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Insects and Arachnids as Bioindicators of Heavy Metal Toxicity in Lahore

Saffora Riaz[1], Noor Virk[1], Farkhanda Manzoor[1], Zulfiqar Ali[2]
[1]Department of Zoology, Lahore College for Women University, Lahore, Pakistan
[2]Department of Zoology, University of the Punjab, Lahore, Pakistan 54590
Correspondence and requests for materials should be addressed to SR (email: riazsaffora@gmail.com)

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Abstract
Heavy metal toxicity is a profound reason for the decline in population of insect pollinators as it transfers through food chain and affect the physiological aspect of the insects. Thus, the heavy metal analysis in different insects such as butterfly (*Pieris rapae*), honey bee (*Apis dorsata*) and arachnid spider (*Pholcus phalangioides*) were examined by using atomic absorption spectrophotometer. The aim of the study was to analyse the contamination and accumulation of heavy metals (Hg, Pb, Cr, Cd, Ni) in insect species as ecological indicator. It was revealed that the mercury (Hg) was in the highest concentration in all the insect samples and the nickel in lowest concentration. Data was statistically evaluated using one way ANOVA and post hoc analysis with significant value 0.05 was applied in case of $p<0.05$ suggested that all insects were good ecological indicator. There was no significant difference in lead, chromium and nickel concentration of honey bees, spiders and butterflies. Honey bees varied significantly $p< 0.02$ with the spiders and butterflies for cadmium. For mercury, the spiders and honey bees were non-significant and the butterflies were significant $p< 0.03$ with the spiders and honey bees. Present study indicated that heavy metals contamination is the major environmental and health concern in Pakistan and insects can be used as bioindicator for environmental pollution.

Keywords: Environmental pollution; Bioindicators; Heavy metal; Lead; Atomic absorption

Introduction
Metal pollution is now a significant issue in many countries, affecting a multitude of environments (Srivastava and Goyal, 2010). Metals are by products of human industry (Khatri et al., 2018) and repeatedly found in such high concentrations that they extent toxic levels in some component parts of ecosystems and thus they can disturb their proper functioning (Shahbazi and Beheshti, 2019). Such negative properties of metal pollution have been revealed for a number of soil-dwelling organisms, including many species of invertebrates (Walker et al., 2012).

Heavy metals are significant group of pollutants that have serious consequence on both human health (Yi et al., 2011) and the health of terrestrial and aquatic communities and ecosystems (Asati et al., 2016). Although mostly metals are essential for the biochemistry and physiology of organisms (e.g., iron, copper and zinc), they become toxic and carcinogenic when the level is significantly higher than the required levels (Skaldina and Sorvari, 2019). Some of these metals are not used by most organisms for any purpose and are toxic at even relatively small concentrations (e.g., Pb, As). Other shows vital physiological roles (e.g., Zn, Cu, Fe, Mn) but can also be harmful when they exceed optimal concentrations. Some metals, such as mercury, plutonium and lead, frequently called as xenobiotic metals, are not recycled by organisms in any biochemical process and can become highly toxic in concentrations further from natural levels (Newman and Clements, 2008). Insects have strong relationship with ecology and are widely used as bioindicators since long time evaluating change in environment (Khatri et al., 2020). It is stated that some insects gathered large amounts of metals in their bodies and thus result in physiological toxicity (Hsu et al., 2006; Heckel et al., 2007).

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Moreover, heavy metals would be transported to organisms on the higher position from insects along food chains (Asghar et al., 2022). In monitoring environmental heavy metal pollution, some predatory insects are used as bio-indicators because of their high abilities of accumulating metals from the ambient environment (Nummelin et al., 2007). Human activities would cause abnormity of the regional metal element geochemistry in soil. Metals in soil would firstly be absorbed by plants, and then assimilated by animals. Butterflies and grasshoppers also have ecological reliability and are sensitive to environmental changes and quality. Insects have been successfully used as bioindicators for environmental pollution and heavy metals contaminations near industrial states and even within urban areas (Parikh et al., 2021).

