# The Effects of Biochar on the Phytoremediation Efficiency of Tobacco *(Nicotiana tabacum)* Plant

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***Abstract***— In order to determine the effects of Biochar on metal uptake and phytoremediation in tobacco plant in heavy metal applied soil, a greenhouse pot experiment was carried out. In the factorial experiment design, the effects of Zn, Cu, N, Pb and Cd metals and Biochar applied to the substrate soil on biomass (dry matter) and metal contents and phytoremediation in tobacco plants were evaluated. The results showed that the Tobacco plant could survive under high heavy metal stress conditions but lost biomass. In metal applications, tobacco plants showed high metal accumulation, metal transfer from soil and especially high Cd phytoextraction efficiency. Biochar applications increased shoot and root biomass, metal accumulation, metal transfer factor, metal uptake and metal phytoextraction efficiency in tobacco plant. The results determined that Biochar has a significant effect to increase the phytoremediation efficiency in polluted soils.

***Keywords***— Biochar; Phytoremediation; Tobacco.

## I. Introduction

Biochar is a porous, carbonaceous product obtained from the pyrolysis of organic substances, and numerous materials can be used as raw materials for its production, including high-carbon sludges, plant materials and organic fertilizers. Typically, biochars have a high cation exchange capacity and an alkaline chemical character. One of the properties of biochars is that they have large surface areas and the ability to adsorb heavy metals [1]. Biochar can also reduce the mobility of heavy metals by changing their redox values [2]. Although Biochar applied to the soil does not cause a decrease in the total heavy metal content of the soil, it reduces the availability and mobility of heavy metals [3] and can reduce the heavy metal concentration in the plant [4].

Recent years, as an alternative to sophisticated traditional technologies for soil remediation, phytoremediation has been highlighted for the efficient and economic removal of heavy metals from soil. Phytoremediation is considered the main and most promising technique to remove heavy metals from the soil and is based on the use of hyperaccumulatory plants that transfer heavy metals to their bodies. A metal polluted soil can directly be used for agricultural purposes by successful phytoremediation. All plants have the potential to extract metals from soil, but some plants termed hyperaccumulators have shown the ability to extract, accumulate and tolerate high levels of heavy metals. In the phytoremediation studies natural hyperaccumulator plants with exceptional metal accumulating capacity, and high-biomass plants accumulating relatively high amounts of the metals are used. It is reported that many hyperaccumulator characteristics with high biomass were found in the tobacco plant, *(Nicotiana tabacum)* [5].

The bioavailability of metals in soil is affected by numerous factors, such as cation exchange capacity, pH values of the soil, excess amounts of fertilizers and chelators. It is widely reported in the literature that biochar enhances plant growth, so biochar has the potential to increase the efficiency of phytoremediators. Although this increase in plant productivity is highly variable according to plant species, it has been measured as approximately 10% [6]. Therefore, Biochar and phytoremediation techniques are considered to have the potential to be used in remediation of heavy metal contaminated soils [7].

The aim of this research was to assess the ability of Biochar on bioavailability and phytoextraction of heavy metals from metal polluted soil by the use of Tobacco plant under greenhouse controlled conditions.

## II. Materials and Method

### A. *Soil charactarization and analysis*

The contaminated soil used in this experiment was sampled from a red mediterranean soil, representative of the major agricultural areas of Turkey Antalya. Experimental 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. The main analytical characteristics of the experimental soil are shown in Table 1 which also shows the pollutant limits of soil permitted by EU legislation [8]. Physical and chemical characteristics of experimental 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 EU [9]. It is evaluated that the characteristics of the experimental soil are suitable for general plant cultures and that it does not contain heavy metals at a level that will adversely affect plant growth. In the experimental soil, available plant nutrients are not sufficient and there is a basic fertilization requirement at the agronomic level for the growth of the Tobacco plant.

