DETERMINATIONS OF HEAVY METALS CONTENTS IN HONEY BY TECHNIQUE OF INDUCTIVELY COUPLED PLASMA SPECTROMETRY

Manzoor Iqbal Khattak[1], Shams-ul-Kinat Manzoor[2], Mahmood Iqbal Khattak[3] and Shaheen Wali[4]
[1&4] Chemistry Department, University of Balochistan, Quetta.
[3]-Khyber Medical University, Peshawar.
[4]-PCSIR Laboratories, Peshawar

ABSTRACT: *honey has been a high-nutrient meal derived from a complex blend of carbohydrates, produced by honeybees as a naturally supersaturated sugar solution. Element's in honey are tiny components that, in high enough amounts, may be harmful to human health. As a result, detecting the metals in honey aids in the product's quality management as a food. The goal of this research was to find out how much metal was in Balochistan honey. This research was carried out in four areas of Khuzdar, a Balochistan province. Samples (n=25) of honey were digested using hydrogen peroxide and nitric acid in microwave-oven then examined by ICP-OES (Inductively coupled plasma–optical emission spectrophotometry. Levels of chromium, nickel, zinc and cadmium levels did not change significantly across locations (P >0.05). In honey samples, a high amount of Zinc (12.5* μg/g-1*) was determined. The metal concentrations were compared to the prescribed dietary limits. Some were higher (lead) than the standard limits, while others were lower (cadmium). Metals are discharged into the environment because of industrial activities, and they enter the food chain via plant absorption of polluted soil or water. Metal concentrations vary depending on a variety of factors, resulting in varying levels of metals in honey. Some management methods, such as food quality control, soil monitoring in agricultural areas, and fertilizer usage restrictions, are advised.*

1. INTRODUCTION

Historically, honey has been a high-nutrient meal derived from a complex blend of carbohydrates, produced by honeybees as a naturally supersaturated sugar solution [1]. In the world, it is only a concentrated form of sugar [2] and because it is employed as a preservative in food, this natural material is very important. More than half of the total solids are made up of sucrose and glucose, lipids, vitamins, amino acids, minerals, phenolic acids and flavonoids. All these factors, as well as nectar supply, affect honey's biochemical characteristics and quality [5-9].

Elements make up a small percentage of honey. The kind of raw floral resources obtained by bees, such as nectar, pollen, and honeydew, determines the nature of these components in honey [10-13]. Metal concentrations in honey are influenced by the flower's elemental composition, as well as its botanical and geographic origin [14, 15]. Maintenance practices, improper processing of honey, emissions from the industrial unit, and smelting pollution are only a few of the external sources of these metals. Pesticides (containing arsenic), fertilizers (having cadmium) and organic mercury may potentially be a source of metals in honey (Figure 1) [14, 16].

Metals in honey may provide a health risk to consumers [17]. Human health may be harmed if unsafe levels of these metals build [18]. Several countries, including Poland [21], Croatia [4], Italy [14], Turkey [22-25], China [17], Slovenia [20], and France [19], have lately studied the quantities of different metals in honey. [17, 14, 19, 24, 25] Many further studies have also been conducted on heavy metal levels in bees and bee products [26-32].

Toxic waste from Iran's mining, smelting and metal treatment sectors is a major problem. When heavy metals contaminate food, water, and the environment, they endanger the health and lives of people and animals across the food chain. Many people in Iran like the taste of honey, although the amount of heavy metal presence in Iranian honey is unknown [17]. Honey quality managing is made even more difficult by the need to identify the presence of heavy metals. The results of this study may help to avoid the problems described above and promote healthy honey consumption. In terms of food safety and public health policy, these results may lead to a better knowledge of the contaminants i.e., air pollution and soil type in honey.

