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EFFECT OF COMPOUND FERTILIZER NPK, LIQUID ORGANIC FERTILIZER NUTRIGREEN AND SOME BIOFERTILIZERS ON THE CONCENTRATION OF HEAVY METALS IN THE LEAVES OF LOCAL ORANGE TRANSPLANTS

Ayad H. Al-Allaf, Nameer N.Hadid and Jassim M. Al-Aareji
Department of Horticulture and Landscaping Design, College of Agriculture and Forestry, University of Mosul, Iraq

ABSTRACT

This study was conducted under Lath House conditions at Department of Horticulture and Landscape Design, College of Agriculture and Forestry, Mosul University, during 2020 growing season, to know the effect of two factors and its combinations on some heavy metals concentrations in the leaves of Local orange (*Citrus sinensis* L.) transplants budded on soure orange (*Citrus aurantium* L.) cultivated on plastic pots filled with 8 kg of media (soil + sheep manuier 3:1). First factor was NPK fertilizer (20:20:20) which were added at two levels (0 and 30 gm.transplant^{-1}). The second factor included five levels of biofertilizers (3 and 6 gm.Biogeain, 5 and 10 gm. Potasiomag and 1gm.Fulzyme.transplant^{-1}) and Nutrigreen organic fertilizer at a concentration of 6 ml.l^{-1} in respect to control. NPK fertilizer were added at two equal doses (15gm.transplant^{-1} in each dose), at 21/3 and 2/5/2020 respectively, biofertilizers were added at 21/3/2020, the organic fertilizer were added at two times (26/3 and 3/5/ 2020) at the amount of 200 ml. in each time. Results indicated that all biofertilizers and organic fertilizer significantly decreased leaves Co, As, Se, Ag and Cr concentrations, While the application of NPK significantly increased leaves As concentration only as compared with control. The best treatment was 30gm NPK +10gm Potasiomag .transplant^{-1} which gave the minimum concentrations of Co, Ag and Cr in the leaves, Meanwhile the minimum leaves As and Se concentrations was in the treatment of 0gm NPK + 10gm Potasiomag .transplant^{-1} .

Keywords Orange, Transplants, Biofertilizer, Organic fertilizer, NPK, Heavy metals .

Introduction

Heavy metals is the idiom used to name of some of metals and semimetals which are associated with water, soil, weather and plants pollution and strees toxicity or ecotoxicity (Duffuse, 2002). Arsenic(As), cadmium(Cd), chromium(Cr), lead(Pb), mercury(Hg), nickel(Ni), coppur(Cu) and zinc(Zn) along with other heavy metals, which are commonly associated with contamination and toxicity to soil plants and microorganisms, (Nagajyoti *et al*., 2010).

