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Investigation of Bioaccumulation Efficiency of Turkish Tobaccos by Evaluating their Heavy Metal Contents in Relation to their Origin

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Abstract: ***Effectiveness and efficiency of exploiting tobacco for bioremediation is examined for cadmium, copper, manganese and zinc. The concentrations of these HMs were determined both in the tobacco plants and soil samples. The collected samples were digested via wet ashing technique by using HNO_3-$HClO_4$ for plant samples and HF-$HClO_4$ for soil samples. Flame atomic absorption spectrometry was used for sample analysis. The regression coefficients were above 0.99 and the detection limits were in the range of 0.03-0.12 mg.L^{-1}. Performance and accuracy of the method were determined and the values found were in agreement with the standard values for the heavy metals analyzed. Bioconcentration factors (BCFs) were calculated. It was found that tobacco plants from cities on the Mediterranean and the Aegean regions had the highest concentration of HMs and BCFs. It was concluded that the characteristics of the soils the tobacco plants are raised influence the metal uptake capacity of the plants, but tobacco can be used as good bioaccumulator for HMs.***

Keywords: Turkish tobacco, bioaccumulation, hyperacumulators, bioconcentration factor, heavy metals

1. Introduction

Increasing world population and its consumption and production style has put a tremendous burden on the ecology and the environment. Major consequences of *laisez faire* understanding was desertification, climate change and the related degrading results on the environment. All elements found in nature are grouped either under metals or non-metals according to different criteria. According to their health effects metals can be classified as nutritionally essential and non-essential. Certain forms and concentrations of metals pose the risk of toxicity. Sometimes toxic metals, which are also called heavy metals (HMs), imitate the action of essential elements in the body and impede metabolic processes to cause illnesses, morbidity and mortality. The physical and chemical properties of HMs (i.e., concentration, oxidation state, etc.) determine the extent to which HMs would be detrimental to human health.

Presence of HMs in the food chain is risky and they enter into the food chain from the soils from which we obtain food. Therefore, cleaning the polluted soils is of utmost necessity. However, neither the chemical nor the physical remediation is economically friendly nor they are technically easy. This brings about the necessity for developing different strategies for eco-friendly, effective and efficient methods for remediation of soils polluted with HMs. At this point the eco-friendliest and most efficient or greenest solution appears to be *phytoremediation which is* the technique that uses plants for decontamination of the environment.

Green plants are known to enrich HMs from polluted environments successfully and among these plants some have the natural ability of accumulating HMs in considerably high amounts. Although much effort has been devoted to exploitation of different plants as bioaccumulators, tobacco despite its high capacity for sorption of HMs, still awaits attention.

Taking this into consideration in this study we targeted at determining effectiveness and efficiency of using tobacco as a bioaccumulator and/or hyperaccumulator for soil remediation.

2. Literature Survey

Increased eco-*un*friendly anthropogenic activity besides unfavorable climate change and desertification rendered most of the soils unfertile and even toxic due to accumulation of heavy metals (HMs). Although HMs naturally occur in the earth's crust activities of mankind have changed their geochemical cycles and biochemical balance to a great extent [1].

Regardless of their atomic mass or density toxic metals are called HMs [2]. Increased level of HMs caused a sharp rise in the incidence of diseases well besides shortage of arable land. It is observed that as HMs pass from lower trophic levels to higher trophic levels (biomagnification) their concentrations increase [3]. Therefore their presence in the body via food-chain is risky. The physical and chemical properties of HMs (i.e., concentration, oxidation state, etc.) determine whether they would be carcinogenic, mutagenic and teratogenic besides causing endocrine and neurological disruption and behavioral changes in humans and they have toxicological effects [4].

Therefore, HMs accumulated in plant and through food chain in the body of both humans and animals (bioaccumulation) since they are not biodegradable [5]. Long term exposure to HMs or exposure to high concentrations of HMs such as Mercury (Hg), Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), Nickel (Ni), and

Zinc (Zn), which are considered to be the most problematic ones regarding their toxicities, can cause severe environmental problems and deleterious health effects in humans [2]. HMs are now considered as an important class of carcinogens [6].

