Ministry of Agriculture and Land Reclamation
Agricultural Research Center

Egyptian Journal of Plant Protection Research Institute
www.ejppri.eg.net

PLANT PROTECTION RESEARCH INSTITUTE PPRI

Estimated of heavy metals pollution by honey bee as bio-indicator

Sherif, A. S. F.; Haitham, Ramadan[2]; Dina, Mohammed Taksira[1] and Marwa, B. M. Gomaa[1]
[1]*Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.*
[2]*Plant Protection Department, Faculty of Agriculture, Tanta University, Egypt.*

ARTICLE INFO
Article History
Received: 18 /11 / 2019
Accepted: 29 / 12 /2019

Keywords:
Heavy metal, pollution, honeybee, environment and biological monitor.

Abstract:

One of the most important global problems at the last few decades is pollution. Honey bee has been examined as bio-indicator to monitor pollutants. The aim of this study is to confirm the importance of foraging honey bee *Apis melliferra* L. (Hymenoptera: Apidae) body as bio accumulator of heavy metals and reflected the pollution in some treated north and middle Egyptian Governorates. This Study was carried out in laboratory of Ministry of Health in 2018. Samples were collected from some north and middle Egypt`s Governorates apiaries. Certain heavy metals were analyzed using Micro plasma [(Inductively Coupled Plasma (ICP)]. the highest concentration mean was ferric (1.038 ppm), but the low was boron (-0.37 ppm). Moreover, Monufia and Alexandria Governorates were the highest concentration's mean in heavy metals pollution (0.32 ppm), while the lowest one was at Ismailia Governorate (0.024 ppm). foraging honey bee *A. melliferra* body could be used as bio-indicator of heavy metals and reflected the pollution.

Introduction

Heavy metal is one of the most environment pollution mirrored. Pollution of soils by transition metals, such as cadmium (Cd), nickel (Ni), zinc (Zn), lead (Pb), copper (Cu), has increased dramatically during the last few decades (Chibuike and Obiora, 2014). Recently the heavy metal pollution affected a lot of health's problem (Alzheimer, anemia, muscles, weakness, cancer, vomiting, diarrhea, headache, cough, nasal ulcer, memory loss……etc.). It's a natural result for intensive used of pesticides and agricultural fertilizers. Wastes, traffic emissions, industrial effluents and industrial chemicals etc. Varied amount of metal pollution exists global now, including Egypt (Moussa and Abdelkhalek, 2007).

The popular way for divulge the heavy metal status in the environment is the chemical analysis. Whoever biological indicators can be used as measure of heavy metal pollution. Biological monitoring within a quality control program involves the systematic use of living beings for obtaining quantitative information on changes in the environment, often due to anthropogenic activities (Bargagli, 1998). The use of insects as bio-indicators in earthly ecosystem, in contrast, has been far less actively embraced. The honey bee, *Apis mellifera* L.

(Hymenoptera: Apidae), has been investigated as bio-indicator to monitor pollutants (Leita *et al*., 1996 and Bogdanov, 2006). Its attractiveness as an ecological detector depends on several features such as high reproductive rate, large flying range since they often forage to 2–3 km away from the apiary, the body is covered with hairs that collect various particles and increase by this mean, close contact with the surrounding environment, sensitive to toxic substances, and the possible use of bee products as indicators for environmental pollution (Porrini *et al*., 2003 and Stark, 2003). This last point is important because bees and bee products can be used as accumulative and reactive indicators (Billalov *et al*., 1992). Contamination of the environment, including Egypt, by metals is now widespread resulting in toxicity (Nordberg *et al*., 2011).

The goal of the present study is to verify the effectiveness of a bio-indicator based method involving the analysis of honey bee workers *A. mellifera* for the determination of the environmental pollution with heavy metals.

Materials and Methods

Study was carried out in laboratory of Ministry of Health in 2018. Honey bee samples were collected from different Governorate`s apiaries, Egypt. with 40 km intervallic distances between each apiary.

1. Samples

(1) Samples were collected from Suez, Ismailia, Alexandria, Beheira, Kafr El Sheikh, Gharbia, Dakahlia, Sharqia, Monufia and Qalyubia.

