Interaction effect of nickel and cobalt in irrigation water on growth, chemical constituents and mineral content of Cypress (*Cupressus Sempervirens, L*) seedling grown on sandy soil

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Abstract: A pot experiment was conducted in two seasons (2013 and 2014) to evaluate the combination effect of nickel and cobalt at 0, 10, 20, 40 and 80ppm of both elements in irrigation water on growth, chemical constituents and mineral content of Cypress (*Cupressus Sempervirens*, L) seedling grown on sandy soil. Results showed that all tested treatments of Ni and Co combinations caused significant increases in stem length and stem diameter of Cypress seedling as compared with control treatment with the exception of 80 ppm Ni+80ppm Co combination treatment for stem length and 40 ppm Ni + 80 ppm Co and 80 ppm Ni combined with 40 or 80 ppm Co for stem diameter. The fresh and dry weight of root, stem and leaves were recorded the highest values by 10 ppm and 10 ppm of Ni and Co followed by 10 and 20 ppm Ni and Co., while the highest concentration of both heavy metals combinations (80 and 80 ppm) led to significant decreases in fresh and dry weight of seedling organs. The obtained results revealed that chlorophyll (a, b), carotenoids and sugars increased with Ni or Co at 10, 20 and 40 ppm concentration as compared with control treatment. However, the highest rate of either Ni or Co (80 ppm) reduced carotenoids, soluble, non- soluble and total sugars content less than control. Total indoles and shoot crude fiber content of Cypress seedling were increased with all Ni or Co treatments. Ni and Co treatments led to increases in leaf N, P, K, Ni and Co content of cypress seedling plant as compared with the control treatment. Moreover, leaf N, P and K content were showed progress increasing with increasing level of both Ni and Co from 10 to 40 ppm.

Keywords: cypress, heavy metals, macro elements, indoles, crude fiber, phenol

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1 Introduction

Common cypress (*Cupressus sempervirens L.*) is a conifer species known as Mediterranean or common cypress, is a medium-sized evergreen tree characterized by a very variable crown shape, from columnar to spread, dark green foliage and small ovoid brown cones. Cypress trees mainly used as an ornamental tree due to its conical crown shape, using for timber, using as a privacy screen, and protection against wind as well. Moreover, it can tolerate and grow in poor, barren, and superficial soils as well as the relatively adaptable to a range of temperatures. For all these reasons, cypress has been introduced in geographic areas that extend far beyond its natural distribution (Bagnoli et al., 2009).

In addition, Nashwa and Hoda (2014) concluded that *Cupressus semervirens,L* trees showed successfully growth under irrigation with treated sewage water which contains higher concentrations from heavy metals. In this

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context, Eid et al., (2015) concluded the success of the growth of cypress seedlings under irrigation conditions with water containing high concentrations of nickel or cobalt up to 80 ppm.

The interactions between nutrients take place when the supply of nutrient affect the uptake or function of another nutrient, thus the interaction can either induce deficiency or toxicity and can modify growth response and there are many specific as well as specific interactions between mineral nutrients of plants (Siedlecka, 1995).

Under the influence of the treated municipal wastewater (TMWW) reuse, Kalavrouziotis et al., (2008) reported that several interactions are taking place between cadmum (Cd), lead (Pb), Nickel (Ni), and cobalt (Co) in the plant tissues of Broccoli plant; they may either increase or decrease the concentration of one of the above mentioned metals or even of the essential elements in the plant parts. Although, Talukder and Sharma (2007), mentioned that Co has been found to interact with many elements and it is antagonistically related with Cd, as at high levels it suppresses its uptake. Similarly, Co has been found to interact antagonistically with Fe, to the extent of causing Fe deficiency (Wallace and Abou-Zamzam, 1989). Also, (Ni), has been reported to be antagonistically related to the Fe leaf level, as low concentrations of Fe are found in the plant, in the presence of high Ni concentrations in the tissues (Foy et al., 1978). On the contrary, Co is synergistically related to Ni, as it has been reported that toxic Co levels of 10-20 mg/kg dry matter, were associated with excess Ni (Anderson et al., 1973).

In addition, Kalavrouziotis et al., (2009) found many statistically significant interactions between Cd, Pb, Co and Ni in the various parts of broccoli plants under irrigation with TMWW such as synergistic interactions of both Co × Pb in the heads at low Co level and Ni × Pb in the leaves, while, antagonistic Co × Pb interaction in the heads.

Also, in this context Rengel (2004) mentioned that heavy metal such as Ni and Co are generally non-toxic and are essential for development and growth of plant, but when their quantity exceeds over the limit will become toxic and inhibit plant growth.

