

Lead induced oxidative stress and development change on *Coriandrum Sativum*

Summary

Lead (Pb) is ubiquitous pollutant in environment which causes many toxic effects, at high and low dose, Lead makes disruptions at all plants by reduction its growth and development of aerial parts and roots. In this study, assessing the capacity of lead to induce a dysfunctioning in development of roots and aerial parts at different concentrations after 21 day and 40 days, biochemical parameters of oxidative stress and morphological change on *Coriandrum sativum* was studied in the present investigation. The seeds were sprayed with a solution containing lead to different concentrations for 40 days with six lead levels (0 as control, 500, 1000, 1500, 2000, 2500 and 3000 mg.l⁻¹). The soil used was characterized; the results show that there is no change in its properties. Parameters such as growth, oxidative damage markers (lipid peroxidation, proline, chlorophyll and hydrogen peroxide contents) were investigated. Roots, sheets and stalks indicate that chlorophyll and carotenoid concentration were significantly decreased at 3000 mg.l⁻¹ Pb than control. Lipid peroxidation and H₂O₂ levels were increased at the dose of 2500 and 3000 mg l⁻¹ compared to control treatment; no difference was noted between 500mg.l⁻¹ and control in all part in plant. Morphological studies show that the group exposed to 3000 mg.l⁻¹ of lead shows a very important development of the tissues of roots and stems compared to control and to the group exposed to 500 mg.l⁻¹ of Pb. changes in morphological structure and non-enzymatic antioxidants have shown that lead exposure causes a significant perturbation on *Coriandrum sativum* Linn seedlings affecting biochemical and physiological processes.

Key words: *Coriandrum sativum*, chlorophyll, Proline, lead, lipid peroxidation.

Introduction

The contamination of soil by heavy metals is one of the most serious environmental problems and has significant implications for human health. Some industrial activities and agricultural practices increase their level in the substrate, and the possible introduction of the elements in the food chain is an increasing human health concern (Cakmak et al., 2000). The accumulation of heavy metals in plants presents a toxic hazard to man, because the cultivated plants are the point of entry into the food chain. Coriander (*Coriandrum sativum* L.), which belongs to the family Apiaceae (Umbelliferae) is mainly cultivated from its seeds throughout the year (Mhemdi et al., 2011), is widely used in the human diet, changing their composition or properties would cause deleterious effects to their consumers.

Engineering industrial techniques used to clean up contaminated soils are expensive and suitable only for small polluted areas (Lutts et al., 2004). Furthermore, these technologies are not only costly, but they also cause soil disturbances and they are not readily accepted by the general public (Perchet, 2008; Saifullah et al., 2009). Phytoremediation has been highlighted as an alternative technique to traditional methodologies, for the removal of heavy metals from soil. Two approaches have been generally proposed for the phytoremediation of heavy metals. The first one is using of natural hyperaccumulator plants with high metal accumulating capacity. However, the research must be pursued at the level of the increase of the assimilation of heavy metals by the vegetation we taking as example the lead. While the second is utilizing of high-biomass plants whereas, relatively high amounts of the metal accumulated by plants is often translocated from the root to the more easily harvestable shoots (Chen et al., 2004 and Manousaki et al., 2007).

Quantities of lead absorbed by the roots depend on the concentration of metal in the soil, but also of its migration capabilities from the ground to the roots and the amount of lead present in the various part of plants depends on the transport from outside of roots toward the inside, and then its translocation to leaves (Patra et al., 2004). Lead contamination in the plant environment is known to cause highly toxic effects on processes such as depression on seed germination (Wierzbicka and Obidzinska, 1998), toxicity of nucleoli (Liu et al., 1994), inhibition of root and shoot growth (Liu et al., 1994), reduction in photosynthesis (Poskuta and Waclawczyk-Lach, 1995), DNA synthesis (Gabara et al., 1992) and inhibition or activation of enzymatic activities (Van Assche and Cliisters, 1990). Lead not only affects plant growth and productivity but also enters into the food chain causing health hazards to man and animals (Seaward and Richardson, 1990).

