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# The effects of increasing doses of nickel and lead applications on some oriental tobacco varieties

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**Abstract:** Heavy metals are hazardous pollutants for humans, animals and plants, when their threshold concentration exceeds. Tobacco can accumulate higher concentrations of heavy metals, and the genotypic differences of tobacco in heavy metal uptake and their growth responses have not been clearly examined. In this study, the effects of nickel (Ni) and lead (Pb) on phytoremediation capabilities were tested in four local Oriental tobacco cultivars (Basma, Akhisar, Sarıbağlar and Dibek). In two pot experiments, nitrogen (N), phosphorus (P) and potassium (P) were applied in fixed doses, while Pb and Ni were applied at 4 different doses (10, 50, 100 and 150 mg $kg^{-1}$). Plants were harvested after 50 days of growing period, and separated into roots, stems, leaves. The biomass values were measured, and Pb and Ni concentrations were quantified in plant organs. Results revealed that Basma cultivar had the highest total biomass value with 16.63 and 15.92 g $pot^{-1}$ for Ni and Pb contents, respectively. While, the lowest total biomass was recorded in Dibek cultivar with 7.09 and 5.71 g $pot^{-1}$ for Ni and Pb, respectively. The biomass, Ni and Pb uptake and accumulation capabilities remained in the following order of cultivars Basma > Akhisar > Sarıbağlar > Dibek. Depending on the application doses, Ni and Pb concentrations of different plant parts (roots, stems and leaves) of all varieties showed significant ($p < 0.01$) increases compared to the control treatments. All of the plant parts of Basma variety had higher Ni and Pb concentrations than the Akhisar, Sarıbağlar and Dibek variety. Nickel concentrations in the roots, stems and leaves of different cultivars were determined to be the least in the control application and the highest in the NPK+150 mg $kg^{-1}$ Ni ($Ni_4$) application. Lead concentrations in the roots, stems and leaves of different cultivars were determined to be the least in the control application and the highest in the NPK+150 mg $kg^{-1}$ Pb ($Pb_4$) application. Basma variety with higher enrichment factor (EF) and bioaccumulation factor (BAF) values were also found to be more effective than the other varieties for phytoremediation of Ni and Pb. The objective of this study was to examine the Ni and Pb uptake, transport and accumulation properties of oriental tobacco with special emphasis on its different varieties.

**Key words**: Tobacco, phytoremediation, nickel, lead, soil, contamination

## 1. Introduction

Heavy metals are considered as hazardous chemicals in the environment, and causing major health problems throughout globe. Heavy metals are one of the main pollutants that affect the plants, animals, and humans at very low concentrations. Heavy metals are not easily degraded in the environment, and could cause severe damages to both living organisms and the environment (Tomás et al., 2012). They are very harmful, because of their nonbiodegradable nature, long biological half-lives and their potential to accumulate in different body parts. Most of the heavy metals are extremely toxic, and even at low concentrations, they can exhibit severe damaging effects in living organisms (Chen et al., 2005; Li et al., 2015). Excessive heavy metals in the human nutrition can be toxic, and can cause acute and chronic diseases (Schmidt, 2003). Food and fodder crops raised on heavy metal contaminated soils have the tendency to accumulate excessive amounts of heavy metals, and pose severe risks to human and animal health (Rattan et al., 2005; Kulhari et al., 2013). Heavy metal concentrations in the soil can lead to enhanced crop uptake and negative affects on the growth and development of plants. Excessive concentrations of high metals in plants lead to oxidative damage which expressively reduces the plant growth and biomass (Lugon-Moulin et al., 2006; Rizwan et al., 2018). Excess Ni induces leaf chlorosis and inhibits plant growth (Leskova et al., 2020). Heavy metal may inhibit the division and proliferation of plant stem cells (Soudek et al., 2010), causing disruptions in plants growth (Mohanpuria et al., 2007). Plants exposed to heavy metals have mutation-like changes in the DNA structure and decreased amount of RNA, soluble proteins and sugars (Zeid et al., 2013). Further, they induce the structural changes in

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chloroplasts, decreases the amount of chlorophylls, and affects the stomatal conductivity of the plant, preventing the continuity of photosynthesis, dramatic changes in related enzyme activities and eventually lowering yield (Tunc and Sahin, 2015). Nowadays, the phytoremediation has received great attention for the remediation of contaminated soil (Huang et al., 2016). Phytoremediation involves the process for treating contaminated area with plants to eliminate pollutants. The basic principle of phytoremediation involves the breaking of contaminant by roots of plants to lesser toxic element or absorption of contaminant, storing it in the stems and leaves of the plant (Kaur et al., 2018). Heavy metal contamination has posed a serious threat to human health and the ecosystem. Therefore, remediation of land contamination is of paramount importance. Phytoremediation is an ecofriendly approach that could be a successful mitigation measure to revegetate heavy metal-polluted soil in a cost-effective way (Yan et al., 2020; Raza et al., 2020).

Phytoremediation is a natural treatment approach to remove organic and inorganic contaminants from the soil and aquatic environments through; 1- immobilizing at roots region, 2- uptake by roots and shoots, and accumulating therein, 3- uptake by roots, transporting to upper parts of the plant, metabolizing or evaporating at roots and leaves (Ghosh and Singh, 2005; Rehman et al., 2017b; Rizwan et al., 2017b; Shah and Daverey, 2020; Antoniadis et al., 2021). The plants to be used for such purposes should reliably be grown under contaminated conditions, able to produce high shoot rates, be deep rooted, fast growing, easy-harvested, and have a high heavy metal accumulation capacity in their shoots. Till date, more than 200 species in the worldwide are being recognized as a tolerant or accumulator of heavy metals, especially for zinc (Zn), nickel (Ni), cobalt (Co), copper (Cu), lead (Pb), and many others (Sun et al., 2015; Tauqeer et al., 2016; Muthusaravanan et al., 2018).

Nickel (Ni) is an essential plant nutrient (Marschner, 1995; Li and Zamble, 2009), but lead are not essential element for tobacco, this metal may be adsorbed and moved to the tissues by transport processes (Oliver and Gregory, 2015).

Tobacco (*Nicotiana tabacum* L.) is recognized as an effective accumulator of metals from the soil, and can accumulate relatively high concentrations in its organs, mainly leaves (Mench et al., 1989; Doroszewska and Bebec, 2004; Vera-Estrella et al., 2017; Rong et al., 2020). It is reported that tobacco is able to produce high biomass rates, easy to find, can be grown over large areas, and can be accepted as hyperaccumulator for some heavy (Vanlı, 2007; Vamerali et al., 2010; Da Silva et al., 2016; Maodzeka et al., 2017; Palusińska et al., 2020). The uptake of heavy metal concentrations in tobacco leaves differs widely, and it mainly depends on the tobacco cultivars/genotypes, soil types or conditions, soil organic matter content, pH of the soil, environment and some other factors (Wagner et al., 1988; Adamu et al., 1989; Tsadilas et al., 2005; Piano et al., 2008; Golia et al., 2009; Zaprjanova et al., 2010; Regassa and Chandravanshi, 2016; Zhao et al., 2020).

Oriental tobacco is produced in a large scale under different climatic and soil conditions, and mainly produced in Turkey. In the present study, 4 different local Oriental tobacco cultivars, which are adapted to different climatic (Aegean, and Blacksea) and soil conditions (alkaline and acid) were evaluated for their phytoremediation capabilities for Ni and Pb.

