Research Article

Heavy metal analysis in mosquitoes, collected from different sites of Lahore

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Abstract | The concentration of heavy metals (Fe, Cu, Zn, Hg, Ni) was measured in mosquitoes collected from three different sites of Lahore (Sadoki, Chungi Amar Sidhu and Kalma Chowk). Mosquitoes were used for heavy metal analysis because they are effective ecological and biological indicator of pollution. Heavy metals were analyzed by atomic absorption spectrophotometer followed by digestion. Descriptive statistics analysis shows the results that the mean concentration for mercury was 40.5426, 31.9116 and 18.6504ppm, respectively. Furthermore, mercury and iron were significant for these sites and all the sites showed the highest level for Mercury (Hg) and lowest level for Nickle (Ni). The *p*-value for Mercury (Hg) and Iron (Fe) was 0.055 and 0.00 respectively which shows significance while the *p*-value for Nickle (Ni), Zinc (Zn) and Copper (Cu) was 0.797, 0.473, 0.260 respectively, showing non-significance.

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1. Introduction

Elements having metallic properties are called heavy metals, irrespective of their atomic mass. Metallic elements have a relatively high density compared to water. There has been an increasing global public health concern associated with ecological contamination due to these metals (Bradl, 2002). Agricultural, industrial, pharmaceutical, geogenic, atmospheric and domestic effluents are sources of heavy metal contamination and environmental pollution. Point source areas influencing environmental pollution are foundries and smelters, mining operations and other such metal-based industrial practices (Starter *et al*., 2010).

Although heavy metals occur naturally in the earth's crust, most environmental heavy metal contamination and pollution is due to anthropogenic activities such as industrial production, mining and smelting operations and agricultural use of metals and metal-containing compounds (Arruti *et al*., 2010). Environmental contaminants affect the immune function of both insects and humans. Heavy metals accumulate easily in insects (Vickerman and Trumble, 2003). Insects found near industrial states have been successfully used as bio-indicators for heavy metal environmental pollution (Chen *et al*., 2005). A bio- indicator is a living organism that gives us an idea of the health of an ecosystem. Among all organisms, insects have contributed to a greater degree to a practical assessment of the sustainability degree (Lopez, 2008). The characteristics of insects, used for the bio-indication of the environment are; their richness and species diversity, easy handling, ecological faithfulness as many species have low tolerance to abiotic factors which creates a relationship between certain insect groups with certain habitat, fragility to small changes and organisms responses for the identification of environmental changes (Tylianakis *et al*., 2004).

Insects which belong to order Diptera and family Sarcophagidae, include flies and mosquitoes which are good bio-monitors of heavy metals on land. Mosquitoes larvae could be used to trace heavy metal pollution. Although mosquitoes transmit diseases, however larvae of *Culiseta subochrea* and *Aedes caspius* may be used as valuable tools for aquatic environmental monitoring (Mausavi *et al*., 2003). High levels of human perturbation and pollution have resulted in lower *Anopheles* mosquito populations in urban environments than in rural environments limiting the role on *Anopheles* mosquito (Mireji *et al*., 2007). Whereas, *Aedes aegypti* is well adapted to survive and develop in the anthropogenic aquatic bodies in urban environments (Huber *et al*., 2008). Their breeding sites can be characterized by heterogeneous and dynamic conditions like varying nutrient content, presence of toxic metals, metal-containing herbicides or other physiological stressors (Mireji *et al*., 2008).

Kitvatanachal *et al*. (2005) performed laboratory investigations on *Culex quinquefasciatus* mosquitoes to examine the effects of lead (Pb) toxicity and lead (Pb) uptake. In lab tests, 0.05, 0.1 and 0.2 mg per litre concentrations of lead nitrate were used. The results showed that hatching, egg production and emergence rates considerably reduced because of Lead (Pb), in comparison with the unexposed group ($p<0.05$) determined by atomic absorption spectrometer (AAS). Hammerschmidt and Fitzgerald (2005) demonstrated a link between loadings of inorganic Hg and accumulation of methylmercury (MeHg) in aquatic biota.