The mechanism of metal carcinogenesis is not known very well since interactions of metals in biological systems are very complex and the metabolic pathways that many metals even the carcinogenic ones follow are similar to those of essential metals. This may be because of the similarity between the binding preferences of the carcinogenic metals and the nutritionally essential ones. Moreover, essential metals can be carcinogenic. For example, while chromium at +3 valance is essential it is a carcinogenic element when it is at +6 valence.

Bioavailability and absorption of metals and metal compounds is profoundly affected by the factor solubility [7].

Chemical species, oxidation state, pH of the medium, presence of other chemical species in the medium and particle size (as small ones can be adsorbed more) are the factors on which determine solubility. This in turn affects its bioavailability and effect on the metabolism and environment. Metal (or metalloid) species are considered "contaminants" when they occur in a form or concentration that causes detrimental effects on humans or on the environment or in media where they are not wanted [1].

Lands contaminated with HMs almost completely lack vegetation and this leads to soil erosion and pollution. Therefore, cleaning the polluted soils is of utmost necessity. However, remediation is neither economically friendly nor is technically easy [2]. Three different major approaches have been employed in remediation: physical, chemical and biological approaches [8]. There are limitations both for the physical and the chemical methods in terms of economic considerations, intensive work, irreversible changes in properties and local microflora of the soil [2]. Above these, chemical methods can cause additional contamination problems. Therefore, eco-friendly and efficient remediation methods should be developed for cleaning soils polluted with HMs.

One of the eco-friendliest and most efficient approaches or green solution is *phytoremediation*- using plants and related soil microbes for decontamination of the environment [9]. Procuring vegetation on contaminated soils has four-fold effect: (1) risk containment (*phytostabilization*). It is an effective mean for preventing erosion and metal leaching. (2) *phytoextraction* of metals that has economic value; (3) sustainable *land management*, where it is possible to improve soil quality by phytoextraction for cultivating crops with higher market value. (4) it is *economically effective* since when compared with other remediation options installation and maintenance expenses are rather low.
Green plants are known to enrich HMs from polluted environments successfully. These plants, which have the natural ability of accumulating HMs more effectively or more selectively than others [10] and in considerably high amounts, are known as bioaccumulators and this process is called phytoextraction [11]. Exploring efficiency and effectiveness of bioaccumulators and hyperaccumulators in soil remediation has attracted much attention. Phytoextraction or HM accumulation capacity of bioaccumulators can be enhanced via genetic modification [12]. The plants, which are of use for phytoremediation should meet certain criteria such as having short life cycles. So that they can be harvested, destroyed or recycled for further use. New crop can be planted for continual remediation. Tobacco is one of them.

The two important parameters, which determine the efficiency of phytoextraction, are the bioconcentration factor (BCF) or the accumulation factor (A) and the translocation factor (TF) [13]. BCF indicates the efficiency of a plant species in accumulating a metal into its tissues from its environment [14]. TF indicates efficiency of the plant in translocating the accumulated metal from its roots to shoots [15].

In this study effectiveness and efficiency of using tobacco as a bioaccumulator and/or hyperaccumulator was determined for Cd, Cu, Mn and Zn. The levels these elements in Turkish tobaccos were determined. Effect of age, grade and origin of tobacco on the HM content of the plant and of the soils, in which they were grown in were investigated. The relation between the BCF and their bioavailability was established by determining the soil HM content.

3. Materials and Methods

Concentration of heavy metals (Cu, Zn, Mn, and Cd) was measured by GBC-904-PBT Flame Atomic Absorption Spectrometer (F-AAS) at the wavelengths 324.8, 213.9, 279.5, and 228.8 nm, respectively. Background correction was made via deuterium lamp. Tobacco samples were dried in WC Heraus Haneau oven at 105 °C until they reached constant weight. Tobacco samples were wet ashed on a Stuart Scientific Hotplate SH 3. Throughout the experiments distilled water obtained from Jencons Autostill 4000X was used.

