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Impact of soil lead pollution and iron foliar application on *Spinacea oleracea* (L.)

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Abstract. The experiments were conducted on spinach plants, *Spinacea oleracea* L. var. balady, at Ministry of Agriculture, Beit-Lahya city, Gaza Strip, Palestine. The experiments aimed to study the effect of soil lead pollution and iron foliar application on spinach plants. Measurements of the lead effects on spinach plants revealed: (a) Growth characters such as root length, shoot height, total leaves area, fresh and dry weights of root, shoot and whole plant were decreased with increasing Pb soil addition. (b) Plant pigments such as chlorophyll a, chlorophyll b and total carotenoids were decreased with the increasing of Pb concentration in the soil. Iron foliar application on spinach plants revealed: (a) Growth characters and plant pigments of plant were increasing with increased concentration of alone iron. (b) Toxicity of Pb on growth characters and its effect on elemental transport and accumulation were reduced when it combined with Fe foliar application.

Key words: lead pollution, iron application, spinach, Gaza Strip, Palestine, iron/ lead interaction.

Introduction. Pollution of agricultural land by heavy metals has imposed an increasingly serious risk to environmental and human health in recent years. With the development of industries and modernization of agriculture, soil pollution has become more increasingly serious. The heavy metal concentrations are so high in soils of many areas that they can poison the soil-plant system, degenerate the soil, and reduce the quality and products of crops. Heavy metals that have a direct impact on plants include Fe, Cu, Mn, Co, Ni, Cd, Pb, and Cr. Soil contamination with heavy metals is becoming a major problem worldwide, leading to losses in agricultural productivity, hazardous effects as they enter the food chain and metal toxicity in plants, humans, and animals. Nonetheless, their availability to plants via soil is determined by natural processes, especially lithogenic and pedogenic, as well as by anthropogenic factors (Sharma & Agrawal 2005; Marica et al 2010; Mazid et al 2010). Among the heavy metals, the use of lead (Pb) during the last 50 years has caused widespread environmental contamination. As lead causes a variety of toxic effects as a major anthropogenic pollutant and has accumulated in different terrestrial and aquatic ecosystems, the fate of lead in the environment is of great concern (Kumar & Tripathi 2008; Verma & Dubey 2003). Among the existing pollutants, lead (Pb) is the major contaminant of the soil (Gratao et al 2005) poising significant environmental problems (Shen et al 2002), including the risk of poisoning for humans and especially children (Lasat 2002).

Biochemical changes in green plants in response to Pb have been reported by several authors: decreases in chlorophyll content (Ewais 1997; Xiong 1997; Kastori et al 1998; Fargasova 2001), carotenoids (Fargasova 2001), proteins (Kevresan et al 2001), nitrate reductase activity (Singh et al 1997; Kevresan et al 2001), lamina and mesophyll thickness, epidermal cell size and diameter of vessels (Kovacevic et al 1999); increases in chlorophyll *a*/*b* ratio (Fargasova 2001) and concentration of phenols (Lummerzheim et al 1995; Lavid et al 2001ab; Olivares 2003).

Lead absorption by roots from the soil occurs via the plasma membrane, probably involving cationic channels such as calcium channels (Romeiro et al 2006). Roots are capable of accumulating significant quantities of this heavy metal and simultaneously restrict its translocation to the shoot (Lane & Martin 1977).

