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EVALUATION OF HEAVY METAL CONCENTRATIONS IN THE XYLEM SAP OF TURKISH PINE (*PINUS BRUTIA* TEN.) AND HONEYDEW OF *MARCHALINA HELLENICA*, GENNADIUS (HEMIPTERA: MARCHALINIDAE), COLLECTED IN WESTERN ANATOLIA, TURKEY

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ABSTRACT

The aim of this study was to investigate the Cr, Mn, Cu, Zn, Cd, and Pb concentrations in samples of the sap from the Turkish pine, *Pinus brutia* Ten., and the honeydew from the insect, *Marchalina hellenica* (Gennadius), both of which are components of the food chain in the production of pine honey, that were collected at different locations in western Anatolia, Muğla province, Turkey. Heavy metal concentrations in the samples were determined using microwave-induced wet combustion and mineralization, followed by inductively coupled plasma mass spectroscopy. The results showed that the Cr, Mn, Cu, Zn, Cd, and Pb concentrations were low in both the sap and honeydew samples. This study represents the first reference on heavy metal concentrations in the sap from *P. brutia* and the honeydew from *M. hellenica* within the region.

KEYWORDS:
Honeydew, Turkish pine xylem sap, *Marchalina hellenica, Pinus brutia* Ten., Heavy metals.

INTRODUCTION

The effects of human activities, such as agriculture, urbanization, industrialization, and transportation, as well as the effects of climate change on vegetation can rapidly manifest. Heavy metals, which can be found in different concentrations in soil, water resources, and air, cause pollution when they exceed certain concentrations [1-4]. Because of high accumulations of heavy metals, there is a possibility of contamination in the soil-to-plant continuum [5]; therefore, it is important to regularly monitor and determine how and to what extent plants and the animals in contact with these plants are affected by these factors. Fifty-four percent of the forested areas in Turkey comprise coniferous forests and, within this proportion, Turkish pine (*Pinus brutia* Ten.) forests are the most widely distributed with 5.9 Mha or 27% of the total forested areas [6]. In Turkey, the insect *Marchalina hellenica* Gennadius (Hemiptera: Marchalinidae), which feeds on pine sap, is distributed in the south Marmara, Aegean, and west Mediterranean regions, which are affected by the Mediterranean climate [7]. Seventy-five percent of the pine honey production areas in Turkey are in Muğla Province in southwestern Turkey, and only a very low percentage is in the Marmara region [6]. The data used in this study were obtained from Muğla Province, which represents a total forested area of 830854.7 ha, 66.41% (538494 ha) of which is Turkish pine forests. Of the Turkish pine forests, 66305.1 ha (12.31%) host *M. hellenica*, and this is where the pine honey is produced [8]. Ninety percent of all pine honey consumed worldwide is produced in Turkey, with the remaining 10% being produced in Greece. To a great extent, the honey exported from Turkey is produced from the pines growing in Muğla Province, but thousands of beekeepers who settled in various regions of Turkey also produce pine honey, which emphasizes the economic importance of beekeeping in Muğla. Thus, the region is of particular importance in terms of both Turkish pine and the pest insect that produces the honeydew; however, the potential tourism industry in the area is intense. This potential industry could threatened the continued existence of all forested areas, and the impacts from the contaminants that result can affect the environment. Plant sap actively transports elements from soils contaminated with heavy metals. *Marchalina hellenica* Gennadius (Hemiptera: Marchalinidae) is a common scale insect species in Turkish pine forests, mainly in the Aegean region, that feeds on pine sap. The insect has been recorded in Turkey, Greece, Italy, and islands in the eastern Mediterranean [9]. The honeydew produced by *M. hellenica* is collected by honeybees to produce pine honey [10]. When *M. hellenica* feeds on the xylem sap of the Turkish pine, there is a high probability that some elements, such as Cr, Mn, Cu, Zn, Cd, and Pb that are present

in the sap could be transported into the insect's secretions. Because the honeybee makes its honey using the *M. hellenica* secretions, there is also a potential risk that these elements could reach the final consumer. In general, several studies have focused on honey production but not enough information is available on the other stages of the food chain. The aim of this study was to fill this gap in the literature by focusing on determining Cr, Mn, Cu, Zn, Cd, and Pb concentrations in different components of the pine honey production process, from the xylem sap to the last step of production using the honeydew.

MATERIALS AND METHODS

The materials examined in this study comprise the xylem sap from the Turkish pine and the honeydew secreted by the insect that feeds on it. The samples were collected from different stations near the road and in the Turkish pine forests that host the insect throughout Muğla province. After collecting the sap and honeydew into sterile eppendorf tubes, the samples were taken to the laboratory and kept at 4°C until analyses. The microwave wet digestion technique was used to dissolve the honeydew and xylem sap samples. A 200-mg sample was placed into a polytetraflouroethylene (PTFE) microwave container, after which 10 mL nitric acid (65%, suprapur, Merck, Germany) was added to the container, and the container was placed into the microwave (200°C, 15 min ramp time, 20 min waiting time, 20 min cooling time). The volume of the dissolved sample was increased to 50 mL using ultrapure water. These liquid samples were then placed into the Agilent 7700x Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent Technologies, Inc., Santa Clara, CA, USA) under the conditions given in Table 1. To draw a calibration curve, 1000 µL AccuTrace Mes-21-1 multi-element calibration solution was mixed for the Cr, Mn, Cu, Zn, Cd, and Pb elements in the tubes. Calibration solutions were prepared from the mixed stock solution at five different concentrations (5, 10, 50, 100, and 200 µg L^{-1}), were inserted into the ICP-MS instrument, and the calibration curve was drawn. The results of the analyses are given as mg kg^{-1} wet weight. To determine the correlations between the metal concentrations in honeydew and Turkish pine sap, the Mann-Whitney U Test was conducted using SPSS v 20 (IBM Corp., Armonk, NY, USA). Statistical significance was determined through the 0.05 alpha level. $P < 0.05$ was determined to be a statistically significant difference between the groups.

