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Growth, chemical constituents and mineral content of Cypress (*Cupressus Sempervirens*, L.*)* seedlings grown on sandy soil as influenced by Nickel and Cobalt in irrigation water

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Abstract: A pot experiment was conducted in two seasons (2013 and 2014) to evaluate the sensitivity of Cypress seedlings grown on sandy soil conditions to various concentrations of both nickel (Ni) and Cobalt (Co) *i.e.* 0, 10, 20, 40 and 80ppm in irrigation water. Results showed that all tested treatments of both heavy metal (Ni or Co) caused significant increases in stem length and stem diameter of Cypress seedling as compared with control treatment with the exception of 80 ppm Ni or Co treatment for stem length and 10, 80 ppm Ni for stem diameter. The fresh and dry weight of root, stem and leaves were significantly increased by 10 and 20 ppm Ni or Co, while raising concentration of both heavy metals to 40 and 80 ppm led to significant decreases in fresh and dry weight of seeding organs in most cases. 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 high 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 seedlings 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 seedlings as compared with 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.
Key words: cypress, heavy metals, Ni, Co, macro elements, indoles, crude fiber, phenol.

Introduction

Cupressus semervirens,L. has been distributed throughout the Mediterranean region since classical times and is an evergreen tree, it grow in a tapering columnar arrived to a height of 20 ft. Its branches are thickly covered with small, imbricate (overlapping like roof tiles), shiny green leaves. The timber is hard, dose grained, and of a fine reddish hue and very durable**.**

The trees of *Cupressus semervirens*,L. is mainly used as ornamental trees due to its conical crown shape, also it can be used for timber, as a privacy screen, and protection against wind. It is relatively adaptable to a range of temperatures, and can tolerate more continental climates. Moreover, it can grow in nutritionally poor soils with different soil characterizations. For all these reasons, it has been introduced in geographic areas that extend far beyond its natural distribution [(1)]. Moreover, [(2)] concluded that *Cupressus semervirens*,L. trees showed successfully growth under irrigation with treated sewage water which contains higher concentrations from heavy metals.

Heavy metals are one of the most important environmental pollutants, thus it have been took a great attention of many researchers **[3]**. In recent years, for low cost sorbets such as algae, fungi, bacteria and plants have investigated for their bio-sorption capacity towards heavy metals **[4]**.

Nickel (Ni) is one of the heavy metals and in the same time is considered an essential trace element for higher plant nutrition **[5]** because of its important in the metalloenzyme urease **[6]** and **[7]** which required for nitrogen metabolism in higher plant **[8]**.

However, exposure to excessive Ni can cause toxic effects on plants as in toxic effect on pollen germination **[9]** or in the inhibition of growth and many parameters of plants **[10]**. Also, excess of Ni causes inhibit a many enzymes and interferes with several aspect of plant biochemistry, such as photosynthesis and pigment synthesis **[11]**.

Cobalt (Co) is essential for Rhizobium which it associates symbiotically with legume roots for N2 fixation, free living nitrogen – fixing bacteria, however, also Co has a direct role in the metabolism of higher plants **[12]**.

Higher concentration of these metals however, may prove toxic and severely interfere with physiological and biochemical functions **[13], [14]** and **[15]**. Also, in this context **[16]** stated 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 they become toxic and inhibit plant growth.

The present study was aimed to find out tolerance capacity of *Cupressus Sempervire* seedlings to various concentrations of nickel (Ni) and cobalt (Co) in irrigation water and its effect on growth, photosynthetic pigments, some chemical components and some mineral elements uptake.

Material and Methods

Two pot experiments were conducted in National Research Centre, Dokki, Giza, Egypt during 2013 and 2014 seasons using *Cupressus sempervirans*, L. seedlings in sandy soil. The physical and chemical Characteristics of the used soil are determined according to **[17]** and presented in Table (1).

Table 1: Physico-chemical properties of used soil.