The present research aimed to study the tolerance capacity of *Cupressus sempervirens, L.* seedlings to different combinations of both Ni and Co concentrations in irrigation water and their effects on growth, photosynthetic pigments, some biochemical constituents and some mineral nutrients content.

2 Material and methods

A pot experiments were conducted in National Research Centre, Dokki, Cairo, Egypt during 2013 and 2014 seasons using *Cupressus sempervirens* L.seedlings in sandy soil. The physical and chemical characteristics of the used soil are determined according to Jackson (1973) and presented in Table 1.

Table 1 Physical-chemical properties of used soil

Mechanical analysis %			Tex.	Physical properties				Macronutrients (mg/100 g)					Heavy metal (mg/kg)	
Sand	Silt	Clay	Sandy	pH	EC (dS/m)	$CaCO_3$ (%)	O.M (%)	P	K	Ca	Mg	Na	Ni	Co
94	3.9	2.1		8.25	0.27	1.67	0.35	0.26	12.09	20.60	7.80	8.74	ND*	ND*

Note: *ND: Not Detectable.

2.1 Plant materials and procedures

One year old seedlings of *Cupressus sempervirens L.* was obtained from the Horticulture Research Institute, Giza. The average length of seedlings was 30-35 cm. On March 25th 2013 and 2014, seedlings were transplanted in 30 cm pots (one plant/pot) filled with sandy soil. The seedlings were arranged in a randomized complete blocks design included 17 treatments, each treatment contained three replicates. Each replicate included 5 plants (i.e. 15 plants per treatment).

All plants received NPK as follows 4.0 g ammonium nitrate 33.5% N, 4.0 g calcium super phosphate 15.5% P_2O_5 and 2.0 g potassium sulphate 48.5% K_2O/pot) in six doses. The plants were fertilized monthly after one month from transplanting.

Nickel as nickel chloride (NiCl) and cobalt as cobalt sulphate ($CoSO_4$) were used after dissolving to prepare the concentration using the distilled water and added as surface irrigation regime began after 30 days from transplanting and repeated twice every week till the end of the season (October 25th 2013 and 2014).

The treatments were represented by all combinations of nickel and cobalt concentrations described in Table 2.

Table 2 Combination of nickel and cobalt concentration treatments

Treatment	Nickel (ppm)	Cobalt (ppm)	Treatment	Nickel (ppm)	Cobalt (ppm)
1-Control	0	0	10	40	10
2	10	10	11	40	20
3	10	20	12	40	40
4	10	40	13	40	80
5	10	80	14	80	10
6	20	10	15	80	20
7	20	20	16	80	40
8	20	40	17	80	80
9	20	80			

The samples have been taken on 25th October 2013 and 2014 at the end of the experiment to determine growth characters, i.e. stem length (cm), stem diameter (mm), fresh and dry weight of seedling organs (g). Also, Chemical constituents of chlorophyll (a), (b) and carotenoids, soluble-non-soluble and total sugars content, total indol, total phenol, crude fiber percentage and minerals N, P, K, Ni, Co.

Chlorophyll (a), (b), and carotenoids content were determined in leaves according to (Saric et al., 1967). Soluble, non-soluble and total sugars content was determined according to (Dubois et al., 1956). Crude fiber percentage was determined in shoot and leaves by method of (Garner, 1949). Total phenols were determined in leaves by using method of (A.O.A.C. 1980). Total indoles were determined in leaves using the method described by (Selim et al., 1978). Nickel and cobalt percentage were determined and measured using the Atomic Absorption Spectrophotometer Zeiss FMD_3 according to (Jackson, 1973).

Total nitrogen determined by using the micro kjeldahl method (A.O.A.C. 1980). Phosphorus, potassium, Ni and Co was extracted by using dry ashing technique according to (Chapman and Pratt, 1978). Phosphorus was photometrical determined using vanadate method and measured by spectrophotometer, while potassium was measured by flam photometer. Ni and Co were measured by Atomic Absorption Spectrophotometer Zeiss FMD_3. Data of the experiments were subjected to statistical analysis, according to the procedure of Snedecor and Cochran (1990), where the means of the studied treatments were compared using least significant differences (L.S.D) test at 0.05 probability levels.

3 Results and Discussion

3.1 Effect of Nickel and Cobalt combinations on growth parameters

3.1.1 Stem length and diameter

As shown in Table 2 as an average of the two seasons, it is clear that there were significant increases in stem length and stem diameter due to all Ni and Co combinations, with the exception of 80 ppm Ni + 80 ppm Co for stem length and 40 ppm Ni +80 ppm Co, 80 ppm Ni+40 ppm Co, 80 ppm Ni +80 ppm Co for stem diameter. The data also appeared that Ni at 20 ppm + Co at 20 ppm combination produced the highest values of stem length and diameter as compared with the control and other treatments.

