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ALLEVIATION OF THE ADVERSE EFFECTS OF LEAD AND CADMIUM ON GROWTH AND CHEMICAL CHANGES OF PEA PLANT BY PHOSPHORUS AND CALCIUM TREATMENTS

By

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ABSTRACT

A study was conducted in The Agricultural Experimental Station Farm, Faculty of Agriculture, Menoufiya University, during the winter seasons of 1998/99 and 1999/2000 to study the effects of lead (Pb) levels (0, 50, 100 and 200 mg l^{-1}) and cadmium (Cd) levels (0, 20, 40 and 60 mg l^{-1}), added as soil treatment at 60 and 75 days after sowing as well as alleviation of the adverse effects of heavy metals by adding PSO_4 and $CaSO_4$ at the rate of 2 mM, respectively. Pot experiments were carried out using sand culture to study the effect of heavy metals on growth characters, chemical composition and yield of pea plant (*Pisum sativum*) cv. Little Marvel.

Results showed that the high levels of Pb and Cd decreased plant height, root length, dry matter content of plant organs, net assimilation rate (NAR), relative growth rate (RGR), leaf area ratio (LAR) and tolerance index (TI).

The chemical analysis of plant leaves showed that all the concentrations of pollutants had deleterious effects on chlorophyll a, b and carotenoids. Moreover, total soluble sugars (TSS), total carbohydrates (TC), total amino acids (TAA), peroxidase (POD) activity and mineral contents (N, P, K) were significantly reduced. Proline

content and membrane integrity were positively increased with all levels of Pb and Cd pollutants, especially at high levels. Cd showed more adverse effect on all parameters than Pb.

Addition of P and Ca to Pb and Cd, respectively, reduced the deleterious effect of pollutants, especially at low concentrations of Pb and Cd.

The best results were observed in reducing accumulation of Pb and Cd in plant organs, especially in fruits. The highest seed yield/plant and seed proteins was recorded under the addition of P and Ca conditions under contamination conditions with Pb and Cd, respectively.

INTRODUCTION

Lead and cadmium are available for uptake by plants from the soil. These metals occur naturally in all soils in at least trace quantities, but their concentrations in soils can be greatly increased by burning of fossil oil, mining, application of sewage sludge in agriculture, application of fertilizers and pesticides, etc.

The uptake and integration of lead and cadmium into plants are affected by almost all environmental factors. Increased concentrations of these metals cause detrimental effects to plants (Wainwright and Woolhouse, 1977).

Cadmium is widely known to be a very hazardous toxic pollutant to various ecosystems (Nriagu, 1980) and one of the most mobile metallic elements in soils (McBride, 1989). The problem in Egypt, as in most developing countries, in the most industrial areas and highways which are located adjacent to the cultivated lands. Consequently, there is a real danger that lead uptake by plants enters human food chain (Wagner, 1993). Moreover, it may accumulate in the soil to reach undesirable level causing plant toxicity and vegetation damage (Aly, 1982).

Cadmium and lead were found to affect the uptake and distribution of nutrient elements in plants (Greger *et al.*, 1991 and Siedleck, 1995), water relations (Barceló and Poschenrieder, 1990 and Ewais, 1997), photosynthesis (Greger *et al.*, 1994 and Krupa and Baszynski, 1995), enzyme activity (Van Assche and Clijsters, 1990), nutrient uptake and distribution (Kovacevic *et al.*, 1999) and membrane functions (Ouariti *et al.*, 1997).

Lead is a major chemical pollutant of the environment and is highly toxic for man. It inhibits the activity of some important enzymes (Rai *et al.*, 1998). Moreover, Stochs and Bagchi (1995) and Taiz and Zeiger (1998) related the toxicity of heavy metals, including Pb to their ability to cause oxidative damage to plant cells. This damage includes enhanced lipid peroxidation, DNA damage and oxidation of protein sulfhydryl groups.

Heavy metal stress to root may be diminished by the phosphate concentration in the rhizosphere. Although the chemical identity in the apoplast varies widely, with some portion being bound to the carboxyl group of pectins and proteins, rather high amount were shown to be precipitated at the cell walls as ortho- or pyrophosphates (Zamdahi, 1976), thus being immobilized.

Culture experiments with seedlings of eight tree species tried to abolish the effect of soil phosphate on Pb uptake (Rolfe, 1973), where insoluble Pb phosphate precipitates in both the soil and in the roots and the seedlings showed visible phosphate deficiency symptoms.

In a previous work, it has been shown that addition of calcium to Cd-treated cultures decreased markedly the inhibition of nitrogenase activity, suggesting a possible antagonistic relationship between Ca^{2+} and Cd^{2+} (Fernandez-Pinas *et al.*, 1995).

Therefore, it appears important to study the effects of pollution not only because of its economic losses resulting from damaging plants and crops, but also because plant injuries may provide an indication for the existing level of pollutants in the environment and, eventually, their impact on human life. Moreover, to study the effect of adding some nutrient elements to reduce the deleterious effect of heavy metal on growth behaviour as well as the yield. The aim of this study is to investigate the toxic effects of lead and cadmium as well as alleviation of their adverse effects with phosphorus and calcium soil amendments, respectively, on the growth and chemical composition of pea plants.

MATERIALS AND METHODS

The present investigation was conducted at the Agricultural Experimental Station Farm, Faculty of Agriculture, Minoufiya University, during 1998/99 and 1999/2000 winter seasons. Pea seeds (*Pisum sativum*) cv. Little Marvel were used in this study. In both seasons, pea seeds were sown on November 12th in clay pots of 30 cm diameter. Each pot was filled with 8 kg dry-acid washed sand and the pots were irrigated with tap water till complete germination. On December 2nd of both seasons, four uniform and healthy seedlings were chosen per each pot.

A nutrient solution of modified Hoagland was prepared according to Epestein (1972). Each pot received 0.5 liter of the solution twice per week. Treatments were arranged in a complete randomized block design with 8 replications for each pollutant.

Pollutant treatments:

Lead (Pb) was used as lead sulphate at the concentrations of 0, 50, 100 and 200 mg l^{-1}. These concentrations were chosen to be in the range of those found in the agricultural soils (25-100 mg l^{-1}) as reported by Aly (1982). Phosphorus was added as phosphorus sulphate (PSO_4) at the concentration of 2 mM with each concentration of lead, to examine its effect on reducing the injurious effect of lead as a metal pollutant (Kahle, 1993).