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TABLE I: THE ANALYTICAL CHARACTERISTICS OF THE EXPERIMENTAL SOIL BEFORE APPLICATIONS

| Parameters | |
|---|---|
| Texture Grade | Loam |
| pH- $H_2O$ (1:5 w/v) | 7.35 |
| $CaCO_3$, % | 23 |
| Organic matter, % | 1,2 |
| Clay,% | 18 |
| CEC, cmol $kg^{-1}$ | 28,2 |
| EC, dS $m^{-1}$ 25°C | 0,88 |
| Total N, % | 0,125 |
| P (ex), mg $kg^{-1}$ | 2,8 |
| K (ex), mg $kg^{-1}$ | 120 |
| Ca (ex), mg $kg^{-1}$ | 1280 |
| Mg (ex), mg $kg^{-1}$ | 255 |
| Total Zn, mg $kg^{-1}$ | 77 (150-300)* |
| Total Cu, mg $kg^{-1}$ | 14 (50-140)* |
| Total Ni, mg $kg^{-1}$ | 12 (30-75)* |
| Total Pb, mg $kg^{-1}$ | 18 (50-300)* |
| Total Cd, mg $kg^{-1}$ | 0,01 (1-3)* |

*: Metal limits in soil, mg $kg^{-1}$ dry wt [9]

Soil texture was determined by the hydrometer method, the soil pH was measured by the $CaCl_2$ method, organic matter content, as determined by the Walkley-Black method, $CaCO_3$ was determined by Scheibler calcimeter, the total Zn, Cu, Ni, Pb and Cd contents of the soil were digested by the aqua regia method (1:3 $HNO_3$/HCl). Total soil metal concentrations were analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

### *B. Preparation of Biochar for Experiment*

Biochar material used in the experiment was obtained from greenhouse wastes (post-harvest leaves, stems and roots of tomato plant) commonly found in Antalya greenhouse regions. Biochar production from greenhouse waste was obtained by pyrolysis of waste biomass, which was ground to 1-5 mm in size, then pyrolized at 300 °C in an oxygen-free medium. The C/N ratio of the biochar used in the research was determined as 175/1 and the pH was 5.87. The particle size of material was less than 3 mm.

### *C. Experimental Design*

A factorial experiment was conducted in randomized complete block design including 2 levels of Biochar (1 control and 1 treatment) and 5 levels (1 control and 4 treatments) of heavy metals with 5 replications. Ten kilograms of air-dried and sieved soil mixture (substrate) were filled into plastic pots. A pot-plate was placed under each pot to prevent leaching. Basic N-P-K fertilization was applied to experimental soil at the rate of 80, 40 and 80 mg $kg^{-1}$ 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 [9].

Biochar were added to pot soil (substrate) at 10 g $kg^{-1}$ rate. A uniform application was obtained by homogenization of the soil. The soil was subsequently incubated in the green house for 2 months before experiment. During this period the soil was irrigated 1-2 times in a week with deionised water to maintain field capacity level of water.

TABLE II. HEAVY METAL TREATMENT LEVELS OF EXPERIMENT

| 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 |

### *D. Plant growth and analysis*

Seeds of Tobacco plant (*Nicotiana tabacum*) were disinfected by sodium hypochlorite solution of 5 % during a few minutes and then rinsed in the distilled water before sowing to soil. The Seeds were germinated in peat+perlite substrate mixture. Then, 2 seedlings of each plant were transplanted into pot soil. All plants were grown under greenhouse environmental conditions. During the experiment, plants were irrigated regularly and treated according to common agrotechnical principles. After 60 days of growth all plants were harvested. Shoots and roots of plant samples were rinsed briefly in deionised water and were dried at 60 ºC in a air-forced oven, ground and then digested in aqua regia (1:3 $HNO_3$/HCl). Total soil and plant metal concentrations were analysed using ICP-MS under optimised measurement conditions, and values were adjusted for oven dried (12 h at 105 °C) material.

### *E. Evaluation parameters and statistical analysis*

Heavy Metal Transfer Factor: Soil-to-plant transfer is one of the key components of human exposure to metals through food chain. Heavy metal transfer factor (TF) is a parameter used to describe the transfer of heavy metals from soil to plant body. The TF of metals in the soil to shoots and roots of the plants was defined as the ratio of the heavy metal concentration in the plants to that in the soil [10].

Theoretical heavy metal transfer factor of harvested plants was calculated using Eq. 1, as follows [11]:

$$TF = \frac{C\ plant}{C\ soil} \qquad (1)$$

where: $C_{Plant}$ is heavy metal concentration in plant tissue, mg kg-1 dry weight; and $C_{Soil}$ is heavy metal concentration in soil, mg kg-1 dry weight.