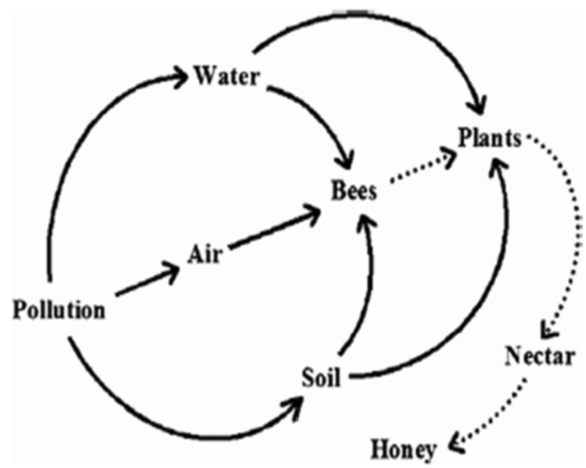

Figure 1 Natural and anthropogenic sources of metals in honey [33].

Numerous scientists have been intrigued by the fact that metal contamination occurs even far from industrial centers and strongly populated economic zones. A. mellifera jemenatica) bodies and wax in four different Khuzdar environmental sampling locations were sampled by the study's authors to determine the levels of metal contamination.

Honey's extensive organic matrix makes mineral analysis challenging. Analysis methods have been developed to identify heavy metals in honey and other sweets. It has recently been discovered that inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and ICP-MASS have been created. Results show that honey samples from Khuzdar had high levels of certain metals and physicochemical properties.

2. EXPERIMENTAL

2.1. Sample collection

Samples were collected in glass jars and transported to the National Center of Excellence Center of Analytical Chemistry at Sind University, Jamshoroo. All honey samples (400 g) were provided by the beekeepers in the study area.

2.2. Apparatus

Samples examined by ICP-OES (Inductively coupled plasma–optical emission spectrophotometry. In a microwave oven, the materials and their preparation were digested (MARS 5, CEM). 15 minutes of 600 W microwave digestion at 120°C, 20 minutes of 600 W microwave digestion at 180°C and 20 minutes of venting were used for the digestion of the materials.

2.3. Reagents and chemicals

Analytical grade reagents were used. Stock solutions of Zn, Ni, As, Pb, Cd, Cr, and Cu (1000 mg/L, ICP standard CertiPUR) acquired from Merck were diluted to make the element standard solutions (Germany). As an internal standard, the 45Sc, 89Y, and 159 Tb solutions were prepared using the identical ICP-OES process. Use the internal standard in regular analysis to compensate for possible long-term drift and correct matrix effects, according to ICP-OES. Hydrogen peroxide (H_2O_2) and concentrated nitric acid (HNO_3) were employed to destroy honey samples (30 percent H_2O_2 pure p.a, Chempur, Poland).

Figure 2 Geographical location of the studied region Khuzdar in the Balochistan province

2.4. Analytical Procedure

It was necessary to weigh and dissolve each sample in PTFE containers exactly 1 gram in 10 milliliter concentrated nitric acid to test for the seven elements mentioned: (HNO_3). In the microwave, the samples were digested [25]. In a closed system, the breakdown of the sample had no contact with the outside world, reducing the risk of contamination. Blank solutions were made using nitric acid. The great sample throughput and sensitivity of ICP-OES allow it to perform multi-element analyses with high precision and accuracy. When applied to honey samples, this method was able to detect the heavy metals of relevance [14]. There have been several studies using atomic absorption and emission spectrometry to identify and analyze heavy metals.

The precision of the analytical method was evaluated by comparing the standard deviation of the repeatability of the experimental results from real samples (S.D). To guarantee accuracy, calibration was utilized (using standard solutions). As an additional step, the ICP-OES technique was utilized to correct matrix effects using the internal standard (45SC, 89Y,159Tb).

Table 1 Instrumental characteristics and settings for ICP-OES

Spectrometer	Agilent 7500ce with ORS
Nebulizer	Micromist
Interface	Interface
RF generator (W)	1550
Argon flow rate (L min^{-1})	0.85
Nebulizer pump (rps)	0.10
Scanning condition	Number of replicate 5, dwelling time 1
Scanning mode	Pulse Reduction gas flow (L min^{-1}):
H_2	3.5
He	4.0
Internal standard	^{45}Sc, ^{89}Y, ^{159}Tb

4.5. Analysis of data

SPSS (v.18) were utilized for analysis of data. T-test and ANOVA were used to analyse the data collected in this study. The p 0.05 significance level was used.

