JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2023, 24(5), 191–200
https://doi.org/10.12911/22998993/161654
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Received: 2023.01.27
Accepted: 2023.03.15
Published: 2023.04.01

Determination of Heavy Metals in Bee Honey as a Bioindicator in the Istog, Drenas and Kastriot Regions

Skender Demaku[1], Arbnorë Aliu[1], Donika Sylejmani[1], Blerina Ahmetaj[1], Jeton Halili[1*]

[1] Faculty of Natural Science and Mathematics, Department of Chemistry, University of Prishtina "Hasan Prishtina", 5, "Nënë Tereze" str., 10000 Prishtina, Kosovo

* Corresponding author's e-mail: jeton.halili@uni-pr.edu

ABSTRACT

The major goal of this research was to employ honey as a bio-indicator to identify the heavy metal levels in bee honey in the Istog, Drenas, and Kastriot regions. As a result, honey was purposely tested in these locations, and two industrial specific areas were chosen for examination and analysis: Kastriot (Graboc) and Drenas (Vrboc), as these are considered to be among the most industrial polluted areas. A honey sample was also taken in the Istog (Vrell, as a clean area) region in order to compare not just honey, but also the area where the bee obtains nectar, as well as the sources of pollution discharge into the environment. Concentrations of heavy metals in honey, (min. and max.) amounted to: Zn (8.705–9.804 mg/kg), Mn (5.620–5.718 mg/kg), Fe (3.635–3.745 mg/kg) and Cu (2.295–2.299 mg/kg). In contrast, lower concentrations of metals, have been observed for: Ni (0.640–1.126 mg/kg), Pb (0.235–0.268 mg/kg), As (0.107–0.199 mg/kg), Cd (0.040–0.058 mg/kg) and Cr (0.025–0.036 mg/kg) while elements such as; Hg, and Co, are almost undetected. The study of hierarchical clusters revealed several groupings of elements with geogenic and anthropogenic origins. The concentrations of heavy metals selected for honey were compared to standards of other countries, such as Poland and other European Union countries. Samples were taken in October 2020 and September 2021. The concentration of heavy metals was determined using inductively coupled plasma optical emission spectrometry, ICP OES.

Keywords: pollution, environment, honey, heavy metals.

INTRODUCTION

The major purpose of this study was to use honey as a bio-indicator to determine the heavy metal levels in bee honey in the districts of Istog, Drenas, and Kastriot in Kosovo. The pollutants that bees acquire from the environment include heavy metals, inorganic chemicals, and radioactive substances, all of which are indicators of regional pollution [Korça et al., 2016]. There are no factories in Istog municipality that contribute considerably to pollution; hence, the environment is clean [REC, 2017]. However, pollution and degradation of environmental assets exist even in this town. Currently, the following are the main sources of air pollution in the municipality of Istog [REC., 2017]: traffic-transport with old motor vehicles, largely without catalysts, light industry, generators, then open municipal landfills with methane and CO_2, landfills of damaged automobiles, scrap collecting dumps, and various operators. On the territory of the municipality of Drenas, significant metal, non-metallic, lead-zinc, iron-nickel, magnesite, and bauxite deposits have emerged as a result of various geological processes [Bajraktari et al., 2019], which play an important role in the Kosovo economy and also have an extremely large effect on environmental pollution.

The impact of coal mining as a raw material for power plants in Kastriot, including mining basins where excavators dig, conveyor belts, precombustion, and disposal operations, all of which are sources of gas-dust pollution and coal particles in the air, water, and land surfaces, [4, 5]. The discharge of gases, such as CO, CO_2, NO_X, N_2O, SO, SO_2 [Bajraktari et al., 2019, Demaku et al., 2019], as well as other air particles in the form of dust from the ash of fossil fuels, such as coal, which also include heavy metals from the "Kosovo" power plants, are possible polluters. In addition to key metals, like zinc, copper, manganese, and chromium that are required for human health and development, honey also gathers trace elements including cadmium, nickel, and lead [Pohl et al., 2012]. Excessive usage of such components, on the other hand, may cause chronic toxicity. A colony of bees can have up to 70 thousand individuals depending on the season. The most numerous in the hive are distinguished queens (mothers), males, and workers. The queen or queens, of which there is only one, are distinguished from ordinary bees by their larger body, longer belly, and shorter wings [Bogdanov et al., 2000, Rico et al., 2009].