The lethal effects of metals can cause damage to insects in several way such as changing the duration of development; reducing the pupation rate, body weight, number of eggs laid, and hatching success; increasing mortality; and leading to declines in population size (Alajmi et al., 2019). The use of honey bees, spiders, and butterflies as a tool for monitoring environmental pollution is due to its effectiveness as an ecological detector is established by several ethological characteristics such as high rate of reproduction, great mobility, large flying range, and numerous bower examinations per day (Girotti et al., 2020). It is also effective as bioindicator because of its morphological characteristics (Murashova et al., 2020). Adelanwa et al. (2017) studied that the accumulation pattern 1510.71±166.83mg/L of copper, and the accumulation was set up to be 872.22±223.96mg/L for lead on day 18. *Salvinia molesta* evidenced to be a good phytoremediator. Azam et al. (2015) evaluated the accumulation of Cd was highest in insect species such as libellulid dragonfly (*Crocothemi sservilia*), acridid grasshopper (*Oxya hyla hyla*), and nymphalid butterfly (*Danaus chrysippus*) followed by Cu, Cr and Ni at $p<0.05$. It was pointed out that the total metal concentration in insects were significantly higher at sites S3 (Mid of Halsi Nala), S9 (End of Halsi Nala) and S1 (Start of Halsi Nala), whereas lowest value was detected at site S6 (Kalra Khasa) located far from industrial area. Perugini et al. (2011) investigated the degree of heavy metal (Hg, Cr, Cd, and Pb) pollution in honeybees (*Apis mellifera*) at their sampling station was 0.52 mg kg^{-1}. The July and September were categorized by the highest concentrations of Pb. Cd also showed statistically significant differences among areas but no statistically significant differences were found for Cr. Laing *et al*. (2002) explored higher Cd, Cu and Zn contents were established in spiders on sites having lower total metal contents in the sediment. These sites were neighboured to the river mouth and were considered by a higher salinity. Between the chloride content of the sediments and the Cd, Cu and Zn content significantly positive correlations were found in P. *piraticus*.

Some studies have revealed a negative influence of heavy metals on pollinator survival and reproduction, particularly for butterflies (Noret et al., 2007; Shu et al., 2009), but other studies suggest a lack of such an influence (Szentgyoergyi et al., 2011). Recent researches on spiders as indicator species has focused on the contamination and gathering of metals (Wilczek et al., 2004). The growth and reproduction can be suddenly reduced due to an increased detoxification effort when spiders are exposed to environmental pollutants, (Wang et al., 2018). Because of the position in the food web and following biological magnification, spiders at the individual level, have excessive potential as biological indicators of metal contamination in soil (Jung et al., 2007).

Materials and methods

Pre weight of insect samples

Placed the insects at room temperature for some time and then weighed the insect samples on microbalance before drying them in oven. This was called as pre weight.

Drying the insect samples

The insect samples were mechanically dried in the oven. The insects were placed separately in the petri dish and labelled them. Spiders were placed in oven at 100-degrees centigrade temperature for about three to four hours. Butterflies and honey bees were placed in oven at 65 degrees centigrade for four to five hours. Took the veils and labelled them according to insects label in the petri dish i.e. butterflies samples as B1, B2 B3.......B10. Honey bees' samples as H1, H2, H3.......H10. Spiders' samples were labeled as S1, S2, S3 ......S10 and placed accordingly.

Post weight of insect samples

Weighed the insect samples again on microbalance after drying them to check the moisture content.

Procedure

Each of the weighed samples was taken into the conical flask with approximately 4 mL of supra pure grade nitric acid and 1mL of perchloric acid. The ratio of mixture of supra pure grade nitric acid and perchloric acid was 4:1respectively. Then it was fitted in the distillation assemblies and the conical flask contact with the hotplate. The sample was digested at 150 centigrade temperature for about 90 minutes. During this process, the sample became transparent at first and then completely disappeared. When the sample was completely digested it indicated the completion of digestion of insect. After cooling for some time, the material is filtered using filter paper in a cylinder. Then poured the liquid samples in the labelled vials. In the same manner digested all the samples. The digested samples were diluted to 10mL using distilled water in the measuring cylinder. Took the falcon tubes and poured the liquid samples in it. For falcon tubes placement the stand was made of the spare box making falcon tubes size holes in it so that our liquid samples were not wasted.

Atomic Absorption Spectrophotometry (AAS)

The final step was heavy metal analysis of samples by atomic absorption spectrophotometer, (a model of Z-5000 Polarized Zeeman, Hitachi company manufactured in Japan) which automatically switch between the flame and graphite. The heavy metals chromium (Cr), cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni) were analyzed.

