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RESPONSE OF LEAD-POLLUTED BORAGE (*Borago officinalis* L.) TO ANTIOXIDANT TREATMENTS

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ABSTRACT

Field experiments were conducted to study the effect of lead (Pb) in different concentrations (0, 100, 200 and 300 ppm) on vegetative growth, flowering, chemical constituents and seed yield of *Borago officinalis*. The study tests also the ability of the antioxidants Ethylenediurea (EDU), Ascorbic acid (AA) and Salicylic acid (SA) to overcome the harmful effects of Pb on the growth and physiological behavior of the polluted plants.

Results showed that Pb treatments negatively affected plant growth represented by shoot length, number of branches and dry weight. Flowering date, flower dry weight, seed yield and fixed oil percentage were also negatively affected by Pb pollution. The endogenous Pb concentration in the analyzed plants increased following the increase in Pb levels in the growth medium. Chl.a, b, and carotenoids were sharply reduced by Pb treatments. Nitrogen, phosphorus and potassium concentrations were decreased dramatically especially at high Pb levels. The activity of peroxidase was positively correlated with the concentration of Pb in leaves and roots. In all cases, EDU, AA and SA treatments seemed to be useful in counteracting the harmful effects exerted by Pb contamination on borage plants by regulating certain enzymes. The results suggested that the measurements of enzyme activities (peroxidase) could serve as early bio-markers to asses the phytotoxicity of Pb polluted soils on *Boragro officinalis*.

INTRODUCTION

Borage (*Borago officinalis*, L.) is a coarse hairy annual herb native to the Mediterranean region and bloom during spring months with showy heavily–clustered blue flowers. Therefore, it is mostly known as "Bee Plant" or "Bee Bread" for its ability to attract bees especially when the field is in shortage of flowering crops (Moftah *et al.*, 1991). Borage has also been grown as an ornamental and nectar plant. Recent interest in borage exists because the seeds contain a high percentage of δ-linolenic acid an unusual fatty acid that has importance as an intermediate in the biosynthesis of prostaglandin. δ-linolenic acid has shown therapeutic promise in the treatment of atopic eczema, diabetes and it may possibly function as a preventive treatment against heart disease and stroke (El-Gengaihi *et al.*, 2000). Moreover, the dietary use of vegetable oil rich in linolenate and free alkaloids as those extracted from borage seeds and flowering (Dodson and Stermitz, 1986) are considered to alleviate hyper-cholesterol and other disorders (Stymne and Stobart, 1986).

Lead (Pb) is the most widespread pollutant emitted from industry, stationary combustion plants and motor vehicles. Consequently, lead could be taken up by plants and thus can enter the human food chain. Moreover, lead may accumulate in the soil at high concentrations, as the case of the industrial countries, causing plant toxicity and vegetation damage (Aly, 1982) because its accumulation in soils affects plant primarily through their root systems. In this regard, Stochs and Bagchi (1995) and Taiz and Zeiger (1998) related the toxicity of heavy metals, including lead, to their ability to cause oxidative damage to plant cells. This damage includes enhanced lipid peroxidation, DNA damage, and the oxidation of protein sulfhydryl groups. In this connection, Marrs (1996) reported that the oxidative stress, caused by heavy metal pollution, is established through a series of redox reactions initiated by hydrogen peroxide (H_2O_2) and other free radicals. Early studies by Stefanov *et al.* (1995) showed that heavy metals affected negatively the antioxidant system within plant tissues, therefore the interest in the usefulness and use of exogenous application of antioxidants for cellular protection against oxidative stress on plants has increased dramatically (Koricheva *et al.*, 1997).

Several compounds were found to act as antioxidants if exogenously applied. Such compounds were found to increase the capacity of plant to tolerate the attack of free radicals generated by heavy metal pollution (Witaker *et al.*, 1990 and Hernandez *et al.*, 1999). In this regard, Rucinska *et al.* (1999) found that at high Pb levels the formation of both free radicals and reactive O_2 species was beyond the capacity of the antioxidant system of lupine plants, which in turn reduced plant growth. Ascorbic acid (Vit. C) is known as antioxidant and growth regulating factor influences many biological processes in plants (Moftah, 2000). Several reporters showed that the application of antioxidants protect plants against heavy metal pollution. Among these antioxidants, ethylene-di-urea (EDU), ascorbate and salicylic acid were found to exert positive effect and overcome the harmful effect of some environmental stress on plant growth. Recent study by Moftah (2000) showed that EDU treatments was useful in counteracting the harmful effects exerted by Pb contamination on tomato and eggplants by regulating certain enzymes. Ascorbic acid acts as co-enzyme and its application was found to increase the nucleic acids and protein contents in plants (Patil and Lall, 1973; Reda *et al.*, 1977 and El-Khayat, 2001). In a study on the effect of ascorbate, Luwe (1996) reported that ascorbate has the ability to prevent the environmental stress damage on plant growth and productivity by regulating cell elongation and expansion. Salicylic acid was found to have an antioxidant effect and could overcome the deleterious effect of different stresses on plant by acting as chelating agent protects the reproductive organs from stress (Oota, 1972). Antioxidants may also increase membrane stability against dehydration in heavy metal-treated plants (Wozny *et al.*, 1995).

The understanding of the effects of heavy metals on plant growth and physiology and the means for detoxification are of great interest to develop plants more tolerant to metal stress. Therefore, the role of antioxidants, ethylenedi-urea (EDU), ascorbic acid (AA) and salicylic acid (SA) was investigated in the present study. The objective of this work is (i) to asses the effect of Pb pollutant on the growth and physiological behavior of Borage (*Borago officinalis*), (ii) to evaluate the nature of susceptibility of Borage to the pollutant, and (iii) to determine the role of the antioxidants (EDU, AA and SA) against Pb pollution.

MATERIALS AND METHODS

Two pot experiments were conducted in the two successive years of 1999/2000 and 2000/2001 at the Nursery of Ornamental Plants, Dept. of Horticulture, Facu. of Agric., Minia Univ. (where plants were grown and growth parameters were determined) and Dept. of Botany, Facu. Of Agric., Menufiya Univ. (where chemical and GLC analysis took place) to investigate the physiological response of lead-polluted *Borago officinalis*, L. to the antioxidants, Ethylenediurea (EDU), ascorbic acid (AA) and salicylic acid (SA).

Soil (clay loamy) was collected from an ordinary agricultural field near Minia town and was analyzed for Pb by Atomic Absorption Spectrophotometer in ammonium acetate extracts (Jackson, 1956). The Pb concentration was found to be 0.11 ppm (desigend as control). Soil was sieved, dried and filled into standard clay pots (30 cm diameter) containing equal weights of soil (6 kg/pot). The physical and chemical analysis of the soil were as follows:

sand (%)	silt (%)	clay (%)	OM (%)	$CaCO_3$ (%)	E.C (m.mh/cm)	pH (1:2 w)	Ex. Ca (mg/100g soil)	Tot N (%)	P (ppm)	K (ppm)
27.1	43.9	29.0	2.3	1.64	0.41	7.98	17.8	0.11	22.0	460.0

DTPA extractable: Fe= 8.69 Zn= 3.00 Mn=25.81

To stimulate soil pollution, the soil was amended with lead acetate $[(CH_3COO)2Pb.3H_2O]$ solutions to produce Pb levels of 100, 200 and 300 mg/L besides control pots that received pure water at same amounts. Soil was watered to field capacity and incubated in the greenhouse for ten days to allow the soil chemical reactions to equilibrate before transplanting, as recommended by De Pasquale *et al.* (1995). Two days before transplanting, EDU and SA solutions were added to each pot in concentration of 500 mg/L and 10 µM, respectively as recommended by the US Department of Agriculture, USDA, (Lee *et al.*, 1992) and Khurana and Cleland (1992).

