**	**Second Season**	**First Season**	**Second Season**
0		276.22 a	321.22 a	51.93 a	44.05 a	11.03 a	13.07 a	8.33 a	7.67 a	7.12 a	6.87 a
100		264.66 b	285.99 b	47.40 b	41.28 b	10.05 b	12.18 b	7.85 a	7.20 a	6.87 a	6.81 a
250		238.43 c	248.20 c	42.68 c	36.43 c	9.03 c	10.83 c	7.16 b	6.37 b	6.44 b	6.33 b
500		193.35 d	219.34 d	38.77 c	32.42 d	7.79 d	9.14 d	6.21 c	5.50 c	5.96 c	5.90 c
	Control	193.10 d	222.18 d	37.90 c	32.59 d	7.74 c	8.87 c	6.45 c	4.95 d	5.06 c	5.02 d
	Halex 2	243.83 c	248.59 c	47.00 b	40.37 b	10.78 a	13.31 a	8.24 a	8.34 a	6.74 b	6.92 b
	Bs	270.29 a	319.06 a	52.62 a	43.20 a	9.74 b	11.79 b	7.53 b	7.04 b	6.89 b	6.56 c
	EM	265.45 b	248.91 b	43.25 b	38.00 c	9.62 b	11.26 b	7.33 b	6.39 c	7.69 a	7.41 a
0	Control	239.59 fg	268.85 d	46.67 cdefg	39.75 cd	9.62 def	10.80 def	7.48 cdef	5.89 ef	5.84 fg	5.57 i
	Halex 2	280.26 c	289.40 c	58.83 a	48.69 a	12.97 a	16.30 a	9.21 a	10.32 a	8.00 ab	7.88 b
	Bs	247.64 ef	273.73 d	48.60 bcdef	40.90 cd	9.67 def	10.95 def	7.69 bcde	6.33 de	6.14 ef	5.79 hi
	EM	337.40 a	352.90 a	53.60 abc	46.84 ab	11.85 b	14.23 b	8.94 ab	8.13	8.49 a	8.23 a
100	Control	224.32 h	232.39 f	39.10 ghi	33.60 ef	8.21 ghi	9.30 fg	7.10 def	4.94 fg	5.24 gh	5.16 j
	Halex 2	264.13 d	265.49 de	48.70 bcdef	42.90 bc	11.12 bc	14.00 b	8.74 abc	8.80 b	6.79 de	7.19 cd
	Bs	299.88 b	344.38 a	57.87 ab	48.40 a	10.83 bcd	13.41 bc	8.15 abcd	8.41 b	7.62 bc	7.39 c
	EM	270.30 d	301.69 b	43.93 defgh	40.20 cd	10.02 cde	12.01 cde	7.39 cdef	6.64 de	7.81 b	7.49 c
250	Control	196.37 j	211.77 g	35.70 hi	30.30 fg	7.10 ij	8.36 gh	6.47 ef	4.88 fg	4.84 hi	4.81 k
	Halex 2	234.53 g	236.29 f	42.20 efgh	37.70 de	10.07 cde	12.18 cd	7.97 abcd	7.67 cd	6.32 ef	6.62 f
	Bs	279.28 c	289.40 c	53.13 abcd	42.90 bc	9.93 cde	12.37 cd	7.38 cdef	7.13 cd	7.12 cd	6.84 ef
	EM	243.53 f	255.33 e	39.67 fgh	34.80 ef	9.00 efg	10.41 ef	6.82 def	5.78 ef	7.49 bc	7.03 de
500	Control	112.10 k	175.72 h	30.13 i	26.72 g	6.02 j	7.02 h	4.76 g	4.10 g	4.33 i	4.53 k
	Halex 2	196.37 j	203.19 g	38.27 ghi	32.17 f	8.97 efg	10.74 def	7.02 def	6.57 de	5.85 fg	5.97 gh
	Bs	254.37 e	268.73 d	50.87 abcde	40.60 cd	8.54 fgh	10.42 ef	6.89 def	6.29 de	6.67 de	6.22 g
	EM	210.55 i	229.70 f	35.80 hi	30.19 fg	7.62 hi	8.39 gh	6.15 f	5.02 fg	6.98 cd	6.87 ef

Values marked with same alphabetical letter(s), within a comparable group of means, do not significantly differ using revised L.S.D. test at 0.05 level.