2. Apparatuses

2.1. Balance

2.2. Microwave

2.3. Digestive

2.4. Micro plasma (Inductively Coupled Plasma (ICP))

3.Procedure:

Samples were collected into plastic packages using gloves, were stored at –18°C before analyses and were dried in microwave for 10 minute at 120^{o}. dried bees (50 g) were placed into vessels with 8 ml of nitric acid (HNO3) and 2 ml of hydrogen peroxide H_2O_2 put in digestive per one hour then leaved it cold for 24 hours, samples were filtrated through 1–2.5 μm filter paper and brought to a final volume of 25 ml with distilled water. Then analysis to determine the following heavy metal (Boron, zinc, ferric, nickel, lead, molybdenum, cadmium, copper, manganese and chromium) in micro plasma (Inductively Coupled Plasma (ICP), is highly sensitive and capable of multi-element trace analysis and ultra-trace analysis, often at the parts-per-trillion level. Testing for trace elements can be performed on a range of materials from super alloys to high purity materials.

Mode of action that coupling to mass spectrometry, the ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration. The concentration of a sample can be determined through calibration with certified reference material such as single or multi-element reference standards ICP-MS. Also lends itself to quantitative determinations through isotope dilution, a single point method based on an isotopically enriched standard. Other mass analysers coupled to ICP systems include double focusing magnetic-electrostatic sector systems with both single and multiple collector, as well as time of flight systems (both axial and orthogonal accelerators have been used).

The concentration of a sample can be determined through calibration with certified reference material such as single or multi-element reference standards. ICP-MS also lends itself to quantitative determinations through isotope dilution, a single point method based on an isotopically enriched standard. Ceased to be discharged, the excess nitric acid was removed by increasing the temperature to 100°C. The solution was carefully evaporated until the perchloric acid began to evaporate. The solution was then cooled, 10 ml of distilled water was added, and the mixture was filtrated through a 1–2.5 µm filter paper and brought to a final volume of 25 ml with distilled water. Microelements in the samples were quantified by atom-absorption spectrometry with an Analyst-400 system (Perkin-Elmer, Waltham, MA, USA) with flame atomization. The following heavy metals were analyzed: Boron (B), Zink (Zn), Nickel (Ni), Molybdenum (Mo), Lead (Pb), Cadmium (Cd), Copper (Cu), Manganese (Mn), Chromium (Cr) and Iron (Fe). All samples were analyzed in duplicate.

Results and discussion

Data in Figure (1) showed the heavy metal (ppm) in honey bee workers samples at the Suez Governorate. It recorded the highest value of heavy metal at ferric (1.65 ppm), while the nickel recorded the lowest value (0 ppm).

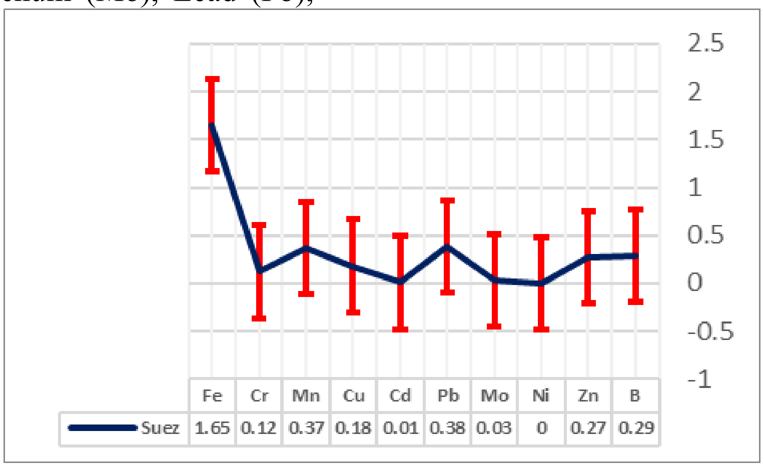

Figure (1): The heavy metal in honeybee worker's samples at the Suez Governorate.

Data in Figure (2) showed that the heavy metal (ppm) in honeybee workers samples at the Ismailia Governorate. It recorded the highest value of heavy metal at ferric (0.91 ppm) while the cadmium and zinc recorded the lowest value (0 ppm).