Lead was recognized as causing oxidative stress in plants; coriander may have a strong resistance to lead, can have an antioxidant activity and inhibit unwanted oxidant processes (Wangensteen et al., 2004; Melo et al., 2005). But little is known about the effects of lead on the physiological processes and the biochemical changes at short and long term of exposed to lead of coriander seedlings. The objective of the present work is to study the effects of different concentrations of lead on leaf, roots and rods of coriander including growth, physiological and biochemical processes such as the different pigments, soluble proteins, lipid peroxidation and hydrogen peroxide contents; and description of morphological changes. The possible mechanisms of Coriander seedlings tolerance of lead stress are briefly discussed in the present study.

25 **Materials and Methods**

26 *Plant material*

27 Coriander (*Coriandrum sativum* L.) seed were used in our experimentation and surface was sterilized with 0.1%
 28 HgCl₂ for the prevention of fungal and bacterial contamination (Young, 1926). In the first, we have tempered
 29 seeds overnight for accelerate germination. We conducted the experiment in 14 pots clean plastic which has a
 30 depth of 20 cm and a diameter of less than 10 cm perforated to allow the water to drain out. Each pot was filled
 31 with 500 g sandy soil, containing compost from vegetable origin added to the sterilized sand (2V of sand / 1V of
 32 compost), soil sample was characterized by pH = 7.8, EC_e = 2.5 dS/m, CaCO₃ = 3.2 %, organic matter = 0.02
 33 %, Clay = 2 %, Silt = 3 % and Sand = 95 %. We have sowed our seeds (70 seeds) to approximately 2.5 cm in
 34 depth and pots were watered to keep moisture content approximately at 60% of water holding capacity during 40
 35 days with solutions of lead acetate at different concentrations (500,1000,1500 , 2000, 2500, 3000 mg.l⁻¹) and a
 36 witness sprayed with distilled water, with 3 replicates. Our seedling was placed in a greenhouse in Oran 1
 37 University in controlled conditions as it is shown on Table 1. After 40 days of lead exposure, the fresh sample
 38 weights was determined and were kept at -80°C for further analyses.

39 *Chlorophyll and carotenoid determination*

40 Fresh biomass (leaves) was homogenized in 80% icecold acetone in the dark and then centrifuged at 10000g for
 41 10 min at 4°C and the supernatant was used for the immediate determination of pigments. Absorbance of the
 42 solution was determined spectrophotometrically at 663, 645 and 480 nM the contents of chlorophyll a, b, and
 43 carotenoid, respectively; with the following equations help of Arnon's formulae (Arnon, 1949), for
 44 quantification of the total chlorophyll, chlorophyll a and chlorophyll b content in an 80% acetone extract:

45 Total chlorophyll = 20.2 (A₆₄₅) + 8.02 (A₆₆₃)
 46 Chlorophyll a = 12.7 (A₆₆₃) - 2.69 (A₆₄₅)
 47 Chlorophyll b = 22.9 (A₆₄₅) - 4.68 (A₆₆₃)
 48 And Carotenoids= (1000A₄₈₀ - 3.27[chl a] - 104[chl b])/227
 49 Chlorophyll and carotenoid concentrations were expressed as mg g⁻¹fresh weight.

50 *Estimation of lipid peroxidation*

51 The level of peroxidation was measured in terms of malondialdehyde (MDA) (a product of lipid peroxidation)
 52 content determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Frozen
 53 shoot was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 8000g
 54 for 20 min and 4.0 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30
 55 min and then quickly cooled on ice bath. The contents were centrifuged at 8000 g for 20 min and the absorbance
 56 of the supernatant was measured at 532 nM and the value for the non-specific absorption at 600 nM was
 57 subtracted. The concentration of MDA was calculated using coefficient of absorbance of 155 mM⁻¹ cm⁻¹. MDA
 58 content expressed as nM g⁻¹ fresh weight.

59 *Determination of hydrogen peroxide*

60 The H₂O₂ concentration was determined according to Loreto and Velikova (2001). Approximately 0.1g of shoots
 61 was homogenized at 4°C in 2 ml of 0.1% trichloroacetic acid (TCA) (w:v). The homogenate was centrifuged at
 62 12000 g for 15 min at 4°C. Then, 0.5 ml of the supernatant was added to 0.5 ml of 10 mM K-phosphate buffer
 63 (pH 7.0) and 1 ml of 1M KI. The H₂O₂ concentration of the supernatant was evaluated by comparing its
 64 absorbance at 390 nM with a standard calibration curve. Hydrogen peroxide concentration was expressed as
 65 μM.g⁻¹ fresh weight.