## 2. Materials and methods

Oriental tobacco cultivars, namely Basma, Akhisar, Dibek and Sarıbağlar were used as plant materials in 2 pot experiments. All experiments were carried out at green house conditions. Sand-perlite mixture was used as the growing media in all the experiments. Some physical and chemical characteristics of soil experiments are given in (Table 1).

Plastic pots were filled with 1.5 kg soil and 75 g perlite. Experiments were separately designed for Ni and Pb. Four doses of (10, 50, 100, and 150 mg $kg^{-1}$) were used for both Pb and Ni treated experiments. Pb treated experiments were arranged as; 1- Control ($Pb_0$), 2- NPK, 3- NPK+10 mg $kg^{-1}$ Pb ($Pb_1$), 4- NPK+50 mg $kg^{-1}$ Pb ($Pb_2$), 5- NPK+100 mg $kg^{-1}$ Pb ($Pb_3$) and 6- NPK+150 mg $kg^{-1}$ Pb ($Pb_4$); Ni experiment were arranged as; 1- Control ($Ni_0$), 2- NPK, 3- NPK+10 mg $kg^{-1}$ Ni ($Ni_1$), 4- NPK+50 mg $kg^{-1}$ Ni ($Ni_2$), 5- NPK+100 mg $kg^{-1}$ Ni ($Ni_3$) and 6- NPK+150 mg $kg^{-1}$ Ni ($Ni_4$). These application doses were selected according to previous studies (Arazi et al., 1999; Angelova et al., 2004; Piano et al., 2008). Relevant concentrations of nutrient and

**Table 1.** Physical and chemical characteristics of experiments soil.

| pH | $CaCO_3$ (%) | Total soluble salt (dS $m^{-1}$) | Organic matter (%) | Texture class | Ni* (mg $kg^{-1}$) | Pb* (mg $kg^{1}$) |
|---|---|---|---|---|---|---|
| 7.80 | 3.07 | 0.20 | 0.50 | Loam | 12.10 | 4.52 |

*: Total extracted with aqua regia

heavy metal solutions were initially mixed with perlite, and then thoroughly mixed with soil and filled into pots. Pb $(NO_3)_2$ was used as a Pb source and $NiSO_4$ $6H_2O$ was used as a Ni source. Except for the control treatment, 150 mg $kg^{-1}$ N, 20 mg $kg^{-1}$ P and 150 mg $kg^{-1}$ K were applied to all treatments. $NH_4NO_3$ was used as N source, $KH_2PO_4$ as P source, and $KH_2PO_4$ and $K_2SO_4$ as K source. Experiments were designed in a randomized block with 3 replications. Initially, 4 seedlings were planted into pots, and after 10 days, they were thinned to 2 seedlings. Plants were harvested as a whole plant (roots+stem+leaves) after 50 days of growth and separated into roots, stems and leaves and biomass values were recorded. All samples were washed so as to remove any adhering soil particles, and rinsed with distilled water, dried at 65–70 °C and grinded. After wet digestion (4:1 $HNO_3$: $HClO_4$ (v/v) (Li et al., 2001; Kacar and İnal, 2008), Pb and Ni concentrations were measured using atomic absorption spectrophotometer (AAS) (Varian AA 220 FS).

### 2.1. Heavy metals hyperaccumulation factors

#### 2.1.1. Enrichment factor (EF)

The EF is calculated as the ratio of metal concentration in the plant to metal concentration in the soil (Lorestani et al., 2011).

#### 2.1.2. Translocation factor (TF)

The translocation factor (TF), also called as the mobilization ratio is the ratio of metal concentration in the shoots to metal concentration in the roots (Lorestani et al., 2011).

#### 2.1.3. Bioaccumulation factor (BAF)

The bioaccumulation factor (BAF) is the ratio of metal concentration from shoots to the soil (Singh et al., 2011).

### 2.2. Statistical analysis

The data was analyzed by analysis of variance (ANOVA) with SPSS statistical program (version 16.0) to determine any statistically significant differences. All treatments were compared with the Duncan's multiple range test ($p \leq 0.05$). The data was interpreted according to $p \leq 0.01$ and $p \leq 0.05$. In the figures, the spread of values is shown as error bars representing standard errors of the means.

## 3. Results and discussion

### 3.1. Biomass

Different concentrations of nickel and lead application have significant effects ($p < 0.01$) on Nickel and lead biomass determined in various plant parts and varieties (Table 2).

It is noteworthy to mention that decreased biomass values were observed in cultivars that were treated with increased application doses of Ni and Pb, and the condition was more distinctive at high doses (Table 3). Significant differences were observed in biomass values of tobacco cultivars with increasing Ni and Pb concentrations and cultivars exhibit different responses against Ni and Pb concentrations. The highest biomass values were determined in NPK application Basma cultuvar (4.01–3.55 g $pot^{-1}$), while the lowest values were witnessed in plants treated with $Ni_4$ (NPK+150 mg $kg^{-1}$ Ni) and $Pb_4$ (NPK+150 mg $kg^{-1}$ Pb) doses. Biomass values were decreased biomass value was observed with the increased doses of Ni and Pb.

The highest biomass value was observed in Basma and the lowest value was recorded in Dibek cultivar. The cultivars Akhisar and Sarıbağlar had biomass values in between them. The differences between cultivars in Pb treatments were similar to the ones in Ni treatments. Biomass value of especially Basma cultivar was found to be higher than the other cultivars in treatments. Dibek cultivar had the lowest biomass value. Akhisar and Sarıbağlar cultivars exhibited similar biomass values with Ni treatments. In both Ni and Pb treatments, the highest biomass values of 4 cultivars were observed in NPK treatments. Decreasing biomass values were observed with increasing Ni and Pb concentrations. With regard to biomass in general, Basma cultivar had the first place and Dibek cultivar had the last place. (Figures 1 and 2).

**Table 2**. Effect of Ni concentrations on biomass of plant parts (g $pot^{-1}$, DW).

| | Cultivars | | | |
|---|---|---|---|---|
| Treatments | Akhisar | Basma | Dibek | Sarıbağlar |
| Control | $1.25^{b}$ | $1.42^{e}$ | $0.80^{cd}$ | $1.09^{d}$ |
| NPK | $3.19^{a}$ | $4.01^{a}$ | $1.99^{a}$ | $2.62^{a}$ |
| $Ni_1$ | $2.72^{a}$ | $3.32^{b}$ | $1.55^{ab}$ | $2.16^{ab}$ |
| $Ni_2$ | $1.58^{b}$ | $2.83^{bc}$ | $1.32^{bc}$ | $1.76^{bc}$ |
| $Ni_3$ | $1.40^{b}$ | $2.66^{c}$ | $0.79^{cd}$ | $1.49^{cd}$ |
| $Ni_4$ | $1.23^{b}$ | $2.39^{c}$ | $0.64^{d}$ | $1.19^{d}$ |

**Table 3**. Effect of Pb concentrations on biomass of plant parts (g $pot^{-1}$, DW).

| Cultivars | | | | |
|---|---|---|---|---|
| Treatments | Akhisar | Basma | Dibek | Sarıbağlar |
| Control | $0.90^{c}$ | $1.00^{d}$ | $0.60^{c}$ | $0.72^{d}$ |
| NPK | $2.27^{a}$ | $3.55^{a}$ | $1.36^{a}$ | $1.78^{a}$ |
| $Pb_1$ | $2.15^{ab}$ | $3.33^{a}$ | $1.13^{ab}$ | $1.58^{ab}$ |
| $Pb_2$ | $1.99^{ab}$ | $3.15^{ab}$ | $1.03^{abc}$ | $1.28^{bc}$ |
| $Pb_3$ | $1.93^{ab}$ | $2.80^{b}$ | $0.90^{bc}$ | $1.20^{bc}$ |
| $Pb_4$ | $1.78^{b}$ | $2.09^{c}$ | $0.69^{c}$ | $1.09^{cd}$ |