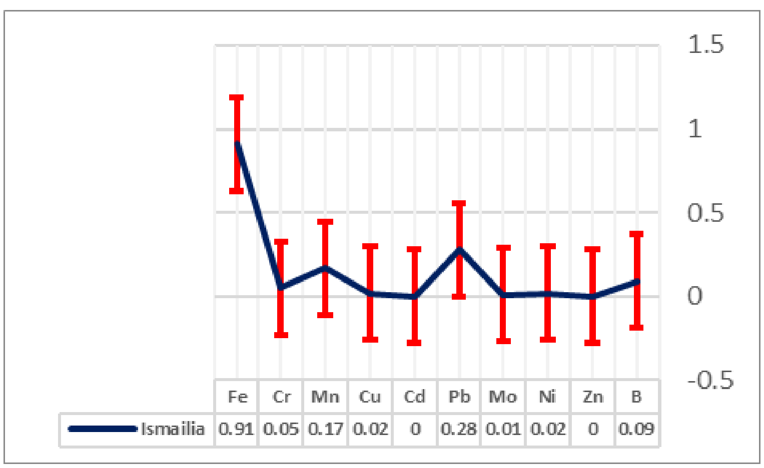

Figure (2): The heavy metal (ppm)in honey bee worker's samples at the Ismailia Governorate

Data in Figure (3) showed the heavy metal (ppm) in honeybee workers samples at the Alexandria Governorate. It recorded the highest value of heavy metal at ferric (1.98 ppm) while the cadmium recorded the lowest value (0 ppm).

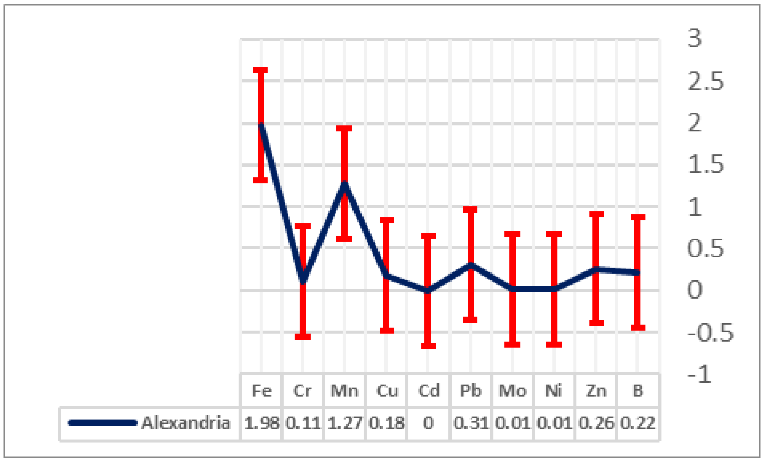

Figure (3): The heavy metal in honey bee worker's samples at the Alexandria Governorate.

Data in Figure (4) showed the heavy metal (ppm) in honeybee workers samples at the Beheira Governorate. It recorded the highest value of heavy metal at ferric (1.1) while the nickel recorded the lowest value (0 ppm).

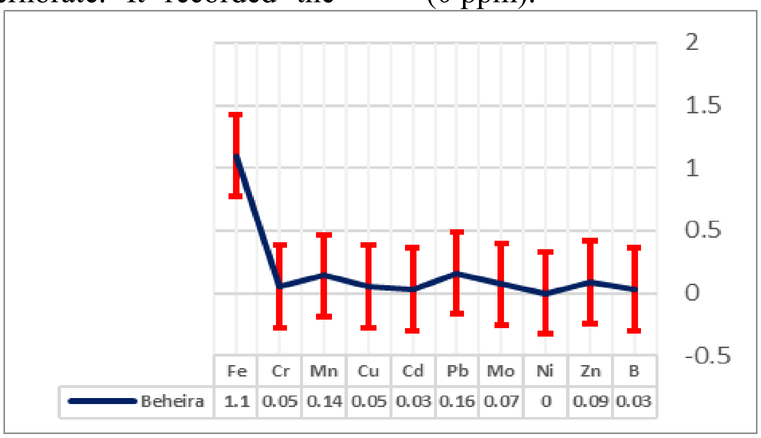

Figure (4): The heavy metal in honeybee worker's samples at the Beheira Governorate.

Data in Figure (5) showed the heavy metal (ppm) in honeybee workers samples at the Kafr Elsheikh Governorate. It recorded the highest value of heavy metal at ferric (2.34 ppm) while the nickel recorded the lowest value (0 ppm).

