THE EFFECTS OF NICKEL APPLICATIONS ON THE GROWTH OF COCKLEBUR (*XANTHIUM STRUMARIUM* L.) PLANT

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Abstract. Nowadays the environmental pollution caused by heavy metals is spreading all over the world, especially where the industry is developing rapidly, and soils are polluted very dramatically and hazardously at a high level. In this study, cocklebur-(*Xanthium strumarium* L.) plants were grown in nickel (Ni) contaminated soil (0, 50, 100, 200 and 400 mg Ni kg^{-1}) under greenhouse conditions for 6 weeks to study the ability of Ni uptake and accumulation of the plants. The Ni treated plants were compared with the control (0 mg Ni kg^{-1}) plant. As a result of that comparison, chlorophyll levels of old and young leaves, dry weights, reduced glutathione (GSH), macronutrient concentrations, such as nitrogen (N), phosphor (P), potassium (K), calcium (Ca) and magnesium (Mg)) and micronutrient concentrations, such as iron (Fe), zinc (Zn), and manganese (Mn) nutrient concentrations of the plants were decreased, whilst the concentrations of Ni and of copper (Cu) increased with increasing dose of Ni application. The results show that the studied plant (*Xanthium strumarium* L.) can be used for the cleaning up of Ni-contaminated soils and is suitable for phytoremediation.
Keywords: *chlorophyll, heavy metal, mineral element, phytoremediation, pollution*

Introduction

Soil pollution is one of the most important environmental problems nowadays. As stated in the previous studies, major soil pollutants, such as heavy metals, excessive use of fertilizers, pesticides, hormones, organic compounds, industrial wastes and accumulation of dangerous substances in the soil, etc. have become a rising problem as a consequence of human activities (Rahman et al., 2005; Daghan, 2007; Karaca and Turgay, 2012; Daghan and Ozturk, 2015).

Environmental pollution, especially caused by heavy metals such as Ni, lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), arsenic (As), zinc (Zn), copper (Cu), has been a research topic and attracted attention of many countries in the world.

Nickel is one of the most commonly found elements in the earth's crust and is located in the 22nd order among the other elements (Kaviani et al., 2017). This element is an essential element for plant growth and development in small doses (Marschner, 1995; Yang et al., 1996; Gheibi et al., 2009). Nickel is a functional component of some enzymes, especially urease (urea amidohydrolase, EC 3.5.1.5) enzyme in the plant. Urease is a metalloenzyme and plays an important role together with nitrogen being the enzyme that catalyzes the hydrolysis of ammonia and carbon dioxide (Dos Reis et al., 2017). Urea is accumulating in the plant tissue under Ni deficiency condition and it causes the toxic effect (Gheibi et al., 2009). Nickel has also function to catalyze enzymes for nitrogen fixation in legumes plants and increased tolerance to plant disease (Gerendás et al., 1999). However, at high concentrations Ni exhibits a toxic effect, negatively affects photosynthesis and membrane functions, and also prevents seed germination, plant growth and development (Parlak, 2016).

Many researchers have focused on the effects of heavy metals on human health and the environment, on the detection of contaminated areas, on cleaning methods and facilities to minimize the damage that these areas have on human health and the environment (Chaney, 1988; Ebbs et al., 1997; Gupta and Gupta, 1998; Karenlampi et al., 2000; Yankov et al., 2000; Lombi et al., 2002; Prasad and Freitas, 2003; Murakami and Ae, 2009; Daghan et al., 2012; Syam et al., 2016; Rehman et al., 2018). Remediation of polluted soil is usually done by excavating the contaminated area and renewing the soil that has been cleaned. However, in recent years, technologies applied directly *in-situ* without digging the earth has gained more attention (Kocaer and Baskaya, 2003).

Phytoextraction is the removal of soil contaminants by plant roots, accumulation in the organs above the soil, and subsequent harvesting of the plants, respectively. This method is especially used on plants to clean up active elements, such as Zn and Cu, or metals not in the nutrient group like Ni, lead (Pb) and cadmium (Cd) (Hamutoglu et al., 2012).

In the literature, many plants such as *Helianthus annuus* L. (Ahmad et al., 2011), *Nicotiana tabacum* L. (Daghan et al., 2012), *Oryza sativa* L., *Zea mays* L., (Murakami and Ae, 2009), *Sebertia acuminate* (Jaffré et al., 2013), *Glycine max* L. (Syam et al., 2016), *Salicornia iranica* (Kaviani et al., 2017) have been tested and used for the phytoremediation of heavy metals from the contaminated soil. Likewise, the success of phytoextraction depends on the detection of suitable plant species that can tolerate and accumulate heavy metals and to produce large biomass using conventional agricultural techniques (Syam et al., 2016).