Borage seeds obtained from the Dept. of Medicinal and Aromatic Plants, Ministry of Agriculture, Dokki, were sown in the nursery beds on Sept. 15^{th} (in both seasons). Eight weeks later, uniform seedlings were transplanted in the pots (one plant/pot). In both seasons, plants designed for ascorbic acid (300 mg/L) were sprayed three times during the growing seasons in two week intervals starting 4 weeks from transplanting. Tween 20 (0.1%) was added as surfactant and spray was done to run off onto plant foliage using hand-held manual atomizer. Control plants received pure water with the surfactant only. A split plot design with three replicates (each replicate contained 15 plants) was followed. The 4 levels of lead (0, 100, 200, and 300 ppm) consisted the main plots (A) while the 4 antioxidant treatments (EDU, AA, SA, and pure water as control) consisted the sub-plots (B). Fertilization was done for all plants at the rate of 5g calcium super-phosphate (15.5% P_2O_5), 5g ammonium nitrate (33.5% N) and 1g potassium sulphate (48% K_2O) per pot. Amounts of fertilizer were divided into 3 equal portions and added after 4, 6 and 8 weeks from transplanting. All other cultural practices were performed as usual. Different measurements were estimated through three stages of growth: a) vegetative growth stage, after 80 days from transplanting: shoot length, number of branches, dry weights of shoots and roots were recorded, by the end of this stage and the beginning of flowering, flowering date (number of days from transplanting to 1st flower appearance) was recorded. b) flowering and fruiting stage, at flowering and beginning of fruiting, after 130 days from transplanting: shoot length, number of branches, dry weights of shoots and flowers per plant were recorded. c) maturity stage, at seed maturation, after 180 days from transplanting: seed yield/plant. At stage (a), photosynthetic pigments in acetone extract(Wettstein, 1957), peroxidase (POD) activity in leaves and roots using guaiacol reagent (Sadasivam and Manicham, 1992) were determined. Pb concentration in leaves and roots were analyzed by Atomic Absorption Spectrophotometer (Perklin-Elmer 560) in nitric and perchloric acids as described by (David, 1958). Total nitrogen in leaves was determined using the modified "Micro-Kjeldahl" method as described by Chapman and Pratt (1978). Phosphorous concentration in leaves was determined colorimetrecally using the stannus chloride method as reported by Troug and Mayer (1949), while potassium in leaves was measured by flame photometry according to Jones and Steyn (1973). While at stage (c), total alkaloids in seeds were estimated according to Masoud *et al.* (1968). At maturity (stage c), fixed oil (%) and fatty acid content of seeds were determined. After extraction in Soxhlet apparatus, the fixed oil derivatives of seeds in the methyl ester, extracts were analyzed by GLC according to Johnson and Davenpart (1971) using Hawlett-Packard 5890A GLC equipped with electron capture detector (ECD), silica capillary column (12m x 0.2 mm i.d), GC held isothermally at 80°C to 150°C at 4°C/min. All chemical analyses were carried out in Botany Dept. at Menufiya Univ. after transferring the black cloth and aluminum foil-wrapped extracts in dry-ice box, while dry materials were transferred in plastic bags.

The obtained data were statistically analyzed using ANOVA test method as described by Snedecor and Cochran (1973) with the aid of (COSTAT) computer program.

RESULTS AND DISCUSSION

Vegetative growth

Data recorded in Tables (1 & 2) showed clearly that all vegetative growth parameters (shoot length, number of branches and dry weights of shoots and roots) were decreased with increasing Pb concentration to reach its minimum value at 300ppm. This decrease was noticed in both, vegetative and flowering stages. It is of interest to notice that the negative effect of Pb on shoot length and number of branches was much more pronounced at the first stage (vegetative stage, Table 1) than that recorded at the second stage (flowering stage, Table 2); while the reverse was true for the weight of the dry matter.

This may be explained by the less dry mater accumulation at the 1st stage thus the effect of Pb is un-noticeable. It is obvious from the data that antioxidant treatments either with EDU, ascorbate, or salicylic acid reduced the deleterious effect of Pb on vegetative characters.

Table (1): Effect of Pb pollution and ethylene diurea (EDU), Ascorbic acid (AA) and salicylic acid (SA) on shoot length, number of branches, dry weight of shoots and roots per plant at first stage after 80 days from transplanting (vegetative growth) of borage plants.

Antioxidant treatments (B)	Lead (Pb) levels (ppm) (A)									
	First season					Second season				
	0	100	200	300	mean	0	100	200	300	Mean
	Shoot length (cm)									
Control	30.20	27.90	25.20	21.00	26.10	28.60	26.40	23.90	19.90	24.70
EDU	33.80	29.90	27.40	24.10	28.80	32.00	28.30	26.00	22.80	27.28
Ascorbic acid	32.60	29.40	26.50	22.80	27.80	30.90	27.80	25.00	21.60	26.33
Salicylic acid	35.10	31.70	28.70	24.90	23.20	33.20	30.00	27.20	23.60	28.50
Mean	32.93	29.70	27.0	23.20		31.18	28.13	25.53	21.98	
LSD (5%)	A= 1.15		B= 0.71		AB= 1.42	A= 1.12		B= 0.69		AB= 1.38
	Number of branches/plant									
Control	13.60	12.40	8.80	4.50	9.83	12.30	11.20	8.00	4.10	8.90
EDU	15.10	13.70	11.50	7.30	11.90	13.70	12.40	10.50	6.70	10.83
Ascorbic acid	14.60	13.20	10.50	7.10	11.35	13.20	11.90	9.50	6.40	10.25
Salicylic acid	16.90	15.10	12.40	8.30	13.18	15.30	13.60	11.30	7.60	11.95
Mean	15.05	13.60	10.80	6.80		13.63	12.28	9.83	6.20	
LSD (5%)	A= 1.33		B= 0.81		AB= 1.62	A= 1.27		B= 0.80		AB= 1.59
	Dry weight of shoot (g/plant)									
Control	165.3	148.8	125.7	105.6	136.4	162.5	146.3	123.6	103.8	134.1
EDU	214.9	165.1	153.1	132.7	166.5	211.3	62.3	150.5	130.4	163.6
Ascorbic acid	208.8	160.3	149.3	130.2	162.2	205.3	157.6	146.8	127.9	159.4
Salicylic acid	222.4	169.8	157.3	136.1	171.4	218.6	166.9	154.7	133.8	168.5
Mean	202.9	161.0	146.4	126.2		199.4	158.3	143.9	124.0	
LSD (5%)	A= 4.86		B= 3.24		AB= 6.48	A= 5.22		B= 3.62		AB= 7.24
	Dry weight of roots (g/plant)									
Control	86.50	73.40	63.10	47.50	67.63	84.30	71.50	61.50	46.30	65.90
EDU	89.20	85.30	77.20	58.60	77.58	86.90	83.10	75.20	57.10	75.52
Ascorbic acid	87.00	83.50	75.30	56.90	75.68	84.80	81.30	73.40	55.50	73.75
Salicylic acid	91.30	86.60	78.90	60.30	79.28	89.0	84.40	76.90	85.80	77.28
Mean	88.50	82.20	73.63	55.88		86.5	80.08	71.78	54.43	
LSD (5%)	A= 3.12		B= 1.97		AB= 3.94	A= 3.00		B= 1.83		AB= 3.66

The obtained results confirm those reported by Gadallah (1995) who showed that the heavy metal toxicity appeared in the reduction of plant height and dry mass accumulation. In addition, Pb pollutant found to accelerate plant respiration and to cause disorder in chloroplast ultra-structure and function (Vassilev *et al.*, 1997). The reduction in chlorophyll content in Pb-treated plants, whether due to destruction of chl. or to interference with its formation, might be, at least partially, accounted for the inhibition of photosynthetic CO_2-fixation (Vassilev *et al.*, 1997) resulting in growth reduction. In a recent study on sunflower, Kastori *et al.* (1998) found that Pb of 10^{-7} – 10^{-3} M reduced plant growth as it retarded cell division and differentiation thus inhibited their elongation in sunflower plants.

Table (2): Effect of Pb pollution and ethylene diurea (EDU), Ascorbic acid (AA) and salicylic acid (SA) on shoot length, number of branches, dry weights of shoots and flowers per plant at second stage after 130 days from transplanting (vegetative growth) of borage plants.