The sources of the heavy metals in the soil could be derived from rocks weathering, fertilizing with organic and chemical fertilizers and the foliar application of fungicides for control diseases in fruit trees (Brunetto *et al*., 2016). All this pollination can reduce plants growth and production especially in young fruit trees (Miotto *et al*,2014). Generally, many plant visible symptom of phytotoxicity for heavy metals includes: leaves chlorosis and necrosis and, subsequently typical symptoms of senescence and abscission and lead to reduced plant vegetative and root growth, (Brechle and Kahle, 1992; Punz and Sieghardt ,1993). Several factors effective on heavy metals toxicity contains a dosage, route of the exposure and the chemical species, as well as the age, gender, genetic, and nutritional status of exposed individuals (Joody,2019). In addition, cumulating of the heavy metals in the fruit plants could have negative effects on the productivity and the composition of the fruits, and finally on the health of human (Brunetto *et al*., 2016). A common practice in fruit nurseries and orchards at every season was the application of chemical fertilizers such as compound NPK fertilizer, and this fertilizer may be the source of several heavy metals, also phosphorus fertilizers contains many heavy metals like Pb, Cd and Hg (Ramalho *et al*., 1999 and Nagajyoti *et al*., 2010). (Alwan and Al-Hamadany, 2012) indicated that urea, ammonium sulphate, ammonium nitrate, potassium sulphate and super phosphate fertilizers contain several heavy metals like Pb, Ni, Co, Cd and Cr, which were accumulated in the soil and plants in the case of use these fertilizers. AlKhaderi (2015) noticed that Cd, Pb and as significantly increased in the leaves of lettuce plants and in the soil of 13 farms in Jordan which were fertilized with 10 kinds of P fertilizers. Joody (2019) indicated that fertilization pomegranate trees by using sewage sludge at a level of 8 kg. tree^{-1} and NPK (50:50:100 gm. tree^{-1}) significantly increased heavy metal (Pb, Cd, Co and Ni) levels in the leaves, as for sheep and poultry manual at a level of 8 kg. tree^{-1} both alone or interactions with sewage sludge significantly decreased leaves heavy metals. The factors influencing the availability of heavy metals are soil pH and the amount and quality of organic matter in the soil (Barancikova and Makavnikov, 2003 and Puchenreiter *et al*., 2005). The bioavailability of heavy metals in the rhizosphere can be influenced by organic acids, root exudates, siderophores and protons. Plants absorb heavy metals from the soil solution preferably in ionic forms like Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Co^{2+}, Cr^{3+} and Cr^{6+} (Brunetto, 2016). One of the ways to decrease the availability of the heavy metals in the polluted soils and its absorption

with the plants is to apply organic matter to these soils (Zaniewicz-Bajkowska *et al*., 2007). (Ferreira *et al*. 2014) and (Ambrosini *et al*., 2015) indicated that the toxicity of heavy metals in the soil cultivated with the fruit trees may be decreased with the application of organic and bio fertilizers. (Brunetto *et al*., 2016) mentioned that young fruit trees fertilized with organic fertilizers and carbuncular mycorrhiza fungi inoculation can reduce the availability and potential for heavy metal toxicity. The goal of ours research is to study the effect of NPK, bio and organic fertilizers on the concentrations of some heavy metals in the leaves of Local orange transplants.

Material and Methods

This work was conducted in the Lath House/Department of Horticulture and Landscape Design/College of Agriculture and Forestry/University of Mosul, during 2020 growing season, to study the effect of two factors and its interactions on the concentrations of some heavy metals in the leaves of Lokal orange (*Citrus sinensis* L.) transplants budded on soure orange (*Citrus aurantium* L.) cultivated on plastic pots filled with 8 kg of media (soil + sheep manuer 3:1). Physical and chemical characteristic of the media were shown in the table (1).

Table 1 : Some physical and chemical characteristic of media (soil + sheep manure 3:1).

Characteristics	Value	Characteristics	Value
Sand(g. kg^{-1})	530.5	Available P (mg. kg^{-1})	3.1
Silt(g. kg^{-1})	242.5	Available K (mg. kg^{-1})	56
Clay(g. kg^{-1})	227.0	Available Ca (mg. kg^{-1})	140
Soil texture	Loamy	Available Mg (mg. kg^{-1})	24
pH	7.0	Available Na (mg. kg^{-1})	56
Organic mater(g. kg^{-1})	8.5	Available Cl (mg. kg^{-1})	113.6
EC(disysimns.$m.^{-1}$)	1.143	$CaCO_3$ (g. kg^{-1})	215.0
Available N(mg. kg^{-1})	98.00	HCO_3^- (mg. kg^{-1})	549.0

*The analysis was carried out at Central Laboratory, Collage of Agricultural and Forestry, Mosul University.

