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Comparative assessment of nutrient composition of *APIS* honey from diversified biogeographical regions of Karnataka, India

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Abstract
Honey is a remarkable food product of honeybees with high-calorific value. The mineral content is highly variable with the species of honeybee, the geographical area and botanical origin. The most medicinal properties of honey along with its flavour depend on its mineral content. Honey samples of *Apis florea*, *Apis mellifera*, *Apis dorsata* and *Apis cerana* were collected from Coorg, Bangalore and Kolar districts of Karnataka. In current study, the quantitative mineral analysis of honey samples were performed by Atomic absorption spectrophotometer and flame photometer. Total ash content was estimated by Ivanov and Chevanakova method (1984). The total ash content ranged between 0.11 to 0.52 per cent. Honey samples of *Apis dorsata* from Coorg showed highest of 0.52 per cent. The F-test and analysis of variance values of total ash content parameter of honey samples were significant at 5% levels. The mineral elements with high frequency were K content 50.68±0.09, Fe 0.09±0.003, Mn 0.18±0.007, Ca 5.87±0.1, Cr 0.012±0.003, Cu 0.06±0.008, P 1.28 ±0.3, Zn 0.15±0.007 ppm in *Apis dorsata* from Coorg. Na content was high in honey samples of *Apis florea* of Kolar with 3.42 ±0.04 ppm. All the mineral contents were within range and varied highly significantly at $p< 0.05$ levels. From present study, it is observed that the Indian honey is good in quality.

Keywords: *Apis* honey, mineral content, total ash content, medicinal properties, honey quality

Introduction
Honey as defined by the Codex Alimentarius Commission (1989) [12] is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant – sucking insects living on parts of plants, which honeybees collect, transform and combine with specific substances of their own store and leave in the honey comb to ripen and mature. The composition and quality of honey also depend on several environmental factors during production such as weather and humidity inside the hive, nectar sugar concentration and treatment of honey during extraction and storage (Joseph *et al.,* 2007) [17]. Honey is an easily digestible food stuff that contains a range of nutritionally important compounds (Celechovoska and Vorlova, 2001) [10]. The major components of honey include various saccharides, water, amino acids, minerals, proteins, vitamins and unstable compounds such as enzymes. Honey is generally considered as a natural and healthy product (Reybroeck, 2003) [32]. Karnataka produces 800 to 900 tons of honey annually. The characteristics of honey from different floral sources influence the commercial value and the consumer preferences (Shripad and Rangaswamy, 2001). Honey also contains minerals and heavy metals, which play important roles in determining honey qualities. The mineral content varies, ranging from 0.04% in pale honey to 0.20% in darker honey (Bogdanov *et al.,* 2007) [8, 34]. Darker honey has comparatively higher mineral content than the lighter honey. The major minerals are mainly derived from the soil and nectar-producing plants, but they may also come from anthropogenic sources, such as environmental pollution. It has been reported that micro- or trace minerals originating from organic or plant sources are important for maintaining human health, while those which originate from inorganic or metallic sources, such as heavy metals, can be toxic (Hernandez *et al.,* 2005; Pohl 2009) [15, 30]. However, due to technological advancements, the determination of inorganic compounds in honey has become simpler, faster, and more economical. Honey has various nutritional, medicinal, and prophylactic (preventative) properties contributed by its various chemical constituents (Da Silva *et al*., 2016) [13]. Nevertheless, in order to yield medicinal effects, honey should be free of any contaminants. Due to this fact and honey utility as a natural, effective, and pleasant sweetener, there is an increasing interest in the study of honey (Mc kee 2003, Lammertyn *et al*., 2004, Bianchi *et al*., 2005) [23, 19, 6]. Honey has also been used as an indicator for a variety of environmental contaminants, including heavy metals, low-level radioactivity, and pesticides (Nalda *et al*., 2005; Bogdanov *et al.*, 2007 & 2013) [26, 8, 34].
The aim of present investigations is to determine essential metals and heavy metals like K, Na, Ca, Mn, Fe Zn, Cu, Cr, P in *Apis* honey samples collected from various regions of Karnataka, India.