Antioxidant treatments (B)	Lead (Pb) levels (ppm) (A)									
	First season					Second season				
	0	100	200	300	mean	0	100	200	300	Mean
	Shoot length (cm)									
Control	74.50	68.90	62.20	51.90	64.38	71.20	65.80	59.40	49.60	61.50
EDU	80.30	73.90	67.60	59.50	69.38	76.70	70.60	64.60	59.90	64.95
Ascorbic acid	78.40	72.50	65.50	56.40	68.68	74.90	69.20	62.60	53.90	65.15
Salicylic acid	86.50	78.00	70.60	61.60	64.18	82.70	74.50	67.40	58.90	70.88
Mean	79.70	73.33	66.48	57.10		76.38	70.30	63.50	54.83	
LSD (5%)	A= 2.63	B= 1.82	AB= 3.64			A= 2.43	B= 1.72	AB= 3.44		
	Number of branches/plant									
Control	18.80	17.10	12.20	6.40	13.63	17.60	16.00	11.40	6.00	12.75
EDU	20.90	18.90	15.90	10.40	16.53	19.60	17.70	14.90	9.80	15.50
Ascorbic acid	20.20	18.20	14.60	10.20	15.80	18.90	17.00	13.60	9.60	14.78
Salicylic acid	23.40	20.90	17.20	11.70	18.30	21.90	19.60	16.10	11.00	17.15
Mean	20.83	18.78	14.98	9.68		19.50	17.58	14.00	8.35	
LSD (5%)	A= 1.41	B= 0.96	AB= 1.92			A= 1.30	B= 0.87	AB= 1.74		
	Dry weight of shoot (g/plant)									
Control	317.2	285.5	241.3	202.7	261.7	313.5	282.2	238.5	200.3	258.6
EDU	412.3	416.8	293.9	254.8	319.5	410.5	315.1	290.3	253.4	317.8
Ascorbic acid	400.6	309.5	288.6	251.8	312.6	397.5	307.1	286.4	249.9	310.2
Salicylic acid	423.0	322.8	300.1	259.3	326.3	419.0	318.7	296.6	256.0	322.6
Mean	390.8	306.0	281.9	242.3		385.1	305.8	278.5	239.9	
LSD (5%)	A= 7.21	B= 5.12	AB= 10.23			A= 6.85	B= 4.98	AB= 9.95		
	Dry weight of flowers (g/plant)									
Control	75.80	49.30	32.00	18.40	43.88	73.20	47.60	30.90	17.80	42.38
EDU	125.3	78.50	65.30	37.80	76.73	121.0	75.80	63.10	36.60	74.13
Ascorbic acid	114.0	73.10	59.20	36.90	60.80	110.1	70.60	57.20	35.70	68.40
Salicylic acid	129.0	79.60	67.30	39.30	78.80	124.0	76.90	65.00	38.00	76.13
Mean	111.0	70.10	55.95	33.10		107.2	67.73	54.05	32.03	
LSD (5%)	A= 2.83	B= 1.81	AB= 3.62			A= 2.69	B= 1.60	AB= 3.54		

The antioxidant treatments seemed to overcome the harmful effects of Pb pollution and improved plant vegetative growth. In this respect, a study by Moftah (2000) showed that EDU improved growth of tomato and eggplants. In early study by Shaddad *et al* (1990), it was found that ascorbic acid counteracted the suppression effect of high salinity levels on seed germination, seedling growth and dry matter increase in lupine and broad bean plants. This increase was accompanied by observed increase in carbohydrates and proteins that were always associated with increase in tissue water-content. In more recent studies, ascorbic acid was found to overcome the deleterious effects of environmental pollution and was involved in root elongation, cell vacuolarization, cell wall expansion and cell elongation (Polle *et al.*, 1995; Luwe, 1996; Noctor and Foyer, 1998). Similar results were reported by Knorzer *et al.* (1999) using salicylic acid.

Dry weight of flowers

Data in same tables showed also that dry weight of flowers was negatively affected by Pb pollution (Table 2). In both growth seasons, dry weight of the flowers showed contrast response to the increase of Pb concentration in the growth media. These findings were in harmony with those reported by El-Ghinbihi (2000) on common bean. Data indicated also that antioxidant treatments alleviated the harmful effect of the pollutant and caused a significant increase in the weight of flowers as compared with untreated plants. The increase in dry weight of flowers/plant as a result of antioxidant treatments may be attributed to the positive effect of antioxidants on increasing number of branches and to their enhancing effect on flower initiation. Similar conclusion was reported by Moftah (2000) using EDU, Raskin (1992) and Khurana and Cleland (1992) using salicylic acid, who found that EDU and salicylic acid stimulate flowering of tomato, eggplants and *Lemna*, respectively. On the other side, ascorbic acid was found to increase vegetation by influencing the synthesis of nucleic acids and enzymes and may slightly increased the dry weight of flowers (Abdel-Halim, 1995 and El-Khayat, 2001). It is clear from data that the effect of the interaction between Pb treatments and anti-oxidants was significant.

Antioxidants were found to increase the capacity of plants to tolerate the attack of free radicals, generated by heavy metal and other environmental pollution (Luna *et al.*, 1994). Therefore, plants under investigation were tolerating relatively high levels of Pb pollutant and their vegetative growth as well as dry weight of flowers were better than those antioxidant untreated-plants. Mishra and Choudhuri (1998) found that Pb decreased shoot/root ratio of rice cultivars differing in their tolerance to heavy metals. They found also that antioxidant treatments alleviated the adverse effect of the heavy metals on plant growth. The effect was more pronounced in the tolerant cultivars than the relatively susceptible ones. Similar results were obtained by Moftah (2000) who found that the antioxidant ethylene-di-urea was useful to counteract the harmful effects exerted by Pb contamination on tomato and eggplant. The possitive effects of ascorbic acid might be due to its involvement in the main metabolic process especially with energy co-enzymes, carbohydrate metabolism and improved biosynthesize activity (El-Khayat, 2001). Salicylic acid has found to increase catalase activity which indicate an activation of the cellular antioxidant system and enzyme level. Salicylic acid has also found to protect soybean cells from herbicide-induced peroxidation in a time concentration-dependent manner and strongly suppressed the herbicide-induced accumulation of protoporphyrin and antagonized the action of peroxidizing herbicides in soybean cells (Knorzer *et al.*, 1999).

Flowering date

Data obtained in Table (3) showed that flowering was delayed by Pb existence in the growth media and there was a positive correlation between the length of the period plant takes to flower and the concentration of Pb in the soil. On the other side, it was clear from the same table that antioxidant treatments decreased the negative effects of Pb on flowering and shortened the period to flower.

Table (3): Effect of Pb pollution and ethylene diurea (EDU), Ascorbic acid (AA) and salicylic acid (SA) on flowering date, seed yield, fixed oil and total alkaloids of borage seeds.

