European Territorial Cooperation Programme
Greece - Italy
2007-2013

INVESTING IN OUR FUTURE
Co-funded by the European Union (ERDF)
and by National Funds of Greece & Italy

Efficient Irrigation Management Tools for Agricultural Cultivations and Urban Landscapes

IRMA

WP6

Specialized research actions

www.irrigation-management.eu

V.01

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Technological Educational Institution of Epirus

http://www.teiep.gr, http://research.teiep.gr

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Olympiaki S.A., Development Enterprise of the Region of Western Greece

http://www.aepde.gr

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Contents

Figures

Tables

Summary

Heavy metals occur naturally as chemical elements in the earth's crust and surface soils in varying concentrations and they readily accumulate in toxic levels. Most of the point sources of heavy metal pollutants are industrial activities and wastes. Heavy metals are transported by runoff water and contaminated water sources including irrigation reservoirs, channels etc.

Cross-contamination of edible parts of vegetables and medicinal plants by heavy metals is an emerging hazard for human health. Research findings have already mentioned that in Greece, farmers have been irrigating their crops with polluted underground water with Ni (Nickelium) and Cr (Chromium) for many years, highlighting a problem that has affected Greek agriculture. European Commission has already set maximum levels for Ni and Cr in water for human consumption (Council Directive 98/83/EC) but not in foodstuffs (Commission Regulation EC 1881/2006) and there is a legal gap about these two emerging hazards in food chain.

In the framework of WP6 of IRMA project, pot experiments conducted in order to study the concentration of Ni and Cr in shoots and roots of Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum L.*) cultivated in a soil never previously polluted with heavy metals and irrigated using different applications of Ni and Cr to the soil through drip irrigation.

Each pot experiment was arranged in a random block design with five treatments and five replicates for each treatment, with a total of 25 pots for each element. Ni was applied as Ni(II), Nickel Chloride Hexahydrate ($NiCl_2$ $6H_20$) in amounts of 0, 5, 10, 20 and 40 mg Ni L^{-1} while Cr was applied as Cr(IV), Potassium Dichromate ($K_2Cr_2O_7$) in amounts of 0, 5, 10, 20 and 40 mg Cr L^{-1}.

The experiments were conducted during spring and summer of 2014. The results are presented in this report. Results show that plants absorbed Ni and Cr in considerable levels. The analysis of the results leaded to the following conclusions that may have practical value, regarding the use of alternative heavy metal contaminated water resources for irrigation: Ni and Cr (as total Ni and Cr) can pass directly through irrigation water to shoots and roots of Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum L.*) depending on the irrigation water concentration of these heavy metals. In addition Lavender and Sweet bush basil cultivated in a soil never previously polluted with heavy metals, irrigated for the first time with different Ni(II) and Cr(IV) concentrations; practically can be found in nature; can be cross contaminated by the irrigation water's content Ni and Cr. The hazard for the transfer of these heavy metals in food chain is evident.

The final outcome is that irrigating with heavy metals contaminated water as alternative to standard water resources is a hazard. This report closes with the wish that these results and conclusions will be a start for further activities by local authorities in Italy and Greece to inform the public about the hazards of heavy metals in food chain.

Introduction

A briefing regarding heavy metals in the environment and the usage of heavy metal contaminated water sources for irrigation

Heavy metals occur naturally as chemical elements in the earth's crust and surface soils in varying concentrations (Alloway and Ayres, 1997). Heavy metals in the environment are very stable; they do not thermodegrade or biodegrade and they readily accumulate in toxic levels (Sharma et. al., 2007). Most of the point sources of heavy metal pollutants are industrial activities and wastes. Sewage and industrial water is a common source for irrigation in some countries as an alternative disposal of waste (Yadav et al., 2002). In most cases, heavy metals are transported by runoff water and contaminated water sources included irrigation reservoirs, channels, etch. To avoid health hazards it is essential to remove these toxic heavy metals from irrigation water or to water plants with this contaminated water under special conditions as alternative water source. The release of large quantities of hazardous materials into the natural environment has resulted in a number of environmental problems and due to their non-biodegradability and persistence, can accumulate in the environment elements such as food chain, and thus may pose a significant danger to human health. Most of the heavy metals in waste waters, but also in irrigation water, are toxic and carcinogenic and cause a serious threat to the human health (Table 1). Of a great concern is their impact into the environment and consequently into soils and irrigation water the through man's agricultural and urban activities (Tom et al., 2014). Furthermore the accumulation of heavy metals in the soil-water-plant system is very important because heavy metals are potentially a health threat due to toxicity result to human life and environment. The anthropogenic input of trace metals can be enhanced by chemical applications such as fertilizers, herbicides, pesticides and applications of animal manure and sewage (Alloway and Ayres, 1997; Montagne et al., 2007). The natural concentration of heavy metals in soils depends primarily on geological parent material composition (Alloway, 1995; Rodriguez et al., 2006), but human activities that involve emitting of large quantities of heavy metals into the environment have dramatically increased natural concentrations in the last century with a secondary effect in the accumulation of heavy metals in plants. The accumulation of heavy metals in crop tissues and transfer of them in soil crop system had well been documented (Dudka et al., 1996; Barman et al., 2000; Kisku et al., 2000, Akoumianakis et al., 2009, Moustakas et al., 2011). Public awareness has been raised on the harmful potential of heavy metals that can accumulate in crops and may end up in human diet through the food chain. Cross-contamination of food by heavy metals is an emerging hazard for human nutrition (Stasinos and Zabetakis, 2013) as there are a lot of proofs are linking food chain, environmental pollution and heavy metal uptake of edible parts of vegetables and medicinal plants (Akoumianakis et al., 2009, Moustakas et al., 2011, Savvas et al., 2013, Barouchas et al., 2014, Akoumianaki-Ioannidou et al., 2015). Many studies have confirmed that heavy metals may accumulate and damage crops or even mankind (Otte et al., 1993; Dudka et al., 1994) and in some cases can cause serious health problems as a result of depletion of some essential nutrients in the body (Arora et. al., 2008). Furthermore the continuously consumption of heavy metal-contaminated food can lead in carcinogenesis (Denkhaus and Salnikow, 2002) For human health, trivalent chromium [Cr(III)] in small amounts is essential nutrient and is poorly bioavailable and presents low ability to enter cells (European Food Safety Authority, 2014), though swallowing large amounts may cause health problems (Zayed and Terry, 2003). Stasinos and

Zabetakis (2013) have previously mentioned that in Asopos regions in Greece, farmers have been irrigating their crops with polluted underground water with Ni and Cr for many years, highlighting a problem that has affected Greek agriculture. European Commission has already set maximum levels for Cr and Ni in water for human consumption (Council Directive 98/83/EC) but not in foodstuffs (Commission Regulation EC 1881/2006) and there is a legal gap about these two emerging hazards in food chain, exposing consumers to toxic elements (Kirkillis et al., 2012). More, a research gap still exists for heavy metals uptake by medicinal and aromatic plants. The IRMA project through irrigation control systems and involving the determination of timing, frequency and duration of each watering event, is trying to answer with the most efficient procedure the critical question of how much and how often to water plants. In this framework evaluation of various approaches regarding irrigation scheduling and provision of relevant recommendations are expected to be of great importance in order to lower the water consumption in agriculture. Furthermore the use of alternative water resources can lower the demands for irrigation water.

In the framework of WP6 of IRMA project, pot experiments conducted in order to study the concentration of Ni and Cr in shoots and roots of Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum L.*) cultivated in a soil never previously polluted with heavy metals and irrigated using different applications of Ni and Cr to the soil through drip irrigation.

Table 1 Sources and toxicological effects of some heavy metals: (Alluri et al,. 2007).

Heavy Metal	Sources	Effects
Cr Chromium	Steel and textile industry	Skin rashes, respiratory problems, haemolysis, acute renal failure, weakened immune systems, kidney and liver damage, alteration of genetic material, lung cancer, Pulmonary fibrosis
Ni Nickel	Effluents of silver refineries, electroplating, zinc base casting and storage battery industries.	Dermatitis, Myocarditis, Encephalopathy, pulmonary fibrosis, cancer of lungs, nose and bone, headache, dizziness, nausea and vomiting, chest pain, rapid respiration.