Results

Humidity and Climate

Environmental factor affects the physiology as well as pathogenicity of insect so humidity and temperature are measured. As the data was collected in different days, seasonal changes occurred along with the weather conditions thus, resulting in change in temperature and humidity. The temperature was 30°C to 38°C. The humidity was varied round about 56-66%. Humidity content in the insects were also considered by taking their weights before and after drying them in the oven as shown in the table 1.

Table 1. Weight (before and after drying) and humidity content in honey bees, butterflies and spiders.

No.	Before drying			After drying			Humidity content		
	Honey bee	Butter flies	Spiders	Honey Bee	Butter flies	Spiders	Honey bee	Butter flies	Spiders
1	0.13g	0.27g	0.03g	0.09g	0.20g	0.02g	0.04g	0.07g	0.01g
2	0.13g	0.29g	0.02g	0.10g	0.21g	0.01g	0.03g	0.08g	0.01g
3	0.14g	0.26g	0.02g	0.12g	0.22g	0.01g	0.02g	0.04g	0.01g
4	0.13g	0.21g	0.03g	0.10g	0.17g	0.02g	0.03g	0.04g	0.01g
5	0.11g	0.24g	0.03g	0.09g	0.19g	0.02g	0.02g	0.05g	0.01g
6	0.11g	0.31g	0.02g	0.09g	0.26g	0.01g	0.02g	0.05g	0.01g
7	0.11g	0.23g	0.03g	0.09g	0.20g	0.02g	0.02g	0.03g	0.01g
8	0.15g	0.24g	0.02g	0.11g	0.20g	0.01g	0.04g	0.04g	0.01g
9	0.12g	0.21g	0.02g	0.09g	0.16g	0.01g	0.03g	0.05g	0.01g
10	0.12g	0.21g	0.02g	0.10g	0.18g	0.01g	0.02g	0.03g	0.01g

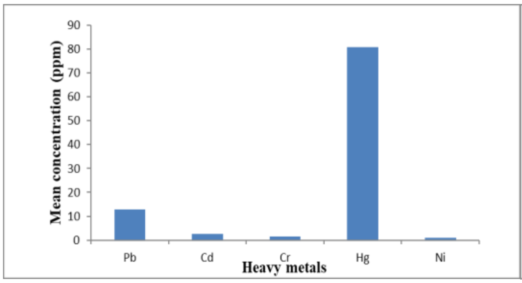
Concentration of heavy metals in insects

Mean concentrations of heavy metals (ppm) and standard deviation analyzed in the butterflies, honey bees and spiders and represented in below mentioned table 2.

Table 2. Mean concentrations of heavy metals (ppm) and standard deviation value in different insects.

No.	Butterflies					Honey bees					spiders				
	Pb	Cd	Cr	Hg	Ni	Pb	Cd	Cr	Hg	Ni	Pb	Cd	Cr	Hg	Ni
1	7.3	2.4	1.3	101.54	0.652	5.3	2.7	0.3	11.581	0.817	6.4	2.7	5	6.417	1.146
2	27.2	2.6	0.2	97.97	0.704	4.9	2.6	0.2	11.616	0.961	7.4	2.6	6.1	10.023	0.726
3	15.7	2.6	3.2	193.37	1.122	5.1	2.7	2.7	16.646	1.196	13.3	2.6	1	9.356	1.197
4	21.1	2.6	2.1	94.78	0.804	5.6	2.7	5.4	17.575	1.095	6.9	2.7	0.5	10.195	0.993
5	11.6	2.6	0.4	50.13	1.812	5.4	2.6	0.5	15.979	1.176	5.6	2.6	1	9.945	0.84
6	10.4	2.7	0.8	104.17	1.029	5.8	2.8	2.6	36.691	1.089	11.4	2.7	1.2	1.789	1.274
7	5.5	2.7	1.4	44.047	0.792	11	2.7	0.3	13.376	1.107	8.3	2.7	1.3	28.5	1.24
8	7.4	2.7	3	35.706	0.57	5	2.8	0.9	10.283	0.987	5.6	2.7	0.9	25.102	1.128
9	4.6	2.7	0.2	51.803	0.937	5.2	2.7	2.4	14.278	0.972	11.8	2.7	0.6	11.472	0.8
10	17	2.7	3.8	34.875	0.645	26.2	2.7	7.5	14.238	0.985	4.2	2.7	0.7	18.877	0.956
Mean	**12.7**	**2.63**	**1.64**	**80.84**	**0.90**	**7.95**	**2.7**	**2.28**	**16.23**	**1.04**	**8.09**	**2.67**	**1.83**	**13.17**	**1.03**
St. Dev.	**7.37**	**0.09**	**1.32**	**48.78**	**0.36**	**6.66**	**0.06**	**2.46**	**7.56**	**0.11**	**2.90**	**0.04**	**1.99**	**8.37**	**0.19**

