

CONCENTRATION OF SOME HEAVY METALS IN DIFFERENT PARTS OF THE TREE AND FRUIT OF WASHINGTON NAVEL ORANGE AS AFFECTED BY FIVE ROOTSTOCKS

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ABSTRACT: *A field experiment was carried out during 2003 and 2004 seasons on 8 years old of Washington Navel orange budded on five citrus rootstocks to study the effect of the rested rootstocks on the concentration of some heavy metals on root, leaf, peel and fruit juice. The obtained results could be summarized as follows:*

- 1. Heavy metals in roots of Sour orange and Troyer citrange rootstocks recorded the highest values of Pb-Se and Ni and moderate values of Cd and Cr. Beside, Volkamer-lemon and Rangpur lime recorded lower values of Pb, Se and Cd and moderate values of Ni with higher values of Cr. Cleopatra mandarin rootstock recorded higher values of Se-Cr and Cd and least values and Ni with moderate values of Pb.*
- 2. Heavy metals in leaves of Washington Navel orange budded on Volkamer lemon and Rangpur lime rootstocks had lower values of Pb-Se and Cd and moderate values of Ni while recorded highest values of Cr. Sour orange and Troyer citrange recorded highest values of Pb and Ni and moderate values of Se and Cd while gave the least values of Cr. Cleopatra mandarin rootstock recorded highest values of Se-Cd and Cr and least values of Ni with moderate values of Pb in their leaves.*
- 3. Heavy metals in fruit peel of Washington Navel orange grown on Volkamer lemon had higher levels of Se-Cd and Ni and lower levels of Pb and Cr. Rangpur lime gave higher values of Pb and Ni and moderate values of Se-Cd and Cr. Troyer citrange recorded lower values of Se-Cd and Ni and moderate values of Pb and Cr, while Sour orange rootstock recorded higher values of Pb and Cr and moderate values of Se-Cd and Ni. Cleopatra mandarin recorded lower values of Pb-Cd and Cr and moderate values of Se and Ni. The differences were significant in most cases. These results are true in the first season, while, the differences were not significant among most tested rootstocks in the second season.*
- 4. All heavy metals in fruit juice of Washington Navel orange recorded negligible concentrations on all tested rootstocks.*
- 5. It could be concluded that roots had more concentration of these metals followed by leaves and much less concentration in fruit peel and juice.*

Also, the amount translocated to fruits seemed to be negligible to cause toxic or harmful effects on human health.

Key Words: *Washington Navel orange – Citrus sinensis – heavy metals – rootstocks – volkamer lemon – Rangpur lime – troyer citrange – Cleopatra mandarin – Sour orange.*

INTRODUCTION

Data obtained during the past few years show a remarkable increase in levels of heavy metals in some Egyptian soils, this has been attributed to several conditions such as industrialization, extensive use of pesticides and fertilizers, which contain heavy metals. Absorption of heavy metals by plants or fruit crops and their distribution in the different parts of the plant were studied by (Darwish *et al.*, 1997, Ahmed *et al.*, 1998; Caselles, 1998 and Maksoud and Haggag (2000), they concluded that most of heavy metals were accumulated in the roots and leaves and the amount transferred to the fruits being small and less toxic for human.

Therefore determining heavy metal concentrations in all fruit parts is considered important for human health because it is used as fresh and as juices. This study is an attempt to put light on the absorption, transport and distribution of heavy metals as affected by different citrus rootstocks and to introduce more information about their concentration in different tree and fruit parts.

MATERIALS AND METHODS

The experiment was carried out on 8-years old trees of Washington Navel orange budded on five different citrus rootstocks in the experimental farm of Sakha Agriculture Research Station, Kafr El-Sheikh Governorate, Egypt during 2003 and 2004 seasons. The tested rootstocks were: Sour orange (*C. aurantium*), Volkamer lemon (*C. volkameriana*), Troyer citrange (*P. trifoliata* x *C. sinensis*), Rangpur lime (*C. aurntifolia* x *C. reticulata*) and Cleopatra mandarin (*C. reshni*). The trees were planted at 5 x 5 meters in a complete randomized block design with three trees plot replicated three times for a total of nine tree per rootstock budded with Washington Navel orange. Mechanical and chemical analysis of experimental field soil was done as shown in Table (1).