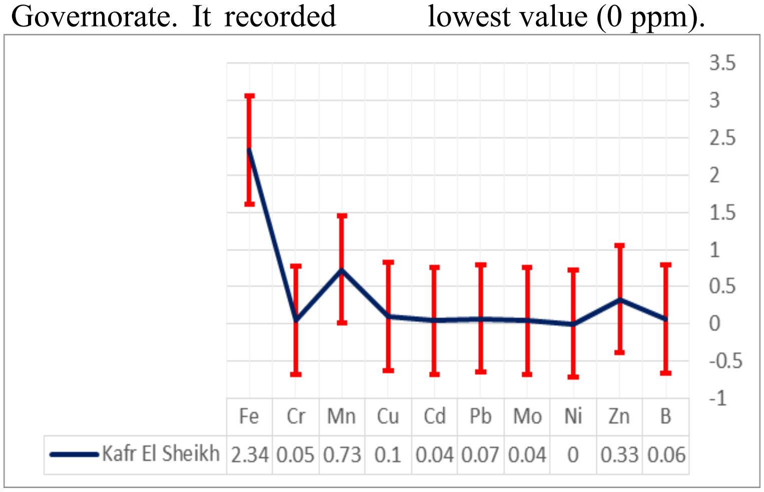

Figure (5): The heavy metal in honey bee worker's samples at the Kafr Elsheikh Governorate.

Data in Figure (6) showed the heavy metal (ppm) in honeybee workers samples at the Gharbia Governorate. It recorded the highest value of heavy metal at ferric (1.35 ppm) while the nickel recorded the lowest value (0 ppm).

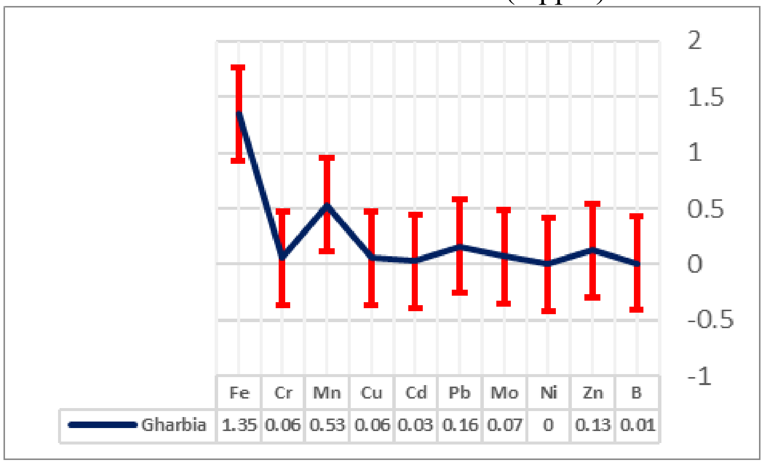

Figure (6): The heavy metal in honeybee worker's samples at the Gharbia Governorate.

Data in Figure (7) showed the heavy metal (ppm) in honeybee workers samples at the Dakahlia Governorate. It recorded the highest value of heavy metal at Ferric (1.72 ppm) followed by manganese (1.06 ppm) while the molybdenum recorded the lowest value (0.03 ppm).

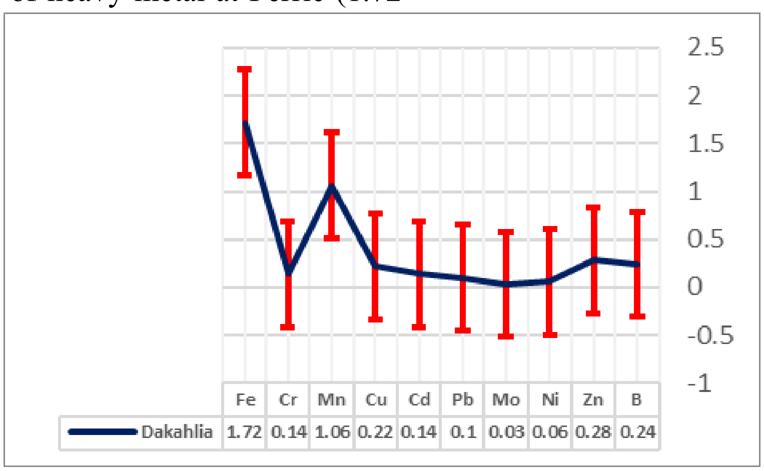

Figure (7): The heavy metal (ppm)in honeybee worker's samples at the Dakahlia Governorate

Data in Figure (8) showed the heavy metal (ppm) in honeybee workers samples at Sharqia Governorate. It recorded the highest value of heavy metal at ferric (0.89 ppm) while the nickel recorded the lowest value (0 ppm).