Materials and Methods

General design of pot experiments

Pot experiments were conducted in a twin span glass-covered greenhouse, W-E oriented, located at the Technological Education Institute of Epirus (Kostakii Campus) 7km SW of the city of Arta in Greece, (**Fig. 1**, latitude 39^o 07′N, longitude 20^o 56′E, altitude 5 m / WGS84) on the coastal area of Western Greece. The greenhouse is equipped with a movable, aluminized shade screen, located horizontally at the level of the eaves, which was used for the reduction of the incoming solar radiation at the level of the plants.

Fig. 1 Aerial view of TEIEP Kostakii Campus (GoogleEarth, 2014)

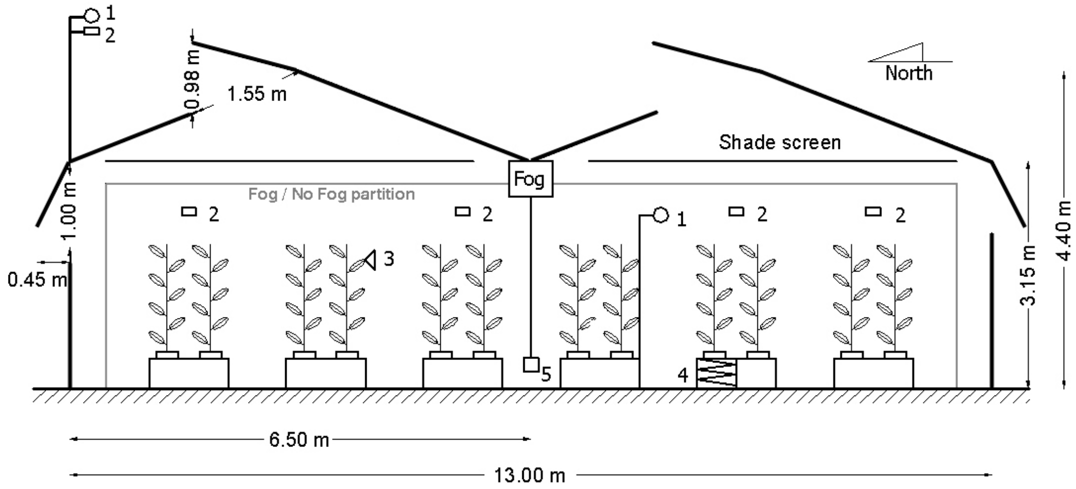

Fig. 2 Cross section of the experimental twin span glass-covered greenhouse

Arta's climate is of Mediterranean type with mild and rainy winters and hot and dry summers with occasional rain events (Fig. 3).

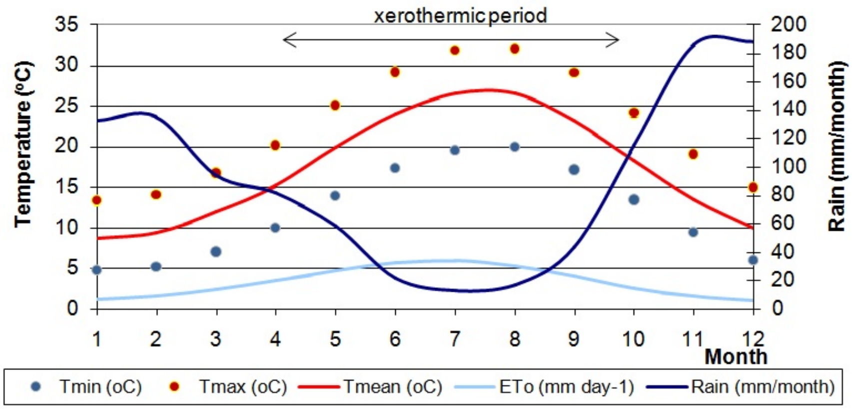

Fig. 3 Omvrothermic diagram of Arta Greece (based on climatic facts of HNMS (2014))

The experiments were targeted to study the concentration of Nickelium (Ni) and Chromium (Cr) in shoots and roots of Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum* L.) as affected by different applications of Ni and Cr to the soil through drip irrigation water. Table 2 shows the general experimental scheme with 5 treatments and 5 replicates.

Table 2 General experimental scheme regarding Nickelium / Chromium cross-contamination in shoots and roots of Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum* L.)

Ni/Cr	REPLICATES	1	2	3	4	5
TREATMENT	mg L-1					
1	0	Ni/Cr11	Ni/Cr 12	Ni/Cr 13	Ni/Cr 14	Ni/Cr 15
2	5	Ni/Cr 21	Ni/Cr 22	Ni/Cr 23	Ni/Cr 24	Ni/Cr 25
3	10	Ni/Cr 31	Ni/Cr 32	Ni/Cr 33	Ni/Cr 34	Ni/Cr 35
4	20	Ni/Cr 41	Ni/Cr 42	Ni/Cr 43	Ni/Cr 44	Ni/Cr 45
5	40	Ni/Cr 51	Ni/Cr 55	Ni/Cr 59	Ni/Cr 63	Ni/Cr 67

General conditions of the heavy metals pot experiments

Heavy metals pot experimental design for Lavender (Lavandula angustifolia)

The experiment was targeted to study the concentration of Nickelium (Ni) and Chromium (Cr) in shoots and roots of Lavender (*Lavandula angustifolia*) as affected by different applications of Ni and Cr to the soil through irrigation water. The experiment was conducted during spring and summer of 2014, started in 14th of May and ended in 3rd of July with harvesting (eight weeks).

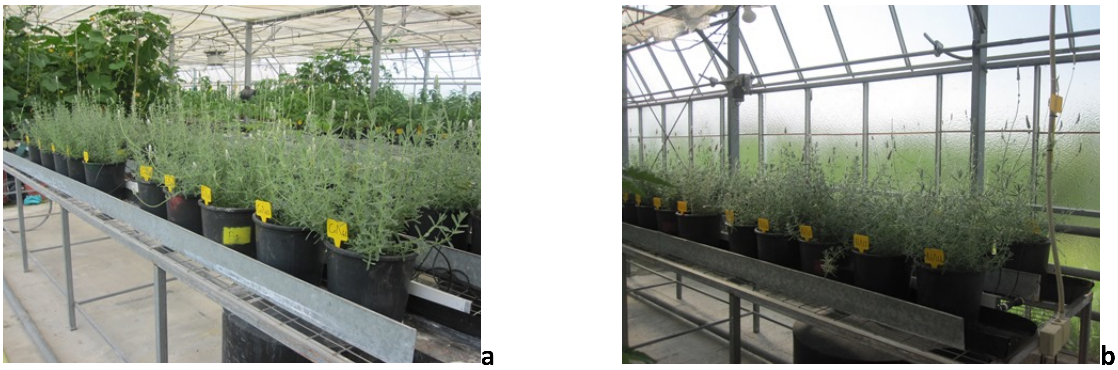

Fig. 4 Heavy metals pot experimental design for Lavender (*Lavandula angustifolia*) (a) Chromium, (b) Nickelium

Four weeks commerce transplants were transplanted to the experimental pots (made of black plastic, 23.5 cm in diameter and 7 L volume) filled with a substrate consisting of 1 peat : 1 perlite : 1 red argillic soil (v/v), with pH 6.9, and Electrical Conductivity 0.3 S m^{-1}. This medium was used in all treatments and in both plants under study. Each pot contained one plant. The pots were arranged in a complete block design with five treatments and five replicates for each treatment, with a total of 25 pots for each element. Nickelium was applied as Nickel(II) Chloride Hexahydrate ($NiCl_2$ $6H_20$) in amounts of 0, 5, 10, 20 and 40 mg Ni L^{-1} and was added once a week with 300 ml of each treatment per pot for the first six weeks (total 3000 ml per pot of each treatment for the whole cultivation period i.e. 7.5 mg Ni for level 5 mg L^{-1}, 15 mg Ni for level 10 mg L^{-1}, 30 mg Ni for level 20 mg L^{-1} and 60 mg Ni for level 40 mg L^{-1}. Chromium(VI) was applied as Potassium Dichromate ($K_2Cr_2O_7$) in amounts of 0, 5, 10, 20 and 40 mg Cr L^{-1} and was added once a week with 300 ml of each treatment per pot for the first six weeks (total 3000 ml per pot of each treatment for the whole cultivation period i.e. 7.5 mg Cr for level 5 mg L^{-1}, 15 mg Cr for level 10 mg L^{-1}, 30 mg Cr for level 20 mg L^{-1} and 60 mg Cr for level 40 mg L^{-1}. Fertilization of the pots was performed approximately once a week with fertigation, using a standard fertilizer solution with 2 mg L^{-1} NO^-_3, 2 mg L^{-1} P, and 2 mg L^{-1} K^+ for each pot. All pots were lined with clear polyethylene bags and the soil moisture was maintained at about field capacity and avoided leaching after irrigation event. Water and fertilizers were supplied via a drip irrigation system. The frequency of irrigation was dependent on solar radiation measured outside the greenhouse with pyranometer. The content of Ni and Cr in the fertilizer was negligible.