In both seasons, all trees received the following fertilization program: 300 gm ammonium sulphate/tree in March + 450 gm ammonium sulphate/tree in June + 200 gm ammonium nitrate/tree and 200 gm potassium sulphate/tree in August.

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Table (1): Mechanical and chemical analysis of experimental field soil.

Mechanical				Chemical			Available ppm			DTPA extractable ppm				
Sand%	Silt%	Clay%	T.Clay	pH	EC	O.M%	N	P	K	Fe	Zn	Pb	Ni	Cd
9.65	32.15	58.20	Clay	8.0	3.35	1.90	18.53	7.78	273.47	20.09	9.97	0.48	0.74	0.19

In this study, four branches of 2 inches in diameter from each replicate were selected in the four directions were tagged for sampling and determination of heavy metals in different plant organs in both seasons.

Determination of heavy metals:

In August of both seasons, 50 spring flush leaves from each replicate were sampled and washed three times with tap water, then washed again with distilled water. Samples were oven dried at 70°C to constant weight, ground, digested with H₂SO₄ and H₂O₂ according to Evenhuis and DeWaard (1980). In September of both seasons, root samples from fibrous roots were taken from each replicate by hand operated well, drilling type soil auger with a cup of 10 cm in diameter to make a hole of 10 cm in diameter and 30 cm depth, then washed several times and oven dried at 70°C to constant weight, ground and digested with H₂SO₅ and H₂O₂ according to Evenhuis and DeWaard (1980). Five mature fruits were taken at random from each tree at harvest time for peel and juice samples. Fruit juice extracted by using hand squeezer, then clarified and dried on hot plate by using low temperature. Fruit peel samples (4 disks from each fruit) were taken for drying and digesting. Both peel and juice samples were dried, and digested with sulphuric acid and hydrogen peroxide according to Evenhuis and DeWaard (1980). In the digested samples of juice, peel, leaves and roots Ni, Pb, Cd, Se and Cr were determined by Perkin Elmer Atomic Absorption spectrophotometer model 2380 Ai according to Jackson and Ulich (1959) and Yoshida *et al.* (1972).

All obtained data were statistically analyzed using a randomized complete block design according to Snedecor and Cochran (1967), and the least significant difference (L.S.D. at 5% and 1% levels) was used to compare the main values.

RESULTS AND DISCUSSION

1. Roots heavy metals content:

Data in Table (2) showed that, both Sour orange and Troyer citrange rootstocks recorded higher values of Pb-Se and Ni levels and moderate values of Cd and Cr levels in their roots with significant differences between

them in both seasons. On the other hand, Volkamer lemon and Rangpur lime rootstocks recorded lower values of Pb, Se and Cd and moderate values of Ni levels. As for Cr levels, higher values were recorded for Volkamer lemon rootstock and the least values in Rangpur lime rootstocks when compared with other tested rootstocks, the differences were significant in most cases in both seasons.

As for Cleopatra mandarin rootstock, data in Table (2) showed higher values of Se-Cr and Cd with least values of Ni and moderate values of Pb when compared with other tested rootstocks. It was clear that, the used rootstock in this study had a significant role in absorbing most heavy metals via their roots. Particularly, Volkamer lemon and Rangpur lime rootstock, assured the least absorption ability in this respect. The differences were significant between both rootstocks and each of the other tested rootstocks, in both seasons. These results find support in the findings of Ennab (2003) and Somaia (2006b) on Washington Navel orange budded on Volkamer lemon and Rangpur lime rootstocks.

Table (2): Concentrations of some heavy metals in the roots of five citrus rootstocks as affected by Washington Navel orange trees during 2003 and 2004 seasons.

