Elemental content profiles in propolis from several cities of Turkey

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ABSTRACT

Background: Macro-element content profiles in propolis that have been previously used in traditional folk medicine have provided enough information to develop a classification of the geological origin of propolis. Within this study, we aim to contribute our research to existing literatüre, particularly through our use of EDXRF spectroscopy, which has not been used to study propolis before. The results of the study led us to conclude that the residues of heavy metals were a limited concentration in Turkish propolis samples.

Objective: The purpose of this study was to investigate the macro-element profiles in Turkish propolis from 18 different cities of Turkey.

Methods: The macro-element of 22 raw propolis samples were investigated using Energy-Dispersive X-ray fluorescence spectrometry.

Results: Turkish Propolis was discovered to be rich with minerals of potassium, sodium which could be more beneficial in human nutrition. Potassium content was at a relatively higher level than other elements in these samples, while calcium content was at alower level in those samples from various regions of Turkey.

Conclusion: The elements of propolis that we studied were distinctive enough to make the discrimination of propolis from different locations in Turkey possible. The quantification by energy-dispersive X-ray fluorescence spectrometry procedures provided good resolution of multi-element analysis in propolis samples.

Keywords: Propolis; element analysis; energy-dispersive X-ray fluorescence spectrometre

INTRODUCTION

Propolis is a naturopathic formulation collected by honeybees from the buds and exudates of conifer trees and plants. The composition of the propolis depends upon the vegetation of the area from which it was collected; however, due to its botanical origins and its resulting variations, there may be difference in the chemical composition of samples from different locations, even those from the same locality [1]. Propolis is used by the bees as a protective barrier in hives. Propolis is collected from plants by honeybees, and has anti-mutagenic and anti-carcinogenic properties in addition to biological and therapeutic effects [2, 3, 4]. Bees use the propolis to protect and reinforce their hives, repair their hive structure, and to cover honeycombs. It kills pathogens, serves as protection against rain due to its stickiness, and prevents undesirable guests from entering the hive [4, 5]. However, not all species of bees produce this bee glue to the same degree [5, 6]. The content of extract and element indicates enviromental differences.

Propolis, a natural product derived from plant resins collected by honeybees, has been used in traditional medicine all over the world for thousands of years. Accordingly, propolis has also gained popularity as a health drink and has been extensively used in foods to improve overall health and prevent diseases [2, 9-11].

However, traces of certain metals can also cause a variety of undesirable changes in propolis preparations during their formation and storage. Among its trace elements, chromium, iron, and zinc were the most common. Trace and macro-element profiles in propolis provided enough information to develop the classification of the geological origin of propolis [7]. The chemical composition of propolis varies greatly and depends directly on the local flora and phenology of the host plants, and indirectly on the locality and time of collection. Thus, the wide variability in the chemical composition of propolis makes it more essential [12]. There have been several studies related to the chemical components of different provinces of Turkish propolis which have provided valuable information on this topic [13-18]. Therefore, there has been significant investigation on the preclinical investigation of propolis in some provinces of Turkey [16, 19, 20-23, 29]. Through this study, we hope to make a contribution to exisitng research literature by using EDXRF spectroscopy, which has not been used for propolis studies thus far.

In this study, we obtained propolis from 22 different provinces to compare the essential element contents of these 22 raw propolis samples in different phyto-geographical regions in Turkey. We used Energy-Dispersive X-ray fluorescence (EDXRF) spectrometry to determine the element content of propolis. The samples were collected from several honey bee farms in Turkey.

MATERIALS AND METHODS

Propolis samples

Propolis samples were collected from several cities of Turkey (Adana, Ankara, Aydın, Bartın, Bilecik, Bolu, Düzce, Burdur, Bursa, Erzurum, Karabük, Kastamonu, Kırıkkale, Muş, Sivas, Trabzon, Van, and Zonguldak), which can be seen on the Turkey map in Figure 1. Materials were obtained from these cities from May 2015 to September 2015. The products were collected from the apiaries of three bee colonies. The samples were combined into an one-pooled propolis. Propolis was collected directly from the hives. Samples were maintained at −20°C before processing.