Bees are vital, because they pollinate flowers by flying from one to the next, which is essential for the development of new plant features. Because honey is made from sugar, which has very little water in its natural state, it can only support a small number of bacteria and germs. Honey degrades only if something is wrong with it. Sugars, organic acids, proteins, enzymes, hormones, yeast, vitamins, and minerals are just a few of the primary and trace ingredients in bee honey. Their presence in the human diet is essential, but excessive levels can be detrimental. Honey is utilized as a bio-indicator to monitor the health of the environment in the places affected by heavy metals [Bogdanov et al., 2000, USEPA, 2007], in addition to its nutritional and therapeutic benefits.

Honey has a pH of 3 to 4.5, which is nearly enough to kill anything that wants to stay in the honey, such as parasites. Honey does not deteriorate, according to [Bogdanov et al., 2000, Rico et al., 2009]. Nectar, the first item collected by bees to generate honey, has high-water content, ranging from 60 to 80 percent. Bees, on the other hand, perform a critical role in eliminating water during the honey-making process by evaporating it with their wings. The chemical makeup of the bee's digestive tract has an impact on honey resistance. When bees expel nectar from their lips to produce honey, these enzymes interact with the nectar to break it down into two products: gluconic acid and hydrogen peroxide [Rico et al., 2009, USEPA, 2007].

Every chemical component plays a crucial part in how nature works and how its many entities function. However, certain elements commonly reach the locations and concentrations where they can negatively impact living things due to natural or human-driven processes. Metals have been demonstrated to have harmful effects on microorganisms [Tahri et al., 2005, Joint, 1981]. Some metals are crucial to the development of plants and are therefore essential to them. However, when they exceed specified quantities, many elements, and even fundamental nutrients, can be hazardous to both farmed and wild plants [Alimentarius, 1998, Aldgini et al., 2019]. Due to industrial progress, humans are also heavily exposed to potentially harmful substances. The focus of this inquiry is on the concentration of specific, possibly harmful components in honey, namely in the Istog, Drenas, and Kastriot of the Republic of Kosovo.

MATERIAL AND METHOD

Sample collection area

A plastic net with a receiver was set at the entrance to each hive, so that honey could be gathered from the bees and transferred to the receiver. The honey samples were then taken straight from the hives using a wooden spoon and kept in plastic containers. The honey samples were collected directly from local beekeepers in three stationary beehives (three samples from each apiary – a total of six samples in the two different time periods).

Table 1 displays the locations of the samples. Istog was the site of the first sampling (M1-Istog-Vrell), Drenas was the site of the second sampling (M2-Drenas-Vrboc), and Kastriot was the site of the third sampling (M3-Kastriot-Graboc), [Korça et al., 2016, Mining, 2009]. The authors took care to take all the collected samples from static hives (like fresh honey) in order for the obtained results to give a more realistic picture of the presence of these metals in the environment, within the research area Figure 1.

Chemical analysis

All chemicals were of analytical grade, stock solutions were purchased from Merck (Germany). The samples were maintained in hermetically sealed plastic containers at 4–5 °C in a cold, dark setting, until analytical measurements were made. The samples were shaken for homogenization, before being heated in a water bath to 60°C for 25 minutes to homogenize.

Table 1. Location of the apiaries [Korça et al. 2016, Mining 2009]

NO	Plot identification	Site description in relation with pollution edge	Distance from the main pollution source (km)
1	Istog (M1. Istog-Vrellë)	Main road of vehicles – easy and heavy transport of various machines	3 km
2	Drenas (M2. Drenas-Vërboc)	Ferronickel industry-road transport	8 km 3 km
3	Kastriot (M3. Kastriot-Graboc)	Electricity industry (IEE), air transport and road transport	2 km 6 km 1 km

Fig. 1. Position of regions on the map and distribution of samples

Following that, 0.5 g of honey was weighed and placed in closed polytetrafluoroethylene (PTFE) vessels, where it was digested with 5 cm^3 HNO_3 (69% V/V, trace pure, Merck, Germany) and 2 cm^3 H_2O_2 (30% V/V, Merck, Germany) and 2 mL H_2O_2 p.a. (30% V/V, Merck, Germany) using a four-stage microware (Analytic Jena TOP wave, Jena, Germany) methodology at temperatures of 150, 170, 200, and 100°C; then, the following program was applied: 5 min up to 150°C, 10 min up to 170°C, 1 min up to 200°C and 1 min down to 100°C, (Table 2) [USEPA. 2007, Joint 1981]. The resulting solutions were then diluted to a volumetric flask capacity of 50 mL and sent off for analysis. The digestion solution was progressively filtered and deposited in a standard 50 ml volumetric flask, which was then diluted with deionized water. After a 24-hour equilibration period, the ICP-OES technique was used to quantify heavy metals in the extracted samples.

