International Journal of Secondary Metabolite
2022, Vol. 9, No. 4, 504–512

https://doi.org/10.21448/ijsm.1082781

Published at https://dergipark.org.tr/en/pub/ijsm

Research Article

Determination of heavy metal concentrations and soil samples of *Betula pendula* and *Populus tremula* in Nemrut Crater Lake

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Abstract: The concentrations of Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb and As heavy metals and the heavy metal accumulation levels of these plants were determined in *Betula pendula* and *Populus tremula* trees, which are the characteristic trees of Nemrut Crater Lake, the second largest crater lake in the world, and the soil samples surrounding them. Heavy metals are considered to be one of the most dangerous and priority pollutants due to their high persistence and toxicity to plant and animal life in the environment. For this reason, the results obtained will contribute to the studies carried out to determine the uptake of heavy metal pollutants in the environment and the self-healing effort of the polluted environments by applying the phytoremediation method.

ARTICLE HISTORY

Received: Mar. 04, 2022
Revised: Oct. 17, 2022
Accepted: Dec. 12, 2022

KEYWORDS

Nemrut Crater Lake,
Betula pendula,
Populus tremula,
Heavy metals.

1. INTRODUCTION

Pollution can be examined in three groups as physical, biological and chemical pollution according to the types of pollutants. The pollution that arises as a result of the mixture of chemical substances consciously or unconsciously thrown into the nature and industrial wastes is chemical pollution. Examples of pollutants that cause chemical pollution are dyes, detergents, pesticides and petroleum products. Heavy metal pollution, which is accepted as a chemical pollution today, is in the first place among other chemical pollutants because they can arise from various sources, are resistant to environmental conditions, and can easily enter the food chain and accumulate in living things in increasing densities (Uzunoğlu, 1999). Heavy metals in these pollutants pose great danger. Exposure to heavy metals and environmental pollution is seen as an increasing problem around the world (Seaward & Richardson, 1989).

Since the beginning of the industrial revolution, heavy metal pollution has become an increasing problem for the environment. Heavy metals are one of the most important factors that cause pollution in wetlands. These metals are one of the main factors that play an important role in the deterioration of the ecological balance, and the development of living things and cause environmental pollution. Metals such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and cobalt (Co) are micronutrients that play an important

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ISSN-e: 2148-6905 /

role in the growth and development of animals and plants. Furthermore, some heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) are elements that are not important for the development of living things (Niess, 1999).

Despite the increase in environmental pollution, the first remediation studies were carried out on a physical and chemical basis. The efficiency of these remediation methods, which are widely used in areas exposed to environmental pollutants, is limited due to the expensiveness, high energy expenditure and side-waste generation (Hamutoğlu *et al*., 2012). Phytoremediation, which means cleaning or restoration of polluted areas with plants, carries a hope against environmental pollution predominantly caused by nano-industry. It is green biotechnology that minimizes, neutralizes or completely cleans and metabolizes contamination factors that cause toxicity with the usage of plants and rhizospheric microorganisms in terrestrial and wetland areas exposed to pollution for various reasons (Prasad, 2003; Prasad & Fertias, 2003; Park *et al*., 2011; Singh &Prasad, 2011; Zhai, 2013; Bayçu *et al*., 2014).

Heavy metals are naturally found in the environment at trace level, in the inanimate realm, in rocks, soil and water, and in plants and animals in the living world. Although natural physical and chemical processes such as erosion of rocks and volcanic activities contribute to heavy metal enrichment in water bodies, human-induced activities have the most important share in the increase in the system (Akbıyık, 2012). Heavy metals are released into the environment in significant quantities as a result of industrial activities such as mining, energy and fuel production, and excessive usage of pesticides and fertilizers (Halim *et al*., 2003; Samarghandi *et al*., 2007). The metal concentration in the soil typically ranges from 1 to 100,000 mg kg^{-1}. High levels of heavy metals cause deterioration of soil quality, decrease in crop yield and quality (Long *et al*., 2002) and therefore cause significant hazards to humans and other organisms (Blaylock & Huang, 2000).