66 *Extraction and assays of soluble proteins*

The proteins have been doses according to the method (Bradford, 1976), briefly 1 g of fresh tissue, was crushed in the presence of sand, in 10 ml of medium of extraction with following composition: phosphate buffer (0.1M, pH 7); K_2HPO_4 to 0.1M; Triton x 100 to 0.1 % ; EDTA and centrifuged at 3000 rpm for 10 min, the supernatant (protein extract) is recovered for the assay of protein and reaction was read to 595 nm and express by mg /g MF (fresh material).

Proline assay

400 Mg of plant material is put into a mortar and chopped with 5 ml of ethanol at 95% followed by three flushing and washes with 5ml of ethanol at 70 %. The final solution is collected in a test tube so that it is decanted during 60 min. two phase are distinguished (a higher phase of light green color and a lower phase of dark green color) (Nguyen and Paquin , 1971). 5 mL of the upper phase are collected, to which are added 2 ml of chloroform and 3 ml of distilled water. After agitation, the solution is maintained at rest for 24 hours in the cold for a good separation. After assay the optical density is read using a colorimeter, the wavelength is 515 nm.

Data analysis

All data were analyzed in three replications and the obtained data were evaluated statistically using Student's test, and least significant difference (LSD) was calculated at $p < 0.05$.

Results

Growth and fresh weight

The results after exposure of seeds to different concentration of lead (500, 1000, 1500, 2000, 2500, 3000 $mg.l^{-1}$) for 20 days, a significant reduction in leaves numbers, by 40%, 58%, 70 ; 74%, 84% and 92% respectively, was obtained compared to control, and the weight of total sheets, rods and roots decrease significantly after poisoned in all groups compared to control by 90%, 87%, 81%, respectively. After 40th days of exposure to different concentration of lead, show a net increase in weight of rods and sheets compared to the control. The numbers of sheets was significantly decreases with increasing the concentration from 500 to 3000, respectively compared to control (Table. 2 and 3). After testing the contaminated soil and the rest of the lead solution used to perform analysis

Chlorophyll and carotenoid

After 20th days, our results shown a significant decrease ($p < 0.05$) in the rate of chlorophyll (a) in all groups exposed to lead (from 500 $mg.l^{-1}$ to 3000 $mg.l^{-1}$) compared to control group by 10.41 %, 24.94 %, 43.27 %, 47.50 % ,65.5% and 76.46 %. Chlorophyll (b) have significantly lower levels compared to control groups by 23.95 %, 55.98 %, 51.39%, 70.89%, 72.56% and 84.95%, respectively. Increased lead exposure causes a significant reduction in carotene levels compared to control from 49.08 % to 94.56 % respectively (Table. 4).

After 40th days, the rate of carotenoid and total chlorophyll decreased significantly at the dose of 2500 $mg.l^{-1}$ and 3000 $mg.l^{-1}$ compared to the control (-27%, -41% and -24%, -34%, respectively) and no difference was noted in the other concentrations.

Lipid peroxidation and hydrogen peroxide

At 20th days of exposed to increased levels of lead (500, 1000, 1500, 2000, 2500 and 3000 $mg.l^{-1}$), we noted a significant increase level of hydrogen peroxide in sheets compared to control. In rods, the level of hydrogen peroxide was higher by 2 to 4 times in all groups exposed to lead compared to control. According our results (fig. 1), we noted that the hydrogen peroxide content was very lower in roots of control, however in contrast the level of hydrogen peroxide was 60 times more important ($p < 0.05$) in stems at 3000 $mg.l^{-1}$ compared to control. After 40 days, the level of hydrogen peroxide is significantly higher (400 times) in the sheets of plant exposed to 3000 $mg.l^{-1}$ of lead compared to control.

Fig. 1, shows a significantly increased content of lipid peroxidation by 1.5 to 2.5 compared to control in sheets after 20 day of plant exposed to 2000 mg.l⁻¹ to 3000 mg.l⁻¹, in rods the level of lipids peroxidation was increased from 1.8 to 5 times in all groups exposed to different concentration of lead compared to control. It has been noted that the rate of TBARS increases each time that the concentration in lead increases, however in roots, after 20 day, we noted that the level of lipids peroxidation was increased by 87.5%, 150%, 250 %, 275% and 300% respectively to a concentration in lead of 1000mg/l, 1500 mg/l, 2000 mg/l, 2500 mg/l and 3000mg/l. after 40 days of exposed to lead, we obtain that the rods and roots indicate a significant increased of level of lipids paroxidation in all groups by 1.2 and 2.7 times compared to control; in sheets, we noted the rate of lipid peroxidation decreases significantly with increasing the rate of lead in the ground, from 1.3 to 3.8 times less.