3. RESULTS AND DISCUSSION

An investigation was carried out to ensure that all the metals recovered from the ore were accurate. There was a high degree of precision with metal recovery rates of 95–100 percent (Table 2). There are several mineral trace elements in honey, which were taken into consideration in this study by utilizing blank samples to ensure accurate findings. Blank samples of honey (compound absence) were identified that were injected previously into heavy metals.

Table 2 and Figure 3 indicate the metal amounts in honey samples. Statistics such as the total number of samples and their means, standard deviations, minimums, and maximums may all be shown. Zinc is the most common metal found in honey samples, with highest concentration of *(12.5* μg/g-1*)* (with a range of 10.9 to 0.9 μg/g-1). Cu, Cr, Cd, Pb and Ni, the other significant metals, have far lower averages than zinc. Cadmium concentrations were highest in the East and lowest in the North. Cadmium concentrations did not change significantly across sites (P=0.110). There were no significant differences ($P > 0.05$) in zinc, nickel, or chromium levels between regions.

There was a statistically significant difference in lead levels for honey samples from various regions (P 0.002), according to ANOVA. A mean lead concentration has been shown as in table-2 which is self- explanatory for researchers in future as base line data reference to the protection of environmental pollution for the purpose of the awareness of the public because the bee's honey is usually are in high altitude places where it is safe from lead pollution. While in light of the earlier research workers on the West and North have the highest and lowest copper concentrations, respectively. P = 0.374 demonstrated no significant differences in copper concentrations among sites.

Table 2 Samples of honey were tested for heavy metal content and recovery

SNo.	Name of Site	Element	Certified Value (µg/g-1)	Measured Value (µg/g-1)	Recovery (%)
1	Pero Uneet (Khuzdar 1)	As	5.67	4.68 ± 0.30	95
2	Pero Uneet (Khuzdar 2)	Cd	0.0 13	0.013 ± 0.001	100
3	Pero Uneet (Khuzdar 3)	Cu	5.64	5.60 ± 0.20	99
4	Pero Uneet (Khuzdar 4)	Pb	0.47	0.45 ± 0.03	97.2
5	Jahl Magsi 1	Zn	12.5	10.9 ± 0.9	88.4
6	Jahl Magsi 2	Cr	0.3	0.29 ± 0.03	99
7	Jahl Magsi 3	Ni	0.91	0.87 ± 0.04	96

