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POLLEN ANALYSIS AND HEAVY METALS DETECTION IN HONEY SAMPLES FROM SEVEN SELECTED COUNTRIES

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Abstract

Honey contains pollen grains derived from the foraging activity of honeybees that reflect the environmental location of the beehives. The variability of honey types produced in a region depends upon the diversity of nectar sources present in the region. Microscopic analysis of the pollen in honey was used to determine its geographical origin. This study describes a simplified method for determining the total amount of pollen grains and the relative frequencies of pollen from various plant sources in honey. In 7 commercially obtained honey samples from Austria, Pakistan, Canada, Germany, Australia, Saudi Arabia and America. Pollen of plant species from 12 families: Brassicaceae, Sapindaceae, Cannabaceae, Convolvulaceae, Myrtaceae, Pinaceae, Cupressaceae, Asteraceae, Moraceae, Fabaceae, Corylaceae and Loranthaceae were identified. In addition to pollen identification, contamination due to heavy metals and their concentration in honey samples were also measured and the results are discussed in this paper.

Introduction

Honey is a natural sweet substance and is produced by honeybees from the nectar of blossoms, from secretion of living parts of plants. Honeybees collect this material, transform and combine it with specific substances of their own, store and leave in the honey comb to ripen and mature (White & Landis, 1980). Freshly extracted honey is a viscous liquid, has a greater density (1.5g/cm3) than water (1g/cm3 at 4ºc), having a strong hygroscopic character, relatively low heat conductivity, low surface tension and various colours that are basically all nuances of yellow amber (Jusbin, 1996). The various chemical components of honey include: carbohydrates that comprise the major portion of honey-about 82% (Hak-Gil *et al*., 1988), and proteins that include a number of enzymes, and eighteen free amino acids, a carboxylic acid group, of which most abundant is proline (White *et al*., 1962). Honey has been claimed (Abdulla & Abdulaziz, 1998) to have therapeutic properties (exhibiting healing powers) in the treatment of digestive, respiratory, cardiac and rheumatic disorders. Several studies have reported honey's immunological, antibacterial, anti-inflammatory, antipyretic properties besides its importance in terms of energy intake. Furthermore, honey has been proved to possess wound healing and analgesic actions (Jusbin, 1996; Abdulla & Abdulaziz, 1998; Pereira *et al*., 1998).

There are four natural resources required by honeybees for survival: water, resin, nectar, and pollen (Seedley 1985). Water is used to cool hive and to dilute honey fed to larvae. Resin is utilized to reinforce hive, seal off decaying wood, and plug up holes. Nectar is the major source of carbohydrates from which honeybees obtain their energy. It is collected by foraging worker bees and is carried back to hive in their honey stomachs. Nectar is usually transferred to hive workers for processing into honey and it can be fed directly to the brood or to adults (Winston, 1987). During nectar gathering, a honeybee consumes 0.5 mg of ripe honey per kilometer of flight. Feeding a bee larva from egg to maturity requires about 142 mg of honey (Winston, 1987).

Pollen is bee’s major source of proteins, fatty substances, minerals, and vitamins. It is essential for the growth of larvae and young adult bees. Honeybees remove pollen from an anther by using their tongue and mandibles. While crawling over flowers, pollen adheres to their "hairy" legs and body. The honeybee combs pollen from her head, body, and forward appendages, mixes it with pollen from her mouth, and transfers it to the corbicula, or "pollen basket", on her posterior pair of legs. When "loaded" with pollen, she will return to her hive. Once at hive, workers pack pollen into the comb. To prevent bacterial growth and delay pollen germination, phytocidal acid is added to the pollen as it is packed into the comb. Other enzymes produced by worker bees are also added which prevent anaerobic metabolism and fermentation thereby enhancing longevity of the stored pollen. Once completely processed for storage, the pollen comb is referred to as "bee bread" (Alfonsus, 1933; Haydak, 1935). Airborne pollen is another potential source of pollen in honey. Sometimes airborne pollen is deposited into ripened honey when it is being removed from a hive by beekeeper.