Soluble protein and Proline levels

The results obtained in Table 5 indicated that the level of soluble protein in leaves was significantly increased by 35% and 60% in groups receive 2000 mg.l⁻¹ and 3000 mg.l⁻¹ respectively compared to control, at 500, 1000, 1500 mg/l dose respectively, we noted a significant decrease 12.5% to 50 %. In rods, the rate of soluble protein was 14.6 and 13.6 times more important in plant receive 2500 mg.l⁻¹ and 3000 mg.l⁻¹ compared to control; in roots, soluble protein was 2 to 4 times more increased in groups receive 1000, 1500, 2000, 2500, 3000 mg.l⁻¹ compared to control. In the Table 6, the soluble protein was significantly higher ($p<0.05$) from 28% to 63% in sheets, rods and roots after irrigation of plant with water contain 3000 mg.l⁻¹ of lead.

At level of leaves, rods and roots (Table. 5 and 6), it was observed that the level of proline increase gradually with increase of lead concentration from 10 to 16 times more compared to control. After 40 days of exposition to lead at different concentration, level of proline was significantly higher from 2 to 8 times more than control, in sheets, rods and roots.

Discussion

Lead is an element not very mobile and has a very high persistence in soil (Saddler and Berthelin, 1998). The plants have many systems of detoxification to limit interaction of these ions with biological molecules; lead induce a range of deleterious effects for the agencies, he disrupts the membrane structures and parietal, modified the statute of waterborne, disrupted the absorption and/or the translocation of essential minerals elements (calcium, manganese, zinc, iron etc....) or further reduced photosynthesis (Seregin and Ivanov, 2001; Sharma and Dubey, 2005). Depending to environment conditions, plants can absorb a part of the lead present in the soil and Pb²⁺ ions broadcast in the root, but are blocked by the physical barrier like endoderme. At the macroscopic scale, the plants exposure to lead induces a reduction in growth of biomass, yields and when stress is too severe, led to the emergence of resprouts necrosis and foliar, chlorotic, or even to the death of the plant (Sharma and Dubey, 2005). It also presents a high affinity for proteins which possess thiol groups or of metal cofactors (metallo-enzymes). However, despite the toxicity certain of these two types of interactions, it is not enough to explain the large variety of deleterious effects observed in plants treated with lead and particularly the genotoxicity induced by this metal (pourrut et al., 2008).

In our results indicate that the treatment with lead induces an increase in production of H₂O₂ and O₂•⁻, we can explain this by fact that it is possible to estimate indirectly the level of ERO production and the generation of oxidative stress, by measuring the activity of antioxidant enzymes, or the rate of lipid peroxidation, which are biomarkers of oxidative stress (Sharma and Dubey, 2005; Chen et al., 2007; Wang and al., 2007). An increase in lipid peroxidation content in coriander grown under Pb stress was observed (Fig. 1). It is possible that increase of MDA concentration in Coriander may be due to a increase in polyunsaturated fatty acid concentration relative to saturated fatty acids, which has also been reported in some plants under stressful conditions (Rucinska and Gwozdz, 2005; Grappa et al., 2007).

The increased of H₂O₂ may be essentially due to reduction of catalase activity, the case of this enzyme is complex, since half of publications reported an inhibition of activity by lead (Verma and Dubey, 2003; Choudhury and Panda, 2004; Seregin and al., 2004; Chen and al., 2007; Dey and al., 2007; Hu and al., 2007; Qureshi and al., 2007; Gopal and Rizvi, 2008), the other half shows an increase of his activity (Reddy and al., 2005; Mishra and al., 2006). This inhibition seems to be due to the species treated, to duration or to intensity of the treatment. The inhibition of catalase activity has been observed during different stress, for all the organisms studied, this inhibition is not a general rule; the origin of this phenomenon is still widely debated. In addition, when the CAT is inhibited, this mechanism is dose-dependent and the catalase is a metallo-enzyme, its inhibition could be due to a direct interaction with the lead (Landberg and Greger, 2002).