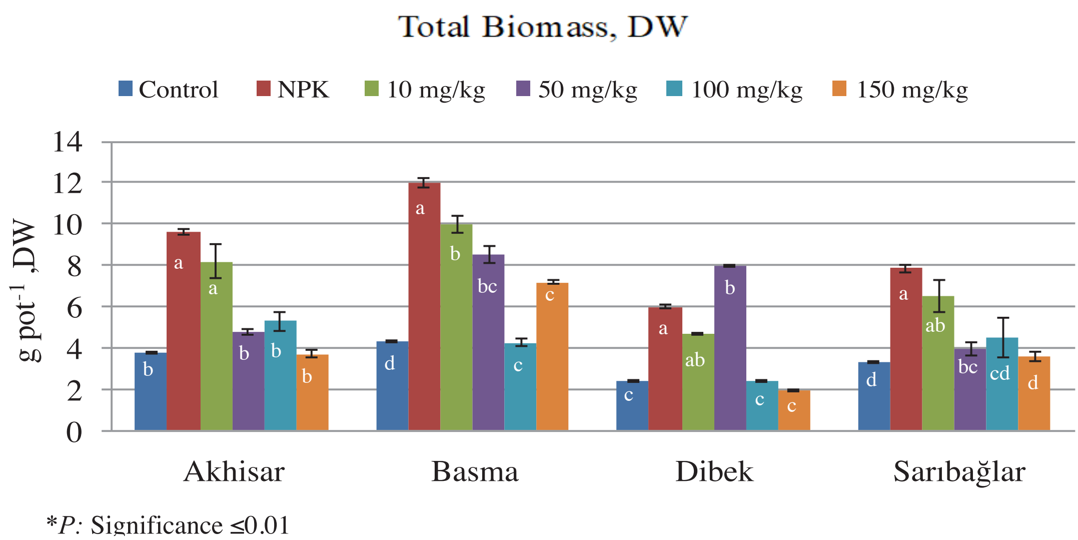


**Figure 1.** Effects of Ni treatments on total biomass value of tobacco cultivars.

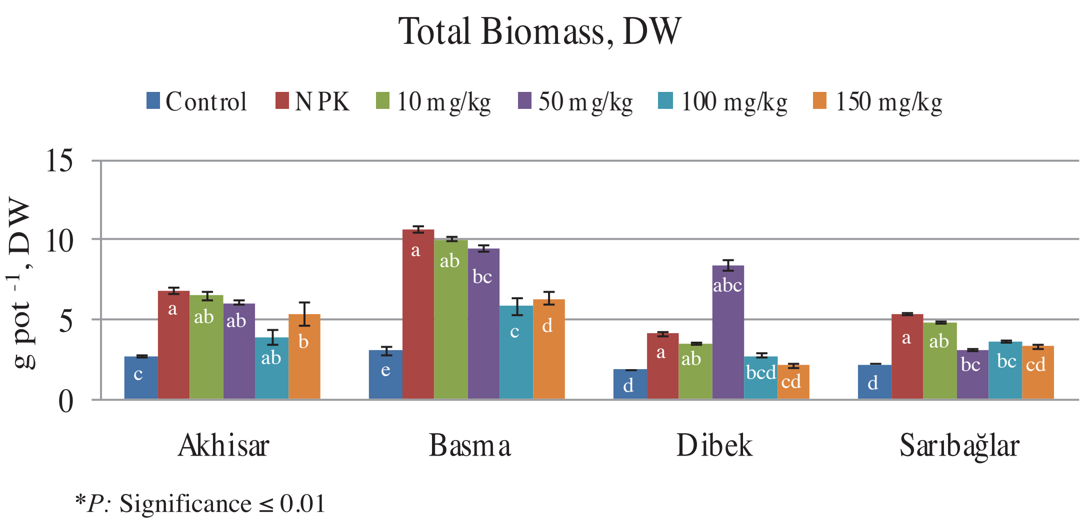


**Figure 2.** Effects of Pb treatments on total biomass value of tobacco cultivars.

Effects of Ni and Pb treatments on biomass value of tobacco cultivars varied based on cultivars and treatment doses. The highest Biomass (root + stem + leaf) values were determined Basma (1.42–4.01 g pot$^{-1}$) and the lowest biomass Dibek (0.64–1.99 g pot$^{-1}$). Decreasing biomass values were observed with increasing Pb treatment doses. Biomass values of Pb treatments varied between 0.60 and 3.55 g pot$^{-1}$. The decrease in biomass values with increasing Pb doses was more distinctive at higher doses. Obviously the addition of Ni and Pb induced a decrease in biomass value with the greatest decrease being noted in the tobacco cultivars after addition of $N_1$ (NPK+10 mg kg$^{-1}$ Ni) and $P_1$ (NPK+10 mg kg$^{-1}$ Pb) doses. Pb accumulation in plant tissue caused plant stress which affected plant root and shoot growth thereby leading to a reduction in biomass (Maodzeka et al., 2017). With increasing Pb applications, it was observed that there were statistically significant decreases in root and green parts dry matter yields (Kınay and Erdem, 2019). Boonyapookana et al. (2005) stated that Pb causes less biomass production in tobacco, *Helianthus annuus*, and *Vetiveria zizanioides*. As indicated by Chen et al. (2009) a linear decrease was observed in biomass values of cultivars with increasing Ni concentrations. Basma was found to be the superior one with respect to biomass accumulation in both experiments. This can be attributed to genetic characteristics, and well-adaptation of this cultivar to the local climatic conditions (Lasat, 2002).

### 3.2. Concentration of plant parts

The increasing doses of Ni application had a statistically significant ($p < 0.01$) effects on the uptake of Ni in various plant parts. Results revealed that Ni concentrations in root, stem and leaves of Basma, Akhisar, Sarıbağlar and Dibek cultivars varied with the application doses (Table 4).

Depending on the application doses, Ni and Pb concentrations of different plant parts (roots, stems and leaves) of all varieties showed significant ($p < 0.01$) increases compared to the control and NPK treatments. All of the plant parts of Basma variety had higher Ni and Pb concentrations than the Akhisar, Sarıbağlar and Dibek variety. In the tobacco varieties examined, it is observed that the Ni concentration (mg $kg^{-1}$) in the root parts varies between 3.73 and 50.19 in Akhisar variety, 3.96 and 65.30 in Basma variety, 3.18 and 33.65 in Dibek variety, and 3.30 and 39.19 in Sarıbağlar variety, depending on the applications. It has been determined that the Ni concentration (mg $kg^{-1}$) in the stem varies between 1.92 and 68.23 in Akhisar tobacco variety, 3.96 and 65.30 in Basma variety, 3.18 and 33.65 in Dibek variety and 3.30 and 39.19 in Sarıbağlar variety. Ni (mg $kg^{-1}$) concentration in the leaf was found to vary between 3.49 and 42.53 in Akhisar tobacco variety, 3.78 and 58.70 in Basma variety, 3.25 and 25.36 in Dibek variety and 3.18 and 33.65 in Sarıbağlar variety. Ni concentrations in the roots, stems and leaves of different cultivars were determined to be the least in the control application and the highest in the $Ni_4$ application. According to Tso (1990), Ni content in tobacco varies from 0.2 to 1.6 mg $kg^{-1}$. However, results from our study point out a significantly higher content for Ni element in cultivars. Statistically significant ($p < 0.01$) differences were found in different plant parts with respect to Pb application concentrations in cultivars (Table 5). The highest lead concentration was determined in roots, and the lowest was found in leaves.