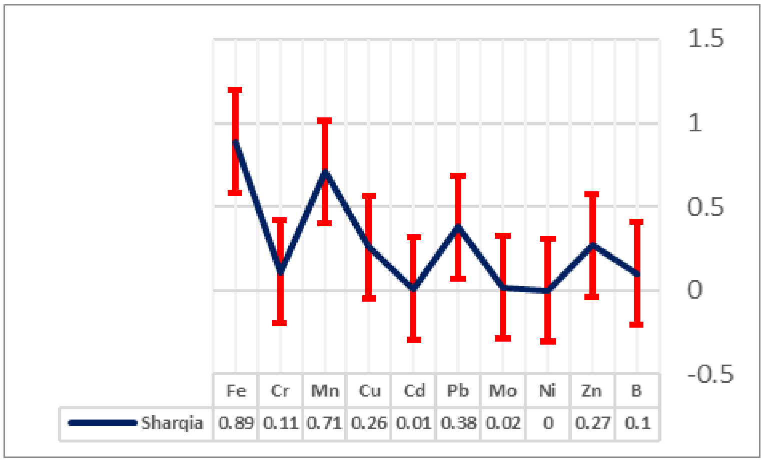

Figure (8): The heavy metal in honeybee worker's samples at the Sharqia Governorate.

Data in Figure (9) showed that the heavy metal (ppm) in honeybee workers samples at the Monufia Governorate. It recorded the highest value of heavy metal at Ferric (2.15 ppm) while the cadmium recorded the lowest value (0.01 ppm).

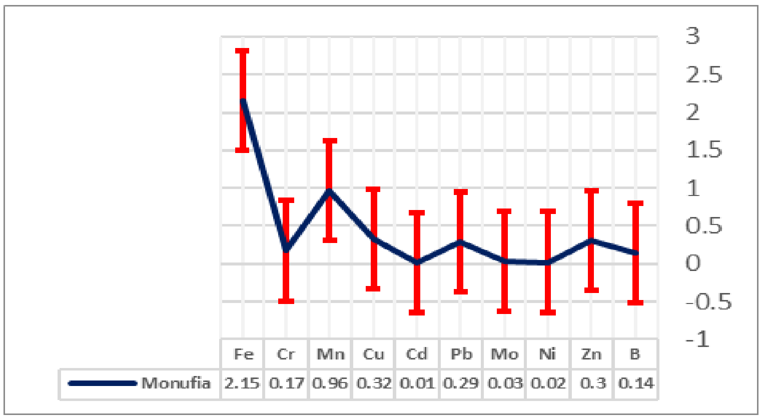

Figure (9): the heavy metal (ppm) in honeybee worker's samples at the Monufia Governorate.

Data in Figure (10) showed the heavy metal (ppm) in honeybee workers samples at the Qalyubia Governorate. It recorded the highest value of heavy metal at ferric (1.29 ppm) while the nickel recorded the lowest value (0 ppm).

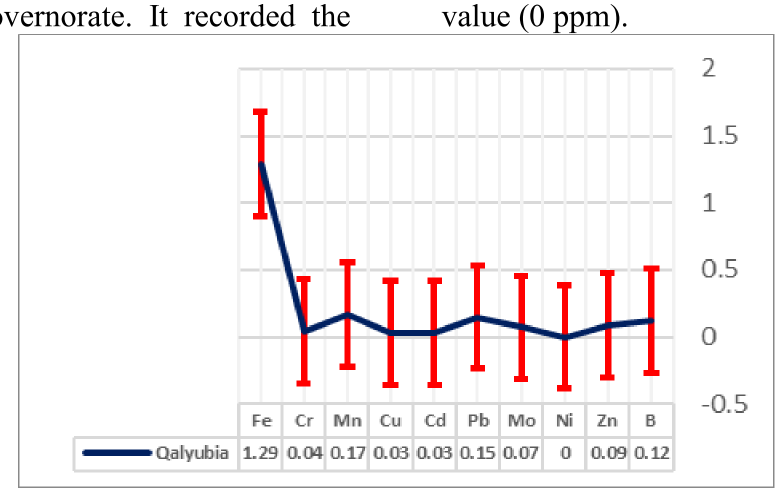

Figure (10): The heavy metal (ppm)in honeybee worker's samples at the Qalyubia Governorate.

From the obtained results showed in Table (1) it could be summarized that, the maximum differences between the stander and treatment of Boron concentration founded at Suez's workers body was (-0.21 ppm). As the minimum concentration founded at Gharbia's workers body was (-0.49 ppm).