Iron (Fe) is essential for plant growth and development (Curie & Briat 2003). In aerobic conditions, soil Fe is usually found as oxihydroxide polymers, which have very low solubility, limiting the Fe supply for plant uptake. Iron deficiency is therefore a yield-limiting factor with major implications for field crop production in many agricultural regions of the world (Hansen et al 2006). Some researchers suggest that excess Fe may result in lower uptake of other essential nutrients, either due to the barrier created by the Fe coatings (Howeler 1973) or due to chemical interactions in the soil (Neue et al 1998). Sahrawat (2004) pointed out the existence of pseudo Fe toxicity (when Fe toxicity symptoms were caused by deficiency of other nutrients) and true Fe toxicity (caused by toxic concentrations of Fe, without any apparent deficiency of other plant nutrients). Lagerwerff & Specht (1970) reported that the concentrations of heavy metals in roadside soil and grass samples from several locations decreased with the distance from vehicular traffic. Contamination was related to the composition of gasoline, motor oil and automobile tires, and to roadside deposition of residues of these materials. Contamination from motor vehicle emissions within 100 m of the roadway has been reported in vegetation (Motto et al 1970; Ward et al 1975), soils (Yassoglou et al 1987), and spider webs (Hose et al 2002). Lead added to fuel as tetra ethyl, to act as a lubricant during combustion, is discharged into the environment in exhaust fumes largely as minute particles of inorganic Pb compounds and about 50 % of this falls within a region of 100 m from the road (Mengel & Kirkby 2001).

Gaza Strip, Palestine is one of the most densely populated areas in the world with limited and deteriorated resources, has already started to suffer the consequences of environment quality deterioration. The situation at the Gaza Strip is below the desired standard, which is attributed to the absence of environmental legislation and public awareness. One of the most important air pollutants near the city center of Gaza are thousands of motor vehicles commuting every day. Therefore, the aim of the present investigation was to study the response of spinach plants (*Spinacea oleracea* L.) to Pb-supplemented soil and Fe-foliar application. Attempts were also made to determine the lead and iron interaction on the spinach plants.

Materials and Methods. The experimental site was located in the open field of the Agriculture Research Center (Ministry of Agriculture), Beit-Lahya City, Gaza Strip, Palestine. Plastic pots with 80 cm length, 20 cm width and 25 cm depth were filled with the air dried soil, obtained from Agriculture Research Center, at 25 kg/pot. Each pot received 22 gram of ammonium sulfate, 18 gram of potassium sulfate and 15 gram of calcium superphosphate. The fertilizers were applied to the plants as soil dressing at three doses/season; the first dose was 15 days after seedling emergence and the second and the third dose were applied 15 days in time intervals. Pb added (treatment) and no Pb added (control) were conducted, and three pots were used as replication for each one, both for the control and the treatment. As regards the Pb treatment, soils of each pot were mixed with a Pb solution in the form of lead acetate, and the final Pb concentrations were 0, 50, 250 and 500 ppm. On January 18^{th} 2007, healthy seeds of spinach were sown into the pots. The pots were divided into 4 groups, the first one received the normal level of fertilizers as mentioned above, but without any other soil addition. The second, third and fourth groups were similar to the first group but the pots received iron as the foliar spray in the leaves of plant before one week of the first and the second harvested samples in the form of iron ethylene di amino tetra acetic acid (Fe EDTA) at the rates (0, 150, 250 and 350 ppm). Regarding to the total treatments, the experiment includes 16 treatments comprised the following amendments as follows: one treatment is a control (without any of metals), 3 treatments with soil Pb pollution at different concentrations, 3 treatments with Fe foliar application at different concentration and 9 treatments consist of a combination of the Pb and Fe at different concentrations as mentioned above.

Finally, plant samples were harvested on February 17^{th} 2007 (plant age = 30 days) and on March 19^{th} 2007 (plant age = 60 days) and washed with tap-water followed by three rinses with deionized water. Afterwards, plants were separated in root, shoot and leaves. Root length (cm), shoot height (cm), fresh and dry weights of the shoot and root (g) and leaves area (cm^2), at all treatments, were estimated according to the methods described by Hunt (1978). Chlorophyll and total carotenoids (mg/g FW) were extracted from the fresh leaves of plants, at the two ages, by acetone 80% and were calorimetrically determined according to the method described by Hoyden (1965).

Regarding to metal determination, all plant samples were soaked in EDTA-Na2 solution (0.01 mol/L) for 15 min to remove the heavy metals adhered at plant surface and then rinsed with deionized water. After drying to constant weight at 105°C for 48 h, all roots, shoots and leaves were milled with mortar and pestle and sieved through a meshes plastic sift, then airproofed and kept in refrigerator at 4°C. Pb, Fe and Zn concentrations of all of the samples (control and different treatments at the two ages) were determined with a flame atomic absorption spectrophotometer (Pu 9100*). According to Snedecor & Cochran (1980), the statistical analysis of the results was done with the use of the factorial experiments and the means of different treatments were compared using the least significant different test (L.S.D) at 0.05 level of probability in the two samples.