A plant that is selected to be used in phytoremediation methods, should have a great amount of metal accumulation in its shoots and harvested parts, be able to tolerate heavy metal that has accumulated, also has to be deep-rooted and fast growing (Daghan et al., 2012).

The aim of this study is to investigate the potential use of *Xanthium strumarium* L., which can grow very easily in nature, has a high amount of roots and shoots, also can be grown in Ni contaminated soil, for heavy metals accumulation and phytoextraction method.

Materials and methods

In this study, *Xanthium strumarium* L., which belongs to the *Asteraceae* family and the Xanthium species was used as plant material. *Xanthium strumarium* L. is an annual plant which has the ability of self-pollinating. Additionally, it can grow even in the most adverse conditions up to 1 m and having advanced pile roots and can be found in almost all regions in Turkey (Cesur and Senkal, 2016).

The Pasakoy soil series of Amik Plain was used in experiments as a soil material (Kılıc et al., 2008). Some physical and chemical properties of the soil were given in *Table 1*.

The air-dried soil sample was sieved from a 4 mm sieve and then 2 kg of soil was filled into each pot. In order to obtain a homogeneous distribution in the soil, increasing doses of (0, 50, 100, 200 and 400 mg kg^{-1}) Ni were applied in the form of $NiSO_4.6H_2O$ prior to planting, and incubated for 3 weeks. Before planting, each pot was fertilizer with the solutions of 200 mg kg^{-1} nitrogen (N) in the form of NH_4SO_4, 100 mg kg^{-1} phosphorus (P) in the form of KH_2PO_4 and 125 mg kg^{-1} potassium (K) with 2.5 mg kg^{-1} iron (Fe) in the form of Fe-EDTA. During the experiment (6 weeks) the pots were

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irrigated with pure water in soil field capacity of 60-80%. The experimental design used was completely randomized design with 3 replications in a factorial arrangement.

Table 1. *The physical and chemical properties of the soil material*

Parameters	Results	References	Parameters	Results	References
Structure	Clay	Bouyoucos (1952)	N	1.12%	Bremner and Mulvaney (1982)
pH	7.56	Soil Survey Staff (1951)	P	19.1 P_2O_5	Olsen et al. (1954)
Salt	0.22%	Soil Survey Staff (1951)	K	77.3 K_2O	Richards (1954)
$CaCO_3$	45.1%	Loeppert et al. (1996)	Available Fe	24.4 mg kg^{-1}	Lindsay and Norvell (1978)
Organic matter	2.55%	Kacar (1995)	Available Cu	3.73 mg kg^{-1}	
Organic carbon	1.48%	Kacar (1995)	Available Mn	69.7 mg kg^{-1}	
Field capacity	32.4%	Alpaslan et al. (1998)	Available Zn	7.40 mg kg^{-1}	
			Available Ni	7.34 mg kg^{-1}	

Before harvesting the leaf chlorophyll contents were measured in 3 replicates by a chlorophyll meter (Konica-Minolta SPAD-502). At the end of the experiment, the plants were harvested about 1 cm above the soil surface and washed with pure water, and then dried in an oven until they reached a constant weight at 65 °C. Afterward, dry weights of the plants were taken and grounded in a grinding mill (Retsch RM 200) for analysis. Test results were evaluated for the whole plant.

The concentrations of Ni, P, K, Ca, Mg, Fe, Zn, Cu and Mn were measured by the inductively coupled plasma atomic emission spectroscopy (ICP-AES Varian Liberty Series II) instrument by dissolving the grounded plant samples in a microwave (MarsXpress CEM) with nitric acid (HNO_3). Nitrogen (N) analysis of plant samples was carried out according to the Kjeldahl method (Bremmer and Mulvaney, 1982).

Nickel content (uptake) of *Xanthium strumarium* L. was calculated as follows (*Eq. 1*):

$$\text{Ni content } (\mu g \text{ plant}^{-1}) = [\text{DW } (g \text{ plant}^{-1}) \times \text{Ni concentration } (\mu g\ g^{-1})] \qquad \text{(Eq.1)}$$

Reduced glutathione (GSH) analysis was performed according to Cakmak and Marschner (1992). The experimental data were evaluated according to the factorial design pattern using the SPSS 22.0 statistical analysis program to determine the significance of levels and grouped by Duncan test according to Bek (1986).

Results and discussion

The results of Ni applications effects on the chlorophyll contents of leaves, dry weight, reduced glutathione (GSH) and Ni concentration of *Xanthium strumarium* L. plant were given in *Table 2*.