Some molecules non-loaded are also able to spread through the plant walls and can thus cause of multiple damage as oxidation of DNA (Britt, 1997), or even the oxidation of proteins including at the level of the cysteines and methionines (Rinalducci et al. 2008; Bartoli et al., 2004), these are the two amino acids that are most sensitive and since they are quite often involved in the fixing of metals or in the catalytic properties of many enzymes and proteins; reactive oxygen species inactivate enzymes and damage important cellular components. ROS are responsible for protein, lipid and nucleic acid modification and are thought to play a major role in ageing and cell death (Jacobson et al., 1996).

The lead exposure leads to a strong inhibition of photosynthesis, the photosynthetic yield, and to a limitation of the rate of assimilation of CO₂. This inhibition is explained by the decrease of the levels of chlorophylls and carotenoids generally found (Kosobrukhov and al., 2004; Gopal and Rizvi, 2008). The chlorophyll b seems more sensitive than chlorophyll a (Kacabova and Natr, 1986 ; Wozny and al., 1995; Vodnik and al., 1999). Singlet oxygen is the first excited electronic state of O₂. Insufficient energy dissipation during photosynthesis can lead to formation of chlorophyll (Chl) triplet state. And the Chl triplet state can react with 3O₂ to give up very reactive singlet oxygen. It has been proved that singlet oxygen formation during photosynthesis can have damaging effect on photosystem I (PSI) and a photosystem II (PSII) and on whole machinery of photosynthesis.

Lead interacts at this level in two ways:

- In a direct manner, by substituting divalent ions related to the metallo-enzymes. This is particularly the case with the δ -aminolevulinate deshydratase (ALAD) which is at the basis of the synthesis of the chlorophylls and whose ion Zn²⁺ is replaced by Pb²⁺;

- In an indirect way, by inducing a deficiency in these divalent ions.

Proline, α -amino acid is an antioxidant and potential inhibitor of programmed cell death and is considered as an indicator of stress; in several plant, the accumulation of proline has been observed as a response to biotic and abiotic stress ((Boguszewska et al. 2010; Torres 2010; Khatamipour and al., 2011). It has been suggested that free proline act as osmoprotectant, a protein stabilizer, a metal chelator, an inhibitor of lipid peroxidation and OH• and ¹O₂ scavenger. Increased proline accumulation appears especially during salt, drought and metal stresses (Trovato et al., 2008). Therefore proline is not only an important signaling molecule, but also an effective ROS quencher. It has been found that the important role of proline is in potentiating pentose-phosphatase pathway activity as important component of antioxidative defense mechanism (Hare & Cress 1997).

Conclusion

Thus, the finding that *Coriandrum sativum* L. shows that it is good plant material for studying other aspects of abiotic stress resistance mechanisms. Based on the present work, it can be suggested that toxic concentrations of lead cause oxidative stress, as evidenced by increased H₂O₂ formation, lipid peroxidation and proline content in coriander plant. In this study, a significant change in different parameters such as growth of shoot and roots, chlorophyll and carotenoid concentrations coupled with lipid peroxidation, protein oxidation and hydrogen peroxide indicated that high Pb levels in nutrient solution produced toxic effects. It was proposed that the reduced growth in Pb of coriander exposed to toxic levels of Pb might be induced by an enhanced production of toxic oxygen species and subsequent lipid peroxidation. Moreover, it was possible to observe that Pb-tolerant plants developed some defense mechanisms against oxidative stress.

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Table 1: General condition of culture in the greenhouse.

Parameters	Brightness L	Temperature °C	Air W/M2	SUNSHINE W/m2	Wind km/h	Humidity %	Side
Day 1	10000	23.9	474	468	0	100	Right
Day 2	9915	15.6	50	51	K/h	100	Left
Day 3	9911	15	50	48	K/h	100	Left
Day 4	9663	15	49	46	K/h	100	Left
Day 5	9410	14.8	50	44	K/h	100	Left
Day 6	9174	14.6	50	43	K/h	100	Left
Day 7	10000	24	474	466	0	100	Right
Day 8	10000	24.9	475	470	0	100	Right
Day 9	8914	14.7	50	43	K/h	100	Left
Day 10	8876	14.3	47	41	K/h	100	Left