Theoretical total metal uptake was calculated using Eq. 2, as follows [12]:

$$Metal\ uptake\ (mg\ pot^{-1}) = C\ x\ W\ x\ n \qquad (2)$$

where: C is heavy metal concentration in plant tissue, mg $kg^{-1}$ dry weight; and W is plant dry weight kg $plant^{-1}$ , and n is number of plant

Theoretical phytoextraction efficiency (%) of harvested plants (shoot and root) was calculated using Eq. 3, as follows [13]:

$$Phytoextraction\ efficiency\ (\%) = \frac{Cp\ x\ W\ x\ n}{Cs\ x\ 10\ kg\ pot-1} \qquad (3)$$

where: Cp is heavy metal concentration in plant tissue, mg $kg^{-1}$ dry weight; and W is plant dry weight kg $pot^{-1}$ ; n is number of plant; Cs is metal concentration of soil mg $kg^{-1}$

One-way ANOVA test ($p \leq 0.05$) calculated using the statistical package SPSS-23 for Windows program were applied to compare the differences in heavy metal concentrations in crops and in evaluation parameters.

## III. RESULTS AND DISCUSSION

### A. *Plant growth and heavy metal concentration of plants*

Heavy metal applications did not have a statistically significant effect on the dry matter value of the tobacco plant., and also no phytotoxicity symptoms were observed by the treatments of heavy metals (Table and Table 4). However, dry matter values of tobacco plants were found to be higher in biochar applications. Although this shows that the tobacco plant is well adapted to high heavy metal levels, it is understood that its development is adversely affected by high heavy metal levels.

TABLE III. SHOOT DRY MATTER (DM) (g POT$^{-1}$) AND HEAVY METAL CONCENTRATION (mg kg$^{-1}$) OF TOBACCO PLANT IN CONTROL AND BIOCHAR TREATMENTS

| | Metal Treatments | DM | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|---|
| Control Tretament | Control | 156 | 23 | 6 | 1 | 1 | 0,04 |
| | 1 | 148 | 143 | 11 | 4 | 12 | 1,23 |
| | 2 | 125 | 123 | 10 | 4 | 20 | 4,96 |
| | 3 | 130 | 195 | 13 | 12 | 23 | 6,23 |
| | 4 | 139 | 232 | 14 | 11 | 48 | 10,37 |
| Biochar Tretament | Control | 340 | 52 | 8 | 1 | 7 | 0,13 |
| | 1 | 337 | 195 | 9 | 8 | 10 | 8,94 |
| | 2 | 354 | 307 | 18 | 12 | 12 | 10,21 |
| | 3 | 343 | 361 | 27 | 20 | 35 | 24,10 |
| | 4 | 346 | 693 | 30 | 26 | 47 | 26,50 |

TABLE IV. ROOT DRY MATTER (g POT$^{-1}$) AND HEAVY METAL CONCENTRATION (mg kg$^{-1}$) OF TOBACCO PLANT IN CONTROL AND BIOCHAR TREATMENTS

| | Metal Treatments | Dry Matte r | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|---|
| Control Tretament | Control | 14 | 44 | 8 | 1 | 2 | 0,07 |
| | 1 | 14 | 198 | 15 | 4 | 12 | 1,42 |
| | 2 | 13 | 156 | 14 | 5 | 47 | 7,58 |
| | 3 | 15 | 184 | 22 | 8 | 52 | 16,31 |
| | 4 | 13 | 386 | 27 | 16 | 58 | 20,59 |
| Biochar Tretament | Control | 22 | 69 | 10 | 2 | 10 | 0,19 |
| | 1 | 21 | 351 | 17 | 16 | 15 | 9,69 |
| | 2 | 23 | 681 | 25 | 18 | 19 | 20,85 |
| | 3 | 21 | 641 | 33 | 19 | 24 | 28,77 |
| | 4 | 22 | 1015 | 54 | 29 | 44 | 37,37 |

It is known that the tobacco plant can contain heavy metal elements at high levels and adapt to levels that can be toxic to many plants. Total metal concentrations both in the shoots and roots of plant were increased by the increasing amounts of metal treatments. Heavy metal concentration of Tobacco plant was determined higher in Biochar treatment than control treatment. In Biochar treatments Zn was relatively the highest accumulating metal. However, the relative increase of Zn and Cd metals was found to be higher than other metals at high metal application levels. Metals accumulated both in shoots and roots in control treatments (no Biochar) were followed as Zn>Pb>Cu>Ni>Cd, but in Biochar treatments this order were followed as Zn>Pb>Cu>Cd>Ni. Metal concentrations of Tobacco in the root tissues was found higher than that of shoots. This shows that the main accumulation site of metals in the tobacco plant is the roots.

