Zagazig J. Agric. Res., Vol. 36 No. (6) 2009 1233-1257

EFFECT OF FOLIAR APPLICATION WITH SOME HEAVY METALS ON WASHINGTON NAVE ORANGE TRANSPLANTS

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Accepted 6/12 /2009

ABSTRACT: A pot experiment was carried out during the two successive seasons of 2005 and 2006 to study the effect of some heavy metals, i.e., lead (Pb), cadmium (Cd) at 200,400,800 and 1200 ppm of each and nickel (Ni) at 50,100,200 and 400 ppm as foliar sprays beside control treatment (without heavy metals), on growth, leaf pigments content as well as some physiological activities and anatomical structure of Washington navel orange transplants onto sour orange rootstock.

The tested treatments of such heavy metals tended to reduce leaf water content, leaf fresh and dry weights, stomatal density, leaf pigments content, thickness of leaf palisade tissue, thickness of stem epidermis and cortex tissues, diameter of stem vascular cylinder, thickness of root epidermis, thickness of root cortex and diameter of the root vascular cylinder. The depressing effect was more pronounced with the higher concentrations of any heavy metal.

On the contrary, the tested heavy metals clearly promoted transpiration rate, concentration of the heavy metal in leaf tissues, leaf proline content, peroxidase and catalase enzyme activity, thickness of leaf midrib tissues, diameter of leaf midrib xylem vessel (mainly with Ni and Pb at 200 ppm) and stem total diameter (especially with higher concentrations of Pb and Ni) as well as pith tissue of stem.

Key words: Navel orange, heavy metals, foliar application, proline, enzymes activity, leaf pigments and anatomical structure.

INTRODUCTION

The presence of heavy metals, such as lead (Pb), cadmium (Cd) and nickel (Ni), in higher plants tissues especially for trees was reported in many investigations. The source of heavy metals correlated mainly with the environmental pollution (Marschner, 1997). Accumulation of Pb, Cu, Zn and Cd was mainly due to aerosols fallings from the atmosphere containing such metals. To some extent, these metals go into the soil and penetrate via the root system into the plants (Angelova *et al.*,1999). Nowak *et al.*(1999) mentioned that in about 90 % of vegetable and fruit samples grown near roads, heavy metals contents were too high, especially Cd and Pb.

The higher concentration of heavy metals in the growing medium were found to reduce leaf water content, leaf fresh and dry weights, respiration rate, stomatal opening and density as well as the thickness of different stem tissues (Bazzas *et al.*, 1974; Poschenrieder *et al.*, 1989; Ibrahim *et al.*, 1994; Cieslinski *et al.*, 1998; Zhang *et al.*, 2001; Veselov *et al.*, 2003; Yang *et al.*, 2004; Radwan, 2007). On the other hand, Sayed (1999) on sunflower plants and Radwan (2007) on mango seedlings found that proline and enzymes activity were increased as leaf Pb and Cd contents were increased.

Therefore, the present investigation was outlined to study the effect of foliar spray of Washington naval orange with Pb, Cd and Ni solutions at different concentrations on growth, leaf water content, stomatal density, leaf pigments, transpiration rate, leaf Pb, Cd and Ni contents as well as peroxidase and catalase activity. In addition, the anatomical features of leaf, stem and root organs were taken into consideration.

MATERIALS AND METHODS

This study was carried out in the nursery of Fac. Agric., Zagazig Univ. in 2005 and 2006, seasons. Healthy two years old orange transplants of Washington navel orange grafted on sour orange root-stock were used. The transplants were planted in 20 liters plastic pots in size equipped with bottom holes (for drainage) and filled with washed sandy soil. The contents of Pb, Cd and Ni in the experimental soil were 0.015, 0.021 and 0.090 ppm, respectively. While tap water used contained 0.045, 0.052 and 0.068 ppm for Pb, Cd and Ni, respectively. The transplants were irrigated twice/ week, with Hoagland solution modified by Johanson *et al.* (1957) Schedule 1. and the soil water content was kept within the range of field capacity. Afterwards, the experimental transplants were kept in a lath house for 1.5 month before the onset of foliar spray treatments.

This experiment included 13 treatments comprised the following heavy metals and concentration: Pb at 200, 400, 800 and 1200 ppm; Cd at 200, 400, 800, 1200 ppm and Ni at 50, 100, 200 and 400 ppm beside control treatment (without heavy metals). The transplants were sprayed three times (April, June and August). This is true in both studied seasons.

Transplants were irrigated with tap water to represent the field capacity of the sandy soil twice/week during the first three months (Feb., March and April) and three times/week during the period extended from May until the end of October. A supplemented mineral nutrition was added to all treatments with irrigation water (once/ week) using modified Hoagland solution.

To evaluate the tested treatments, samples were taken from the experimental transplants on late September; i.e., 180 days after the onset of the considered treatments.

Schedule 1. Composition of modified Hogland solution nutrients

Compound*	Molecular weight	Concentration of stock solution	Volume of solution ml/ lot final solution	Element
KNO_3	101.00	1.00 M	6.0	K,N
$(CaNO_3)2.4H_20$	235.16	1.00 M	4.0	Ca, N
$(NH_4)_2H_2PO_4$	115.08	1.00 M	2.0	P
$MgSO_4.7\ H_2O$	246.49	1.00 M	1.0	Mg, S
H_3BO_3	61.84	25.00 mM	--	B
$MnSO_4.H_2O$	169.01	2.00 mM		Mn
$ZnSO_4.7H_2O$	287.55	2.00 mM	1.0	Zn
$Cu\ SO_4.5H_2O$	249.71	0.50 mM	--	Cu
$H_2MoO_4(85\%MoO_3)$	161.79	0.50 mM	--	Mo
Ferric citrate solution composed of 10g citric acid.	---	---	1.0	Fe

***A combined stock solution was prepared containing all micronutrients, except (Fe^{++}).**

The following parameters were used to determine the effect of tested treatments:

Leaf characteristics

In each season, a sample of 25 mature leaves of spring growth cycle (about six months old) was collected from the medium position of the shoots and taken immediately to the laboratory to determine: leaf fresh weight (gm), leaf dry weight (gm) and leaf water content (%).