Pollens are the essential tools in the analyses of honey. Different type of pollens are used to indicate floral nectar sources utilized by bees to produce honey, relative pollen frequency is often used to verify and label a honey sample as to the major and minor nectar sources. This information has important commercial value because honey made from some plants commands a premium price. Even non-premium grades of honey require certain types of verification because they must be correctly labeled before marketed. Identifying and quantifying the pollen in honey samples is one of the best ways to determine the range of nectar types used to produce a honey, and therefore label correctly, based on actual foraging resources. Another reason that pollen analyses of honey are often required is to identify geographical source of origin (Vaughn & Bryant, 2001).

The pollens from the combination of wind and insect-pollinated taxa found in a honey sample will often produce a pollen spectrum that is unique for the specific geographical region where it was produced. Because of trade agreements, import tariffs, and legal trade restrictions, most of the leading honey-producing nations of the world require accurate labeling of honey before it can be sold. This is especially true for the European Economic Union that has strict labeling regulations for honey products since 1974 (Anon., 2001).

Honey possesses numerous nutritional, healing, and prophylactic (preventative) properties. These are a direct consequence of its chemical composition.

In order to have a beneficial effect honey must be free of any contaminating agents, any contaminants such as heavy metals present in honey above the admitted levels by pollution standards, are threats to human health. 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 (McKee, 2003).

The detection of compounds, not previously found in honey imports by the European Union has generated increased control of residues that might be present in honeys (Danty, 2003). 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. Several studies have indicated that bees and their products may be used as biological indicators of the environmental pollution present in the area where they fly (Fernández *et al*., 1994; Buldini *et al*., 2001; Bogdanov *et al*., 2003; Celli & Maccagnani, 2003).

Therefore it is important to take into account the type of equipment used to produce honey as well as the quality of the equipment used to store honey after harvesting as the possible sources of honey contamination with heavy metals. Contact with stainless steel surfaces during harvesting, processing and/or preparation of honey for the market, can generate high Cr content, due to the corrosive effect of honey acidity. Likewise, storing honey in galvanized containers can be a source of Zn contamination (González *et al*., 2000; Bogdanov *et al*., 2003).

This study was carried out to identify and measure the concentration of heavy metals present in honey samples from Pakistan, Australia, Canada, USA, Austria, Saudi Arabia and Germany. Plant species were also identified from pollen sculpturing found in honey samples, and to point out adulteration in honey from possible different sources.

Materials and Methods

Honey samples from 7 countries viz., Pakistan, Australia, Canada, USA, Austria, Saudi Arabia and Germany were purchased from the market and examined for different pollen types, their identification, and for heavy metal detection. Pure honey was brought from Soon Valley (Khushab) as reference sample.

Sample prepation: Pollen analyses of the honey samples were carried out by dilution method. Four dilutions 0.5ml, 1.0ml, 1.5ml and 2.0ml were prepared for each honey sample in 50 ml of double distilled water. Three duplicates of each dilution were examined. Slides without any dilution for each sample were also prepared. Pollen grains were stained with 1% safaranine mixed in glycerine jelly (Erdtman, 1956).

Atomic absorption spectroscopy: Atomic absorption spectrophotometer (AAS) Model VARIAN spectra AA240 was used to determine heavy metals concentration. The instrument was calibrated and standardized with different working standards. After making sure that the instrument was properly calibrated and results of standards were in the confidence limit, concentration of metals in each sample was measured individually.

Results

All the available data about pollens, concentration of heavy metals in honey were analyzed and documented. Results are presented in Tables 1-3 and Fig. 1.

Pollen analysis in honey: Pollens grains of 15 plant species belonging to 12 families were identified in 7 samples of honey from different countries.