Mechanical analysis %			**Tex.**	**Physical properties**				**Macronutrients (mg/100 g)**					**Heavy metals (mg/Kg)**	
Sand	Silt	Clay	Sandy	pH	EC (mS/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*

*ND: Not Detectable

Plant Materials and Procedures:

One year old seedlings of *Cupressus sempervirans*, L. was obtained from the Horticulture Research Institute, Giza. The average heights of seedlings were 30-35 cm. On March 25^{th} 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 9 treatments, each treatment contained 3 replicates. Each replicate included 5 plants (i.e. 15 plants per treatment).

Basic dressing:

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

Nickal as nickel chloride (NiCl) and cobalt as cobalt sulphate ($CoSO_4$) were used after dissolving to prepare to determined 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).

Concentrations used were as follows:

1. Control	2. Ni (10 ppm)
3. Ni (20 ppm)	4. Ni (40 ppm)
5. Ni (80 ppm)	6. Co (10 ppm)
7. Co (20 ppm)	8. Co (40 ppm)
9. Co (80 ppm)	

Data recorded:

The samples have been taken on 25th October 2013 and 2014 to determine the following:

Growth characters:

1. Stem length (cm).
2. Stem diameter (mm).
3. Fresh weight of plant organs (g).
4. Dry weight of plant organs (g).

Chemical constituents:

1. Chlorophyll (a), (b) and carotenoids.
2. Soluble-Non-soluble and total sugars content.
3. Total-indol.
4. Total-phenol.
5. Crude fiber percentage.
6. Minerals N, P, K, Ni and Co.

Chlorophyll (a), (b) and carotenoids contents were determined in leaves according to [(18)]**.** Soluble, non-soluble and total sugars content was determined according to [(19)]**.** Crude fiber percentage was determined in shoot and leaves by method of [(20)]**.** Total phenols were determined in leaves by using method [(21)]**.** Total indoles were determined in leaves using the method described by [(22)]**.**

Plant nutrients were determined as follows:

Total nitrogen by using the micro kjeldahl method [(21)]. Phosphorus, potassium and micronutrients were extracted by using dry ashing technique according to [(23)]. Phosphorus was photometrically determined using vanadate method and measured by spectrophotometer, while potassium was measured by flam photometer. Nickel and cobalt percentage were determined using the Atomic Absorption Spectrophotometer Zeiss FMD_3 according to [(17)].

Data of the experiments were subjected to statistical analysis, according to the procedure of [(24)] where the means of the studied treatments were compared using L.S.D test at 0.05 probability levels.

Results and Discussion

1. Effect of Nickel and Cobalt on growth parameters:

1.1 Stem length:

Results in Table (2) show that all tested treatments of both heavy metal (Ni or Co) caused significant increases in stem length as an average of two seasons as compared with control with the exception only of Ni treatment at 10 ppm. Data in the same table revealed that stem length increased with increasing Ni or Co rate from 10 to 20 or 40 ppm then decreased with increasing Ni or Co rate to 80 ppm, however very slightly increases between 20 and 40 ppm rate and insignificant increases in stem length in concern of Ni and Co, respectively. Also, it was clearly surpassing to Co treatment on Ni treatment in enhancing stem length and the highest stem length value was obtained from 40 ppm Co treatment.

1.2 Stem diameter:

Data in Table (2) showed that stem diameter of cypress seedlings was significantly increasing by all treatments of Ni and Co concentrations as compared with control as an average of two seasons with the exception of 10 and 80 ppm Ni treatments. Referring to the Ni and Co effects, results clearly indicated to superiority of Co treatments at all rates which led to significant increases. The increasing concentration from 10 to 40 ppm Co led to significant increases with increasing Co concentration then significantly decreases with increasing concentration to 80 ppm as compared with 40 ppm Co. The highest value of stem diameter attained from 40 ppm Co, while in the concern of Ni the significant increases attained from 10 to 20 ppm then significantly decreased with increasing concentration to 40 and 80 ppm.

1.3 Fresh weight of seedling organs:

Table (2) shows that root, stem and leaves fresh weight of cypress seedlings were significantly increased by all treatments of Ni and Co as an average of two seasons as compared with control treatment with the exception of 40, 80 ppm Ni and 10 ppm Co in concern of root fresh weight. Results of the same table indicated that seedling organs i.e. roots, stem and leaves were significantly increased with increasing Ni rates from 10 to 20 ppm then decreased by 40 and 80 ppm. However, root and stem fresh weight were signicantly increased with increasing Co rates from 10 to 40 ppm, then decreased with 80 ppm while leaves fresh weight was significantly increased with increasing Co rates from 10 to 20 ppm and decreased with 40 and 80 ppm.