The experiment was carried out in the Randomized Complete Block Design (R.C.B.D.) at two factors, first was NPK fertilizer (20:20:20) which were added at two levels (0 and 30 gm. $transplant^{-1}$), Meanwhile the second factor included five levels of biofertilizers (3 and 6 gm.Biogeain, 5 and 10 gm. Potasiomag and 1 gm. Fulzyme.$transplant^{-1}$) and Nutrigreen organic fertilizer at a concentration of 6ml.L^{-1} in respect to control. Biogeain bio-fertilizer contain two kindes of bacteria(*Azotopacter chroococcum* + *Azosperillium brasilense*)at a concentration of $1*10^6$/ml(cfu), Potasiomag contain $1*10^6$/ml(cfu) *Bacillus circulars* bacteria and Fulzyme biofertilizer contain *Bacillus subtilis* and *Pseudomonas pudida* bacteria at a concentration of $2*10^{18}$/ml(cfu) and some enzymes like Protease, Amylase, Chitinase and Lipase, Meanwhile Nutrigreen contain 50% of 19 amino acids.NPK fertilizer were added at two equal doses (15gm.$transplant^{-1}$ in each dose), at 21/3 and 2/5/2019 respectively, biofertilizers were added at 21/3/2019 by mixing it with transplants soil, Meanwhile the organic fertilizer were added at two times (26/3 and 3/5/ 2019) at the amount of 200 ml. in each time.

On the 15th of July, 20 leaves from the periphery of each experimental unit under care were collected randomly. The leaves were obtained from the third to six leaves of shoots of transplants. To be easily transported to the laboratory, the leaves were placed in polyethylene bags. These leaves were cleaned and washed with tap water many times, then washed again with HCl 0.01 *N* and rinsed with distilled water to remove any residue from the spray. The leaf samples were air dried and then moved at 70^o C to the oven until a constant weight was reached (Jim and Stein, 2011). The dry matter of leaves was used to define the concentration of heavy metals in the leaves. 0.5gm of dry-ashed samples were taken for digestion using a mixture of concentrated leaves. H_2SO_4 with $HClO_4$ (4:1), to determine Co, As, Se, Ag and Cr by Atomic Absorption in accordance with the mentioned techniques (Bhargava and Raghupathi, 1999). All data has been tabulated and computer-based statistically analyzed using the SAS software (SAS, 2002). The variations between different treatment means were tested with Duncan Multiple Range test at 0.05 level (Duncan, 1955).

Results and Discussion

Cobalt (Co)

The application of all concentrations of bio-fertilizers (Biogeain, Potasiomag and Fulzyme) and organic fertilizer (Nutrigreen) significantly decreased the concentration of leaves Co as compared with control treatment (Tabel, 2). Potasiomag at a concentration of 10 gm.$trans^{-1}$ gave the lowest values (0.321 mg. kg^{-1}dry.wt), while the highest values (0.461 mg. kg^{-1}dry.wt)was resulted from the control treatment ,other treatments gave in between results, while the application of NPK fertilizer unsignificantly effected on leaves Co, but the interaction between bio and organic fertilizers with NPK significantly effected on leaves Co, The lowest values (0.305 mg.kg^{-1}dry.wt) was in the treatment of 30gm NPK +10gm Potasiomag. $transp.^{-1}$, while the highest values (0.467 mg. kg^{-1}dry.wt)was resulted from the control treatment.

Arsenic (As)

Results in table(3) showed that the application of bio-fertilizers (Biogeain, Potasiomag and Fulzyme) and organic fertilizer (Nutrigreen) in all concentrations significantly decreased leaves As concentration as compared with control treatment. Potasiomag at a concentration of 5 gm.$transp.^{-1}$ gave the lowest values (2.24 mg.kg^{-1}dry.wt)followed closely without any significant difference by 10 gm.$transp.^{-1}$ of Potasiomag , while the highest values (3.72 mg. kg^{-1}dry.wt)was resulted from the control treatment, other treatments gave in between results. The application of NPK fertilizer significantly effected on leaves As, the treatment of 30gm NPK.$transp.^{-1}$ significantly dominated over control treatment. The interaction between bio and organic fertilizers with NPK significantly effected on leaves As, The lowest values (2.18 mg. kg^{-1}dry.wt) was in the treatment of 0gm

NPK +10gm Potasiomag .transp.$^{-1}$, while the highest values (3.94 mg.kg^{-1}dry.wt) was resulted from the interaction between 30gm NPK + 0gm of Bio and Organic fertilizers.transp.$^{-1}$.