The Pb content of tobacco cultivars increased depending on the applied heavy metal concentrations. Pb concentration (mg $kg^{-1}$) in the roots of Akhisar tobacco variety was determined between 2.61 and 81.84, 3.87 and 97.05 in Basma, 2.36 and 63.54 in Dibek, 2.48 and 76.65 in Sarıbağlar variety. According to tobacco types, Pb concentration in the stem varied between 2.01 and 53.84 in Akhisar variety, 2.08 and 60.63 in Basma, 1.84 and 41.58 in Dibek, 1.89 and 44.10 in Sarıbağlar variety. The Pb concentration in leaf content varied between 3.49 and 42.53 in the leaves of Akhisar variety, 3.78 and 58.70 in Basma, 3.25 and 25.36 in Dibek and 3.45 and 36.03 in Sarıbağlar variety. Pb concentrations in the roots, stems and leaves of different cultivars were determined to be the least in the control application and the highest in the $Pb_4$ application. Ni and Pb concentrations of the roots in both experiments were determined higher than the stem and leaves. Tobacco roots also accumulated Co and Ni more than did the stem (Liu et al., 2019). This situation has been reported by Boonyapookana et al. (2005), shoot Pb concentrations for most plants are generally low since little Pb is transferred from the root to shoot. Lugon-Moulin et al. (2004) stated that Pb depends largely on the soil characteristics, the type and variety of tobacco, as well as the place of cultivation. Maodzeka et al. (2017) reported tobacco Pb concentration varies according to genotypes

**Table 4**. Ni concentration (mg $kg^{-1}$) in different parts of tobacco cultivars.

| | | Control | NPK | $Ni_1$ | $Ni_2$ | $Ni_3$ | $Ni_4$ |
|---|---|---|---|---|---|---|---|
| Root | Akhisar | $3.44^d$ | $3.92^d$ | $7.13^d$ | $33.10^c$ | $73.67^b$ | $88.44^a$ |
| | Basma | $3.65^d$ | $4.10^d$ | $8.21^d$ | $40.52^c$ | $82.11^b$ | $108.42^a$ |
| | Dibek | $3.07^d$ | $3.21^d$ | $6.41^d$ | $27.64^c$ | $56.67^b$ | $65.83^a$ |
| | Sarıbağlar | $3.33^d$ | $3.61^d$ | $6.80^d$ | $30.50^c$ | $67.66^b$ | $74.78^a$ |
| | | * | * | * | * | * | * |
| Stem | Akhisar | $1.92^d$ | $2.10^d$ | $5.30^d$ | $27.96^c$ | $43.53^b$ | $68.23^a$ |
| | Basma | $2.02^d$ | $2.25^d$ | $6.95^d$ | $32.00^c$ | $55.43^b$ | $85.44^a$ |
| | Dibek | $1.86^d$ | $2.05^d$ | $4.11^d$ | $21.57^c$ | $34.09^b$ | $51.92^a$ |
| | Sarıbağlar | $1.87^d$ | $2.11^d$ | $4.16^d$ | $24.97^c$ | $38.14^b$ | $58.54^a$ |
| | | * | * | * | * | * | * |
| Leaf | Akhisar | $3.73^d$ | $4.41^d$ | $6.73^d$ | $18.67^c$ | $39.02^b$ | $50.19^a$ |
| | Basma | $3.96^d$ | $4.76^d$ | $10.16^d$ | $26.18^c$ | $50.02^b$ | $65.30^a$ |
| | Dibek | $3.18^d$ | $3.78^d$ | $5.89^d$ | $15.55^c$ | $22.48^b$ | $33.65^a$ |
| | Sarıbağlar | $3.30^d$ | $3.88^d$ | $6.28^d$ | $16.93^c$ | $26.95^b$ | $39.19^a$ |
| | | * | * | * | * | * | * |

*p: Significance ≤ 0.01.

**Table 5**. Pb concentration (mg kg$^{-1}$) in different parts of tobacco cultivars.

| | | Control | NPK | Pb$_1$ | Pb$_2$ | Pb$_3$ | Pb$_4$ |
|---|---|---|---|---|---|---|---|
| Root | Akhisar | 2.61$^{e}$ | 3.92$^{de}$ | 8.20$^{d}$ | 30.76$^{c}$ | 61.81$^{b}$ | 81.84$^{a}$ |
| | Basma | 3.88$^{d}$ | 4.08$^{d}$ | 8.37$^{d}$ | 35.52$^{c}$ | 74.06$^{b}$ | 97.05$^{a}$ |
| | Dibek | 2.36$^{e}$ | 3.43$^{de}$ | 6.94$^{d}$ | 20.68$^{c}$ | 53.28$^{b}$ | 63.54$^{a}$ |
| | Sarıbağlar | 2.48$^{e}$ | 3.14$^{de}$ | 7.08$^{d}$ | 23.34$^{c}$ | 59.99$^{b}$ | 76.65$^{a}$ |
| | | * | * | * | * | * | * |
| Stem | Akhisar | 2.01$^{d}$ | 2.07$^{d}$ | 4.21$^{d}$ | 16.06$^{c}$ | 36.78$^{b}$ | 53.84$^{a}$ |
| | Basma | 2.08$^{d}$ | 2.13$^{d}$ | 5.49$^{d}$ | 22.91$^{c}$ | 45.66$^{b}$ | 60.63$^{a}$ |
| | Dibek | 1.84$^{d}$ | 1.99$^{d}$ | 3.25$^{d}$ | 12.46$^{c}$ | 27.24$^{b}$ | 41.58$^{a}$ |
| | Sarıbağlar | 1.89$^{d}$ | 2.05$^{d}$ | 4.03$^{d}$ | 13.96$^{c}$ | 31.61$^{b}$ | 44.10$^{a}$ |
| | * | * | * | * | * | * | * |
| Leaf | Akhisar | 3.49$^{d}$ | 5.56$^{d}$ | 6.35$^{d}$ | 16.18$^{c}$ | 30.06$^{b}$ | 42.53$^{a}$ |
| | Basma | 3.78$^{e}$ | 5.76$^{de}$ | 8.37$^{d}$ | 20.98$^{c}$ | 44.82$^{b}$ | 58.70$^{a}$ |
| | Dibek | 3.25$^{d}$ | 4.66$^{d}$ | 5.57$^{d}$ | 11.42$^{c}$ | 19.25$^{b}$ | 25.36$^{a}$ |
| | Sarıbağlar | 3.45$^{d}$ | 4.96$^{d}$ | 6.16$^{d}$ | 12.79$^{c}$ | 23.11$^{b}$ | 36.03$^{a}$ |
| | * | * | * | * | * | * | * |

*p: Significance ≤ 0.01.

and cultivars. Shoot Pb concentrations for most plants are generally low since little Pb is transferred from the root to shoot. Likewise, previous study reports the highest Pb concentration accumulation in plant roots (Wilde, 2005). The highest Pb concentration was revealed in the root, while the lowest in the leaves. Similar results were also reported by Del Piano et al. (2008) in tobacco plants. Lead is only sparingly soluble in solution, and even at highly contaminated sites, Pb in the soil solution is often less than 4 mg/L (Cunningham and Berti, 2000). Likewise, another study also revealed that low transportation rate of Pb from roots to stems and leaves is due to the formation of stable complexes with amino acids which might indicate reduced transportation of this ion from the root (Mengel and Kirkby, 2001). Nicotianamine can significantly enhance plant tolerance to high Ni, as shown by the overexpression of a nicotianamine synthase from barley or from the metal hyperaccumulator Noccaea caerulescens in tobacco or Arabidopsis, respectively (Kim et al., 2005, Pianelli et al., 2005).