TABLE 1
ICP-MS instrument conditions.

Radiofrequency power	1550 W
RF matching	2.1 V
Sample depth	8 mm
Carrier gas	0.95 l/min
Dilution gas	0 l/min
S/C temperature	2°C
Nebulizer type	MicroMist

RESULTS AND DISCUSSION

No previous studies on the presence of heavy metals in the *M. hellenica* honeydew have been conducted in the region; therefore, this study represents the first reference. Plants carry heavy metals from the roots to the shoots by transporting them in the xylem and phloem. Primary transport in the xylem, retranslocation in the phloem, and transfer from the xylem into the phloem are important processes for the distribution of an element within a plant [11]. Plants accumulate heavy metals in their roots and shoots in concentrations higher than those in the soil [12]. Because high concentrations of heavy metals can be found in the various honeys obtained from bee hives, particularly those next to highways and near steel plants [13], honey can be used as an environmental marker [14]. The levels of Cr, Mn, Cu, Zn Cd, and Pb concentration found in our results from Turkish pine xylem sap and honeydew are summarized in Table 2. According to the results, no statistically significant differences in heavy metal concentrations were found between honeydew and pine sap ($p > 0.05$).

TABLE 2
Mean heavy metal concentrations in the samples (mg kg^{-1} ± s.d.).

	Cr	Mn	Cu	Zn	Cd	Pb
Honeydew (n = 18)	0.022 ± 0.005	0.039 ± 0.032	0.047 ± 0.019	0.334 ± 0.02	0.001 ± 0.000	0.012 ± 0.002
Turkish pine sap (n = 34)	0.048 ± 0.031	0.069 ± 0.053	0.042 ± 0.034	0.317 ± 0.081	0.001 ± 0.000	0.015 ± 0.006

Note: s.d.= standard deviation.

Turkish pine bark can also be used to monitor heavy metal contamination [15]. In a previous study conducted in Izmir on Turkish pine bark, 5.0–20.0 mg kg^{-1} Cr, 75–375 mg kg^{-1} Mn, 2.25–15.63 mg kg^{-1} Cd, and 50–375 mg kg^{-1} Pb were detected [16]. In another study, it was reported that the bark of the Turkish pine effectively accumulates Pb [17]. Sawidis et al. [18] detected 2.2–8.1 mg kg^{-1} Cu, 13.1–29.7 mg kg^{-1} Zn, and <1.5–7.3 mg kg^{-1} Pb in Turkish pine leaves. No previous studies on Turkish pine sap were found in the literature. In our study, 0.048 mg kg^{-1} Cr, 0.069 mg kg^{-1} Mn, 0.042 mg kg^{-1} Cu, 0.317 mg kg^{-1} Zn, 0.001 mg kg^{-1} Cd, and 0.015 mg kg^{-1} Pb were detected in Turkish pine sap.

Although there have been no studies on honeydew, there are studies on honeydew honey. In a study conducted in Poland on honeydew honey (n = 2), 4.31 mg kg^{-1} Zn, 0.027 mg kg^{-1} Cd, and 0.037 mg kg^{-1} Pb were detected [13]. In the honeydew honey from Italy, 1.70 mg kg^{-1} Mn, 1.87 mg kg^{-1} Zn, 4.40 mg kg^{-1} Cu, 0.0027 mg kg^{-1} Cd, and 0.09 mg kg^{-1} Pb were detected [19]. In a study by Uren et al. [20] on honeydew honey in Turkey, the results showed 0.752 mg kg^{-1} Mn, 1.05 mg kg^{-1} Cu, and 0.011 mg kg^{-1} Cd. In current study, 0.33 mg kg^{-1} Zn, 0.001 mg kg^{-1} Cd, and 0.012 mg kg^{-1} Pb was found in the honeydew.

The tolerable upper intake levels of heavy metals specified by the World Health Organization and Institute of Medicine, Food and Nutrition Board are as follows: Cr: 250 µg day^{-1}, Mn: 11 mg day^{-1}, Cu: 10 mg day^{-1}, Zinc: 40 mg day^{-1}, and Pb: 25 µg kg^{-1} $week^{-1}$ [21, 22]. According to these values, the heavy metal concentrations found in the samples of plant sap and honeydew in our study do not pose a significant risk for human health.

CONCLUSIONS

The heavy metal concentrations determined in the Turkish pine sap and honeydew samples were found to be lower than the tolerable upper intake levels. No statistically significant differences were found in heavy metal concentrations between the Turkish pine sap and honeydew samples. This study reports the first reference on the evaluation of Cr, Mn, Cu, Zn, Cd, and Pb concentrations in Turkish pine sap and honeydew.

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