Stomatal density

Frequency of stomata/ mm^2 on the lower surface of mature leaf were counted. Leaves of the same age and located on the third node from the shoot base were used. The Xantopren method, described by Stino *et al.* (1974) was used for determination of number of stomata / 1 mm^2 of the lower blade surface.

Transpiration rate

On the middle of July, Aug. and Sept., of each season, transpiration rate (gm H_2O/ Dec^2/hr.) was determined by the Phytometer method described by El- Sharkawi (1969) and Mohsen (1975).

Leaf Pb, Cd and Ni contents

They were determined by the Atomic Absorbed Thermo Jarral ash AA- Scani methods (Paker, 1974).

Leaf proline content

It was determined in fresh leaves according to the method described by Bates *et al.* (1973).

Peroxidase enzyme activity

It was carried out following the method described by Purr (1950).

Catalase enzyme activity

It was carried out according to the method described by Feinstein (1949).

Leaf photosynthetic pigments

Leaf samples were taken to determine chlorophyll-a, chlorophyll-b, total chlorophylls (a+b) content, as well as carotenoides. The method described by Wettestein (1957) was adopted.

Histological traits

Leaf: Samples were collected from the mature leaves in Sept., using three plants at random from each treatment. Samples were washed then dried with plotting paper and, then immediately billed and fixed in FAA. Afterwards samples were dehydrated with normal butyl alcohol and paraffin wax (m.p. 58-60C°) for infiltration and embedding (Johansen, 1940). Transfer section of 15 micron was cut using a rotory microtone. Saffranin (0) and fast green (FCF) technique was used in staining, then washed in absolute ethanol and cleared in xylol and mounted in Canada balsam (Johansen, 1940). Slides were oven dried at 30 °C for one week then prepared to scrutinization.

Main stem and roots: Five roots and shoots of half cm long and about 1 cm in diameter were prepared directly for sectioning. The samples were immediately cut by a sliding microtome with a thickness of about 20 micron (M). The sections were placed in 50 % ethanol having few drops of saffranin and left overnight. Dehydration was followed up to absolute ethanol. The sections were stained by fast green (FCF), then washed in absolute ethanol and cleared in xylol and mounted in canada bealsam (Franklin, 1945). Five replicated sections were microscopically examined for each of the roots and shoots.

The complete randomized block design was followed throughout the whole work. Four concentrations were investigated for each heavy metal beside control, each concentration was represented by three replicates and each replicate comprised of three pots (Snedecor and Cochran, 1968). The data were subjected to analysis of variance and the new LSD was used to compare between means (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

Leaf Fresh and Dry Weights

From Table 1 it is clear that, the leaf fresh weight was, generally, higher with the lower heavy metals concentration than with the higher ones. This was particularly obvious with Ni, as control leaf was 3.91 and 4.92 gm in the two seasons against only 3.00 and 3.93 gm, respectively with the uppermost concentration (400 ppm). A nearly similar trend was observed with leaf dry weight.

Leaf Water Content

The data Table, 1 also show that leaf water content was, in most cases, higher in control leaves then decreased gradually to reach minimal values with the higher concentration of heavy minerals. As such, the leaf water content was 40.63 and 39.65% in control leaves, while fell to 40.38 and 33.51 % with Pb at 1200 ppm, 36.58 and 30.82% with Cd at 1200 ppm and 30.42 and 26.75 % with Ni at 400 ppm. It seems that Ni severely depressed leaf water content compared with the other tested heavy metals (Pb and Cd).

Many investigators, working on different plants, reported the depressive effect of heavy metals on fresh and dry weight as well as

Table 1. Effect of some lead (Pb), cadmium (Cd) and nickel (Ni) treatments on leaf fresh weight, leaf dry weight, leaf water content, stomatal density and transpiration rate of Washington navel orange transplants (2005 and 2006 seasons)

Concentration		Leaf fresh weight (g/ leaf)		Leaf dry weight (gm/leaf)		Leaf water content (%)		Stomatal density (/mm^2)	Transpiration rate (gm H_2O Dec.^{2}/hr.)
Metal	ppm	2005	2006	2005	2006	2005	2006	Av. Two seasons	Av. Two seasons
	0.0 Control	3.91	4.92	2.40	3.41	40.63	39.65	240.33	0.22
Pb	200	4.11	5.21	2.38	3.48	42.10	39.14	238.33	0.81
	400	4.01	5.11	2.11	3.11	40.31	37.60	120.66	0.88
	800	3.85	4.96	2.09	3.09	40.64	33.17	127.66	1.06
	1200	3.42	5.42	2.03	2.00	40.38	33.51	158.33	1.10
Cd	200	4.00	5.01	2.37	3.47	40.76	34.10	222.66	0.22
	400	3.82	4.93	2.40	3.41	37.19	32.40	225.33	0.38
	800	3.61	4.72	2.10	3.11	37.85	30.73	204.66	0.52
	1200	3.21	4.32	2.03	2.92	36.58	30.82	230.66	0.82
Ni	50	3.62	4.73	2.35	3.45	37.05	32.56	214.66	0.20
	100	3.12	4.23	2.90	3.11	35.10	27.05	238.00	0.18
	200	3.01	4.11	2.04	3.01	32.21	26.39	230.00	0.28
	400	3.00	3.93	2.03	2.65	30.42	26.75	212.33	0.30
New L.S.D. 0.05		0.01	0.10	0.03	0.07	0.85	1.26	21.42	0.11

