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Bioaccumulation and analysis of heavy metals, minerals, and trace elements in the soil, pollen, nectar, and honey samples from Majha region, Punjab, India

Vishu Verma, Randeep Singh and Vijay Kumar

Abstract
Heavy metals are taken into consideration as a few of the most crucial and potentially harmful pollutants. Eleven heavy metals (Se, Zn, Cd, Ca, Fe, Cu, Ni, Pb, K, Mn, and Na) were determined in soil, pollen, nectar, and honey samples collected from four districts (Amritsar, Gurdaspur, Pathankot, and Tarn Taran Sahib) of Majha Region, Punjab, India. Metal concentrations were measured using a microwave plasma-atomic emission spectrometer (Agilent 4200 MP-AES). The measurement of heavy metals in honey is highly relevant for both quality assurance and dietary purposes. The findings showed that honey from contaminated areas contained more heavy metals than honey from clean areas. This has to do with the pollution that is produced by industrial activity in contaminated areas. Heavy metals can possibly be hazardous to pollinator species that depend on these plants for nectar and pollen, in addition to having an adverse effect on plant productivity and survival. As a result, heavy metals have a significant negative impact on forager bee survival, brood growth, and species diversity. The present study concluded that the honey bee (*Apis mellifera*), which reflects the presence and amount of heavy metals in plants near apiary sites, is a useful marker for monitoring environmental pollution with heavy metals. Additionally, the bioaccumulation factor (BAF) of heavy metals was estimated in consideration of soil to pollen, soil to nectar, and nectar to honey. The accumulation of Zn, Ca, and Cu in honey is high due to water pollution, and forager bees collecting water leads to an increase in the concentration of these metals in honey. The bioaccumulation factor of Cd was not calculated due to samples below the detection limit (BDL). Comparing the studied heavy metal concentration with the literature, we concluded that local environmental factors influence the difference in metal concentration.

Keywords: Bioaccumulation, heavy metals, honey, honey bee, microwave plasma-atomic emission spectrometer

1. Introduction

The practise of managing honey bee colonies in order to attain the desired objectives is known as beekeeping. Traditionally, four species of honey bee are recognised within the genus *Apis* (Lindauer, 1952), including the eastern honey bee, *Apis cerana* (Fabricius, 1793); the dwarf honey bee, *Apis florae* (Fabricius, 1787); the gaint honey bee, *Apis dorsata* (Fabricius, 1793); and the western honey bee, *Apis mellifera* (Linnaeus, 1758). Honeybees (*Apis mellifera*) are among the most important living organisms that are affected by environmental conditions and have a great ability to sense environmental changes; they are considered a biological indicator of many toxic environmental factors that exist in nature (Celli *et al*., 2003) [8]. Honeybees may come into contact with the metal mostly when foraging for nectar and pollen from plants where the heavy metal had previously accumulated and in water sources. (Porrini *et al*., 2003) [19].

Heavy metals and metalloids can accumulate in soil and contaminate it through atmospheric deposition, land application of fertilisers, animal manures, sewage sludge, pesticides, emissions from rapidly expanding industrial areas, and disposal of high-metal wastes (Maurya *et al*., 2018) [17]. Minerals originate in the soil, are transported into plants through the roots, and get into honey via nectar, although they may also come from anthropogenic sources, such as environmental pollution, or be influenced by beekeeping practises and honey processing (Bogdanov *et al*., 2008) [3]. Honey bee accretions are related to air, water, and soil; they go from flower to flower, touch branches and leaves, drink water from pools, and their hairy bodies collect aerosol particles.

Pollens are essential tools in the analysis of honey. Different types of pollen were used to indicate floral nectar sources utilised by bees to produce honey.

Honeybees use their tongue and mandibles to remove pollen from an anther. Pollen attaches to their hairy bodies and legs as they crawl across flowers (Bibi *et al*., 2008) [2].

The main source of carbohydrates for honeybees to get their energy from is nectar. It is collected by foraging worker bees and carried back to the 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) [25].

Honey is a naturally sweet material that honey bees make from the nectar of flowering plants and the secretions of live plant components. Honeybees gather this material, alter it by combining it with particular substances of their own, then store it and allow it to develop in the honeycomb (White and Landis, 1980) [24]. Honey bees (*Apis mellifera*) forage on a wide range of plants and water sources in different places, and therefore they strongly interact with the environment around the hive. Due to this characteristic, it was suggested that honeybees, honey, and other associated products could serve as possible bio-indicators to track pollution near the hive (Devillers and Pham-Delegue, 2002) [11].

According to the United States Environmental Protection Agency and the International Agency for Research on Cancer (IARC), heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are also classified as known or probable human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans (Paul *et al*., 2012) [18]. The main objective of this study was to determine the heavy metal pollution in soil, pollen, nectar, and honey samples as well as the bioaccumulation factor of those pollutants.

2. Materials and Methods

2.1. Sampling

Sampling of soil, pollen, nectar, and honey was done in four districts belonging to the Majha region of Punjab. Soil samples were collected from the fields with flora and were taken at a depth of 15 cm. Samples of fresh pollen were collected with the help of pollen traps. Approaching to meet the sedentary beekeepers and honey samples were collected from the hives randomly.

Nectar and honey samples were collected directly from beehive colonies and transferred freshly in plastic centrifugal tubes of 15 ml. Unwanted materials such as wax, dead bees, and comb pieces were removed by straining the samples through cloth before analysis.

2.2. Reagents and Chemicals

All chemicals and reagents used in the current study were of analytical grade. Concentrated 69.72% nitric acid (HNO_3) and concentrated 70% per chloric acid ($HClO_4$) were used for the preparation of the di-acid mixture (4:1). Deionized water (chemically pure with conductivity 1.5 µs/cm and below) was used for dilution of sample and intermediate metal standard solutions prior to analysis and rinsing glassware and sample bottles.

2.3. Equipment's and Apparatus

An electronic balance, 100 ml round-bottomed flasks, culture tubes, volumetric flasks (25 ml, 50 ml, 100 ml, 250 ml, and 1000 ml), measuring cylinders, digestion flasks (250 ml), culture tubes (100 ml), filter paper, Whattman No. 1, and other glassware were used for this experiment.

2.4. Procedure for sample preparation by acid digestion method

The samples collected from the different locations were analysed. Dry material was taken in a 250 ml digestion flask. Weigh 1g for honey samples over the analytical balance. 15 ml of a diacid mixture ($HNO_3:HClO_4 = 4:1$) was added to it. The mixture was digested in a digestion chamber (a fume hood chamber) for 2-3 minutes until it became colourless. Evaporate until yellow or red fumes with an acrid odour first appear, and then white, dense fumes of perchloric acid appear. The contents were diluted to about 30 ml with distilled water, filtered through Whatman filter paper No. 1, and transferred to a 50 ml volumetric flask for a final volume of 50 ml with distilled water in a measuring cylinder. The digested sample is then transferred to a 60 ml culture tube and marked with the sampling number. For further analysis of heavy metal concentrations, were done with microwave plasma-atomic emission spectrometer (MP-AES 4200).