Mosquitoes (Diptera: *Culicidae*) have aquatic lifestyles stages which might be beneficial indictors of Hg contamination or MeHg accumulation in aquatic ecosystems. Nummelin *et al*. (2007) investigated heavy metal concentration in various predatory insects collected from close to a steel factory and from control sites. Atomic absorption spectrophotometer (AAS) was used to analyse ants (*Formicidae*), antlion larvae (*Myrmeleontidae*), dragon fly larvae (*Odonata*) and water-striders (*Gerridea*). Metal concentration in insects close to the manufactory was higher while the water-striders had higher cadmium (Cd) concentration in control area. Discriminant analysis showed that all insect groups could be used as heavy metal indicators Variations in Iron (Fe) were easily detected by water-striders. Antlions were effective in accumulating Manganese (Mn) and cadmium (Cd) while ants were efficient in accumulating Manganese (Mn) only. Water-striders are poor in accumulating Lead (Pb) however, antlions and ants are effective.

According to Mireji *et al*. (2010), heavy metal pollution in diverse habitats is swiftly increasing. Mosquitoes species such as the Anopheles, which is a major malaria vector species (Diptera: *Culicidae*) seemed to tolerate metal contaminated aquatic habitats, despite their normal proclivity for ‘clean’ water. Atafar *et al*. (2010) reported that the global natural ecosystems are negatively impacted by human interventions and heavy metals pollution of aluminium, cadmium, chromium, lead, mercury, nickel and zinc in the environment. The toxicity of heavy metals is increasing in the air, water and soil, therefore becoming part of natural biogeochemical cycle (Lee *et al*., 2006). Nasirian *et al*. (2014) evaluated the quantity of some trace metals in mosquito larvae in Shadegan International Wetland from Iran. Azam *et al*. (2015) studied the accumulation and contamination of heavy metals (Cd, Cr, Cu, Ni and Zn) in ecology of industrial area in district Gujrat of Punjab, Pakistan.

2. Materials and Methods

The present study was conducted from March, 2018 to May, 2018 for the heavy metal analysis in mosquitoes from three different sites of Lahore, Sadoki, Kalma chowk and Chungi Amar Sidhu. The sample size was five for each site. The heavy metals which were analyzed are iron (Fe), zinc (Zn), copper (Cu), Nickle (Ni) and Mercury (Hg). The data was analyzed statistically by SPSS and ANOVA. Duncan's test was also applied as shown in graphs and tables.

2.1. Study Area and Sampling

Mosquitoes were collected from three sites in Lahore which is the second largest city of Pakistan. Vehicular exhaust emission is the main contribution to heavy metal environmental pollution in Lahore. The sites chosen for survey and study were Sadoki, Chungi Amar Sidhu and Kalma Chowk. Five samples were collected from each site thus the total number of samples was fifteen. The mosquitoes were selected to be used as bio indicators due to their ubiquitous nature and relatively easy collection, ecological importance, aquatic life stages (larval and pupal stages), and sensitivity to natural and anthropogenic sources of heavy metals. As mosquito growth occurs during the larval stage while feeding on microorganisms and particulate organic detritus thus, accumulation during the aquatic life stages is the primary source of heavy metals in mosquito populations. Therefore the adult mosquitoes provide a simple and useful measure of local heavy metal contamination.

2.2. Collection of Mosquitoes

Mosquitoes were collected using net trap then they were enclosed in the glass vials. The mosquitoes collected from each of these three sites (Kalma Chowk, Chungi Amar Sidhu, Sadoki) and were kept separately in their respective vial.

2.3. Weighing and Drying

Before drying the mosquitoes they were pre-weighed on the digital micro-balance. Microwave oven (Thermoline Scientific) was used to dry the samples at the temperature of +60ºC for 2 hours and after that they were post weighed. Three groups of samples of these three sites were weighed about 0.002g each.

2.4. Digestion

Mosquitoes were treated chemically for digestion. Each sample of these three groups was heated separately in 5 ml of concentrated nitric acid for 2 hours at 50ºC and after that for 6 to 8 hours at 130ºC on a magnetic stirrer hot-plate (PCSIR). After heating in 5ml of concentrated nitric acid, 5ml of hydrogen per oxide was added and heated again for an additional 5 hours. The solution was poured into the samples using a single channel CAPP auto-clavable (100-1000µl) micro-pipette. During the digestion process, the sample became transparent at first and then completely disappeared. This disappearance indicated the completion of digestion process.

2.5. Filtration

After digestion, samples were filtered by using Whatman uniflo 0.2µ pore syringe filter paper which is used for rapid syringe filtration of small volume samples. After filtration, the samples were diluted with 10ml of distilled water.