Antioxidant treatments (B)	Lead (Pb) levels (ppm) (A)									
	First season					Second season				
	0	100	200	300	mean	0	100	200	300	Mean
	Flowering date (days to flower from transplanting)									
Control	86.40	88.60	90.80	93.60	89.85	85.80	88.00	90.20	93.10	89.28
EDU	87.40	87.00	88.60	89.70	88.18	86.70	86.40	88.20	88.70	87.50
Ascorbic acid	87.60	87.30	88.90	90.20	88.50	87.10	86.60	88.20	89.20	87.78
Salicylic acid	82.60	84.00	86.90	87.50	85.25	82.10	83.60	86.40	87.00	84.78
Mean	86.00	86.73	88.80	90.25		85.43	86.15	88.25	89.50	
LSD (5%)	A= 1.14		B= 0.74		AB= 1.48	A= 1.05		B= 0.64		AB= 1.28
	Seed yield (g/plant)									
Control	3.52	2.29	1.49	0.86	2.04	3.41	2.22	1.44	0.83	1.98
EDU	5.73	3.80	3.18	1.86	3.65	5.58	3.66	3.07	1.83	3.58
Ascorbic acid	4.98	3.33	2.76	1.65	3.18	4.82	3.22	2.67	1.59	3.08
Salicylic acid	5.53	3.65	3.04	1.73	3.49	5.35	3.54	2.94	1.67	3.38
Mean	4.94	3.28	2.62	1.53		4.79	3.18	2.53	1.48	
LSD (5%)	A= 0.25		B= 0.15		AB= 0.30	A= 0.22		B= 0.13		AB= 0.26
	Fixed oil of seeds (%)									
Control	24.13	17.48	10.12	6.00	14.43	24.69	17.89	10.35	6.14	12.27
EDU	27.12	25.66	21.81	13.24	21.96	27.75	26.29	22.41	14.13	22.67
Ascorbic acid	25.81	22.82	18.92	11.75	19.83	26.39	23.34	19.35	11.98	20.27
Salicylic acid	26.12	24.20	20.85	12.30	20.87	26.64	24.78	21.31	12.59	21.33
Mean	25.80	22.55	17.93	10.82		26.37	23.08	18.36	11.21	
LSD (5%)	A= 1.28		B= 0.78		AB= 1.56	A= 1.35		B= 0.81		AB= 1.62
	Total alkaloids in seeds mg/g dwt (at second stage)									
Control	4.11	2.17	1.10	0.70	2.02	4.02	2.12	1.08	0.69	1.98
EDU	5.33	4.09	2.57	1.68	3.42	5.21	4.00	2.52	1.63	3.34
Ascorbic acid	4.91	3.89	2.30	1.50	3.15	4.80	3.80	2.26	1.46	3.08
Salicylic acid	5.63	4.45	2.70	1.78	3.64	5.51	4.35	2.65	1.73	3.56
Mean	5.00	3.65	2.17	1.42		4.89	3.56	2.13	1.38	
LSD (5%)	A= 0.71		B= 0.35		AB= 0.69	A= 0.62		B= 0.31		AB= 0.63

The best effect in this matter was reported with the use of salicylic acid followed by EDU then ascorbic acid treatment. Early studies showed that salicylic acid triggered flowering in several species (Khurana and Maheshwari, 1987). Salicylic acid was found to have florigenic effect and to induce floral bud formation in plants belonging to different families (Khurana and Cleland, 1992). Although the mechanism by which salicylic acid induces flowering is not well known, one hypothesis suggests that salicylate induces flowering by acting as a chelating agent, because the free hydroxyl group in its structure confers metal chelating activity (Raskin, 1992). This view is supported by the fact that chelating agents can induce flowering (Oota, 1972 and Seth *et al.*, 1970). Nevertheless, the benzoic acid involved in the structure of calicylate molecule was found to have a florigenic activity (Fujioka *et al.*, 1985). The iron chelating properties of salicylate could, however, explain the inhibitory effect of salicylic acid on the ethylene-flowering enzyme, because iron is an essential co-factor for the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene (Raskin, 1992).

Seed yield

Data recorded in Table (3) confirmed those reported by El-Ghinbihi (2000) and indicated that seed yield/plant was decreased significantly by increasing Pb concentrations in the soil to reach lowest value at 300 ppm. At this value the seed yield was decreased by about 70% as compared with untreated plants during the 1st or 2nd season. While EDU, ascorbate and salicylic acid treatments significantly increased the yield as compared with untreated plants. As shown in the table, treatments seem to alleviate the negative effects of Pb and make a progressive improvement on the yield. In this respect, the best results were obtained from the EDU followed by salicylate then ascorbate treatment. The positive effect of antioxidants on seed yield was more pronounced at higher Pb pollution level (300 ppm) than at low level. The increase in seed yield by nearly 116%, 101% and 91% as a result of treatment with EDU, SA and AA, respectively was recorded at 300 ppm of Pb at the 1st growth season as compared with untreated plants. At 2nd season results followed the same trend as that in the 1st season. It is clear that the effect of the interaction between Pb and other treatments on seed yield/plant was significant in both seasons. This increase in the total seed yield/plant by antioxidant treatments may be attributed, directly, to the enhancing effect on fruit setting and the increment in number of flowers and seeds/plant. The positive effect of plant antioxidants on plant growth may contribute also to the increase in plant productivity. The stimulating effects of antioxidants on seed yield of plants under heavy metal pollution was also reported in earlier studies by Clarke (1983) and Moftah (2000), using EDU; El-Khayat (2001), using ascorbic acid; and Khurana and Cleland (1992), using salicylic acid.

Fixed oil

It is obvious from the data obtained in Table (3) that the fixed oil concentration in seeds was considerably decreased by increasing Pb level in the soil. The reduction in fixed oil was linear with the increase of Pb pollution. On the other side, it is clear that the presence of EDU in the growing media seems to reduce the negative effect of the Pb, thus increased the fixed oil even at high level of the pollutants. In addition, treating lead polluted-plants with ascorbic acid or salicylic acid decreased also the harmful effect of Pb and enhanced the fixed oilo concentration in seeds of treated plants. The interaction between Pb treatments and antioxidants seems to be significant as shown in the same table. The negative effect of heavy metal contamination on fatty acids and oil content of many species was reported in early studies by Creissen *et al.* (1994) while the positive effects of natural chemicals and antioxidants on minimizing this harmful effects were discussed by (De Pasquale *et al*, 1995 and Moftah, 2000).

Total alkaloids

Data recorded in Table (4) clearly showed that the content of total alkaloids in seeds was decreased with introducing Pb in the soil. The decrease was taking linear form with increasing Pb level. Antioxidants, on the other side, alleviated the negative effect of Pb and enhanced the production of alkaloids so that alkaloid contents were increased in antioxidant-treated plants more than untreated plants. Data recorded in the same table indicated that the effect of the interaction between lead concentrations and antioxidant treatments on the total alkaloid content was significant. The present results concerning total alkaloid contents in borage plants are in complete harmony with those reported with Dodson and Stermitz (1986) fractions were also reported by Huong *et al.* (1998).

Table (4): Effect of Pb pollution and ethylene diurea (EDU), Ascorbic acid (AA) and salicylic acid (SA) on chlorophyll a, b and carotenoids of borage leaves.

Antioxidant treatments (B)	Lead (Pb) levels (ppm) (A)									
	First season					Second season				
	0	100	200	300	mean	0	100	200	300	mean
	Chl a in leaves (mg/g dwt)									
Control	3.490	3.039	2.324	1.119	2.493	3.485	3.035	2.321	1.117	2.490
EDU	3.499	3.349	3.214	2.319	3.095	3.494	3.345	3.210	2.315	3.091
Ascorbic acid	3.475	3.330	3.187	2.290	3.071	3.470	3.326	3.183	2.286	3.066
Salicylic acid	3.588	3.519	3.250	2.350	3.177	3.583	3.514	3.246	2.346	3.172
Mean	3.513	3.309	2.994	2.020		3.508	3.305	2.990	2.016	
LSD (5%)	A= 0.160 B= 0.101 AB= 0.203					A= 0.150 B= 0.098 AB= 0.195				
	Chl b in leaves (mg/g dwt)									
Control	2.188	1.719	1.164	0.967	1.510	2.181	1.714	1.160	0.964	1.505
EDU	2.204	2.181	1.880	1.569	1.959	2.197	2.175	1.874	1.564	1.953
Ascorbic acid	2.200	2.185	1.840	1.530	1.939	2.193	2.179	1.83	1.525	1.932
Salicylic acid	2.360	2.220	1.920	1.610	2.028	2.352	2.214	1.920	1.605	2.023
Mean	2.338	2.076	1.701	1.419		2.231	2.071	2.696	1.415	
LSD (5%)	A= 0. B= 0.092 AB= 0.184					A= 0.110 B= 0.071 AB= 0.142				
	Carotenoids in leaves (mg/g dwt)									
Control	1.132	0.996	0.919	0.881	0.982	1.126	0.991	0.914	0.876	0.977
EDU	1.149	1.119	1.064	1.027	1.090	1.143	1.114	1.058	1.020	1.084
Ascorbic acid	1.145	1.112	1.054	1.015	1.082	1.139	1.106	1.048	1.010	1.076
Salicylic acid	1.168	1.148	1.085	1.046	1.112	1.162	1.142	1.079	1.039	1.106
Mean	1.149	1.094	1.031	0.992		1.143	1.088	1.025	0.986	
LSD (5%)	A= 0.051 B= 0.030 AB= 0.061					A= 0.042 B= 0.027 AB= 0.053				