Cadmium (Cd) was used as cadmium chloride in the nutrient solution at the concentrations of 0, 20, 40 and 60 mg l^{-1}. Calcium was added as calcium sulphate ($CaSO_4$) at the concentration of 2 mM for each concentration of cadmium with the aim of reducing the deleterious effect of cadmium as a metal pollutant (Fernandez-Pinas *et al.*, 1995).

Pollutants and other additives were applied twice at the vegetative growth stage (40 days after sowing), followed by the second application, 15 days later (55 days after sowing) in the nutrient solution.

Two-plant samples were successively taken at random from every treatment throughout the whole course of development, starting 70 days after sowing and then two weeks later (i.e., 70 and 85 days after sowing).

At each sampling time, five plants were taken out carefully from the pots using a stream of water to minimize loss of root system, and were then cleaned from any adherent dirt using wet muslin cloth, and the following data were recorded:

I. Growth analysis:

In the laboratory, plants were separated into leaves, stems and roots, and the following growth parameters were determined: plant height (cm), root length (cm), number of leaves per plant, dry matter (g) of plant organs (70°C, using a hot-air oven for 72 hours). The data of dry weight and leaf area were used to determine the following growth parameters:

- Leaf area ratio (LAR) as m^2/kg dry weight= Leaf area (LA)/ Dry weight of plant
- Relative growth rate (RGR) as mg/g/day = $\text{Log } W_2 - \text{Log } W_1 / t_2 - t_1$
- Net assimilation rate (NAR) as g/m/day = $(W_2 - W_1)(\text{Log } A_2 - \text{Log } A_1) / (A_2 - A_1)(t_2 - t_1)$

where:

W_1 and W_2 are the dry weight of whole plant at time 1 (t_1) and time 2 (t_2), respectively.

A_1 and A_2 are the leaf areas (cm^2) at t1 and t2, respectively.

RGR and NAR were calculated according to the method of Buttery and Buzzell (1974).

- Tolerance index (TI) of each plant against each of the metals was calculated according to Hertstein and Jager (1985) as follows:

TI = mean root length of 8 plants in metal solution / mean root length of 8 plants in control solution.

II. Chemical analysis:

On the first sampling time (70 days after sowing), four plants were randomly collected from each treatment, fresh leaves were analyzed for:

Photosynthetic pigments: Fresh leaves were analyzed for chlorophyll pigments concentration by using the method of Witham *et al.*(1971).

Peroxidase (POD) activity (expressed as optical density/g fresh weight after 2 min) was measured in fresh leaves according to the method described by Fehrman and Dimond (1967).

Membrane integrity: The loss of membrane integrity in leaf tissue subjected to heavy metals stress was studied in term of the leakage of solutes, and absorption at Ultraviolet wave length of 273 nm which was determined following the method of Leopold *et al.* (1981).

Proline content was measured in fresh leaves using 5-sulphosalycilic acid and acid ninhydrine reagent following the method of Bates *et al.* (1973).

Total carbohydrates and soluble sugars concentrations in the dry leaves (mg/g dry weight) were determined using the colorimetric method of Dubois *et al.*(1956).

Total amino acids were determined in dry leaves as described by the method of Rosen (1959).

N, P and K: Nitrogen was determined using micro-Kjeldahl method. Phosphorus and potassium were estimated colorimetrically and by Flame photometer, respectively (A.O.A.C., 1975).

Pb and Cd analysis in leaves, stems and roots were determined by atomic absorption spectroscopy and expressed as mg l^{-1} according to Cottenie *et al.*(1982).

III. Yield components:

At the end of plant life, all plants from each treatment were carefully removed and kept in shady place until complete drying, then the following data were recorded: number of pods per plant, number of seeds per pod (mean 20 pods), weight of seeds per plant (g).

The concentrations of protein, Pb and Cd were determined in the dry seeds using the above mentioned method and total protein content was calculated by multiplication of N x 6.25.

Statistical analysis:

All data were subjected to statistical analysis according to the procedures outlined by Snedecor and Cochran (1973). The analysis was done using a statistic computer program (COSTAT).

RESULTS AND DISCUSSION

1. Vegetative growth:

Data presented in Table (1) indicate clearly that plant height, root length and number of leaves were significantly decreased with increasing both Pb and Cd concentrations. In the first season, the highest concentrations of Pb and Cd were highly serious in reducing plant height by 42 and 51%, respectively compared with control plants, with the observation that Cd was more deleterious than Pb pollutant.

The effect of those pollutants in reducing plant height might be due to its effect on the meristematic activity and, consequently, retardation of vertical expansion and longitudinal growth. Similar results were obtained by Gadallah (1995) and El-Shintinawy and El-Ansary (2000).

The same table showed that root length was seriously reduced by Pb and Cd, especially at high levels by 56 and 63%, respectively compared to the control plants, in the first season. Stiborova *et al.*(1987)

found that high levels of lead were toxic to the meristimatic regions of barley roots.

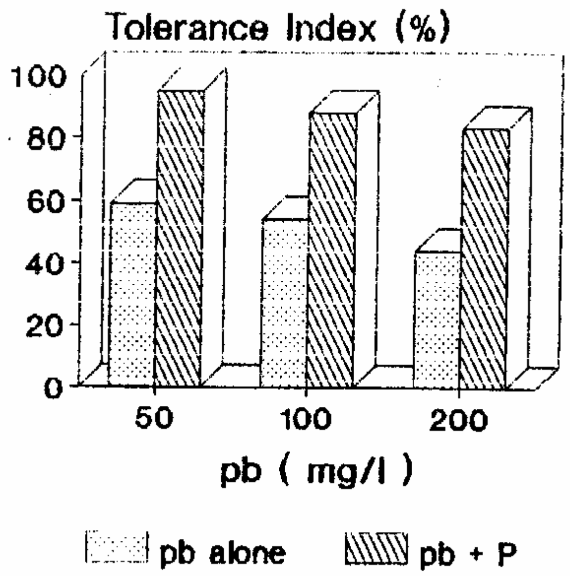
The negative effect of Cd on root growth might be attributed to the inhibitory effect on the vegetative growth, thus reduction in the nutrient supply to other plant organs might occur (Poschenrieder *et al.*, 1989 and Van Noordwijk *et al.*, 1995).