***Heavy metals pot experimental design for Sweet bush basil (Ocimum basilicum* L.)**

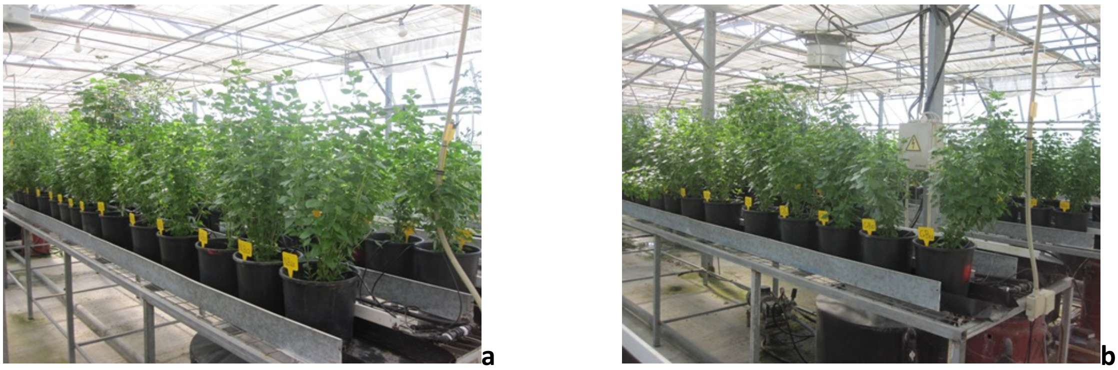

Fig. 5 Heavy metals pot experimental setup for Sweet bush basil (*Ocimum basilicum* L.) (a) Chromium, (b) Nickelium

The experiment was targeted to study the concentration of Nickelium (Ni) and Chromium (Cr) in shoots and roots of sweet bush basil (*Ocimum basilicum L*) as affected by different applications of Ni and Cr to the soil through irrigation water. The experiment was conducted in the spring of 2014, started in 14th of May and ended in 3d of July with harvesting (eight weeks). Four weeks commerce transplants were transplanted to the experimental pots (made of black plastic, 23.5 cm in diameter and 7 L volume) filled with a substrate consisting of 1 peat : 1 perlite : 1 red argillic soil (v/v), with pH 6.9, and Electrical Conductivity 0.3 S m^{-1}. This medium was used in all treatments and in both plants under study. Each pot contained one plant. The pots were arranged in a randomized complete block design with five treatments and five replicates for each treatment, with a total of 25 pots for each element. Nickelium was applied as Nickel(II) Chloride Hexahydrate ($NiCl_2\ 6H_20$) in amounts of 0, 5, 10, 20 and 40 mg Ni L^{-1} and was added once a week with 300 ml of each treatment per pot for the first six weeks (total 3000 ml per pot of each treatment for the whole cultivation period i.e. 7.5 mg Ni for level 5 mg L^{-1}, 15 mg Ni for level 10 mg L^{-1}, 30 mg Ni for level 20 mg L^{-1} and 60 mg Ni for level 40 mg L^{-1}. Chromium(VI) was applied as Potassium Dichromate ($K_2Cr_2O_7$) in amounts of 0, 5, 10, 20 and 40 mg Cr L^{-1} and was added once a week with 300 ml of each treatment per pot for the first six weeks (total 3000 ml per pot of each treatment for the whole cultivation period i.e. 7.5 mg Cr for level 5 mg L^{-1}, 15 mg Cr for level 10 mg L^{-1}, 30 mg Cr for level 20 mg L^{-1} and 60 mg Cr for level 40 mg L^{-1}. Fertilization of the pots was performed approximately once a week with fertigation, using a standard fertilizer solution with 2 mg L^{-1} NO^-_3, 2 mg L^{-1} P, and 2 mg L^{-1} K^+ for each pot. All pots were lined with clear polyethylene bags and the soil moisture was maintained at about field capacity and avoided leaching after irrigation event. Water and fertilizers were supplied via a drip irrigation system. The frequency of irrigation was dependent on solar radiation measured outside the greenhouse with pyranometer. The content of Ni and Cr in the fertilizer was negligible.

The concept of the automated scheduled irrigation system

Irrigation scheduling includes the determination of both frequency and duration of irrigation events in order to maintain soil moisture within desirable limits. The goal of irrigation is to restore the water that has been "consumed" through evapotranspiration to a level close to field capacity. In some special cases (i.e. saline soil conditions) more water is provided in order to create an optimum root environment.

Fig. 6 The automation of irrigation in the experimental greenhouse of TEIEP

Plant analysis

At the end of the experiments, approximately eight weeks after transplanting, shoots and roots were harvested. All the plant parts were oven-dried at 50°C to constant weight, ground in a Retsch Mixer Mill model MM 200 and passed through a 250 µm plastic sieve. Plant parts smaller than 250 µm in diameter (0.5 g) from each plant species and pot, were placed in porcelain beakers and ashed at 550°C. The residue was dissolved in 5 ml of 6N HCl and transferred to 100 ml volumetric bottles. The clear solutions were analyzed by ICP-OES (Thermo Scientific iCAP 6000). The operating conditions were: Nebulizer Gas flow rates: 0.5 L min^{-1}; Auxiliary Gas Flow: 0.5 L min^{-1}; Plasma Gas Flow: 15 L min^{-1}; Pump rate: 45 rpm; ICP RF Power: 1100 W. Aliquots of an ICP multi element 100 mg L^{-1} standard solution (Panreac) containing the analyzed elements, were used in the preparation of calibration solution. Working standard solutions were prepared by dilution of the stock standard solution to desired concentration in 1% HNO_3. The ranges of the calibration curves (6 points) were selected to match the expected concentrations for all the elements of the sample studied by ICP-OES. The correlation coefficient r^2 obtained for all cases was 0.9999. The detection limits (LOD) were calculated as the concentrations of an element that gave the standard deviation of a series of ten consecutive measurements of blank solutions.

Statistical analysis

The influence of Ni and Cr application in Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum* L.) was evaluated by 2-way analysis of variance (ANOVA). Statistical analysis was carried out with STATISTICA^{TM} Ver. 8.0 (StatSoft 2008) version 8 for all the parameters studied. All data *were analyzed with a one-way ANOVA* and subjected to Duncan's multiple range test *($p \leq 0.05$) for the mean treatment separation and comparison. S*tatistical significance of the effects due to treatments with Ni and Cr were determined.

Results and Discussion

Heavy metals pot experiment for Lavender (Lavandula angustifolia)

Effects of Ni contaminated irrigation water on the accumulation of Ni in shoots and roots

With no Ni addition in irrigation water, Nickelium concentrations in shoots fluctuated between 0.936 and 1.072 mg kg^{-1}. Nickelium accumulation in shoots increased with increasing Ni addition to the irrigation water (Fig. 7).