Sample Preparation (EEP) of Propolis

10 gr of propolis samples were dissolved in 100 ml of 96% ethanol and incubated for 24 h at 60°C. After incubation, the sample was centrifuged for 10 min at 4000 rpm. The supernatan was filtered and evaporated to dryness in a rotavapor.

Element analysis

EDXRF is an excellent non-destructive method to determine the elemental content of a sample. The elemental concentration of the samples was determined using a Skyray EDX3600B spectrometer equipped with an Oxford Rh anode X-ray tube. The spectrometer has a SSD detector made in Germany which has 145 ± 5 eV energy resolutions. This spectrometer is capable of 0.05% measurement precision, analytical range of elements from Sodium to Uranium, and ppm-99.99% analysis range. Moreover, 24 elements can be analyzed simultaneously using it. The standardization of the samples was conducted using Panalytical AXIOS Advanced WDXRF. In the work of standardization, the IQ+ program was used since this program gives the semi-quantitative results with 95-90% precision for all materials. The standard curves were drawn at the EDX3600B spectrometer using the standard values which were obtained from the IQ+ program and uploaded to the system. The concentration of the samples was determined by the system using these standard curves. It is possible to measure the sample with solid or liquid. Accordingly, current samples were measured as a solid target by being pressed at a five tone hydraulic press that compressed the sample powder into a solid thick pellet of 40 mm diameter using a boric acide (H3BO3-powder) as a protective cover.

Heavy Metal Analysis

Studies were performed at a constant temperature of 25°C to be representative of environmental conditions. All propolis samples were lyophilized and dried. Next, dried samples were added to 5 ml püre nitric acid and 5 ml hydrogen perocside. This mixture was heated for 6 hours. The solution was cooled and filtered. Then the solution was analyzed using Atomic Adsorption Spectrometer (GBC 933 AAA). The amount of heavy metal (μg/kg) was also calculated (Matin, 2014).

Statistical analysis

The differences in element composition in the 22 raw propolis samples from Turkey were evaluated by non-parametric Kruskal Wallis test of one-way analysis of variance (ANOVA). $p<0.05$ was considered significant. SPSS 10 software was used for analyses.

RESULTS AND DISCUSSION

Propolis is an important apicultural product with various chemical compositions and several pharmacological and nutritional applications. Moreover, it is a mixture of compenents collected by bees discovered to have diverse biological properties. The content of propolis has been discovered to be linked to the normal surrounding habitat, weather, and season collection. Diversity in chemical composition of propolis reflect the floral richness and the climate differences in the country [24].

The different polyphenol concentrations in raw material are essential for standardization and categorization of raw material propolis. Geographical molecular marker is also important for analyzing the location where each propolis sample was taken from [11, 25-26]. The composition of propolis is dependent on the flora, season, and time of the collection [27]. Consequently, it could be suggested that the element content may help determine additional qualities of propolis samples. However, apart from the analysis of natural propolis constituents, elements and toxic contaminants such as heavy metals can also be a subject of chemical control [12].

In this study, the aim was to evaluate the micro/macro elements and heavy metal contents of raw propolis samples originating from different locations of Turkey using energy-dispersive X-ray fluorescence (EDXRF) spectrometry. Thus, as seen in Figure 1, propolis samples collected from different regions of Turkey were analyzed.