3.1.2 Fresh and dry weight of seedling organs

Results in Table 3 as an average of the two seasons showed that root, stem and leaves fresh and dry weight of seedlings were significantly increased only by some combination at low concentrations of both Ni and Co, while Ni at 40 ppm combined with Co at 40 or 80 ppm as well as Ni at 80 ppm combined with all Co concentration led to significant reduction in fresh and dry weight of seedling organs. Also leaves revealed significant decreases with most Ni and Co combinations treatments.

In addition, the highest values of root and stem for fresh and dry weight were obtained with Ni at 10 ppm + Co at 10 ppm combination, while the combination of Ni at 40 ppm + Co at 10 ppm resulted the highest values of leaves fresh and dry weight as compared with control or other combinations of Ni and Co combination treatments.

The enhancing of the growth parameters due to Ni and Co combination treatments at the low concentrations of both metals may be attributed to the activation of some enzymes and photosynthesis in plants. On the contrary with high concentrations of Ni and Co combinations caused reduction of plant growth characters such as fresh and dry weight of roots and shoot. Similar results were reported by Athar and Ahmad (2002), Khan and Khan (2010), Jaleel et al., (2009), who reported that the higher levels of Ni or Co resulted in reduction of, shoot growth, which suggests that heavy metals inhibit root and shoot growth directly by inhibiting cell division or cell elongation, or a combination of both. Furthermore, excess Ni or Co has been shown to decrease content of essential nutrients in plant tissues which may reduce the biosynthesis of these metalloenzymes by causing deficiencies of these essential nutrients (Gajewaska, et al., 2006).

These obtained results indicate that the growth of cypress seedlings under the low and medium concentrations of cobalt and nickel is improved. It can be said that these plants can grow naturally and well when cultivated and irrigated with water of lower quality, which contain these heavy metals at medium concentrations such as wastewater or industrial drainage and thus, good water can be provided for the irrigation of plants eaten by humans, animals or plants sensitive to those heavy metals, especially under conditions of lack of fresh water.

Table 3 Interaction effect of nickel and cobalt in irrigation water on some growth parameter of Cypress seedling (Average of the two seasons)

Treatments		Stem length (cm)	Stem diameter (mm)	Fresh weight of seedling organs (g)			Dry weight of seedling organs (g)		
Nickel (ppm)	Cobalt (ppm)			Root	stem	Leaves	Root	stem	Leaves
Control		45.89	6.50	28.75	61.94	83.27	13.12	20.32	35.71
10	10	78.38	12.0	43.76	90.51	101.34	20.59	37.19	42.55
	20	81.26	12.80	38.74	81.24	87.81	18.19	31.33	36.87
	40	89.3	11.50	35.40	80.20	86.99	17.06	23.61	39.34
	80	70.2	10.20	28.10	77.40	61.40	14.50	23.20	22.30
20	10	95.88	14.90	31.52	63.85	78.36	14.81	28.75	32.89
	20	104.36	15.30	28.36	77.73	83.58	13.36	30.04	35.07
	40	73.8	10.50	37.40	77.50	72.23	12.89	31.59	30.90
	80	66.5	7.40	31.20	70.20	59.40	9.70	31.80	19.30
40	10	63.9	10.80	30.20	71.40	119.89	9.63	34.97	44.23
	20	60.5	11.30	30.80	74.30	105.39	13.86	20.13	36.32
	40	55.2	7.00	27.30	60.20	63.26	8.60	16.95	24.48
	80	50.1	6.10	20.20	52.10	60.80	6.60	19.20	24.20
80	10	51.2	9.70	16.50	44.30	49.00	7.20	13.50	22.20
	20	52.8	7.30	15.40	43.80	47.50	6.30	13.0	20.10
	40	50.1	5.10	13.20	34.70	45.45	4.40	10.5	18.30
	80	48.9	5.00	10.0	30.10	44.35	3.20	9.20	16.00
LSD 5%		3.90	0.50	1.40	2.69	11.23	0.43	0.87	4.01

3.2 Effect of Nickel and Cobalt combinations on some chemical constituents

3.2.1 Chlorophyll (a), (b), carotenoids and sugars content

The obtained results as an average of the two seasons as shown in Table 4 (1) revealed that the highest values of chlorophyll (a, b), found when the plants were treated with combination of Ni at 40 ppm + Co at 10 ppm concentration while the highest values of carotenoids, soluble, non-soluble and total sugars content of leaves were found when the plants were treated with combination of Ni at 20 ppm + Co at 40 ppm concentration as compared with control or other treatments. However high concentrations of both metals combinations reduced Chlorophyll (a), (b), carotenoids, soluble, non- soluble and total sugars content less than control treatment. Excess Ni has been reported to cause damages to the photosynthetic apparatus and decreases chlorophyll content (chlorophyll a, b, total chlorophyll and chlorophyll a/b ratio) (Gajewaska et al., 2006).