The effect trend as previous shown in shoot and root length was clearly presented in shoot and root dry weight as well as number of leaves per plant. In this respect, the No. of leaves and dry mass were less affected by lowest metal levels (Table 1). Cd was more suppressive than Pb in pea plants. These results are in conformity with those of Aidid and Okamato (1992) working on *Impatiens balsamina*, where Pb (0.5 mM) and Cd (0.1 mM) suppressed stem cell elongation.

Heavy metal stress to roots may be diminished by phosphate concentration in the rhizosphere. Although their chemical identity in the apoplast varies widely, with some portion being bound to the carboxyl group of pectins and proteins, rather high amount was shown to be precipitated at the cell walls as ortho- or pyrophosphates (Kahle, 1993), thus being immobilized. Culture experiments with seedlings of eight tree species tried to isolate the effect of soil phosphate on Pb uptake (Rolfe, 1973). The seedlings showed visible phosphate deficiency symptoms after 60 days, due to the formation of insoluble Pb phosphate precipitates both in the soil and in the roots. The present data show clearly that addition of PSO_4 at 2 mM concentration inhibits the deleterious effect of Pb, especially at low concentration, all the growth parameters (plant height, root length and dry weight) recorded high values compared with untreated plants, especially root length. Burton *et al.*(1986) reported that the interaction between heavy metal
Control values were used for both pollutant experiments and PSO_4 may be independent antagonist, additive or synergistic. Thus, the pattern of uptake by roots of several tree species was changed. On the other hand, there might be a number of different mechanisms for cell damage by cadmium, and an involvement of Ca^{2+} in protection against Cd^{2+}

Table (1): Effect of heavy metals (Pb and Cd) and addition of P and Ca on growth characteristics of pea plants in the growing seasons of 1998/99 and 1999/2000.

Treatments	Plant height (cm)	Root length (cm)	No. of leaves	Dry weight (g) Shoot	Dry weight (g) Root	Dry weight (g) Total dry weight
1998/99						
Control	42.65	15.75	9.48	5.81	1.239	7.049
Pb (mg l^{-1})						
50 Pb	37.50	9.25	7.20	4.95	1.210	6.160
100 Pb	30.10	8.50	6.16	3.99	1.112	5.102
200 Pb	24.50	6.90	5.01	3.67	1.091	4.761
50 Pb + P	41.50	14.90	8.51	5.25	1.235	6.485
100 Pb + P	38.90	13.86	7.35	4.86	1.212	6.072
200 Pb + P	36.80	13.01	6.81	4.32	1.181	5.501
L.S.D$_{0.05}$	1.13	1.05	0.85	0.43	0.0112	0.85
Cd (mg l^{-1})						
20 Cd	38.00	10.10	7.00	5.00	1.184	6.184
40 Cd	30.80	7.35	6.20	4.40	1.121	5.521
60 Cd	21.10	5.68	4.20	3.80	1.091	4.891
20 Cd + Ca	41.00	14.36	7.10	5.30	1.218	6.518
40 Cd + Ca	37.20	12.91	6.90	4.95	1.195	6.145
60 Cd + Ca	35.10	11.85	5.20	4.51	1.158	5.668
L.S.D$_{0.05}$	1.33	1.14	0.75	0.61	0.0112	0.84
1999/2000						
Control	57.50	16.75	14.50	5.97	1.233	7.203
Pb (mg l^{-1})						
50 Pb	53.70	9.61	13.10	4.89	1.211	6.101
100 Pb	50.10	8.53	11.00	3.92	1.110	5.030
200 Pb	46.00	6.80	9.00	3.71	1.080	4.790
50 Pb + P	56.20	15.51	13.90	5.52	1.221	6.741
100 Pb + P	52.90	14.81	12.85	4.95	1.202	6.152
200 Pb + P	50.00	13.92	12.40	4.46	1.160	5.616
L.S.D$_{0.05}$	1.12	1.03	0.43	0.33	0.011	0.84
Cd (mg l^{-1})						
20 Cd	52.10	10.50	12.50	5.10	1.214	6.314
40 Cd	40.00	7.60	10.80	4.50	1.132	5.632
60 Cd	31.90	6.00	8.10	3.90	1.081	4.981
20 Cd + Ca	55.10	15.65	13.10	5.60	1.221	6.821
40 Cd + Ca	49.20	13.92	11.80	5.00	1.211	6.211
60 Cd + Ca	47.60	12.69	9.60	4.60	1.182	5.782
L.S.D$_{0.05}$	1.35	1.06	0.51	0.33	0.012	0.81



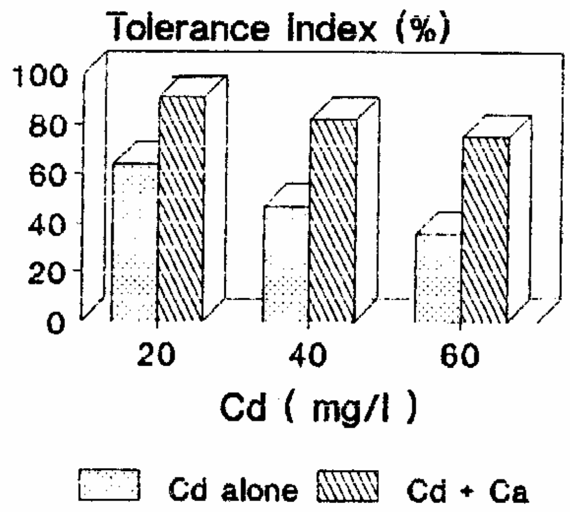


Fig.(1): Tolerance Index (%) as affected by pb and Cd as well as addition of P and Ca, in 1998/99.

inhibition of important physiological functions remain as an open-possibility (Fernandez-Pinas *et al.*, 1995).

The present data (Table 1) show that addition of Ca in nutrient solution with Cd was found to extend a detoxifying role towards Cd inhibition of physiological processes. Ca at the concentration of 2 mM was able to greatly decrease the toxic effect of Cd on the growth of pea (Table 1). Our results show that addition of Ca had a negative effect on Cd accumulation, suggesting an antagonestic interaction between Cd and Ca. It has been suggested that this antagonism could be explained by a competition phenomenon due to their very similar ionic radii (Gipps and Coller, 1982).