Photosynthetic pigments

Chl a, b and carotenoid concentrations in plant leaves were significantly decreasing with increasing Pb concentration (Table 4). The lowest pigment values were recorded at 300ppm of Pb. The reductions in chl. a, b and carotenoids at 300 ppm of Pb were 68, 56 and 22%, respectively compared with untreated plants. Photosynthetic pigments have often been shown as one of the main sites of the toxic Pb and other HM actions in many plant species such as cucumber, safflower and some common bean varaieties (Fodor *et al.*, 1998; Sayed, 1999; and El-Ghinbihi, 2000). Thus, the decrease in chls and carotenoid contents appears to be one of the first visible bio-markers of Pb toxicity. The possible mechanism of Pb toxicity on chl pigments were attributed to inhibiting the biosynthesis of the aminolevulinic acid (ALA), a precursor of chl (Thomas and Singh, 1996) and/or stimulating the activity of chlorophyllese and chlorophyll degradation (Abdel-Basset *et al.*, 1995). In recent study, Lagriffoul *et al.* (1998) reported that Pb can alter chl biosynthesis by inhibiting protochlorophyllide reductase through interfering the sulfhydryl site on the enzyme. The decrease in carotenoid concentration occurred by Pb application may also lead to decrease chl a and chl b because carotenoids prevent chl photodestruction. The inhibition of Pb on Fe uptake and transport to plant leaves may result also in reducing chl synthesis and cause chlorosis (Fodor *et al.*, 1998). The obtained results were in harmony with those reported by Sayed (1999) on safflower and Koriesh (2001) on *Prosopis juliflora*.

All antioxidant treatments seemed to overcome the harmful effect of Pb and improved the photosynthetic pigment concentrations in leaves. Best results in this concern were obtained from salicylic acid followed by EDU then ascorbic acid treatment. It has been found that the antioxidants had the potential to protect the enzymes involved in chl synthesis against oxidative damages that might occur from HM or other environmental pollution (Lee *et al.*, 1992 and Foyer *et al.*, 1994). In recent study by Hernandez *et al.* (1999) the beneficial of antioxidants in protecting chl and carotenoids from oxidative stress, as one of the mechanism, was reported. The protective function of antioxidants on carotenoids against oxidative damage of Pb might be at least partially occurred for the protection of chl against destruction (Koricheva *et al.*, 1997). Similar results were obtained by Moftah (2000) using EDU, Calatayud *et al.* (1999) using ascorbic acid and Raskin (1992) using salicylic acid.

Data showed that there was a significant effect from the interaction between Pb treatments and antioxidants on the concentration of the photosynthetic pigments. This effect was recorded at both experimental seasons.

Lead concentration

It was obvious from data in Table (5) that Pb concentration in leaves and roots were increased progressively with increasing Pb level in the soil. It was clear that the concentrations of Pb in roots were much higher than those found in leaves. This may be due to the existence of pb close to roots in the growth media. This increase in Pb in plant tissues with increasing Pb in the soil may be one of the reasons that decreased the growth of plants with Pb pollution. These results are in harmony with those reported by Ahmed (2001) on *Tagetes patula* and Koriesh (2001) on *Prosopis julifora*.

Antioxidants seemed to decrease Pb concentrations in both leaves and roots. The most reduction in leaf Pb was recorded at salicylic acid followed by EDU and finally ascorbic acid treatment. It seems obvious that the application of EDU, AA and SA increased the ability of the plants to prevent the translocation of excess Pb to shoots and/or the ability to detoxify the metal after it has been absorbed. Therefore, antioxidant treated plants could survive excess Pb in the growth medium as compared to untreated ones. This mechanism might be achieved by the high capacity of antioxidant treated plants to immobilizing the heavy metal ions in their roots (Das *et al.*, 1997). Similar results were reported by Moftah (2000) using EDU on tomato and eggplants. The interaction between Pb levels in the soil and antioxidant treatments showed a significant effect on the concentration of Pb in leaves.

Table (5): Effect of Pb pollution and ethylene diurea (EDU), Ascorbic acid (AA) and salicylic acid (SA) on peroxidase activity and Pb concentrations in leaves and roors of borage plants.

Antioxidant treatments (B)	Lead (Pb) levels (ppm) (A)									
	First season					Second season				
	0	100	200	300	mean	0	100	200	300	mean
	Peroxidase activity in leaves (IU)*									
Control	0.457	0.596	0.410	0.330	0.448	0.507	0.663	0.455	0.366	0.498
EDU	0.525	0.700	0.475	0.380	0.520	0.586	0.779	0.527	0.421	0.578
Ascorbic acid	0.521	0.693	0.472	0.377	0.516	0.578	0.770	0.524	0.418	0.573
Salicylic acid	0.532	0.716	0.486	0.389	0.531	0.593	0.798	0.540	0.431	0.591
Mean	0.508	0.675	0.460	0.368		0.566	0.753	0.512	0.409	
LSD (5%)	A= 0.014	B= 0.011	AB= 0.023			A= 0.017	B= 0.013	AB= 0.026		
	Peroxidase activity in roots (IU)									
Control	0.539	0.610	0.467	0.376	0.498	0.596	0.673	0.515	0.413	0.549
EDU	0.622	0.710	0.542	0.435	0.577	0.688	0.782	0.598	0.479	0.637
Ascorbic acid	0.618	0.706	0.539	0.432	0.574	0.682	0.778	0.593	0.474	0.632
Salicylic acid	0.635	0.724	0.554	0.446	0.059	0.706	0.799	0.611	0.491	0.652
Mean	0.602	0.686	0.524	0.420		0.668	0.758	0.579	0.464	
LSD (5%)	A= 0.015	B= 0.012	AB= 0.024			A= 0.017	B= 0.014	AB= 0.028		
	Pb (mg/kg dwt leaves)									
Control	0.161	21.12	58.66	66.35	36.57	0.181	21.51	59.25	66.95	36.97
EDU	0.150	17.28	45.16	51.45	28.51	0.162	17.63	45.61	51.93	28.83
Ascorbic acid	0.156	18.60	47.53	53.43	29.93	0.171	18.84	48.05	53.91	30.24
Salicylic acid	0.142	16.28	43.16	49.45	27.26	0.158	16.58	43.49	49.78	27.50
Mean	0.152	18.32	48.63	55.17		0.68	18.64	49.10	55.64	
LSD (5%)	A= 1.46	B= 0.94	AB= 1.88			A= 1.64	B= 1.11	AB= 2.22		
	Pb (mg/kg dwt roots)									
Control	2.89	33.41	67.24	75.63	44.79	3.08	33.82	67.53	76.26	45.17
EDU	10.19	39.07	73.20	81.32	50.95	10.47	39.55	73.52	81.99	51.38
Ascorbic acid	10.73	40.35	74.85	83.67	52.40	10.73	41.94	76.17	85.47	53.58
Salicylic acid	9.25	38.10	72.45	79.35	49.79	9.21	38.35	72.66	80.01	50.06
Mean	8.27	37.89	71.94	79.99		8.37	38.42	70.22	78.68	
LSD (5%)	A= 2.13	B= 1.41	AB= 2.82			A= 2.25	B= 1.55	AB= 3.01		

* IU = International unit for enzyme activity measurements (Sadasivam and Manicham, 1992).

Peroxidase activity

Data recorded in Table (5) indicated clearly that peroxidase activity in leaves and root tissues was decreased at Pb concentrations higher than 100ppm, while at 100ppm the activity of enzyme was increased by about 30% in leaves and 13% in roots as compared with the untreated plants in both seasons. It was suggested that at higher Pb concentration the formation of both free radicals and reactive O_2 species are beyond the capacity of the endogenous antioxidant system, thus peroxidase activity decreased at high Pb levels (Rucincka *et al.*, 1999). Similar results were obtained by Moftah (2000) and El-Gamal (2000).