Table (2): Effect of Nickel and Cobalt on some growth parameter of Cypress seedlings (Average of two seasons)

Treatments		Stem length (cm)	Stem diameter (mm)	Fresh weight of seedling organs (g)			Dry weight of seedling organs (g)		
				Root	stem	leaves	root	stem	leaves
Control		45.89	6.50	27.83	56.51	81.26	12.36	20.91	34.10
Ni	10 ppm	47.38	6.60	29.54	58.94	84.54	15.17	21.79	35.49
	20 ppm	58. 46	10.90	36.87	69.83	91.37	16.93	25.83	39.26
	40 ppm	57.36	9.10	20.31	61.30	83.45	9.63	22.98	35.03
	80 ppm	33.81	7.30	16.20	52.80	75.00	6.30	19.20	28.10
Co	10 ppm	73.83	11.20	28.37	73.85	92.41	13.30	27.31	38.81
	20 ppm	79.64	12.40	32.97	78.54	95.88	15.46	29.05	40.23
	40 ppm	81.59	14.20	38.20	81.40	93.54	19.04	23.49	37.37
	80 ppm	38.70	10.30	30.30	73.20	66.30	10.30	15.40	25.20
LSD 5%		2.03	0.85	1.62	1.98	2.84	1.47	1.37	1.56

1.4 Dry weight of seedling organs:

Table (2) shows that root, stem and leaves dry weight of Cypress seedlings were significantly increased by 10 and 20 ppm Ni application then gradually decreased with increasing the concentration to 40 and 80 ppm. On the other hand root dry weight was gradually significant increased with increasing Co concentration up to 40 ppm then decreased with 80 ppm, while stem and leaves dry weight were significantly increased by 10 and 20 ppm concentration then decreased with 40 and 80 ppm. Data obviously revealed superiority of Co than Ni metal whereas the highest values of seedling organs were obtained by 40 ppm Co for root and 20 ppm Co for stem and leaves.

Enhancing growth characters in response to Ni and Co treatments at the low concentrations of both metals, i.e. 10 and 20ppm of Ni and 10 to 40ppm of Co may be due to activation some enzymes and photosynthesis in plants. Vice versa with high concentrations of Ni or Co caused reduction of plant growth characters such as fresh and dry weight of roots and shoot. The obtained results agreed with [25], [7] and [26] who showed that the influence of higher levels of Ni or Co resulted in depressed shoot growth, which suggests that heavy metals inhibit root and shoot growth directly by inhibiting cell division or cell elongation or both.

2. Effect of Nickel and Cobalt on some chemical constituents:

2.1 Chlorophyll (a), (b), carotenoids and sugars content:

The obtained results of average of two seasons as shown in Table (3-1) revealed that chlorophyll (a, b), carotenoids and sugars increased when the plants were treated with Ni and Co at 10, 20 and 40 ppm concentration as compared with control treatment. However the high rate of both Ni and Co (80 ppm) reduced carotenoids, soluble, non- soluble and total sugars content less than control. The highest values of chlorophyll (b), carotenoids and sugars content was gained from Ni treatment at 40 ppm, while 20 ppm Co treatment gave the highest chlorophyll content. These results are in harmony with the finding and illustrated by [27], [28], [7] and [29] they found inhibition of chlorophyll content with increasing concentrations of heavy metals such as Ni and Co. Also, [30] 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 (3-1): Chlorophyll (a, b), carotenoids and sugars content in leaves of Cypress seedlings as affected by Nikel and Cobalt treatments (Average of two seasons)