2. Tolerance against metals in solutions:

The tolerance of pea roots against the Pb and Cd metals was investigated (Fig. 1). Plant differences due to the effects of single metals are obvious. The concentrations of Pb and Cd caused an almost complete reduction of root growth. The Cd treatment allowed a slight amount of root production compared with Pb. Calculated tolerance indices of pea plant against the metals tested were generally low, meanwhile, Cd tolerance indices were lower than with Pb, especially at high levels of pollutants (Fig. 1).

Addition of both P and Ca to the nutrient medium containing Pb and Cd, respectively, increased TI of roots. This treatment showed high root tolerance index, especially at high concentrations of pollutants. The need for such investigations had been suggested by Coughtry and Martin (1979), Cox and Hutchinson (1979) and Hertstein and Jager (1985).

3. Growth analysis:

Data in Table (2) show that the highest levels of Pb decreased significantly RGR, NAR and LAR of treated pea plants by 71, 42 and 18%, respectively, comparing with untreated plants. Whereas, addition of PSO_4 to the nutrient medium protected the plants from the harmful effect of Pb.

Table (2): Effect of heavy metals (Pb and Cd) and addition of P and Ca on physiological parameters of pea plants 70-85 days after sowing in the growing seasons of 1998/99 and 1999/2000.

Treatments	1998/99			1999/2000		
	RGR mg g^{-1} d^{-1}	NAR mg g^{-1} d^{-1}	LAR m^2 kg^{-1} $plant^{-1}$	RGR mg g^{-1} d^{-1}	NAR mg g^{-1} d^{-1}	LAR m^2 kg^{-1} $plant^{-1}$
Control	105	0.268	24.1	99	0.386	22.7
Pb (mg l^{-1})						
50 Pb	80	0.208	22.0	81	0.351	21.4
100 Pb	71	0.170	20.2	72	0.325	19.2
200 Pb	30	0.156	19.7	32	0.301	18.9
50 Pb + P	91	0.179	24.0	92	0.359	22.5
100 Pb + P	82	0.175	23.1	83	0.345	21.6
200 Pb + P	74	0.171	22.2	75	0.068	20.7
L.S.D$_{0.05}$	5	0.021	1.8	16	0.331	1.82
Cd (mg l^{-1})						
20 Cd	68	0.167	23.2	69	0.214	21.5
40 Cd	59	0.157	21.3	61	0.201	20.1
60 Cd	53	0.150	18.2	55	0.194	18.0
20 Cd + Ca	89	0.260	24.0	91	0.285	22.9
40 Cd + Ca	86	0.244	22.3	89	0.241	22.1
60 Cd + Ca	81	0.207	21.6	85	0.202	20.6
L.S.D$_{0.05}$	18	0.120	1.95	20	0.038	1.71

It was, also, clearly observed that Pb was more toxic than Cd in reducing RGR, NAR and LAR (50, 44 and 25%, respectively compared with the control plants) in the first season. Data in the second season almost show the same trend.

A growth analysis is an useful method for evaluation of the main factors limiting plant growth. In Cd-treated plants, this analysis was rarely made. Greger *et al.*(1991) and Landberg and Greger (1994) proved that Cd inhibited relative growth rate (RGR) in sugar beet plants. Also, Abo-Kassem *et al.*(1995) reported that the toxic effect of Cd on RGR of wheat plants is due to net assimilation rate (NAR) retardation rather than to leaf area ratio (LAR) inhibition. The main factor limiting plant growth was NAR inhibition due to decrease in photosynthesis (Vassilev *et al.*, 1998), who reported that one of the reasons for the reduced photosynthetic rate was the lower plastid pigment content which accelerates respiration.

4. Photosynthetic pigments:

Data in Table (3) show that chlorophyll contents decreased as Pb and Cd levels were increased, particularly at high concentrations. It was clear that the data of the second season followed the same trend of the first one.

Addition of P and Ca to the nutrient medium abolished the harmful effect of Pb and Cd, on photosynthetic pigments content (Table 3). The harmful effect of Pb on photosynthetic pigments due to the reduction in chlorophyll content may be attributed to the inhibition of chlorophyll biosynthesis in treated plants as suggested by some published reports, which indicated that Pb accumulate in chloroplast disorganizing their ultrastructure and decreasing the biosynthesis of chloroplasts (Lukaszak and Poskuta, 1998 and Zaman and Zereen, 1998)

Plastid pigments have been shown as one of the main sites of the toxic Cd action. It was established that Cd decreased chlorophyll content in many plant species; e.g., tomatoes (Baszynski *et al.*, 1980), wheat and cucumber (Malik *et al.*,1992), maize (El-Enany,1995) and beans (Krupa and Baszynski,1995). The reduced chlorophyll content in Cd-treated plants is due to inhibitions of its biosynthesis (Stobart *et al.*, 1985) and/or activation of its enzymatic degradation (Somashekaraiah, 1992). Lang *et al.*(1995) related the decreased chlorophyll content to the Cd^{2+}-induced iron deficiency in plants, and Prasad (1995) related the closure of stomata by Cd^{2+}.

Table (3): Effect of heavy metals (Pb and Cd) and addition of P and Ca on photosynthetic pigments of pea plants (mg/g d.wt.) of pea leaves in the growing seasons of 1998/99 and 1999/2000.

Treatments	1998/99				1999/2000			
	Chl A	Chl b	Chl a+b	carotenoids	Chl a	Chl b	Chl a+b	Carotenoids
Control	5.73	3.88	9.61	2.25	5.91	3.68	9.59	2.31
Pb (mg l^{-1})								
50 Pb	4.92	2.98	7.90	1.79	5.10	2.86	7.96	1.82
100 Pb	4.41	2.83	7.24	1.59	4.85	2.65	7.50	1.62
200 Pb	4.01	1.57	5.58	1.42	4.41	1.61	7.02	1.49
50 Pb + P	5.82	3.81	9.63	2.29	5.62	3.91	9.53	2.32
100 Pb + P	5.12	3.73	8.85	2.16	5.21	3.84	9.05	2.21
200 Pb + P	4.57	3.08	7.65	2.26	4.91	3.12	8.03	2.28
L.S.$D_{0.05}$	0.62	0.51	0.81	0.21	0.61	0.75	0.62	0.40
Cd (mg l^{-1})								
20 Cd	4.40	2.81	7.21	1.78	4.80	2.71	7.51	1.81
40 Cd	3.66	1.52	5.18	1.16	3.91	1.58	5.49	1.21
60 Cd	3.02	1.31	4.23	1.02	3.52	1.39	4.91	1.09
20 Cd + Ca	5.29	3.22	8.51	2.87	5.55	3.32	8.87	2.88
40 Cd + Ca	5.00	2.70	7.70	2.50	5.22	2.78	8.00	2.59
60 Cd + Ca	4.89	2.61	7.59	2.45	5.01	2.60	7.61	2.48
L.S.$D_{0.05}$	0.44	0.58	1.55	0.45	0.42	0.22	0.66	0.51