Figure1 showed that the mercury (Hg) is in the highest concentration about 80.8% in butterflies collected from the Model town park, Lahore. The lowest concentration was found for nickel (Ni) about 0.9% in butterflies. Thus, the heavy metal concentration in butterflies in the increasing order was Hg>Pb>Cd>Cr>Ni or 80.8>12.8>2.6>1.6>0.9.



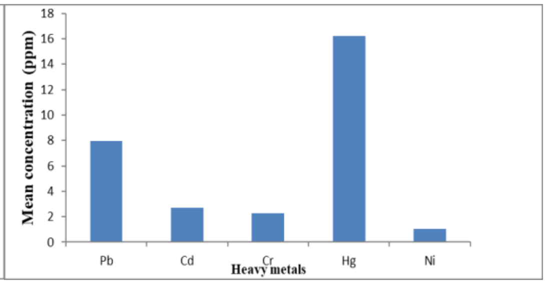


Fig. 1 **Fig. 2**

Fig. 1. Graph showing Heavy metals (Pb, Cd, Cr, Hg and Ni) concentration in butterflies collected from the Model Town Park, Lahore.

Fig. 2. Bar graph shows Heavy metals (Pb, Cd, Cr, Hg and Ni) concentration in honey bees collected from the Model Town Park, Lahore.

Figure 2 showed that the mercury (Hg) is in the highest concentration about 16.2% in honey bees collected from the Model town park, Lahore. The lowest concentration was found for nickel (Ni) about 1% in butterflies. Thus, the heavy metal concentration in honey bees in the increasing order was Hg>Pb>Cd>Cr>Ni (16.2>7.95>2.7>2.28>1.0). Figure 3 showed that the mercury (Hg) is in the highest concentration about 13.2% in spiders collected from the University of the Punjab, Lahore. The lowest concentration was found for nickel (Ni) about1% in spiders. Thus, the heavy metal concentration in butterflies in the increasing order was Hg>Pb>Cd>Cr>Ni (13.2>8.0>2.7>1.8>1.0). Figure 4 showed that lead was detected in elevated level in butterflies and lowest level in the honey bees. Butterflies>spiders>honey bees (12.78>8.09>7.95). Figure 5 depicted the elevated level of cadmium (Cd) in honey bees and minimum level in butterflies. Honey bees>spiders>butterflies (2.70>2.67>2.63).

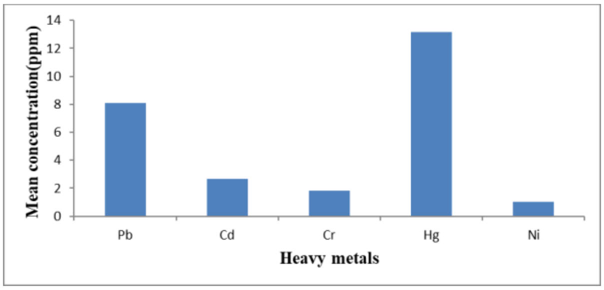
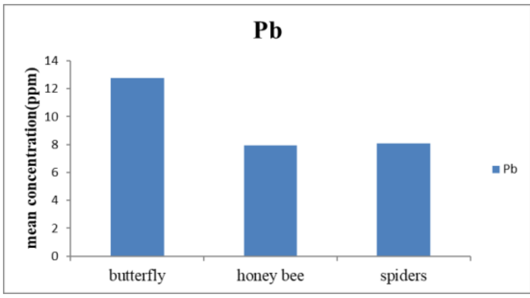


Fig. 3. Bar graph shows Heavy metals (Pb, Cd, Cr, Hg and Ni) concentration in spiders collected from the University of the Punjab, Lahore.