Data indicated also that all antioxidant treatments increased enzyme activity either in leaves or roots of treated compared with untreated plants. The slightly higher peroxidase activity in root tissues, compared to leaves, may reflect the high activity of enzymes in those organs exist in high levels of pollutant. In this regard, most increase in the activity of peroxidase was found at 100 ppm of Pb with antioxidant treatments. It seems that the increase in peroxidase activity in the presence of antioxidants or low level of HM is probably a mean of heavy metal detoxification and plant protection. In this regard, Creissen *et al.* (1994) reported that under heavy metal pollution, peroxidase and catalase activities increased as a way of accommodation to metal stress. These enzymes were found to provide antioxidant protection and preserve membrane integrity. Therefore, the antioxidant treatments tend to maintain the activity of the enzymes at high levels. Scalet *et al.* (1995) showed that peroxidase activity increased as an early response to environmental stress and might provide cells with resistance against the formation of H2O2, which is formed in chloroplasts when plants are exposed to heavy metal pollution. These results are in full agreement with those obtained by Moftah (2000) using EDU on tomato and eggplants, Knorzer *et al.* (1999) using salicylic acid on soybean and Blokhina *et al* (2000) using ascorbic acid on *Iris germanica*. The interaction between Pb and antioxidants affected significantly the peroxidase activity in both leaves and roots.

Elemental concentration

Nitrogen, phosphorous, and potassium concentrations in leaves were linearly decreased with increasing Pb levels in the soil (Table 6). The decrease in elements content in plant tissues as affected by Pb pollution was statistically significant. It is of interest to notice that the effect of Pb on the elements was not the same. In this respect, K was more affected by the pollutant than N which, in turn, was affected more than P. These results might reflect the interference of Pb to the absorption of the nutrients by the plant. The inhibitory effect of heavy metal pollution on elemental content of plant tissues may be due to the action of the pollutants on the uptake and translocation of the elements within plant roots (El-Ghinbihi, 2000). The obtained results are in accordance with those obtained by Aly (1982) who found that P and K concentrations of pepper and jews mallow leaves were decreased when plants were treated with lead. The reduction in P and K concentration was reported in coconut leaves treated with lead by Biddappa *et al.* (1987).

Antioxidant treatments, on the other side, seemed to improve the percentage of nutrient elements within leaf tissues. In this respect all antioxidants have positive effects on the uptake of elements as compared with untreated plants. The stimulating effect of antioxidants on N concentration could be explained as N_2 absorption and nitrogenous compounds such as polyamines were increased in antioxidant-treated plants (Kramer and Wang, 1989; Lee *et al.*, 1992). In this respect, the stimulating effect of antioxidants on plant growth and root expansion, consequently improving photosynthetic rate, may improve the uptake of nutrient elements such as N, P, and K (Moftah, 2000). The interaction between Pb levels and antioxidant treatments on the concentration of nutrient elements was significant in the two growth seasons.

Table (6): Effect of Pb pollution and ethylene diurea (EDU), Ascorbic acid (AA) and salicylic acid (SA) on nitrogen, phosphorus and potassium concentrations (%) in borage leaves.

Antioxidant treatments (B)	Lead (Pb) levels (ppm) (A)									
	First season					Second season				
	0	100	200	300	mean	0	100	200	300	mean
	Nitrogen (%) in leaves									
Control	1.963	1.325	0.850	0.500	1.160	1.952	1.318	0.845	0.497	1.153
EDU	2.310	1.960	1.682	0.998	1.738	2.297	1.950	1.672	0.992	1.728
Ascorbic acid	2.127	1.943	1.667	0.985	1.681	2.115	1.932	1.657	0.979	1.671
Salicylic acid	2.440	1.983	1.729	1.039	1.798	2.454	1.973	1.719	1.032	1.795
Mean	2.210	1.803	1.482	0.881		2.205	1.793	1.473	0.875	
LSD (5%)	A= 0.13 B= 0.084 AB= 0.168					A= 0.12 B= 0.075 AB= 0.15				
	Phosphorus (%) in leaves									
Control	0.357	0.245	0.160	0.090	0.213	0.348	0.239	0.156	0.088	0.208
EDU	0.460	0.362	0.314	0.191	0.332	0.448	0.353	0.306	0.187	0.324
Ascorbic acid	0.430	0.359	0.311	0.189	0.322	0.419	0.350	0.303	0.185	0.314
Salicylic acid	0.498	0.369	0.320	0.199	0.347	0.485	0.360	0.312	0.195	0.338
Mean	0.436	0.334	0.276	0.167		0.425	0.326	0.269	0.164	
LSD (5%)	A= 0.031 B= 0.022 AB= 0.045					A= 0.027 B= 0.019 AB= 0.038				
	Potassium (%) in leaves									
Control	2.651	1.829	1.152	0.596	1.557	2.521	1.739	1.096	0.567	1.481
EDU	2.682	2.537	1.608	0.847	1.919	2.550	2.412	1.530	0.806	1.825
Ascorbic acid	2.670	2.481	1.573	0.829	1.888	2.539	2.359	1.497	0.789	1.796
Salicylic acid	2.699	2.589	1.641	0.864	1.948	2.567	2.462	1.561	0.822	1.853
Mean	2.676	2.359	1.494	0.784		2.544	2.243	1.421	0.746	
LSD (5%)	A= 0.13 B=0.056 AB= 0.117					A= 0.11 B= 0.049 AB= 0.098				

Fatty acid contents

All levels of Pb decreased the fatty acid contents in seeds of Pb-treated plants (Table 7). The most affected fatty acid was stearic followed by linolenic while linoleic was not substantially affected. In contrary, palmitic showed an increase with Pb treatments. In the other side, all antioxidant treatments have stimulated the biosynthesis of fatty acid and increased their levels in plant seeds. The highest stimulating effect of antioxidants were shown at highest level of Pb (300ppm) at which palmitic, linoleic and linolenic showed most increase as compared with control and other Pb levels. This response might reflect the potential effect of antioxidants on the negative effect of heavy metals on the biological system of plants. The negative effects of heavy metals and othe environmental pollutants on oils and fatty acid fractions of many oily seeds were reported by Raskin (1992). The positive effects of antioxidants and other natural products on these constituents were also discussed by Creissen *et al.* (1994) and Moftah (2000).

Table (7): Effect of Pb pollution and ethylene diurea (EDU), Ascorbic acid (AA) and salicylic acid (SA) on fatty acid concentrations (%) of borage seeds.

Treatment	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Total
Control	10.70	3.41	14.89	36.11	24.61	89.72
EDU	11.10	2.91	17.10	38.91	22.70	82.72
Ascorbic acid	11.09	3.21	17.88	34.70	25.11	91.99
Salycilic	11.76	3.82	15.58	37.92	23.50	92.60
Pb100	11.81	2.49	18.51	29.61	22.01	84.43
Pb200	13.39	0.47	13.81	37.79	16.10	81.56
Pb300	13.29	0.46	13.68	36.66	16.11	80.20
Pb100 +EDU	10.80	3.43	14.90	36.12	24.63	89.88
Pb100 + Ascorbic acid	10.60	3.39	14.88	36.10	24.58	89.55
Pb100 + Salycilic	10.85	3.44	14.91	36.13	24.66	89.99
Pb200 +EDU	11.83	2.51	18.75	29.87	22.26	85.22
Pb200 + Ascorbic acid	11.81	2.41	18.65	29.80	22.19	84.86
Pb200 + Salycilic	11.92	2.59	18.82	29.92	22.34	85.59
Pb300 +EDU	13.49	0.47	13.95	37.99	16.30	82.12
Pb300 + Ascorbic acid	13.59	0.45	13.93	37.85	16.24	82.06
Pb300 + Salycilic	13.39	0.59	14.15	38.05	16.49	82.67

REFERENCES

Abdel-Basset, R.; A.A. Issa and M.S. Adam (1995). Chlorophyllase activity: effects of heavy metals and calcium. Photosynthetica, 31: 421-425.