the water content of leaves and the whole biomass (Paivake, 1983; Veresolgou *et al.*, 1987; Salim *et al.*, 1988; Azpiazu, 1989; Khristodorov *et al.*, 1989; Abdel-Aal and Abdel-Nasser, 1995; Begonia *et al.*, 1998; Michalska *et al.*, 1998; Cieslinski *et al.*, 1998; Zhang *et al.*, 2001; Radwan, 2007).

Leaf Stomatal Density

As shown in Table 1, the highermost stomatal density was recorded by the control (240.33 stomata /mm^2).The concentrations of 200ppm Pb, 200 and 400 ppm Cd and 100 and 200 ppm Ni were statistically equal to control in this respect. However, the least stomatal density values were recorded by Pb at 400 and 800 ppm (120.66 and 127.66 stomata /mm^2) Cd at 800 and1200 ppm (204.66, 230.66 stomata /mm^2, respectively) and by Ni at 400 ppm (212.33 stomata /mm^2).

Available literature concerning the effect of heavy metals on leaf stomatal density are scarce.

Transpiration Rate

Table 1 also demonstrates that leaf transpiration rate was gradually increased as the concentration of the heavy metal was increased. As such, control leaves recorded only (0.22 gm H_2O/$Dec.^2$./hr.). A gradual promotion of transpiration rate was observed with the higher levels of tested heavy metals 1.1, 0.82 and 0.30 gm H_2O/$Dec.^2$/hr for Pb 1200 ppm, Cd 1200 ppm and Ni 400 ppm, respectively. It seems that Ni was of insignificant effect in promotion of transpiration as compared with Pb or Cd metals.

Bazzaz *et al.* (1974) reported that relatively low concentration of Pb, Cd and Ni inhibited photosynthesis and transpiration of detached sunflower leaves.

Leaf Pb, Cd and Ni Contents

From Table 2, a gradual increase in leaf Pb, Cd and Ni content was observed as the tested concentrations of sprayed solutions were increased. The transplants sprayed with 0.0 Pb indicated leaf Pb content of only 2.7 ppm in the average two seasons with the control followed by a gradual promotion with increasing sprayed concentration to reach 79.3 ppm in the average two seasons with sprayed solution of 1200 ppm. The same trend was clear with Cd with values of 1.1 ppm (control) and 54.9 ppm in the average two seasons with spray solution of 1200 ppm.

Table 2. Effect of some lead (Pb), cadmium (Cd) and nickel (Ni) treatments on Pb, Cd and Ni contents, proline amino acid, peroxidase and catalase enzymes in leaf of Washington navel orange transplants (2005 and 2006 seasons)

Concentration		Pb (ppm)	Cd (ppm)	Ni (ppm)	Proline contnet (M. mole /gm FW)		Peroxidase enzyme (M. mole H_2O_2/gm FW)		Catalase enzyme (M. mole H_2O_2/gm FW)	
Metal	**ppm**	**Av. Two seasons**			**2005**	**2006**	**2005**	**2006**	**2005**	**2006**
	0.0 Control	2.7	1.1	1.5	1.13	0.97	9.25	8.89	14.60	12.73
Pb	**200**	14.1	11.3	6.6	1.15	1.02	10.65	9.49	15.34	12.93
	400	29.4	24.3	8.8	1.45	1.15	11.33	10.11	15.30	13.14
	800	42.9	27.3	12.3	1.63	1.35	11.81	10.55	15.70	13.35
	1200	79.3	33.1	16.1	1.80	1.56	11.92	10.87	16.22	14.38
Cd	**200**	4.5	9.8	3.7	1.24	1.08	10.23	9.57	13.51	11.22
	400	17.2	26.3	9.1	1.42	1.18	10.98	10.22	14.17	12.21
	800	24.2	45.9	14.1	1.61	1.52	11.45	10.69	14.68	12.80
	1200	30.1	54.9	26.1	1.70	1.65	12.02	11.01	15.12	13.62
Ni	**50**	11.1	5.3	25.0	1.33	1.18	10.42	9.12	15.27	14.98
	100	13.0	11.6	41.0	1.39	1.24	11.03	10.63	15.17	13.22
	200	14.2	21.5	51.5	1.47	1.32	11.53	11.02	15.82	13.74
	400	15.1	27.7	54.0	1.50	1.41	11.98	11.48	16.20	14.25
New L.S.D. 0.05		2.15	2.14	2.01	0.10	0.11	0.12	0.13	0.10	0.10

The same trend was noticed with Ni; the control leaf Ni content was only 1.5 ppm in the average two seasons, while amounted gradually to reach 54 ppm with Ni spray at 400 ppm.

The results were logic, and expected and reflected the effect of increasing concentration of the heavy metal on its level in leaf and merely made a positive relation.

Many literature reports declared that increasing heavy metals in environment surrounding the plant (in soil, irrigation water or air) caused considerable promotion in the content of these metals in different plant organs (leaf, stem, root or fruit) (Abdel - Aal and Abdel Nasser,1995; Zray *et al*., 1995;Canet *et al*., 1997; Angelova *et al*., 1999; Nowak *et al*., 1999).