Results and Discussion. There was a reduction in plant height (root length and shoot height), both at 30 and 60 days, with increasing concentrations of Pb either alone or in combination with any of the three concentrations of iron foliar application (Table 1). Similar results were observed by Tandy et al (2005) for *Helianthus annuus* growing in nutrient solution with Pb. Table 1 also reveals that the difference in height for plants from the zero and highest lead treatments (500 ppm) increased with time. This suggests that, over time, Pb must have interfered more strongly with the metabolic processes of the plant.

According to Kosobrukhov et al (2004), application of Pb brings about a considerable decrease in dry mass accumulation of different plant parts. In *Vetiver zizanioides* and *V. nemoralis* the biomass accumulation of plants was reduced with increasing Pb concentrations (Chantachon et al 2004). Nevertheless, for spinach plants we observed a reduction in total leaf area, fresh weight and dry weight of whole plant with increasing concentration of Pb concentration (Table 1). The decrease in biomass under heavy metal stress has been reported earlier (Krovacevic et al 1999; Pandey & Sharma 2002). The reduction of these growth character is in accordance with Romeiro et al (2006). Similar results were reported by Yang & Lee (1990) on lettuce, Monem et al (1991) on tomato, Chen et al (1991) on rice. Lang et al (1998) suggested that, Pb at (10mM) in the soil retarded the leaf growth. On the other hand, Zaman & Zereen (1998) demonstrated that growth was inhibited in radish tip seedlings grown for 30 days in a silt loam soil containing Pb (0-2000 ppm), the degree of inhibition varied with contaminant concentration. Furthermore, Liu et al (2000) studied the effects of different concentrations of lead nitrate on indian mustard and reported that root growth decreased progressively with increasing concentration of Pb in solutions, the seedlings exposed to 10^{-3} M Pb exhibited substantial growth reduction. On the other hand, beetroot (*Beta vulgaris* var. *saccharifera*) grown in hydroponics with Pb showed an increase in root dry mass without any change in shoots, even in 2 mM Pb, resulting in an increased root to shoot ratio (Larbi et al 2002). The inhibition of root growth may be due to a decrease in calcium in root tips, leading to a decrease in cell division or cell elongation (Haussling et al 1988; Eun et al 2000). The inhibition of growth of shoot may be due to a decrease in photosynthesis, in upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability (Sharma & Dubey 2005).

Data in Table 1 indicated that, both at 30 and 60 days, significant increases in all of the studied growth characters were recorded by the plants treated with the three different levels (150, 250 and 350 ppm) of Fe foliar application either alone or combined with lead sol addition, with some exceptions. Similar results were reported by many workers (Smith et al 1984) on cucumber and (Saker et al 1996) on bean plants.

It is clear form the results in Table 2 that, lead soil addition either alone or in combination with iron foliar application significantly decreased the chlorophyll a concentration, both at 30 and 60 days. The above treatments was insignificantly affected the chlorophyll b concentration. Despite, decreases in photosynthesis were relatively small, reaching statistical significance only in plants grown with high concentration of lead (Romeiro et al 2006).

Table 1

Growth characters of spinach plants after growing for 30 and 60 days under the impact of Pb soil addition and Fe foliar application

