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ASSESSMENT OF HEAVY METALS IN HONEY BY ATOMIC ABSORPTION SPECTROMETER

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ABSTRACT

Heavy metals in honey are of interest mainly for quality control and nutritional aspect along with the determination of environmental contamination.The objective of this work was to determine distribution of the levels of selected heavy metals like Cadmium (Cd), Chromium (Cr), Arsenic (As), Mercury (Hg) and Lead (Pb) in honey samples by Atomic Absorption Spectrometer (AAS). A total of 20 samples were collected from different regions in Karnataka of which 15 samples were multifloral and five unifloral. The analysis revealed the presence of Lead (4.2ppm), Chromium (0.14ppm) and Cadmium (0.76ppm) in the collected samples. Contamination by Mercury and Arsenic was found to be below detection level. The presence of these toxic metals in bee honey is an evidence of micropolluting agents in the environment. These findings helped us to conclude that the acceptable level of heavy metals present in the honey of the study area has no negative impact on the living organisms. Honey produced in different regions of Karnataka state do not completely lack heavy metals but they are at satisfactory level and good quality for human consumption.

Keywords: Honey, Heavy metals, Cadmium, Chromium, Arsenic, Mercury, Lead.

INTRODUCTION

Honey is a natural sweet substance produced by honeybees from the nectar of blossoms or secretions of plants parts, which they collect, transform and combine with specific substances, and store in honey combs [(1)] . The chemical composition of honey explains the numerous nutritional, healing and prophylactic properties. Honey contains sugar, proteins, vitamins, minerals, enzymes, polyphenols and flavonoids [(2)]. Because of this unique and

complex nature, honey is proved to be useful in the treatment of burns, wounds, skin ulcers, in the treatment of external eye diseases as an antioxidant and as antimicrobial [3] . In order to have a beneficial effect honey must be free from any contaminating agents [4]. Heavy metals present above the admitted levels by pollution standards are a threat to human health [5]. The current international honey market trend, regarding quality is more demanding. Therefore, it is necessary to promote all feasible activities in order to produce residue free honey. The raw materials for honey production is obtained by honeybees from external environment, therefore it also contains pollutants characteristic of relevant environment [6]. Honeybees cover larger areas and contact innumerable surfaces during their foraging activity, hence they have been studied as biomonitors of environmental contamination, with analysis of honey as a possible indicator of the magnitude of such contamination [7]. Honeybees and their products can thus supply suitable amount of biological material to be easily sampled and analyzed throughout the year [8]. Heavy metals present in the atmosphere may be deposited on the hairy bodies of bees and may be brought back to the hive with pollen, or absorbed together with the nectar of the flowers, or through water and honeydew. Heavy metals in honey are of interest mainly for quality control and nutritional aspects along with the determination of the level of toxic substances in the environment. The nectar, from which the honey is made, may contain heavy metals absorbed by the roots from the polluted soil. High levels of metals are undesirable because of their known or supposed toxicity and can be a source of health hazards to human beings. Therefore, high concern exists about the contribution of these ingredients to the total dietary intake of trace metals that may be present as contaminants.

Heavy metals are emitted in a continuous manner by various natural and anthropological sources. Since they are not degraded, they are continuously kept “in play”, thus entering the physical and biological cycles [9]. Among many pollutants accumulating in the environment, there are elements of toxic properties like Cadmium, Chromium, Lead, Mercury and Arsenic may cause vascular diseases, kidney or bone damage, irregular functioning of human and animal reproductive system. They can easily penetrate to the cell membranes and internal organs as well as cause denaturation of proteins in the blood or mucous membranes and penetrate to the tissues [10]. Heavy metals are dangerous because of many reasons, firstly during processing of food the metals doesn’t decompose, on the contrary their concentration referred to the mass unit increases. Secondly, the metals tend to accumulate in the human organism, and may slow or even block the intracellular biochemical processes. Thirdly, the majority of the metals possess carcinogenic and mutagenic properties, after they are

assimilated it is very difficult to remove them from the human system [11]. Palynological analysis was performed to determine the type of honeys studied. The aim of the study was to determine the contamination level of chosen toxic elements like Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), and Mercury (Hg) in unifloral and multifloral honey samples collected directly from bee colonies of several places in Karnataka and also to find a link between the amount of heavy metals found in the samples from the possibly contaminated area and the samples from the pollution free areas. Since honey is a nutritional resource that depends on biotic and abiotic factors around the beehives, the presence of heavy metals could be related to its geographical and botanical origin [12]. As the consumption of honey is increasing because of its multiple health promoting effects, the metal contaminants in the honey need to be evaluated [13]. The concentration of different metals has been widely documented in honey from many countries [14-17]. Determination of heavy metals in honey is of high interest mainly for quality control and nutritional aspect [18].