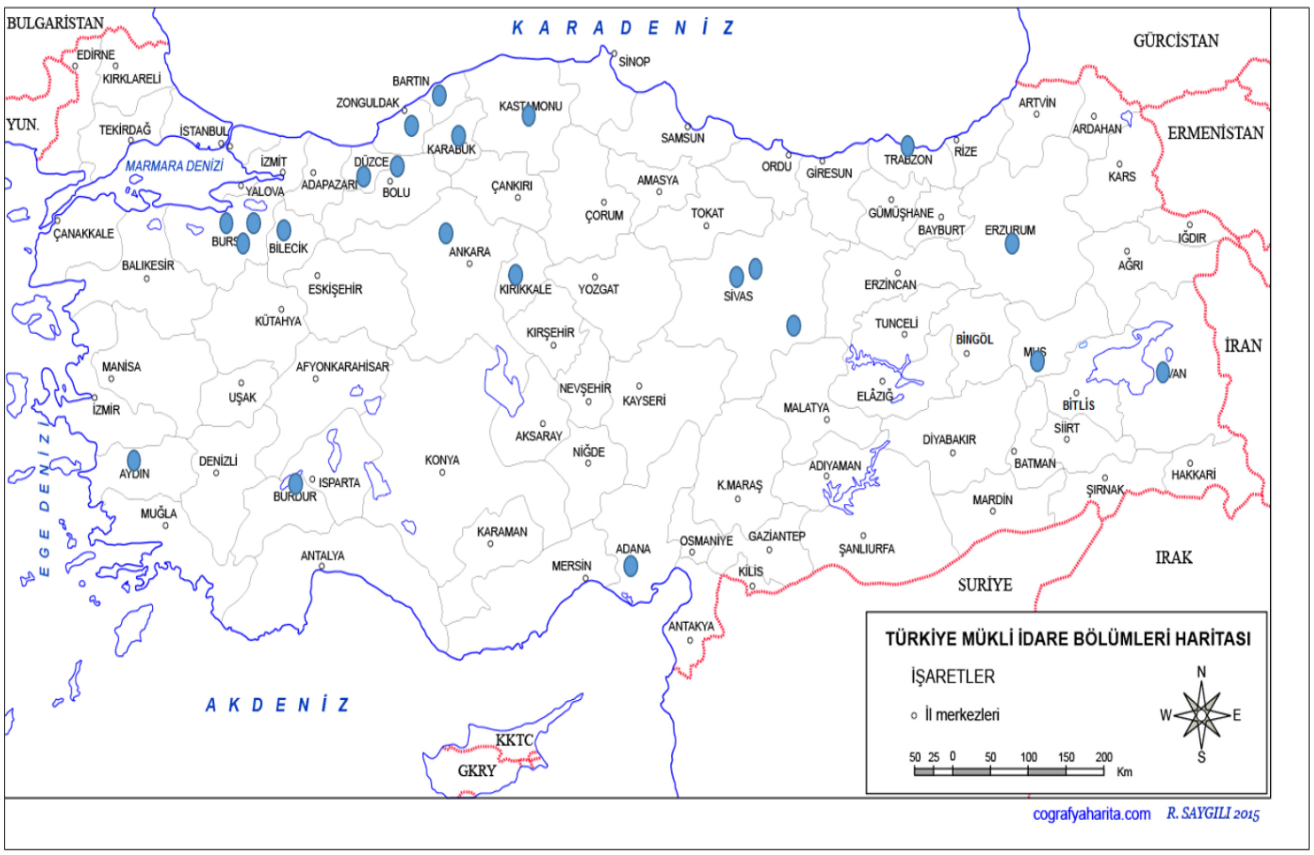

Figure 1. Turkey map shows the cities where the propolis samples were collected

Obtained samples have a wide distribution in Turkey and have been cultivated by farmers from private lands for centuries [28].

Propolis produced by bees is a 50-70% mixture of resins and balsams, 30-50% wax, 5-10% polen, and 10% essential oils, also being mixed with the bee's salivary excretions. Propolis wax contains amino acids, with the major amino acids being glutamic acid. The average of wax in raw Turkish propolis is 41% ± 22.2 and the average of moisture in it is 2.1% (Table 1).

Table 1. Physicochemical properties of 22 raw propolis samples from various region of Turkey

	Wax %	Ash %	Moisture %
Average	41.0	1.7	2.1
Std. Dev.	22.2	1.0	1.1
Minimum	5.3	0.2	0.5
Maximum	75.9	4.2	3.1

The samples and reagents were prepared daily in order to avoid losses and contamination. In most of the analyzed samples, Si, Na, Mg, Al, K, Fe, Cu, Zn and Mn were observed.

Table 2. Concentration of elements in propolis (N: 22, %) by using Energy-Dispersive X-ray fluorescence (EDXRF) spectrometry.