These elements, which are one of the most important inorganic impurities that mix with soil and water and accumulate in these environments, and are defined as pure substances consisting of the same kind of atoms, can cause many environmental and human health problems such as microbial activity, soil fertility, biological diversity and even poisoning in living things through the food chain (Vanlı and Yazgan, 2008).

Studies are carried out to determine the toxic heavy metal contents, biomonitor and hyperaccumulator properties of plants, and as a result of these studies, the evaluation of the usage of plants within the scope of phytoremediation in terms of human and environmental health gains importance with each passing day. Therefore, the aim of this study is to contribute to the studies carried out to determine the uptake of heavy metal pollutants in the environment and the self-healing effort of the polluted environments by determining the concentrations of Mn, Fe, Cu, Zn, Pb and As heavy metals in *Betula pendula* and *Populus tremula* trees, which are the characteristic trees of Nemrut Crater Lake, and in the soil samples surrounding them, by applying phytoremediation method. Moreover, it is predicted that the plant sap obtained from *Betula sp.* species, which is an important food and medicine plant throughout history, will come to the fore as a functional food and food additive for a healthy life in the coming period (GKGM, 2016). Therefore, determining the heavy metal concentration in different organs of Betula pendula will help to know the effects on human health of these species, which are thought to be used as food and food additives.

2. MATERIAL and METHODS

2.1. Study area

The Nemrut Caldera is one of the most characteristic and original landforms of Turkey in terms of volcanic activities, which was formed as a result of the explosion of Mount Nemrut on the highest peak of the Van and Bitlis Plains and shows the structural features of a great caldera collapse. Nemrut Crater ranks first in Turkey, fourth in Europe and sixteenth in the world. Nemrut Crater Lake, with a diameter of about 6 km, is the second in the world after Mount Mazama Crater Lake in Oregon, USA. Nemrut Caldera Natural Monument was taken under protection with many protection status until today due to its richness and virginity in terms of its formation and, accordingly, its geological, geomorphological and hydrogeological features, and in terms of floristic and visual landscape values. These are the First Degree Natural Site, Natural Monument, Tourism Area and Wetland-RAMSAR Area having International Importance (Figure 1).