Treatment		Chlorophyll (a)	Chlorophyll (b)	Carotenoids	Total sugars (%)		
		(mg/g F.W)			Soluble sugars	Non-Soluble sugars	Total sugars
Control		0.58	0.23	0.76	41.30	3.80	45.10
Ni	10 ppm	0.59	0.27	0.87	43.70	3.90	47.60
	20 ppm	1.49	1.03	1.63	55.30	4.14	59.44
	40 ppm	1.34	1.18	1.83	58.70	4.53	63.23
	80 ppm	0.21	0.62	0.45	35.60	2.31	37.91
Co	10 ppm	0.69	0.34	0.96	44.60	3.54	48.14
	20 ppm	1.75	0.74	1.11	50.70	3.21	53.91
	40 ppm	1.21	0.98	1.37	53.80	3.35	57.15
	80 ppm	0.85	0.64	0.36	37.30	2.40	39.70

2.2 Indoles, Phenols and Crude fiber content:

Table (3-2) illustrates that total indoles and shoot crude fiber content of *Cupressus sempervirens,* L. seedlings were increased with all Ni or Co treatments, however, leaves crude fiber content was slightly increased or decreased by some treatments of both heavy metals as compared with control. On the other hand total phenol content obviously decreased due to Ni or Co treatment compared with control and the lowest values were obtained from the higher rate of 80 ppm Ni or Co. In addition, total content of indoles and crude fiber were clearly increased with increasing rate of either Ni or Co from 10 to 20 or 40 ppm then decreased with 80 ppm and the highest values of indoles were attained from Co at 40 ppm, while Ni at 40ppm and 20 ppm gave the highest crude fiber content of shoot and leaves, respectively. Similar results were obtained by [28].

Table (3-2): Indoles, Phenols and Crude fiber of cypress seedlings as affected by Nikel and Cobalt treatments (Average of two seasons).

Treatments		T. Indoles	T. phenols	Crude fiber (%)	
		(mg/g/F.W leaves)		Stem	Leaves
Control		18.34	6.32	24.30	30.52
Ni	10 ppm	22.35	1.39	37.90	32.11
	20 ppm	26.35	2.45	44.80	33.45
	40 ppm	20.41	1.28	66.34	20.23
	80 ppm	16.30	0.87	60.10	12.18
Co	10 ppm	20.82	1.41	50.37	28.45
	20 ppm	24.43	3.42	55.75	31.70
	40 ppm	27.45	3.84	50.73	21.35
	80 ppm	21.20	0.73	47.20	14.28

2.3 Mineral element contents:

Data in Table (4) revealed that Ni and Co treatments led to increasing in leaf N, P, K, Ni and Co content of cypress seedling content as compared with control treatment. Moreover, leaf N, P and K content showed progress increasing with increasing level of both Ni and Co from 10 to 40 ppm then depressed with the highest level, 80 ppm. On the other hand, leaf Ni and Co content was increased with increasing levels of both Ni and Co treatments till 80 ppm. The highest values of leaf N, P and K content were obtained from 40 ppm Co treatment, while 80 ppm Co treatment caused the highest values of leaf Ni and Co accumulation. The activation and inhabitation of mineral elements uptake due to heavy metals concentration levels were similar obtained by [(7)]. Also [(30)] reported that increasing Ni concentration in growth medium (10 ppm) led to high accumulation of Ni in plant. In addition, [(31)] 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.

Table (4): Mineral elements content in leaves of Cypress seedlings as affected by Nikel and Cobalt treatments (Average of two seasons).

Treatments		Macronutrients content (mg/g dry weight)			Heavy metals content (mg/kg dry weight)	
		N	P	K	Ni	Co
Control		3.56	0.13	4.21	0.09	0.11
Ni	10 ppm	7.89	0.18	4.69	0.73	0.12
	20 ppm	8.79	0.21	7.34	1.31	0.12
	40 ppm	9.37	0.23	7.46	1.95	0.13
	80 ppm	6.30	0.18	3.61	2.30	0.22
Co	10 ppm	8.79	0.25	7.23	0.90	0.73
	20 ppm	9.45	0.25	8.35	0.80	0.75
	40 ppm	9.69	0.28	8.91	0.80	0.89
	80 ppm	6.10	0.16	5.41	2.50	0.93

Conclusion:

The results indicated the success of planting cypress trees seedlings under conditions of irrigation water containing concentrations of nickel or cobalt up to 80 ppm and therefore, it is possible to providing quantities of fresh water to irrigate edible plants and plants which are sensitive to those concentrations of nickel and cobalt.

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