Leaf Proline Content

As shown in Table 2 leaf proline content was significantly increased with increasing the concentration of all used metals compared with control. That was true in both seasons of study. Control leaves contained only 1.13, 0.97 M. mole proline/g FW of the leaf in the two seasons. However, the values were gradually increased to reach 1.80 and 1.56, 1.70 and 1.65, 1.5 and 1.41 M. mole proline/gm FW in the two seasons with the uppermost tested concentration of the concerned heavy metals; i.e., 1200 ppm Pb, 1200 ppm Cd and 400 ppm Ni, respectively.

The amino acid proline usually increased in cases, when the plant suffers from a stress. The toxicity of tested heavy metals could be considered as a stress factor. Literature reports in this respect are not available.

Leaf Peroxidase Enzyme Activity

Table 2 also indicated significant increment in peroxidase activity with the rise in concentration of any considered heavy metal. This was obvious in the two experimental seasons. Thus, control leaves recorded only 9.25 and 8.89 M. mole H_2O_2/ gm FW in the two seasons, while gradually amounted to reach 11.92 and 10.87 M. mole H_2O_2/ gm FW with Pb 1200 ppm, 12.02and 11.01 M. mole H_2O_2 gm FW with Cd 1200 ppm and 11.98 and 11.48 M.mole H_2O_2/ gm FW with Ni 400 ppm, in the first and second seasons, respectively.

El-Mosallamy *et al*. (2007) on lupine and Radwan (2007) on mango seedlings reported that peroxidase activity was, generally, increased in leaf tissues by application of Pb and Cd without significant differences among concentrations.

Leaf Catalase Enzyme Activity

Table 2 also shows catalase activity in leaves treated with the concerned heavy metals. A nearly similar trend was observed as noticed with peroxidase enzyme. The least values were recorded by the control (14.60 and 12.73 M. mole H_2O_2/ gm FW in the two seasons). However, with Cd the least values came from the concentrations of 200 ppm (13.51and 11.22 M. mole H_2O_2/ gm FW in the two seasons. Anyhow, the uppermost values were, in most cases, recorded by the high concentration, i.e. 16.22 and 14.38 M.mole H_2O_2/ gm FW with Pb 1200 ppm,15.12and 13.62 M. mole H_2O_2/ gm FW with Cd 1200 ppm and 16.20 and 14.25 M. mole H_2O_2/ gm FW with Ni 400 ppm, in the first and second seasons, respectively.

In this concern, El-Mosallamy *et al*. (2007) on lupine plants found that catalase and peroxidase activities were significantly increased up to 1% sewage sludge, while they were significantly decreased with sewage sludge application at 2 %. Radwan (2007) on mango seedlings reported that catalase activity in leaves was less affected by Pb or Cd concentrations as compared with peroxides enzyme.

On the other hand, Wahdan (2001) found that phosphorylase and peroxidase activities in *Brassica oleracea* var. Capitata L. were reduced by 38 and 27 %, respectively, after 5 weeks of treatment with 1.44 uM Pb.

Leaf Photosynthetic Pigments

Table 3 illustrates the effect of tested heavy metals treatments on chl.a, chl.b, total chls. (a+b) and carotenoides in the two experimental seasons.

All considered leaf pigments indicated the same trend in response to the tested heavy metals. The general direction was: uppermost values linked with the control with gradual reduction as the concentration of any tested metal were increased. For example, the control recorded 1.25and 1.14 mg/gm FW for chl.a in the two seasons, i.e., with 1200 ppm Pb the values decreased to 0.52 and 0.67 mg/gm FW in the two seasons. The corresponding values with Cd at 1200 ppm were

Table 3. Effect of some lead (Pb), cadmium (Cd) and nickel (Ni) treatments on leaf pigments contents (mg/gm FW) in transplants of Washington navel orange (2005 and 2006, seasons)

Concentration		Chl. a		Chl. b		Chls. (a+b)				Carotenoids			
Metal	ppm	2005	2006	2005	2006	2005	R.V.	2005	R.V.	2005	R.V.	2006	R.V.
	0.0 Control	1.25	1.14	0.91	0.79	2.16	100	1.93	100	0.71	100	0.65	100
Pb	200	1.01	0.95	0.65	0.55	1.66	77	1.50	78	0.64	90	0.59	91
	400	0.95	0.89	0.56	0.50	1.51	70	1.39	72	0.59	83	0.56	86
	800	0.73	0.78	0.45	0.35	1.18	55	1.13	59	0.52	73	0.49	75
	1200	0.52	0.67	0.39	0.31	0.91	42	0.98	51	0.49	69	0.42	65
Cd	200	0.99	0.92	0.72	0.70	1.71	79	1.62	84	0.66	93	0.61	94
	400	0.97	0.90	0.59	0.50	1.56	72	1.40	73	0.63	89	0.56	86
	800	0.68	0.75	0.38	0.31	1.06	49	1.06	55	0.55	77	0.51	78
	1200	0.57	0.54	0.31	0.30	0.88	41	0.84	44	0.52	73	0.48	74
Ni	50	0.98	0.91	0.71	0.69	1.69	78	1.60	83	0.67	94	0.62	95
	100	0.96	0.95	0.57	0.48	1.53	71	1.43	74	0.64	90	0.59	91
	200	0.67	0.73	0.36	0.30	1.03	48	1.03	53	0.56	78	0.48	74
	400	0.55	0.56	0.30	0.28	0.85	39	0.84	52	0.53	75	0.47	72
New L.S.D. 0.05		0.10	0.10	0.13	0.13	0.11	--	0.12	--	0.08	--	0.02	--

R.V. = relative value in relation to control as 100.