Addition of P and Ca treatments, on the other hand, seemed to reduce the harmful effect of Pb and Cd pollutant, respectively on growth parameters. A considerable observation was that the promising effect of P and Ca in alleviating the deleterious effect of Pb and Cd pollution was much more pronounced at relatively higher concentrations of the heavy metal. In this regard, data in Tables (1 and 2) showed that percentage increase in RGR, NAR and LAR, resulted from P and Ca treatments, were higher at 200 and 60 mg l^{-1} of Pb and Cd, respectively. An early study by Zamdahi (1976) showed that plant tolerance to heavy metal stress can be achieved by means of phosphate treatment. Whereas, their chemical identity in the apoplast and thus the heavy metal being immobilized.

5. Carbohydrate fractions:

The negative effects of all concentrations of Pb and Cd on total soluble sugars (TSS) and total carbohydrates (TC) were indicated in Table (4). In this concern, Pb pollutant was more effective in reducing the TSS and TC than Cd effect. Moreover, the addition of P to polluted nutrition solution with Pb and Ca with Cd significantly altered the hazard effect of both heavy metals in reducing TCC and TC.

The negative effect of Pb on TSS content might be attributed to its deleterious effect on the rate of sugar biosynthesis and flow of photoassimilates (Poskuta *et al.*, 1987). Moreover, the inhibitory effect of Pb on the photosynthetic enzyme ribulose bisphosphate carboxylase (RuBpc), reported by Salisbury and Ross (1992). Cd-treated plants had less TSS and TC than the control and this may be attributed to the inhibition of photosynthetic activity and CO_2 assimilation (Greger and Orgen, 1991) as the dry weight also decreased (Table 1). However, at 2 mM Ca level in the nutrient medium, the concentrations of TSS and TC in plants subjected to different levels of Cd were higher than those of plants subjected to Cd only.

Ca is known to reduce the toxicity of Cd to plants (Hosono *et al.*, 1979; Greger and Bertell, 1992 and Mclaughlin *et al.*,1998). Moreover, Ramalho *et al.*(1995) reported that Ca is very important to the plant metablism in general and for photosynthetic processes in particular. Moreover, Ca ion bounds to photosystem II and stabilisation of chlorophyll and apoprotein from light harvesting II (Tanaka *et al.*, 1992; Katoh and Han, 1993 and Schikler and Caspi, 1999).

6. Total amino acids and proline content:

It can be clearly noticed from the data presented in Table (4) that Pb significantly depressed TAA content in pea leaves with increasing

Table (4): Effect of heavy metals (Pb and Cd) and addition of P and Ca on total soluble sugars (TSS), total carbohydrates (TC) (mg glucose/g dry weight), total amino acids (TAA) (mg leucine/g dry weight) and proline (μg leucine/g dry weight) of pea plant leaves in the growing seasons of 1998/99 and 1999/2000.

Treatments	1998/99				1999/2000			
	TSS (mg/g d.wt.)	TC (mg/g d.wt.)	TAA (mg/g d.wt.)	Proline (μg/g d.wt.)	TSS (mg/g d.wt.)	TC (mg/g d.wt.)	TAA (mg/g d.wt.)	Proline (μg/g d.wt.)
Control	11.9	26.85	53.97	125	12.36	28.09	56.82	130
Pb (mg l^{-1})								
50 Pb	9.21	24.50	45.12	501	9.50	25.10	44.20	510
100 Pb	8.55	22.20	36.42	529	8.31	21.90	34.17	533
200 Pb	6.90	19.95	33.08	576	7.19	18.39	31.29	380
50 Pb + P	10.90	25.10	50.15	131	11.90	26.40	54.10	142
100 Pb + P	10.12	24.60	47.71	152	11.00	25.83	49.39	165
200 Pb + P	9.45	23.70	41.96	167	10.50	23.36	46.50	179
L.S.D$_{0.05}$	0.92	1.70	2.45	14.6	0.35	1.82	2.51	13.2
Cd (mg l^{-1})								
20 Cd	9.80	23.10	36.46	313	9.70	22.77	38.33	320
40 Cd	9.10	21.45	34.03	494	8.45	20.88	35.78	460
60 Cd	8.50	19.10	32.52	567	7.60	19.60	33.61	520
20 Cd + Ca	11.20	24.57	51.71	107	11.59	25.89	51.95	110
40 Cd + Ca	10.10	23.75	48.84	126	10.83	22.98	46.86	131
60 Cd + Ca	9.50	21.50	46.15	137	9.80	20.99	44.50	150
L.S.D$_{0.05}$	1.10	1.85	2.21	15.8	0.52	1.91	2.31	16.1

concentration of Pb in nutrient medium. Moreover, Cd showed more serious effect in reducing TAA in pea leaves. Whereas, addition of P to Pb and Ca to Cd mostly reduced the harmful effect of both Pb and Cd on reducing TAA. The decrease in TAA content could be due to a metabolic disorder leading to inhibition in protein synthesis. In accordance to this hypothesis, Delhaize *et al.*(1989) and El-Shintinawy and El-Ansary (2000) observed a decrease in the rate of protein synthesis in *Datura innoxia* treated with Cd. Moreover, Ewais (1997) reported that Pb decreased total soluble protein content in shoots.

Concerning the accumulation of proline as affected by Pb and Cd application, there was a highly significant induction in this respect. Pb was more effective in proline content than Cd, especially at low levels. Whereas, addition of P to Pb and Ca to Cd in nutrient solution altered this effect. The negative effect of Pb and Cd on water uptake, water movement and transpiration was observed (Poschenrieder *et al.*, 1989; Alia, 1991; Costa and Morel, 1994; Chen and Kao, 1995 and Marchiol *et al.*, 1996), established that Cd was a strong inducer of proline accumulation. Moreover, Shah and Dubey (1997/98) and Vassilev *et al.*(1998) suggested that proline played the role of enzyme protectant.