2.5. Statistical Analysis

The analysis of chemical characteristics and heavy metal content of different honey samples was done in triplicate, and the data are presented as mean±S.D and calculated by using Microsoft Excel 2021. One way ANOVA followed by Tukey's HSD test as post hoc was used to compare the means of chemical properties in different types of honey among the studied sites. Differences at $p<0.05$ were considered statistically significant. Statistical analysis was done with the help of ICAR WASP 1.0 and Minitab version 19 (Pennsylvania, USA) computer software programmes.

(a) (b) (c) (d)

Fig 1: (a) Soil sample (b) Pollen sample (c) Collection of fresh nectar and honey from bee hive frames (d) Honey sampling (e) Collection of pollen with the help of pollen traps (f) Heavy metals detection by using microwave plasma atomic emission spectroscopy (MP-AES 4200) (g) Hot plate used for heating the samples (h) Electronic balance used for weighing samples (i) Fume hood used for sample digestion (j) Nectar sample (k) Sample digestion in culture tube.

Table 1: List of sampling site and GPS coordinates with surrounding flora

Sr. No.	Sampling site in Punjab according to district wise	Village of sampling	Longitude and latitude	Surrounding Flora
1.	Amritsar	Mattewal (MT)	31°41`11.3``N 75°08`43.5``E	*Brassica* spp.
		Dharmu Chak (DC)	31°39`03.1``N 75°11`36.6``E	*Brassica* spp.
2.	Gurdaspur	Aliwal Araian (AA)	31°49`45.2``N 75°07`12.2``E	*Brassica* spp.
		Kala Nangal (KN)	31°50`60.0``N 75⁰12`57.6``E	*Brassica* spp.
3.	Pathankot	Kiri Khurd (KK)	32°15`59.37`N 75°30`03.6``E	*Litchi chinensis* *Mangifera indica*
		Kotli Muglan (KM)	32°14`06.3``N 75°33`27.5``E	*Litchi chinensis* *Mangifera indica*
4.	Tarn Taran Sahib	Kot Dharm (KD)	31°28`13.9``N 74°51`06.1``E	*Brassica* spp.
		Amarkot (AK)	31°12`53.3``N 74°37`10.2``E	*Brassica* spp.

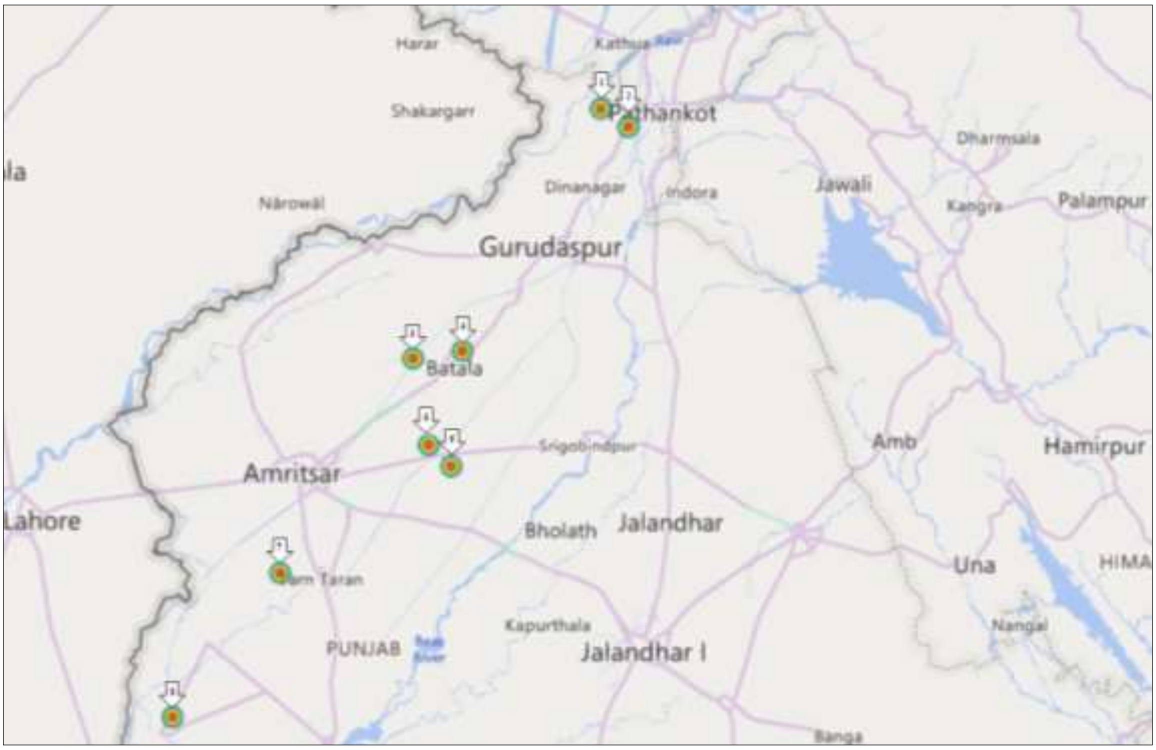


Fig 2: Sampling sites of different districts of Majha Region, Punjab. (1: Kiri Khurd, 2: Kotli Muglan, 3: Aliwal Araian, 4: Kala Nangal, 5: Mattewal, 6: Dharmu Chak, 7: Kot Dharm, 8: Amarkot)

3. Results and Discussion

Heavy metals Se, Zn, Cd, Ca, Fe, Cu, Ni, Pb, K, Mn, and Na were analysed in soil, pollen, nectar, and honey samples by using a microwave plasma-atomic emission spectrometer. The results obtained after thorough analysis showed the presence of heavy metal content in honey samples within permissible limits. The bioaccumulation factor of Cd was not calculated due to samples below the detection limit (BDL). The accumulation of Zn, Ca, and Cu in honey is high due to water pollution, and forager bees collecting water leads to an increase in the concentration of these metals in honey.

3.1. Bioaccumulation factor (BAF) of heavy metals, minerals, and trace elements in the soil, pollen, nectar, and honey samples

The bioaccumulation factor for each element, BAF_{SP}– (soil to pollen), BAF_{SN}– (soil to nectar) and BAF_{NH}– (nectar to honey), was calculated in the present study.

The bioaccumulation factor (BAF) of heavy metals was also calculated w.r.t. soil to pollen, soil to nectar, and nectar to honey. The values of bioaccumulation factors from soil to pollen ranged from 1.13–3.13, 0.73–2.27, 0.85–1.65; 0.40–2.19; 0.75–2.00; 0.47–1.55; 0.66–1.50; 0.64–1.29; 0.71–1.33; and 0.47–0.71 for Se, Zn, Ca, Fe, Cu, Ni, Pb, K, Mn, and Na, respectively. On the other hand, the values of bioaccumulation factors from soil to nectar ranged from 0.40–0.73, 0.73–2.13, 0.98–1.53, 0.51–1.36, 0.75–1.75, 0.38–1.22, 0.50–1.00, 0.34–0.75, 0.57–1.33, and 0.54–1.35 for Se, Zn, Ca, Fe, Cu, Ni, Pb, K, Mn, and Na, respectively, and the values of bioaccumulation factors from nectar to honey ranged from 0.16–1.02, 0.56–1.57, 0.95–1.47, 0.45–1.27, 0.75–1.50, 0.58–1.57, 0.66–2.00, 0.60–1.26, 0.66–2.00, and 0.68–1.23 for Se, Zn, Ca, Fe, Cu, Ni, Pb, K, Mn, and Na, respectively, The bioaccumulation factor of Cd from soil to pollen, soil to nectar, and nectar to honey was not calculated due to below-detection limits (BDL) in soil, pollen, nectar, and honey samples.