	Mg	**Na**	**Al**	**Si**	**S**	**K**	**Ca**	**Fe**	**Mn**
Ankara -Kızılcahamam	0	0.13	0.08	0.63	0	0.51	0	0.02	0.21
Kırıkkale	0.02	0.19	0.12	0.99	0.15	**0.91**	0	0.03	0.22
Sivas -Divriği	0	0.11	0.05	0.44	0.10	0.14	0	0.02	0.22
Sivas-Zara	0.03	**0.30**	0.11	1.89	**0.29**	0.50	0.35	0.02	0.23
Sivas	0	0.22	0.08	1.26	0	0.14	0	0.03	0.21
Burdur	0	0.20	0.06	1.23	0	0.45	0.03	0.03	0.20
Bursa-Kemalpaşa	0	0.08	0.09	0.23	0	0.03	0	0.02	0.23
Bursa- Mudanya	0	0.07	0.08	0.13	0.12	0.06	0	0.02	0.21
Bilecik	0	0.09	0.09	0.04	0	0.07	0	0.02	0.19
Adana	0	0.22	0.11	1.20	0.26	0.56	0.15	0.02	0.22
Aydın	0	0.16	0.09	0.76	0	0.17	0	0.03	0.24
Bolu-Yeniçağ	0	0.06	0.04	0.13	0	0.18	0	0.02	0.22
Düzce-Gölkaya	0	0.11	0.06	0.59	0.04	0.07	0	0.02	0.21
Çaycuma-Çiftlik	0.11	0.09	0.07	0.27	0	0.16	0	0.02	0.23
Zonguldak-Ereğli	0	0.07	0.08	0.01	0.30	0.11	0	0.02	0.21
Bartın	0.09	0.10	0.09	0.36	0	0.35	0	0.02	0.24
Karabük	0.24	0.07	0.10	0.01	0	0.24	0	0.02	0.22
Kastamonu	0	0.11	**0.14**	0.36	0.24	0.23	0	0.02	0.21
Trabzon	0.50	0.04	0.09	0.01	0	0.52	0	0.02	0.22
Erzurum	0.14	1.45	0.02	**11.56**	0	0	0	0.02	0.18
Van	0	0.26	0.12	1.90	0	0.14	**0.76**	0.03	0.19
Muş	0	0.41	0.09	3.17	0	0.63	0.62	0.03	0.21

In this study, Ca was low in content in the samples except for Sivas-Zara 0.34 %, while higher levels of K in propolis was collected from Kırıkkale 0.9 %, Adana 0.6 %, and Trabzon, Ankara-Kızılcahamam, Sivas-Zara 0.5% are shown in the Table 2. In another study by Doğan et al., 2006, higher levels of Na was observed in propolis from Artvin, İzmir and Bursa region. Mg was similar content with the ranges of their study [20].

Silicon is a crucial element for human health that has proved to suppress many illnesses [29], in addition to the fact that Si content is quite high in Erzurum, Muş and Sivas as Erzurum 11%, Muş 3%, Sivas-Zara 1.8 % in the current results is promising for propolis. Furthermore, the fact that the 0.5% Mg content was found in the sample collected from Trabzon is remarkable, as it was 0.2 % in Karabük and propolis samples from other provinces. Thus, it suggests that all cities have their own characteristic elements in propolis, with environmental conditions being effective in them.

Therefore, the concentration of heavy metals in propolis can reflect the contamination of the environment originating from emissions. Sulfur content of propolis was quite high in Zonguldak Ereğli 0.30%, Sivas–Zara 0.29%, Adana 0.26%, Kastamonu 0.24%, in addition to decreasing in other provinces which are shown in Table 2; this is remarkable as it indicates there is environmental pollution in those provinces [30]. Propolis may be used as an indicator of environmental contamination.

Table 3. Non-Parametric statistics used to calculate mean and standard deviation of elements in 22 raw propolis samples from different regions of Turkey.

Elements	N	Mean %	Std. Deviation
Na	22	0.2064	0.29240
Al	22	0.0845	0.02773
Si	22	1.2350	2.43748
S	22	0.0682	0.10813
K	22	0.2805	0.23868
Ca	22	0.0868	0.21180
Fe	22	0.0277	0.00456
Mn	22	0.2145	0.01535

Non-Parametric statistics were used to calculate mean and standard deviation for elements in samples (Table 3). Potassium content was at a higher level (mean: 0.2805±0.2386 %) in samples, while calcium concent was at a lower level (mean: 0.0868±0.21180) in those from the studied regions of Turkey.