Figure 1. General view photos from the research area

2.2. Plant Materials

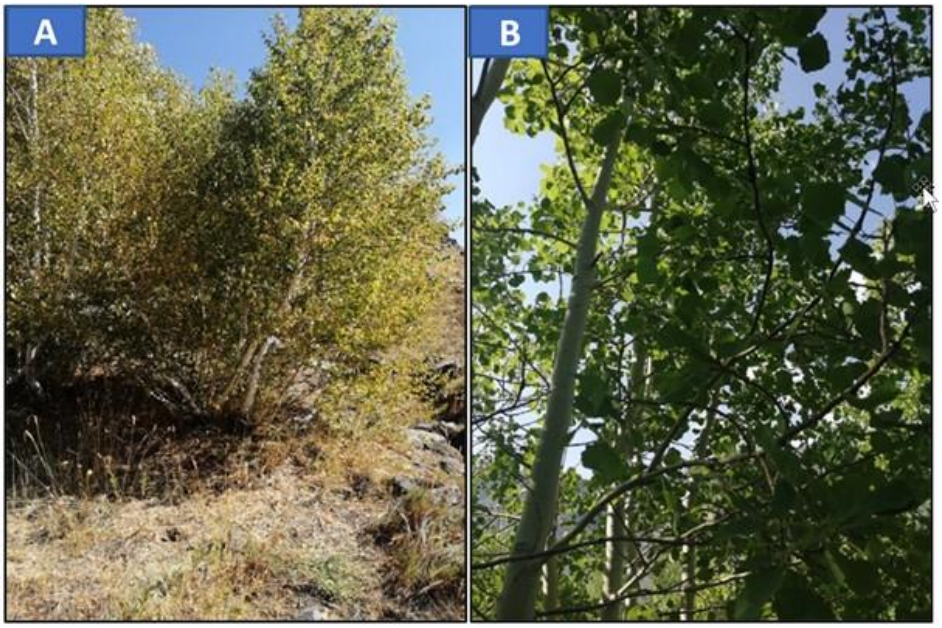
2.2.1. *Betula pendula* Roth

B. pendula is a member of the Betulaceae family. It is a deciduous tree with a smooth stem that grows naturally in Turkey (Figure 2a). It can be up to 15-30 m in length in the environments where it grows naturally. Their stems have a snow-white thin bark. The shoots are thin and slender, hanging down, bare but with abundant resin glands (Anşin and Özkan, 1993). Male flower seeds are cylindrical, 8-10 cm long, in terminal (tip) or lateral catkins (chatons). Male flowers that form in autumn are in bundles 2-4 cm long. The flowers, which are covered with scales, spend the winter months in this way and bloom in March-May (Korkut *et al*., 2010).

2.2.2. *Populus tremula* L. subsp. *tremula*

P. tremula is a member of the Salicaceae family. Although their homeland is unknown, they show natural distribution in many areas from Western Europe, North Africa to the interior of Asia, Siberia and Japan. This tree species is a Class I forest tree that can grow up to 25 m in height, has a cylindrical trunk, dense branches, and a broad conical top (Yaltırık, 1993). The shells are greenish-gray, shiny and flat. Shoots and buds are red chestnut color. It is also shiny as if it was polished. The buds are terminal and spirally arranged on the shoots. The flower-bearing buds are large, plump and blunt-tipped, while the leaf buds are pointed, smaller, light and sticky. The leaves on the long shoots (especially on the log shoots) are egg-shaped with a pointed tip, and the bottom sides are heart-shaped. The leaf margins are irregularly double-row toothed, and the undersides are soft hairy. The leaves on the short shoots are 3-7 x 3-7 cm in size, almost equal in length to the width, circular, blunt-tipped, and the bottom side is in the form of a light heart. The upper surface of the leaves is dark green, the lower surface is grayish green and bare, the edges of the leaf blade are toothed. The petiole is long (6-8 cm), and since it is pressed from the sides, it causes the leaf blade to sway even in a light wind. The petiole scar is in the form of a circle slice and there are three vascular bundle scars on it (Kayacık, 1981; Yaltırık, 1993). Since the petioles of this tree are printed on the sides, the leaves sway even in the slightest wind. That's why the tree is called aspen (Figure 2b).

Figure 2. General view of the studied plant species. A) *Betula pendula*, B) *Populus tremula*



2.3. Heavy Metal in Sediment and Plant Analysis

Some vegetative organs (bark, root, leaf) of *B. pendula* and *P. tremula* trees, which form the climax vegetation of the Nemrut Caldera chosen as the study area, were taken under suitable seasonal conditions. While taking plant samples, soil samples surrounding these plants were taken appropriately. The purpose of obtaining the appropriate vegetative organs such as bark, leaves and roots of these trees, which are the subject of the study, was to determine separately the heavy metal contents accumulated in the underground and above-ground organs of the plant and to make comparisons between these organs. After this process, these plant parts were washed to avoid contamination with tap water, and then passed through the ELGA PURELAB-Q DV25 brand pure water device in the Environmental Engineering Laboratory, and these parts were laid on separate filter papers to be dried. After drying, the plant samples, which were wrapped in filter papers in the MST55 incubator in the same laboratory, were kept for 24 hours at 50 ^{0}C - 80 ^{0}C, and at the end of 24 hours, these samples were placed in separate sealed bags and put into cabinets. Furthermore, the preliminary processes of soil samples taken from 2 different areas where these trees are concentrated were carried out in our Environmental Engineering Laboratory. Soil samples were laid on filter papers and aerated for two weeks. The samples were first subjected to the combustion process in the microwave device, 0.5 g sample was taken and 6 mL 65% HNO_3 and 2 mL 30% H_2O_2 were added. The burned samples were diluted 50 times with 2% HNO_3 (200 µM sample + 9800 µM 2% HNO_3) for reading in the ICP*MS device. In the reading made in the ICP*MS device, the peroxide and nitric acid used in the dilution in the microwave were also subjected to the burning process as a blank and read as a special blank and this value was deducted from the results in the ICP reading. 50-times dilutions were calculated in the ICP device and the results were transferred to an excel file.