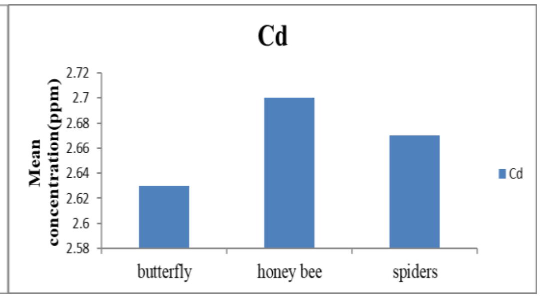
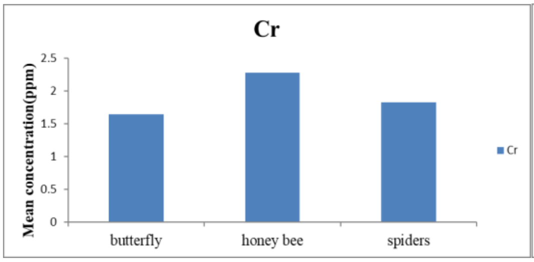


Fig. 4 **Fig. 5**

Fig. 4. Lead (Pb) concentration in different insects collected from Model Town Park, Lahore and University of the Punjab.

Fig. 5. Cadmium (Cd) concentration in different insects collected from Model Town Park, Lahore and University of the Punjab.

Figure 6 showed that chromium (Cr) was detected in elevated level in honey bees and minimum level in butterflies. Honey bees>spiders>butterflies (2.28>1.83>1.64). Figure 7 depicted the elevated level of mercury (Hg) in butterflies and in minimum level in spiders. Butterflies>honey bees>spiders (80.8>16.2>13.2).



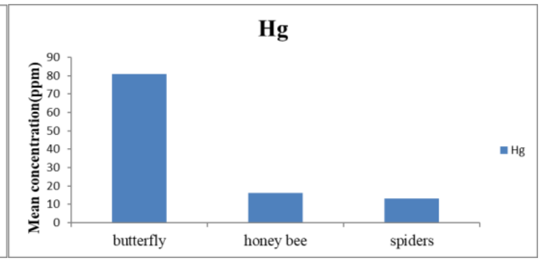


Fig. 6 **Fig. 7**

Fig. 6. Chromium (Cr) concentration in different insects collected from Model Town Park, Lahore and University of the Punjab.

Fig. 7. Mercury (Hg) concentration in different insects collected from Model Town Park, Lahore and University of the Punjab.

Figure 8 indicated elevated level of nickel (Ni) in honey bees and in minimum level in butterflies. Honey bees>spiders> butterflies (1.04>1.03>0.91).

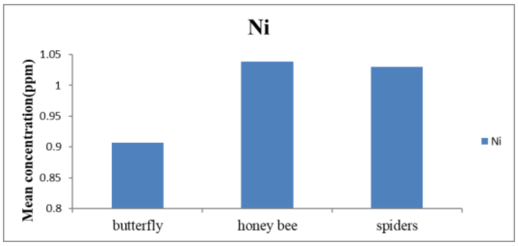


Fig. 8. Nickel (Ni) concentration in different insects collected from Model Town Park, Lahore and University of the Punjab.

Data was statically analyzed using one-way ANOVA with SPSS 15.0 with significance value of 0.05, post hoc was applied in case of $p < 0.05$. The interpretation for lead was honey bees, spiders and butterflies were non-significant There was no significant difference in lead, chromium and cadmium concentration of honey bees, spiders and butterflies. Honey bees varied significantly with the spiders and butterflies. For mercury, the spiders and honey bees were non-significant and the butterflies were significant with the spiders and honey bees. For nickel, the butterflies, spiders and honey bees were found non-significant. No significant difference was found in overall heavy metal concentration of different insects. These were the following consideration of Duncan's test for Pb, Cd, Cr, Hg and Ni.

Table 3. Duncan's test for lead

Insects	N	Subset for alpha = 0.05
		1
Honey bee	10	7.9500
Spiders	10	8.0900
Butterfly	10	12.7800
Sig.		.099

The interpretation for lead was honey bees, spiders and butterflies were non-significant.

Table 4. Duncan's test for cadmium

Insects	N	Subset for alpha = 0.05	
		1	2
Butterfly	10	2.6300	
Spiders	10	2.6700	2.6700
Honey bee	10		2.7000
Sig.		.228	.363

For cadmium the butterfly and spiders were non-significant and spiders and honey bees were also non-significant. Honey bees were significantly with the spiders and butterflies.