Materials and Methods

Study areas

The present study areas of Karnataka were of Coorg district, Bangalore district and Kolar district.

Procurement of *Apis* honey samples

One hundred and fifty six *Apis* honey samples of *Apis florea*, *Apis mellifera*, *Apis cerana Apis dorsata*, were harvested from various geographical areas of Coorg, Kolar and Bangalore, Karnataka during 2019. Each honey sample was first filtered with a sterile mesh to remove debris. All the samples were collected and transported in sterile sealed bottles or screwed cups with authentic labels. Four replications of bottles for each sample were kept under storage at 2-8 ^{0}C until tested as per the method proposed by Nzeako and Hamdi (2000) [29].

Determination of total ash content in honey

5 to10g of honey was accurately weighed in a silica or platinum dish whose weight was pre-determined. Few drops of pure olive oil were added to prevent the spattering of honey. Then, it was heated carefully over a low flame until swelling ceases. It was then kept in the muffle furnace at 600 ± 20 ^{0}C till white ashes obtained. The dish was cooled in a desiccator and weighed (Ivanov and Chevanakova, 1984).
The ash content in honey sample was calculated by using the following formula:

$$\text{Ash (\% by mass)} = \frac{100x\ (m_2\text{-}m)}{(m_1\text{-}m)}$$

m= mass in g. of empty dish
m_1= mass in g. of dish with 5-10ml of honey
m_2=mass in g. of dish with ash
The results were expressed as mg/Kg.

Analysis of minerals in Honey Samples

5 g of each sample was weighed using an analytical balance, transferred into a beaker, digested using nitric/perchloric acid and filtered into a 50 ml volumetric flask. Distilled water was used to make the solution to the mark. For the determination of calcium, strontium was added to reduce interferences from aluminum and phosphorous (A. Mbiri *et al* 2011) [1]. Potassium and Sodium was determined using flame photometer. Calcium, Iron, Zinc, Copper, Phosphorus, Manganese and Chromium were determined using Atomic absorption spectrometer (Mudasar Manzoor *et al.*, 2013 and Rodriguez-otero *et al., 1*994) [25, 31]. Data of all mineral contents of honey samples were analyzed by Analysis of Variance (ANOVA) along with F test, highly significant values were determined by using F table ($p \leq .005$).

Results

The mineral composition of *Apis* honey samples of Karnataka showed significant variations.
The total ash content of honey collected from Coorg varied between 0.20 and 0.52 per cent (Fig.1). The honey samples of *Apis florea* of Coorg district ranged from 0.20 to 0.48 per cent, *Apis cerana* between 0.25 and 0.49 per cent, *Apis mellifera* from 0.26 to 0.49 per cent and *Apis dorsata* from 0.25 to 0.52 per cent.