In addition, similar results were found and illustrated by Zengin and Munzuroglu (2005), Dubey and Pandey (2011), Kadhim (2011) and Jaleel et al., (2009), they found inhibition of chlorophyll content with increasing concentrations of heavy metals such as Ni and Co. Also, Pandey (2011) reported that increasing Ni concentration in growth medium led to reduction in chlorophyll a, b and total chlorophyll content of water lettuce plant; however the low level (0.01ppm) stimulates photosynthetic content.

Table 4.1 Interaction effect of nickel and cobalt in irrigation water on chlorophyll (a, b), carotenoids and sugars content in leaves of Cypress seedlings (Average of two seasons)

Treatments		Chlorophyll (a)	Chlorophyll (b)	Carotenoids	Sugars (%)		
Nickel (ppm)	Cobalt (ppm)	(mg/g fresh weight)			Soluble sugars	Non-Soluble sugars	Total sugars
Control		0.58	0.23	0.76	41.30	3.80	45.10
10	10	2.21	0.85	1.02	45.20	2.89	48.09
	20	2.36	0.93	1.05	47.30	3.74	51.04
	40	2.00	1.12	1.13	50.30	4.25	54.55
	80	0.80	0.65	0.31	33.20	1.85	35.05
20	10	1.89	1.05	1.24	48.70	4.49	53.19
	20	1.34	1.12	1.47	49.90	4.34	54.24
	40	1.75	1.19	1.63	60.20	5.21	65.41
	80	0.75	0.55	0.28	30.10	1.87	31.97
40	10	2.51	1.21	1.16	48.90	4.48	53.38
	20	0.83	0.63	1.05	33.20	2.39	35.59
	40	0.64	0.28	0.93	30.80	2.41	33.21
	80	0.62	0.50	0.21	28.30	1.34	29.64
80	10	0.41	0.22	0.66	31.30	2.31	33.61
	20	0.40	0.18	0.60	27.20	1.84	29.04
	40	0.21	0.14	0.51	20.40	1.72	22.12
	80	0.18	0.14	0.47	16.50	1.70	18.20
LSD 5%		0.38	0.12	0.12	2.72	0.19	1.34

3.2.2 Indoles, Phenols and Crude fiber content

As an average of the two seasons data in Table 4.2 showed that, total indole content of leaves was significantly increased by Ni and Co in combinations only at 10 and 20 ppm of Ni combined with all concentration of Co (10, 20, 40, 80 ppm) while, significantly reduction was resulted from high Ni concentrations (40 and 80 ppm) combined with all Co concentrations (10, 20, 40 and 80 ppm). On the contrary phenol leaves content significantly decreased with 10 and 20 ppm of Ni combined with all concentration of Co (10, 20, 40, 80 ppm) and significantly increased with high Ni concentrations (40 and 80 ppm) combined with all Co concentrations (10, 20, 40 and 80 ppm) as compared with the control treatment. The highest values of leaves indoles and phenols content were obtained from 20 ppm Ni + 40 ppm Co and 40 ppm Ni + 40 ppm Co combinations treatments, respectively compared with the control and other treatments.

In concern of stem and leaves crude fiber contents, it is worth to note that stem crude fiber content was significantly increased with all combination treatments of Ni and Co as compared with the control treatment. However, leaves crude fiber content was only significantly increased by 20 ppm Ni combined with 20, 40 or 80 ppm Co. The highest values of stem and leaves crude fiber content were attained by 20 ppm Ni + 20 ppm Co and 20 ppm Ni + 40 ppm Co combination treatments, respectively as compared with the control and other treatments. Similar results were obtained by Kadhim (2011) and Jaleel et al., (2009).