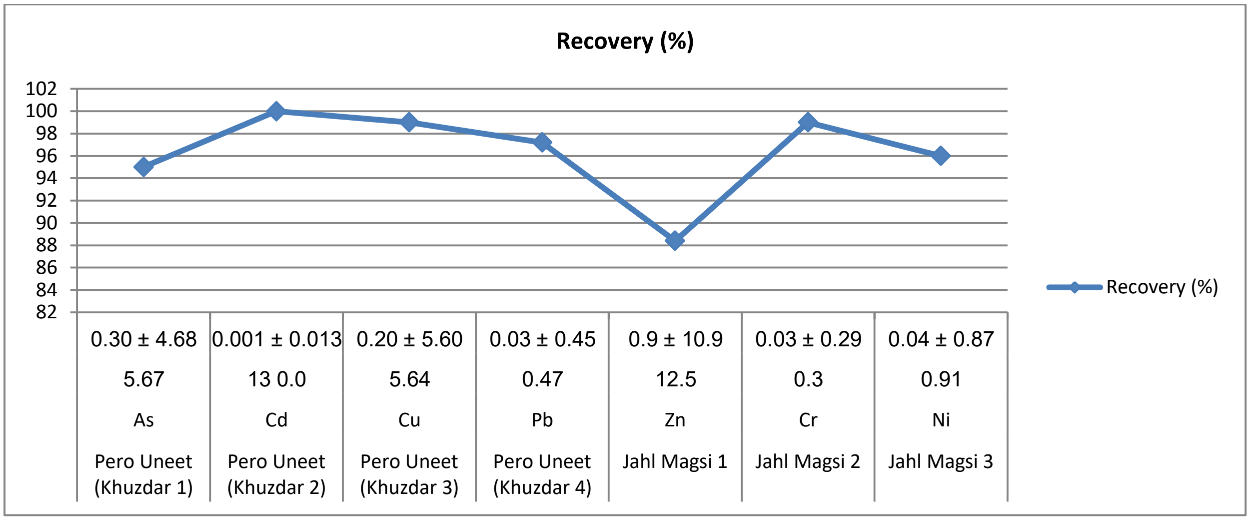

Figure-3. Honey samples were tested for heavy metal concentrations and recoveries.

The typical adult's body weight is used to calculate the preliminary tolerable daily intake (PTDI) of copper, which is 3 mg [34]. (60 kg body weight). For all living things, including humans, to thrive, copper must be present. However, much copper in the body may have detrimental health effects. However, this study's mean copper level was significantly lower than that found in previous surveys in New Zealand (2500µg/g-1) [38], Ireland (0.2mg/100g-1) [37], Italy (16300–182000 µg/g-1), China (32998 µg/g-1) [17], Turkey's Black Sea Region (9750–35600 µg/g-1) [23]. A possible explanation for this discrepancy is variations in the places where we researched, such as differences in fertilizer usage or shifts in plant-growing practices.

On average, cadmium contents ranged from well under the legal limit of 200000 g µg/g-1 to much beyond that limit of 1,36 to 125.88 gkg-1 [40]. In this study, the concentration of cadmium was recorded lower than observed in Italy (305000 µg/g-1). These levels were substantially higher as recorded in earlier studies in Turkey (0380–2030 µg/g-1) [14], Poland (1.5 µg/g-1), Romania (15 µg/g-1) [42], Chinese (1340 µg/g-1) (15) and Macedonia (3630 µg/g-1) [43]. Due to discrepancies between our findings and those of other surveys, this may be due to differences in the regions studied in other studies. It enters the food chain via plant absorption of contaminated soil or water because of cadmium being released into the environment through a number of industrial processes. There are several variables that influence the cadmium contents found in honey samples from various regions, and these differences may be traced back to the different sites.

There were 205400 µg/g-1 and 935480 µg/g-1 respectively, of lead in the honey samples collected from the South and East, according to our findings. A total of 507.58 gkg-1 of lead was found in honey samples collected from all four regions, above the FAO/WHO/1984 recommended limit of 300000 µg/g-1 in the last two concentrations. Items and places may contain lead, which is why it is important to be aware of it. As soon as it is released into the atmosphere, lead is absorbed by plants in the soil at the point of emission. This means that some foods, such as honey, may have a higher percentage of lead in them. Honey from the East has higher amounts of lead than honey from any other part of the country, according to this study. Lead may have been absorbed by plants that provide nectar for bees because of soil contamination. Aside from the negative impact on human metabolism, lead may cause several health issues, such as cancer and heart disease. A serious approach to the problem is thus required.

A lead concentration of 0100–200000 µg/g-1was found in several samples of Rumanian honey by Antonescu and Mateescu [44], however, this was well below the limits stipulated by the Codex Alimentarius' latest guidelines [45].

From China, more than three times as much lead (33980 μg/g-1) was found in honey samples compared to Croatia, New Zealand, Turkey, Poland, Romania, Taiwan and mainland China with an average 652000, 17, 17600, 0048, 29 and 7 μg/g-1. The lead amounts found in this study were much lower when compared with Italian honey (2370000 μg/g-1) and Slovenia (5940 μg/g-1).