Growth character		30 days					60 days				
	Treatment	Control	Fe1	Fe2	Fe3	Mean	Control	Fe1	Fe2	Fe3	Mean
Root length (cm)	Control	10.6	11.0	11.2	11.3	11.1	19.1	21.2	17.5	18.5	19.1
	Pb1	9.3	8.3	8.6	9.5	8.9	16.2	15.5	16.8	15.8	16.0
	Pb2	7.3	7.3	8.0	9.2	7.9	14.2	15	15.9	16.7	15.4
	Pb3	6.9	6.7	7.7	7.1	7.1	12.7	14.6	15.9	16	14.8
	Mean Fe	8.5	8.3	8.9	9.3		15.5	16.6	16.5	16.7	
	L.S.D 0.05	Pb = 2.2 Fe = NS Pb*Fe=3.1					Pb = 0.95Fe = 0.95 Pb*Fe=1.30				
Shoot height	Control	14.9	19.3	16.4	20.9	17.9	28.0	30.3	32.7	37.7	32.1
	Pb1	12.7	17.3	16.1	18.3	16.1	25.7	29.4	30.7	27.8	28.4
	Pb2	10.5	16.5	13.9	16.5	14.3	22.5	24.8	25.8	29.5	25.6
	Pb3	9.5	10.5	11.8	13.2	11.3	19.1	23.7	25.3	27.8	24.0
	Mean Fe	11.9	15.9	14.6	17.2		23.8	27.0	28.6	30.7	
	L.S.D 0.05	Pb = 4.5 Fe = 4.5 Pb*Fe=6.4					Pb = 6.4Fe = 6.4 Pb*Fe=9.1				
Leaves area	Control	287.0	450.6	381.8	488.3	401.9	915.8	999.0	1062.3	1315.4	1073.1
	Pb1	284.2	318.5	308.5	398.6	327.5	749.3	965.7	999.0	1115.7	957.4
	Pb2	277.7	286.0	308.8	362.2	308.7	649.4	834.2	849.2	1032.3	841.2
	Pb3	213.4	249.2	258.5	247.1	242.0	582.8	765.9	932.4	932.4	803.4
	Mean Fe	265.6	326.1	314.4	374.0		724.3	891.2	960.7	1098.9	
	L.S.D 0.05	Pb = 71.1 Fe = 71.1 Pb*Fe=101.1					Pb = 120.7Fe = 120.7 Pb*Fe=171.5				
Fresh weight	Control	11.5	12.6	13.0	14.3	12.8	29.7	33.0	36.9	39.3	34.7
	Pb1	9.3	11.2	11.2	12.8	11.2	24.7	29.5	33.1	32.7	30.0
	Pb2	8.2	10.0	11.0	11.4	10.1	22.0	29.1	31.2	29.8	28.0
	Pb3	7.7	9.1	10.1	9.9	9.2	17.2	22.1	25.0	25.9	22.5
	Mean Fe	9.2	10.7	11.3	12.1		23.4	28.4	31.5	31.9	
	L.S.D 0.05	Pb = 2.7 Fe = 2.7 Pb*Fe=3.8					Pb = 1.7Fe = 1.7 Pb*Fe=2.4				
Dry weight	Control	0.862	1.353	1.147	1.467	1.207	2.75	3.00	3.20	3.95	3.22
	Pb1	0.850	0.957	0.927	1.197	0.983	2.25	2.90	3.00	3.35	2.88
	Pb2	0.840	0.859	0.927	1.091	0.929	1.95	2.51	2.95	3.10	2.63
	Pb3	0.668	0.748	0.776	0.727	0.730	1.75	2.30	2.85	2.81	2.43
	Mean Fe	0.805	0.979	0.944	1.120		2.18	2.68	3.00	3.30	
	L.S.D 0.05	Pb = 0.209 Fe = 0.209 Pb*Fe=0.297					Pb = 0.30Fe = 0.30 Pb*Fe=0.43				

Pb1=50ppm, Pb2=250ppm, Pb3= 500ppm, Fe1= 150ppm, Fe2 =250ppm Fe3=350 ppm.

Decreases in carotenoid concentrations were statistical significance in all spinach plants grown with the effect of lead or the combination with iron. As the carotenoids protect chlorophyll from photo-oxidative destruction (Middleton & Teramura 1993), a differential reduction in carotenoids under excess of different heavy metals might be a reason for the differential decrease in chlorophyll being greater in heavy metal starved plants.