Table 4.2 Interaction effect of nickel and cobalt in irrigation water on Indoles, Phenols and Crude fiber of cypress seedling (Average of two seasons)

Treatments		Total indoles	Total phenols	Crude fiber (%)	
Nickel (ppm)	Cobalt (ppm)	(mg/g leaves fresh weight)		Stem	Leaves
Control		18.34	6.32	24.3	30.52
10	10	22.89	2.23	48.70	22.41
	20	28.93	4.32	49.80	25.62
	40	30.32	4.64	47.05	22.82
	80	25.30	0.65	41.30	20.33
20	10	33.21	5.21	58.73	26.93
	20	35.05	5.83	70.41	37.51
	40	43.41	2.71	62.70	37.92
	80	31.30	0.60	50.1	33.78
40	10	19.45	2.09	35.6	20.21
	20	15.21	10.31	35.8	18.30
	40	8.01	10.42	35.6	15.70
	80	7.77	0.43	30.2	13.47
80	10	8.15	9.41	40.2	15.20
	20	8.13	9.21	45.3	13.30
	40	7.21	8.10	55.8	11.10
	80	7.0	7.40	58.6	9.80
LSD 5%		2.82	0.56	1.34	3.12

The results of plant analysis also showed an improvement in the plant chemical content under the low and medium concentrations, of nickel and cobalt combinations, which reflects the improved physiological performance of the plant.

3.2.3 Mineral element contents

Mineral elements content of cypress seedling leaves are presented in Table 5. Macronutrients, i.e. nitrogen (N) and phosphorus (P) were significantly increased by Ni at 10, 20 and 40 ppm combined with all Co concentration rates (10, 20, 40 and 80 ppm) and the highest values of N and P content were attained from 20 ppm Ni +40 ppm Co combination treatment. On the other hand, K content was significantly increased with 10 and 20 ppm Ni combined with all concentration rates of Co treatments, except 20 ppm Ni + 80 ppm Co combination treatment. The highest values of N and P content were obtained by Ni at 20 ppm plus Co at 40 ppm, while highest K leaves content resulted from Ni at 20 ppm + Co at 10 ppm combination treatment as an average of two seasons. Similar findings were found by Jaleel et al., (2009), who reported that increasing Co levels in growth medium had negative effect on N, P and K content of maize plant.

Table 5 Interaction effect of nickel and cobalt in irrigation water on mineral elements content in leaves of Cypress seedling (Average of two seasons)

Treatments		Macronutrients content (mg/g dry weight)			Heavy metal content (mg/kg dry weight)	
Nickel (ppm)	Cobalt (ppm)	Nitrogen	Phosphorus	Potassium	Nickel	Cobalt
Control		3.56	0.13	4.21	0.09	0.113
10	10	7.32	0.29	7.31	1.39	0.531
	20	8.58	0.26	8.43	1.10	0.834
	40	9.18	0.29	8.88	1.05	0.952
	80	7.30	0.20	6.21	1.81	1.820
20	10	10.35	0.33	8.98	1.89	1.270
	20	10.62	0.36	8.97	1.45	1.434
	40	11.53	0.37	7.43	1.18	1.743
	80	5.10	0.17	3.21	2.31	1.930
40	10	5.63	0.18	4.25	1.95	2.290
	20	6.43	0.18	4.11	1.83	2.841
	40	7.17	0.23	4.09	1.77	3.080
	80	4.30	0.18	2.41	2.45	2.100
80	10	2.34	0.11	3.61	1.43	2.100
	20	2.30	0.07	3.60	1.68	2.500
	40	1.68	0.07	3.00	4.11	5.200
	80	1.60	0.04	2.80	4.25	8.300
LSD 5%		0.53	0.03	0.28	0.27	0.51

Regarding Ni and Co content of leaves the data obviously showed that all combinations of Ni and Co concentrations significantly increased leaves content from those minerals (Ni and Co) and the maximum Ni and Co content in leaves of cypress seedlings were recorded at 80 ppm Ni + 80 ppm Co combination treatment.

The activation and inhabitation of mineral elements uptake due to heavy metals concentrations were similar obtained by Wallace and Abou-Zamzam (1989). Also, Pandey (2011) reported that increasing Ni concentration in growth medium (10ppm) led to high accumulation of Ni in plant. In addition, Gajewaska et al., (2006) stated that high Ni levels in plants reduce the rate of metabolic activities and decreased water and nutrient uptake in plant, which were found in our obtained results.

These results showed that N, P, K concentrations were improved under the low and medium concentrations of nickel and cobalt combinations, which is explained as the activation of plant uptake of these nutrients and increased utilization, thus reducing the additives of these major nutrients.

4 Conclusion

The results indicated to the possibility success of planting cypress seedlings under conditions of irrigation water containing nickel with concentrations up to 80 ppm together with cobalt concentration up to 40 or 80 ppm with no or low negative on cypress seedlings growth. Further studies should be conduct with another heavy metals combination or under irrigation with low quality water such as drainage and wastewater or sewage water to save providing quantities of fresh water to irrigate edible plants and plants which are sensitive to those concentrations of heavy metals.

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