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Effect of Exposure to Heavy Metals on Some Physiological and Morphological Characteristics of Washington Navel Orange Nursery Trees

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Abstract: This study was carried out during two successive seasons of 2016 and 2017 to study the effect of some heavy metals, i.e., lead (Pb), cadmium (Cd) at 200, 400, 600 and 1200 ppm of each and nickel (Ni) at 50, 100, 200 and 400 ppm as foliar sprays beside control (Check) treatment (tap water), on growth, leaf pigments content as well as some physiological activities and anatomical structure of Washington navel orange nursery trees on to sour orange rootstock. These transactions reduced leaf water content, leaf fresh and dry weights, stomatal density, leaf pigments content, the depressing effect was more pronounced with the higher concentrations of any heavy metal. However, the tested heavy metals clearly promoted transpiration rate and increased concentration of the heavy metal in leaf tissues, leaf proline content, peroxidase and catalase enzyme activity.

Key words: Navel orange · Heavy metals · Foliar application · Proline · Enzymes activity and leaf pigments

INTRODUCTION

Composting can be defined as the process in which of organic waste treatment by aerobic microorganisms, as such, it comprises three major phases: mesophilic and thermophilic stages and cooling (the compost stabilization stage) [1]. It can reduce the solid waste volume by 40-50%, pathogens are destroy by the metabolic heat generated by the thermophilic phase, degrade a big number of hazardous organic pollutants and make available a final product that can be used as a soil improvement or organic fertilizer [2, 3]. If the final product contains high heavy metals concentration, it may be noxious to soil, plants and human health. Heavy metals uptake by plants and successive accumulation in human tissues and biomagnifications through the food chain causes both human health and environment concerns [4].

Soil heavy metal pollution has become a worldwide environmental issue that has attracted considerable public attention largely from the increasing concern for the security of agricultural products [5]. Globally, there are 5 million sites of soil pollution covering 500 million ha of land, in which the soils are contaminated by different heavy metals or metalloids, with the present soil concentrations higher than the geo-baseline or regulatory levels [6]. Heavy metal pollution in soil has a combined worldwide economic impact estimated to be in excess of US$10 billion per year [7].

Immobilization, soil washing and phytoremediation techniques are frequently listed among the best demonstrated available technologies (BDATs) for remediation of heavy metal-contaminated sites [8]. In spite of their cost-effectiveness and environment friendliness, field applications of these technologies have only been reported in developed countries. In most developing countries, these are yet to become commercially available technologies possibly due to the inadequate awareness of their inherent advantages and principles of operation. With greater awareness by the governments and the public of the implications of contaminated soils on human and animal health, there has been increasing interest amongst the scientific community in the development of technologies to remediate contaminated sites [9]. In developing countries with great population density and scarce funds available for environmental restoration, low-cost and ecologically sustainable remedial options are required to restore contaminated lands so as to reduce the associated risks, make the land resource available for agricultural production, enhance food security and scale down land tenure problems.

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The source of heavy metals correlated mainly with the environmental pollution [10]. Accumulation of Pb, Cu, Zn and Cd was mainly due to aerosols failings from the atmosphere containing such metals. To some extent, these metals go into the soil and penetrate via the root system into the plants [11]. Nowak *et al*. [12] 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 was found to reduce leaf water content, leaf fresh and dry weights, respiration rate, stomatal opening and density [13-20]. On the other hand, Sayed [21] on sunflower plants and Radwan [20] on mango nursery trees found that proline and enzymes activity were increased as leaf Pb and Cd contents were increased.

Therefore, the present investigation was outlined to shady the effect of foliar spray of Washington navel 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.

MATERIALS AND METHODS

The present study was carried out during two successive seasons 2016 and 2017 on healthy two years old nursery trees of Washington navel orange grafted onto sour orange rootstock were used in the nurseries International Company in Giza. The nursery trees were planted in 25 liters plastic pots in size good drainage, ventilation and filled with washed sandy soil. Horticultural practices for nursery trees were carried out. They were irrigated twice/ week, with Hoagland solution modified by Johanson *et al.* [22] Table 1 and the soil water content was kept within the range of field capacity. Afterwards, the experimental nursery trees were kept in greenhouse covered with plastic 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, 600 and 1200 ppm; Cd at 200, 400, 600, 1200 ppm and Ni at 50, 100, 200 and 400 ppm beside control (check treatment without heavy metals).

The nursery trees were sprayed three times (April, June and August). This is true in both studied seasons. Nursery trees were irrigated with normal 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 nursery trees on late September; i.e., 180 days after the onset of the considered treatments.