0.57 and 0.54 mg / gm FW in the two seasons. With Ni 400ppm the values were 0.55 and 0.56 mg/gm FW in the two seasons. The same trend was noticed for chl.b, total chls. (a+ b) and carotenoids.

The depressive effect of heavy metals, particularly at higher concentration, on leaf photosynthetic pigments was in agreement with Paivake (1983) on garden pea, Kacabova and Nart (1986) on spring barley, Stiborova and Ditrichova (1987) on barley and maize, Ali (1991, 1992) on orange tree, Zaman and Zereen (1998) on radish plants, Sayed (1999) on sunflower and Radwan (2007) on mango seedlings.

Histological Traits on Transplant organs

Leaf

Calculated data in Table 4 and Figs.1 and 2 illustrate the thickness of leaf blade and leaf midrib as affected by the tested treatments, the leaf blade thickness was always more thin with the lower concentrations of any tested heavy metal; i.e., Pb 200 ppm, Cd 200 ppm and Ni 50 ppm. More thick blades resulted from the control and the higher concentrations of any tested heavy metal; i.e. Pb 1200 ppm, Cd 1200 ppm and Ni 400 ppm.

The leaf midrib Fig. 3 indicated another pattern of response, the least values (739M) came from the control, while the uppermost midrib thickness was recorded by Pb at 200 ppm (1029.0M), Cd 1200 ppm (1095.0M) and Ni 400 ppm (970.0M).

Thickness of the upper and lower epidermis was not significantly affected by the tested heavy metals treatments.

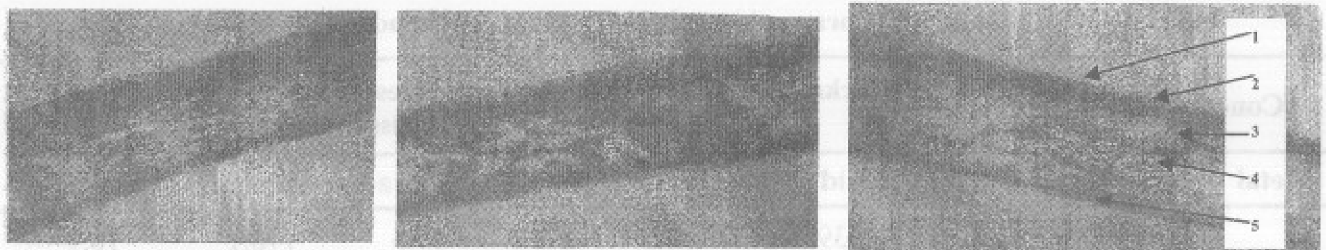
The palisade cells revealed significant response to the tested treatments. The uppermost thickness was recorded by the control (69.3M). Gradual and significant reductions were observed with the tested heavy metals concentrations which recorded 43.0 M with Pb 1200 ppm, 56.1M with Cd 1200 ppm and 49.5 with Ni 400 ppm.

Thickness of spongy tissue revealed significant response to the tested Pb, Cd and Ni concentrations Fig. 4. Pb at 200 ppm and Ni at 50 ppm induced significant reduction (162.3 and 166.5M) as compared to control (178.1M), while the uppermost value came from Ni at 400 ppm (196.3 M.).

Table 4. Effect of some lead (Pb), cadmium (Cd) and nickel (Ni) treatments on anatomical structure of leaves in Washington navel orange transplants (Av. of 2005 and 2006 seasons)

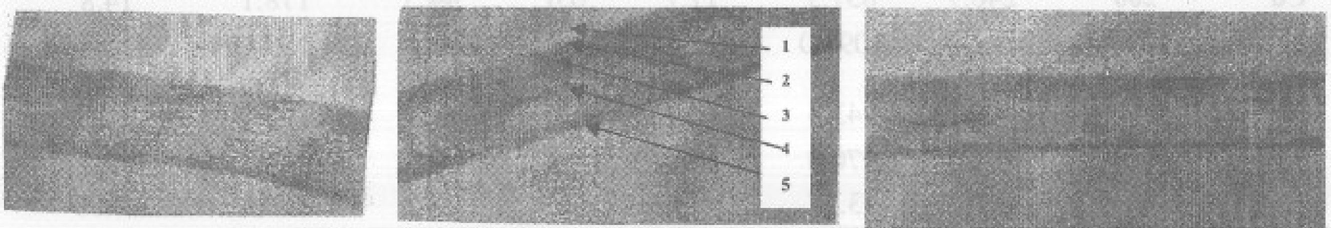
Concentration		Total leaf thickness (M*)		Thickness of epidermis (M*)		Thickness of mesophyll tissue (M*)		Diameter of midrib xylem vessel (M*)
Metal	ppm	Blade	Midrib	Upper	Lower	Palisade	Spongy	
	0.0 Control	267.4	739.0	11.7	8.3	69.3	178.1	18.1
Pb	200	226.4	1029.0	11.6	6.6	44.9	163.3	29.7
	1200	247.4	920.7	9.9	6.5	43.0	188.0	18.1
Cd	200	240.9	851.3	11.7	6.6	44.5	178.1	14.8
	1200	264.0	1095.0	11.6	6.6	56.1	189.7	14.8
Ni	50	244.2	943.8	10.0	5.0	62.7	166.5	24.7
	400	264.0	970.0	10.0	8.2	49.5	196.3	26.4
New L.S.D. 0.05		8.29	13.77	NS	NS	3.42	4.38	2.08

