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Macro and Essential Trace Elements in *Apis cerana-indica* Honey from Garhwal Himalaya, India

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Abstract

Quantification of macro and trace elements is of quite an interest to evaluate the nutritional level of honey and for characterizing the bee products in view of safety implications. The aim of present study was set to estimate the macro and essential trace elements of twenty four honeys from some localities of district Pauri, Garhwal Himalaya. Potassium and sodium concentrations were determined by using flame photometer and calcium, magnesium, iron, zinc, copper and manganese were studied by atomic absorption spectrophotometer. Results revealed that potassium was most abundant macro element and in the trace elements, iron and zinc were found to be most common. Statistically analyzed samples were found significantly different ($p<0.05$). Copper showed high and positive correlation with Zinc ($r=0.884$) as compared to others, while indicated least and negative correlation with sodium which was $r=-0.007$.

1. Introduction

Honey is the natural, easily digestible food processed by diverse types of *Apis* species using nectar, plant exudates and furthermore dew components. *Apis* belongs to Hymenopteran group that generally works in an eco-friendly environment and provides many services, as pollinator (Przybylowski and Wilczynska, 2001), besides producing honey and other products. Beekeeping with *Apis cerana-indica* is an age-old practice still taken by inhabitants in rural areas (Gaur and Nawani, 1989; Gaur and Tiwari, 2000; Tiwari *et al*., 2013). *Apis* species feed in their 1-2 km territory around their hives and in doing so interact with air, soil and water in the locality.

Nutritional level of honey varies generally with organic, topographical, extraction and storage conditions. Carbohydrates in form of fructose and glucose comprise about 90-95% of the honey dry matter and other diverse mixes, such as organic acids, proteins, amino acids, minerals, phenolic compounds, and vitamins (Ayoub *et al*., 2009). Honey contains different quantities of minerals ranging from 0.02 to 1.03 g/100g, with potassium being the most abundant element comprising approximately one third of the total mineral contents (Bogdanov *et al*., 2007), followed by calcium, magnesium, sodium, sulfur, and phosphorus. Trace elements include iron, copper, zinc, and manganese and play a critical role in biological systems such as general metabolism, germination, circulatory systems and influence reproduction as catalysts of various biochemical and physiological reactions (Staniskien *et al*., 2006).

Mineral elements of honey are of quite an interest not only for quality control but also as bio-indicators of environmental contamination. Terrestrial systems such as air, soil, water, and plants have a great influence on the chemical composition of derived products. The minerals make their way into the honey through soil (Stankovska *et al*., 2008), thus information on the metal profile appears suitable for categorizing honey for its nutritional value. In this paper an attempt was made to estimate macro and essential trace elements of honey samples collected from *Apis cerana-indica* colonies in some localities of Khirsoo block of district Pauri, Garhwal Himalaya.

2. Material and Methods

The study was carried out in district Pauri, Garhwal Himalaya, India, during 2016. District Pauri is located between 29^0 20'-29^0 75' N latitude and 78^0 10'-78^0 80' E longitude at an elevation from 600–1700 m (Fig. 1). The dominant plant species visited by bees for nectar and pollen in study area were *Aegle marmelos*, *Phyllanthus emblica*, *Bombax ceiba*, *Toona ciliata*, *Syzygium cumini*, *Bauhinia variegata*, *Adhatoda zeylanica*, *Indigofera cassioides*, *Murraya koenigii*, *Citrus* spp., *Quercus semecarpifolia*, *Rhododendron arboreum*, *Aesculus indica*, *Myrica esculenta*, *Juglans regia*, *Berberis*