Table 5: Duncan's test for chromium

Insects	N	Subset for alpha = 0.05
		1
Butterfly	10	1.6400
Spiders	10	1.8300
Honey bee	10	2.2800
Sig.		.503

For chromium, the butterflies, spiders and honey bees were non-significant.

Table 6: Duncan's test for mercury

Insects	N	Subset for alpha = 0.05	
		1	2
Spiders	10	13.1676	
Honey bee	10	16.2263	
Butterfly	10		80.8415
Sig.		.815	1.000

For mercury, the spiders and honey bees were non-significant and the butterflies were significant with the spiders and honey bees.

Table 7: Duncan's test for nickel

Insects	N	Subset for alpha = 0.05
		1
Butterfly	10	0.9067
Spiders	10	1.0300
Honey bee	10	1.0385
Sig.		0.271

For nickel, the butterflies, spiders and honey bees were non-significant. The above study indicated that these insect groups were potential indicators of metal contamination and can be used in biomonitoring.

Discussion

Heavy metals are an important class of pollutants having major damaging effects on human health as well as ecosystems (Alengebawy et al., 2021). The primary focus of the present study was on the analysis of the commonly studied heavy metal pollutants including Cd, Cr, Hg, Pb and Ni and Zn in the three taxa of insects i.e., butterflies, honey bees and spiders through Atomic Absorption Spectrophotometry.

Biological indicator species are species that can be measured as substitutes for environmental health and levels of pollution before adverse environmental influences become recognizable (Parikh et al., 2021). It describes to the accumulation of metals, emphases on the response of the indicator species to determine, for example, the rate and range of metal accumulation in the body. A great deal of consideration has been given to the effects of metal pollution on insects (Singh et al., 2022). In observing environmental heavy metal pollution, some predatory insects are used as bio-indicators because of their high capabilities of accumulating metals from the ambient environment (Nummelin et al., 2007). The present study on the heavy metal analysis of butterflies showed that the mercury (Hg) was in the highest concentration about 80.8% in butterflies collected from the Model town park, Lahore and the lowest concentration was found for Nickel (Ni) about 0.9% in butterflies as shown in figure 1. Thus, the heavy metal concentration in butterflies was higher for mercury (80.8ppm) followed by lead (12.8ppm), cadmium (2.6ppm), chromium (1.6ppm) and nickel (0.9ppm). In contrast to this study the (Azam et al., 2015) observed the site and species dependent metal accumulation patterns for Cd, Ni, Cr, Zn, and Cu in butterfly. Significantly elevated concentrations were found for Cd, Cr, and Cu in "Mid of Halsi Nala" > "End of Nala" > "End of Nala" and it thus confirms the expectation that animal body's burden reflects site pollution ($p < 0.05$). The accumulation pattern for copper was 1510.71±166.83mg/L and the accumulation was set up to be 872.22±223.96mg/L for lead in *Salvinia molesta* (Adelanwa et al., 2017).

Honeybees (*Apis mellifera)* have great potential for detecting and monitoring environmental pollution, given their wide-ranging foraging behaviour. Bees are one of the most important groups of pollinators in the temperate zone. Although heavy metal pollution is recognized to be a problem affecting large parts of the world, we currently lack insights into the effects of heavy metals on wild bee survival and reproduction. The present study on the heavy metal analysis of honey bees showed that the mercury (Hg) was in the highest concentration about 16.2% in honey bees collected from the Model town park, Lahore. The lowest concentration was found for Nickel (Ni) about 1% in butterflies. Thus, the heavy metal concentration in honey bees was higher for mercury (16.2ppm) followed by lead (7.95ppm), cadmium (2.7ppm), chromium (2.28ppm) and nickel (1.0ppm) as shown in the figure 2. The levels of Cd, Cr, and Pb found in the samples of honey bees were reported (Perugini et al., 2011) and showed contrast with the present results, Cr was in the highest mean concentrations (0.74 mg kg^{-1}) with respect to the tested heavy metals ($p<0.01$). Considering the location of the sampling stations, no significant difference was detected among the different sites ($p>0.05$), though the absolute highest value of 5.07 mg kg^{-1} was found in the sampling station 1 during the month of July.