Pollen spectra of honeys revealed a variety of not only nectariferous but also nectarless sources available to bees and included taxa whose presence indicated their origin. The amount and diversity of pollen present in honey is usually related to vegetation, climate and geographical location of beehive. The pollen composition of the honeys studied revealed important information on the flora of that region. *Eucalyptus fibrosa* pollens were most frequently observed in Saudi Arabian, Australian, Germans, Austrian and in Pakistani samples. *Pinus roxburghii* pollens were present in Austrian and Pakistani samples. *Albizia julibrissin* pollens were identified in Saudi Arabian and Austrian honey samples. *Raphanus raphanistrum* pollens were present in Saudi Arabian and Austrian samples. *Broussonetia papyrifera* pollens were seen only in Pakistani sample. *Ipomoea indica* pollens were observed in Germans and Canadian samples. *Amyema miquelii* pollens were present in Australian and Germans honey**.** *Dodonea viscosa* pollens were identified only in Germany honey. *Corylus avellana* pollens were found in Germans Australian samples. *Melilotus indica* pollens were seen in Germans Australian and Saudi Arabian honey samples. *Calendula arvensis* and *Xanthium strumarium* pollens were present only in Saudi Arabian honey samples. *Eucalyptus fibrosa, Melilotus indica* and *Corylus avellana* pollens were present in higher concentration. *Brassica rapa* pollens were identified only in Canadian honey sample.

The results of this study indicate that iron was on highest concentration followed by zinc, lead, copper, manganese and cobalt. The lowest iron concentration was found as 4.345 ppm in honey sample from Australia and the highest concentration was found as 7.433 ppm in honey sample from Pakistan.

The lowest manganese level was 0.064 ppm in honey sample from America and the highest manganese level was 0.140 ppm in honey sample from Germany. The lowest zinc content was found 0.137 ppm in Canadian sample and the highest zinc content 0.471 ppm was in honey sample from Germany. The minimum copper concentration observed was 0.023 ppm in honey sample from Saudi Arabia and maximum 0.498 ppm in Austrian honey sample. The lowest lead content 0.02 ppm was in Australian honey and the highest lead content was 1.81 ppm in honey sample from Saudi Arabia. Lead data of honey samples from Saudi Arabia were much higher than other six samples.

Table 1. Pollen grains of plants found in 7 honey samples.

S. No	Name of pollen	Family of pollen
1.	Raphanus raphanistrum L.	Brassicaceae
2.	*Dodonea viscosa* L.	Sapindaceae
3.	*Cannabis sativa* L.	Cannabaceae
4.	*Brassica rapa* L.	Brassicaceae
5.	*Iipomoea indica* B.	Convolvulaceae
6.	*Eucalyptus fibrosa* F. Muel.l	Myrtaceae
7.	*Pinus roxburghi* Sargent.	Pinaceae
8.	*Juniperus coahuilensis* M.	Cupressaceae
9.	*Xanthium strumarium* L.	Asteraceae
10.	*Calendula arvensis* L.	Asteraceae
11.	*Broussonetia papyrifera* (l.) Vent	Moraceae
12.	*Melilotus indica* L.	Fabaceae
13.	*Albizia julibrissin* Durazz.	Fabaceae
14.	*Corylus avellana* L.	Corylaceae
15.	*Amyema miquelii* L.	Loranthaceae

Discussion

The pollen composition of honey studied revealed important information on the flora of the region. In seven honey samples from Australia, Pakistan, Canada, Germany, Austria, Saudi Arabia, and America, pollen types of 15 plant species from 12 families: Brassicaceae, Sapindaceae, Cannabaceae, Convolvulaceae, Myrtaceae, Pinaceae, Cupressaceae, Asteraceae, Moraceae, Fabaceae, Corylaceae and Loranthaceae were identified. In American honey no pollens were observed as it was possibly a monoclonal honey, and may be the producing company had filtered it through a mesh of less than 4.5µ before marketing so that no pollen could be seen.

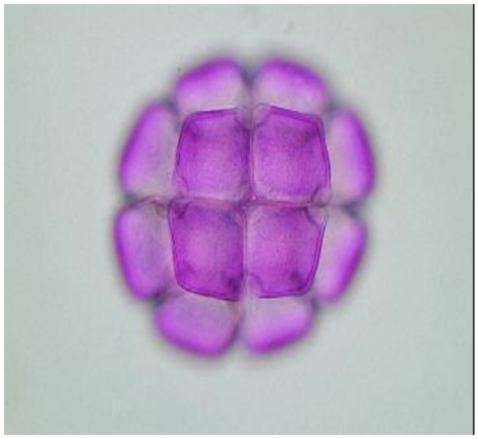
Table 2. Number of pollen grains found in different dilutions of honey samples from 7 countries belonging to different plant species.