Instrumentation and statistical analyses

By the application of inductively coupled plasma–atomic emission spectrometry (ICP-OES, Optima 2100-DV- Perkin Elmer), the concentrations of the following 11 elements were determined: As> Cd> Co> Cr> Cu> Fe> Hg> Mn> Ni> Pb and Zn.

Table 2. Temperature digestion

Step	1	2	3	4
Temperature (°C)	150	170	200	100
Ramp (min)	2	4	2	1
Hold (min)	5	10	15	10

Two spiked blanks and two method blanks were processed simultaneously for each group of analytical samples. The Minitab Software program was used to calculate and present statistical charts, which are shown separately for each element parameter.

RESULTS AND DISCUSSION

Heavy metals results

The presented values of the concentration of chemical elements, analyzed in the honey in three sampling sites (six sample site) were shown in tabular, graphical, PCA and dendrogram way [Alimentarius 1998], where the concentrations of these metals are reflected in different values Table 3. The following table (Table 4) is a summary of all results in the form of statistical analyses of the measurements made on honey samples in three sampling points (M1-M3/ October/ 2020 and September/ 2021). Mean, standard deviation, variance, minimum, maximum, Q1, Q3, median, and range are used to present the data; As> Cd> Co> Cr> Cu> Fe> Hg> Mn> Ni> Pb and Zn.

The figures above (Fig. 2–6) are constructed using the concentrations of elements in honey samples. The diagrams are separated into sections for each scenario, displaying the greatest concentration of each element in the tested samples.

If the highest concentrations of elements in honey (October 2020) are compared, it can be observed that the concentration of Zn is maximum in the measured samples, followed by Mn, Fe and Cu, continuing with a very small difference to the other elements.

Table 3. Results of chemical analysis (October 2020 and September 2021) of elements concentration in honey (mg/kg)

Elements	October 2020			September 2021		
	M1. Istog	M2. Drenas	M3. Kastriot	M1. Istog	M2. Drenas	M3. Kastriot
As	0.077	0.093	0.107	0.084	0.098	0.119
Cd	0.040	0.027	0.031	0.058	0.033	0.035
Co	n.d	n.d	n.d	n.d	n.d	n.d
Cr	0.025	0.015	0.018	0.036	0.023	0.029
Cu	2.295	1.105	1.040	2.299	2.111	1.084
Fe	3.635	1.395	1.480	3.745	2.485	1.749
Hg	n.d	n.d	n.d	n.d	n.d	n.d
Mn	5.620	3.770	0.700	5.718	3.993	0.774
Ni	0.640	0.340	0.055	0.665	1.126	0.356
Pb	0.235	0.115	0.245	0.268	0.138	0.262
Zn	8.705	1.920	1.851	9.804	2.390	2.245

Note: *E** – elements (variables), *M** – samples site, n.d* – no detection.

Table 4. Descriptive statistics of heavy metals presented in honey samples, (M1-M3. October 2020 and September 2021)

October 2020 variable	Mean	Mean	St. Dev	Min.	Q1	Median	Q3	Max.
M1 (Istog)	2.36	1.03	3.08	0.03	0.06	0.64	4.63	8.71
M2 (Drenas)	0.976	0.419	1.257	0.015	0.060	0.340	1.657	3.770
M3 (Kastriot)	0.614	0.232	0.696	0.018	0.043	0.245	1.260	1.851
September 2021 variable	Mean	Mean	St. Dev	Min.	Q1	Median	Q3	Max.
M1 (Istog)	2.52	1.13	3.38	0.04	0.07	0.67	4.73	9.80
M2 (Drenas)	1.377	0.479	1.437	0.023	0.066	1.126	2.438	3.993
M3 (Kastriot)	0.739	0.268	0.804	0.029	0.077	0.356	1.417	2.245