M* : Micron , NS (Not significant)



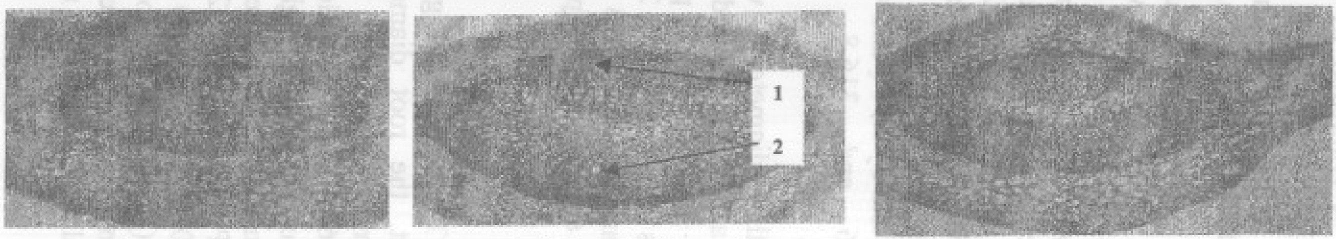
a (control) b (pb at 200 ppm) c (pb at 1200 ppm)

Fig. 1 (a, b and c). T.S in leaf blade thickness of Washington navel orange transplants treated with (pb) at at 0.0 control, 200 and 1200 ppm (Notice: 1- Cuticle 2-upper epidermis 3-palisade tissue 4- spongy tissue 5- lower epidermis tissue) (X=200)



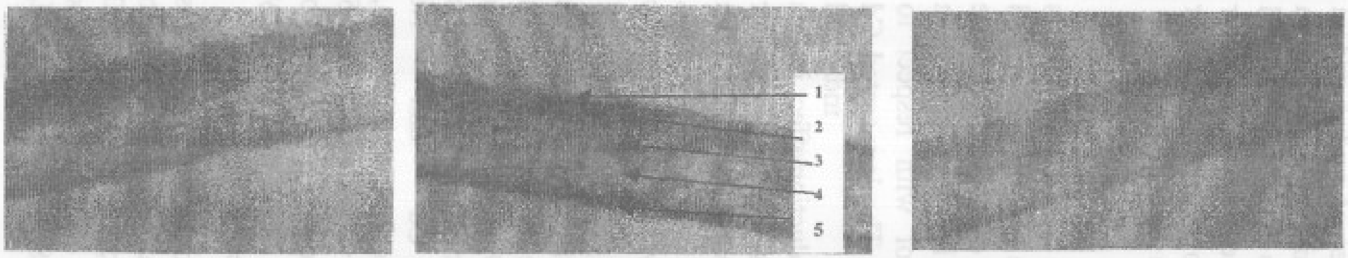
a (control) b (Cd at 200 ppm) c (Cd at 1200 ppm)

Fig. 2 (a ,b and c). T.S in leaf blade thickness of Washington navel orange transplants treated with (Cd) at 0.0 control, 200 and 1200 ppm) Notice :1- Cuticle 2-upper epidermis 3-palisade tissue 4- spongy tissue 5- lower epidermis tissue) (X=200)



a (control) b (pb at 200 ppm) c (pb at 1200 ppm)

Fig. 3 (a ,b and c). T.S in leaf midrib thickness of Washington navel orange transplants treated with (pb) at 0.0 control, 200 and 1200 ppm) (Notice: Diameter of midrib 1- phloem and 2- xylem vessels) (X=200)



a (control) b (Ni at 50 ppm) c (Ni at 400 ppm)

Fig. 4 (a, b and c). T.S in leaf blade thickness of Washington navel orange transplants treated with (Ni) at 0.0 control, 50 and 400 ppm) (Notice: 1- Cuticle 2-upper epidermis 3- palisade tissue 4- spongy tissue 5- lower epidermis tissue) (X=200)

The diameter of midrib xylem vessel was significantly affected in this respect. Pb at 200 ppm tented to increase xylem vessel diameter compared to control. Cd at 200 or 1200 ppm gave the same results.

Stem

From Table 5 and Fig. 5, it is clear that the uppermost tested concentrations of Pb and Ni were superior to the lower concentration and the control with respect to stem diameter. The low tested Cd concentration indicated insignificant reductioneffect in this respect. Thus, stem diameter reached 2450 M with Pb 1200ppm and 2574.0 M with Ni 400 ppm, while was only 2112.0 M with the control.

Thickness of the epidermis was higher with the control (9.9 M), Cd 200 ppm (9.9) and Ni 50 ppm (8.2M)as compared with the uppermost Cd and Ni concentration (1200 and400 ppm, respectively), which recorded 6.5 and 4.9 M respectively. All Pb concentrations failed to induce any significant effect in this respect.

Thickness of cortex tissue was significantly affected only by Pb concentrations; Pb at 200 or 1200 ppm recorded clearly lower cortex thickness (108.9 and 111.8 M, respectively) as compared to the control (125.4 M). Cd and Ni treatments failed to show significant effect in this concern.

Diameter of the vascular cylinder was, generally, larger with the control (396.0 M) than with the tested heavy metals treatments. Thus, diameter of the vascular cylinder was depressed by Pb 200 ppm (188.0M), by Cd 200 and 1200 ppm (316.8 and 303.6 M, respectively) and by Ni 50 and 400 ppm (270.6 and 316.8 M, respectively).