Abdel-Halim, S.M. (1995). Effects of some vitamines as growth regulators on growth, yield and endogenous hormones of tomato plants during winter. Egypt. J. Appl. Sci., 10: 322-344.

Ahmed, M.M. (2001). Effect of some heavy metas on some Tagetes species. M.Sc. Thesis, Facu. Agric., Cairo Univ.

Aly, E.A. (1982). Physiological studies on the contamination and toxicity of some plants by certain heavy metals. Ph.D. Thesis, Facu. Agric., Cairo Univ.

Biddappa, C.C.; H.N. Khon; O.P. Joshi and P. Mainzandan (1987). Effect of root feeding of heavy metals on the lead concentration. Plant and Soil, 10: 295-297.

Blokhina, O.B.; E. Virolainen; K.V. Fagerstedt; A. Hoikkala; K. Wahala and T.V. Chirkova (2000). Antioxidant status of anoxia-tolerant and intolerant plant species under anoxia and re-aeration. Physiol. Plant., 109: 396-403.

Calatayud, A.; V.I. Deltora; A. Abadia; J. Abadia and E. Barreno (1999). Effects of ascorbate feeding on chlorophyll fluorescence and xanthophyll cycle components in the lichen *Parmelia quercina* (Willd.) Vainio exposed to atmospheric pollutants. Physiol. Plant., 105: 679-684.

Clarke, B.B. (1983). The impact of lead, cadmium and ozone on foliar symptom development, mineral composition, growth and yield of woody and herbaceous plants. Diss. Abst. Inter., 43: 3088.

Chapman, H.D. and P.F. Pratt (1978). Methods of analysis for soil, plant and water. Dept. Agric. Sci., Univ. California, pp 112-129.

Creissen, G.P.; E.A. Edwards and P.M. Mullineaux (1994). Glutathione reductase and ascorbate peroxidase. In Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants. Eds. C.H. Foyer and P.M. Mullineaux. Pp 344-361. CRC Press, Boca

Das, P.; S. Samantaray and G.R. Rout (1997). Studies on lead and cadmium toxicity in plants. Environ. Pollu., 98: 29-36.

David, D.J. (1958). Determination of zinc and other metals in plants by atomic absorption spectroscopy. Analyst., 83: 655-661.

De Pasquale, R.; A. Rapisarda; M.P. Germano; S. Ragusa; S. Kirjavainen and E.M. Galati (1995). Effects of cadmium on growth and pharmacologically active constituents of the medicinal plant *Coriandrum sativum* L. Water, Air and Soil Pollu., 84: 147-157.

Dodson, C.D. and F.R. Stermitz (1986). Pyrrolizidine alkaloids from Borago officinalis seeds and flowers. J. Nature Products, 49: 727-728.

El-Gamal, S.M.A. (2000). Physiological response of some plants to some sources of pollution. Ph.D. Thesis, Facu. Agric. Minufiya Univ.

El-Gengaihi, S.; H.E. Wahba and A.M. Raffaat (2000). The response of borage plants (*Borago officinalis*, L.) to different nitrogen sources and rates. Egypt. J. Hort., 27: 409-423.

El-Ghinbihi, F.H. (2000). Growth, chemical composition and yield in some common bean varieties as affected by different cadmium and lead levels. Minufiya J. Agric.Res., 25: 603-625.

El-Khayat, A.S.M.(2001). Physiological effects of tryptophane, thiamine and ascorbic acid on *Hibscus sapdariffa*, L. plants. The 5th Arabian Hort. Conf., Ismailia, Egypt, March 24-28, 2001, 11: 251-263.

Fodor, F.; E. Cseh; A. Varga and G. Zaray (1998). Lead uptake, distribution and remobilization in cucumber. J. Plant Nutr., 21:1363-1373.

Foyer, C.H.; M. Lelandais and K.J. Kunert (1994). Photooxidative stress in plants. Physiol. Plant., 92: 696-717.

Fujioka, S.; I. Yamaguchi; N. Murofushi; N. Takahoshi and S. Kaihara (1985). The role of benzoic acid and plant hormones in flowering of *Lemma gibba*. Plant Cell Physiol., 26: 655-659.

Gadallah, M.A.A. (1995). Effects of cadmium and kinetin on chlorophyll content, saccharides and dry matter accumulation in sunflower plants. Biol. Plant., 37: 233-240.

Hernandez, L.E.; A. Campillo; A. Jimenez; J.J. Alarcon and F. Sevilla (1999). Response of antioxidant systems and leaf water relation to NaCl stress in pea plants. New Phyto., 141: 241-251.

Huong, D.T.; M.T. Martin; T. Sevenet and M. Pais (1998). Pyrrolizidine alkaloids from Amphorogyne spicata. J. Nat. Prod., 61: 1444-1446.

Jackson, M.L. (1956). Soil Chemical Analysis. Englewood Cliff, N.J.

Johnson, A.R. and J.B. Davenpart (1971). Biochemistry and Methodology of Lipids. P. 35, John Wiley and Sons Inc. New York.

Jones J.B. and W.J.A. Steyn (1973). Sampling handling and analyzing plant tissue samples. Soil Testing and Plant Analysis. Soil sci. Amer. Inc., 249-270.

Kastori, R.; M. Plesnicar; Z. Sakac; D. Pankovic and I. Arsenijevic (1998). Effects of excess lead on sunflower growth and photosynthesis. J. Plant Nutr., 21: 75-85.

Khurana, J.P. and C.F. Cleland (1992). Role of salicylic acid and benzoic acid in flowering of a photoperiod-insensitive strain *Lemma paucicostata* L. P61. Plant Physiol., 100: 1541-1546.

Khurana, J.P. and S.C. Maheshwari (1987). Floral induction in *Wolffia microscopica* by non-inductive long days. Plnt Cell Physiol., 24: 907-912.

Knorzer, O.C.; B. Lederer; J. Durner and P. Boger (1999). Antioxidative defence activation in soybean cells. Physiol. Plant., 107: 294-302.

Koricheva, J.; S. Roy; J.A. Vranjic; E. Haukioja; P.R. Hughes and O.Hanninen (1997). Antioxidant responses to simulated acid rain and heavy metal deposition in birch seedlings. Environ. Pollu., 95: 249-258.

Koriesh, E.M. (2001). Studies on treated sewage water. I- Irrigation trees with treated sewage water. The 5th Arabian Hort. Conf., Ismailia, Egypt, March 24-28, 2001. 11: 329-336.

Kramer, G.F. and C.Y. Wang (1989). Correlation of reduced chilling injury and oxidative damage with increased polyamine levels in zucchini squash. Physiol. Plant., 76: 479-484

Lagriffoul, A.; B. Mocquot; M. Mench and J. Vangronsveld (1998). Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stressed enzymes in young maiz plants (*Zea mays* L.).

Lee, E.H.; G.F. Kramer; R.A. Rowland and M. Agrawal (1992). Antioxidants and growth regulators counter the effects of O_3 and SO_2 in crop plants. Agric. Ecosys. Environ., 38: 99-106.

Luna, C.M.; C.A. Gonzalez and V.S. Trippi (1994). Oxidative damage caused by excess copper in oat leaves. Plant Cell Physiol., 35: 11-15.

Luwe, M. (1996). Antioxidants in the apoplast and symplast of beech (*Fagus sylvatica* L.) leaves: seasonal variation and responses to changing ozone concentrations in air. Plant cell Environ., 19: 321-328.

Marrs, K.A. (1996). The functions and regulation of glutathione s-transferases in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 47: 127-158.

Masoud, A.N.; L.A. Sciuchetti; N.A. Farnsworth; R.N. Blomster and W.A. Meer (1968). Effect of gibberrilic acid on growth, alkaloid production and VLB content of *Catharanthus roseus*. J. Pharm. Sci., 57: 589-593.

Mishra, A. and M.A. Choudhuri (1998). Amelioration of lead and mercury effects on germination and rice seedling growth by antioxidants. Biol. Plant., 41: 469-473.