Table 2: Plant morphology modification after 20 days to lead-exposed at different level

Concentration mg/l	Length (cm)	Number of Sheets	Weight (g)		
			Sheets	Rods	Roots
Control	11.5	113	1.641	1.315	0.563
500	7.04	67	0.458	1.115	0.347
1000	4.02	47	0.337	0.675	0.331

1500	3.07	33	0.284	0.259	0.102
2000	2.6	29	0.155	0.164	0.84
2500	1.5	17	0.122	0.077	0.75
3000	1.01	9	0.117	0.36	0.34

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Table 3: Plant morphology modification after 40 days to lead-exposed at different level

Concentration Mg/l	Length Cm	Number of sheets			Weight (g)		
		Green	Clear Green	Yellow	Sheets	Rods	Roots
0	23	595	0	0	17.961	5.726	2.828
500	19	567	158	0	15.460	5.222	3.443
1000	17.5	487	182	28	15.470	5.594	4.671
1500	18	334	298	131	17.635	5.377	3.847
2000	18.5	266	184	141	18.814	5.433	4.635
2500	19	158	398	157	19.323	5.245	4.213
3000	17.8	165	390	149	17.524	5.317	5.423

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Table 4: Effect of lead on different pigments in *Coriandrum sativum* L.

	20th Day				40th Day			
	Chlorophyll mg.g ⁻¹ Fresh tissue weight				Chlorophyll mg.g ⁻¹ Fresh tissue weight			
	a	b	Total	Total Carotenoid	a	b	Total	Total Carotenoid
Control	9,22±1.12	7,18±1.22	16,4±2.55	5,99±2.85	7,47±0.99	3,6±0.44	11,07±1.13	2,95±0.75
500	8,26±0.73	5,46±2.43	13,72±2.10	3,05±1.25	7,23±1.57	3,4±0.97	10,63±1.55	2,71±0.61
1000	6,92±1.43	3,16±0.91	10,08±1.98*	1,73±1.97	7,13±1.62	3,23±0.34	10,36±1.92	2,69±0.54
1500	5,23±1.29*	3,49±1.09*	8,72±1.21*	1,73±2.44	6,81±2.55	3,16±1.07	9,96±2.40	2,6±0.16
2000	4,84±0.81*	2,09±1.46*	6,93±2.45*	1,35±1.11*	6,26±2.09	2,93±0.81	9,19±1.41	2,42±0.45
2500	3,18±1.32*	1,97±1.66*	5,15±1.68*	0,48±0.22*	5,7±1.84	2,63±0.71	8,33±1.34	2,13±0.72
3000	2,17±0.75*	1,08±0.99*	3,25±2.59*	0,32±0.14*	5,01±1.24*	2,21±0.42*	7,23±1.75*	1,73±0.64

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(*) the average of three replication are significantly different compared to control at p<0.05.