The tobacco and soil samples were ground in agate ball-grinder sieved through 0.15 mm Nylon sieve and were kept in air-tight polypropylene (pp) bottles or bags before storage in vacuum desiccators. For filtering Cole Palmer Whatman® black ribbon filter papers were used. All glassware and the bottles were soaked in 10% HNO3 solution for 48 h before usage.

Analytical reagent grade nitric acid (HNO_3, 65 %), hydrogen peroxide (H_2O_2, 30 %), hydrochloric acid (HCl, 37 %), perchloric acid ($HClO_4$,70 %), hydrofluoric acid (HF, 48 %) were obtained from Merck, Darmstadt, Germany. All of the solutions were prepared with distilled water. The stock metal solutions (1,000 mg/L) were prepared from metal foils or powders with 99.99 % purity which were obtained from Merck, Darmstadt, Germany and were kept in PP bottles. Copper and Manganese solutions were prepared by dissolving 1.000 g of the metals in 50 mL of 6 M HNO_3

upon heating and then the solution was diluted to 1 liter in a volumetric flask with ultrapure water. Zinc and Cadmium solutions were prepared by dissolving 1.000 g of the metal in 20 ml of 5 M HCl and diluted to 1.0 liter in a volumetric flask with distilled water. Working solutions were prepared daily from the stock solutions via appropriate dilutions with distilled water when the analysis was to be performed.

Accuracy studies for zinc, manganese, and cadmium were carried out as mentioned in our previous study with peach leaves with the name "Certified Reference Material GBW 08501-Peach Leaves prepared in China" was employed. For accuracy studies for copper, "Certified Reference Material NIST (National Instituteof Standards and Technology) SRM (Standard ReferenceMaterial) 1547; NIST, Gaithersburg, MD, USA-PeachLeaves" was employed.

3.1. Description of study areas

Tobacco leaves and soil samples were taken from different parts and cities of Turkey where tobacco production has an important role in the agricultural economy. These cities, which are namely, from Bafra, Samsun and Trabzon, which are on the Black Sea side in the north, Bursa, which is on the Marmara sea side in the northwest, from Izmir, which is on the Aegean Sea side on the west, and from Hatay which is on the Meditarrenean Sea on the south east are presented in Fig. 1.

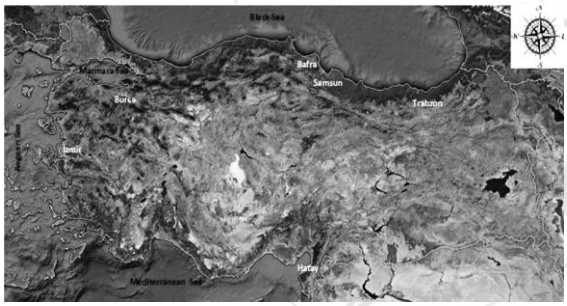

Figure 1: Locations from where the tobacco leaf samples and the soil samples were collected

As can be seen Figure 1. Black Sea is surrounded by Europe in the north and in the west, by Anatolia in the south and by the Caucasus in the east. However, while it is connected to the Atlantic Ocean through Marmara sea, Bosphorus and Dardanelles straits and the Mediterranean sea it is also connected to the Sea of Azov through the Strait of Kerch. The hydrological exchange between the Mediterranean sea and the Black sea is a two way process. The outflow from the Black Sea is cooler and less saline, and it floats over the warm, more saline Mediterranean inflow. Due to the significant difference in salinity (87 % of the entire sea body)there is a remarkable difference in the density and there is anoxic layer (without oxygen) below the surface waters which also contains high levels of hydrogen sulphide. The Marmara sea, which is an inland sea lying completely within the borders of Turkey, connects the Black Sea to the Aegean Sea via the Bosphorus and Dardanelles sraits and is often considered as a part of the Mediterranean Sea. In the Marmara although sea the surface salinity, which is about 22 ppt close to that of the Black sea, in the bottom of the sea it is around 38 ppt which is similar to that of the Mediterranean Sea. The water with high density salinity in the bottom water does not migrate to the surface. Moreover, the rivers draining into the Marmara sea reduces the salinity of the sea, but their influence is less than the influence of the rivers on the Black Sea.