The pith diameter was significantly affected by Pb and Ni treatments. The largest pith diameter was recorded by Pb 200 and 1200 ppm (990 and 1023 M, respectively and by Ni 50 ppm (1165 M.).

Root

Data in Table 6 and Fig. 6 disclose that, the root diameter gave uppermost value with the control (2666.4 M); the least values came from the highest concentration of any tested heavy metal; i.e., Pb 1200 ppm (2329.8 M.), Cd 1200 ppm (2181.3 M.) and Ni 400 ppm (2023.0 M.).The lower concentration of any tested heavy metal revealed in between root diameter.

Table 5. **Effect of some lead (Pb), cadmium (Cd) and nickel (Ni) treatments on anatomical structure of stem Washington navel orange transplants (Av. of 2005 and 2006 seasons)**

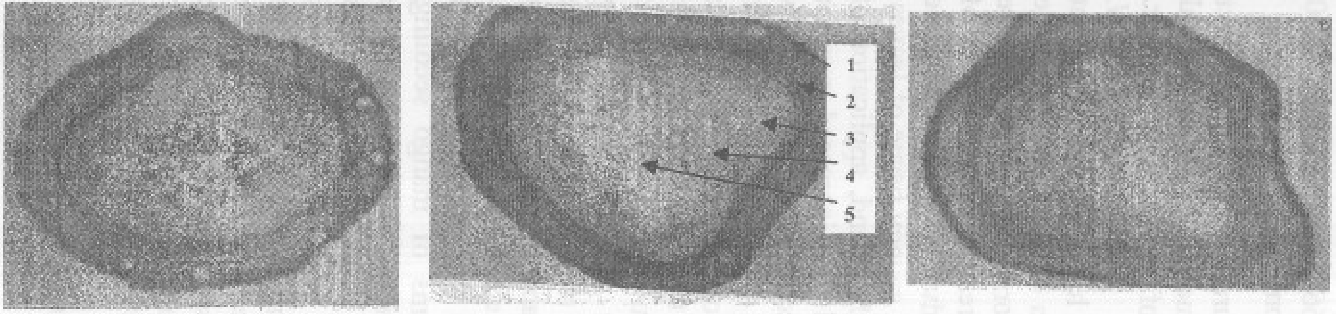
Concentration		Stem diameter (M*)	Thickness (M*)		Diameter of vascular cylinder (M*)	Diameter of pith (M*)
Metal	ppm		Epidermis	Cortex		
	0.0 Control	2112.0	9.9	125.4	396.0	874.5
Pb	200	2002.0	8.2	108.9	188.0	990.0
	1200	2450.0	9.9	111.8	445.5	1023.0
Cd	200	2072.0	9.9	122.1	316.8	841.5
	1200	1949.1	6.5	118.7	303.6	726.0
Ni	50	2095.0	8.2	135.3	270.6	1165.0
	400	2574.0	4.9	112.1	316.8	953.8
New L.S.D. 0.05		46.05	2.2	13.24	36.11	49.18

M* : Micron

Table 6. Effect of some lead (Pb), cadmium (Cd) and nickel (Ni) treatments on anatomical structure of root tissues of Washington navel orange transplants (Av. of 2005 and 2006 seasons)

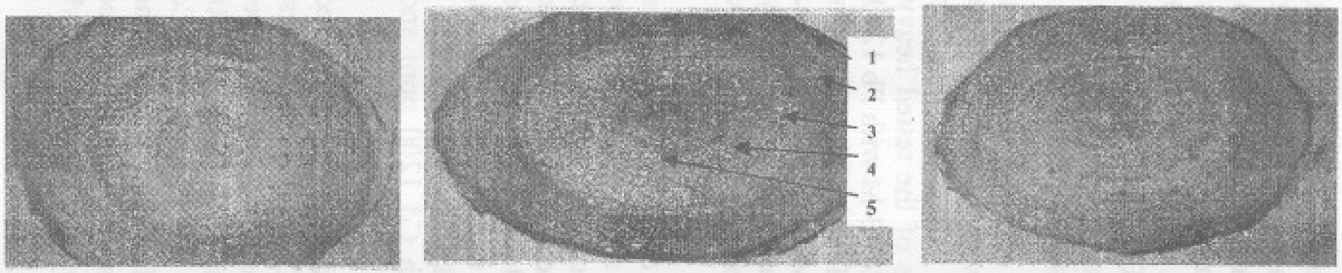
Concentration		Root diameter (M*)	Thickness (M*)		Diameter of vascular cylinder (M*)	Diameter of pith (M*)
Metal	ppm		Epidermis	Cortex		
	0.0 Control	2666.4	23.1	340.0	815.0	46.2
Pb	200	2514.6	20.4	333.3	757.0	409.2
	1200	2329.8	13.2	267.3	580.8	102.3
Cd	200	2425.5	21.4	277.2	741.5	174.9
	1200	2181.3	19.8	241.0	742.5	105.6
Ni	50	2178.0	21.4	207.9	706.2	72.6
	400	2023.0	18.1	178.2	699.6	66.0
New L.S.D. 0.05		42.15	2.17	32.14	49.72	28.08

M* : Micron



a (control) b (Ni at 50 ppm) c (Ni at 400 ppm)

Fig. 5 (a ,b and c). **T.S in stem of Washington navel orange transplants treated with (Ni) at 0.0 control, 50 and 400 ppm) (Notice: 1- epidermis 2- cortex 3- phloem 4- xylem 5- pith diameter) (X=200)**



a (control) b (Cd at 200 ppm) c (Cd at 1200ppm)

Fig. 6 (a ,b and c). **C.S in root of Washington navel orange transplants treated with (Cd) at 0.0 control, 200 and 1200 ppm) (Notice: 1- Epidermis 2- cortex 3- phloem 4- Diameter of vascular cylinder and 5- pith cells in the center of section) (X=200)**

The epidermis thickness was significantly affected by Pb, Cd at 1200 ppm and Ni at 400 ppm treatments: more thick epidermis resulted from the control, Pb 200 ppm, Cd at 200 ppm and Ni at 50 ppm as compared with the highest concentrations of heavy metals.