As can be seen on *Table 2*, the Ni applications cause a reduction in dry weights of *Xanthium strumarium* L. plant and likewise, chlorophyll contents were also decreased with those applications ($p \leq 0.01$).

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Table 2. *The effects of different doses of Ni on the chlorophyll content, dry weight, GSH and Ni concentrations of Xanthium strumarium L. (n = 3)*

	Ni ($mg\ kg^{-1}$)	**Chlorophyll (SPAD Unit)**		**Dry weight ($g\ plant^{-1}$)**	**GSH ($\mu g\ mL^{-1}$)**	**Ni concentration ($mg\ kg^{-1}$)**
		Old leaf	**Young leaf**			
Xanthium strumarium L.	0	30.7 a	32.5 a	6.61 a	266 b	5.03 e
	50	28.6 b	31.6 ab	6.50 a	281 ab	10.7 d
	100	28.5 b	30.3 b	5.88 b	269 b	21.7 c
	200	28.4 b	31.4 ab	5.61 bc	336 a	49.5 b
	400	22.4 c	32.4 a	5.25 c	225 b	101 a
Dose	F	53.2**	5.92**	21.0**	4.82*	3411**

**$p \leq 0.01$ statistically significant within error bounds
*$p \leq 0.05$ statistically significant within error bounds

The chlorophyll content showed a decrease in the old and young leaves of *Xanthium strumarium* L. plant. The lowest chlorophyll contents were measured at 400 mg kg^{-1} Ni application dose of old and young leaves (respectively 22.4 and 32.4 SPAD unit). On the contrary, a decrease was observed with increasing Ni doses according to control groups. The dry weights of the plant were varied between 6.61-5.25 g $plant^{-1}$. Nickel applications were decreased plant dry weights compared to the control plant ($p \leq 0.01$). Similar results were reported by Parlak (2016). The researcher has investigated the effect of Ni treatment (0, 25 and 50 µM) on the growth and biochemical properties of wheat *(Triticum aestivum* L.). He found that the chlorophyll content of plants decreased with Ni applications. Rahman et al. (2005) were investigated the effect of increased doses of Ni (0, 1.0, 10 and 100 µM) on the growth and composition of barley plants. They reported that the barley plant was generally adversely affected Ni application and the lowest chlorophyll content was measured at 100 µM Ni treatment.

The increased application doses of Ni on the plant resulted with the lowest GSH concentration in the control group as 266 µg mL^{-1} and the highest as 336 µg mL^{-1} at 200 mg Ni kg^{-1} dose in the plant (*Table 2*). The GSH concentrations were decreased with Ni applications (*Table 2*). The lowest GSH concentration (266 µg mL^{-1}) was obtained at the control group while the highest GSH concentration (336 µg mL^{-1}) was obtained at 200 mg Ni kg^{-1} application. Daghan et al. (2012) reported an enhancement of glutathione concentrations in transgenic and non-transgenic tobacco plants, which different Ni doses were applied to the soil.

The Ni concentration of the *Xanthium strumarium* L. plant increased (5.03-101 mg kg^{-1}) with increasing Ni doses (*Table 2*). According to Alloway (1995), the critical concentration of Ni was obtained in tissues of the plant in the range of 10-100 mg kg^{-1}. As Kaviani (2017) stated in a previous research by using *Salicornia iranica* plant, augmented Ni (0, 50, 250, and 500 mg Ni kg^{-1}) doses in the soil resulted in increased Ni concentration in the plants.

The Ni contents were increased with Ni application ($p \leq 0.01$) and the highest Ni content (530 µg $plant^{-1}$) was obtained at 400 mg Ni kg^{-1} application (*Fig. 1*). Khan and Khan (2010) studying the effects of increased Ni application (0, 10, 50, 100, 200 and 400 mg kg^{-1}) on growth and yield of chickpea plant. They found that the Ni content of the root, stem, and leaf of plants was increased with Ni applications.

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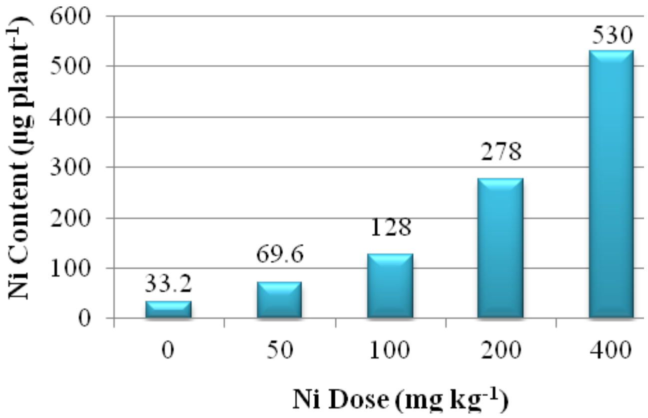

***Figure 1.** The effect of increasing Ni application on plant Ni content (n = 3)*

The effects of increasing doses of Ni on N, K, Ca and Mg concentrations of the plant were found statistically significant ($p \leq 0.01$). Compared to the control application the macro (N, P, K, Ca and Mg) element concentrations of plants were reduced with Ni applications (*Table 3*).