The decreased concentration of the chloroplastic pigments may be an outcome of reduced synthesis and/ or enhanced oxidative degradation of these pigments by the imposed oxidative stress (Pandey et al 2009). It has been suggested that lead can alter photosynthesis through effects on stomata or directly on mesophyll cells in which both photochemical and biochemical reactions can be affected (Kosobrukhov et al 2004). In barley seedlings, elevated levels of Pb in the soil reduce photosynthesis either through reduced carboxylase activity or through effects on the metabolites of the Calvin cycle (Stiborova et al 1987). Consequently, the effect of Pb is perceived as a decrease in growth and development of the plant.

Regarding to the effect of the iron foliar applications (150, 250 and 350 ppm) either alone or combined with lead soil addition on chlorophyll a of spinach plants, both at 30 and 60 days, it was significantly and gradually increased at all levels as compared with control iron untreated plants. Concerning to the chlorophyll b concentration of spinach plant, in the two samples, no constant trend could be detected by the plants treated with iron foliar application at the all levels when compared with control- iron untreated plants. As well as, concerning the effect of iron foliar application either alone or in combination with lead soil addition on carotenoids concentration, inconstant trend could be obtained by the plants supplied with iron foliar application at the all levels when compared with control untreated iron plants. These results may be attributed to the role played by Fe in many physiological processes within the plant which consequently promoting plant growth. Interference of heavy metals with normal iron metabolism is known to induce physiological iron deficiency which is expressed in the form of chlorosis due to decreased concentration of chloroplastic pigments (Pandey et al 2009). Excess of divalent cationic heavy metals compete with iron for uptake (Pandey & Sharma 2002) by binding with biomolecules of which iron is a constituent.

Table 2

Plant pigments concentration of spinach plants after growing for 30 and 60 days under the impact of Pb soil addition and Fe foliar application

Plant Pigment	Treatment	30 days					60 days				
		Control	Fe1	Fe2	Fe3	Mean	Control	Fe1	Fe2	Fe3	Mean
Chlorophyll a	Control	4.01	3.90	4.06	4.41	4.09	4.37	4.82	5.28	5.17	4.91
	Pb1	3.44	3.29	4.20	3.96	3.72	3.69	4.14	4.50	4.46	4.20
	Pb2	3.22	3.64	3.49	3.98	3.58	3.14	4.76	4.39	4.59	4.22
	Pb3	3.22	3.93	4.23	4.07	3.86	2.86	3.48	3.95	4.05	3.58
	Mean Fe	3.47	3.69	3.99	4.10		3.51	4.30	4.53	4.57	
	L.S.D 0.05	Pb = 0.457 Fe = 0.457 Pb*Fe=0.650					Pb = 0.348Fe = 0.348 Pb*Fe=0.495				
Chlorophyll b	Control	1.560	1.425	1.415	1.370	1.443	1.627	1.387	1.547	1.773	1.583
	Pb1	1.435	1.330	1.430	1.235	1.358	1.240	1.420	1.173	1.293	1.282
	Pb2	0.915	1.295	1.180	1.685	1.269	1.157	1.373	1.313	1.333	1.294
	Pb3	1.035	1.415	1.575	1.330	1.339	1.420	1.550	1.570	1.837	1.594
	Mean Fe	1.236	1.366	1.400	1.405		1.361	1.433	1.401	1.559	
	L.S.D 0.05	Pb = NS Fe = NS Pb*Fe=0.452					Pb = 0.289Fe = NS Pb*Fe=0.410				
Carotenoids	Control	2.095	1.535	1.395	1.610	1.659	1.570	1.503	1.927	1.630	1.658
	Pb1	1.520	1.290	1.910	2.095	1.704	1.827	1.207	1.360	1.463	1.464
	Pb2	1.505	1.205	1.535	1.750	1.499	1.460	1.567	1.453	1.497	1.494
	Pb3	2.045	1.775	2.005	1.380	1.801	1.243	1.413	1.620	1.687	1.491
	Mean Fe	1.791	1.451	1.711	1.709		1.525	1.423	1.590	1.569	
	L.S.D 0.05	Pb = 0.263 Fe = 0.263Pb*Fe=0.374					Pb = 0.179Fe = NS Pb*Fe=0.254				

Pb1=50ppm, Pb2=250ppm, Pb3= 500ppm, Fe1= 150ppm, Fe2 =250ppm Fe3=350 ppm.