Name of country	Plants species	Dilutions in 50 ml of double distill water				Without dilution
		0.5 ml	1.0 ml	1.5 ml	2.0 ml	
Austria (A) honey sample	*Juniperus coahuilensis*	4	10	15	26	48
	Eucalyptus fibrosa	2	4	7	12	22
	Pinus roxburghii	1	2	4	7	13
	Albizia julibrissin	1	3	4	8	14
	Raphanus raphanistrum	1	1	2	3	5
Pakistan (B) honey sample	*Broussonetia papyrifera*	0	1	3	4	9
	Pinus roxburghii	0	1	1	3	7
	Eucalyptus fibrosa	0	0	2	2	5
	Cannabis sativa	2	4	5	7	9
Canada (C) honey sample	*Brassica rapa*	0	1	3	6	8
	Iipomoea indica	0	0	0	2	5
Germany (D) honey sample	*Amyema miquelii*	5	8	14	25	37
	Dodonea viscosa	5	7	12	20	31
	Corylus avellana	0	1	2	4	6
	Melilotus indica	0	1	3	7	9
	Eucalyptus fibrosa	5	10	21	33	48
	Iipomoea indica	0	1	3	6	11
Australia (E) honey sample	*Corylus avellana*	5	11	16	23	43
	Melilotus indica	3	7	10	21	38
	Eucalyptus fibrosa	7	15	25	35	58
	Amyema miquelii	5	9	12	21	37
	Eucalyptus fibrosa	7	13	21	32	47
	Melilotus indica	9	16	27	36	49
	Albizia julibrissin	1	2	3	3	5
	Calendula arvensis	4	6	8	12	20
	Xanthium strumarium	1	3	5	8	13
	Raphanus raphanistrum	2	3	5	7	11
Saudi Arabia (F) honey sample	*Eucalyptus fibrosa*	7	13	21	32	47
	Melilotus indica	9	16	26	37	49
	Albizia julibrissin	1	2	3	3	4
	Calendula arvensis	4	6	8	12	20
	Xanthium strumarium	1	3	5	8	13
	Raphanus raphanistrum	2	3	5	7	11
USA (G) honey sample	No pollens were observed					

Table 3. Heavy metals concentration parts per million (ppm) in 7 honey samples.

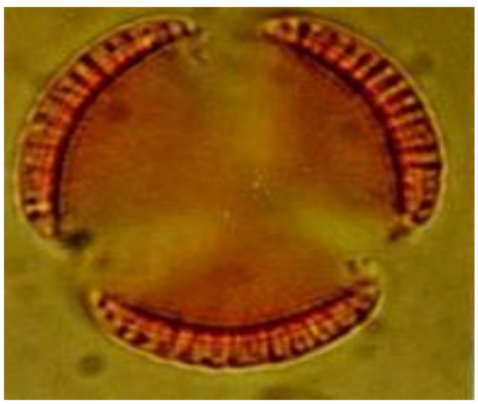
Samples	Pb	Cu	Fe	Co	Zn	Mn
A	0.28	0.498	4.717	0.007	0.2992	0.081
B	0.17	0.494	7.433	0.013	0.3011	0.075
C	0.04	0.026	5.269	0.014	0.1368	0.070
D	0.12	0.164	6.301	0.001	0.4717	0.140
E	0.02	0.033	4.345	0.018	0.2194	0.341
F	1.81	0.023	5.088	0.007	0.2110	0.086
G	0.24	0.032	4.467	0.012	0.1903	0.064

Key: A: Austria, B: Pakistan, C: Canada, D: Germany, E: Australia, F: Saudi Arabia, G: USA