3.2. Occurrence of heavy metals, minerals, and trace elements in the honey samples

Selenium was found in almost all the honey samples, with the maximum value observed in Kotli Muglan (0.39±0.02 mg/kg) and the minimum value at Kala Nangal (0.03±0.05 mg/kg). In honey bees, selenium content in the bee body depends on Se concentrations in plants and nectar. Its impacts on survival and foraging behaviour could drastically lower the bee colony's productivity and longevity, which would decrease pollination effectiveness (Hladun *et al*., 2013) [16]. The highest content of iron in honey samples was observed at Dharmu Chak (2.58±0.93 mg/kg), and the minimum content was observed at Mattewal (1.03±0.14 mg/kg). Secondary oxides that have been absorbed or precipitated into soil as mineral particles and iron-organic matter complexes are the main sources of iron in soils that are utilised by plants. Fe exists in two oxidation states: ferrous iron (Fe^{2+}), which is reduced, and ferric iron (Fe^{3+}), which is oxidised (Guerinot and Kim, 2007) [13].

Non-significant variations in the concentrations of Zn, Ca, Cu, Ni, Pb, K, Mn, and Na were observed in honey samples. Zn concentrations, reduced weight gain, and a prolonged larval period resulted in a significantly lower relative growth rate (RGR), which indicated surviving insects may allocate more energy from foods for detoxification than for growth (Zhan *et al*., 2020) [26]. Calcium is present in soil in various forms, *viz*., mineral particles, $CaCO_3$, simple salts, exchangeable calcium, etc. The amendment of lime (Ca $(OH)_2$) is one of the major sources of Ca in soils used to reclaim soils with a pH less than 5.5 (Gupta and Abrol, 1990) [14]. Iron-organic matter complexes and secondary oxides that have been absorbed or precipitated in the soil as mineral particles are the main sources of iron in soils that are utilised by plants. Fe can exist in either its reduced form as ferrous iron (Fe^{2+}) or its oxidised form as ferric iron (Fe^{3+}) (Guerinot and Kim, 2007) [13]. Copper is added to agricultural soils via manure applications, sewage sludge, mineral fertilisers, and pesticides. Cu occurs in manure through animal feed, like roughage, concentrate, and especially Cu-containing additives (Hayat *et al*., 2021) [15].

The major sources of nickel in the environment are industrial waste materials, lime fertiliser, and sewage sludge. Ni enters the soil mainly through anthropogenic activities such as mining, smelting, and the application of some organic amendments (Cempel, 2006) [9]. The major sources of Pb are the metal smelting and storage battery manufacturing industries. Ingestion of polluted soil is the most dangerous way to be exposed to soil lead. Generally speaking, plants don't take up or store lead. Leafy vegetables and root crops are more likely to have higher concentrations (Tiwari *et al*., 2013) [22]. As indicators and members of the food chain, lead concentrations in insects were studied for bioaccumulation (Zhuang *et al*., 2009) [27]. Lead enrichment has been recorded in the food chain of insect herbivores to their predator (the ladybird beetle, *Coccinella septempunctata*) (Butt *et al*., 2018) [5]. In the soil, the principal sources of K are minerals such as feldspars (particularly orthoclase) and micas, which release potassium in the weathering process. Application of potassium fertilisers also increases the concentration in the soil (Bertsch and Thomas, 1985) [1]. Manganese plays an important role in physiological processes such as photosynthesis and the detoxification of superoxide free radicals in plants. Manganese sulphate (MnSO4) is highly water-soluble and suited for soil or foliar application in Mn-deficient soils (Rashed *et al*., 2019) [21]. Sodium is a beneficial element for plants and is required for several vital functions, but high levels of Na are detrimental to plant growth. The majority of Na pockets in soil are formed by the concentrated runoff of pesticides, fertilisers, and other soil amendments (Choudhary and Kharche, 2018) [10].

Table 2: Occurrence of heavy metals, minerals and trace elements in soil samples from different sites of Majha Region, Punjab

Sr. No.	Elements	Amritsar		Gurdaspur		Pathankot		Tarn Taran Sahib		F_{Cal}	HSD	Permissible Limit (WHO)
		Mattewal	Dharmu Chak	Aliwal Araian	Kala Nangal	Kiri Khurd	Kotli Muglan	Kot Dharm	Amarkot			
1.	Se	0.88^{c}± 0.08	0.72^{cd}±0.12	0.57^{de}±0.05	0.44^{e}±0.21	0.73^{cd}±0.18	1.17^{b}±0.23	0.88^{d}±0.13	0.77^{d}±0.12	23.77*	0.24	-
2.	Zn	0.16±0.04	0.15±0.01	0.15±0.04	0.14±0.02	0.19±0.05	0.18±0.01	0.11±0.07	0.20±0.10	1.48^{NS}	-	0.60
3.	Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	0.02
4.	Ca	20.36^{a}±1.27	19.91^{a}±0.69	19.11^{a}±0.08	20.36^{a}±1.10	20.63^{a}±0.54	20.25^{a}±0.09	13.46^{b}±6.63	21.64^{a}±0.41	3.21*	4.22	-
5.	Fe	2.86^{ab}±1.20	3.05^{ab}±0.32	3.79^{a}±0.78	2.81^{abc}±0.41	1.77^{cd}±0.36	2.09^{bcd}±0.28	1.27^{d}±0.92	2.12^{bcd}±0.15	5.14*	1.06	-
6.	Cu	0.08±0.01	0.08±0.01	0.08±0.01	0.07±0.01	0.07±0.02	0.07±0.00	0.04±0.03	0.08±0.01	1.95^{NS}	-	10.0
7.	Ni	0.12±0.04	0.11±0.04	0.16±0.12	0.09±0.04	0.17±0.02	0.17±0.10	0.18±0.02	0.12±0.02	0.75^{NS}	-	10.0
8.	Pb	0.03±0.01	0.03±0.00	0.03±0.00	0.02±0.01	0.03±0.00	0.03±0.01	0.02±0.01	0.04±0.00	1.77^{NS}	-	2.00
9.	K	20.32^{a}±3.83	21.62^{a}±3.59	21.58^{a}±3.52	18.51^{ab}±3.77	17.10^{abc}±3.21	14.26^{bc}±2.03	11.93^{c}±4.63	18.19^{ab}±1.74	3.05*	5.90	-
10.	Mn	0.06±0.01	0.06±0.01	0.07±0.01	0.05±0.01	0.05±0.01	0.05±0.00	0.03±0.02	0.06±0.00	1.78^{NS}	-	-
11.	Na	7.03±0.32	6.61±1.21	7.08±0.45	5.59±1.79	7.56±0.51	7.50±0.57	7.71±0.81	8.40±0.57	2.55^{NS}	-	-