3. RESULTS

The concentrations of *B. pendula* and *P. tremula* trees, which are the characteristic trees of Nemrut Crater Lake, the second largest crater lake in the world and of Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb and As heavy metals in the soil samples surrounding them and the heavy metal accumulation levels of these plants were determined (Table 1, Table 2). Although the limit values of heavy metal amounts (Table 3, Table 4) for plants in polluted and uncontaminated soils in the studies in the international literature differ, as a result of our analysis, the heavy metal concentrations in the parts of both plants were found well below these values. One of the most important reasons for this is that the natural environment in which these two plants, which are the subject of our study, are located, is free from many pollutants. While it was determined that the amount of Fe (34.79 mg kg^{-1}) measured only in the root part of the *B. pendula* was above the Fe limit value accepted by FAO/WHO in plants, it was found to be normal for other reference values in the literature. When the results obtained from soil samples (Table 3) were compared with the limit values for heavy metals in the soil pollution control regulation and Table 4, it was seen that all heavy metals except Fe are within the limit values. It was determined that the Fe value in the soil taken from both stations (T1, T2) was well above the limit values.

Table 1. Heavy metal values (mg/kg) in the organs of investigated species

Betula pendula			*Populus tremula*			Heavy metals
Leaf	Bark	Root	Leaf	Bark	Root	
0.05111	0.01783	0.6312	0.03946	0.00917	0.3793	Cr
39.170	3.487	21.51	3.631	1.433	5.05	Mn
11.320	1.196	34.790	8.269	1.142	42.62	Fe
0.05962	0.02082	0.2085	0.04614	0.06233	0.5073	Ni
0.2464	0.148	0.1256	0.2176	0.1049	1.673	Cu
8.505	4.245	1.380	4.124	4.679	1.898	Zn
0.00788	0.00483	0.02146	0.05763	0.04721	0.1754	Cd
0.0141	0.00124	0.03382	0.00957	0.00127	0.0456	Pb
0.00586	0.00126	0.01474	0.00814	0.00136	0.0333	As

Table 2. Soil sample values (mg/kg) were taken from the study area.

Soil samples	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb	As
Soil1 (T1)	0.3046	12.41	414	0.168	0.9308	3.149	1.101	0.4961	0.270
Soil2 (T2)	0.3667	16.670	576.9	0.221	0.1847	3.856	2.991	0.7054	0.391

Table 3. Reference values for normal and toxic concentrations of heavy metals in plants according to FAO/WHO & Allen (1989).

Heavy Metals	Necessary upper value for soil (mg/kg) (FAO/WHO, 1996).	Allowable value for plants (mg/kg) (FAO/WHO, 1996).	Allowable value for plants (mg/kg) Allen (1989).
Cd	0.8	0.05	0.01-0.3
Zn	50	50	10-100
Cu	36	5	25-40
Cr	100	0.5	0.05 – 0.5
Pb	85	2	2-20
Mn	70	5	50-500
Ni	35	5	5
Fe	4.5	30	40-500
Ar	20	0.2	*

Table 4. Reference values for normal and toxic concentrations of heavy metals in plants (Misra &Mani, 1991; Kastori *et al*., 1997; Schulze *et al*., 2005; Kabata-Pendias, 2007).