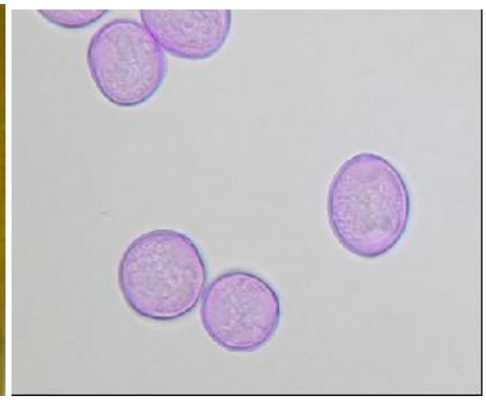


A. *Albizia julibrissin*.

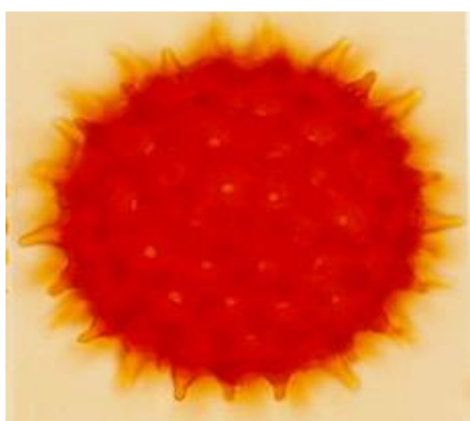
B. *Amyema miquelii*.



C. *Raphanus raphanistrum.*

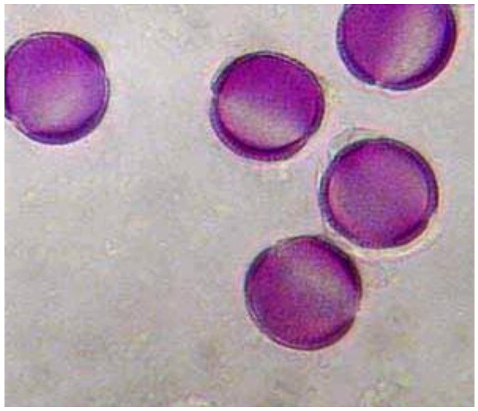


D. *Broussonetia papyrifera.*

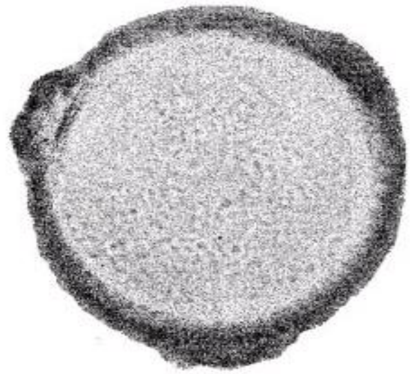


E. *Ipomoea indica*.

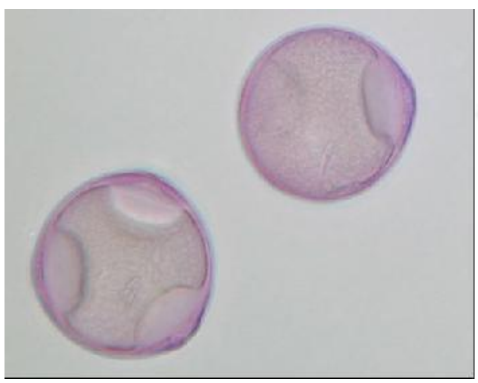
Fig. 1. Photomicrographs of pollen grains identified in honey samples.