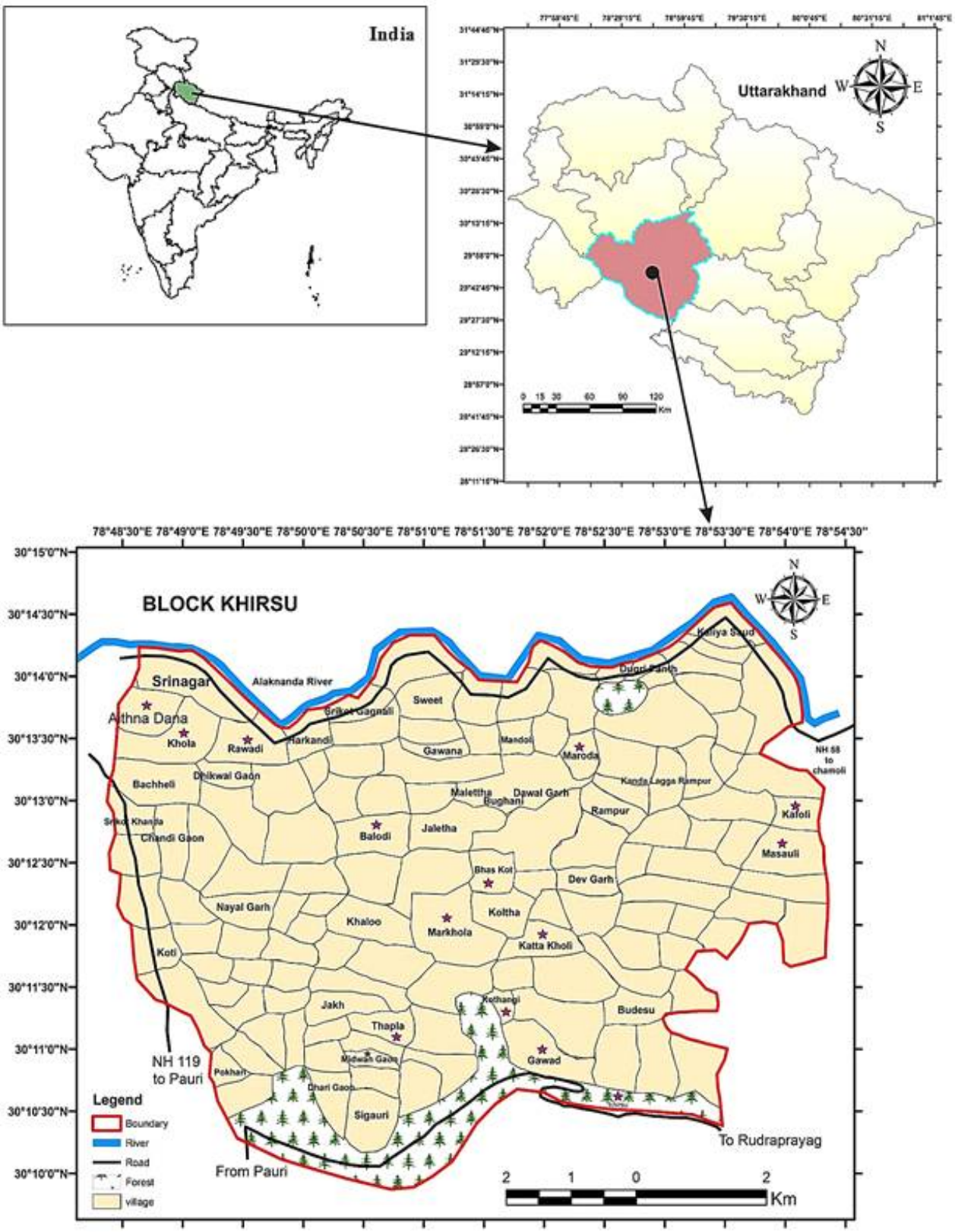

Fig. 1: The map of study area with marked locations of the analyzed honey samples.

Table 1: Mineral element composition of honey samples collected from various villages.