Mean ± S.D., BDL= Below Detection Limit, NS= Non-Significant, HSD= Honest Significantly Difference

Table 3: Occurrence of heavy metals, minerals and trace elements in pollen samples from different sites of Majha Region, Punjab

Sr. No.	Elements	Amritsar		Gurdaspur		Pathankot		Tarn Taran Sahib		F_{Cal}	HSD
		Mattewal	Dharmu Chak	Aliwal Araian	Kala Nangal	Kiri Khurd	Kotli Muglan	Kot Dharm	Amarkot		
1.	Se	1.53^{a}±0.04	1.49^{ab}±0.13	1.47^{ab}±0.03	1.38^{ab}±0.11	1.32^{b}±0.11	1.33^{c}±0.05	1.42^{a}±0.04	1.53^{a}±0.04	24.23*	0.17
2.	Zn	0.21^{ab}±0.06	0.15^{bc}±0.02	0.11^{c}±0.01	0.11^{c}±0.01	0.15^{bc}±0.05	0.14^{bc}±0.02	0.25^{a}±0.09	0.18^{abc}±0.01	3.08*	0.08
3.	Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-
4.	Ca	20.44±0.48	20.23±0.95	19.52±0.03	19.48±0.16	19.79±0.37	22.98±6.17	22.34±5.93	18.51±0.16	0.73^{NS}	-
5.	Fe	1.98±0.82	1.62±0.22	1.55±0.22	1.23±0.25	2.89±1.88	1.70±0.18	2.79±0.61	1.46±0.29	1.85^{NS}	-
6.	Cu	0.09±0.02	0.07±0.01	0.06±0.01	0.06±0.01	0.09±0.03	0.07±0.01	0.08±0.01	0.07±0.00	1.60^{NS}	-
7.	Ni	0.07±0.02	0.16±0.08	0.12±0.05	0.14±0.06	0.08±0.01	0.08±0.01	0.10±0.01	0.08±0.03	1.48^{NS}	-
8.	Pb	0.03^{a}±0.01	0.03^{a}±0.01	0.03^{b}±0.00	0.03^{b}±0.00	0.03^{b}±0.00	0.02^{c}±0.01	0.03^{b}±0.00	0.03^{b}±0.00	4.38*	0.01
9.	K	13.96±0.69	14.36±2.74	13.38±1.87	13.26±5.49	14.32±4.02	9.32±2.51	14.41±5.90	10.67±1.17	0.75^{NS}	-
10.	Mn	0.07±0.01	0.06±0.01	0.05±0.01	0.05±0.01	0.04±0.01	0.04±0.01	0.04±0.02	0.07±0.03	1.34^{NS}	-
11.	Na	3.46^{c}±0.44	3.44^{c}±0.10	3.33^{c}±0.22	3.53^{c}±0.60	3.95^{bc}±0.26	5.16^{ab}±1.49	4.87^{ab}±1.03	6.02^{a}±0.70	5.42*	1.29

Mean ± S.D., BDL= Below Detection Limit, NS= Non-Significant, HSD= Honest Significantly Difference

Table 4: Occurrence of heavy metals, minerals and trace elements in nectar samples from different sites of Majha Region, Punjab

Sr. No.	Elements	Amritsar		Gurdaspur		Pathankot		Tarn Taran Sahib		F_{Cal}	HSD
		Mattewal	Dharmu Chak	Aliwal Araian	Kala Nangal	Kiri Khurd	Kotli Muglan	Kot Dharm	Amarkot		
1.	Se	0.54^{bc}±0.08	0.36^{cd}±0.05	0.35^{cd}±0.02	0.18^{d}±0.04	0.34^{cd}±0.27	0.80^{a}±0.04	0.65^{ab}±0.10	0.49^{bc}±0.11	8.01*	0.20
2.	Zn	0.17±0.05	0.32±0.13	0.14±0.04	0.16±0.06	0.14±0.11	0.15±0.07	0.13±0.03	0.18±0.01	1.99^{NS}	-
3.	Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-
4.	Ca	21.60±5.44	28.46±0.21	26.25±5.61	22.61±5.40	23.30±6.79	22.49±4.57	20.71±0.62	21.34±0.21	1.08^{NS}	-
5.	Fe	2.25±0.63	3.76±1.67	1.94±0.52	1.68±0.33	1.38±0.81	1.95±0.43	1.73±0.36	2.22±0.59	2.54^{NS}	-
6.	Cu	0.09±0.02	0.06±0.01	0.07±0.02	0.07±0.02	0.08±0.05	0.08±0.04	0.07±0.02	0.08±0.03	0.22^{NS}	-
7.	Ni	0.08±0.01	0.34±0.27	0.10±0.04	0.11±0.02	0.07±0.05	0.09±0.04	0.07±0.01	0.09±0.01	2.36^{NS}	-
8.	Pb	0.02±0.01	0.03±0.01	0.03±0.00	0.02±0.01	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.01	1.35^{NS}	-
9.	K	9.72±1.03	7.47±3.74	10.80±5.42	10.06±1.30	4.99±2.35	7.58±0.85	9.61±1.38	8.38±1.66	1.14^{NS}	-
10.	Mn	0.05^{ab}±0.01	0.06^{a}±0.01	0.04^{b}±0.01	0.04^{b}±0.01	0.03^{b}±0.01	0.04^{b}±0.01	0.04^{b}±0.01	0.04^{b}±0.01	2.85*	0.01
11.	Na	4.84^{bc}±0.51	6.61^{a}±1.54	6.17^{ab}±0.93	7.60^{a}±1.22	4.35^{c}±0.39	6.49^{a}±0.85	4.22^{c}±0.10	4.63^{c}±0.27	6.35*	1.49

Mean ± S.D., BDL= Below Detection Limit, NS= Non-Significant, HSD= Honest Significantly Difference

Table 5: Occurrence of heavy metals, minerals and trace elements in honey samples from different sites of Majha Region, Punjab.