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 (g), leaf dry weight (g) 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.* [23] was used for determination of number of stomata/mm^2 of the lower blade surface.

Transpiration Rate: On the middle of July, Aug. and Sept., of each season, transpiration rate (g H_2O/ Dec^2/hr.) was determined by the Phytometer method described by El- Sharkawi [24] and Mohsen [25].

Leaf Pb, Cd and Ni Contents: They were determined by the Atomic Absorbed Thermo Jarral ash AA- Scani methods [26].

Leaf Proline Content: It was determined in fresh leaves according to the method described by Bates *et al.* [27].

Peroxidase Enzyme Activity: It was carried out following the method described by Purr [28].

Catalase Enzyme Activity: It was carried out according to the method described by Feinstein [29].

Leaf Photosynthetic Pigments: Leaf samples were taken to determine chlorophyll-a, chlorophyll- b, total chlorophylls (a+b) content, as well as carotenoids. The method described by Wettestein [30] was adopted.

Table 1: Composition of modified Hogland solution nutrients

Compound*	Molecular Weight	Concentration of stock solution	Volume of solution mu 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
$MgSO_4$-$7H_2O$	246.49	1.00 M	1.0	Mg, S
H_3BO_3	61.84	25.00mM	--	B
$MnSO_4$-H_2O	169.01	2.00 mM		Mn
$ZnSO_4.7H_2O$	287.55	2.00mM	1.0	Zn
Cu SO_4-$5H_20$	249.71	0.50mM	--	Cu
$H_2MoO_4(85\%MoO_3)$	161.79	0.50 mM	--	Mo
Ferric citrate solution composed of l0g citric acid.	-------	-------	1.0	Fe

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

Statistical Analysis: Data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran [31].

RESULTS AND DISCUSSION

Leaf Fresh and Dry Weights: From Table 2 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 g in the two seasons against only 3.00 and 3.93 g in, respectively with the uppermost concentration (400 ppm). A nearly similar trend was observed with leaf dry weight.

Leaf Water Content: The data in Table, 2 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 metals. 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 the water content of leaves and the whole biomass [32-39, 16, 17, 20].

Leaf Stomatal Density: As shown in Table 2, the higher most stomatal density was recorded by the control 240.33 stomata /mm^2. The concentrations of 200 ppm Pb, 200 & 400 ppm Cd and 100 & 200 ppm Ni were statistically equal to control in this respect. However, the least stomatal density values were recorded by Pb at 400 & 600 ppm 120.66 and 127.66 stomata /mm^2 Cd at 600 and l200 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 2 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 g 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 g 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.* [13] 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 3 a gradual increase in leaf Pb, Cd and Ni content was observed as the tested concentrations of sprayed solutions were increased. The nursery trees sprayed with 0.0 Pb indicated leaf Pb content of only 2.7 ppm in the average of two seasons with the control followed by a gradual promotion with increasing sprayed concentration to reach 79.3 ppm in the average of 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.

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 reflected the effect of increasing concentration of the heavy metal on its level in leaf and merely made a positive relation.

Table 2: Effect of 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 nursery trees (2016 and 2017 seasons)

Concentration		Leaf fresh weight (g/leaf)		Leaf dry weight (g/leaf)		Leaf water content (%)		Stomatal density (mm^2)	Transpiration rate (g H_2O Dec^2/hr.)
Metal	ppm	2016	2017	2016	2017	2016	2017	Av. two seasons	Av. two seasons
Control	0.00	3.91	4.92	2.40	3.41	40.63	39.65	240.33	0.22
	200	4.11	5.21	2.38	3.48	42.10	39.14	238.33	0.81
Pb	400	4.01	5.11	2.11	3.11	40.31	37.60	120.66	0.88
	600	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
	200	4.00	5.01	2.37	3.47	40.76	34.10	222.66	0.22
Cd	400	3.82	4.93	2.40	3.41	37.19	32.40	225.33	0.38
	600	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
	50	3.62	4,73	2.35	3.45	37.05	32.56	214.66	0.20
Ni	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

Table 3: Effect of 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 nursery trees (2016 and 2017 seasons)