Hyperaccumulator plants including tobacco releases organic chelates for metal extraction (Chen et al., 2003; Evangelou et al., 2006; Duarte et al., 2007). The use of heavy metal chelators have been introduced to soil to make Pb more bioavailable for plant uptake. The addition of synthetic chelators has been shown to increase soil Pb mobility and plant uptake (Boonyapookana et al., 2005). Root secretions may affect the heavy metal uptake and translocations. Rhizosphere acidification and release of root exudates contribute to the absorption of several heavy metals (Lasat, 2002). Some root morphological traits, such as pattern of root density, maximum depth and specific root length, are considered to be crucial for adaptation to stress conditions (Fitter et al., 1991). Metal transfer from soil to plants depends on the parameters like soil type, soil pH, tobacco plant types, geographical location and fertilizers with varying chemical compositions (Golia et al., 2008). Researchers have shown that for effective uptake to occur, metals need to be solubilized in the rhizosphere, and then moved across the root–cell plasma membrane for subsequent transport into the xylem (Robinson et al., 2003).

Plants take up heavy metals from the soil via root to shoot transport. The roots receive metal either by symplastic transport or by apoplastic transport (Thakur et al., 2016; Ling et al., 2017). Heavy metals enter through intercellular spaces (apoplast) in apoplastic transport and through specific ion channels or carriers in symplastic transport (Chaudhary et al., 2018). Chemical changes are known to alter metal bioavailability and speciation in the growing medium, contributing to improved metal uptake by plant roots (Chiu et al., 2005).Various compounds have been studied for their ability to mobilize metals and enhance metal accumulation in plants, including chelating agents (Chen and Cutright, 2001; Grčman et al., 2003), organic acids (Chen et al., 2003; Evangelou et al., 2006), and amino acids (Singer et al., 2007). Plant growth can enhance the mobility and bioavailability of metals in soil by decreasing soil pH and root exudates (Majewska et al., 2011). Plants release organic acids, including oxalic acids, fatty acids and citric acids through the roots, which can change the soil pH in cooperation with the microbial interactions in rhizosphere, resulting in dissolution of metals in soils, and makes the metals more bioaccessible to plants (Pan et al., 2018). Common organic acids are gluconic acid, tartaric acid, oxalic acid, and citric acid, humic acid, malic acid, and oxalic acid. These acids are also reported to enhance phytoremediation by increasing the uptake of nutrients and heavy metals by the plants (Gómez-Garrido et al., 2018; Yang et al., 2018). Plants accumulate Cd, Pb, and Ni from soils at different levels depending on plant species, genotypes within the same species, soil pH, and organic matter content (Antonious et al., 2017).

The reason for higher accumulation of Pb in roots could be a defense mechanism that the plant has developed to protect its stem, fruit, and shoots from Pb toxicity (Yerli et al., 2020). Similarly, Zaprjanova et al. (2010) have reported decreasing levels of Pb concentrations from roots to leaves in tobacco. Tso (1990) reported that Pb concentrations in tobacco leaves and stems may reach up to 200 and 19 mg $kg^{-1}$, respectively. Piano et al. (2008) stated that limited Pb transport happens from roots to other plant parts. Golia et al. (2003) reported different responses of tobacco cultivars to heavy metal concentrations [Zn, Cu, Ni, Cd, manganese (Mn)], and their response order was Burley > Oriental > Virginia. Angelova et al. (2004) reported that the distribution of the heavy metal in the organs of tobacco plants has a selective character, which decreases in the following order: leaves > capsules > stems > seeds > roots.

Differences in the metal concentration in tobacco leaves examined seem to imply that different types of tobacco have different responses to metal accumulation (Golia et al., 2007). Zaprjanova et al. (2010) reported that Pb concentration in tobacco leaves and blossoms, and Cd concentration in the leaves increased linearly with the increase of the total element's content in the soil. The natural concentration of Pb in plants grown in unpolluted regions is in the range of 0.1–10 mg $kg^{-1}$ (Kabata-Pendias, 2011). In this study, control, NPK and $Pb_1$ (NPK+10 mg $kg^{-1}$ Pb) applications lowered this reference range, but $Pb_2$ (NPK+50 mg $kg^{-1}$ Pb), $Pb_3$ (NPK+100 mg $kg^{-1}$ Pb) and $Pb_4$ (NPK+150 mg $kg^{-1}$ Pb) applications significantly showed higher content of Pb element value.

### 3.3. Heavy metals hyperaccumulation factors

Enrichment factor (EF) increased with nickel and lead application dose and the smallest value was obtained in control and the highest value in $Ni_4$ (NPK+150 mg $kg^{-1}$ Ni) and $Pb_4$ (NPK+150 mg $kg^{-1}$ Pb) applications. EF was noticed for the cultivars in the following order; Basma > Akhisar > Sarıbağlar > Dibek. Likewise, the translocation factor (TF) of the cultivars also differed with the Ni and Pb applications (Table 6).

The highest TF values were obtained in Basma and Akhisar varieties in $Ni_1$ (0.85) and $Ni_2$ (0.85) applications. In Pb applications, the highest TF value has been obtained in the control (0.77) application (Table 7). It has been reported that the highest TF values determined in roots compared to stem and leaves is due to the lower transfer ability of Pb from roots to leaves (Proshad et al., 2020).

**Table 6.** Heavy metals hyperaccumulation factors of nickel.

| Enrichment factor (EF) | | | | | | |
|---|---|---|---|---|---|---|
| Cultivars | control | NPK | $Ni_1$ | $Ni_2$ | $Ni_3$ | $Ni_4$ |
| Akhisar | 0.75 | 0.86 | 1.58 | 6.59 | 12.91 | 17.10 |
| Basma | 0.79 | 0.91 | 2.09 | 8.15 | 15.50 | 21.41 |
| Dibek | 0.67 | 0.74 | 1.36 | 5.35 | 9.36 | 12.51 |
| Sarıbağlar | 0.70 | 0.79 | 1.42 | 5.98 | 10.97 | 14.25 |
| Translocation factor (TF) | | | | | | |
| Akhisar | 0.56 | 0.54 | 0.74 | 0.85 | 0.59 | 0.77 |
| Basma | 0.55 | 0.54 | 0.85 | 0.78 | 0.67 | 0.78 |
| Dibek | 0.60 | 0.63 | 0.64 | 0.78 | 0.60 | 0.78 |
| Sarıbağlar | 0.56 | 0.58 | 0.61 | 0.82 | 0.56 | 0.78 |
| Bioaccumulation factor (BAF) | | | | | | |
| Akhisar | 0.15 | 0.17 | 0.44 | 2.31 | 3.59 | 5.63 |
| Basma | 0.16 | 0.18 | 0.57 | 2.64 | 4.58 | 7.06 |
| Dibek | 0.15 | 0.16 | 0.33 | 1.78 | 2.81 | 4.29 |
| Sarıbağlar | 0.15 | 0.17 | 0.34 | 2.06 | 3.15 | 4.83 |