Sr. No.	Elements	Amritsar		Gurdaspur		Pathankot		Tarn Taran Sahib		F_{Cal}	HSD	Permissible Limit
		Mattewal	Dharmu Chak	Aliwal Araian	Kala Nangal	Kiri Khurd	Kotli Muglan	Kot Dharm	Amarkot			
1.	Se	0.31^{ab}±0.14	0.37^{ab}±0.08	0.22^{b}±0.19	0.03^{c}±0.05	0.32^{ab}±0.04	0.39^{a}±0.02	0.22^{b}±0.03	0.26^{ab}±0.05	4.23*	0.16	0.10
2.	Zn	0.21±0.08	0.18±0.05	0.18±0.02	0.18±0.05	0.22±0.03	0.18±0.04	0.19±0.04	0.20±0.02	0.30^{NS}	-	-
3.	Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	-	-	0.20
4.	Ca	27.31±5.14	27.29±5.31	30.34±0.25	24.51±4.93	27.29±4.07	28.33±5.28	30.59±1.36	24.47±2.33	0.95^{NS}	-	60.0
5.	Fe	1.03^{b}±0.14	2.58^{a}±0.93	1.67^{b}±0.25	1.55^{b}±0.47	1.55^{b}±0.11	2.49^{a}±0.40	1.20^{b}±0.33	1.62^{b}±0.30	4.65*	0.76	-
6.	Cu	0.10±0.01	0.09±0.02	0.09±0.01	0.08±0.02	0.11±0.03	0.09±0.02	0.08±0.01	0.06±0.01	2.41^{NS}	-	-
7.	Ni	0.11±0.02	0.07±0.02	0.07±0.01	0.07±0.01	0.11±02	0.07±0.02	0.07±0.01	0.09±0.02	2.18^{NS}	-	-
8.	Pb	0.03±0.00	0.03±0.00	0.02±0.01	0.02±0.01	0.04±0.01	0.03±0.01	0.02±0.01	0.04±0.01	1.90^{NS}	-	0.10
9.	K	5.87±0.54	8.26±0.66	7.94±1.28	6.74±0.77	6.29±1.69	8.11±0.55	7.08±0.21	7.39±1.59	2.17^{NS}	-	52.00
10.	Mn	0.06±0.03	0.04±0.01	0.04±0.01	0.07±0.02	0.06±0.01	0.06±0.02	0.04±0.02	0.07±0.01	0.89^{NS}	-	-
11.	Na	4.12±0.50	4.60±0.85	4.91±0.14	5.22±0.82	4.47±0.46	4.49±0.44	5.18±0.56	5.35±1.41	1.03^{NS}	-	4.00

Mean ± S.D., BDL= Below Detection Limit, NS= Non-Significant, HSD= Honest Significantly Difference

Table 6: Heavy metal concentrations in honey from different locations of the world

Elements	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Se	Ca	K	Na	Unit	References
Majha Region, Punjab, India	0	-	-	0.06	1.03	0.04	0.07	0.02	0.18	0.03	24.47	5.87	4.12	μg g^{-1}	This Study
	0	-	-	0.11	2.58	0.07	0.11	0.04	0.22	0.39	30.59	8.26	5.35		
Egypt	0.01	2.50	-	1.75	58.00	0.50	4.10	4.20	9.30	-	-	-	-	μg g^{-1}	Rashed and Soltan (2004) [20]
Spain	-	-	-	0.53	-	0.13	-	-	1.33	-	-	-	-	μg g^{-1}	Fernandez-Torres *et al*. (2005) [12]
	-	-	-	2.11	-	9.47	-	-	7.82	-	-	-	-		
Poland	0.01	-	0.05	1.37	9.46	10.43	0.50	0.12	19.30	-	-	-	-	μg g^{-1}	Bulinski, Wyszogrdzka-koma, and Marzec (1995) [4]
Italy	0.70	-	2.70	140	914	19.80	8.04	141	414	-	-	-	-	μg g^{-1}	Caroli *et al*. (2000) [7]
Turkey	0.50	-	-	0.02	0.27	0.02	3.20	-	0.06	-	-	-	-	μg g^{-1}	Uren, Serifoglu, and Sarikahya (1998) [23]
Romania	0	-	-	0.50	10	-	-	0.20	6.20	-	-	-	-	μg g^{-1}	Carmen and Cristina (2001) [6]

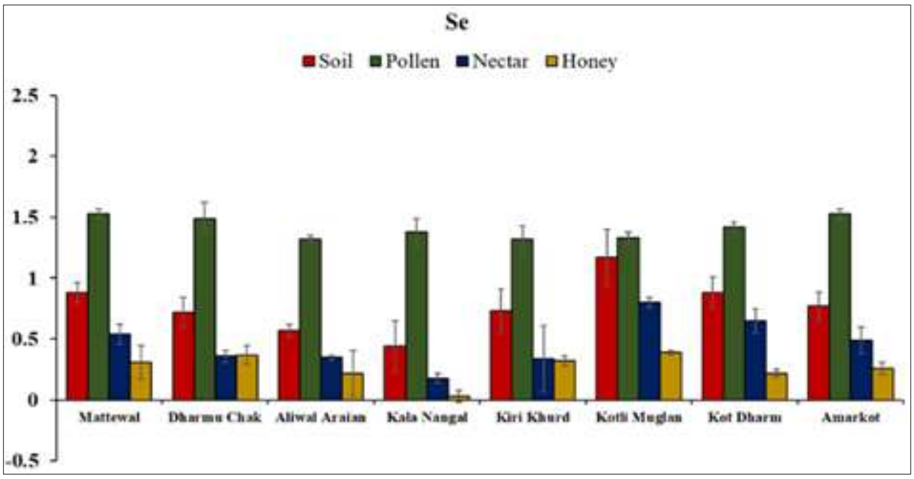


Fig 3: Comparison of selenium in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

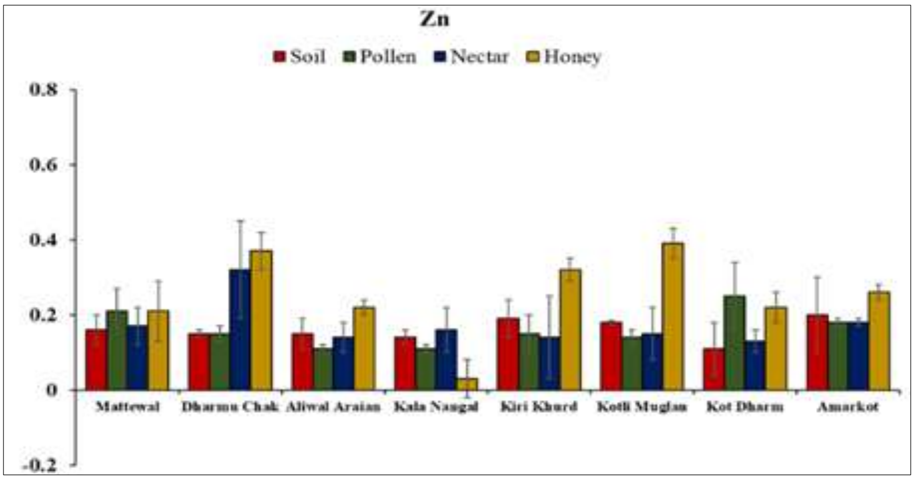


Fig 4: Comparison of zinc in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

