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Accumulation of Polyphenols and Flavonoids in Tobacco Plant (*Nicotiana Tabacum*) Grown in Heavy Metal Applied Soil

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Abstract: *In the greenhouse experiment tobacco plants were treated with heavy metals (Zn, Cu, Ni, Pb and Cd) to estimate changes of polyphenols and flavonoids levels. Increasing levels of heavy metals (till to ten fold of soil limit values of metals) were applied experimental soil have increased heavy metal contents of plant. In particular, tobacco plant has absorbed Ni and Cd metals at relatively high levels. Dry matter amounts of plant were not changed by treatments. Tobacco plant grown in soil treated with high levels of heavy metals in the experiment produced a high biomass in the short vegetation period and did not show phytotoxicity even at the highest metal levels. Heavy metals applied to the soil increased the total polyphenols and flavonoid amounts in the tobacco plant. Increase in the polyphenol and flavonoid content of tobaco was more pronounced in the Ni, Pb and Cd metal applications. Research findings showed that tobacco plant is well adapted to heavy metal stress conditions with high phenolic substance production.*

Keywords: *Tobacco, Heavy Metals, Polyphenols, Flavonoids*

1. Introduction

Today, increasing agricultural, urban and industrial activities cause soil, water and air to be polluted with heavy metals. Heavy metal pollution in agricultural soils is one of the most serious environmental and public health problems and has significant harmful effects on human health and ecosystems. Biocides, various organic and mineral fertilizers which are used extensively in agricultural soils, various industrial emissions and air pollutants are among the common sources of heavy metals. Heavy metals cannot be degraded in nature like organic substances and their concentrations in the soil may increase continuously depending on the emission sources and can be transported through the food chain. Therefore, heavy metals are a major concern for environmental health due to their toxicity, extensive resources, non-biodegradable properties and accumulation behavior [1].

In recent years, phytoremediation technique has come to the fore as an alternative to sophisticated and costly technologies in the remediation of metal-contaminated soils. In phytoremediation studies, hyperaccumulator plants that have high metal absorption capacity and create high biomass in a short time are used. However, many plants defined as hyperaccumulator generally only accumulate certain elements, tend to grow slowly, have low biomass, and are compatible with different climates and ecologies, creating difficulties in the selection of phytoremediation plants. [2, 3]. The main strategy for the phytoremediation is to detect plants from nature those have a high biomass and high metal hyperaccumulating properties. The tobacco plant (Nicotiana tabacum) can live in different ecological regions with its many varieties, can form high biomass vegetatively in a short time, and can adapt to difficult soil and climatic conditions. It has been reported that the tobacco plant also has high hyperaccumulatory properties [4].

Exposure of plants to heavy metals can lead to many adverse effects that inhibit cellular processes [5]. One of the early responses of plants to heavy metal stress is the production of reactive oxygen species (ROS) in plant cells [6]. In terms of heavy metal effects, ROS can be produced directly by Cu, Pb, Cr and As and indirectly by Cd in plant tissues [7]. The effect of ROS can cause damage at physiological and biochemical levels. Therefore, plants have developed different mechanisms against these stress factors [8].

In case of an imbalance between ROS production and degradation, plants have developed a defense system consisting of various enzymatic antioxidants. [9]. Plant cells also produce low molecular non-enzymatic antioxidants that are involved in ROS removal. Depending on the chemical structure, phenolic compounds are divided into several groups: simple phenols, benzoic acids, phenylpropanoids and flavonoids [8, 10].

Phenolic compounds are classified as beneficial antioxidants that can remove ROS in plants exposed to stress factors [11]. Some phenolic compounds can reduce the toxic effects of heavy metals by increasing plant tolerance, and increasing the biosynthesis of phenolic compounds in plants exposed to heavy metal stress helps protect plants from oxidative stress [12, 13]. Flavonoids are among the main secondary metabolites synthesized in almost all parts of plants as part of plant-environment communication [14]. Flavonoids enhance the process of metal chelation, which can reduce the level of harmful hydroxyl radicals in plant cells, and perform a protective function under stress conditions by neutralizing radicals before they damage cells [15]. Therefore, the concentration of phenolic compounds in plant tissues is considered a good indicator that allows researchers to estimate the range of tolerance to stress factors that occur in plants [16].