### B. *Metal transfer factor (TF) of plants*

TF is an important phytoremediation factor that shows the transport of a metal to the plant and is related to the availability of the metal and the uptake power of the plant. TF of Tobacco plant in treatments were decreased by the increasing amounts of metal treatments (Table 5 and Table 6). Biochar applications increased the TF value of the tobacco plant. The highest TF value among metals was determined in Cd metal. This indicates that Tobacco plant have adapted to accumulate heavy metals without any physiological disorder in natural conditions. TF of metals in Tobacco was followed Cd>Zn>Cu>Ni>Pb order. There was no significant difference in metal transfer factor values of shoot and root tissues for all metals. This results also show the remarkable ability of Cd accumulation in Tobacco plant and also effects of Biochar applications on metal availability and metal transfer to plant.

TABLE V. SHOOT METAL TRANSFER FACTOR OF TOBACCO PLANT IN CONTROL AND BIOCHAR TREATMENTS

| | Metal Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control Tretament | Control | 0,41 | 0,61 | 0,13 | 0,08 | 3,48 |
| | 1 | 0,38 | 0,07 | 0,05 | 0,05 | 0,35 |
| | 2 | 0,14 | 0,02 | 0,01 | 0,01 | 0,42 |
| | 3 | 0,12 | 0,01 | 0,02 | 0,01 | 0,26 |
| | 4 | 0,07 | 0,01 | 0,01 | 0,01 | 0,30 |
| Biochar Tretament | Control | 1,11 | 0,97 | 0,18 | 0,49 | 13,78 |
| | 1 | 0,59 | 0,07 | 0,10 | 0,04 | 3,19 |
| | 2 | 0,42 | 0,05 | 0,04 | 0,01 | 1,09 |
| | 3 | 0,26 | 0,04 | 0,04 | 0,01 | 1,29 |
| | 4 | 0,24 | 0,03 | 0,03 | 0,01 | 0,94 |

TABLE VI ROOT METAL TRANSFER FACTOR OF TOBACCO PLANT IN CONTROL AND BIOCHAR TREATMENTS

| | Metal Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control Tretament | Control | 0,72 | 0,76 | 0,18 | 0,10 | 6,38 |
| | 1 | 0,48 | 0,08 | 0,05 | 0,05 | 0,41 |
| | 2 | 0,16 | 0,03 | 0,01 | 0,04 | 0,65 |
| | 3 | 0,10 | 0,02 | 0,01 | 0,02 | 0,70 |
| | 4 | 0,10 | 0,01 | 0,01 | 0,01 | 0,59 |
| Biochar Tretament | Control | 1,01 | 1,00 | 0,20 | 0,59 | 16,74 |
| | 1 | 0,76 | 0,09 | 0,18 | 0,07 | 2,81 |
| | 2 | 0,64 | 0,05 | 0,07 | 0,01 | 1,80 |
| | 3 | 0,31 | 0,04 | 0,03 | 0,01 | 1,26 |
| | 4 | 0,25 | 0,03 | 0,03 | 0,01 | 1,08 |

### C. *Metal uptake of plants*

As expected, metal uptake of plants was increased by the increasing amounts of metal applications in control and Biochar treatments. Metal uptake of Tobacco plant treated with Biochar was found higher than control treatments (Table 7 and Table 8). Total metal uptake amount was determined as highest for Zn metal in Biochar treatments. Metal uptake rate of Zn and Cd were increased about more than 10 to 100 fold by the treatments compared to control. Metal content of shoot tissues was determined higher than roots. In all treatments metal uptake amount was determined for metals in Zn>Pb>Cu>Cd>Ni order.