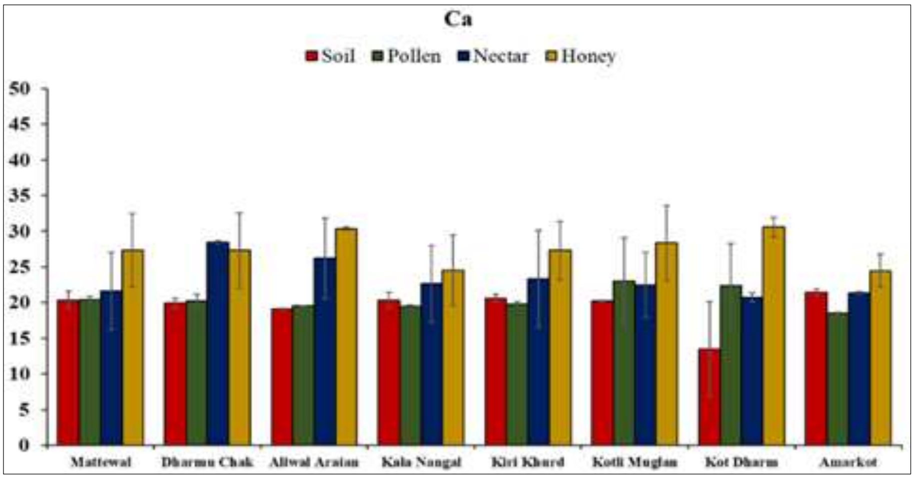


Fig 5: Comparison of calcium in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

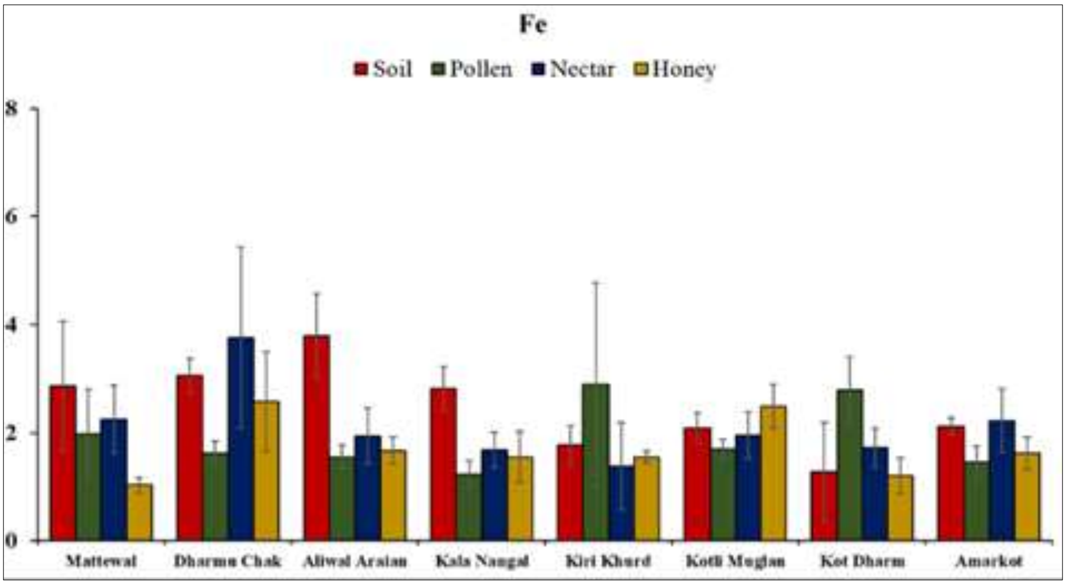


Fig 6: Comparison of iron in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

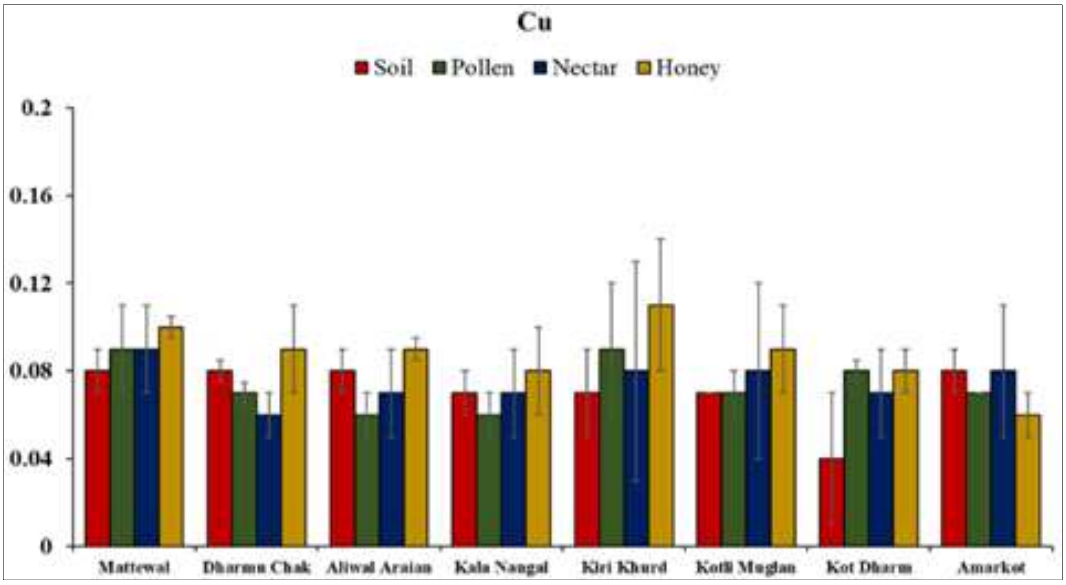


Fig 7: Comparison of copper in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

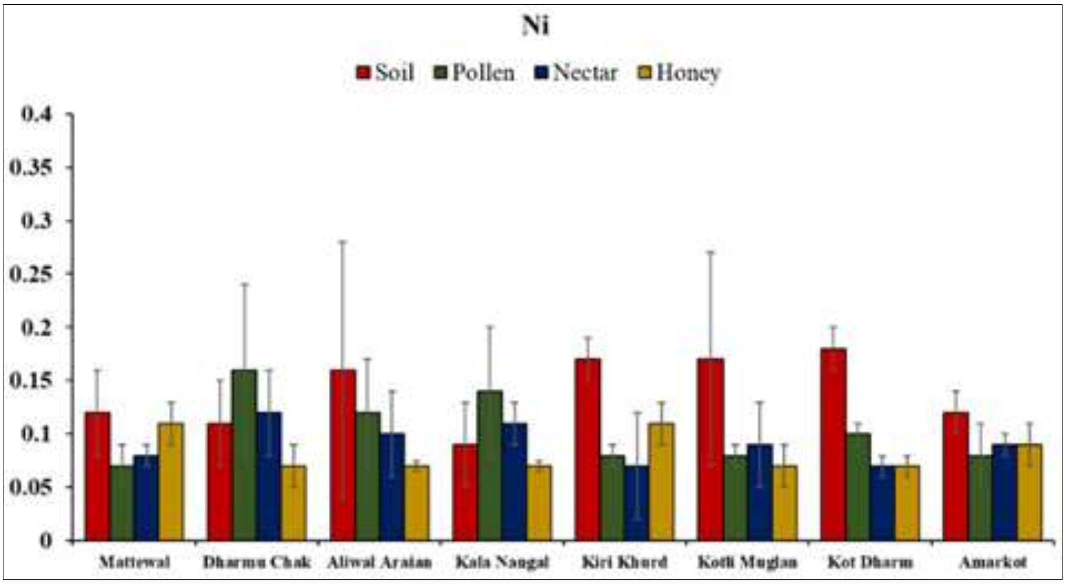


Fig 8: Comparison of nickel in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