**Table 7.** Heavy metals hyperaccumulation factors of lead.

| Enrichment factor (EF) | | | | | | |
|---|---|---|---|---|---|---|
| Cultivars | control | NPK | $Pb_1$ | $Pb_2$ | $Pb_3$ | $Pb_4$ |
| Akhisar | 1.79 | 2.55 | 4.15 | 13.93 | 28.46 | 39.42 |
| Basma | 2.15 | 2.64 | 4.91 | 17.56 | 36.40 | 47.87 |
| Dibek | 1.86 | 2.23 | 3.48 | 9.85 | 22.07 | 28.86 |
| Sarıbağlar | 1.73 | 2.24 | 3.82 | 11.08 | 25.37 | 34.68 |
| Translocation factor (TF) | | | | | | |
| Akhisar | 0.77 | 0.52 | 0.51 | 0.52 | 0.59 | 0.65 |
| Basma | 0.53 | 0.52 | 0.65 | 0.65 | 0.61 | 0.62 |
| Dibek | 0.77 | 0.58 | 0.46 | 0.60 | 0.51 | 0.65 |
| Sarıbağlar | 0.76 | 0.65 | 0.56 | 0.59 | 0.52 | 0.57 |
| Bioaccumulation factor (BAF) | | | | | | |
| Akhisar | 0.44 | 0.45 | 0.93 | 3.55 | 8.13 | 11.91 |
| Basma | 0.46 | 0.47 | 1.21 | 5.06 | 10.10 | 13.41 |
| Dibek | 0.40 | 0.44 | 0.71 | 2.75 | 6.02 | 9.19 |
| Sarıbağlar | 0.41 | 0.45 | 0.89 | 3.08 | 6.99 | 9.75 |

The TF values for heavy metals are usually less than one as reported for most elements in the previous study by Liu et al. (2019). A hyperaccumulator is defined by EF or TF when it is more than one (Lorestani et al., 2011). Bioaccumulation factor (BAF) increased with nickel and lead application dose and the smallest value was obtained in control and the highest value in $Ni_4$ (NPK+150 mg $kg^{-1}$ Ni) and $Pb_4$ (NPK+150 mg $kg^{-1}$ Pb) applications and follwing order; Basma > Akhisar > Sarıbağlar > Dibek.

The BAF indicates the potential of a plant to take up heavy metals from the soil, while the TF represents the transport of the element from roots to leaves. Most of the plants trapped heavy metals in their roots and this might be an evolutionary strategy to protect the shoots from heavy metal toxicity. As a result, the TF values for heavy metals are usually less than one, as reported by Liu et al. (2019). BAF values increased with increasing Ni and Pb concentrations, and a positive linear relationship between soil and plant metal concentrations has been reported in many previous studies (Liu et al., 2016; Saha et al., 2016). BAF value varies depending on soil properties (McGrath and Zhao, 2003). It has been stated that the BAF value of the leaf in alkaline soil is higher than acidic soil (Liu et al., 2016). Both the EF and TF should be considered to evaluate whether a plant is metal hyperacumulator or not (Iqbal et al., 2020). It has been stated that the TF differs from metal to metal, and from plant to plant (Proshad et al., 2020). Higher BAF values of Basma might reflect the genetic differences due to root secretion and adaptation of the variety to the growing environment. Basma had naturally developed an adaptation mechanism to grow under acidic conditions where heavy metal solubility is high.

## 4. Conclusion

In present study, Ni and Pb uptake and accumulation capacities in different oriental tobacco cultivar were tested to determine of the phytoremediation potential. Higher Ni and Pb concentrations were determined mostly in roots rather than stems and leaves. While the highest biomass values were observed in $Ni_1$ (NPK+10 mg $kg^{-1}$ Ni), and $Pb_1$ (NPK+10 mg $kg^{-1}$ Pb) treatments. Biomass values of cultivars under $Ni_4$ (NPK+150 mg $kg^{-1}$ Ni) and $Pb_4$ (NPK+150 mg $kg^{-1}$ Pb) treatment doses decreased significantly. Basma cultivar recorded the highest biomass yield other cultivars under increased Ni and Pb concentrations. Further, the highest EF and BAF were also found in Basma cultivar. Thus, Basma cultivar can be a better choice for phytoremediation when compared to other cultivars. Growing conditions like soil pH and genetic properties of Basma variety should be considered as important parameters for phytoremediation.

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## References


Adamu CA, Mulchi CL, Bell PF (1989). Relationships between soil pH, clay, organic matter and CEC and heavy metal concentrations in soils and tobacco. Tobacco Science 33: 96-10.

Angelova V, Ivanov K, Ivanova R (2004). Effect of chemical forms of lead, cadmium, and zinc in polluted soils on their uptake by Tobacco. Journal of Plant Nutrition 27 (5): 757-773.

Antoniadis V, Shaheen SM, Stark HJ, Wennrich R, Levizou E et al. (2021). Phytoremediation potential of twelve wild plant species for toxic elements in a contaminated soil. Environment International 146: 106233.

Antonious GF, Turley ET, Kochhar TS (2017). Testing bioaccumulation of Cd, Pb, and Ni in plants grown in soil amended with municipal sewage sludge at three kentucky locations. JSM Environmental Science & Ecology 5 (1): 1039.

Arazi T, Sunkar R, Kaplan B, Fromm H (1999). A tobacco plasma membrane calmodulin-binding transporter confers Ni2+ tolerance and Pb2+ hypersensitivity in transgenic plants. The Plant Journal 20 (2): 171-182.

Boonyapookana B, Parkpian P, Techapinyawat S, DeLaune RD, Jugsujinda A (2005). Phytoaccumulation of lead by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), and vetiver (*Vetiveria zizanioides*). Journal of Environmental Science and Health Part A 40 (1): 117-137.

Chaudhary K, Agarwal S, Khan S (2018). Role of phytochelatins (PCs), metallothioneins (MTs), and heavy metal ATPase (HMA) genes in heavy metal tolerance. In: Prasad R (editor). Mycoremediation and Environmental Sustainability. Cham, Switzerland: Springer, pp. 39-60.

Chen H, Cutright T (2001). EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere 45 (1): 21-28.

Chen YX, Lin Q, Luo YM, He YF, Zhen SJ et al. (2003). The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere 50 (6): 807-811.

Chen Y, Wang C, Wang Z (2005). Residues and source identification of persistent organic pollutants in farmland soils irrigated by effluents from biological treatment plants. Environment International 31 (6): 778-783.

Chen C, Huang D, Liu J (2009). Functions and toxicity of nickel in plants: Recent advances and future prospects. Clean 37: 304-313.

Chiu KK, Ye ZH, Wong MH (2005). Enhanced uptake of As, Zn, and Cu by Vetiveria zizanioides and Zea mays using chelating agents. Chemosphere 60 (10): 1365-1375.

Cunningham, SD Berti WR (2000). Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-lead issue. In: Terry N, Banuelos GS (editors). Phytoremediation of Contaminated Soil and Water. Boca Raton, FL, USA: CRC Press, pp. 359-376.

Da Silva CP, De Almeida TE, Zittel R, De Oliveira Stremel TR, Domingues CE et al. (2016). Translocation of metal ions from soil to tobacco roots and their concentration in the plant parts. Environmental Monitoring and Assessment 188: 663.

Del Piano L, Abet M, Sorrentino C, Barbato L, Sicignano M et al. (2008). Uptake and distribution of lead in tobacco (*Nicotiana tabacum* L.). Journal of Applied Botany and Food Quality 82: 21-25.

Doroszewska T, Bebec A (2004). Variation for cadmium uptake among Nicotiana species. Genetic Resources and Crop Evolution 21: 323-333.

Duarte B, Delgado M, Caçador I (2007). The role of citric acid in cadmium and nickel uptake and translocation, in Halimione portulacoides. Chemosphere 69 (5): 836-840.