***Table 3.** The effect of different doses of Ni on N, P, K, Ca and Mg concentration of Xanthium strumarium L. (n = 3)*

	Ni	N	P	K	Ca	Mg
	(mg kg^{-1})	**(g kg^{-1})**				
Xantium strumarium L.	0	32.2 a	10.3 a	57.5 a	33.9 a	1.26 a
	50	28.7 b	10.2 ab	48.4 b	30.7 b	1.22 b
	100	28.2 b	9.74 bc	48.3 b	29.5 b	1.20 bc
	200	27.6 bc	9.62 c	47.8 b	28.7 b	1.19 c
	400	26.2 c	9.55 c	46.6 b	28.7 b	1.19 c
Dose	F	22.5**	4.91*	55.7**	11.9**	12.1**

**$p \leq 0.01$ statistically significant within error bounds
*$p \leq 0.05$ statistically significant within error bounds

Ahmad et al. (2011), investigated the phytotoxic effect of macro and micronutrients on the yield and macro and microelement concentration of *Helianthus annuus* L. plants with Ni applications (0, 10, 20, 30 and 40 mg L^{-1}). They observed a decrease in concentrations of N, P, K, Ca, Fe, Zn, Cu and Mn elements by the increase of Ni doses, on the other hand Ahmad et al. (2009), reported that increased Ni (10, 20, 30, 40, 50 and 60 mg L^{-1}) applications have been associated with the effect of 5 different sunflowers (*Helianthus annuus* L.) seeds, indicating a decrease in Mg and K contents due to increased Ni dose.

It was observed that the effects of Ni applications on microelement concentrations of *Xanthium strumarium* L. plant were statistically significant ($p \leq 0.01$) (*Table 4*).

When the rise in Ni doses was compared with the control group on the microelements (Fe, Zn, and Mn) of the plants, it was found that the concentration of these elements was decreased, while the Cu element concentration was increased (*Table 4*). Previous studies have shown that high amount of Ni applications can reduce the

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amount of Fe (Chen et al., 2009; Pandey and Sharme, 2002). Rahman et al. (2005) reported that microelements (Fe, Zn and Mn) reduced in other applications except for 1.0 µM Ni, whilst investigating the effect of Ni doses (0, 1.0, 10 and 100 µM) on the growth and composition of barley plants.

Table 4. *The effect of different doses of Ni on Fe, Zn, Cu, and Mn concentrations in Xanthium strumarium L. (n = 3)*

	Ni	**Fe**	**Zn**	**Cu**	**Mn**
	(mg kg^{-1})	**(mg kg^{-1})**			
Xantium strumarium L.	0	49.0 a	15.7 a	43.0 c	116 a
	50	48.3 a	15.3 ab	45.3 c	111 ab
	100	45.7 a	13.7 bc	45.7 c	107 b
	200	45.0 a	12.9 c	54.3 b	107 b
	400	35.0 b	10.9 d	63.3 a	106 b
Dose	F	11.1**	13.06**	15.7**	5.58**

**$p \leq 0.01$ statistically significant within error bounds

Conclusion

Increasing doses of Ni used in this study induced a decrease in chlorophyll content and dry weight of plants. The main causes of this decrease are thought to be the accumulation of the Ni element in plant tissues at toxic levels and the negative effects of metabolisms that allow the plant to grow and develop.

The effect of Ni applications on GSH concentration according to the control group was varied between 225-336 µg mL^{-1}, which was exhibited the highest at 200 mg Ni kg^{-1} application, while the lowest Ni content was at 33.2 µg plant^{-1} and the highest 530 µg plant^{-1} with 400 mg Ni kg^{-1}.

The content of Ni has increased with varying dose applications, and no chlorosis or necrosis has been observed in the plant. *Xanthium strumarium* L. plant is found to be an important step for investigating the biochemical and physiological responses of plants against Ni toxicity and suitable for increasing the phytoremediation efficiency.

In addition, *Xanthium strumarium* is a native and aggressive weed in many countries. Although the species can be used in phytoremediation, it absolutely should be grown on controlled conditions.

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