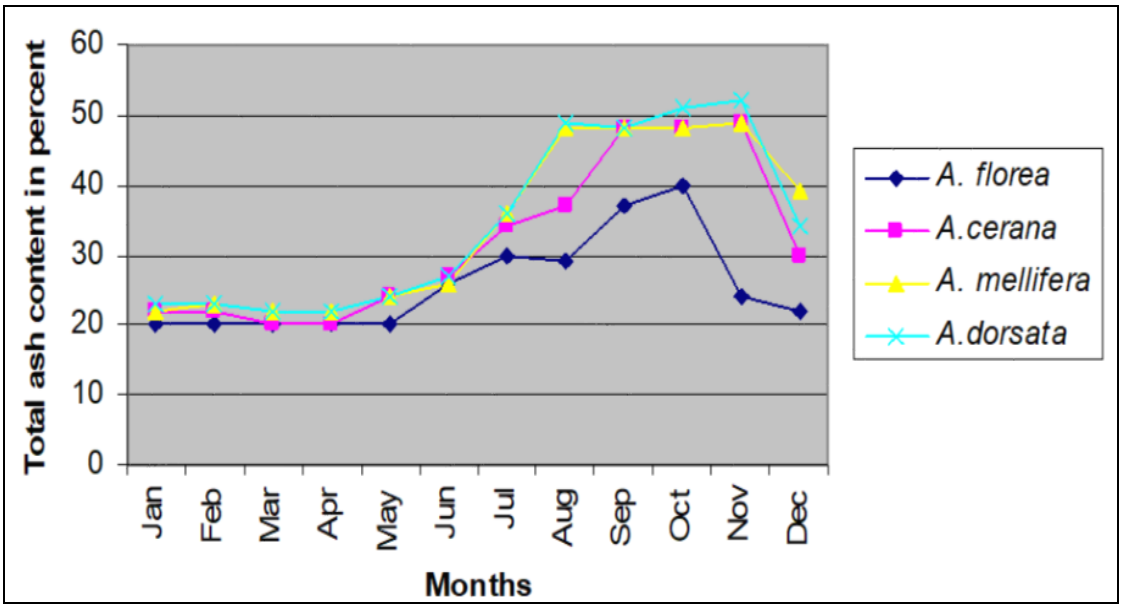

Fig 1: Showing the total ash content of *Apis* honey samples from Coorg during 2019.

The honey samples of Bangalore district ranged from 0.14 to 0.35 per cent (Fig.2). The honey samples of *Apis florea* of Bangalore district ranged from 0.14 to 0.35 per cent, *Apis cerana* between 0.14 and 0.26 per cent, *Apis mellifera* from 0.14 to 0.26 per cent and *Apis dorsata* from 0.14 to 0.26 per cent.

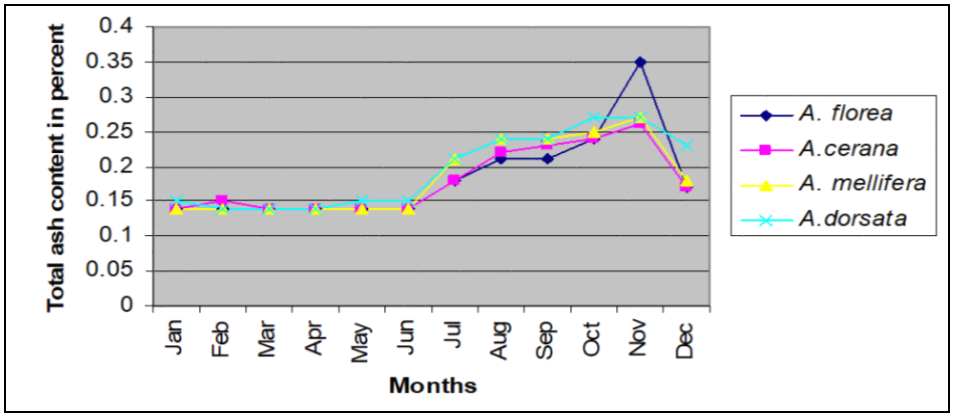

Fig 2: Showing the total ash content of *Apis* honey samples from Bangalore during 2019.

The honey samples of Kolar district ranged from 0.11 to 0.26 per cent (Fig.3). The honey samples of *Apis florea* of Kolar district ranged from 0.11 to 0.26 per cent, *Apis cerana* between 0.11 and 0.26 per cent, *Apis mellifera* from 0.11 to 0.26 per cent and *Apis dorsata* from 0.12 to 0.26 per cent.