Rootstock	Pb ppm	Se ppm	Cd ppm	Cr ppm	Ni ppm	Pb ppm	Se ppm	Cd ppm	Cr ppm	Ni ppm
	2003					2004				
Sour orange	4.50	6.60	5.28	6.42	8.80	4.60	6.48	5.96	6.55	8.44
Volkamer lemon	3.42	5.70	4.12	7.53	7.47	3.97	5.37	5.17	7.79	7.38
Troyer citrange	4.58	6.26	6.66	6.45	7.34	4.88	6.16	6.63	6.20	6.96
Rangpur lime	3.69	5.92	4.54	6.09	7.26	3.61	5.82	4.48	6.13	6.97
Cleopatra mandarin	3.52	6.81	7.55	7.69	6.81	3.98	6.68	7.53	7.69	6.71
L.S.D. 5%	N.S	0.71	0.49	0.30	0.30	0.51	0.76	0.36	0.20	0.33
1%	N.S	1.01	0.66	0.43	0.42	0.70	0.02	0.49	0.28	0.46

2. Leaves heavy metals content:

Data in Table (3) showed that, the highest values of Pb and Ni were recorded in leaves of Washington Navel orange trees budded on Sour orange and Troyer citrange rootstocks. While, recorded the least values of Cr and moderate values of Se and Cd. As for Volkamer lemon and Rangpur lime

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rootstocks the data recorded lower levels of Pb-Cd and Se in their leaves, on the other hand, both rootstocks recorded moderate values of Ni, with higher values of Cr on Volkamer lemon rootstock. Moderate values of Cr in leaves on Rangpur lime rootstock when compared with the other tested rootstocks with significant differences in most cases. Data in Table (3) recorded significantly higher values of Se-Cd and Cr with least values of Ni and moderate values of Pb for Cleopatera mandarin rootstocks when compared with the other tested rootstocks. These results assure that the used rootstock plays an important role in the distribution of heavy metals in the different parts of plant. These results are in harmony with those obtained by Haggag and El-Kobbia (1989) on Washington Navel orange, Caséllés *et al.* (1998) on lemon and Somaia (2006a,b) on Valencia and Washington Navel orange grown on different rootstocks.

Table (3): Concentrations of some heavy metals in the leaves of Washington Navel orange trees as affected by five citrus rootstocks during 2003 and 2004 seasons.

Rootstock	Pb ppm	Se ppm	Cd ppm	Cr ppm	Ni ppm	Pb ppm	Se ppm	Cd ppm	Cr ppm	Ni ppm
	2003					2004				
Sour orange	3.33	5.83	3.25	5.30	7.59	3.79	5.39	3.77	6.47	7.29
Volkamer lemon	2.67	4.25	1.99	6.50	5.98	2.27	4.44	2.86	6.56	6.23
Troyer citrange	3.11	5.28	4.39	5.61	6.22	3.73	5.23	3.47	6.14	6.80
Rangpur lime	2.18	4.38	2.52	5.84	6.11	2.89	4.48	2.58	6.05	7.01
Cleopatra mandarin	2.91	6.91	5.76	6.73	5.56	2.26	4.90	4.76	5.99	5.34
L.S.D. 5%	0.18	0.48	0.33	0.29	0.56	0.38	0.24	0.38	0.28	0.29
1%	0.24	0.69	0.45	0.38	0.70	0.54	0.32	0.43	0.38	0.39

3. Fruit peel heavy meals content:

Data in Table (4) showed that, Pb and Cr values in fruit peel of Washington Navel orange budded on Sour orange rootstocks were significantly higher while Se and Cd and Ni were moderate in both seasons when compared with the other tested rootstocks.

As for Volkamer lemon rootstock, the data recorded higher values of Se-Cd and Ni and lower values of Pb and Cr with significant differences in most cases only in the first season. In the second season, results recorded highest level of Se and Ni and least level of Cd and Cr while recorded moderate

values of Pb without significant differences in most cases when compared with the other tested rootstocks. As for Troyer citrange rootstock, data in Table (4) showed lower values of Se-Cd and Ni and moderate values of Pb and Cr with significant differences when compared with other tested rootstocks only in the first season. In the second season, the differences were not significant in most cases. Moreover, data on Washington Navel grown on Rangpur lime rootstock recorded higher level of Pb and Ni and moderate values of Se-Cd and Cr with significant differences in some cases in the first season.