Moftah, A.E. (2000). Physiological responses of lead polluted tomato and eggplant to the antioxidant ethylene diurea. Minufiya J. Agric. Res., 25: 933-955.

Moftah, A.E.; F.A. Attia and A. Abdu (1991). Response of borage (*Borago officinalis*, L.) plants grown under saline conditions to atonic and kinetin. Minufiya J. Agric. Res., 16: 1215-1235.

Noctor, G. and C.H. Foyer (1998). Ascorbate and glutathione keeping active oxygen under control. Ann. Rev. Plant Physiol. Plant Mol. Biol., 49: 249-279.

Oota, Y. (1972). The response of *Lemna gibba* G3 to a single long day in the presence of EDTA. Plant Cell Physiol., 13: 575-580.

Patil, B.N. and S.B. Lall (1973). Effect of presowing treatment with L-ascorbic acid and gibberrelic acid on growth and physiological constituents of wheat. Botanique, 4: 5770. [C.F. Biol. Abst. 57: 34].

Polle, A.; G. Wieser and W.M. Harranek (1995). Quantification of ozone influx and apoplastic ascorbate content in needles of Norway spruce trees (*Picea abies* L. Karst.) at high altitudes. Plant Cell Environ., 18: 681-688.

Raskin, I. (1992). Role of Salicylic acid in plants. Ann. Rev. Plant. Physiol. Plant Mol. Biol., 43: 439-463.

Reda, F.; M. Fadl; R.S. Abdel-All and A.E. Moursi (1977). Studies on *Ammi visnaga* L. The effect of thiamin and ascorbic acid on growth and chromone yield. Egypt. J. Farm Sci., 18: 19-27.

Rucinska, R.; S. Waplak and E.A. Gwozdz (1999). Free radical formation and activity of antioxidant enzymes in lupin roots exposed to lead. Plant Physiol. Biochem., 37: 187-194.

Sadasivam, S. and A. Manicham (1992). Peroxidase, determination and electrophorasis. In: Biochemical Methods for Agricultural Science. Wiley Eastern Limited, New Delhy, pp 59-74.

Sayed, S.A. (1999). Effects of lead and kinetin on growth and some physiological components of safflower plant. Growth Reg., 29: 167-174.

Scalet, M.; R. Federico; M.C. Guido and F. Manes (1995). Peroxidase activity and polyamine changes in response to ozone and simulated acid rain in Aleppo pine needels. Environ. Exper. Bot., 35: 417-425.

Seth, P.N.; R. Venkatarman; S.C. Maheshwari (1970). Studies on the growth and flowering of a short-day plant, *Walffia microscopica*. II. Role of metal ions and chelates. Planta, 90: 349-359.

Shaddad, A.; A.F. Radi; A.M. Abdel-Rahman and M.M. Azooz (1990). Response of seeds of *Lupinus termis* and *Vicia faba* to the interactive effect of salinity, ascorbic acid or pyridoxine. Plant and Soil, 122: 177-183.

Snedecor, G.W. and W.G. Cochran (1973). Statistical Methods, 6^{th} Ed. The Iowa State Univ. Press. Ames, Iowa, USA.

Stefanov, K.L.; K.A. Seizova; S.D. Pandev; N.V. Yanishlieva; E.M. Marinova; L.A. Tyankova; L.L. Kuleva and S.S. Popov (1995). Effect of lead ions on lipids and antioxidant complex activity of *Capsicum annuum* L. leaves, pericarp and seeds. J. Sci. Food Agric., 67: 259-266.

Stochs, S.J. and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med. 18: 321-336.

Stymne, S. and A.K. Stobart (1986). Biosynthesis of linolenic acid in cotyledons and microsomal preparations of the developing seeds of common borage (*Borago officinalis*). Biochem. J., 240: 385-393.

Taiz. L. and E. Zeiger (1998). Heavy metal stress and homeostasis. In: Plant Physiology. Sinauer Assoc., Mas., USA, p 116.

Thomas, R.M. and V.P. Singh (1996). Reduction of cadmium-induced inhibition of chlorophyll and carotenoid accumulation in *Cucumis sativus* L. by uniconazole (S.3307). Photosynthetica, 32: 145-148.

Trough, E. and A.H. Mayer (1949). Improvements in the Denige's colorimetric method for phosphorus and arsenic. Ind. Eng. Chem. Anal., 1: 136-139.

Vassilev, A.; I. Yardanov and T. Tsonev (1997). Effects of Cd on the physiological state and photosynthetic activity of young barley plants. Photosynthetica, 34: 293-302.

Wettstein, D.V. (1957). Chlorophyll, lethal und der subminkroskovische Form wechsel der Plastiden. Exptl. Cell Res., 12:427- 433.

Witaker, B.D.; E.H. Lee and R.A. Rowland (1990). EDU and ozone protection foliar glycrolipids and steryl lipids in snap bean exposed to O_3. Physiol. Plant., 80: 286-293.

Wozny, A.; J. Schneider and E. Gwozdz (1995). The effect of lead and kinetin on greening barley leaves. Biol Plant., 37: 541-552.

الاستجابات الفسيولوجية لنباتات البوراج (خبز النحل) النامية فـــى بيئـــة ملوثـــة بالرصاص للمعاملة بمضادات الأكسدة

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أجرى هدا البحث خلال عامى ١٩٩٩/٢٠٠٠ و٢٠٠٠/٢٠٠١ لدراسة امكانية التغلب علـى التأثيرات الضارة الناتجة عن نمو نباتات البوراج (خبز النحل) فى تربة ملوثة بالرصـــاص. فقـد تمت زراعة النباتات فى اصص تحت معدلات مختلفة من الرصـــاص (صفــر ، ١٠٠ ، ٢٠٠ ، ٣٠٠ جزء في المليون) ثم عوملت التربة قبل نقل الشتلات الى الأصص بيومين بمضاد الأكســـدة ايثيلين ثنائى اليوريا بمعدل ٥٠٠ مجم/لتر او حمض الساليسليك بمعدل ١٠ ميكرومولر، كلا علـــى حده. اما المعاملة الثالثة فكانت رش النباتات بحمض الأسكوربيك بمعدل ٣٠٠مجم/لتر بعـــد نقـل الشتلات بأربعة اسابيع، وذلك لدراسة تأثير تلك المعاملات على النمو والتركيب الكيماوى والأزهار ومحصول البذور وقد اوضحت النتائج المتحصل عليها مايلى:

اثرت المعاملة بالرصاص تأثير سلبى على صفات النمو ممثلة فى اطوال النباتات وعـــدد الأفرع ووزنها الجاف خاصة عند التركيزات العالية من الرصاص. ازداد تركيز الرصاص داخـل انسجة النباتات المعاملة وكانت الزيادة كبيرة عند التركيز ٣٠٠ جزء في المليون كمـــا اوضحـــت النتائج نقص نسبة صبغات البناء الضوئى مثل الكلوروفيـــل وايضـــا الكـــاروتينويدات بالمعاملـــة بالرصاص تحت اى مستوى وخاصة المستويات العالية. أدت المعاملة بالرصاص ليضا الى نقـص تركيز النتروجين والفوسفور والبوتاسيوم. ارتفع نشاط انزيم البيروكبسيديز فى كـــل مـــن الأوراق والجذور فى النباتات المعاملة بالرصاص. وتأخر التزهير ونقص وزن الأزهـــار ووزن البـــذور ونسبة الزيت فى النباتات المعاملة بمقارنتها بغير المعاملة. فى كل الحـــالات ســـابقة الذكـــر أدى استخدام مضاد الأكسدة الى الأقلال من التأثيرات الناتجة من التلوث بالرصاص حيـــث ادى الـــى تحسن نمو النباتات عند نموها فى بيئات تحتوى عليه ربما نتيجة لحماية بعض الأنزيمات الهامـــة فى الخلية النباتية من التأثير السلبى الناتج عن التلوث بالرصاص. وكانت احسن المعـــاملات هـــى استعمال الأيثيلين ثنائى اليوريا او حمض الساليسيليك.