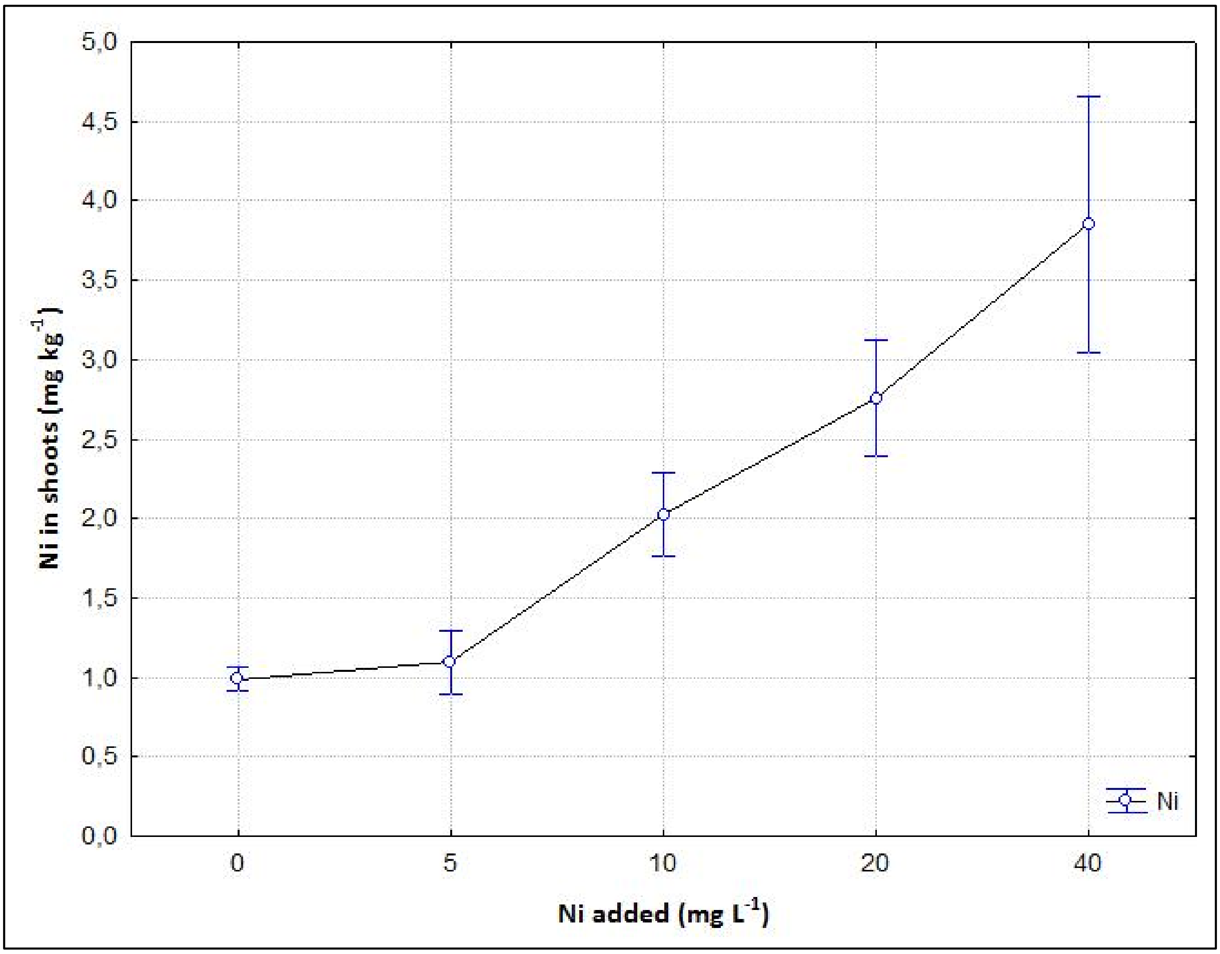

Fig. 7 Plot of means and conf. intervals (95.00%) for Ni concentration in shoots of Lavender as affected by Ni in irrigation water

The highest Ni accumulation in shoots was 3.853 mg kg^{-1} at 40 mg L^{-1} Ni addition. Duncan's multiple range test ($p \leq 0.05$) for the mean treatment - Ni (mg L^{-1}) added – show statistical significant differences due to Ni addition in irrigation water except between the 1st and the 2nd treatment (Table 3).

Table 3 Duncan means for groups in homogeneous subsets for Ni concentration in shoots of Levanda as affected by Ni in irrigation water

Duncan test; variable Ni (LEV_Ni_SHOOTS) Homogenous Groups, alpha = .05000 Error: Between MS = .10116

Treatment	Ni added (mg L^{-1})	Ni in shoots (mg kg^{-1})	1	2	3	4
1	0	0.987	****			
2	5	1.096	****			
3	10	2.023		****		
4	20	2.757			****	
5	40	3.853				****

With no Ni addition in irrigation water, Nickelium concentrations in roots fluctuated between 4.811 and 8.378 mg kg^{-1}. Nickelium accumulation in roots increased with increasing Ni addition to the irrigation water (Fig. 8). The highest Ni accumulation in roots was 21.337 mg kg^{-1} at 40 mg L^{-1} Ni addition. Duncan's multiple range test ($p \leq 0.05$) for the mean treatment - Ni (mg L^{-1}) added – show statistical significant differences due to Ni addition in irrigation water with an exception between 2nd, 3d treatments and 4th, 5th treatments (Table 4).

Table 4 Ducan means for groups in homogeneous subsets for Ni concentration in roots of Levanda as affected by Ni in irrigation water

Duncan test; variable Ni (LEV_Ni_ROOTS) Homogenous Groups, alpha = .05000 Error: Between MS = 4.7852

Treatment	Ni added (mg L^{-1})	Ni in shoots (mg kg^{-1})	1	2	3	4
1	0	6.080			****	
2	5	10.631	****			
3	10	12.126	****			
4	20	19.124		****		
5	40	21.337		****		

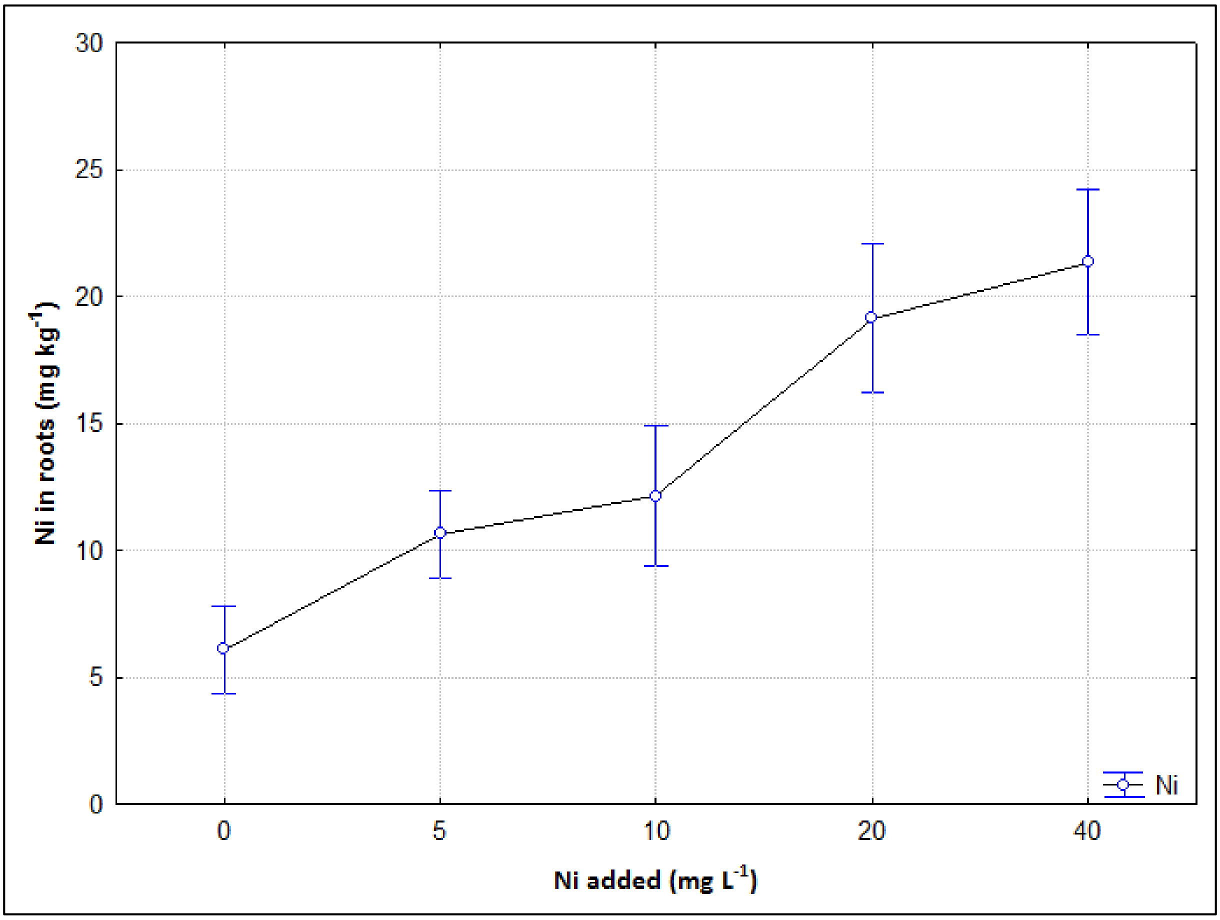

Fig. 8 Plot of means and conf. intervals (95.00%) for Ni concentration in roots of Lavender as affected by Ni in irrigation water