Data in Table (4) showed that, peel taken from fruit trees on Cleopatra mandarin rootstock had significantly lower values of Pb-Se-Cd and Cr and moderate values of Ni only on the first season. However, the concentration of all heavy metals seemed to be traces in fruit peel of Washington Navel orange grown on the five tested rootstocks. These results find support in those obtained by Darwish *et al.* (1997), Maksoud and Haggag (2000) and Ennab (2003) on Balady and Washington Navel orange fruits.

Table (4): Concentrations of some heavy metals in the fruit peel of Washington Navel orange trees as affected by five citrus rootstocks during 2003 and 2004 seasons.

Rootstock	Pb	Se	Cd	Cr	Ni	Pb	Se	Cd	Cr	Ni
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
	2003					2004				
Sour orange	0.029	0.029	0.019	0.062	0.018	0.026	0.031	0.018	0.051	0.019
Volkamer lemon	0.019	0.035	0.022	0.037	0.021	0.024	0.034	0.015	0.037	0.021
Troyer citrange	0.024	0.023	0.016	0.048	0.015	0.020	0.026	0.020	0.044	0.016
Rangpur lime	0.031	0.030	0.017	0.043	0.019	0.024	0.030	0.017	0.042	0.017
Cleopatra mandarin	0.019	0.025	0.016	0.036	0.018	0.025	0.034	0.019	0.041	0.018
L.S.D. 5%	0.003	0.005	0.003	0.004	0.003	0.003	0.003	N.S	0.008	N.S
1%	0.004	0.007	0.004	0.005	0.004	0.004	0.004	N.S	0.010	N.S

4. Fruit juice heavy metals content:

The data indicated that the used rootstock did not affect the concentration of most heavy metals in the juice. This result was true in both seasons. Although some heavy metals such as Cr record higher values than the other as shown in Table (5). However, the concentration of all heavy metals were

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always the least in fruit juice when compared with those in peel or leaves and roots.

In general, the results in Tables (2, 3, 4, 5) clear that root had more concentration of heavy metals (Pb, Se, Cd, Cr and Ni) followed by leaves, and the least concentration of these metals were recorded in fruit peel and juice.. In this respect, Haggag and El-Kobbia (1989) on Washington Navel orange reported that leaves had much higher levels of Ni, Pb and Cd as compared with those determined in fruit peel, pulp and juice.

These results may be due to one or more of these reasons: (1) Soil pH plays a role in uptake of heavy metals of plants as reported by Lurbano *et al.* (1986). They cleared that heavy metals concentration seemed to be decreased in wheat seedlings due to the increase of soil pH. Obviously, pH values of the experimental soil of this study was high (pH = 8) as presented in Table (1). Subsequently, little heavy metals absorption was expected. (2) Organic mater increase the fixation of heavy metals in soils as ions or silicate or chalets, this makes heavy metals less available for absorbing by plant (Salama and Khalifa, 1993 on Balady guava nurslings). (3) Ca plays an important role for the integrity of selective ion transport mechanisms and also inhibits the absorption of heavy metals by plant roots. Maksoud and Haggag (2000) found that Ca had effective role on diminishing accumulation on heavy metals in leaves and fruits of Washington Navel orange trees. In other study, on the same trees, results showed higher Ca content in roots of Volkamer lemon and Rangpur lime when compared with other rootstocks (Somaia, 2008). These results may explain, why both of Volkamer lemon and Rangpur lime rootstocks absorbed less amount of most heavy metals via their roots when compared with all tested rootstocks. (4) The absorption of Se-Cd-Cu-Pb and Ni by plant roots is very little and also their mobility in plant is considered very low as recorded by Darwish *et al.* (1997).

In conclusion, Volkamer lemon and Rangpur lime seemed to be the best rootstocks among the five tested ones for reducing most of heavy metals in the juice of Washington Navel orange and this result attain the aim of this study.

Table (5): Concentrations of some heavy metals in the fruit juice of Washington Navel orange trees as affected by five citrus rootstocks during 2003 and 2004 seasons.