Selenium (Se)

Selenium(Se) concentration in orange leaves significantly decreased with the application of all concentrations of Biogeain, Potasiomag, Fulzyme and Nutrigreen as compared with control treatment (Tabel 4). Potasiomag at a concentration of 10 gm.transp.$^{-1}$ gave the lowest values (0.359 mg.kg^{-1}dry.wt) followed closely without any significant difference by other treatments of bio and organic fertilizers, while the highest values (0.491 mg.kg^{-1}dry.wt) was resulted from the control treatment, Meanwhile the application of NPK fertilizer unsignificantly effected on leaves Se, but the interaction between bio and organic fertilizers with NPK significantly effected on leaves Se, The lowest values (0.349 mg.kg^{-1}dry.wt) was in the treatment of 0gm NPK +10gm Potasiomag.transp.$^{-1}$, while the highest values (0.486 mg.kg^{-1}dry.wt) was resulted from the interaction between 30gm NPK + 0gm of Bio and Organic fertilizers.transp.$^{-1}$.

Silver (Ag)

Results in table(5) showed that the application of Biogeain,Potasiomag,Fulzyme and Nutrigreen in all concentrations significantly decreased leaves Ag as compared with control treatment, the treatment of of 10 gm.trans^{-1} of Potasiomag gave the lowest values (0.0364 mg.kg^{-1}dry.wt) followed closely without any significant difference by, Potasiomag(5 gm.transp.$^{-1}$), Biogeain (6gm.trans.$^{-1}$), Fulzyme (1gm.trans.$^{-1}$) and Biogeain (3gm.trans.$^{-1}$) respectively , while the highest values (0.0593 mg.kg^{-1}dry.wt)was resulted from the control treatment. The application of NPK fertilizer unsignificantly effected on leaves As but the interaction between bio and organic fertilizers with NPK significantly effected on leaves Ag, The lowest values (0.0364mg.kg^{-1}dry.wt) was in the treatment of 30gm NPK +10gm Potasiomag.transp.$^{-1}$, while the highest values (0.0662 mg.kg^{-1}dry.wt) was resulted from the interaction between 30gm NPK + 0gm of Bio and Organic fertilizers .transp.$^{-1}$.

Chromium (Cr)

Cr concentration in orange leaves significantly decreased with the application of Biogeain, Potasiomag, Fulzyme and Nutrigreen as compared with control treatment (Table 6). Potasiomag at a concentration of 10 gm.transp.$^{-1}$ gave the lowest values (14.523mg.kg^{-1}dry.wt)followed closely without any significant difference by Potasiomag (5 gm.trans.$^{-1}$) and Nutrigreen (6 ml.L^{-1}) respectively, while the highest values (20.331mg.kg^{-1}dry.wt) was resulted from the control treatment, Meanwhile the application of NPK fertilizer unsignificantly effected on leaves Cr, but the interaction between bio and organic fertilizers with NPK significantly effected on leaves Se , The lowest values (14.343 mg.kg^{-1}dry.wt) was in the treatment of 30gm NPK +10gm Potasiomag.transp.$^{-1}$, while the highest values (21.326 mg.kg^{-1}dry.wt)was resulted from the interaction between 30gm NPK + 0gm of Bio and organic fertilizers.transp.$^{-1}$.

Table 2 : Effect of NPK,Bio and organic fertilizers on leaves cobalt (Co) concentration(mg. kg^{-1}dry.wt.) of local orange transplants budded on soure orange rootstock.*

Levels of Bio and Organic fertilizers	NPK levels (gm.transplant^{-1})		
	0	30	Means
Control(0 gm.trans.$^{-1}$)	0.456 a	0.467 a	0.461 a
Biogeain(3gm.transp.$^{-1}$)	0.365 b	0.340 b-d	0.353 bc
Biogeain(6 gm.transp.$^{-1}$)	0.338 b-d	0.321 cd	0.329 c
Potasiomag(5 gm.transp.$^{-1}$)	0.313 cd	0.332 b-d	0.329 c
Potasiomag(10 gm.transp.$^{-1}$)	0.337 b-d	0.305 d	0.321 d
Fulzyme(1 gm.transp.$^{-1}$)	0.359 b	0.344 bc	0.351 bc
Nutrigreen(6 ml.L^{-1})	0.362 b	0.364 b	0.363 b
Means	0.361 a	0.353 a	

*Means of each factor and their combination having the same letters are unsignificantly different at 0.05 level.