Concentration		Pb (ppm)	Cd (ppm)	Ni (ppm)	Proline content (M. mole /g FW)		Peroxidase enzyme (M. mole H_2O_2/g FW)		Catalase enzyme (M. mole H_2O_2/g FW)	
Metal	ppm	Av. Two seasons			2016	2017	2016	2017	2016	2017
Control	0.00	2.7	1.1	1.5	1.13	0.97	9.25	8.89	14.60	12.73
	200	14.1	11.3	6.6	1.15	1.02	10.65	9.49	15.34	12.93
Pb	400	29.4	24.3	8.8	1.45	1.15	11.33	10.11	15.30	13.14
	600	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
	200	4.5	9.8	3.7	1.24	1.08	10.23	9.57	13.51	11.22
Cd	400	17.2	26.3	9.1	1.42	1.18	10.98	10.22	14.17	12.21
	600	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
	50	11.1	5.3	25.0	1.33	1.18	10.42	9.12	15.27	14.98
Ni	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.ll	0.12	0.13	0.10	0.10

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 i.e. leaf, stem, root or fruit [37, 40, 41, 11, 12].

Leaf Proline Content: As shown in Table 3 leaf proline content was significantly increased with increasing the concentration of all used metals compared with control. This 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 & 1.56, 1.70 & 1.65, 1.5 & 1.41 M. mole proline/g 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 3 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/ g FW

Table 4: Effect of lead (Pb), cadmium (Cd) and nickel (Ni) treatments on leaf pigments contents, (mg/g FW) in nursery trees of Washington navel orange (2016 and 2017, seasons)

Concentration		Chl. A		Chl. b		Chls. (a+b)				Carotenoids			
Metal	ppm	2016	2017	2016	2017	2016	R.V.	2017	R.V.	2016	R.V.	2017	R.V.
Control	0.00	1.25	1.14	0.91	0.79	2.16	100	1.93	100	0.71	100	0.65	100
	200	1.01	0.95	0.65	0.55	1.66	77	1.50	78	0.64	90	0.59	91
Pb	400	0.95	0.89	0.56	0.50	1.51	70	1.39	72	0.59	83	0.56	86
	600	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
	200	0.99	0.92	0.72	0.70	1.71	79	1.62	84	0.66	93	0.61	94
Cd	400	0.97	0.90	0.59	0.50	1.56	72	1.40	73	0.63	89	0.56	86
	600	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
	50	0.98	0.91	0.71	0.69	1.69	78	1.60	83	0.67	94	0.62	95
Ni	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	---

RV = relative value in relation to control as 100

in the two seasons, while gradually amounted to reach 11.92 and 10.87 M. mole H_2O_2/ g FW with Pb 1200 ppm, 12.02 and 11.01 M. mole H_2O_2 g FW with Cd 1200 ppm and 11.98 and 11.48 M. mole H_2O_2/ g FW with Ni 400 ppm, in the first and second seasons, respectively.

El-Mosallamy *et al.* [42] on Lupine plant and Radwan [20] on mango nursery trees 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 3 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/ g FW in the two seasons. However, with Cd the least values came from the concentrations of 200 ppm 13.5l and 11.22 M. mole H_2O_2/ g 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/ g FW with Pb 1200 ppm, 15.l2 and 13.62 M. mole H_2O_2/ gin FW with Cd 1200 ppm and 16.20 and 14.25 M. mole H_2O_2/g FW with Ni 400 ppm, in the first and second seasons, respectively.

In this concern, El-Mosallamy *et al.* [42] on lupine plants found that catalase and peroxidase activities were significantly increased up to 1% sewage sludge, while they were significantly decreased with the treated sewage sludge application at 2%. Radwan [20] on mango nursery trees reported that catalase activity in leaves was less affected by Pb or Cd concentrations as compared with peroxides enzyme.

On the other hand, Wahdan [43] found that phosphorylase and peroxidase activities in *Brassica oleracea* L. var. Capitata were reduced by 38 and 27 %, respectively, after 5 weeks of treatment with 1.44 µM Pb.

Leaf Photosynthetic Pigments: Table 4 illustrates the effect of tested heavy metals treatments on chl.a, chl.b, total chls. (a+b) and carotenoids 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 was increased. For example, the control recorded 1.25 and 1.14 mg/g FW for chl.a in the two seasons, i.e., with 1200 ppm Pb the values decreased to 0.52 and 0.67 mg/g FW in the two seasons. The corresponding values with Cd at 1200 ppm were 0.57 and 0.54 mg/g FW in the two seasons. With Ni 400 ppm the values were 0.55 and 0.56 mg/g 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 [32] on garden pea, Kacabova and Nart [44] on spring barley, Stiborova and Ditrichova [45] on barley and maize, Zaman and Zereen [46] on radish plants, Sayed [21] on sunflower and Radwan [20] on mango nursery trees.

The obtained results in this investigation should be considered as a simulation of the environmental pollution resulting from the use of 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.

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