Effects of Cr contaminated irrigation water on the accumulation of Cr in shoots and roots

With no Cr addition in irrigation water, Chromium concentrations in shoots fluctuated between 0.179 and 1.098 mg kg^{-1}. Chromium accumulation in shoots increased with increasing Cr addition to the irrigation water (Fig. 9). The highest Cr accumulation in shoots was 5.561 mg kg^{-1} at 40 mg L^{-1} Cr addition. Duncan’s multiple range test ($p \leq 0.05$) for the mean treatment - Cr (mg L^{-1}) added – show statistical significant differences due to Cr addition in irrigation water with an exception between 1st, 2nd treatments and 4th, 5th treatments (Table 5).

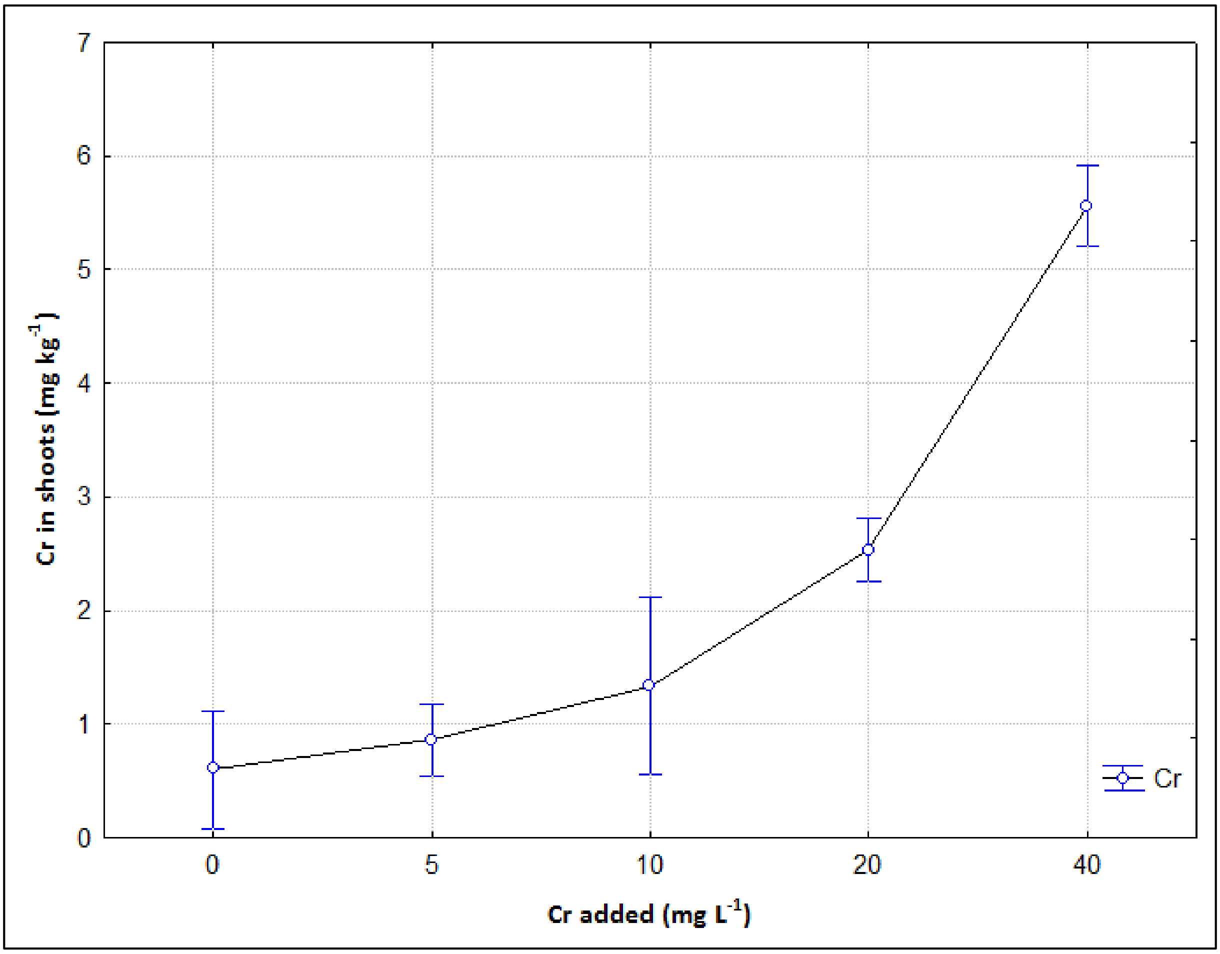

Fig. 9 Plot of means and conf. intervals (95.00%) for Cr concentration in shoots of Lavender as affected by Cr in irrigation water

Table 5 Ducan means for groups in homogeneous subsets for Cr concentration in shoots of Lavendrer as affected by Cr in irrigation water

Duncan test; variable Cr (LEV_Cr_SHOOTS) Homogenous Groups, alpha = .05000 Error: Between MS = .013430

Treatment	**Cr added (mg L^{-1})**	**Cr in shoots (mg kg^{-1})**	**1**	**2**	**3**	**4**
1	0	0.603	****			
2	5	0.858	****	****		
3	10	1.336		****		
4	20	2.530			****	
5	40	5.561				****

With no Cr addition in irrigation water, Chromium concentrations in roots fluctuated between 7.523 and 11.860 mg kg^{-1}. Chromium accumulation in roots increased with increasing Cr addition to the irrigation water (Fig. 10). The highest Cr accumulation in roots was 45.331 mg kg^{-1} at 40 mg L^{-1} Cr addition. Duncan's multiple range test ($p \leq 0.05$) for the mean treatment - Cr (mg L^{-1}) added - show statistical significant differences due to Cr addition in irrigation water with an exception between 1st, 2nd , 3d treatments (Table 6).

Table 6 Ducan means for groups in homogeneous subsets for Cr concentration in roots of Lavender as affected by Cr in irrigation water

Duncan test; variable Cr (LEV_Cr_ROOTS) Homogenous Groups, alpha = .05000 Error: Between MS = 8.9721						
Treatment	**Cr added (mg L^{-1})**	**Cr in shoots (mg kg^{-1})**	**1**	**2**	**3**	**4**
1	0	9.166	****			
2	5	12.532	****			
3	10	13.244	****			
4	20	22.779		****		
5	40	45.331			****	