The Aegean Sea resembles an elongated gulf of the Mediterranean Sea. The surface water circulates in a counter-clockwise gyre. The hypersaline water from the Mediterranean sea moves northward toward the Black sea. Then when it meets the less dense water from the Black Sea it sinks below the inflow coming from the Black Sea and then it flows through the Dardanelles strait into the Marmara sea [16]. The salinity in the bottom of the sea is about 39% [17].

The Mediterranean Sea is connected to the Atlantic Ocean through the strait of Gibraltar. It is surrounded by Europe and Anatolia on the north, by North Africa on the south and by the Levant on the east. In the west it is connected to the Atlantic Ocean by the Strait of Gibraltar, in the east to the Sea of Marmara and the Black Sea via the Dardanelles and the Bosporus straits and in the southeast to the Red Sea through the Suez Canal. Mediterranean is characterized by its general feature that evaporation is much more than precipitation and river runoff. Evaporation and hence the salinity is high in its eastern half where Turkey is located since water level decreases.

3.2. Sampling of tobacco leaves

The tobacco leaves were sampled and their moisture content was determined as it is mentioned in our other work. The moisture content was also determined and reported [18].

3.3. Soil sampling

Soil samples were collected from the same places where tobacco leaves were collected at a depth of50 cm and contamination from vegetation is avoided. They were stored in PP containers at 4 °C. Followingly, their moisture content was determined by drying them at 100-105 ^{o}C in oven until constant weight is attained. Then they were ground, homogenized by using an agate ball-grinder and sieved through 0.15 mm Nylon sieve.

3.4. Heavy metal determination in soil samples

The HM contents of the soil samples were determined according to the PR NF ISO 14869-1 standard. 0.5 g of dried soil samples were placed in Teflon beakers. 4 ml of distilled water was added onto to them to moisten them. Following this 10 ml of hydrofluoric (HF) and 3 ml of perchloric ($HClO_4$) acids were added. Then the mixtures were allowed to decant for 12 h. The solutions were evaporated until dryness at 150 ^{o}C. After filtration through 0.45 mm filters, wet digests were redissolved and the final volume was made 100 ml with 2% (v/v) HCl solution [19]. HMs in soil samples were determined via F-AAS.

3.5. Data processing

The determination of bioconcentration factor (BCF) is performed with Equation 1.

BCF % = $(C_{HM\,in\,leaves}/C_{HM\,in\,soil}) \times 100$ (1)

$C_{HM\ in\ leaves}$: concentration of HMs in plant tissue; $C_{HMin\ soil}$: concentration of HMs in soil.

4. Results and discussion

4.1. Calibration studies

Heavy Metal Content of Turkish Tobacco Leaves of different origin, years and grades were determined in another study we made [18]. It was observed that Mn concentrations in tobacco leaves gathered from Trabzon region and copper and zinc content of the leaves from Bursa and Balıkesir were found to be higher than those of other regions. Correlation could not be found between the HM content and the production year and grade of tobacco leaves. Only in the tobacco leaves of Bafra, which is in the Black Sea region, Mn, Cu, and Zn concentration increased with the age/production year of tobacco leaves [18].

4.2. Precision study of soil samples

Quality control was made via analysis of standard metal solutions and duplicate analysis (for the fraction soluble in water). The purchased metal standard solutions were used to validate the accuracy of the method [6].

Accuracy studies for Zn, Mn and Cd were made by analysis of Certified Reference Material GBW 08501-Peach Leaves Prepared In China while for Cu Certified Reference Material NIST (National Institute of Standards and Technology) SRM (Standard Reference Material) 1547; NIST, Gaithersburg, MD, USA-Peach Leaves was analyzed. 0.500 g of five parallel certified reference peach leaves were digested according to the same procedure which was exploited for the analysis of the tobacco leaves [18]. The moisture content of the peach leaves was also determined according to the same procedure employed for the tobacco leaves. Mean recovery data obtained was made in another stuy [18].

Soil samples obtained from the same sampling sites from which the tobacco leaves were collected were analyzed for the same HMs and results are presented in Table 1.