Zinc concentration recorded the upper differences between the stander and treatment of founded at Kafr El Sheikh's workers body was (0.32 ppm). As the lower concentration founded at Ismailia's workers body was (-0.01 ppm). Moreover, the maximum differences between the stander and treatment of Nickel founded at Dakahlia's workers body was (0.04 ppm). As the minimum concentration founded at Suez, Beheira, Kafr El Sheikh, Gharbia, Sharqia and Qalyubia's workers body was (-0.02 ppm). The upper differences between the stander and treatment of Molybdenum founded at Kafr El Sheikh was (-0.03). No differences between the stander and treatment of molybdenum founded at Beheira, Gharbia, Qalyubia's workers body was (0 ppm). As the lower differences between the stander and treatment of Molybdenum founded at Ismailia and Alexandria's workers body was (-0.06 ppm). The maximum differences between the stander and treatment of Lead founded at Suez and Sharqia's workers body was (0.37 ppm). As the minimum concentration of Lead founded at Dakahlia's workers body was (0.09 ppm). The upper differences between the stander and treatment of Cadmium founded at Dakahlia's workers body was (0.139 ppm). As the lower concentration of Cadmium founded at Ismailia and Alexandria's workers body was (-0.001 ppm). The Monufia's workers body recorded the high differences between the stander and treatment at Copper (0.31 ppm). As the Ismailia's workers body recorded the low concentration at Copper (0.01 ppm). The high differences between the stander and treatment of Manganese founded at Alexandria's workers body was (1.25 ppm). As the low concentration of Manganese founded at Ismailia's workers body was (-0.15 ppm). The maximum differences between the stander and treatment of Chromium founded at Monufia's workers was (0.12 ppm). As the low concentration of Chromium founded at Qalyubia's workers body was (-0.01 ppm). The high differences between the stander and treatment of Ferric founded at Kafr El Sheikh's workers body was (1.84 ppm). As the low concentration of Ferric founded at Sharqia's workers body was (0.39 ppm).

Table (1): Heavy metal differences between the Egyptian stander and treatments in honey bee worker's samples from several Egyptian Governorates.

Governorates	B	Zn	Ni	Mo	Pb	Cd	Cu	Mn	Cr	Fe	mean
Suez	-0.21	0.26	-0.02	-0.04	0.37	0.009	0.17	0.35	0.07	1.15	0.24
Ismailia	-0.41	-0.01	0	-0.06	0.27	-0.001	0.01	-0.15	0	0.41	0.024
Alexandria	-0.28	0.25	-0.01	-0.06	0.3	-0.001	0.17	1.25	0.06	1.48	0.32
Beheira	-0.47	0.08	-0.02	0	0.15	0.029	0.04	0.12	0	0.6	0.05
Kafr El Sheikh	-0.44	0.32	-0.02	-0.03	0.06	0.039	0.09	0.71	0	1.84	0.26
Gharbia	-0.49	0.12	-0.02	0	0.15	0.029	0.05	0.51	0.01	0.85	0.12
Dakahlia	-0.26	0.27	0.04	-0.04	0.09	0.139	0.21	1.04	0.09	1.22	0.28
Sharqia	-0.4	0.26	-0.02	-0.05	0.37	0.009	0.25	0.69	0.06	0.39	0.16
Monufia	-0.36	0.29	0	-0.04	0.28	0.009	0.31	0.94	0.12	1.65	0.32
Qalyubia	-0.38	0.08	-0.02	0	0.14	0.029	0.02	0.15	-0.01	0.79	0.08
Mean	-0.37	0.192	-0.009	-0.068	0.218	0.029	0.132	0.591	0.04	1.038	

B: Boron, Zn: Zink, Ni: Nickel, Mo: Molybdenum, Pb: Lead, Cd: Cadmium, Cu: Copper, Mn: Manganese, Cr: Chromium and Fe: Iron.

From the obtained results showed in Figure (11), it could be summarized that, at Suez governorate, the high element's differences between the stander and treatment (1.15 ppm) found in ferric element. As the low element (-0.21 ppm) found in boron element. At Ismailia Governorate the high element's differences between the stander and treatment (0.41 ppm) found in ferric element. As the low element was -0.41 ppm found in boron. However, Alexandria Governorate high element's differences between the stander and treatment (1.48 ppm) found in ferric element. As the low differences was (-0.28 ppm) found in boron. Moreover, Beheira Governorate high element's differences between the stander and treatment (0.6 ppm) founded in ferric element. As the low differences was (-0.47) ppm found in boron element. As, the Kafr El Sheikh Governorate high element's differences between the stander and treatment (1.84 ppm) founded in ferric element. As the low was (-0.44) founded in Boron element. However, the Gharbia Governorate high element's differences between the stander and treatment was (0.85 ppm) founded in Ferric element. As the low differences was (-0.49 ppm) founded in Boron element. Dakahlia Governorate high element's differences between the stander and treatment was (1.22 ppm) founded in ferric element. As the low was (-0.26 ppm) founded in Boron element.