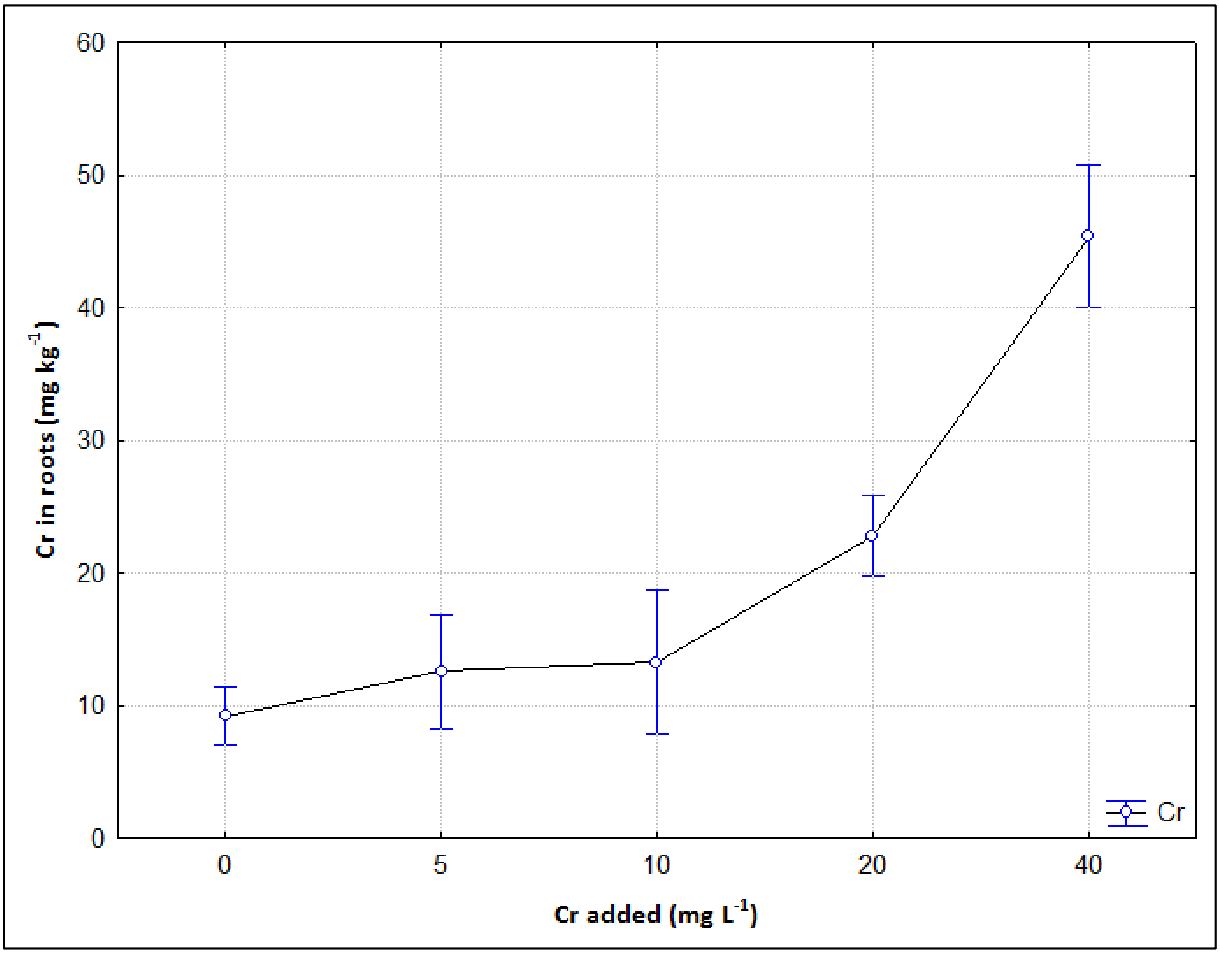

Fig. 10 Plot of means and conf. intervals (95.00%) for Cr concentration in roots of Lavender as affected by Cr in irrigation water

Heavy metals pot experiment for Sweet Bush Basil (Ocimum basilicum L.)

Effects of Ni contaminated irrigation water on the accumulation of Ni in shoots and roots

With no Ni addition in irrigation water, Nickelium concentrations in shoots fluctuated between 0.618 and 1.036 mg kg^{-1}. Nickelium accumulation in shoots increased with increasing Ni addition to the irrigation water (Fig. 11). The highest Ni accumulation in shoots was 1.829 mg kg^{-1} at 40 mg L^{-1} Ni addition. Duncan's multiple range test ($p \leq 0.05$) for the mean treatment - Ni (mg L^{-1}) added – show statistical significant differences due to Ni addition in irrigation water except between 1st, 2nd and 3d, 4th treatments (Table 6).

Table 7 Ducan means for groups in homogeneous subsets for Ni concentration in shoots of Sweet Bush Basil as affected by Ni in irrigation water

Duncan test; variable Ni (BAS_Ni_SHOOTS) Homogenous Groups, alpha = .05000 Error: Between MS = ,04477

	Ni added (mg L^{-1})	**Ni in shoots (mg kg^{-1})**	**1**	**2**	**3**	**4**
1	0	0.780	****			
2	5	0.901	****			
3	10	1.236		****		
4	20	1.243		****		
5	40	1.829			****	

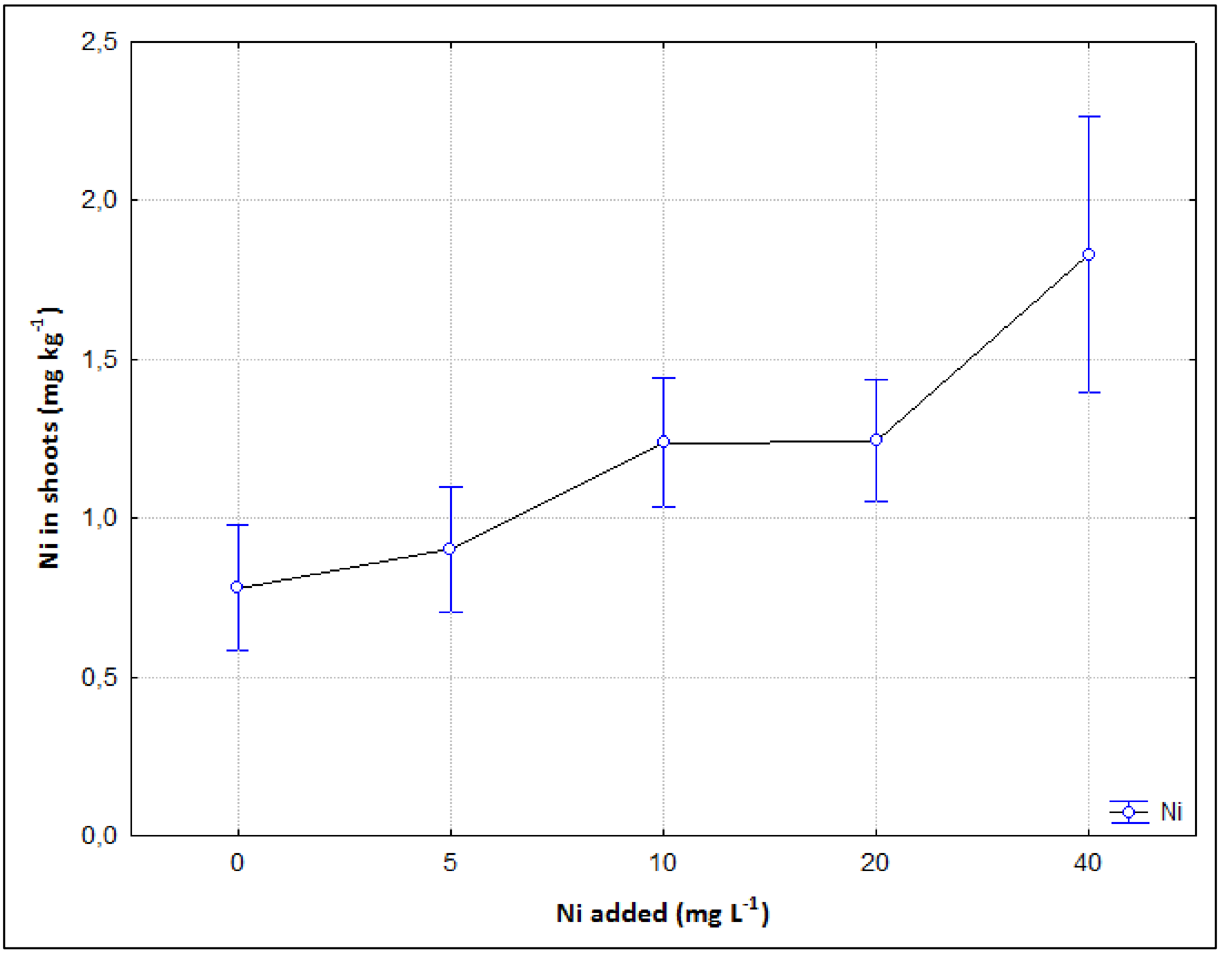

Fig. 11 Plot of means and conf. intervals (95.00%) for Ni concentration in shoots of Sweet Bush Basil as affected by Ni in irrigation water

With no Ni addition in water, Nickelium concentrations in roots fluctuated between 3.757 and 7.741 mg kg^{-1}. Nickelium accumulation in roots increased with increasing Ni addition to the irrigation water (Fig. 12). The highest Ni accumulation in roots was 63.549 mg kg^{-1} at 40 mg L^{-1} Ni addition. Duncan's multiple range test ($p \leq 0.05$) for the mean treatment - Ni (mg L^{-1}) added – show statistical significant differences due to Ni addition in irrigation water with an exception between 1st, 2nd and 2nd, 3d treatments (Table 8).