Analysis of heavy metals in the polyphagous, ground-dwelling spiders the mercury (Hg) was in the highest concentration about 13.2% in spiders collected from the University of the Punjab, Lahore. The lowest concentration was found for Nickel (Ni) about1% in spiders. As figure 3 showing that the heavy metal concentration in spiders was higher for mercury (13.2ppm) followed by lead (8.0ppm), cadmium (2.7ppm), chromium (1.8ppm) and nickel (1.0ppm). This was supported by the study of (Zhang et al., 2009) in which the mercury was greatly accumulated than the cadmium and lead in the carnivorous insects as expected when the food chain stretched to the secondary consumers. The concentration factors of the carnivorous insect (spiders) food chain was7.88ppm for mercury 0.48ppm and for cadmium 0.57ppm for lead. Thus, resulted that the concentration factors depended on the metal and insect species of food chains.

The concentration of lead in three insect taxa revealed that the lead was in elevated level in butterflies (12.78) and lowest level in the honey bees (7.95) as shown in figure 4. The concentration of lead in the butterflies, spiders and honey bees were 12.78ppm and 8.09ppm and 7.95ppm. The lead (Pb) concentration recorded in honey bees in different studies in different years that contrast the present results in ug/g were 0.19–1.67 (Steen et al., 2012), 0.52–1.00, 0.64–1.25 (Conti and Botre, 2001), 0.58–0.62, 0.27–1.4 (Fakhimzadeh and Lodenius, 2000), 0.15–0.55, 0.45–0.95 (Porrini et al., 2002), 0.28–0.29 and 0.64–1.01 (Roman, 2005).

Irrespective of the site, the lowest lead concentration was detected in the web-building L. *triangularis*, where the metal level was almost three times lower than in remaining species. . (Wilczek and Babczynska, 2000) The highest Pb concentration was found in the hepatopancreas of the wandering P. *amentata* from Losien, and was on average twice as high as in the organs of web-building species: M. *segmentata*, A. *labyrinthica* and in both species of Araneidae and exceeded by 8 times the Pb concentration in the hepatopancreas of Linyphiidae. The pattern of

Pb accumulation in ovaries of the analysed species was similar as stated for the hepatopancreas. The highest concentration of the metal was detected in the ovaries of web-building A. *labyrinthica* and wandering spider's P. *amentata* from Katowice Steelworks area. Only in L. *triangularis* was the level of Pb higher in specimens from the reference site.

The figure 5 showing the cadmium (Cd) accumulation detected in three insect taxa exhibited that it was in elevated level in honey bees (2.70ppm) and minimum level in butterflies (2.63ppm). The concentration of cadmium detected in the honey bees, spiders and butterflies were 2.70ppm, 2.67ppm and 2.63ppm respectively. The cadmium (Cd) concentration in microgram per gram found by different scientists in honey bees were 0.05–0.75 ($\mu g\ g^{-1}$) (Steen et al., 2012), 2.89–3.43(Conti and Botre, 2001) 2.87–4.23 (Conti and Botre, 2001),0.03–0.18, 0.05–1.2 (Fakhimzadeh and Lodenius, 2000), 0.14–0.16 and 0.10–0.17 (Roman, 2005).

Ground spider's P. *amentata* and web-building spiders L. *triangularis* from Beskid SlaskiMts accumulated significantly more cadmium in the hepatopancreas than individuals of same spider species from Katowice Steelworks area. The highest Cd concentration was detected in the hepatopancreas of the web-building M. *segmentata* from Łosień and of the ground-living P. *amentata* from the reference site. However, only in case of M. *segmentata* was the accumulation of cadmium from Silesia twice as high as in spiders from the reference site. The lowest level of the metal was found in the hepatopancreas of A. *labyrinthica* and L. *triangularis* collected in Katowice Steelworks area (Wilczek and Babczynska, 2000).

The elevated chromium (Cr) concentration was detected in honey bees and minimum level was detected in butterflies in heavy metal analysis through Atomic Absorption Spectrophotometer (AAS). The accumulation level of chromium in honey bees, spiders and butterflies was 2.28ppm, 1.83ppm and 1.64ppm respectively as shown in figure 6. The chromium (Cr) concentration in microgram per gram found by different scientists in honey bees were 0.15–0.28 (Steen et al., 2012), 0.054–0.080, 0.052–0.116 Sites (Conti and Botre, 2001), 0.1–3.6, 0.1–1.2 (Porrini et al., 2002), 0.05–0.18, 0.16–0.23 (Roman, 2005).