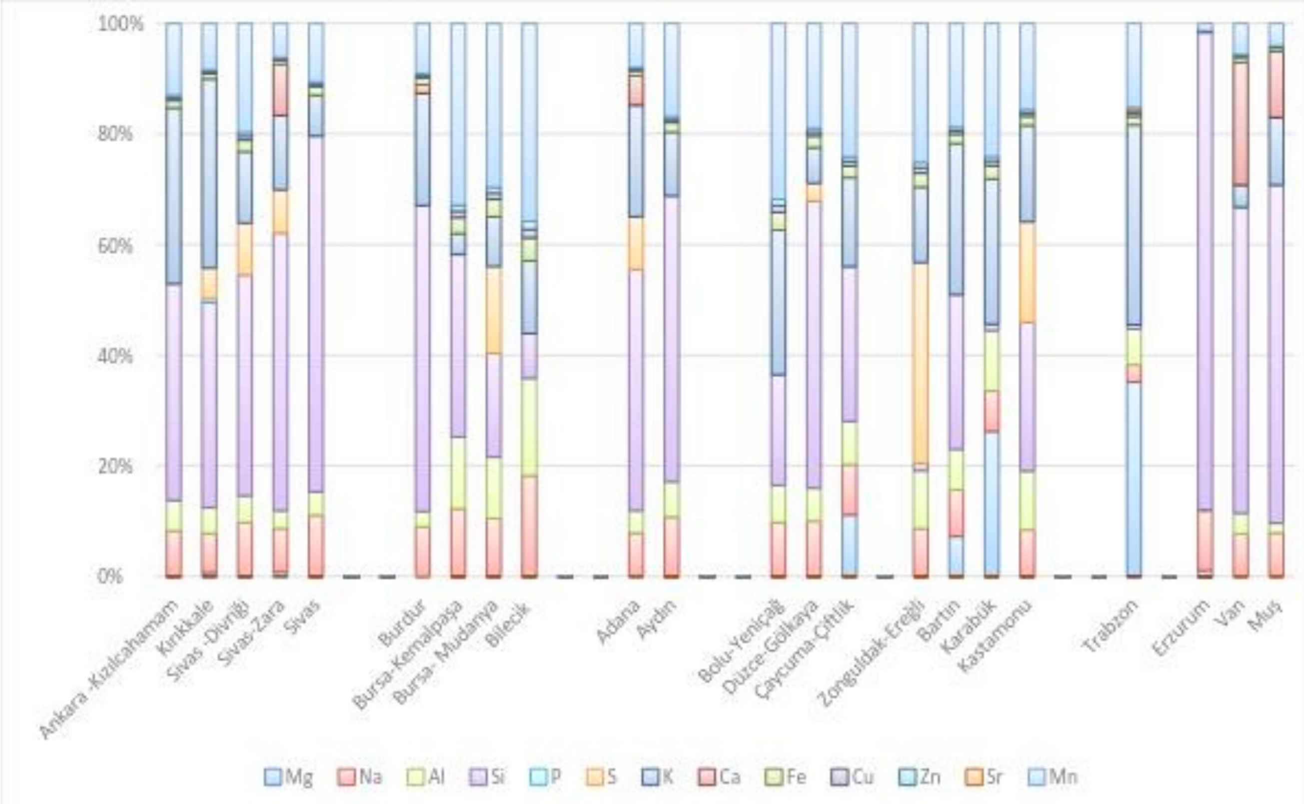

Figure 2. Percentage of component in 22 raw propolis samples from different cities in Turkey

The copper content was at 0.0081 to 0.0082%. Cu and Zn contents in propolis were within safe limits [30], and compared well with levels in foods from Turkish Food Codex, though Zn contents were high (Figure 2).

Propolis can contain heavy metals that can significantly affect the biological properties of derived product cDCA [31]. We revealed Al (mean: 0.845±0.02773) and Fe contents (mean: 0.0277±0.0456) were at lower levels than in the previous report [19]. However, it included high levels of Mn (mean: 0.2145±0.01535) at low levels or the absence of heavy metals; as a result, propolis may be used as an indicator of environmental contamination [7].