EXPERIMENTAL

Twenty fresh honey samples were collected from different places in Karnataka. The honey samples were collected in tightly closed glass containers and stored at room temperature till analysis. Honey consists mostly of organic matrix which contains trace metallic constituents. In atomic absorption analysis the metals must be separated from the organic matrix followed by extracting the metal in mineral acid for aspiration. Five grams of honey sample was taken into a silica dish and placed on a hot plate then heated slowly until it was completely charred. The dish was then transferred to muffle furnace at a temperature 450°C for ash. When the ashing was completed, it was cooled and extracted with minimum amount of HCl and evaporated to dryness. Further it was extracted again with 10 ml of 25% HCl, filtered and made up to 50 ml with HCl. This solution was used for the determination of metals by using a Flame Atomic Absorption Spectrometer Buck scientific model 210 VGP [19]. Air-Acetylene flame was preferred for the determination of the five selected elements (Cr, Cd, Hg, Pb, As). The temperature of the air-acetylene flame was approximately 2300°C. Standard stock solutions of the respective metals were also prepared. All the samples were analyzed in triplicates. The wavelength (nm) of the emission peak of the five elements analyzed and the respective working range were measured are: As - 193.7 nm for 50-200 ppm; Pb – 217.0 nm for 2-10 ppm; Cr – 357.9 nm for 2-8 ppm; Cd – 228.8 nm for 0.5-2.0 ppm; Hg – 253.7 nm for 100-400 ppm.

RESULTS AND DISCUSSION

Table 1. Heavy metal concentrations in analyzed honey samples

Place	Botanical Origin	Lead (Pb)	Mercury (Hg)	Arsenic (As)	Cadmium (Cd)	Chromium (Cr)
Sirsi	Multifloral	2.1 ± 0.046	BDL	BDL	0.2 ± 0.4	0.12 ± 1.2
Karwar	Multifloral	2.4 ± 0.049	BDL	BDL	0.33 ± 0.1	0.07 ± 0.6
Bangalore	Unifloral (Eucalyptus)	4.2 ± 0.019	BDL	BDL	0.45 ± 0.5	0.05 ± 0.2
Rayalpadu	Multifloral	ND	ND	ND	0.01 ± 1.2	ND
Sulya	Multifloral	0.2 ± 0.013	BDL	BDL	0.76 ± 0.2	0.06 ± 0.4
Mysore	Multifloral	1.6 ± 0.023	BDL	BDL	0.34 ± 0.5	0.05 ± 0.4
Doddaballapur	Multifloral	ND	ND	ND	ND	0.01± 0.8
Belgaum	Multifloral	0.7 ± 0.09	BDL	BDL	0.26 ± 0.1	0.14 ± 0.2
Ajjampura	Multifloral	0.2 ± 0.013	BDL	BDL	0.1 ± 0.1	0.14 ± 0.2
Kumbalgodu	Multifloral	ND	ND	ND	0.005 ± 0.4	ND
Bijapur	Multifloral	0.9 ± 0.011	BDL	BDL	0.1 ± 0.2	0.09 ± 0.5
Siddapur	Unifloral (Sapindus)	1.9 ± 0.013	BDL	BDL	0.1 ± 0.2	0.07 ± 0.5
Honnavar	Multifloral	3.2 ± 0.023	BDL	BDL	0.23 ± 0.1	0.09 ± 0.5
Devanahalli	Unifloral (Pongamia)	1.5 ± 0.011	BDL	BDL	0.27 ± 0.5	0.12 ± 0.8
Ankola	Unifloral (Mangifera indica)	2.3 ± 0.019	BDL	BDL	0.29 ± 1.2	0.11 ± 0.9
Chintamani	Multifloral	ND	ND	ND	ND	ND
Puttur	Unifloral (Rubber)	3.7 ± 0.019	BDL	BDL	0.32 ± 0.8	0.06 ± 0.9
Nagarahole	Multifloral	ND	ND	ND	ND	ND
Medikere	Multifloral	ND	ND	ND	ND	ND
Bandipur	Multifloral	ND	ND	ND	ND	ND
Minimum Detection Levels		**0.01ppm**	**0.04ppm**	**0.01ppm**	**0.004ppm**	**0.0004ppm**

ND: No Detection, BDL: Below Detection Level, Concentrations are in ppm.
Data presented as mean ± standard deviation.