The levels of Cr found in samples coming from sites 2, 3, 4, and 5 vs. 6 and 7 did not show significant differences ($p>0.05$), although the sampling sites were located in different regions (Perugini et al., 2011). Independently from the origin of samples, Cr was reported to be the highest mean concentration (0.97 $mg\ kg^{-1}$) in bees collected during August, while the lowest concentration was found in October (mean value of 0.33 $mg\ kg^{-1}$. These bees also reported the Cd lowest mean value (0.04 $mg\ kg^{-1}$). The temporal trend for Cd was not of particular interest. This metal did not show any statistically significant difference of distribution in comparison among months ($p >0.05$). For Pb, independently from the sampling month, honeybees caught in the hives located near Ciampino airport (site number 8) showed a significant difference ($p<0.01$), with a lead mean value of 0.52 $mg\ kg^{-1}$, compared with all other bees coming from the sites 1, 3, 6, and 7 but not with those of sites 2, 4, and 5 ($p>0.05$). Pb concentrations did not show peak values in a particular month; on the contrary, they were distributed in a homogeneous manner during all months ($p>0.05$) (Perugini et al., 2011). Mercury is one of the greatest toxic heavy metals and known as environmental pollutant. Due to the movement and abundance of mercury, the effectiveness of insects as bio monitors for metal pollution. In butterflies the mercury (Hg) was detected in elevated level and in minimum level in spiders as shown in figure 7. The concentration of mercury in butterflies, honey bees and spiders were 80.8ppm, 16.2ppm and 13.2ppm respectively. The suggested reason for higher level of mercury was the mercury sources in the sampling areas.

Data was procured in this study showed that the source of mercury in the environment was water irrigated to the fields, canal water and industrial wastes (Fayiga et al., 2018). The waste from the industries moved to the canal water and thus that polluted water was irrigated to the fields. The pollutants were assimilated by the roots of the pants, transferred to the herbivorous insects and from them to the secondary consumers and thus disturbed the whole ecosystem by the most common mercury sources that were the pesticides, fertilizers, industries etc. It was reported in support by (Zhang *et al.*, 2009) that concentration factors varied with metals and food chains.

Spiders and mantis mainly feed on *Locusta migratoria manilensis* and *Acrida chinensis* in summer grassland. Mercury, cadmium and lead concentrations of spiders were 1.48 mg kg kg^{-1}, 3.04 mg $kg^{-1}$and 1.93 mg kg^{-1} times of those of *Locusta migratoria manilensis*, respectively, but lower than those of *Acrida chinensis*. Mercury, cadmium and lead concentrations of mantis were 21.55 mg kg^{-1}, 1.13 mg kg^{-1} and 7.92 mg kg^{-1} times of those of *Locusta migratoria manilensis*, respectively; 3.54 mg kg^{-1}, 0.28 mg kg^{-1} and 1.53 mg kg^{-1} times of those of *Acrida chinensis*, respectively. It demonstrated that mantis accumulated metals more easily than spiders (Zhang et al., 2009).

The figure 8 showed the lowest concentration of nickel (Ni) in the butterflies than other insect species and highest in the honey bees. The concentration of nickel metal in the honey bees, spiders and butterflies was 1.04ppm, 1.03ppm and 0.91ppm respectively. The (Ni) concentration in micro gram per litre documented by different studies in different years in honey bees were 0.19–0.47 (Steen et al., 2012), 0.12–0.42, 0.13–0.43 (Porrini et al., 2002), 0.27–0.42 and 0.36–0.50 (Roman, 2005). The nickel sources in the environment of Lahore were vehicles, nickel plated material, steel and jewelry. The proposed reason for lowest level of nickel was the less sources of nickel in that particular environment.

Conclusion

The focus of the present study was on the analysis of the commonly studied heavy metal pollutants and it was concluded that the mercury (Hg) was in the highest concentrations and the Nickel (Ni) was found in the lowest concentration in all three insect taxa. The proposed reason was the fertilizers used, water irrigation and air pollution in the sampling area. Although there was no significant difference found in all the three insects under observation. Metals were essential for the biochemistry and physiology of insects but if it was higher in concentration than the required level it may cause damaging effects such as change in the duration of development, number of eggs laid and increasing mortality, thus leading to decline in number of population size. Steps should be taken to minimize the accumulation by which mortality can be diminished so that the insects can enhance the survival with contamination at the expense of additional energy.

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SR, NV, FM and ZA conceived the concept, wrote and approved the manuscript.

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