At Sharqia Governorate the high element's differences between the stander and treatment (0.69 ppm) founded in manganese element. As the low was (-0.4 ppm) founded in boron element. While Monufia Governorate high element's differences between the stander and treatment (1.65 ppm) founded in ferric element. As the low was (-0.36 ppm) founded in boron element. While the Qalyubia Governorate high element's differences between the stander and treatment (0.79 ppm) founded in ferric element. As the low was (-0.38) founded in boron element.

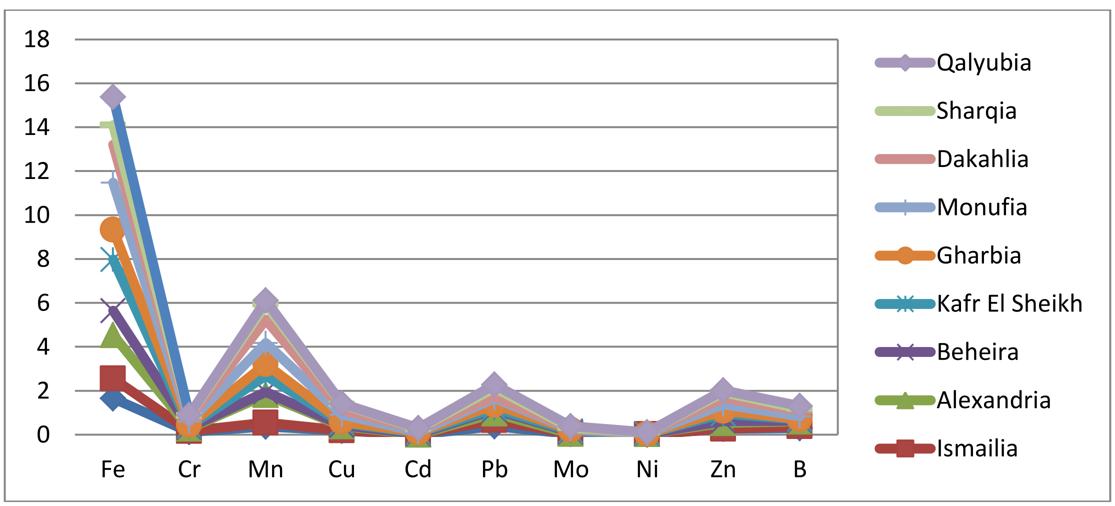

Figure (11): Heavy metal mean in honeybee worker's samples from several Egyptian Governorates.

From the obtained data the following conclusion can be recommending; the most suitable north and meddle Egypt treatments governorates for breeding honeybee was Ismailia. On the other hand, Monufia and Alexandria Governorates heavy not recommended for breeding honey bee. It was observably that, the Ferric element recorded the highest concentration at all Governorate's samples except at Dakahlia. While the boron is the lowest element concentration. Many authors discussed this question and found that, Functions in polluted environment, plant products used by honey bee may also be contaminated and as a result also a part of these pollutants will accumulate in it as revealed by Accorti *et al.* (1990); Balestra *et al.* (1992) and Roman (1997). Krantzberg and Stokes (1990) found that, Pb, Fe, Cd, Cu, Ni, Zn, and occasionally, Al accumulated in the mid gut and anal papillae of chironomids, with the greatest frequency of detection occurring in the mid gut of larvae collected from the mere heavily contaminated site. Kuterbach and Walcott (1986) reported that the accumulated iron rich granules in the fat body of adult honey bees. Conti and Botre (2001) found the concentrations of Cd and Pb in honey bee workers ranged between 2.87- 4.23 ppm for Cd and 0.61- 1.25 ppm for Pb. The concentrations of Cu, Zn and Cd reported herein were higher than those reported by Birge and Price (2001). These differences may be attributed to the varying degrees of heavy metals contaminations at each location (Zhou *et al*., 1994). Trace elements in insects were found on the surface of their chitinous exoskeleton or incorporated into their body tissues (Hare, 1992).

Studies of Leita *et al.* (1996) show a high content of zinc and cadmium on the body surface of insects because of atmospheric fallout (atmospheric fallout), whereas a lead accumulated in the body. Iron-containing granules were found in fat cells and in columnar cells of the midgut of adult worker honey bees (Raes *et al.,* 1989).