The metal-accumulation characteristic of tobacco plants and its adaptation to survive in wide climatic and geographical regions are considered as a useful material that can be used to understand the physiological mechanisms related to resistance to abiotic stresses. There is a lack of information on the effect of heavy metals on the antioxidant system of plants with potential for use in phytoremediation. It is assumed that heavy metals applied at various concentrations will cause a different reaction to oxidative stress in the tobacco plant. In this study, it was aimed to reveal the response of tobacco plant to stress caused by heavy metals in the soil.

2. Materials and Methods

2.1. Experimental Studies

This study was carried out on the trial material included in the previous phytoremediation study of the author and colleagues [17]. A greenhouse pot experiment was carried out to research polyphenol and flavonoid contents of Nicotiana tabacum treated with heavy metals. Tobacco seeds (Nicotiana tabacum L. Çelikhan var.) which cultivated commonly in southeast of Turkey were obtained from of Adıyaman region, Turkey. Seeds were disinfected by sodium hypochlorite solution of 5 % during a few minutes and then rinsed in the distilled water before sowing to soil.

A red mediterranean soil was used in the experiment. Soil was air dried, siewed by 2 mm then mixtered by perlite at the rate of 30 percent and 20 % peat to maintain slighty texture in the pot medium. Analytical characteristics of experimental soil was presented in Table 1.

Physical and chemical characteristics of greenhouse soil mixture studied before the experiment are well within the accepted normal range of agronomic values, and the heavy metal concentrations are below the levels indicated by the European Union [18].

Basic N-P-K fertilization was applied to experimental soil at the rate of 100, 50 and 100 kg ha^{-1} of N (as NH_4NO_3), P (as KH_2PO_4) and K (as K_2SO_4). Heavy metals Zn, Cu, Ni, Pb and Cd were added to experimental soil as metalic salt solutions (as $Zn(NO_3)_2$, $CuSO_4$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $Cd(NO_3)_2$, respetively) as in Table 2. Metal concentrations were designed to maintain beginning from maximum till to 10 fold of maximum metal limits of European Union [18]. Soil and metals were incubated field capacity of water at least 3 months before experiment.

A factorial experiment was conducted in randomized complete block design including 5 levels of heavy metals with 5 replications. The Seeds were germinated in peat+perlite substrate mixture. Then, 3 seedlings of each plant were transplanted in every pot containing 10 kg soil. During the experiment, the plants were watered regularly and treated according to common agrotechnical principles. After 60 days of growth all plants were harvested from soil surface. Plant samples were dried at 60 ºC in a forced-air oven, ground with agitate mortar and then digested in aqua regia (1:3 HNO_3/HCl). Total metal concentrations were analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

TABLE I: The analytical characteristics of the experimental soil before applications [17].

Parameters		Metal limits in soil, mg kg^{-1} dry wt [7]
Texture	Loam	
pH- H_2O (1:5 w/v)	7.25	
$CaCO_3$, %	5,26	
Organic matter, %	8.25	
Clay,%	6,5	
CEC, cmol kg^{-1}	18,4	
EC, dS m^{-1} 25°C	0,65	
Total N, %	1.28	
P (ex), mg kg^{-1}	8.7	
K (ex), mg kg^{-1}	74	
Ca (ex), mg kg^{-1}	658	
Mg (ex), mg kg^{-1}	124	
Zn, mg kg^{-1}	65.2[1]	150-300
Cu, mg kg^{-1}	10,5	50-140
Ni, mg kg^{-1}	7,8	30-75
Pb, mg kg^{-1}	22,6	50-300
Cd, mg kg^{-1}	0,01	1-3

[1]:Total concentrations,

TABLE II. Heavy metal treatment levels of experiment [17].