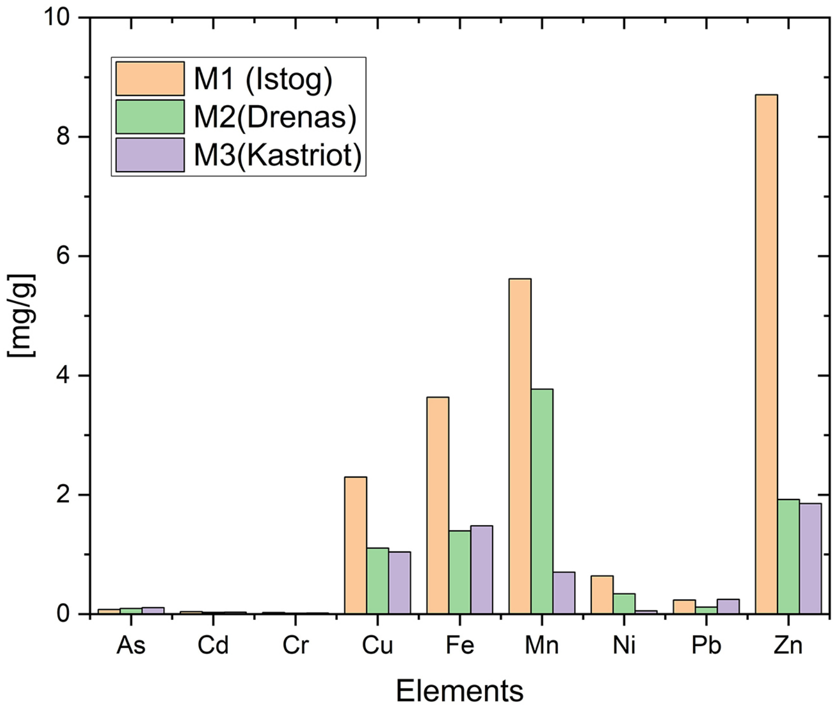

Fig. 2. The presence of heavy metals in honey samples (mg/kg) (October 2020)

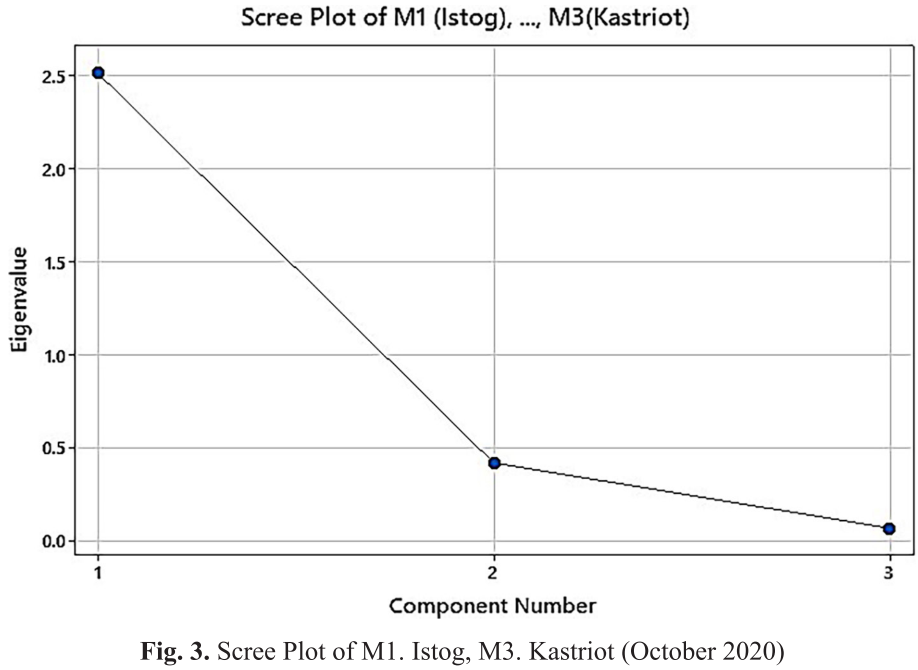

Fig. 3. Scree Plot of M1. Istog, M3. Kastriot (October 2020)

A cluster analysis with PCA method (Principal Competent Analysis) revealed three distinct groups of elements in honey (Figure 3–5) and (Figure 7–9). The first group was made up of Zn and Mn with of natural origin, whereas the second group was of anthropogenic origin, such as; Fe and Cu (the coal processing activities) and third group, contained Ni and Pb and other elements which were in extremely low values.

On the basis of the presented results in the analyzed samples, and on some European standards (ES, Polish legislation) [Aldgini et al., 2019, Intawongse et al., 2006] (EU legislation) for the maximum allowed values of chemical elements in honey, we have concluded that; the concentration of chemical elements (heavy metals) analyzed in honey as bio indicators, in the considered sampling sites, do not pose a high risk to human