Honey sample number	Collection site (Villages name)	Potassium (ppm)	Calcium (ppm)	Sodium (ppm)	Magnesium (ppm)
HS-Rai-01	Rawadi	8.3^{a}±0.76	1.54^{a}±0.39	2.09^{b}±0.61	0.44^{b}±0.08
HS-Ait-02	Aithna Dana	8.49^{ab}±1.1	0.53^{b}±0.28	2.5^{bc}±0.7	0.76^{ac}±0.3
HS-Mar-03	Maroda	5.54^{c}±0.6	2.65^{ac}±0.4	0.37^{d}±0.12	0.84^{a}±0.1
HS-Gaj-04	Gajeli	10.43^{f}±0.75	1.62^{a}±0.14	3.5^{a}±0.8	0.81^{a}±0.04
HS-Kaf-05	Kafoli	5.36^{c}±0.7	2.35^{ad}±0.5	1.51^{be}±0.42	0.36^{bd}±0.11
HS-Mus-06	Musoli	10.45^{d}±0.86	0.49^{b}±0.11	4.19^{af}±0.26	0.78^{a}±0.06
HS-Mus-07	Musoli	13.83^{dg}±0.12	3.09^{cde}±0.3	5.43^{g} ±0.69	1.05^{ae}±0.12
HS-Kho-08	Khola	11.75^{ef}±0.75	6.28^{f}±0.4	3.52^{ah}±0.61	2.86±0.32
HS-Kho-09	Khola	15.31^{g}±0.49	3.65^{eg}±0.31	2.72^{bhi}±0.43	1.27^{e}±0.23
HS-Bal-10	Balodi	7.90^{a}±0.98	6.47^{f}±0.62	1.62^{bn}±076	1.04^{ae}±0.12
HS-Bal-11	Balodi	2.70^{h}±0.76	2.21^{a}±0.25	1.83^{bj}±0.29	1.52^{ef}±0.26
HS-Bha-12	Bhash kot	14.14^{d}±0.87	9.98±0.45	5.40^{g}±0.53	0.87^{a}±0.07
HS-Mar-13	Markhola	13.75^{dg}±0.2	8.64^{h}±0.7	3.10^{aci}±0.13	1.09^{ae}±0.12
HS-Mar-14	Markhola	13.66^{d}±0.85	2.75^{ae}±0.75	3.98^{aik}±0.25	0.023^{g}±0.005
HS-Cha-15	Chakoli	12.81^{de}±0.63	6.76^{fi}±0.4	8.56^{l}±0.62	0.510^{bc}±0.17
HS-Kot-16	Kothangi	15.74^{g}±1.44	3.57^{egj}±0.81	4.63^{fgkm}±0.3	2.59^{h}±0.22
HS-Kot-17	Kothangi	13.6^{d}±0.84	4.87^{k}±0.65	5.6^{g}±0.46	0.01^{g}±0.001
HS-Gaw-18	Gawad	13.59^{d}±0.69	7.42^{il}±0.67	2.83^{ab}±0.24	2.44^{h}±0.11
HS-Gaw-19	Gawad	8.81^{b}±2.44	4.22^{gjk}±0.29	1.10^{ejnd}±0.12	0.7^{ab}±0.13
HS-Gaw-20	Gawad	13.04^{dc}±1.01	2.27^{a}±0.26	4.3^{amo}±0.5	0.13^{dg}±0.02
HS-Khi-21	Khirsoo	13.84^{dg}±0.94	8.63^{h}±0.32	5.0^{go}±0.23	1.13^{ae}±0.04
HS-Tha-22	Thapla	13.75^{dg}±0.99	6.84^{fl}±0.12	8.0^{l}±0.8	$.28^{bg}$±0.03
HS-Mid-23	Midwan gaon	2.66^{h}±0.8	0.62^{b}±0.12	6.68±0.4	1.71^{f}±0.22
HS-Mid-24	Midwan gaon	12.69^{de}±0.9	3.85^{gj}±0.3	3.86^{aim}±0.65	0.4^{b}±0.12
Average		**10.93±3.8**	**4.22±2.76**	**3.8±2.09**	**0.98±0.7**

Mean±SD for triplicate, mean values in same column with same letters are non-significantly different ($p<0.05$).

aristata, *Lyonia ovalifolia*, *Litsea monopetala, Prunus cerasoides*, *Prunus persica*, *Pyrus pashia*, *Pyracantha crenulata*, *Prinsepia utilis*, *Rosa moschata*, *Rubus ellipticus*, *R. niveus*, *Brassica* spp. and *Raphanus sativus* etc. The common bee species *Apis cerana-indica* is found in domesticated hives. Honey samples were collected from sixteen villages of the area at the time of extraction and brought to laboratory for further analysis. All the samples were filtered through cheese cloth to remove debris and stored in Borosil bottles for analysis. Names of honey samples were assigned based on respective villages from where these were collected (Fig. 1).

One gm of honey sample was dissolved in 12 ml of analytical-reagent of diacid (nitric acid 65% and perchloric acid 30%) and mixed followed by digesting up to the complete dryness. To digest, samples were diluted and volume brought up to 100 ml with double distilled water. Mineral constituents Ca, Mg, Mn, Fe, Zn, Co were determined by Atomic Absorption Spectrometer and K, Na by flame photometer.

2.1. Statistical analysis

All measurements were carried out in triplicates and presented as mean ± standard deviation. Pearson's correlation and one way ANOVA was done using SPSS.