Metals	Metal treatments, mg kg^{-1}				
	Control	1	2	3	4
Zn	0	300	750	1500	3000
Cu	0	140	350	700	1400
Ni	0	75	250	500	750
Pb	0	300	1000	2000	3000
Cd	0	3	10	20	30

2.2. Determination of plant stress parameters on plant material

The ethanol hydrochloric acid mixture method was used for the extraction of phenolic substances [19]. Total polyphenol content in plant was determined according to the method of Folin-Ciocalteu phenol reagent and contents expressed as mg EAG g^{-1} DW [20]. The flavonoid content in plant was determined by the aluminum trichloride method [21] and the flavonoid contents were expressed as mg EQ g^{-1} DW.

2.3. Statistical Analysis

ANOVA test ($p \leq 0.05$) calculated using the statistical package SPSS-23 for Windows program were applied to compare the differences in parameters.

3. Results and Discussion

3.1. Plant growth and heavy metal concentration of plants

Dry matter values of tobacco plant did not change significantly with heavy metal applications and also no phytotoxicity was observed by the treatments (Table 3). This shows that plants are well adapted to stress conditions, even to ten fold of maximum soil metal concentration limits. However, Zn, Cu, Ni, Pb and Cd concentration in tobacco plant were increased by the increasing amounts of metal treatments. Compared with the control treatment, Ni and Cd metals gave the highest change relatively at increasing concentrations of metals (Figure 1). Although each of the heavy metals is applied till to 10 fold starting from their threshold limit value, the fact that Ni and Cd metals are found in relatively higher concentrations in the plant compared to the control application shows that the tobacco plant takes Ni and Cd metals more effectively without plant toxicity.

TABLE III. Dry matter (g pot^{-1}) and heavy metal concentration (mg kg^{-1}) of tobacco plant [17]

Treatments	Dry matter	Zn	Cu	Ni	Pb	Cd
Control	152,3	37,73	5,357	0,559	3,142	1,050
1	157,6	177,05	6,678	6,456	8,709	5,811
2	165,4	385,09	11,679	10,529	11,857	12,363
3	148,4	383,23	18,319	11,050	17,577	20,371
4	150,2	517,21	20,820	20,910	33,218	23,446

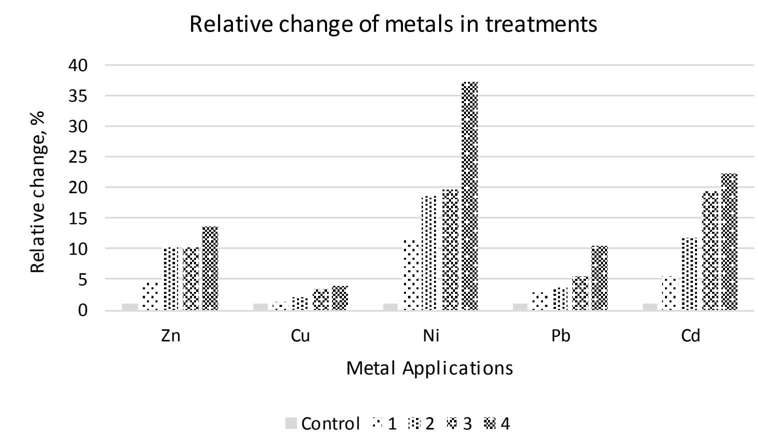

Fig.1. Relative change of heavy metals in tobacco plant in metal treatments

3.2. Variation in Plant Stress Parameters in Metal Treatments

Heavy metals applied to the soil increased the total polyphenol content of the tobacco plant (Figure 2). The polyphenol content in the plant generally increased in relation to the increased metal applications to the soil. Depending on the metal applications, the increase in the polyphenol content of the plant was more pronounced in the Ni, Pb and Cd metal applications. Polyphenol content of tobacco plant showed higher values in Ni and Cd applications. This situation is in parallel with the high level of Ni and Cd uptake of the tobacco plant from the soil. The findings support the hypothesis that plants exposed to abiotic stress metabolically synthesize high levels of polyphenol.