According to Codex Alimentarius [45] and Commission Regulation [48], the mean arsenic level (11870 μg/g-1) was determined in all honey samples, which is far below the maximum permitted value (10000-500000 μg/g-1). Even though there were only a few measurements in the literature. Our figure is higher than those reported in Siena County (6590 μg/g-1) and Italy (0500 μg/g-1) [14,35]. In between New Zealand, Slovenian and Croatian honey's a considerable variation were examined [38]). Arsenic in honey may come from a variety of sources, including nonferrous metallurgy, industry, and agrochemicals such as arsenic-based fertilizers and pesticides. Food quality monitoring and minimizing the use of arsenic-based pesticides are among the proposed control measures.

An average of 12–15 mg of zinc per day is advised for adults [51–53]. This analysis found the highest and lowest Zn levels in the West at 6638550 μg/g-1, while the North had the lowest at 122860 μg/g-1. On average the lower Zn level were reported from Italy (3205000 μg/g-1) [39], Siena County (1820gkg-1) [14], China (132950 μg/g-1), Slovenia (3.61mgkg-1) [20], New Zealand (11800 μg/g-1) and Taiwan (0996 μg/g-1). Keeping honey in galvanized containers might lead to Zn contamination [55]. According to some experts, the kind of flowers that bees nectar from may be the principal cause of Zn contamination in honey [15]. Zinc is an essential component of the human body, but excessive levels may have detrimental health effects. The need for quality control of food products arises from this.

According to our results, the average nickel content in honey samples collected from all four regions was 651780 μg/g-1. There are more industrial nickel sources in the East, which might explain why the nickel mean in that area is higher. Many factors influence the quantity of nickel in food, including the nickel source and the distance from the contamination source. Throughout the soil profile, nickel may be found in the air, water, and soil. According to the FAO/WHO Expert Committee on Food Additives, a daily nickel intake limit of 5000 μg/g-1 body weight has been set for nickel (JEFCA). Prior studies reported no nickel content in the samples tested [43,56,57].

Chromium (Cr) is one of the 14 most dangerous heavy metals, according to the Environmental Protection Agency (EPA) [58]. The most prevalent method of exposure for the general population is via the consumption of chromium-containing food [59]. Adults are advised to consume between 30 and 100 grams of protein each day. The most prevalent non-occupational route of chromium exposure is via the consumption of food. Foods may include chromium trivalent, which is assumed to be present [60]. In this study, the level of Cr (172370- 1220300 μg/g-1) was determined on average. The chromium concentration of honey from the north was the greatest. An intake of approximately 0.15 mg of chromium (III)/kg of body weight per day, or 10 mg per person per day, was estimated to be safe by the EVM, but the WHO recommended that chromium supplementation not exceed 250 g per day [61]. Based on these guidelines and data, the chromium amounts in the honey samples tested are considered to be within an acceptable range. This study's findings can't be compared to those of others since there isn't much research on the topic. A total of four places were surveyed, and the average amounts of heavy metals were calculated. All heavy elements, except arsenic, showed significant regional variations (P 0.05).

A lot of people like their tea with honey as a sweetener. The overall amount of heavy metals consumed should be taken into consideration since black tea includes heavy metals [62,63].

4. CONCLUSION

Metals were found in all types of honey, and the quantities of metals varied by region owing to a number of causes, according to the research. According to the data, the greatest and lowest quantities were found for zinc and arsenic, respectively. Geological and geochemical conditions may have an impact on honey's chemistry. Regional differences may be exacerbated by factors such as proximity to industry, soil type, fertilizer usage, and plant-growing practices. In order to establish a standard metal concentration threshold based on body weight and honey intake, the further honey study is required. Many criteria used in this study were based on a person's daily or weekly metal intake, but there was no data on how much honey that individual consumed on a daily basis.

5. RECOMMENDATIONS

The following recommendations were made on the basis of present findings, food products quality should be controlled by the authorities to prevent hazardous contaminants which are harmful to human health. The safety of the water that is being irrigated for the purpose of agriculture should be thoroughly checked by the water management authorities. Limiting the use of fertilizer which is hazardous in nature, because honey's corrosive action may cause chromium and zinc contamination when it comes into touch with stainless steel surfaces and galvanized containers, it's best to store honey in suitable containers.

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