Concerning the effect of lead soil addition on Pb concentration in spinach plant, Table 3 shows the significantly and gradually increases in Pb concentrations, both at 30 and 60 days, with the three different rates of lead soil addition either alone or combined with iron foliar application when compared with control– untreated plant or control lead untreated plants, respectively. The variations of Pb accumulation in all the tested plants were greater under the Pb treatment than under the control. In the control, the Pb concentration was 5.5 ppm. Under the Pb treatment, the highest Pb concentrations in shoots were 72 ppm and 84 ppm at 30 and 60 days, respectively. Respect the normal range of Pb in plants, Bowen (1997) reported that normal concentration of Pb in edible vegetables range between 0.20 - 20 ppm. Also, Cottenie et al (1979) suggested that, the normal range of Cu, Ni, Pb and Cd were 6 - 15, 0 - 8, 2 - 14 and 0 - 2 ppm respectively but the phototoxic content of them were 20 > 80 > 60 and >100 respectively. Therefore, it can be suggested that Pb concentration in the all treatments were up to the normal range but do not reach to phototoxic content level. The increase in metal accumulation in different plant parts and its increases with longer exposure is in accordance with Pandey & Sharma (2002) and Erdei et al (2002). The higher accumulation of metals in roots than leaves and stem was probably due to its rapid absorption by the roots and its slow translocation to shoot (Nada et al 2007). Similar results were reported by Liu et al (2000) and Curylo et al (1998) on carrot plants. Moreover, our results revealed that Pb concentration in the spinach shoot was mostly related to its concentration in soil. This result is in agreement with Liu et al (2000) working on carrot, reported that lead concentration of leek shoots and roots increased with increasing soil contamination with lead. In addition, results were reported by Fageria (2000), the uptake of lead in cereals increased with increasing soil lead. The castor bean plants were able to accumulate large quantities of Pb, especially in roots, demonstrating the high capacity for Pb absorption and accumulation of that organ (Romereio et al 2006). Similar results were obtained for *Carex rostrata, Eriophorum angustifolium* and *Phragmites australis* grown in hydroponics (Stoltz & Greger 2002). A high capacity for Pb retention in the roots with restricted translocation to the shoots was reported for *Helianthus annus* L. (Romeiro 2005), *Pinus radiate* (Jarvis & Leung 2002), spinach (Tsen et al 2002) and *Prosopis* spp. (Aldrich et al 2004).

Regarding to the effect of iron foliar application on lead concentration in spinach shoots, the results in Table 3 revealed that, significant decreases in Pb concentration were recorded by the spinach shoot supplied with the three different rates of iron in combination with any of the three different rates of lead soil addition. The decrease percentage of lead was 15.7, 13.4 and 24.6 %, at the three iron concentration, respectively for the 30 days and 9.6, 16.6 and 18.9 % for the 60 days, when compared with control- Fe untreated plants. In addition, low value of Pb was accumulated in plants treated with highest level of iron foliar application (350 ppm) when combined with lead soil addition in the two samples. Thus, it may be suggested that, decreasing of Pb concentration on spinach plant by using and increasing iron foliar application supported the assumption that there are antagonistic relationship between Fe and Pb accumulation in plants. Thus, it is important to mention that using iron foliar application was very important tool to reduced Pb concentration of spinach shoots. In this respect, Boisson et al (1999) reported that the mobility of heavy metals in soil is influenced by several factors including pH, adsorption on minerals such as, phosphate rock and iron oxides and complexion to inorganic or organic legends (Neubaver et al 2002).