7. Peroxidase (POD) activity:

Pb and Cd exert their toxicity through inactivation of enzymes, possibly through reaction with SH-groups of proteins (Fuhrer, 1982). The data presented in Table (5) clearly proved that both Pb and Cd decreased POD activity in pea leaves, whereas the addition of P and Ca to Pb or Cd, respectively, in nutrient medium raised POD activity. Lee *et al.*(1976) and Fuhrer (1982) reported that Cd induced a decrease in POD activities in soybean and bean leaves. It has been postulated by several investigators that the action of peroxidase located in cell walls would be to confer regidity to the cell wall and prevent later expansion involved in growth (Gardiner and Cleland, 1974 and Fry, 1986). Thus, Cd- and Pb-induced inhibition in root growth of rice seedlings is likely to result from cell wall tightening processes related to formation of cross-linkages among cell wall polymers (Chen and Kao, 1995).

It is suggested that, at higher Pb and Cd concentrations, the formation of both free radicals and reactive O_2 species is beyond that capacity of the endogenous antioxidant system, thus POD activity

Table (5): Effect of heavy metals (Pb and Cd) and addition of P and Ca on the activity of peroxidase (POD) (optical density after 2 min) and membrane permeability* (%) in the growing seasons of 1998/99 and 1999/2000.

Treatments	Peroxidase activity (O.D after 2 min)		Membrane permeability (%)	
	1998/99	1999/2000	1998/99	1999/2000
Control	1.791	1.931	20	19
Pb (mg l^{-1})				
50 Pb	1.341	1.562	26	24
100 Pb	1.123	1.261	31	30
200 Pb	0.651	0.693	40	38
50 Pb + P	1.521	1.512	21	20
100 Pb + P	1.257	1.487	23	23
200 Pb + P	1.118	1.121	25	26
$L.S.D_{0.05}$	0.232	0.213	1.4	1.5
Cd (mg l^{-1})				
20 Cd	1.157	1.211	28	29
40 Cd	0.790	0.826	36	35
60 Cd	0.680	0.611	50	51
20 Cd + Ca	1.390	1.451	22	21
40 Cd + Ca	1.261	1.329	24	23
60 Cd + Ca	1.072	1.131	27	28
$L.S.D_{0.05}$	0.251	0.243	1.9	2.0

decreased at high Pb levels (Rucinska *et al.*, 1999). In this regard, Creissen *et al.*(1994) reported that, under heavy metal pollution, POD and catalase activities increase as a way of acclimation to metal stress. These enzymes were found to provide antioxidant protection and preserve membrane integrity. Therefore, the addition of P and Ca treatments tended to maintain the activity of the enzyme at high level. In more recent work, Scalet *et al.*(1995) showed that POD activity increased as an early response to environmental stress and might provide cells with resistance against the formation of H_2O_2, which is formed in chloroplasts when plants are exposed to heavy metal pollution.

8. Membrane integrity:

The present investigation shows that there was a marked reduction in membrane permeability gradual with increasing Pb and Cd concentrations in nutrient medium. The maximum reduction recorded with highly Pb and Cd concentrations. Meanwhile, elements (P and Ca) additions repaired the membrane damage caused by heavy metals. Kahle (1993) reported that plant cell membranes are considered the primary sites of metal injury. The metal-induced changes in membrane properties not only affect K^+ anf H^+ extrusion but also the function of membrane carriers and ion channels as well as the permeability of cell membranes to water. Membrane damage affected by Pb and Cd may be due to lipid peroxidation mediated by activated oxygen radicals (hydrogen peroxide, hydroxyl and superoxide radicals) and could be quenched by the induction of specific enzyme like peroxidase superoxide dismutase and catalase (De Vos and Schat,1981). The increased peroxidase and hydrolytic enzyme activities might initiate senescence (Prasad, 1995 and Devi *et al.*, 1996). Aidid and Okamoto (1992) suggested that the electrogenic H^+-pump might be selectivity damaged and the passive permeability of the membrane might be changed as affected by lead. Many studies showed that even low concentrations of Pb could cause severe ultrastructural damage by interference with the structural integrity of the organells such as chloroplasts and mitochondria (Buwalada *et al.*, 1992), in addition to inhibiting metabolic processes by direct reduction of enzyme activities (Ouariti *et al.*, 1997).

It is well documented that Cd inhibits plant growth (Foy *et al.*, 1978) but the degree of inhibition depends on the Ca level (Greger and Bertell, 1992). Cd^{2+} may compact with Ca^{2+} or prevent it from being utilized in Ca^{2+}-dependent processes, leading to diminished growth. Mukherji and Mukerji (1979) showed that Cd^{2+} may inhibit cell elongation. Ca^{2+} is needed for the formation of middle lamella at cell division and has an important role in the maintenance of membrane integrity (Alam, 1993) and its absence causes a less of selectivity and other adverse effects in the plasma membranes.

Table (6): Effect of heavy metals (Pb and Cd) and addition of P and Ca on nitrogen (N), phosphorus (P) and potassium (K) as (%) of pea leaves in the growing seasons of 1998/99 and 1999/2000.

Treatments	1998/99			1999/2000		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Control	2.20	0.50	3.31	2.81	0.48	3.97
Pb (mg l^{-1})						
50 Pb	2.00	0.39	3.00	2.41	0.38	3.66
100 Pb	1.90	0.37	2.49	2.29	0.35	2.31
200 Pb	1.65	0.32	2.39	2.17	0.31	2.92
50 Pb + P	2.18	0.49	3.23	2.67	0.47	3.89
100 Pb + P	1.95	0.47	3.25	2.54	0.46	3.62
200 Pb + P	1.78	0.45	3.10	2.41	0.43	3.21
$L.S.D_{0.05}$	0.21	0.03	0.30	0.11	0.03	0.33
Cd (mg l^{-1})						
20 Cd	1.81	0.35	2.75	2.10	0.37	3.51
40 Cd	1.60	0.31	2.51	1.81	0.34	3.11
60 Cd	1.31	0.27	2.13	1.62	0.30	2.75
20 Cd + Ca	2.00	0.46	3.11	2.62	0.46	3.85
40 Cd + Ca	1.90	0.42	2.91	1.95	0.44	3.52
60 Cd + Ca	1.71	0.40	2.75	2.10	0.41	3.00
$L.S.D_{0.05}$	0.18	0.03	0.28	0.12	0.03	0.34