Thickness of cortex tissue was much bigger with the control (340 M) than with the higher concentrations of the tested heavy metals; i. e., 267.3 M with Pb 1200 ppm 241.0 M with Cd 1200 ppm and 178.20 M with Ni 400 ppm. The lower level of each heavy metal recorded in between cortex thickness.

Diameter of vascular cylinder followed the same trend as cortex tissue. The control revealed the greatest diameter (815.0 M), while the least values came from the higher heavy metals concentration, i. e., 580.8 M with Pb 1200 ppm , 742.5 M with Cd 1200 ppm and 699.6 M with Ni 400 ppm .

The pith diameter was affected by Pb and Cd only. The highest values came from the lower concentration; i. e., 409.2 M with Pb 200 ppm and 174.9M with Cd 200 ppm. The control and the higher Ni concentrations recorded lower pith values; i. e., 46.2 M, 72.6 and 66M for the control , 50 and 400 ppm for Ni , respectively.

Literature reports on the effect of heavy metals on the anatomical features of plants are meager. However, El-Mosallamy *et al.* (2007) on lupine plants found that application of 2 % sewage sludge or irrigation with 100 % sewage water increased the thickness of blade, midveinal bundle, palisade tissue and spongy tissue of leaves. On the other hand, application of sewage sludge increased number of both cortical layer and cortex thickness of stem. Also, Radwan (2007) on mango seedlings reported that lead or cadmium application in irrigation water, especially at 1000 ppm clearly decreased total leaf blade thickness (lower and upper epidermis, palisade and spongy tissues) as compared with untreated (control). Also, stems of mango seedlings treated with high concentration of Pb or Cd (1000 ppm) revealed an abnormal and compacted tissues and a decrease in number of xylem vessels and modularly rays in xylem tissue as well as the other stem tissues, i.e., phloem and pith. The root appeared to be more sensitive to heavy metals treatments than shoots.

The obtained results in this investigation should be considered as a simulation of the environmental pollution resulting from the use of sewage sludge, sewage water, some industrial composts as well as from the heavy metal containing aerosols falling from the atmosphere and fuel burning by motors and factories; all these sources cause the contamination of soil and plants.

The most hazard effects of the tested heavy metals in the present work were the depressions in leaf weight and leaf photosynthetic pigments which are the source of assimilates in plants as well as the changes in transpiration rate, enzymes activity and anatomical features of the leaves, stem and roots.

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تأثير الرش الورقي ببعض المعادن الثقيلة على شتلات البرتقال بسرة واشنطن

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أجريت هذه الدراسة على شتلات البرتقال بسرة واشنطن والمطعومة على اصل النارنج ونامية في أصص خلال موسمي ٢٠٠٥، ٢٠٠٦ لدراسة تأثير الرش الورقي ببعض المعادن الثقيلة وهي الرصاص، والكادميوم بتركيزات (٢٠٠، ٤٠٠، ٨٠٠، ١٢٠٠ جزء فى المليون لكل منهما)، والنيكل بتركيز (٥٠، ١٠٠، ٢٠٠، ٤٠٠ جزء فى المليون) بالإضافة الى الكنترول على النمو، ومحتوى الأوراق من الصبغات، وبعض الأنشطة الفسيولوجية وأيضا التركيب التشريحي لأجزاء الشتلة (ورقة - ساق - جذر).

أوضحت النتائج أن الرش بالمعادن الثقيلة المختبرة أدت إلى انخفاض محتوى الأوراق من الرطوبة، و الوزن الطازج، والوزن الجاف لها، وكذلك كثافة الثغور الموجودة على السطح السفلي للأوراق، وأظهرت النتائج أيضا تأثيرا مثبطا للرش بالمعادن الثقيلة على محتوى الأوراق من الصبغات وعلى سمك النسيج العمادي للورقة، سمك طبقة البشرة وطبقة القشرة و قطر الاسطوانة الوعائية في الساق، كما قللت هذه المعاملات من العناصر الثقيلة المختبرة سمك طبقة البشرة، وسمك طبقة القشرة وقطر الاسطوانة الوعائية في الجذور وكان التأثير المثبط أكثر وضوحا كلما زاد التركيز للرش من المعادن الثقيلة المستخدمة.

وعلى العكس فان المعادن الثقيلة المختبرة أدت إلى زيادة معدل النتح، و محتوى المعادن الثقيلة المختبرة داخل الورقة، وكذلك محتوى الأوراق من الحمض الأمينى البرولين، ونشاط إنزيمى البيروكسيديز والكتاليز كما سببت معاملات المعادن الثقيلة زيادة سمك العرق الوسطى للورقة و قطر الوعاء الخشبي للعرق الوسطى للورقة (خاصة مع النيكل والرصاص بتركيز ٢٠٠ جزء فى المليون) أما في الساق فقد سببت المعادن الثقيلة زيادة قطر الساق (خاصة مع التركيزات المرتفعة من الرصاص و النيكل)، وكذا نخاع الساق فى موسمى الدراسة.