Table 3

Lead, iron and zinc concentration (ppm) of spinach shoot after growing for 30 and 60 days under the impact of Pb soil addition and Fe foliar application

Metal concentration	Treatment	30 days					60 days				
		Control	Fe1	Fe2	Fe3	Mean	Control	Fe1	Fe2	Fe3	Mean
Lead	Control	5.47	4.94	8.10	5.05	5.89	7.30	6.70	8.70	6.10	7.20
	Pb1	40.85	31.60	34.20	26.90	33.39	65.00	55.00	52.00	60.00	58.00
	Pb2	46.00	39.50	42.50	36.00	41.00	79.00	69.00	62.00	58.00	67.00
	Pb3	72.00	62.50	57.50	56.00	62.00	84.00	82.00	73.50	68.50	77.00
	Mean Fe	41.08	34.64	35.58	30.99		58.83	53.18	49.05	48.15	
	L.S.D 0.05	Pb = 4.87 Fe = 4.87 Pb*Fe= 6.92					Pb = 6.10Fe = 6.10Pb*Fe= 8.68				
Iron	Control	63.0	108.0	111.0	104.0	96.5	79.0	136.5	129.5	125.5	130.5
	Pb1	72.0	115.0	111.0	88.0	96.5	63.0	128.0	107.0	113.5	116.1
	Pb2	64.5	108.0	112.0	94.0	94.6	63.0	109.5	118.5	127.0	118.3
	Pb3	82.5	83.0	97.5	90.0	88.3	68.5	111.0	110.0	127.0	116.0
	Mean Fe	70.5	103.5	107.8	94.0		68.3	121.2	116.2	123.2	
	L.S.D 0.05	Pb = 9.1Fe = 9.1Pb*Fe= 13.0					Pb = 13.1Fe = 13.1Pb*Fe= 18.7				
Zinc	Control	42.0	49.0	60.0	52.0	50.7	50.0	44.0	48.0	55.0	49.2
	Pb1	49.5	36.0	57.5	57.0	50.0	52.0	39.0	45.0	58.0	48.5
	Pb2	46.0	51.0	58.0	47.0	50.5	49.5	62.0	60.0	64.0	58.8
	Pb3	43.5	29.0	41.5	37.5	37.9	42.0	36.0	54.0	60.0	48.0
	Mean Fe	45.2	41.2	54.2	48.3		48.3	45.2	51.7	59.25	
	L.S.D 0.05	Pb = 7.81Fe = 7.81 Pb*Fe= 11.2					Pb = 9.0Fe = 9.0Pb*Fe= 12.8				

Pb1=50ppm, Pb2=250ppm, Pb3= 500ppm, Fe1= 150ppm, Fe2 =250ppm Fe3=350 ppm.

Significant decreases in Fe accumulation have been recorded at the spinach plants with Pb increasing treatments, when compared with control untreated plants (Table 3). Moreover, the results revealed that low values of Fe concentrations were recorded by treated shoot of the plants with any of the three levels of Pb combined with any of the three different levels of iron foliar application when compared with corresponding treatment sprayed with iron foliar application alone, with some exceptions. In this connection, low value of Fe was accumulated by the plants supplied with highest level of lead (500 ppm) in combined with iron foliar application of the two samples. In this respect, it may be suggested that, inhibiting effects of Fe concentration by using and increasing lead soil addition indicated antagonistic relationship between these two elements (Pb and Fe). On the other hand, from the results, it can be suggested that the several detrimental effects attributed to lead soil addition on all of the studied growth characters might be partially due to decreases in iron concentration.

The range of iron concentration in the treated plants, with three different rates of alone iron (150, 250 and 350 ppm) was 104 - 111 ppm in the first sample and was 125.5 – 136.5 ppm in the second sample, while the normal iron levels in plants it is reported to be 50 – 250 ppm (Das 2000 and Cottenie et al 1979). Therefore, it can be suggested that iron concentration in spinach plants grown under experiment were within the normal range regardless Fe or Pb levels application.