Rootstock	Pb	Se	Cd	Cr	Ni	Pb	Se	Cd	Cr	Ni
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
	2003					2004				
Sour orange	0.0029	0.0020	0.0015	0.0058	0.0051	0.0025	0.0022	0.0013	0.0048	0.0054
Volkamer lemon	0.0015	0.0012	0.0014	0.0043	0.0054	0.0018	0.0020	0.0011	0.0043	0.0052
Troyer citrange	0.0032	0.0017	0.0016	0.0050	0.0051	0.0027	0.0018	0.0016	0.0045	0.0051
Rangpur lime	0.0017	0.0018	0.0012	0.0049	0.0052	0.0028	0.0018	0.0016	0.0049	0.0054
Cleopatra mandarin	0.0018	0.0011	0.0011	0.0037	0.0053	0.0024	0.0017	0.0013	0.0042	0.0055
L.S.D. 5%	0.0002	0.0004	0.0003	0.0003	N.S	0.0004	N.S	0.0006	N.S	N.S
1%	0.0003	0.0005	0.0003	0.0004	N.S	0.0006	N.S	0.0008	N.S	N.S

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تركيز العناصر الثقيلة في أجزاء مختلفة من الشجرة والثمرة لأشجار البرتقال أبو سره المطعومة على خمسة أصول مختلفة

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الملخص العربي

أجريت هذه الدراسة خلال عامي ٢٠٠٣-٢٠٠٤م على أشجار برتقال أبوسره (عمرها ٨ سنوات) والمطعومة على خمسة أصول مختلفة هي ليمون الفولكاماريانا - ليمون الرانجبور التروير سيترنج - اليوسفي كيلوباترا - النارنج والتي تم زراعتها في مزرعة التجارب البحثية بسخا - كفرالشيخ - مصر وذلك لدراسة تأثير الأصول على امتصاص ونقل العناصر الثقيلة في أجزاء الشجرة والثمرة وقد بينت النتائج أن:

١- العناصر الثقيلة في جذور الأصول المختبرة أعطت النتائج التالية: أصول النارنج والتروير سيترنج سجلت أعلى القيم في الرصاص والسيزيوم والنيكل وقيم متوسطة من الكاديوم والكروم أما الفولكاماريانا وليمون الرانجبور فقد سجلوا قيم منخفضة من الرصاص والسيزيوم والكاديوم وقيم متوسطة من النيكل بينما سجلت قيم مرتفعة من الكروم في جذورها في حين سجل أصل اليوسفي كيلوباترا تركيزات مرتفعة من السيزيوم والكاديوم والكروم وتركيزات منخفضة من النيكل في حين سجل تركيزات متوسطة من الرصاص وكانت هذه النتائج مؤكدة إحصائياً.

٢- تركيز العناصر الثقيلة في الأوراق في أشجار البرتقال أبوسره المطعومة على أصلى الفولكاماريانا وليمون الرانجبور كانت منخفضة بالنسبة

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للرصاص - السيزيوم والكاديوم وكان متوسط بالنسبة للنكل بينما التركيز كان عالى بالنسبة للكروم فى حين سجل أصلى النارنج والتروير سيترنج قيما مرتفعة من الرصاص والنكل وقيم متوسطة من السيزيوم والكاديوم أما الكروم فقد سجل مستويات منخفضة عن باقى العناصر فى أوراق الأشجار.

٣- العناصر الثقيلة (الرصاص - السيزيوم - الكاديوم - الكروم - النكل) فى قشرة ثمار البرتقال أبوسره المطعومة على أصلى الفولكاماريانا وليمون الرانجبور كانت منخفضة وذلك بالمقارنة بباقى الأصول.

٤- تركيز العناصر الثقيلة فى العصير كان قليل بالمقارنة بباقى الأجزاء وكانت أقل المستويات مع أصول الفولكاماريانا وليمون الرانجبور واليوسفى كيلوباترا.

٥- مما سبق يمكن استخلاص أن العناصر الثقيلة تراكمت فى الجذور وأن الكمية التى وصلت إلى الثمار صغيرة جدا وخاصة عصير الثمار وكانت أقل من أن تسبب سمية تضر بصحة الإنسان.