Heavy metals	Normal Concentration (mg / kg)	Toxic Concentration (mg / kg)
Pb	5.0-10.0	30.0-300.0
Cd	0.05-0.2	5.0-30.0
Cu	5.0-30.0	20.0-100.0
Cr	0.1-0.5	5.0-30.0
Ni	0.1-5.0	10.0-100.0
Mn	15-100	400
Fe	50-250	>500
As	10-60	<2

4. DISCUSSION and CONCLUSION

Bioconcentration factor (BCF), this value is used to evaluate the metal accumulation in plant regions or the transfer of metals from the soil to the plant root (Chen *et al*., 2004) and to calculate this value, the plant root metal concentration (mg/kg) is divided by the soil metal concentration (mg/kg). The translocation factor (TF), which is important, indicates the degree of phytoremediation of the species (Zhao & Duo, 2015) and is used to evaluate metals transferred from soil-fed plant roots to the plant stem. Additionally, this value represents the plant's ability to absorb and transfer metals from the sediment and then store it in the above-ground parts (Wei *et al*., 2002). Transport factor (TF) is an indicator of the movement of heavy metals from the root to the upper organs and is calculated by dividing the heavy metal concentration in the stem of the plant to that in the root (Padmavathiamma & Li, 2007). If TF> 1, the plant transferred the metal from the root to the stem. If BCF>1, it is called a plant accumulator (Baker, 1989). The TF and BCF values we obtained as a result of our study are shown in Table 5 and Table 6.

Table 5. TF and BCF values of *Betula pendula*.

Heavy Metals	Translocation Factor (TF)	Bioconcentration Factor (BCF)	
		Aerial parts	Root
Cr	0.1092	0.06894	0.6312
Mn	19.776	42.657	21.51
Fe	0.3597	12.516	34.79
Ni	0.3858	0.08044	0.2085
Cu	3.1401	0.3944	0.1256
Zn	9.2391	12.75	1.38
Cd	5.9226	0.01271	0.02146
Pb	0.4535	0.01534	0.03382
As	0.4830	0.00712	0.01474

It can be said that the TF values of Mn, Cu, Zn and Cd metals in *Betula pendula* plant were greater than 1, especially these metals accumulate excessively in the aboveground organs and the plant showed hyperaccumulator properties in terms of these metals. When we looked at the BCF values, we can think that this plant has accumulator properties since BCF>1 for Mn and Zn metals.

Table 6. TF and BCF values of *Populus tremula*.

Heavy Metals	Translocation Factor (TF)	Bioconcentration Factor (BCF)	
		Aerial parts	Root
Cr	1.2820	0.04863	0.3793
Mn	1.0027	5.064	5.05
Fe	0.2208	9.411	42.62
Ni	0.2138	0.10847	0.5073
Cu	0.1927	0.3225	1.673
Zn	4.6380	8.803	1.898
Cd	0.5977	0.10484	0.1754
Pb	0.2379	0.01085	0.0456
As	0.2852	0.0095	0.0333

When the TF and BCF values of the *Populus tremula* were examined, while TF>1 in terms of Cr, Mn and Zn metals, the only metal with BCF>1 was Zn. Therefore, we can say that this plant has an accumulator feature in terms of Cr, Mn and Zn metals.

Consequently, it was seen that the heavy metal concentrations obtained from the soil samples in this study did not exceed the allowable heavy metal accumulation limit values in the soil in Turkey and in the world and were at an acceptable level. When the data of the plant samples were examined, it was determined that there was heavy metal accumulation, but we can say that it was within the heavy metal limit values accepted for the plants obtained as a result of many studies in the literature with the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO). Only the amount of Fe (34.79 mg kg^{-1}) measured in the root part of the *Betula pendula* plant was determined to be above the Fe limit value accepted by FAO/WHO in plants. It is known that plants with hyperaccumulators that accumulate in the above-ground organs can be used successfully in phytoextraction, and those that accumulate in their roots can be used successfully in phytostabilization processes and can naturally clean the metal pollution in the environment. Due to the limited number of similar studies with *Betula pendula* and *Populus tremula* species, and for the first time, the amount of heavy metal concentrations they can accumulate in their bodies were shown in detail in this study, and these findings will make an important contribution to the literature in phytoremediation applications.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Sukru Hayta: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing -original draft. **Elif Fırat**: Methodology, Supervision, and Validation.

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