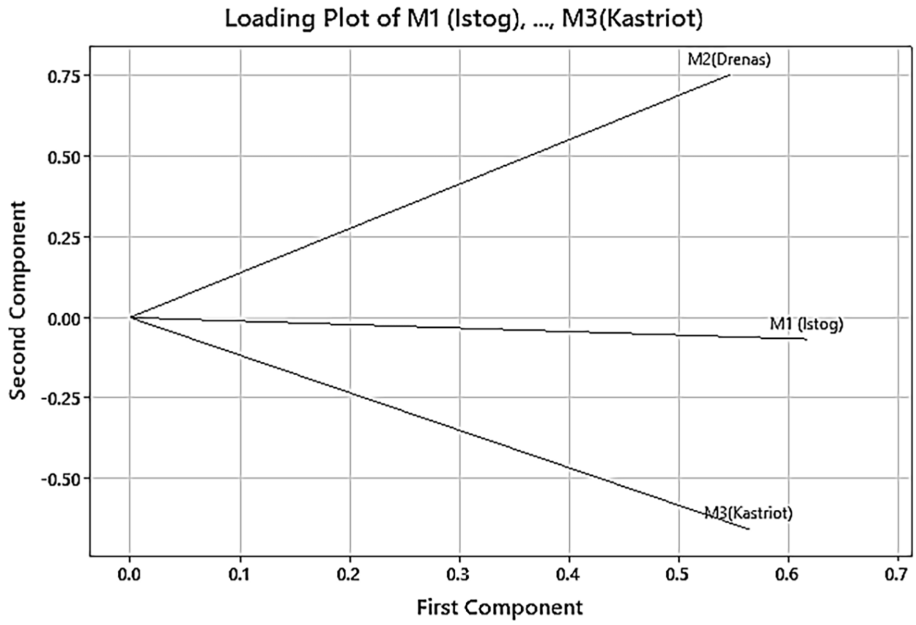

Fig. 4. Loading Plot of M1. Istog,…, M3. Kastriot (October 2020)

health (consumer), although most European standards stipulate that the honey for consumption should have no presence of heavy metals at all.

In the concrete case of this research, in almost three sampling sites, there is a noticeable presence of chemical elements, especially Zinc (8.705–9.804 mg/kg) and Manganese (5.62–5.718 mg/kg) in the first sampling site (M1-Istog-Vrella), where the values vary as they increase; for instance: iron (3.635–3.745 mg/kg), copper (2.295–2.299 mg/kg), and cadmium (0.040–0.058 mg/kg) show higher values, compared with other elements analyzed. Although this region can be considered as the cleanest place, different values of heavy metals have been recorded in the analyzed sample, and the values presented, show a kind of alarm, signaling pollution of the environment even in this research area. Moreover, in the second sampling site: (M2-Drenas-Vërboc), higher values of elements were noted: Manganese (3.770–3.993 mg/kg) and Zinc (1.920–2.390 mg/kg) but also iron (1,395–2.485 mg/kg) and copper (1,105–2.111 mg/kg), represent a slight deviation, compared to other elements, analyzed in honey samples, in three different sampling sites (in six honey samples site).

In turn, in the third sampling site: (M3-Kastriot-Graboc), a slight increase of elements is

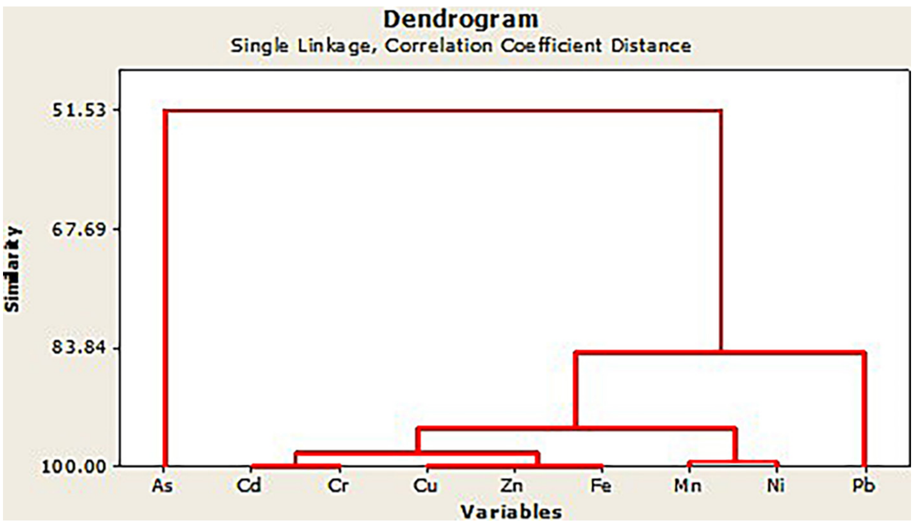

Fig. 5. Hierarchical cluster dendrogram for honey samples (October 2020)