F. *Brassica rapa*.

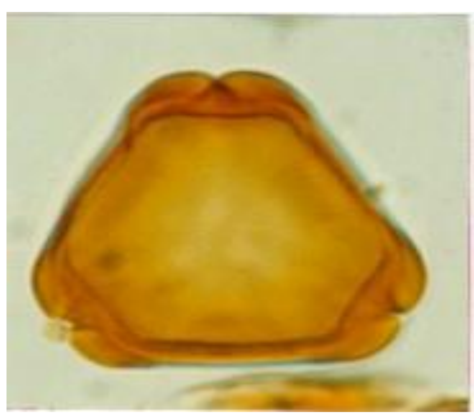


G. *Cannabis sativa*.



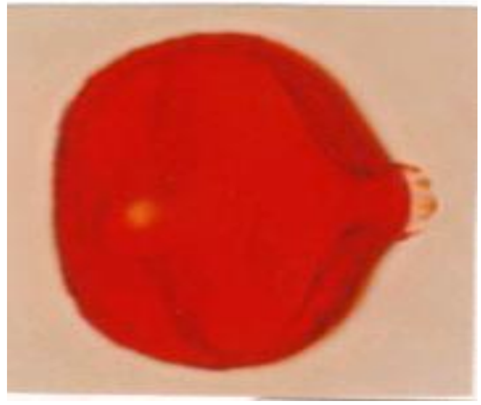
H. *Corylus avellana*.

I. *Dodonea viscosa*.

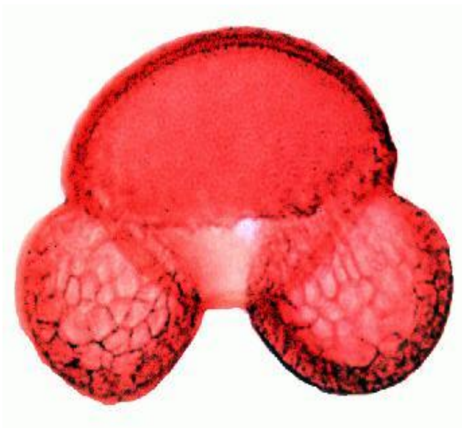


J. *Eucalyptus fibrosa*.

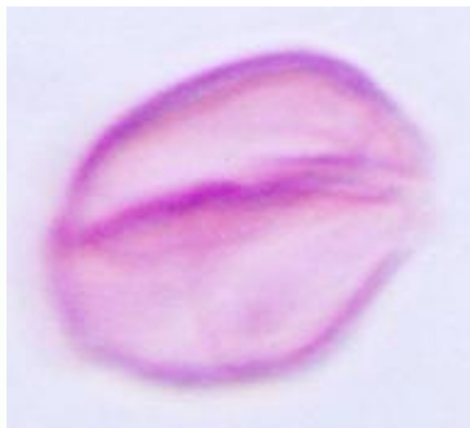
K. *Juniperus coahuilensis*.



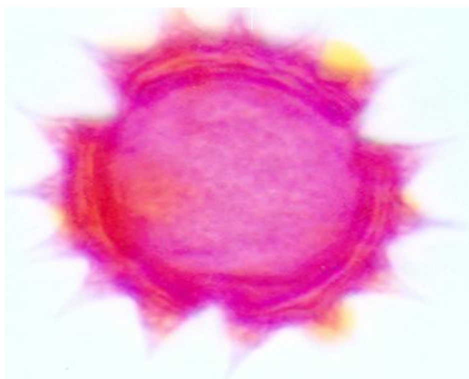
L. *Melilotus indica*.



M. *Pinus roxburghii*.



N. *Xanthium strumarium*



O. *Calendula arvensis*.

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies *via* food, drinking water and air as trace elements, some heavy metals (e.g., copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, when present at higher concentrations can lead to poisoning.

Heavy metals were analyzed and measured by atomic absorption spectrophotometer. The results of this study indicate that iron had highest concentration followed by zinc, lead, copper, manganese and cobalt but these concentrations were not very high except concentration of lead which was highest in Saudi Arabian honey sample. The possible reason could be due to the higher concentration of lead in the air from oil extraction and vehicular exhaust emissions. Moisture content in honey increases due to hygroscopic nature of honey and moisture from surrounding atmosphere is absorbed as a source of pollution.

This study point out some possible sources of honey pollution of heavy metals and identifies 15 pollen types present in different honey samples and indicates regional vegetation. Further studies should be undertaken to help in finding out possible sources of heavy metal pollution and vegetation of the area from where the honey originated.

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