Table 3 : Effect of NPK, Bio and organic fertilizers on leaves arsenic(As) concentration (mg. kg^{-1}dry.wt.) of local orange transplants budded on soure orange rootstock.*

Levels of Bio and Organic fertilizers	NPK levels (gm.transplant^{-1})		
	0	30	Means
Control (0 gm.transp.$^{-1}$)	3.50 b	3.94 a	3.72a
Biogeain (3gm.transp.$^{-1}$)	2.64 c	2.50 cd	2.57b
Biogeain (6 gm.transp.$^{-1}$)	2.68 c	2.62 c	2.65b
Potasiomag (5 gm.transp.$^{-1}$)	2.22 e	2.27 de	2.24c
Potasiomag (10 gm.transp.$^{-1}$)	2.18 e	2.60 c	2.39c
Fulzyme (1 gm.transp.$^{-1}$)	2.54 cd	2.68 c	2.61b
Nutrigreen (6 ml.L^{-1})	2.67 c	2.78 c	2.73b
Means	2.63 b	2.77a	

*Means of each factor and their combination having the same letters are unsignificantly different at 0.05 level.

Table 4 : Effect of NPK,Bio and organic fertilizers on leaves selenium(Se) concentration (mg. kg^{-1}dry.wt.) of local orange transplants budded on soure orange rootstock.*

Levels of Bio and Organic fertilizers	NPK levels (gm.transplant^{-1})		
	0	30	Means
Control (0 gm.transp.$^{-1}$)	0.469 a	0.486 a	0.491 a
Biogeain (3gm.transp.$^{-1}$)	0.377 b	0.373 b	0.375 b
Biogeain (6 gm.transp.$^{-1}$)	0.365 b	0.375 b	0.370 b
Potasiomag (5 gm.transp.$^{-1}$)	0.355 b	0.366 b	0.361 b
Potasiomag (10 gm.transp.$^{-1}$)	0.349 b	0.369 b	0.359 b
Fulzyme (1 gm.transp.$^{-1}$)	0.374 b	0.367 b	0.371 b
Nutrigreen (6 ml.L^{-1})	0.374 b	0.376 b	0.375 b
Means	0.384 a	0.387 a	

*Means of each factor and their combination having the same letters are unsignificantly different at 0.05 level.

Table 5 : Effect of NPK,Bio and organic fertilizers on leaves silver(Ag) concentration(mg.kg^{-1}dry.wt.) of local orange transplants budded on soure orange rootstock.*

Levels of Bio and Organic fertilizers	NPK levels (gm.transplant^{-1})		
	0	30	Means
Control (0 gm.transp.$^{-1}$)	0.0523 b	0.0662 a	0.0593 a
Biogeain (3gm.transp.$^{-1}$)	0.0428 c	0.0366 de	0.0397 bc
Biogeain (6 gm.transp.$^{-1}$)	0.0400 cd	0.0371 de	0.0385 bc
Potasiomag (5 gm.transp.$^{-1}$)	0.0368 cde	0.0363 de	0.0375 bc
Potasiomag (10 gm.transp.$^{-1}$)	0.0381cde	0.0347 e	0.0364 c
Fulzyme (1 gm.trans.$^{-1}$)	0.0387 cde	0.0368 de	0.0377 bc
Nutrigreen (6 ml.L^{-1})	0.0410 cde	0.0391 cde	0.0401 b
Means	0.0416 a	0.0410 a	

*Means of each factor and their combination having the same letters are unsignificantly different at 0.05 level.