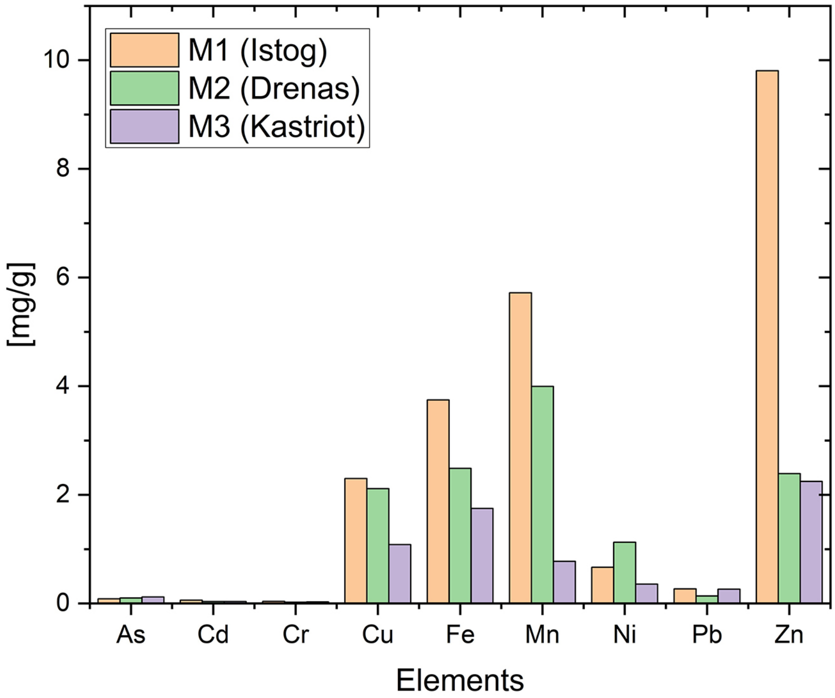

Fig. 6. The presence of heavy metals in honey samples (mg/kg) September 2021

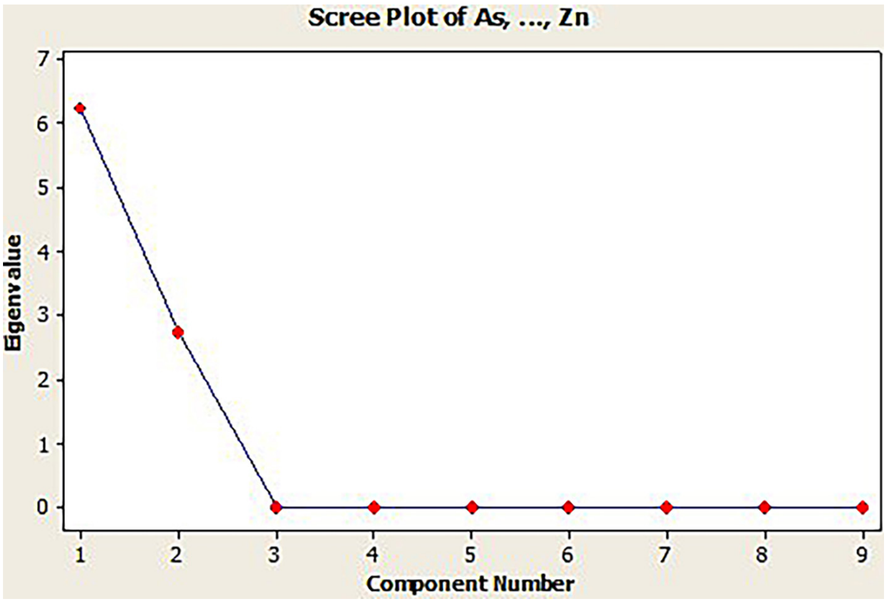

Fig. 7. Scree Plot of M1. Istog, M3.Kastriot (September 2021)

observed: iron (1,480–1.749 mg/kg) then copper (1,040–1.084 mg/kg) and zinc (1.851–2.245 mg/kg), while the other elements, in almost all three sampling sites, are approximately in comparable values, and do not pose an acute risk if this honey is used for consumption by living organisms.