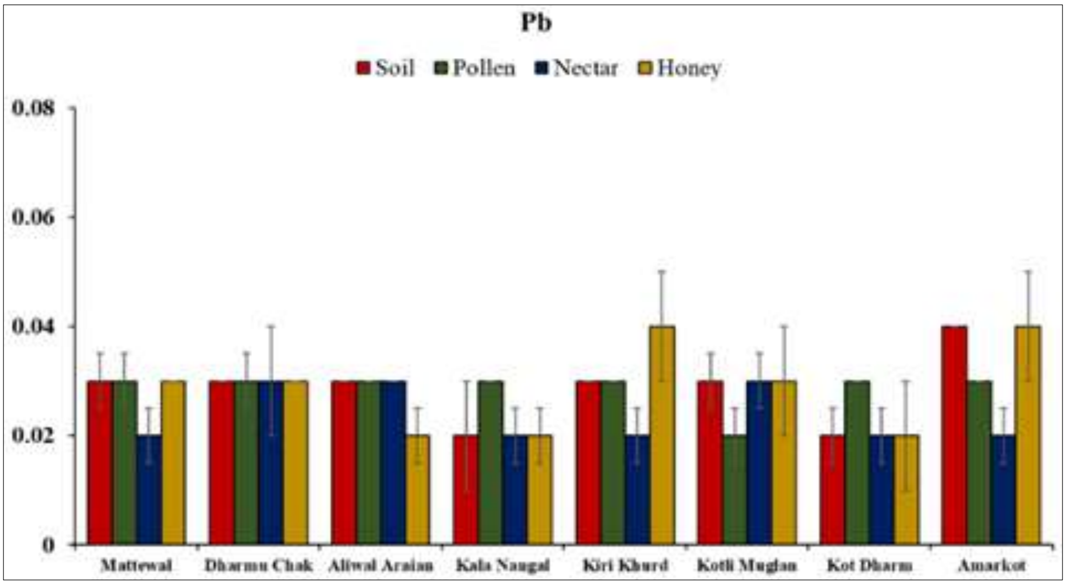


Fig 9: Comparison of lead in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

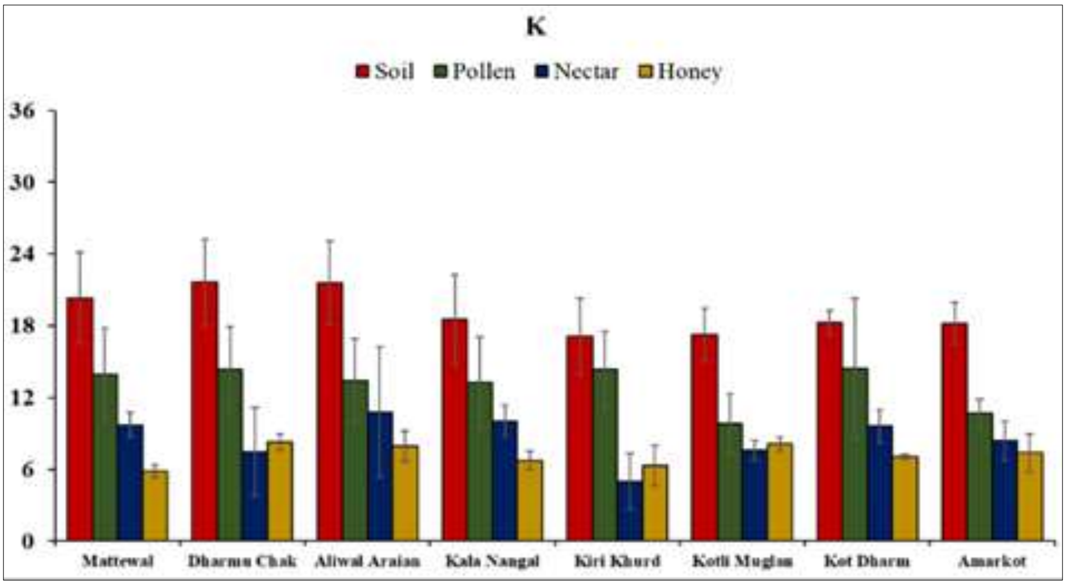


Fig 10: Comparison of potassium in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

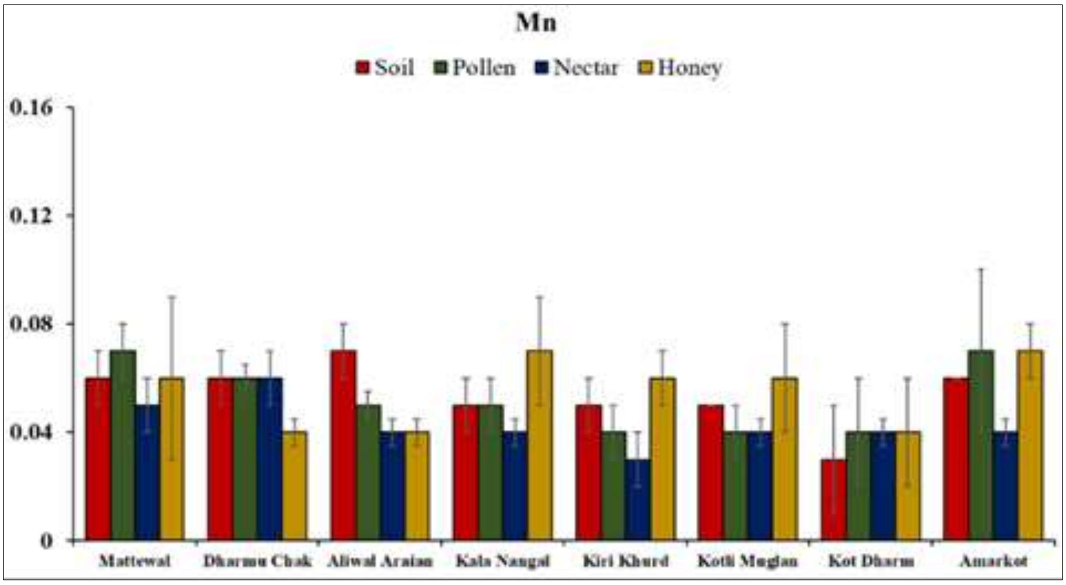
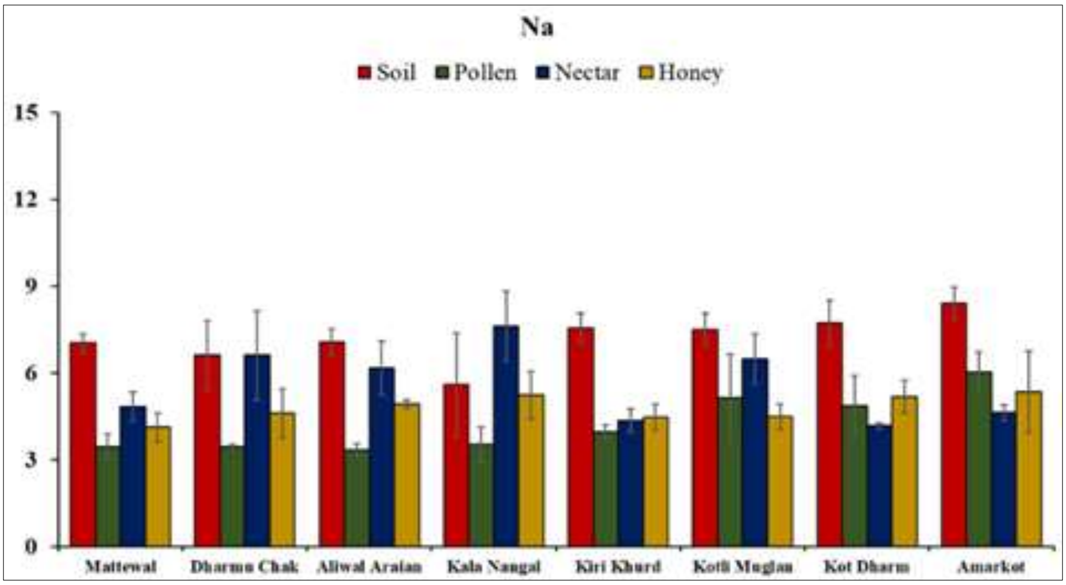