Table 6 : Effect of NPK,Bio and organic fertilizers on leaves chromium(Cr) concentration(mg.kg^{-1}dry.wt.) of local orange transplants budded on soure orange rootstock.

Levels of Bio and Organic fertilizers	NPK levels (gm.transplant^{-1})		
	0	30	Means
Control (0 gm.transp.$^{-1}$)	19.336 b	21.326 a	20.331 a
Biogeain (3gm.transp.$^{-1}$)	16.473 c	15.936 cde	16.205 b
Biogeain (6 gm.transp.$^{-1}$)	16.253 cd	15.263 def	15.758 bc
Potasiomag (5 gm.transp.$^{-1}$)	14.900 ef	14.520 f	14.710 d
Potasiomag (10 gm.transp.$^{-1}$)	14.703 f	14.343 f	14.523 d
Fulzyme (1 gm.transp.$^{-1}$)	16.226 cd	14.836 ef	15.531bc
Nutrigreen (6 ml.L^{-1})	15.343 def	15.013 ef	15.178 cd
Means	16.176 a	15.891 a	

*Means of each factor and their combination having the same letters are unsignificantly different at 0.05 level.

Data present in tables (2-6) indicated that the application of all concentration of bio-fertilizers (Biogeain, Potasiomag and Fulzyme) and organic fertilizer (Nutrigreen) significantly decreased the concentration of heavy metals (Co, Zr, Se, Ag and Cr) in the leaves of Local orange transplants as compared with control, Potasiomag at a concentration of 10 gm.transp.$^{-1}$, gave the lowest concentrations of these metals in the leaves as compared with other treatments ,except As, the lowest values of this metal was in the treatment of Potasiomag at a concentration of 5 gm.transp.$^{-1}$ followed closely without any significant difference by 10 gm.transp.$^{-1}$ of Potasiomag .These results may be attributed to the role of the addition of bio and organic fertilizers may contribute to increase the content of soil organic matter , this may lead to complexation with heavy metals which presents in the soil (Casali *et al*., 2008). On the other hand , the mineralization of organic waste with the application of bio-fertilizer which contain several kinds of bacteria produces molecules into the soil solution that can complex heavy metals and reduce their bioavailability and therefore the toxicity (Brunetto *et al*., 2014). Zaniewicz-Bajkowska *et al*. (2007) indicated that one of the ways to counteracts negative effects of soil contamination with heavy metals is to apply organic matter into polluted soil. Also Ferreira *et al* (2014) and Ambrosini *et al.* (2015) mentioned that the toxicity of heavy metals in the soil cultivated with fruit trees can be reduced with the application of organic and bio fertilizers. Brunetto *et al*. (2016) mentioned that the addition of organic fertilizers and inoculation of young plants with arbuscular mycorrhizal fungi can decrease the availability and the potential of heavy metal toxicity to fruit trees.

The application of NPK significantly increased leaves As concentration only as compared with control. AlKhaderi (2015) noticed that As significantly increased in the leaves of lettuce plants and in the soil of 13 farms in Jordan which were fertilized with 10 kindes of P fertilizers.The best treatment was 30gm NPK +10gm Potasiomag .transp.$^{-1}$

which gave the minimum concentrations of Co,Ag and Cr in the leaves , Meanwhile the minimum leaves As and Se concentrations was in the treatment of 0gm NPK + 10gm Potasiomag .transp^{-1}.

Conclusion

This study demonstarted that the concentrations of heavy metals (Co, As, Se, Ag and Cr) in the leaves of Local orange transplants significantly decreased with the application of all concentrations of bio-fertilizers (Biogeain, Potasiomag and Fulzyme) and organic fertilizer (Nutrigreen), Potasiomag at a concentration of 10 gm.transp.$^{-1}$, gave the lowest concentrations of these metals in the leaves as compared with other treatments. The application of NPK alone unsignificantly effected on leaves Co, Se, Ag and Cr, Meanwhile As in the leaves significantly increased with the application of NPK fertilizer as compared with control treatment.

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