It can be emphasized that the presence of Zn in the honey that has been taken for research, in the region of Istog, may come as a reason of natural factor (geological factors) [Intawongse et al., 2006, Korça and Demaku, 2021] or in one form another, has been transmitted in different ways, during seasonal changes, (via: air, water, soil and plants) where vegetation [Demaku et al., 2022, Spirić et al., 2019], in this case vegetation, has absorbed matter during the exchange of various sorption processes, from the root to the flower petal, or various leaves [Yue et al., 2019, Ahmad et al., 2010]. Moreover, such elements as: Mn, Fe and Cu, which have shown

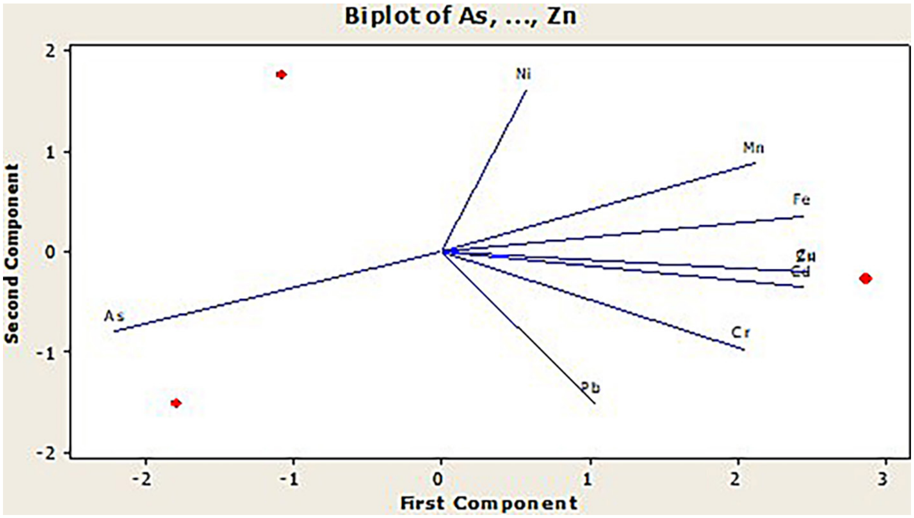

Fig. 8. Bi plot of (As, Zn) for: M1. Istog,…, M3. Kastriot (September 2021)

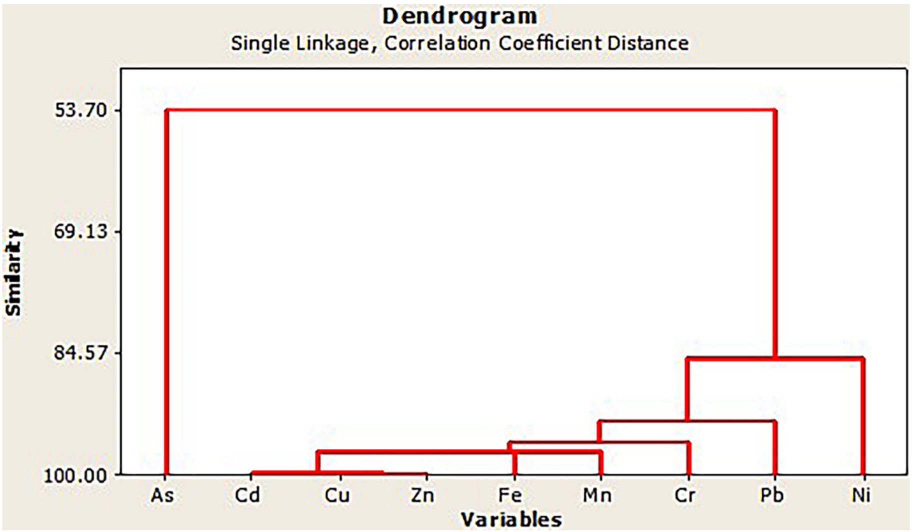

Fig. 9. Hierarchical cluster dendrogram for elements in honey (September 2021)

more higher values, in (M1.Istog-Vrell, in the time period, September 2021) may have geological origins, and may be layered on flower petals, even from direct emission of various gases, [Kalbande et al., 2008, Šajn et al., 2013] which are released by the burning of lubricants, by various moving-transport machinery, or in this case, iron may originate, even from large scrap metal dumps, near municipal and regional roads, [Kalbande et al., 2008, Šajn et al., 2013] near the shores of rivers.

The link between the elements and the two samples (Istog and Kastriot) in September 2021 is shown in the biplot in Figure 7–8. The biplot's observations lead us to the conclusion that Cu and Zn are positively linked with PC1, which accounts for the bulk of the data's variation. This shows that the concentrations of Cu and Zn in the samples from Istog and Kastriot in September 2021 have a significant link. As Mn and PC1 have a negative correlation, samples with high Mn concentrations may not be the same as those with high Cu and Zn concentrations. The second-largest amount of variation in the data is explained by PC2, which has a negative correlation with Pb. Hence, samples with high Pb concentrations are thought to be different from ones with high Cd values.