3. Results

Macro elements such as potassium, calcium, sodium, and magnesium are presented in Table 1 and essential trace elements iron, zinc, copper, and manganese in Table 2. Honey samples were categorized as per World Health Organization (1996). In the study

Table 2: Trace mineral element composition in honey samples collected from various villages.

Honey sample number	Iron (ppm)	Zinc (ppm)	Copper (ppm)	Manganese (ppm)
HS-Rai-01	0.54[a]±0.01	0.12[a]±0.01	ND	ND
HS-Ait-02	0.78[a]±0.01	0.62[ab]±0.10	ND	ND
HS-Mar-03	1.45[ab]±0.38	0.64[acg]±0.12	0.007[a]±0.0006	ND
HS-Gaj-04	1.17[ac]±0.19	2.20±0.26	ND	ND
HS-Kaf-05	0.553[a]±0.13	2.85±0.31	0.012[a]±0.0009	0.00116[a]±0.0001
HS-Mus-06	1.59[abcd]±0.23	1.36[d]±0.37	0.008[a]±0.001	ND
HS-Mus-07	1.167[d]±0.35	0.75[aef]±0.03	0.013[a]±0.01	0.0046[a]±0.001
HS-Kho-08	1.78[be]±0.16	3.68[f]±0.07	0.237±0.04	ND
HS-Kho-09	2.70[f]±0.2	1.14[cdf]±0.2	ND	0.013[a]±0.002
HS-Bal-10	1.46[de]±0.4	1.09[bdeg]±0.07	ND	0.025[a]±0.003
HS-Bal-11	0.741[a]±0.2	0.021[a]±0.001	0.030[a]±0.008	0.92±0.05
HS-Bha-12	3.31±0.2	0.86[ad]±0.07	0.0053[a]±0.001	0.0038[a]±0.0001
HS-Mar-13	4.20±0.26	5.35[h]±0.13	0.311[bc]±0.02	0.006[a]±0.0002
HS-Mar-14	0.62[a]±0.25	0.50[a]±0.08	0.0019[a]±0.0009	ND
HS-Cha-15	0.517[a]±0.1	.18[a]±0.015	ND	0.004[a]±0.0005
HS-Kot-16	5.15[g]±0.13	3.67[f]±0.2	0.324[b]±0.02	ND
HS-Kot-17	0.922[a]±0.24	0.13[a]±0.03	ND	ND
HS-Gwa-18	2.41[f]±0.4	5.24[h]±0.2	0.291[c]±0.007	ND
HS-Gwa-19	1.46[ade]±0.2	0.98[ad]±0.2	ND	ND
HS-Gwa-20	0.68[a]±0.05	0.03[a]±0.004	0.0074[a]±0.0007	ND
HS-Khi-21	7.60±0.84	4.30±0.26	0.32[b]±0.02	0.005[a]±0.0007
HS-Tha-22	1.12[ad]±0.11	1.20[dei]±0.18	ND	0.001[a]±0.0004
HS-Min-23	4.92[f]±0.9	1.32[d]±0.28	ND	0.002[a]±0.0007
HS-Min-24	0.67[a]±0.07	0.76[afgi]±1.02	0.003[a]±0.0004	ND
Average	**1.98±1.81**	**1.62±1.64**	**.066±0.12**	**.041±0.18**

*ND= Not detected

potassium content ranged from 2.66 (Sample, 23) to 15.74 (Sample, 16) with an average of 10.93 ppm. Sodium varied from 0.37 (Sample, 3) to 8.8 (Sample, 22) with an average of 3.8 ppm. Calcium content of honey fluctuated from 0.49 (Sample, 2) to 9.98 (Sample, 12) with an average of 4.22 ppm. Magnesium content of honey differed from 0.01 (Sample, 17) to 2.86 (Sample, 8), with an average of 0.98 ppm.

The iron content of honey varied from 0.54 (Sample, 1) to 7.6 (Sample 21), with an average of 1.98 ppm. Zinc content of honey differed from 0.02 (Sample 11) to 5.35 (Sample 13) with an average of 1.62 ppm. Copper content varied from 0.001 (Sample 14) to 0.32 (Sample 21) with an average of 0.066 ppm and manganese content of honey ranged from 0.001 (Sample 22) to 0.92 (Sample 11) with an average of 0.041 ppm.