Evangelou MWH, Ebel M, Schaeffer A (2006). Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*. Chemosphere 63 (6): 996-1004.

Fitter AH, Strickland TR (1991). Architectural analysis of plant root systems. 2. Influence of nutrient supply on architecture in contrasting plant species. New Phytologist 118 (3): 383-389.

Ghosh M, Singh SP (2005). A review on phytoremediation of heavy metals and utilization of its byproducts. Applied Ecology and Environmental Research 3: 1-8.

Golia EE, Mitsios IK, Tsadilas CD (2003). Concentration of heavy metals in burley, virginia and oriental tobacco leaves in the Thessaly region of central Greece. Communications in Soil Science and Plant Analysis 36: 200-230.

Golia EE, Dimirkou A, Mitsios IK (2007). Accumulation of metals on tobacco leaves (primings) grown in an agricultural area in relation to soil. The Bulletin of Environmental Contamination and Toxicology 79: 158-162.

Golia EE, Dimirkou A, Mitsios IK (2008). Levels of heavy metals pollution in different types of soil of central Greece. Bulletin of Environmental Contamination and Toxicology 80: 206-210.

Golia EE, Dimirkou A, Mitsios IK (2009). Heavy-metal concentration in tobacco leaves in relation to their available soil fractions. Communications in Soil Science and Plant Analysis 40 (1-6): 106-120.

Gómez-Garrido M, Mora Navarro J, Murcia Navarro FJ, Faz Cano Á (2018). The chelating effect of citric acid, oxalic acid, amino acids and Pseudomonas fluorescens bacteria on phytoremediation of Cu, Zn, and Cr from soil using Suaeda vera. International Journal of Phytoremediation 20 (10): 1033-1042.

Grčman H, Vodnik D, Velikonja-Bolta S, Lestan D (2003). Ethylenediaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. Journal of Environmental Quality 32 (2): 500-506.

Huang D, Hu C, Zeng G, Cheng M, Xu P et al (2016). Combination of Fenton processes and biotreatment for wastewater treatment and soil remediation. Science of The Total Environment 574: 1599-1610.

Iqbal M, Ahmed S, Rehman W, Menaa F, Ullah MA (2020). Heavy metal levels in vegetables cultivated in Pakistan soil irrigated with untreated wastewater: preliminary results. Sustainability 12 (21): 8891.

Kabata-Pendias A (2011). Trace Elements in Soils and Plants. 4th ed. Boca Raton, FL, USA: CRC Press.

Kacar B, İnal A (2008). Bitki Analizleri. Ankara, Turkey: Nobel Yayın Dağıtım (in Turkish).

Kaur R, Bhatti SS, Singh S, Singh J, Singh S (2018). Phytoremediation of heavy metals using cotton plant: a field analysis. Bulletin of Environmental Contamination and Toxicology 101 (5): 637-643.

Kim S, Takahashi M, Higuchi K, Tsunoda K, Nakanishi H et al. (2005). Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. Plant Cell Physiology 46 (11): 1809-1818.

Kınay A, Erdem H (2019). The effects of ıncreasing doses of lead applications on growth, Pb and microelement concentrations of tobacco varieties. Turkish Journal of Agriculture-Food Science and Technology 7 (12): 2083-2088 (in Turkish).

Kulhari A, Sheorayan A, Bajar S, Sarkar S, Chaudhury A et al. (2013). Investigation of heavy metals in frequently utilized medicinal plants collected from environmentally diverse locations of north western India. Springerplus 2: 676.

Lasat MM (2002). Phytoremediation of toxic metal: a review of biological mechanisms. Journal of Environmental Quality 31: 109-120.

Leskova A, Zvarik M, Araya T, Giehl RFH (2020). Nickel toxicity targets cell wall-related processes and PIN2-mediated auxin transport to ınhibit root elongation and gravitropic responses in arabidopsis. Plant and Cell Physiology 61 (3): 519-535.

Li Y, Zamble DB (2009). Nickel homeostasis and nickel regulation: an overview. Chemical Reviews 109: 4617-4643.

Li P, Lin C, Cheng H, Duan X, Lei K (2015). Contamination and health risks of soil heavy metals around a lead/zinc smelter in southwestern China. Ecotoxicology and Environmental Safety 113: 391-399.

Ling T, Gao Q, Du H, Zhao Q, Ren J (2017). Growing, physiological responses and Cd uptake of corn (*Zea mays* L.) under different Cd supply. Chemical Speciation & Bioavailabilit 29 (1): 216-221.

Liu H, Wang H, Ma Y, Wang H, Shi Y (2016). Role of transpiration and metabolism in translocation and accumulation of cadmium in tobacco plants (*Nicotiana tabacum* L.). Chemosphere 144: 1960-1965.

Liu H, Wang H, Zhang Y, Wang H, Yang J et al. (2019). Comparison of heavy metal accumulation and cadmium phytoextraction rates among ten leading tobacco (*Nicotiana tabacum* L.) cultivars in China. International Journal of Phytoremediation 21 (7): 699-706.

Lorestani B, Cheraghi M, Yousefi N (2011). Accumulation of Pb, Fe, Mn, Cu and Zn in plants and choice of hyperaccumulator plant in the industrial town of Vian, Iran. Archives of Biological Sciences 63 (3): 739-745.

Lugon-Moulin N, Zhang M, Gadani F, Rossi L, Koller D et al. (2006). Critical review of the science and options for reducing cadmium in tobacco (*Nicotiana tabacum* L.) and other plants. Agronomy for Sustainable Development 26 (3): 111-180.

Majewska M, Kurek E, Slomka A (2011). Effect of plant growth on total concentrations of Zn, Pb and Cd, and their distribution between operational fractions in the upper layer of a 100-year-old zinc-lead waste heap. Polish Journal of Environmental Studies 20 (3): 591-597.

Marschner H (1995). Mineral Nutrition of Higher Plants. 2nd ed. New York, USA: Academic Press.

McGrath SP, Zhao FJ (2003). Phytoextraction of metals and metalloids from contaminated soils. Current Opinion in Biotechnology 14 (3): 277-282.

Mench M, Tancogne J, Gomez A, Juste C (1989). Cadmium bioavailability to *Nicotiana tabacum* L. *Nicotiana rustica* L. and *Zea mays* L. grown in soil amended or not amended with cadmium nitrate. Biology and Fertility of Soils 8: 48-53.

Mengel K, Kirkby EA (2001). Principles of Plant Nutrition.5th ed. Dordrecht, Netherlands: Springer.

Maodzeka A, Hussain N, Wei L, Zvobgo G, Mutemachani M et al. (2017). Elucidating the Physiology and biochemical responses of different tobacco (*Nicotiana tabacum*) genotypes to Pb toxicity. Environmental Toxicology and Chemistry 36: 175-181.

Mohanpuria P, Rana NK, Yadav SK (2007). Cadmium induced oxidative stres influence on glutathione metabolic genes of Camellia sinensis (L.). Environmental Toxicology 22 (4): 368-374.

Muthusaravanan S, Sivarajasekar N, Vivek JS, Paramasivan T, Mu Naushad et al. (2018). Phytoremediation of heavy metals: mechanisms, methods and enhancements. Environmental Chemistry Letters 16 (4): 1339-1359.

Oliver M, Gregory PJ (2015). Soil, food security and human health: A review. European Journal of Soil Science 66 (2): 257-76.

Pan C, Chen J, Wu K, Zhou Z, Cheng T (2018). Heavy metal contaminated soil imitation biological treatment overview. IOP Conference Series: Materials Science and Engineering 301: 012113.