Table 4. The comparision of differences for elements in 22 raw propolis samples from various region of Turkey by using Kruskal-Wallis test.

	Mg	Na	Al	Si	S	K	Ca	Fe	Mn
Chi-Square	4,311	16,880	0,195	17,103	3,438	8,280	12,118	9,610	8,411
df	5	5	5	5	5	5	5	5	5
Asymp. Sig.	0,506	0,005	0,999	0,004	0,633	0,141	0,033	0,087	0,135

A Kruskal-Wallis test was performed to compare differences in elements of 22 raw propolis samples from various regions of Turkey. According to the Kruskal-Wallis test results asymp. sig. value for Na, Si, and Ca was $P < 0.05$. Therefore, differences for these elements were statistically significant (Table 4).

Ca was at a high levels (frequency: 2> median) in samples from Meditarian region and Eastern Anatolia but at a low level (frequency: 8≤ median) in samples from Black Sea region. Na was at a high level (frequency: 4> median) in samples from middle Anatolia but was at a low level (frequency: 8≤ median) in samples from Black Sea region. Si was at a high level (frequency 4> median) in samples from middle Anatolia but it was at a low level (frequency: 7≤ median) in samples from the Black Sea region. These propolis samples were rich in minerals of K and Na, and can therefore be potentially more beneficial in human nutrition. The obtained results indicated there was no pollution in the provinces where these samples were collected.

The chemical content may be used to determine additional qualities of propolis samples. However, in addition to the analysis of natural propolis constitutents, elements and toxic contaminants such as heavy metals should also be a subject of chemical control [12].

In this study, the concentrations of three representative heavy metals (cadmium, chromium, and lead) were measured using atomic absorption spectroscopy. Samples were collected from six different sampling points: from six different geografic regions of Turkey. All apiaries employed for this study were specifically constructed without any metal parts in order to avoid the risk of contamination of the assayed materials. Lead (23.81-518.24 μg/kg), arsenic (17.88- 573.36 mg/kg), and cadmium (1542-2441 μg/kg) were found in these propolis samples (Table 5). Conti and Botre (2000) [32] determined lead as 1.06-4.32 (μg/kg) and cadmium density as 0.62-6.59 (μg/kg).

Table 5. Heavy metal levels of propolis from six cities of Turkey

Provinces	Pb (μg/kg)	As (μg/kg)	Cd (μg /kg)
Zonguldak	518.24	127.16	1817.00
Ankara	42.14	41.26	1542.00
Bursa	843.13	573.36	2385.00
Adana	104.46	23.71	2437.00
Muş	57.57	17.88	2441.00
Kastamonu	23.81	52.69	1783.00

Overall, experimental data revealed statistically significant differences between the background levels of heavy metals recorded from the reference sites. According to the results of the statistical analysis, significant differences were found between propolis samples in heavy metal values such as arsenic ($p = 0.030 < 0.05$) and cadmium ($p = 0.002 < 0.5$). As a result, cadmium has the highest values in propolis. In this study, propolis has high levels of heavy metals, as in previous studies [33]. These results indicate that propolis can be considered representative bioindicators of environmental pollution.

Final results indicate the usefulness of including metals in the characterization of propolis samples according to their origin, and could thereby be regarded as an indication of environmental pollution in the collection area in order to evaluate the potential of propolis for the development of new drugs.

The element patterns of propolis we have studied were sufficiently distinctive to make the discrimination of propolis from different locations in Turkey. However, it is possible this variation was influenced by different mineral composition of plants and numerous environmental factors (e.g., soil pH, humidity, mobility of trace elements, etc.).

The results obtained from this work allowed us to conclude that the residues of heavy metals were a limited concentration in Turkish propolis samples. Their chemicals will not be determined until NMR analysis is conducted. Therefore, it is necessary to obtain additional structural confirmation to establish a more specific database of wild propolis from Turkey.

List of Abbreviation: EDXRF- energy-dispersive X-ray fluorescence spectrometre; NMR-Nuclear magnetic resonance; ANOVA-one-way analysis of variance

Competing interests: All authors have no competing of interest.

Author's contributions: All authors contributed to this study.

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