TABLE VII. SHOOT METAL UPTAKE OF TOBACCO PLANT, mg $POT^{-1}$

| | Metal Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control Tretament | Control | 3 | 0,7 | 0,1 | 0,1 | 0,01 |
| | 1 | 18 | 1,4 | 0,5 | 1,4 | 0,14 |
| | 2 | 17 | 1,3 | 0,5 | 2,5 | 0,61 |
| | 3 | 26 | 1,7 | 1,4 | 2,8 | 0,75 |
| | 4 | 33 | 2,0 | 1,4 | 6,2 | 1,34 |
| Biochar Tretament | Control | 10 | 1,5 | 0,2 | 1,3 | 0,03 |
| | 1 | 37 | 1,8 | 1,3 | 1,8 | 1,60 |
| | 2 | 62 | 3,7 | 2,2 | 2,3 | 1,91 |
| | 3 | 71 | 5,2 | 3,6 | 6,3 | 4,36 |
| | 4 | 137 | 5,8 | 4,7 | 8,5 | 4,84 |

TABLE VIII. ROOT METAL UPTAKE OF TOBACCO PLANT, mg $POT^{-1}$

| | Metal Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control Tretament | Control | 0,5 | 0,08 | 0,01 | 0,01 | 0,001 |
| | 1 | 2,0 | 0,14 | 0,04 | 0,11 | 0,013 |
| | 2 | 1,4 | 0,11 | 0,04 | 0,39 | 0,063 |
| | 3 | 2,0 | 0,23 | 0,08 | 0,51 | 0,163 |
| | 4 | 3,5 | 0,23 | 0,14 | 0,50 | 0,175 |
| Biochar Tretament | Control | 0,8 | 0,12 | 0,01 | 0,13 | 0,001 |
| | 1 | 3,9 | 0,20 | 0,20 | 0,18 | 0,120 |
| | 2 | 8,0 | 0,30 | 0,23 | 0,24 | 0,285 |
| | 3 | 6,8 | 0,36 | 0,23 | 0,27 | 0,345 |
| | 4 | 11,3 | 0,62 | 0,36 | 0,52 | 0,480 |

### D. *Phytoextraction efficiency (PE) of plants*

PE rates of Tomato plant were decreased by the applications of increasing amounts of metals (Table 9). At control treatment Cd metal has the highest rate of PE value in control and Biochar treatments. In all treatments Cd has the highest PE values. PE values determined at the higher rates for Biochar than control. This indicates that Biochar affect the ability of phytoextraction for all examined metals, especially for Cd metal in soil remediation.

TABLE IX. PHYTOEXTRACTION EFFICIENCY OF TOBACCO PLANT, %

| | Metal Treatments | Zn | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| Control Tretament | Control | 1,35 | 1,12 | 0,28 | 0,20 | 23,40 |
| | 1 | 1,20 | 0,13 | 0,07 | 0,09 | 0,86 |
| | 2 | 0,48 | 0,05 | 0,02 | 0,03 | 0,99 |
| | 3 | 0,38 | 0,03 | 0,04 | 0,02 | 0,59 |
| | 4 | 0,25 | 0,02 | 0,02 | 0,02 | 0,56 |
| Biochar Tretament | Control | 2,58 | 2,35 | 0,68 | 1,28 | 88,21 |
| | 1 | 1,43 | 0,17 | 0,22 | 0,12 | 6,84 |
| | 2 | 1,06 | 0,14 | 0,11 | 0,03 | 2,48 |
| | 3 | 0,60 | 0,10 | 0,09 | 0,05 | 2,66 |
| | 4 | 0,59 | 0,06 | 0,07 | 0,04 | 1,78 |

## IV CONCLUSION

It has been observed that tobacco plants can grow without showing phytotoxicity at high heavy metal levels, but are affected in terms of biomass value. On the other hand, it has been observed that the tobacco plant has a high metal metal accumulation capacity, metal transfer from the soil and especially high Cd phytoextraction efficiency. Biochar applications increased metal accumulation, metal transfer factor, metal uptake and metal phytoextraction efficiency in tobacco plant. The results showed that Biochar applications can be used as a promising and environmentally friendly soil conditioner to increase phytoremediation efficiency in polluted soils. However, studies on biochar need to be tested in open field soils with different texture, pH and lime values.

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