The result of heavy metal concentrations of 20 honey samples from different places of Karnataka are presented in Table 1, of which 15 were multifloral and five unifloral. From the result obtained, the predominant heavy metal observed was Lead followed by Cadmium and Chromium whereas Arsenic and Mercury were found to be below detection level. In the present study, 13 samples were contaminated with Lead ranging from 0.2 to 4.2 ppm, among which eight and five were multifloral and unifloral respectively. High concentration of Lead was detected from the honey samples collected from urban areas like Bangalore (4.2 ppm) followed by Puttur (3.7 ppm), Honnavar (3.2 ppm), Karwar (2.4 ppm), Sirsi (2.1 ppm) Siddapur (1.9 ppm) and Mysore (1.6 ppm). Low concentration of Lead was detected from the rural sites like Sulya, Doddaballapur, Belgaum, Ajjampura, Bijapur, Devanahalli and

Ankola. Lead is one of the most widespread metal pollutants, can reach human system through air, water and food. This metal has no beneficial role in human metabolism and produces a progressive toxicity and can cause health disorders [20]. In the present investigation, 15 honey samples were found to be contaminated with Cadmium. The concentration ranged from the lowest level of 0.005 ppm from Kumbalgudu sample to highest of 0.76 ppm in sample collected from Sulya, among which 10 were multifloral and 5 were unifloral. Similarly like Lead, Cadmium is also an undesirable element and its toxic properties can cause health disorders. The Chromium contamination was recorded in 5 unifloral and 9 multifloral honey samples. Honey samples from places such as Belgaum and Ajjampura (0.14ppm), Devanahalli (0.12ppm), Ankola (0.11ppm), Honnavar and Bijapur (0.09ppm) showed low Chromium content. High contamination of Lead, Cadmium and Chromium were reported from Rome and Province [21]. In the present study it was noticed that most of the samples were contaminated with three heavy metals such as Lead, Cadmium and Chromium. However the levels of contamination was within permissible limits with respect to Cadmium and Chromium whereas three samples from Bangalore, Puttur and Honnavar contaminated with Lead exceeded the permissible limit as given by the Press Information Bureau, Government of India, 2010, Ministry of Health and Family Welfare.

It is interesting to know that all the five unifloral samples were contaminated by the selected metals in the order Pb > Cd > Cr. The presence of Lead, Cadmium and Chromium in bee honey is an evidence of micropolluting metals in the environment. Arsenic and Mercury are elements of strong toxic properties may interfere with the physiological functions and cause diseases in humans. However contamination by Arsenic and Mercury was found to be below detection level. Honey samples collected from the bee hives of *Apis cerana* from places like Doddaballapur, Rayalpadu and Kumbalgodu exhibited low levels of heavy metals, whereas honey samples from Nagarahole, Madikeri and Bandipur were free from analysed metal contamination. Based on the assessment of heavy metal contamination, the levels of heavy metals are comparatively more in urban areas than forest and rural areas. This might be because of pollution from the vehicular traffic, heavy industries, and other anthropogenic activities. Honey can be an indicator of environmental pollution [22]. Accumulation of these heavy metals in plant tissues is hazardous for the food chain and may cause damages to human beings and animals [23]. Atmospheric fallout, contaminated water and chemicals from pesticides and fertilizers are some major cause of heavy metal contamination in the plant [24]. The present study concludes that though the concentration of Lead was found most prominent

in few honey samples, the majority of the samples were below the permissible values and it fits the National standards. The levels of other metals like Cadmium, Chromium, Arsenic and Mercury were non-significant. The present investigation of heavy metals in honey samples of Karnataka revealed that the study area is found to be less contaminated with heavy metals. The region has considerable area of natural forests and plantation crops. Also the farmers are switching over to organic agriculture. Thus honey samples produced in different regions of Karnataka state do not completely lack heavy metals but are at acceptable limits and satisfactory level for safe human consumption.

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