The presence of even small peaks collected from whole honey bee workers and their tissues (mid gut, hind gut and fat body) compared to the control, may indicate the ability of honey bee workers to accumulate these toxic metals in their tissues. Moreover, it may emphasize the possibility of using whole honey bee workers and their tissues (mid gut, hind gut and fat body) as biomarkers for environmental pollution with heavy metals.

References

Accorti, M.; Guardini, R.; Modi, G. and Persano-Oddo, L. (1990): Urban pollution and honey bees. Apicoltura, 6: 43- 55.

Balestra, V.; Celli, G. and Porrini, C. (1992): Bees, honey, larvae and pollen in biomonitoring of atmospheric pollution. Aerobiologia, 8(1): 122- 126.

Bargagli, R. (1998): Trace element in terrestrial plants. An ecophysiological approach to biomonitoring and biorecovery. Springer Verlag, Berlin, New York, 324.

Billalov, F.S.; Kolupaev, B.I.; Kotov, Yu. S. ; Muharamova, S.S. and Srebneva, L. (1992): Bee products and environmental control, Pchelovodstvo, 12: 4-6.

Birge, W.J. and Price, D.J. (2001): Analysis of metals and Polychlorinated biphenyl (PCB) residues in honey bees, honey and pollen samples collected from the Paducah gaseous diffusion plant and other areas. Division of Waste Management. Kentucky Department for Environmental Protection.

Bogdanov, S. (2006): Contaminants of bee products. Apidologi, (37):1–18.

Chibuike, G.U. and Obiora, S.C. (2014): Heavy metal polluted soils: Effect on plants and bioremediation methods. Applied and Environmental Soil Science. doi: 10.1155/2014/752708

Conti, M.E. and Botre, F. (2001): Honey bees and their products as potential bioindicator of heavy metal contamination. Environmental Monitoring and Assessment, 69 (3): 267–282.

Hare, L. (1992): Aquatic insects and trace metals: Bioavailability, bioaccumulation and toxicity. Critical Reviews in Toxicology, 23:327-369.

Krantzberg, G. and Stokes, P. M. (1990): Metal and tissues distribution in larvae of *Chironomus* with reference to X-ray microprobe analysis. Archives of Environmental Contamination and Toxicology, 19: 84-93.

Kuterbach, D. A. and Walcott, B. (1986): Iron containing cells in the honeybee (*Apis mellfiera*). I. Adult morphology and physiology. Journal of Experimental Bioloev., 126: 375-387.

Leita, L.; Muhlbachova, G.; Cesco, S.; Barbattini, R. and Mondini, C. (1996): Investigation on the use of honey bees and honey bee products to assess heavy metals contamination. Environmental Monitoring and Assessment, 43: 1–9.

Moussa, M.I. and Abdelkhalek, A. M. (2007): Meteorological analysis for black cloud (Episodes) formation and its monitoring by remote sensing. Journal of Applied Sciences Research, 3(2): 147-154.

Nordberg, G.F.; Fowler, B.A.; Nordberg, M. and Friberg, L. (Eds.) (2011): Handbook on the Toxicology of Metals. Amsterdam, Boston: Academic Press.

Porrini, C.; Sabatini, A.; Girotti, S.; Ghini, S.; Medrzycki, P.; Grillenzoni, F.; Bortolotti, L.; Gattavecchia, E. and Celli, G. (2003)**:** Honey bees and bee products as monitors of the environmental contamination. Apiacta, 38: 63-70.

Raes, H.; Bohyn, W.; De Rycke, P. H. and Jacobs, F. (1989): Membrane - bound iron-rich granules in fat cells and midgut cells of the adult honey bee (*Apis mellifera* L.). Apidologie, 20:327-337.

Roman, A. (1997): Bees and bee products as bioindicator of environmental contamination in the area of influence of industry of copper (LGOM) and cement lime (Opole). A.R. Scientific Papers of Wroclaw, Animal Husbandry, 323:175 – 196.

Stark, J.A. (2003): The honey-bee (*Apis mellifera* L.) as monitor of short – and long-term environmental and ecological changes, XXXVIII-th Apimondia International Apicultural Congress, Ljubljana.

Zhou, Q.; Wu, Y. and Xiong, X. (1994): Compound pollution of Cd and Zn and its ecological effect on rice plant. Chinese journal of applied Ecology, 5: 428-441.