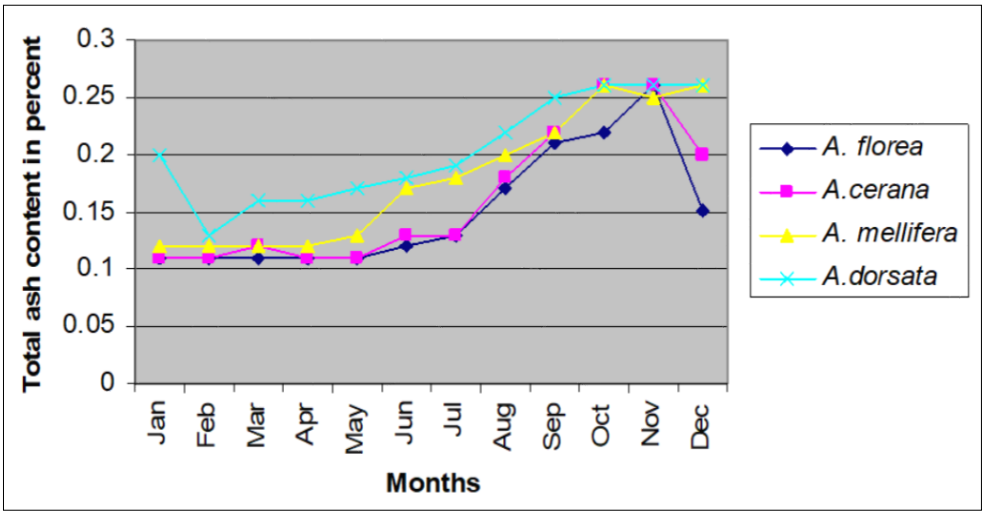

Fig 3: Showing the total ash content of *Apis* honey samples from Kolar during 2019.

The F-test and analysis of variance values of total ash content parameter of honey samples from Coorg, Bangalore as well as Kolar were significant at 5% levels. Mineral contents of various *Apis* honey samples of Coorg during investigation period showed highest ppm values for Potassium content of *Apis* honey and the least recorded value was of chromium. However, the vlues were significant at 5 percent levels (Table 1).

Table 1: Mineral Constituents of *Apis* honey samples from Coorg, Karnataka during 2019.

Mineral Type	**Honey Samples**			
	Apis florea **(ppm**±S.E)	***Apis cerana*** **(ppm**±S.E)	***Apis mellifera*** **(ppm**±S.E)	***Apis dorsata*** **(ppm**±S.E)
Potassium	34.80±0.05	46.78±0.06	49.11±0.06	50.68±0.09
Iron	0.06±0.003	0.08±0.001	0.08±0.008	0.09±0.003
Manganese	0.12±0.005	0.17±0.008	0.16±0.002	0.18±0.007
Magnesium	1.54±0.1	1.22±0.1	1.09±0.2	1.35±0.2
Calcium	3.6±0.8	4.21±0.5	4.33±0.3	5.87±0.1
Sodium	2.15±0.5	3.01±0.6	3.02±0.6	3.09±0.7
Chromium	0.011±0.001	0.011±0.005	0.011±0.002	0.012±0.003
Copper	0.02±0.001	0.04±0.007	0.05±0.003	0.06±0.008
Phosphorus	1.13±0.2	1.22±0.4	1.24±0.5	1.28±0.3
Zinc	0.08±0.001	0.09±0.005	0.08±0.004	0.15±0.007

Significant at $p < 0.05$ levels

Mineral contents of various *Apis* honey samples of Kolar during investigation period showed highest ppm values for Potassium content of *Apis* honey and the least recorded value was of chromium. However, the values were significant at 5 percent levels (Table 2).

Table 2: Mineral Constituents of *Apis* honey samples from Kolar, Karnataka during 2019.

Mineral Type	Honey Samples			
	Apis florea **(ppm**±S.E**)**	***Apis cerana*** **(ppm**±S.E**)**	***Apis mellifera*** **(ppm**±S.E**)**	***Apis dorsata*** **(ppm**±S.E**)**
Potassium	38.08±0.02	41.95±0.01	45.37±0.03	47.98±0.04
Iron	0.02±0.01	0.03±0.01	0.04±0.01	0.05±0.01
Manganese	0.08±0.003	0.12±0.002	0.14±0.004	0.16±0.001
Magnesium	1.12±0.01	1.08±0.01	1.03±0.02	1.01±0.008
Calcium	2.1±0.03	2.39±0.05	3.94±0.05	4.74±0.04
Sodium	3.42±0.04	3.02±0.08	2.52±0.03	2.88±0.02
Chromium	0.005±0.001	0.009±0.001	0.010±0.001	0.010±0.001
Copper	0.01±0.001	0.02±0.001	0.03±0.001	0.03±0.001
Phosphorus	0.53±0.05	0.85±0.07	0.92±0.03	0.99±0.04
Zinc	0.03±0.001	0.06±0.001	0.02±0.001	0.09±0.001

Significant at $p< 0.05$ levels

Mineral contents of various *Apis* honey samples of Bangalore during investigation period showed highest ppm values for Potassium content of *Apis* honey and the least recorded value was of chromium. However, the values were significant at 5 percent levels (Table 3).