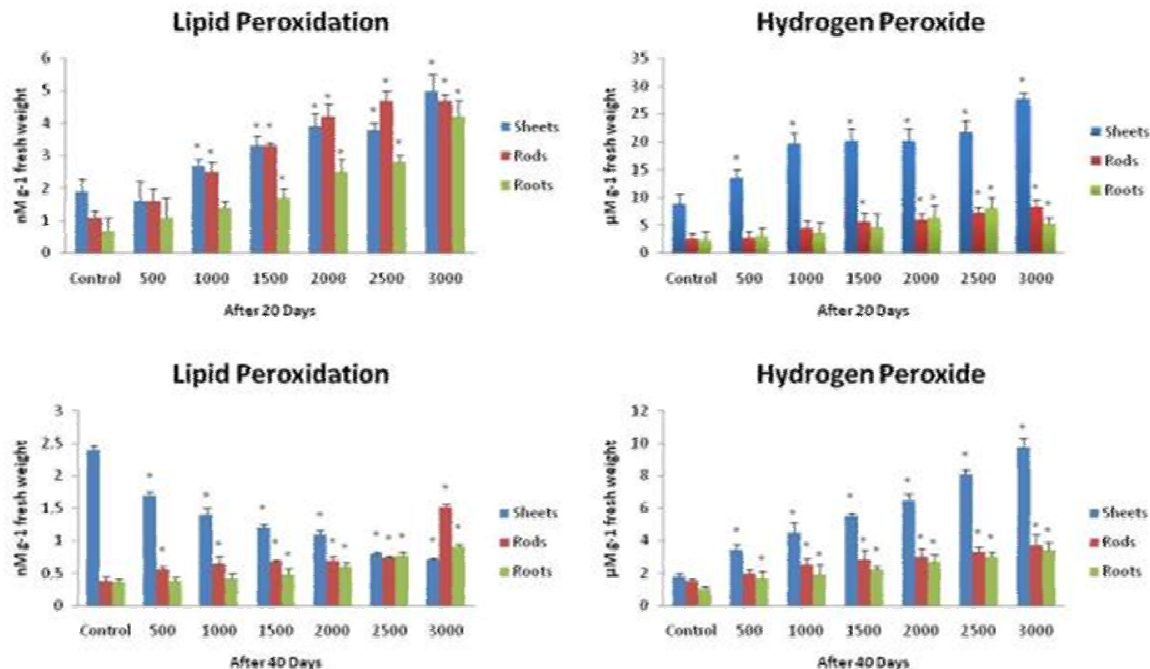


Figure 1 : Effect of different lead level on lipid peroxidation and hydrogen peroxide of *Coriandrum sativum* L. ((*) the average of three replication are significantly different compared to control at p<0.05.)

Table 5: Effect of lead on soluble proteins (μg/g fresh tissue weight) and proline (mM.g⁻¹) in *Coriandrum sativum* L. after 20 days

	Sheets		Rods		Roots	
	Soluble Protein	Proline	Soluble Protein	Proline	Soluble Protein	Proline
Control	114,8±6.4	1,78±0.45	8,4±1.14	1,11±0.61	103,6±10.2	0,56±0.42
500	58,8±4.8*	2,28±0.65	16,8±1.01*	2,94±0.35*	98,0±9.5	1,33±0.54
1000	67,2±5.7*	3,28±0.49*	47,6±3.21*	3,83±0.73*	224,0±20.2*	1,94±0.96
1500	98,0±4.9*	4,67±0.46*	22,4±2.22*	4,94±1.04*	302,4±34.3*	4,72±1.40*
2000	151,2±15.5*	6,33±1.07*	25,2±2.71*	5,89±0.89*	355,6±40.1*	9,67±2.20*
2500	112,0±16.4	10,94±2.22*	123,2±15.51*	6,94±1.55*	291,2±35.5*	12,56±2.75*
3000	179,2±25.5*	12,78±3.33*	114,8±13.43*	9,00±2.22*	431,3±55.5*	16,89±4.61*

((*) the average of three replication are significantly different compared to control at p<0.05.

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Table 6: Effect of lead on soluble proteins ($\mu\text{g/g}$ fresh tissue weight) and proline (mM.g^{-1}) in *Coriandrum sativum* L. after 40 days

	Sheets		Rods		Roots	
	Soluble Protein	Proline	Soluble Protein		Soluble Protein	Proline
Control	30,8 \pm 4.5	0,009 \pm 0.001	28,0 \pm 2.22	1,01 \pm 0.29	19,6 \pm 3.12	1,1 \pm 0.44
500	61,6 \pm 6.6*	0,014 \pm 0.004	22,4 \pm 1.75*	1,72 \pm 0.42	19,6 \pm 4.42	2,3 \pm 0.82
1000	58,8 \pm 3.7*	0,019 \pm 0.003*	14,0 \pm 0.95*	1,90 \pm 0.72	14,0 \pm 2.71	2,7 \pm 0.42*
1500	50,4 \pm 3.1*	0,021 \pm 0.007*	8,4 \pm 1.40*	3,51 \pm 1.21*	14,0 \pm 3.65	3,0 \pm 0.75*
2000	75,4 \pm 7.4*	0,021 \pm 0.005*	11,2 \pm 0.75*	4,85 \pm 1.53*	11,2 \pm 1.71*	4,0 \pm 1.09*
2500	58,8 \pm 5.2*	0,022 \pm 0.006*	25,2 \pm 2.33	6,50 \pm 1.95*	19,6 \pm 3.10	5,3 \pm 1.17*
3000	81,2 \pm 8.8*	0,027 \pm 0.004*	30,8 \pm 4.41	8,31 \pm 2.12*	25,2 \pm 2.73	7,4 \pm 1.54*

344 (*) the average of three replication are significantly different compared to control at $p < 0.05$.

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