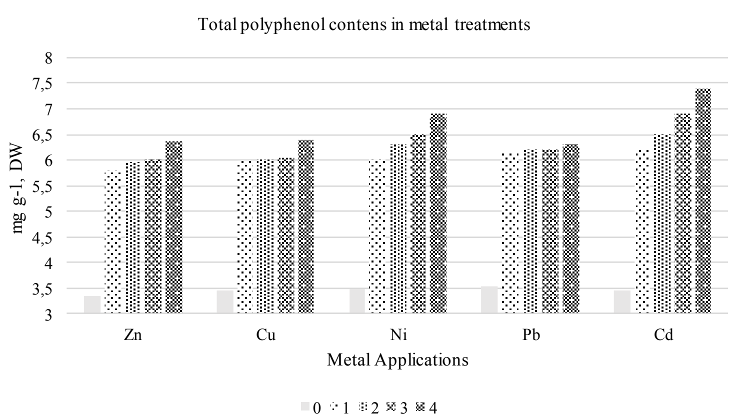

Fig. 2. Total polyphenol contents of tobacco plant in metal treatments

As with the polyphenol content, heavy metals applied to the soil increased the total flavonoid content of the tobacco plant (Figure 3). With the partial exception of some applications of Zn and Cu metals, flavonoid content in the plant increased generally in relation to increased metal applications to the soil. The increase in the flavonoid content of the plant with metal applications was more pronounced in Ni, Pb and Cd metal applications. The flavonoid content of the tobacco plant showed higher values in Ni, Pb and Cd applications. Unlike the polyphenol content, higher flavonoid content was obtained in the plant compared to Zn and Cu metals in Pb applications to the soil. The high flavonoid content in Ni and Cd metal applications is in parallel with the high Ni and Cd uptake of the tobacco plant from the soil.

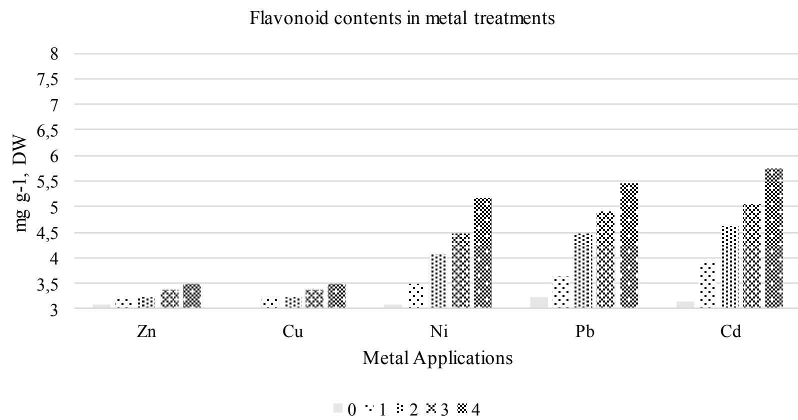

Fig. 3. Flavonoid contents of tobacco plant in metal treatments

The increase in the amount of total polyphenols and flavonoids of tobacco plant due to heavy metal applications supports the thesis that phenolic substance production may be a defense mechanism of the plant against heavy metal stress. The high level of polyphenol accumulation in tobacco plants under heavy metal stress is consistent with studies on different plants [12, 13, 22, 23].

4. Conclusion

Research findings showed that tobacco plant is well adapted to heavy metal stress conditions with high phenolic substance production. It is desired that the phytoremediation plant selected in the rehabilitation of metal-contaminated soils is resistant to abiotic toxicity, forms high biomass and has a high metal absorption capacity. Tobacco plant grown in soil treated with high levels of heavy metals in the experiment produced a high biomass in the short vegetation period and did not show phytotoxicity. Metal content increased with increasing metal applications to the soil. In particular, tobacco plant has absorbed Ni and Cd metals at relatively high levels. The high total polyphenol and flavonoid values of the tobacco plant under metal stress make it an alternative and promising plant in phytoremediation applications.

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