Table 3: Mineral Constituents of *Apis* honey samples from Bangalore, Karnataka during 2019.

Mineral Type	Honey Samples			
	Apis florea **(ppm**±S.E**)**	***Apis cerana*** **(ppm**±S.E**)**	***Apis mellifera*** **(ppm**±S.E**)**	***Apis dorsata*** **(ppm**±S.E**)**
Potassium	32.94±0.63	36.52±0.55	40.11±0.73	40.32±0.71
Iron	0.01±0.001	0.04±0.001	0.04±0.001	0.06±0.001
Manganese	0.09±0.001	0.10±0.005	0.11±0.007	0.11±0.009
Magnesium	1.01±0.53	1.01±0.63	1.09±0.19	1.03±0.14
Calcium	1.42±0.22	1.58±0.43	2.77±0.11	3.86±0.16
Sodium	2.83±0.15	2.99±0.22	2.41±0.32	2.33±0.27
Chromium	0.002±0.001	0.002±0.001	0.006±0.001	0.009±0.001
Copper	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001
Phosphorus	0.44±0.11	0.32±0.13	0.75±0.16	0.83±0.19
Zinc	0.04±0.001	0.02±0.001	0.01±0.001	0.08±0.001

Significant at $p< 0.05$ levels

Discussions

In the present analysis of total ash content in *Apis* honey ranged from 0.20 and 0.52 percent. Similar findings were reported by Rodrigues *et al*, (1994) of 0.48 percent in honey samples from spain. Bonvehi and Coll (1993) [9] reported 0.06 to 0.39 percent of average ash content in French lavender honey of Spain. Anass *et al* (2003) [2] analysed average ash content with 0.16 to 0.44 percent in Eucalyptus honey. Joseph *et al* (2007) [17] reported 0.66 percent of ash content in Sudano Guinean honey.

The major minerals of honey recorded were potassium, iron, manganese, magnesium, calcium, sodium, phosphorus, zinc and chromium. The highest content being potassium with 50.63±0.09 ppm in *Apis dorsata* honey of Coorg and least with 32.94±0.63 ppm in *Apis florea* honey of Bangalore. The values of K in this study were less than the values recorded in earlier studies where mean values in honey were found to be 2310, 1774 and 3166 ppm from Kicevo, Zulia and Tenerife respectively (Machado De Melo *et al*, 2018: Frias *et al.*, 2008; Betzabé Sulbarán de Ferrer *et al.*, 2004) [20, 14, 4]. The lower values of K content in present samples may be influenced by climatic factors, storage conditions or lowest values of NPK in that particular area.

The mineral chromium content is also highest in *Apis dorsata* honey of Coorg with 0.012±0.003 ppm and 0.002±0.001ppm was recorded least in *Apis cerana and Apis florea* honey samples from Bangalore. Similar correlated results were reported by Mossel (1998) [24], Karbournioti and Drimjias (1997) [18] in Australia, Greek and grass land honey respectively. All honey samples were recorded lower contents of Cr as compared to other minerals (heavy metals) in our study.

The mineral iron content is highest in *Apis dorsata* honey of Coorg with 0.09±0.003 ppm and 0.01±0.001ppm was recorded least in *Apis florea* honey samples from Bangalore. The Fe content was recorded highest in the honey sample *Apis mellifera* with a value of 2.800 ppm from hills of Jammu and Kashmir and the lowest level was recorded in the honey sample *Apis cerana* from hills of Tamil Nadu with a value of 0.69 ppm (Mudasar

Manzoor *et al.,* 2013) [25]. In our study the values of Fe recorded were lower than those values of 13.5 and 3.37 ppm reported earlier in Zulia and Tenerife (Frias *et al.,* 2008) [14], Mudasar Manzoor *et al.,* 2013 [25] and closer to values reported earlier by A. Mbiri, *et al* 2011 [1], ranging between 0.08 and 0.59 ppm.