One way ANOVA establish that element significantly differed in mean content of analyzed samples and Pearson's correlation (Table 3) was used to find a possible correlation between the contents of analyzed samples.

4. Discussion

Study demonstrated that potassium was the most abundant component with an average of 10.93±3.8 ppm. Various workers (Gonzalez-Miret *et al*., 2005; Rehman *et al*., 2008; Adenekan *et al*., 2014; Ummi and Jimoh, 2015) also reported potassium as the most abundant mineral which highly depends on the botanical origin, extraction strategy and on storage conditions (Balasubramanyam and Reddy, 2003). In the present study, the potassium content was found in accordance with Adenekan *et al*. (2014) who found 8.08

ppm potassium in honey. Dominant nature of potassium in honey makes it more beneficial for consumption by humans especially for children as potassium regulates acid-alkaline balance in the blood and is involved in the transmission of nerve impulses, activates the functions of several enzymes and the muscular function of the heart. It also has a positive effect on the function of skin and kidneys (Adenekan *et al*., 2014). We found that the potassium is positively correlated with calcium while negatively correlated with manganese (Table 3). The second most abundant element in the study was calcium with an average of 4.22±2.76 ppm, similar to findings reported by Almeida-Anacleto and Marchini (2004). Sodium content with an average of 3.8±2.09 ppm was found in the study, similar value was also reported by Sahney and Kumar (2017) they observed sodium content of honey ranging from 4.77±3.31 to 7.714±0.025 ppm from Allahabad, India. The levels of magnesium 0.98±0.7 ppm during the study was lowest as comparable to reported by Liberato *et al*. (2013) who reported 13.53 ppm magnesium content in Brazilian honey. Calcium and Magnesium were found to be positively correlated with iron, zinc and copper, while sodium was found to be positively correlated with calcium (Table 3).

Essential trace element, iron content with an average of 1.98±1.81 ppm, was found in line with earlier report by Liberato *et al*. (2013) who reported 1.58 ppm iron in honey from Brazil. Iron was found to be positively correlated with zinc and copper (Table 3). During the study, zinc 1.62±1.64 ppm was found higher than <0.01 ppm lower limit set by European Commission (2002) and Codex Alimentarius (2001) and also quite higher than earlier report by Laberato *et al*., (2013) who reported 0.56 ppm zinc content in honey. Table 3 also depicts that a good correlation (r=0.889) exists between zinc and copper, similar findings were found previously by Tosic *et al*. (2017) who found r=0.777 between zinc and copper and concluded that these metals are naturally present in the soil. During study copper content 0.066±0.12 ppm was lower than the earlier report by Laberato *et al*. (2013) who reported 0.43 ppm followed by manganese with an average of 0.041±0.18 ppm. Manganese is a heavy metal and an essential micronutrient (Sarwar *et al*., 2010), its lower limit proves honey is of good quality and safe for consumption. Zinc is essential in our diet, as it has direct impact on digestion but in its adorable limit if it exceeds in food it result into toxic effects. The study revealed that all samples were of good quality and safe for human consumption.

Most of analyzed samples showed significant difference ($p<0.05$) with each other while various samples showed non-significant difference, are marked with same letters (Table 1-2).

The correlation coefficient (Table 3) showed positive and significant (always lower than $P<0.05$ or 0.01 level) correlation between variables. In overall, copper showed high and positive correlation with zinc (r=0.884) as compared to others while least and negative correlation with sodium which was -0.007.

5. Conclusions

Present study concludes that honey samples of *Apis cerana-indica* compares favourably with earlier authors around the world and coincide with the lower limit of international standards of mineral contents. We also conclude that *Apis cerana-indica* honey is dominant in potassium content which enhances the nutritional quality of honey for medicinal and other purposes.

Table 3: The values of correlation coefficient (r) between investigated essential and trace mineral elements.

Mineral elements	Potassium	Sodium	Calcium	Magnesium	Iron	Zinc	Copper	Manganese
Potassium	1	0.410**	0.506**	0.005	0.207	0.261*	0.366**	−0.442**
Sodium		1	0.271*	−0.095	0.189	−0.078	−0.007	−0.207
Calcium			1	0.166	0.391**	0.449**	0.488**	−0.142
Magnesium				1	0.472**	0.601**	0.660**	0.147
Iron					1	0.597**	0.675**	−0.139
Zinc						1	0.884**	−0.206
Copper							1	−0.065
Manganese								1

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

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