9. N, P and K contents:

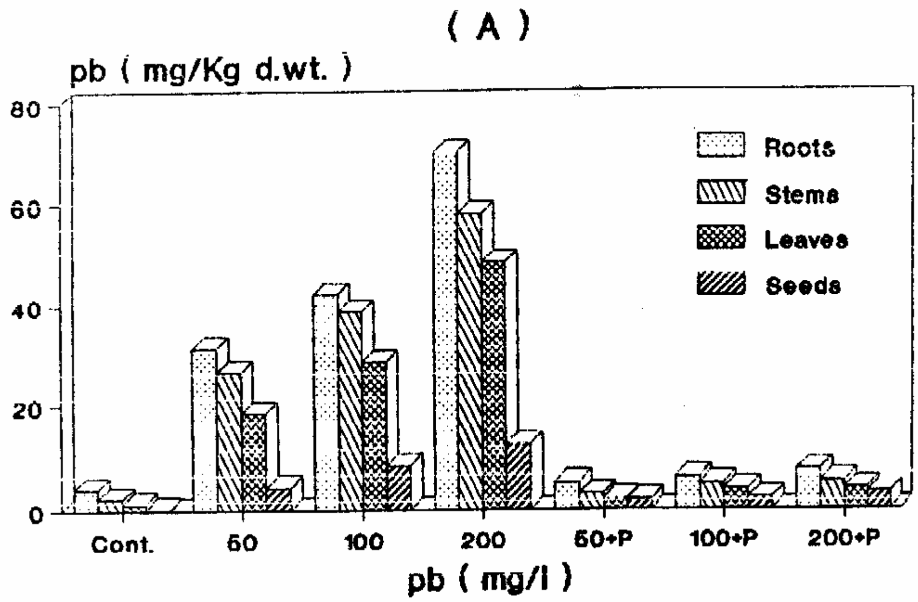
Data recorded in Table (6) show that N, P and K concentrations were negatively affected by Pb and Cd treatments. However, it was evident that Cd was more hazardous than Pb treatment.

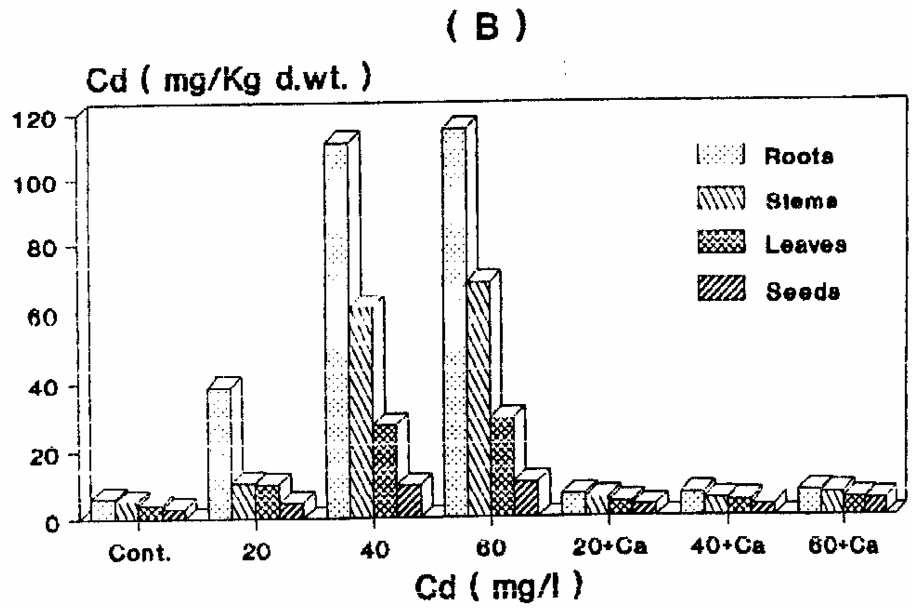
It was clear that high levels of heavy metals showed more adverse negative effects than the lower ones. The highly negative effects of Pb polluted soil on N, P and K were also reported by Kahle (1992) and Hlusek and Richter (1992). This reduction was attributed to the inhibitory effect of the heavy metals on the uptake and translocation of these elements by plant roots. Moreover, the effect of the pollutants on element content might be ascribed to their indirect factors such as their effects on the water uptake and transpiration and, consequently, decreasing mineral uptake and translocation by plants (Kahle, 1993 and Fodor *et al.*, 1996), who reported that nitrogen fixation was shown to be inhibited in response to increasing Cd and Pb supply. The obtained results in accordance with those obtained by Hernandez *et al.*(1995), who found that K concentration was decreased in both root and shoot of pea seedlings exposed to cadmium.

Data presented in the same table clearly prove that application of P and Ca at 2 mM significantly reversed the harmful effects of Pb and Cd, respectively on mineral content of pea leaves. Greger and Bertell (1992) reported that Cd causes a decrease in the Ca concentration in the roots, and since Ca is needed in growth processes of plant and high nutrient (Ca^{2+}) concentration diminishes the effect of Cd^{2+} activity.

10. Bioaccumulation of Pb and Cd in plant organs:

Application of Pb and Cd caused highly significant increase of their content in roots, stems, leaves as well as fruits (Fig. 2, a & b). Their contents increased with increasing metal doses. Most of the heavy metals were accumulated in roots and less amounts were translocated into the shoots. The order of metal accumulation in stem and leaves, for Pb and Cd. The translocation of Pb and Cd to the aerial parts was limited and the fruits showed the lowest content (Fig.2,a&b).





Fig(2): Bioaccumulation of pb and Cd in organs of pea plants as affected by pb and Cd as well as addition of P and Ca, in 1998/99 season.

Table (7): Effect of heavy metals (Pb and Cd) and addition of P and Ca on yield and its components of pea plants in the growing seasons of 1998/99 and 1999/2000.