Palusińska M, Barabasz A, Kozak K, Papierniak A, Maślińska K et al. (2020). Zn/Cd status-dependent accumulation of Zn and Cd in root parts in tobacco is accompanied by specific expression of ZIP genes. BMC Plant Biology 20: 37.

Pianelli K, Mari S, Marques L, Lebrun M Czernic P (2005). Nicotianamine over accumulation confers resistance to nickel in Arabidopsis thaliana. Transgenic Research 14: 739-748.

Proshad R, Zhang D, Uddin M, Wu Y (2020). Presence of cadmium and lead in tobacco and soil with ecological and human health risks in Sichuan province, China. Environmental Science and Pollution Research 27 (15): 18355-18370.

Rattan RK, Datta SP, Chhonkar PK, Suribabu K, Singh AK (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater: a case study. Agriculture, Ecosystems and Environment 109 (3-4): 310-322.

Raza A, Habib M, Kakavand SN, Zahid Z, Zahra N et al. (2020). Phytoremediation of Cadmium: Physiological, Biochemical, and Molecular Mechanisms. Biology 9 (7): 177.

Regassa G, Chandravanshi BS (2016). Levels of heavy metals in the raw and processed Ethiopian tobacco leaves. Springer Plus 5 (1): 1-9.

Rehman MZ, Rizwan M, Ali S, Ok YS, IshaqueW et al. (2017b). Remediation of heavy metal contaminated soils by using Solanum nigrum: a review. Ecotoxicology and Environmental Safety 143: 236-248.

Rizwan M, Ali S, Adrees M, Ibrahim M, Tsang DC et al. (2017b). A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. Chemosphere 182: 90-105.

Rizwan M, Ali S, Rehman MZ, Rinklebe J, Tsang DC et al. (2018). Cadmium phytoremediation potential of Brassica crop species: a review. Science of The Total Environment 631-632: 1175-1191.

Robinson B, Green S, Mills T, Clothier B, Van der Velde M et al. (2003). Phytoremediation: Using plants as biopumps to improve degraded environments. Australian Journal of Soil Research 41 (3): 599-611.

Rong Q, Zhong K, Huang He, Li C, Zhang C et al. (2020). Humic acid reduces the available cadmium, copper, lead, and zinc in soil and their uptake by tobacco. Applied Sciences 10 (3): 1077.

Schmidt U (2003). Enhancing phytoextraction: the effects of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. Journal of Environmental Quality 32 (6): 1939-1954.

Saha N, Rahman MS, Jolly YN, Rahman A, Sattar MA et al. (2016). Spatial distribution and contamination assessment of six heavy metals in soils and their transfer into mature tobacco plants in Kushtia District, Bangladesh. Environmental Science and Pollution Research 23 (4): 3414-3426.

Shah V, Daverey A (2020). Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil. Environmental Technology & Innovation 18; 100774.

Singer AC, Bell T, Heywood CA, Smith JAC, Thompson IP (2007). Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant Alyssum lesbiacum: Evidence of histidine as a measure of phytoextractable nickel. Environmental Pollution 147: 74-82.

Singh KP, Mogan D, Sinha S Dalwani R (2004). Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. Chemosphere 55 (2): 227-255.

Singh J, Upadhyay SK, Pathak RK, Gupta V (2011). Accumulation of heavy metals in soil and paddy crop (Oryzasativa), irrigated with water of Ramgarh Lake, Gorakhpur, UP, India. Toxicological & Environmental Chemistry 93 (3): 462-473.

Sun Y, Li Y, Xu Y, Liang X, Wang L (2015). In situ stabilization remediationf cadmium (Cd) and lead (Pb) co-contaminated paddy soil using bentonite. Applied Clay Science 105: 200-206.

Soudek P, Katrusakova A, Sedlacek L, Petrova S, Koci V et al. (2010). Effect of heavy metals on inhibition of root elongation in 23 cultivars of flax (*Linum usitatissimum* L.). Archives of Environmental Contamination and Toxicology 59 (2): 194-203.

Tauqeer HM, Ali S, Rizwan M, Ali Q, Saeed R et al. (2016). Phytoremediation of heavy metals by Alternanthera bettzickiana: growth and physiological response. Ecotoxicology and Environmental Safety 126: 138-146.

Thakur S, Singh L, Wahid ZA, Siddiqui MF, Atnaw SM et al. (2016). Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. Environmental Monitoring and Assessment 188 (4): 206.

Tomás J, Árvay J, Tóth T (2012). Heavy metals in productive parts of agricultural plants. Journal of Microbiology, Biotechnology and Food Sciences 1: 819-827

Tsadilas CD, Karaıvazoglou NA, Tsotsolis NC, Stamatiadis S, Samaras V (2005). Cadmium uptake by tobacco as affected by liming, N form and year of cultivation. Environmental Pollution 134: 239-246.

Tso TC (1990). Production nutrition-minor elements and heavy metals, In: Physiology and Biochemistry of Tobacco Plant. Beltsville, MD, USA: Ideals, pp. 313-368.

Tunc T, Sahin U (2015). The changes in the physical and hydraulic properties of a loamy soil under irrigation with simpler-reclaimed wastewaters. Agricultural Water Management 158: 213-224.

Vamerali T, Bandiera M, Mosca G (2010). Field crop for phytoremediation of metal contaminated land. A Review. Environmental Chemistry Letters 8: 1-17.

Vanlı Ö (2007). Removal of Pb, Cd, B elements from soil by chelate assisted phytoremediation method. MSc, İstanbul Technical University, İstanbul, Turkey (in Turkish with an abstract in English).

Vera-Estrella R, Gomez-Mendez MF, Amezcua-Romero JC, Barkla BJ, Rosas-Santiago P et al. (2017). Cadmium and zinc activate adaptive mechanisms in Nicotiana tabacum similar to those observed in metal tolerant plants. Planta 246 (3): 433-451.

Wagner GJ, Sutton TG, Yergan R (1988). Root control of leaf cadmium accumulation in tobacco. Tobacco Science 32: 88-91.

Wilde EW (2005). Phytoextraction of lead from firing range soil by Vetiver grass.Chemosphere 61 (10): 1451-1457.

Yan A, Wang Y, Tan SN, Yusof MLM, Ghosh S et al. (2020). Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Frontiers in Plant Science 11: 359.

Yang P, Zhou XF, Wang LL, Li QS, Zhou T et al. (2018). Effect of phosphate-solubilizing bacteria on the mobility of insoluble cadmium and metabolic analysis. International Journal of Environmental Research and Public Health 15 (7): 1330.

Yerli C, Çakmakcı T, Şahin Ü, Tüfenkçi Ş (2020). The effects of heavy metals on soil, plant, water and human health. Turkish Journal of Nature and Science 9 (Special Issue): 103-114 (in Turkish with an abstract in English).

Zaprjanova P, Dospatliev L, Angelova V, Krasimir I (2010). Correlation between soil characteristics and lead and cadmium content in the aboveground biomass of Virginia tobacco. Environmental Monitoring and Assessment 163: 253-261.

Zeid IM, Ghazi SM, Nabawy DM (2013). Alleviation of heavy metals toxicity in waste water used for plant irrigation. International Journal of Agronomy and Plant Production. 4 (5): 976-983.

Zhao M, Li T, Yu H, Zhang X, Zheng Z et al. (2020). Fractionation and chemical structure of dissolved organic matter in the rhizosphere associated with cadmium accumulation in tobacco lines (*Nicotiana tabacum* L.). Environmental Science and Pollution Research 27: 17794-17803.