The mineral manganese content is highest in *Apis dorsata* honey of Coorg with 0.18±0.007 ppm and 0.08±0.003ppm was recorded least in *Apis florea* honey samples from Kolar. The highest concentration of Mn was found in *Apis dorsata* from Western Ghats of Tamil Nadu with a value of 1.126 ppm reported by Mudasar Manzoor *et al.,* 2013 [25]. Mahmood Ahmed *et al.,* (2016) [22] reported 0.73 to 0.97 ppm Mn in the Pakistan honey samples. The mineral calcium content is highest in *Apis dorsata* honey of Coorg with 5.87±0.1ppm and 1.42±0.22 ppm was recorded least in *Apis florea* honey samples from Bangalore. The highest Ca value of present honey samples may be influenced by the time of extraction from the comb in relation to ripening process by the bees, tempature conditions, seasons or geographical zone.

The mineral sodium content is highest in *Apis florea* honey of Kolar with 3.42±0.04 ppm and 2.15±0.5 ppm was recorded least in *Apis florea* honey samples from Coorg. 122.8 to 181.7 ppm was reported by Mahmood Ahmed *et al.,* (2016) [22] in the Pakistan honey samples. Mudasar Manzoor *et al.,* (2013) [25] reported 25.17 ppm of Na in *Apis cerana* honey samples from Tamilnadu and Kashmir.

The mineral copper content is highest in *Apis dorsata* honey of Coorg with 0.06±0.008 ppm and 0.01±0.001ppm was recorded least in *Apis florea* honey samples from Bangalore and Kolar *Apis mellifera, Apis cerana Apis dorsata* honey samples of Bangalore. The highest concentration of Cu was recorded in honey sample *Apis dorsata* from Western Ghats of Tamil Nadu with a value of 0.624 ppm while the lowest concentration of Cu was recorded from honey sample *Apis mellifera* from Jammu and Kashmir with value of 0.275 ppm (Mudasar Manzoor *et al.,* 2013) [25]. The concentration of Cu in present samples were lower, compared to the values recorded in Swiss and Tenerife honey which were 0.88 and 1.28 ppm (Stefan *et al.,* 2007) [34] and has a closer values reported by A. Mbiri,*et al* (2011) [1] ranging between 0.02 and 0.03.

The mineral zinc content is highest in *Apis dorsata* honey of Coorg with 0.15±0.007 ppm and 0.01±0.001 ppm was recorded least in *Apis mellifera* honey samples from Bangalore. In *Apis* honey samples, Ciobanu Radukscu (2016) reported 0.987 mg/kg of Zn, Berinde and Michnea (2013) [3] reported 1.09 to 1.39 range of Zn in *Apis* honey.

The mineral phosphorus content is highest in *Apis dorsata* honey of Coorg with 1.28±0.3 ppm and 0.32±0.13 ppm was recorded least in *Apis cerana* honey samples from Bangalore. The range is in accordance with the results reported by Bhushanam & Madhusudan (2017) [5] and Nguyen *et al*., (2018 & 2019). Mahajan (1984) analyzed honey from Shimla and reported that dark colored honey have more minerals than light colored honey of *Apis cerana*. Though, the quantity of minerals was less, they play a vital role in determining the color, medicinal and nutritional value of honey. From present study, it is observed that the Indian honey is good in quality.

Conclusions

The present study concludes that the mineral analysis of honey is essential for nutritional quality and safety of honey with regards to the contents of major, minor and trace elements. Of all the minerals tested in the honey samples, *Apis dorsata* honey from Coorg have high contents of K, Fe, Mn, Ca,Cr, Cu, P and Zn as compared to the rest of the honey samples, while the Na content is high in *Apis florea honey* from Kolar. The results of the present study reveal that Indian honey quality with respect to the concentration of these mineral elements in safety baseline levels for human consumption and for medicinal applications as well.

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