Treatments	1998/99				1999/2000			
	No. of pods /plant	No. of seeds /pod	Seed yield /plant (g)	Seed protein (%)	No. of pods /plant	No. of seeds /pod	Seed yield /plant (g)	Seed protein (%)
Control	14.0	6.3	30.99	18.9	12.0	5.88	40.28	19.5
Pb (mg l^{-1})								
50 Pb	6.8	4.6	20.60	17.0	6.2	3.80	20.10	18.0
100 Pb	5.0	3.3	10.65	16.5	4.0	3.10	10.48	17.5
200 Pb	3.0	2.3	10.34	15.9	3.3	2.90	10.23	17.1
50 Pb + P	13.0	6.3	30.60	18.6	11.2	4.81	30.70	19.1
100 Pb + P	11.5	5.5	30.00	18.0	10.0	4.20	30.24	18.6
200 Pb + P	10.1	4.6	20.76	17.6	9.7	3.10	20.96	17.9
$L.S.D_{0.05}$	2.82	2.91	1.74	1.2	2.56	2.82	1.66	1.3
Cd (mg l^{-1})								
20 Cd	4.6	4.0	11.77	16.5	9.5	3.55	10.35	16.9
40 Cd	4.0	3.7	11.14	15.0	6.2	3.26	10.14	15.5
60 Cd	3.1	2.8	11.00	14.3	4.1	2.91	10.01	14.8
20 Cd + Ca	13.3	6.7	30.31	17.9	11.7	4.91	30.31	18.1
40 Cd + Ca	10.0	5.3	20.99	16.8	9.8	4.80	30.19	17.3
60 Cd + Ca	9.5	4.6	20.61	16.3	9.1	4.21	20.91	16.8
$L.S.D_{0.05}$	3.2	2.54	1.53	1.3	2.6	2.43	1.52	1.4

The most important results reported herein showed the maximum reduction in accumulation of Pb and Cd in all parts of pea plants by P and Ca presented in nutrient solution. This supports the results of DE Pasquale *et al.*(1995),Ewais (1997) and Gromova (1997).

Generally, roots act as a barrier to movement of toxic heavy metals through the soil-plant system. These results are in accordance with those reported by El-Kobbia and Ibrahim (1988), Metwally and Rabie (1989), Kahel (1992), Wierzbicka (1998) and Angelova *et al*. (1999). The results indicate that the potential hazards associated with food chain transfer of P and Ca applied to Pb and Cd are substantially lower than equivalent Pb and Cd treatments, and that the hazards do not increase over time (Lehoczky *et al*., 1998).

11. Yield and its components:

Data presented in Table (7) illustrated that the high concentration of Pb and Cd decreased No. of pods/plant, No. of seeds/pod, seed yield/plant as well as seeds total protein content of pea plants. In this concern, Cd was more harmful in reducing pea yield than Pb. Whereas, the addition of nutrients P and Ca, inactivated Pb and Cd, respectively, which have toxic effect on pea yield. These results are in accordance with Xian (1989), Aery and Sarkar (1991) and Nagoor and Vyas (1998), who reported that higher Pb and Cd concentrations significantly decreased the yield of kidney beans and protein content in wheat plants.

Finally, it can be concluded that the addition of P and Ca reduced the deleterious effect of heavy metals Cd and Pb. Moreover, this addition seems to counteract the toxic effect of both Pb and Cd, respectively. Accumulation and transport of heavy metals and low molecular mass organic compounds are considered as either compartible solutes or stress markers which can also be modified by the presence of P and Ca.

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الملخص العربى

تقليل الآثار الضارة لكل من الرصاص والكادميوم على كل من النمو والتغيرات الكيميائية بواسطة الفوسفور والكالسيوم لنبات البسلة

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أجرى هذا البحث بمزرعة كلية الزراعة – جامعة المنوفية فى الموسم الشتوى لعامى ١٩٩٨ /٩٩ – ١٩٩٩/٢٠٠٠ بهدف دراسة تأثير كل من الرصاص (صفر، ٥٠، ١٠٠، ٢٠٠ ملليجرام/لتر) وكذلك الكادميوم (صفر، ٢٠، ٤٠، ٦٠ملليجرام/لتر) بالإضافة إلى تقليل الأثر الضار للعناصر الثقيلة بإضافة الفوسفور والكالسيوم بتركيز ٢ملليمول لكل منهما على التوالى ودراسة تأثير ذلك على النمو والتغيرات الكيميائية وتراكم العناصر الثقيلة والمحصول لنبات البسلة صنف Little Marvel وذلك فى مزرعة رملية. وقد أستخدم الماء كمعاملة مقارنة – وقد تم إستخدام هذه الملوثات بإضافتها مع محلول التغذية على مرتين (٤٠ و ٥٥يوم من الزراعة). وقد بينت النتائج مايلى:

• وجد أن التركيزات العالية لكل من الرصاص والكادميوم أدت إلى نقص معنوى فى طول النبات – طول الجذر – الوزن الجاف للأعضاء النباتية وكذلك نقص معنوى فى معدل النمو النسبى (RGR) وصافى معدل التمثيل (NAR) ونسبة المساحة الورقية (LAR) وكذلك معدل مقاومة الجذور (TI).

• وجد أيضا أن جميع الملوثات أثرت تأثيرا معنويا على تركيز كل من كلورفيل أ وكلورفيل ب وأيضا الكاروتينات بالإضافة إلى أن التركيزات العالية أدت إلى نقص واضح فى كل من السكريات الكلية والذائبة والأحماض الأمينية الكلية.

• كذلك حدث نقص واضح فى نشاط إنزيم البيروكسيديز ومحتوى الأوراق من العناصر (ن – فو – بو) بالمقارنة بالنباتات الغير معاملة.

• كما لوحظ زيادة واضحة فى محتوى الأوراق من البرولين ودرجة نفاذية الجدر الخلوية وخاصة مع التركيزات العالية من الملوثات.

• وجد أن إضافة كل من الفوسفور والكالسيوم للمحاليل الملوثة بالرصاص والكادميوم على التوالى أدت إلى عكس التأثير الضار لهذه العناصر وخاصة عند التركيزات المنخفضة من الملوثات وكانت أفضل النتائج هو تقليل تراكم كل من الرصاص والكادميوم فى جميع أعضاء نبات البسلة (أوراق – جذور – ساق) وخاصة الثمار.

• إلى جانب زيادة محصول النبات من البذور ومحتواها من البروتين سجلت أحسن النتائج بإضافة كل من الفوسفور والكالسيوم تحت ظروف التلوث بالرصاص والكادميوم على التوالى.