Significant increases in iron concentration were recorded by the spinach shoot sprayed with the three different rates of iron either alone or in combination with lead soil addition when compared with control untreated plants or plant supplied with lead soil addition alone. In this connection, it is important here to mention that, high value of Fe was accumulated by the plants when supplied with iron (250 ppm) and (350 ppm) in combination with lead soil addition in the first and second samples (Table 3). Therefore, it can be suggested that, there are increases in iron concentration by using and increasing iron foliar application. Perez et al (1995) reported that ferrous sulfate significantly increased Fe contents of citrus leaves. Moreover, El Fouly et al (1998) found that, applying a mixture of Fe and Zn and Mn as chelate (EDTA) increased the contents of Zn, Fe and Mn in cucumber leaves. In addition, El Shafey et al (1986) working on spinach pants, mentioned that leaf soluble and assimilated Fe were significantly increased with increasing Fe rates. Moreover, Banuls et al (2003) working in citrus found that Fe and Mn concentration in leaves increased as a result of Fe application (Fe chelate at the rate of 3 g Fe per tree). Adams et al (2000) grew soybean plants in nutrient solution, Fe was supplied over a wide range of concentration to induce a range of deficiency symptoms to derive critical concentration level for Fe.

There was a reduction in Zn concentration, both at 30 and 60 days, with increasing concentrations of Pb, when compared with the plants treated with the lowest rate of lead soil addition (Table 3). The lowest value of Zn was accumulated by the plants treated with the highest level of lead (500 ppm) either alone or combined with the lowest iron foliar application, in the two samples. Therefore, it can be suggested that, the low values of Zn concentration which recorded with increasing lead soil addition of spinach shoot indicating antagonistic relationship between Pb and Zn absorption and/or translocation. On the other hand, at 30 days sample, high value of Zn was accumulated by the plants treated with the middle rate of iron either alone or in combination with the middle rate of lead. The lowest value of Zn was detected by the plants supplied with the lowest level of iron in combination with the highest level of lead. In addition, at 60 days sample, it was clear that, highest value of Zn was obtained by the plants supplied with middle rate of lead in combination with the highest level of iron, and the lowest concentration of Zn recorded by the plants supplied with highest level of lead when combined with the lowest level of iron.

Regarding to the effect of iron foliar application on zinc concentration in spinach plant, the results in Table 3 revealed that, as the mean value non-significant and significant increases in zinc concentration were recorded by the spinach shoot supplied with the middle and the highest rates of iron foliar application, while significantly decreases in zinc concentration were recorded by the spinach shoot supplied with the lowest rate of iron foliar application in combination with lead soil addition as compared with control untreated iron plants or plant supplied with lead soil addition alone, with some exceptions. This result is in agreement with El Fouly et al (1998), reported that spraying cucumber plants, with a mixture of Fe and Zn increased the contents of Zn, Fe in cucumber leaves. Moreover, Perez et al (1995) suggested that the ferrous sulfate significantly increased Zn contents of citrus leaves. In addition, Banuls et al (2003) working in citrus found that Zn concentration in leaves increased as a result of Fe application.

Based on the results, it could be concluded that, over time, Pb must have interfered more strongly with the metabolic processes of the spinach plant. Consequently, the effect of Pb is perceived as a decrease in growth and development of the plant. Results may be attributed to the role played by Fe in many physiological processes within the plant which is consequently promoting plant growth. Interference of heavy metals with normal iron metabolism is known to induce physiological iron deficiency which is expressed in the form of chlorosis due to decreased concentration of chloroplastic pigments. Excess of divalent cationic heavy metals, Pb and Zn, compete with iron for uptake by binding with biomolecules of which iron is a constituent. It can be suggested that the several detrimental effects attributed to lead soil addition on all of the studied growth characters might be partially due to decreases in iron concentration. Decreasing of Pb concentration on spinach plant by using and increasing iron foliar application supported the assumption that there are antagonistic relationship between Fe and Pb accumulation in plants. Thus, it is important to mention that using iron foliar application was very important tool to reduce Pb concentration of spinach shoots. Based on the previous results, it can be suggested that the several detrimental effects attributed to Pb soil addition on the all of the studied growth characters of spinach plants might be directly or indirectly due to decreases in Zn concentration which might have negative effects on plants. It may be concluded that, the effect of Fe foliar application in combination with Pb soil addition was different from the individual effects of every metal alone on the concentration of Zn in spinach shoot.

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