Table 8 Ducan means for groups in homogeneous subsets for Ni concentration in roots of Sweet Bush Basil as affected by Ni in irrigation water

Duncan test; variable Ni (BAS_Ni_ROOTS) Homogenous Groups, alpha = ,05000 Error: Between MS = 78,731						
	Ni added (mg L^{-1})	**Ni in shoots (mg kg^{-1})**	**1**	**2**	**3**	**4**
1	0	6.190	****			
2	5	12.912	****	****		
3	10	19.047		****	****	
4	20	27.048			****	
5	40	63.549				****

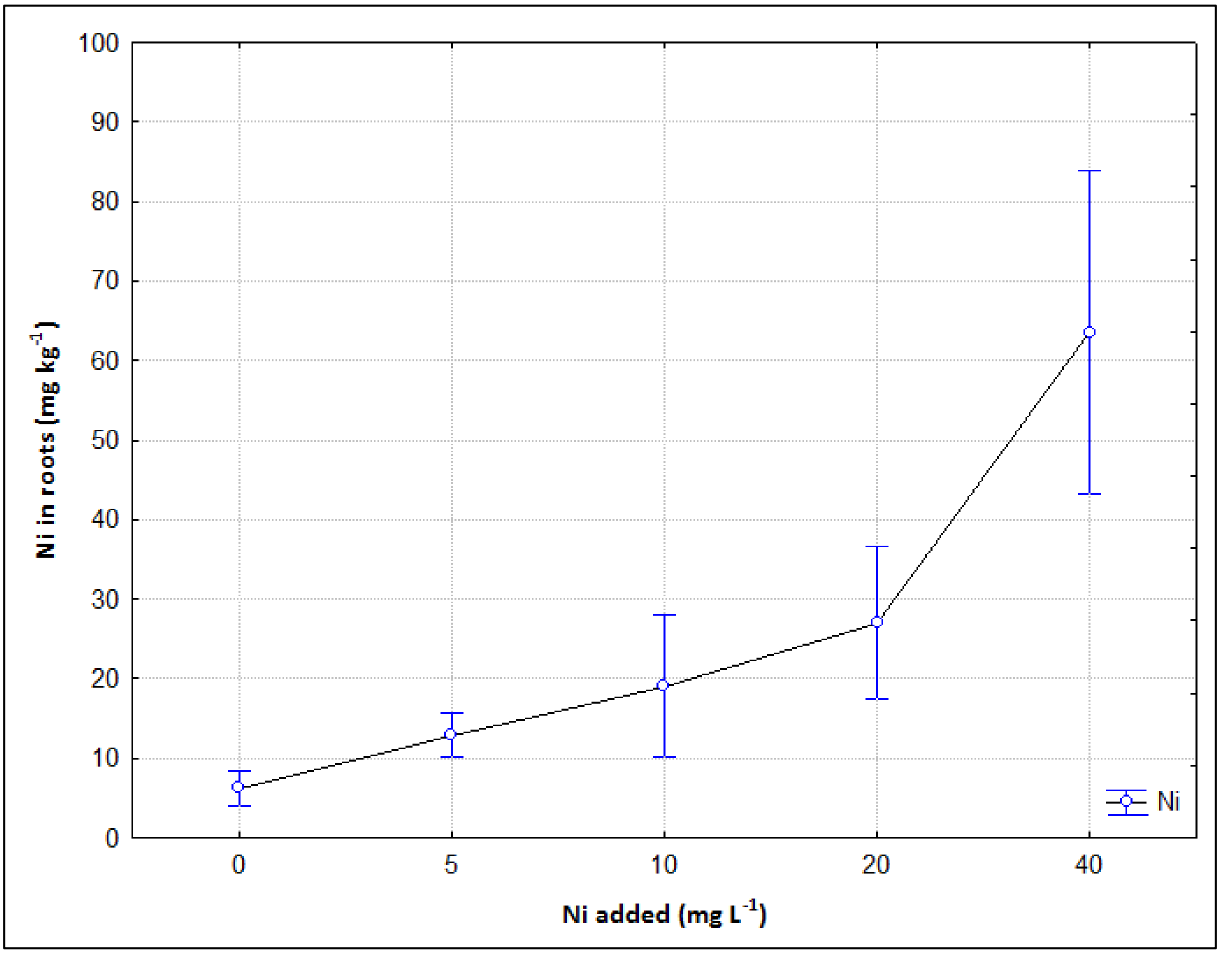

Fig. 12 Plot of means and conf. intervals (95.00%) for Ni concentration in roots of Sweet Bush Basil as affected by Ni in irrigation water

Effects of Cr contaminated irrigation water on the accumulation of Cr in shoots and roots

With no Cr addition in irrigation water, Chromium concentrations in shoots fluctuated between 0.558 and 0.819 mg kg^{-1}. Chromium accumulation in shoots increased with increasing Cr addition to the irrigation water (Fig. 13). The highest Cr accumulation in shoots was 1.485 mg kg^{-1} at 40 mg L^{-1} Cr addition. Duncan’s multiple range test ($p \leq 0.05$) for the mean treatment - Cr (mg L^{-1}) added – show statistical significant differences due to Cr addition in irrigation water with an exception between 1st, 2nd treatments and 3d, 4th, 5th treatments (Table 9).

Table 9. Ducan means for groups in homogeneous subsets for Cr concentration in shoots of Sweet Bush Basil as affected by Cr in irrigation water.

Duncan test; variable Cr (BAS_Cr_SHOOTS) Homogenous Groups, alpha = .05000 Error: Between MS = .02714						
	Cr added (mg L^{-1})	**Cr in shoots (mg kg^{-1})**	**1**	**2**	**3**	**4**
1	0	0.671		****		
2	5	0.829		****		
3	10	1.444	****			
4	20	1.479	****			
5	40	1.485	****			