Fig 11: Comparison of manganese in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab



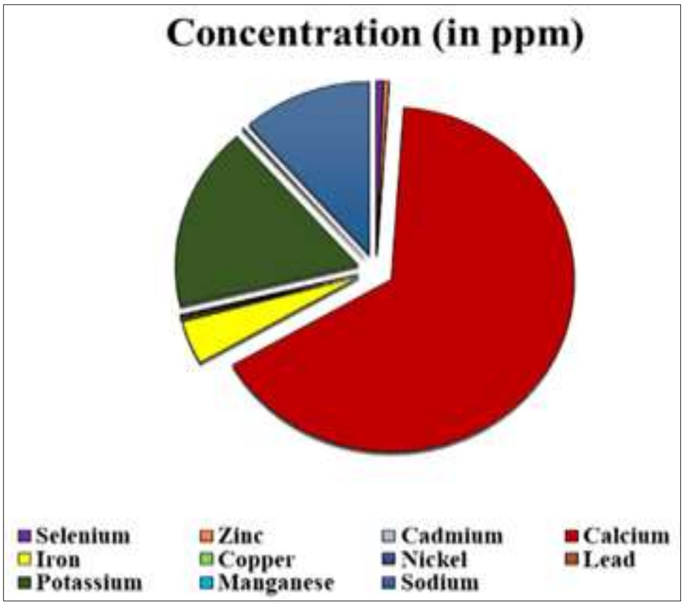


Fig 13: Comparison of manganese in soil, pollen, nectar and honey samples from different locations of Majha Region, Punjab

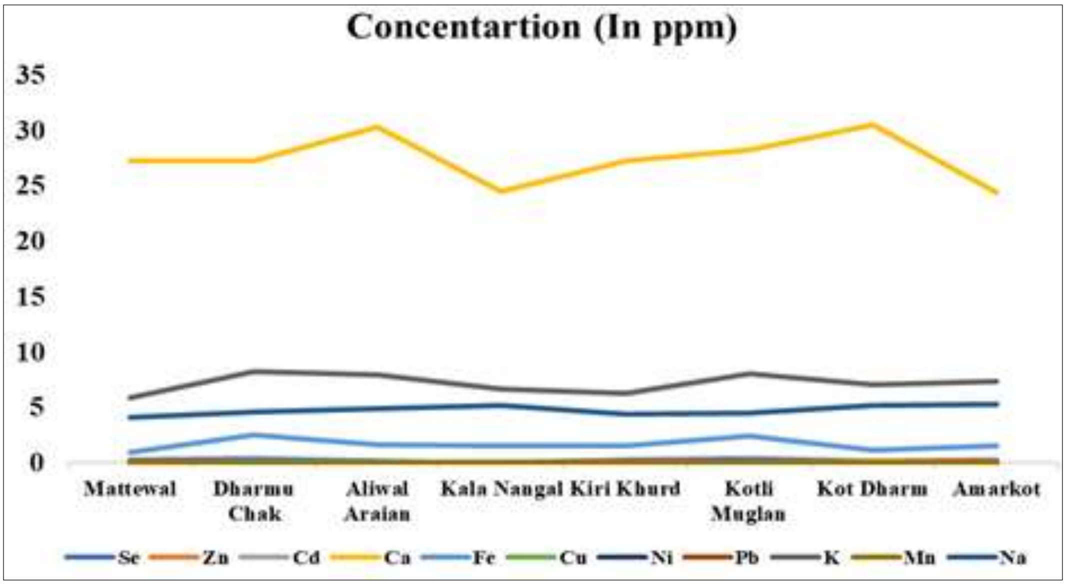


Fig 14: Concentration of heavy metals, minerals and trace elements in honey samples from different sites of Majha Region Punjab Punjab

4. Conclusion

Overall, the present study concluded that the honey bee is a good marker for monitoring environmental pollution with heavy metals and reflects the presence and quality of heavy metals in plants surrounding the apiary areas. Due to the cultural practises followed and other anthropogenic activities going on around agricultural fields, there is a rise in heavy metal contamination in the soil. The agricultural crops grown in these areas bio-accumulate these heavy metals from the soil into their different aerial and flowering parts. From pollen and nectar, this heavy metal contamination is transferred to honey during nectar collection and pollination by honey bees. Therefore, the present study was carried out to evaluate the concentrations of eleven different elements, including alkali metals (Na and K), alkaline earth metals (Ca), transition metals (Mn, Fe, Ni, Cu, Zn, and Cd), post-transition metals (Pb), and non-metals (Se), in soil, pollen, nectar, and honey samples from eight different sites in four districts (Amritsar, Gurdaspur, Pathankot, and Tarn Taran Sahib) of Majha Region, Punjab. The bio-accumulation factors of the studied metal concentrations in the BAF_{SP}– (soil to pollen), BAF_{SN}– (soil to nectar), and BAF_{NH}– (nectar to honey) systems provide explanations for the movement of heavy metals from soil to plant and from plant to honey. Comparing the observed heavy metal concentrations with the research papers and literature, we concluded that the difference in metal concentration depends on the local environmental conditions. Air pollution comes mostly from planning activities, industrial activities, and traffic. It can affect soil, flowers, nectar, and honey, where heavy metals enter the composition of honey from these sources. The accumulation of Zn, Ca, and Cu in honey is high due to water pollution, and forager bees collecting water leads to an increase in the concentration of these metals in honey. The bioaccumulation factor of Cd was not calculated due to samples below the detection limit (BDL).

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