Overall, the biplot's indicates that different groups of samples may be distinguished based on their element concentrations, with Cu and Zn being the most crucial elements for doing so. The biplot also reveals that there are variations in the elemental concentrations between the samples from Istog and Kastriot. For instance, compared to the samples from Istog, the samples from Kastriot contain higher quantities of Mn and Fe. The correlations of the dendrogram in Figure 9 (September 2021) demonstrate that the distribution of heavy metals in honey is extremely heterogeneous due to the existence of a wide range of plants that can absorb various elements in varying amounts through their root systems [Kalbande et al., 2008, Šajn et al., 2013]. Dust has a significant impact on honey, because bees graze in areas like dumps where they can obtain water or other resources that may be significantly contaminated with heavy metals, which can pollute honey, [Kalbande et al., 2008, Šajn et al., 2013].

As well, in the sampling points at the point: (M2.Drenas-Vërboc-especially in the second time period, September 2021) as the most higher values, the following elements are presented: Mn < Fe < Zn and Cu, while other elements, such as: Cd < As < Co < Cr < Hg < Ni and Pb, are almost values of trace elements, which do not pose a permanent risk, with their concentration in gelatinous biochemical materials, or even in the food chain aspect.

It can be concluded that the presence of these elements (especially like, Fe: 2.485 mg/kg and Ni: 1.126 mg/kg) in bee honey as bio indicators (M2.Drenas-Vërboc, in the time period September 2021) can come from natural resources, as well as from human activity, and with special emphasis, from the industrial activity of the factory ‘’New Ferronickel’’, which has been operating for two decades now in the Drenas region. As such, this factory causes pollution in the environment, air, water and Earth, and when compounded by industrial pollution, pollution from urban, pollution from households, and other pollution, then the impact of pollution on the environment, will be much larger.

Even in the third sampling site: (M3. Kastriot-Graboc-September 2021) in the analyzed material (bee honey as a bio indicators), with more pronounced concentration, the following elements are presented: Zn – 2.245 mg/kg, Fe – 1.749 mg/kg, Ni – 0.356 mg/kg and Cu – 1.084 mg/kg. The authors think that the Kastriot region (i.e., including the village of Graboc) is constantly endangered by industrial pollution, pollution that comes from ash dumps, road and air traffic, from ‘’TC Kosovo’’ (chimneys) and wastewater (used in the manufacturing industry of TPP Kosovo), [Demaku et al., 2022].

CONCLUSIONS

Considering the outdated mechanism of various manufacturing industries in Kosovo, it can be concluded that different gases are released into the air, outside any European criteria and standards, including gases such as: CO, CO_2, SO, SO_2, NO_X, N_2O, and many different types of toxic gases, which pollute the natural environment. Moreover, the Earth is endangered by various pollutions of manufacturing industries, air and land traffic, artificial fertilizers and various households.

Even aquatic environments, such as: rivers, lakes, streams, lagoons, wells and many other environments e.g. catchments, are endangered and polluted by various potential pollutants, ranging from: household pollution, urban pollution, industrial pollution, etc. Extremely high concentrations of Zn: 8.705–9.804 mg/kg < Mn: 5.620–5.718 mg/kg < Fe: 3.635–3.745 mg/kg < Cu: 2.295–2.299 mg/kg and Ni: 0.640–1.126 mg/kg, were found in the honey which was collected, around the industrial area, in the town of Drenas and Kastriot as well as in Istog area (especially high values of; Zn and Mn). Cluster analyses revealed 2 groups of elements, related to sources of pollution, and also 1 group that is usually expected to be of geogenic origin. The correlations between types of samples for each selected potentially toxic element were mostly weak and moderate. There were, however, important correlations indicating some pollution transfer from one environmental compartment to another. Since this is the first research on honey in the region of Istog, Drenas and Kastriot, more research should be conducted in order to more thoroughly evaluate the contamination situation of the set natural products.

Clearly, a strong source of pollution is tailings dumps, as fine particles are transported from them toward the surrounding environment. To prevent the spread of pollution, they should be covered with unpolluted soil or concrete. Another possibility is to transport the tailings into the cavities created in more remote areas during gravel excavation, and then to cover them with soil. To date, there have been no uniform criteria regarding the purity of 100% of honey from potential various pollutants, and there are no unifications between EU countries (Belgium, Germany or even Spain) regarding the classified regions for the extraction of pure honey. Almost every country has its own basic/minimum criteria in determining the purity and different ingredients in honey.

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