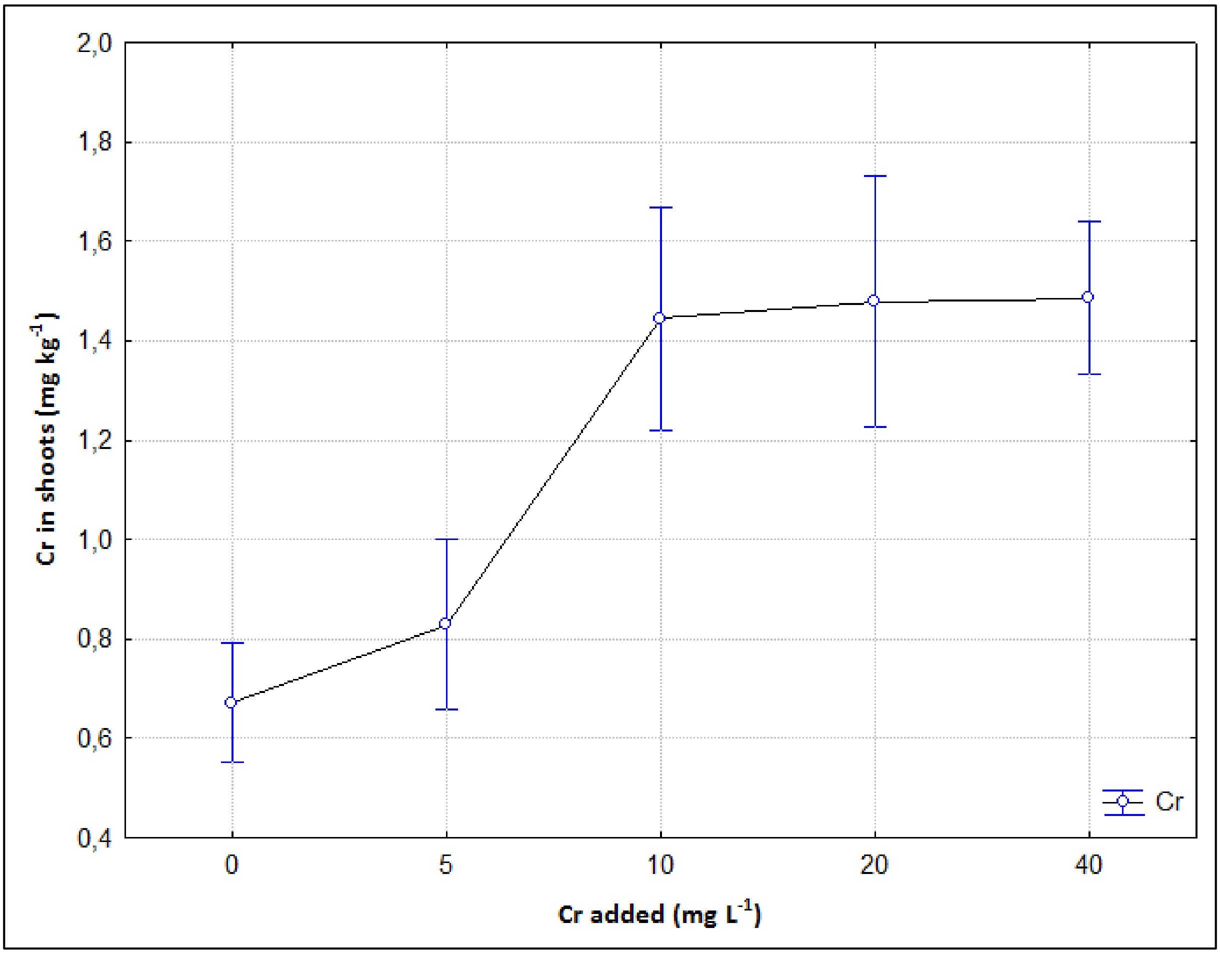

Fig. 13 Plot of means and conf. intervals (95.00%) for Cr concentration in shoots of Sweet Bush Basil as affected by Cr in irrigation water

With no Cr addition in irrigation water, Chromium concentrations in roots fluctuated between 5.334 and 10.063 mg kg^{-1}. Chromium accumulation in roots increased with increasing Cr addition to the irrigation water (Fig. 14). The highest Cr accumulation in roots was 117.280 mg kg^{-1} at 40 mg L^{-1} Cr addition. Duncan's multiple range test ($p \leq 0.05$) for the mean treatment - Cr (mg L^{-1}) added - show statistical significant differences due to Cr addition in irrigation water with an exception between the 1st and the 2nd treatment (Table 10).

Table 10 Ducan means for groups in homogeneous subsets for Cr concentration in roots of Sweet Bush Basil as affected by Cr in irrigation water

Duncan test; variable Cr (BAS_Cr_ROOTS) Homogenous Groups, alpha = .05000 Error: Between MS = 52.301						
	Cr added (mg L^{-1})	**Cr in shoots (mg kg^{-1})**	**1**	**2**	**3**	**4**
1	0	7.077	****			
2	5	14.967	****			
3	10	28.595		****		
4	20	54.668			****	
5	40	117.280				****

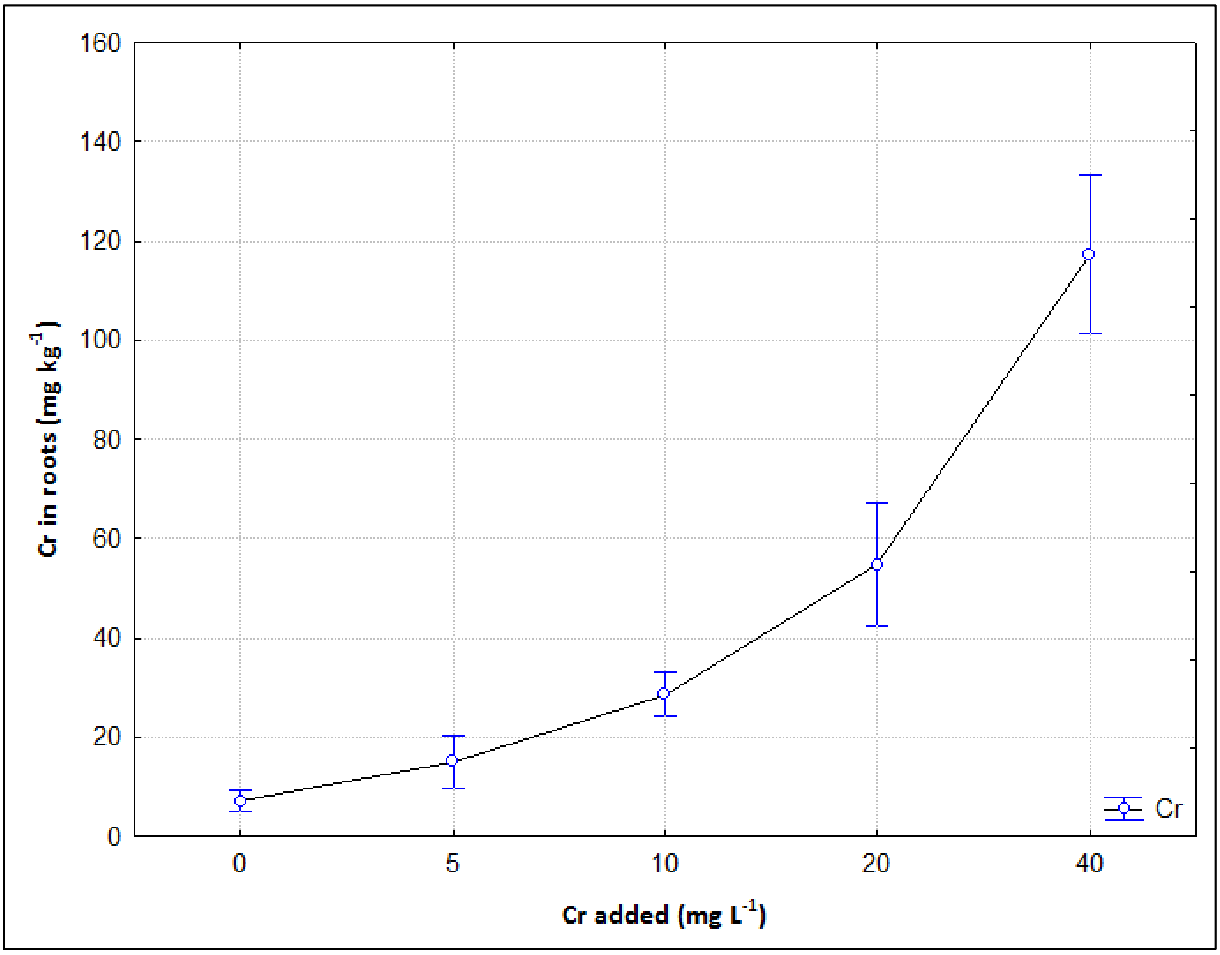

Fig. 14. Plot of means and conf. intervals (95.00%) for Cr concentration in roots of Sweet Bush Basil as affected by Cr in irrigation water.

Conclusions and Recommendations

The objectives of the study were to examine the cross-contamination of Nickelium (Ni) and Chromium (Cr) in shoots and roots of Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum* L.) as affected by different applications of Ni and Cr to the soil through irrigation contaminated water.

The results showed that plant absorbs Ni and Cr in considerable levels. The analysis of the results leads to the following conclusions that may have practical value, regarding the use of alternative heavy metal contaminated water resources for irrigation:

Ni and Cr (as total Ni and Cr) can pass directly through irrigation water to shoots and roots of Lavender (*Lavandula angustifolia*) and Sweet bush basil (*Ocimum basilicum* L.) depending on the irrigation water concentration of these heavy metals.

Lavender and Sweet bush basil cultivated in a soil never previously polluted with heavy metals, irrigated for the first time with different Ni(II) and Cr(IV) concentrations; practically can be found in nature; can be cross contaminated by the irrigation water's content Ni and Cr. Even if irrigation water does not contain Ni(II) and Cr(IV) heavy metals, total Ni and Cr can be transferred from soil to plant, as a result of an easily absorbed bioavailable element that is containing in soil derived from the parent material during pedogenesis processes.

The results show cross-contamination of Lavender and Sweet bush basil because of the use of of irrigation water polluted by Ni(II) and Cr(IV) heavy metals.

This report, closes with the wish that these important results and conclusions, will be a start for further activities by local authorities in Italy and Greece to inform the public about the hazards of heavy metals in food chain.

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