Table 1: Heavy Metal Content of Soil Samples

Origin	*HM concentration (mg/kg) in the soil samples*			
	Cd	*Zn*	*Cu*	*Mn*
Hatay	1,000	244,0	33,00	1253
Trabzon	BLD	170,0	119,2	1,030
Bafra	2,300	20,50	43,30	24,60
İzmir	1,900	320,0	38,00	1274
Samsun	0,100	59,10	33,70	6,600

4.3. Determination of bioconcentration factor

Bioconcentration factor (BCF) of tobacco plants is calculated and the results are presented in Table 2.

Table 2: *Bioconcentration Factor (BCF) for tobacco plants*

Origin	*Year of Production*	*BCF (%) for HMs Investigated*			
		Cd	*Zn*	*Cu*	*Mn*
Hatay	1992 (AG)	175,0	15,20	124,8	3,400
Hatay	1993 (AG)	-	10,50	36,70	1,700
Hatay	1994 (AG)	85,00	14,80	71,50	1,700
Hatay	1995 (AG)	-	19,50	77,90	3,800
İzmir	1992 (AG)	38,90	10,30	92,40	7,500
İzmir	1992 (BG)	57,90	14,50	88,90	6,100
Bursa	1996 (AG)	-	14,40	66,80	3,700
Bursa	1998 (AG)	-	10,40	163,8	2,800
Bursa	1997 (BG)	84,00	18,20	255,0	3,000
Trabzon	1995 (KP)	-	38,80	49,80	6388
Trabzon	1996 (KP)	-	15,80	17,40	9709
Trabzon	1997 (KP)	-	70,20	56,30	5874
Trabzon	1998 (KP)	-	31,00	37,30	7262
Trabzon	1998 (AG)	-	18,10	34,50	2262
Bafra	1995 (KP)	-	204,3	83,90	225,7
Bafra	1996 (KP)	55,20	206,2	108,4	325,6
Bafra	1997 (KP)	86,20	236,0	80,20	414,1
Bafra	1998 (AG)	60,30	225,3	86,20	253,3
Samsun	1997 (AG)	9,400	936,5	0,800	79,80

According to the results obtained the tobacco leaves obtained from Bafra and Trabzon, which are all in the Black Sea region of Turkey, have the highest bioconcentration factors. The salinity of Black Sea is less than that of the Mediterranean and the Aegean Seas. The pH of seawater is in the 7.5 to 8.4 range, which is very mildly basic. However, as the salts in seawater are more concentrated than they currently are, which is the case in Hatay, Izmir and Bursa and is in decreasing order, then the pH would be higher. On the other hand, at such high pH values most of the metal ions would precipitate as their hydroxides. The low concentration of HMs in tobacco plant in Hatay and Izmir cities may be due to this. On the other hand, the HM concentration in the soil of these cities is higher than the HM concentration in the plant giving relatively low BCFs. Duly, the BCFs obtained via tobacco plants obtained from the cities in the Black Sea region is very high. However, there were some plants in which the concentration of HMs was below limit of detection. Therefore, BCF values for these samples could not have been reported.

5. Conclusions

The precision, accuracy, and recovery studies, it was concluded that the accuracy and the precision of the digestion processes both for the tobacco leaves and the soil samples were efficient and acceptable for all metals except cadmium. The method based on wet sample digestion using HNO_3+$HClO_4$ for tobacco leaves and on $HClO_4$ for soil samples were found to be appropriate.

The results of the study showed that tobacco plant is a good bioaccumulator for HMs and depending on the soil and water characteristics they are grown they can be used as efficient hyperaccumulator for heavy metals and especially for manganese. It was also observed that the metal concentration did not show significant change with the age and grade of the plant, but there was a significant correlation

between the locality and HM content of the plant. The HM concentration in the plant and thus the bioconcentration efficiency and the plant's ability to be a good bioaccumulator was observed to be higher in regions where the salinity and thus pH was higher.

6. Future Scope

Further efforts need to be put in investigating interaction among the soil parameters such as salinity, pH, and the effect of climate with the bioaccumulation efficiency of the tobacco plant.

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