2.6. Atomic Absorption

Samples were analysed for heavy metals by atomic absorption spectrophotometer (AAS 8010 fully automatic flame or graphite furnace AAS) which automatically switched between the flame and graphite. The heavy metals which were analysed are copper (Cu), Zinc (Zn), mercury (Hg), Iron (Fe), and Nickle (Ni). Among these metals copper (Cu), Zinc (Zn) and Iron (Fe) were analysed by flame AAS (atomic absorption spectrophotometer) while Nickel (Ni) and mercury (Hg) were analysed by graphite furnace AAS (Atomic absorption spectrophotometer).

3. Results

Heavy metal analysis by atomic absorption depicting each metal in the collected samples is represented by figures and tables provided below. Mercury content in mosquitoes was found to be the highest at Sadoki. The order of heavy metals contents was as follows;

Hg >Fe >Cu >Zn >Ni
40.5426 > 7.44 >2.88 >2.54 >0.616

At site 2, Chungi Amar Sidhu the mercury content was found to be the highest while zinc concentration was the lowest. The order of heavy metals contents was as follows;

Hg >Fe >Cu >Ni >Zn
31.9116 >5.06 >0.84 >0.6896 >0.44

At site 3, Kalma Chowk the mercury content was highest while nickel content was the lowest. The order of heavy metals contents was as follows;

Hg >Fe >Zn >Cu> Ni
18.6504 >6.44 >4.78 >0.7 >0.613

Table 1: Mean Concentration (ppm) of heavy metals in mosquitoes collected from Site 1 (Sadoki)

No. of Samples	**Heavy metals**				
	Fe	**Zn**	**Cu**	**Ni**	**Hg**
1.	7.8	-0.7	1.4	0.991	34.385
2.	6.3	11.1	0.9	0.632	44.341
3.	5	-1.1	1.7	0.406	49.817
4.	8.3	3.3	9.5	0.306	38.105
5.	9.8	0.1	0.9	0.745	36.065
Mean(ppm)	7.44	2.54	2.88	0.616	40.5426

Table 2: Mean Concentration of heavy metals in mosquitoes collected from site 2 (Chungi Amar Sidhu)

No. of samples	**Heavy metals**				
	Fe	**Zn**	**Cu**	**Ni**	**Hg**
1.	5.7	-1.1	1.1	0.614	36.549
2.	7.7	-0.1	1.9	0.451	32.939
3.	2.3	-2.8	0.8	0.57	39.152
4.	3.6	4.3	0.2	0.951	26.207
5.	6	1.9	0.2	0.862	24.711
Mean(ppm)	5.06	0.44	0.84	0.6896	31.9116

Table 3: Mean Concentration (ppm) of heavy metals in mosquitoes collected from site 3 (Kalma Chowk)

No. of samples	**Heavy metals**				
	Fe	**Zn**	**Cu**	**Ni**	**Hg**
1.	5.8	0	0.3	0.617	20.923
2.	9.3	11.6	1.1	0.647	18.499
3.	4.3	-0.7	0.4	0.641	18.086
4.	8	14.1	0	0.659	16.96
5.	4.8	-1.1	1.7	0.501	18.784
Mean(ppm)	6.44	4.78	0.7	0.613	18.6504

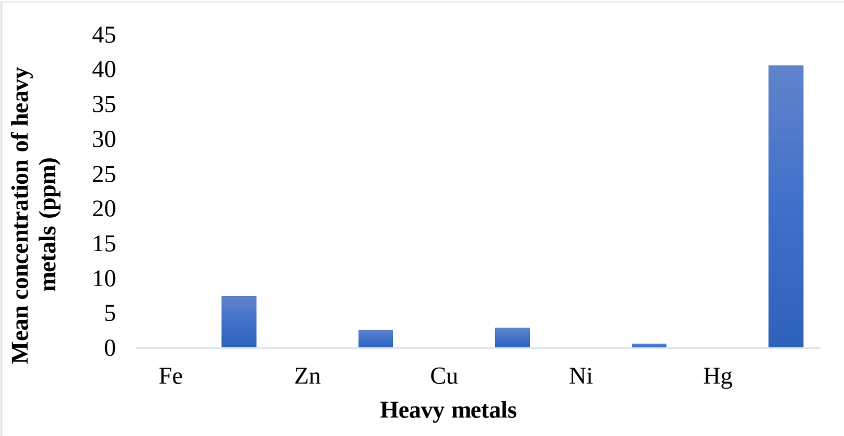

Figure 1: Heavy metal concentration (ppm) at site 1

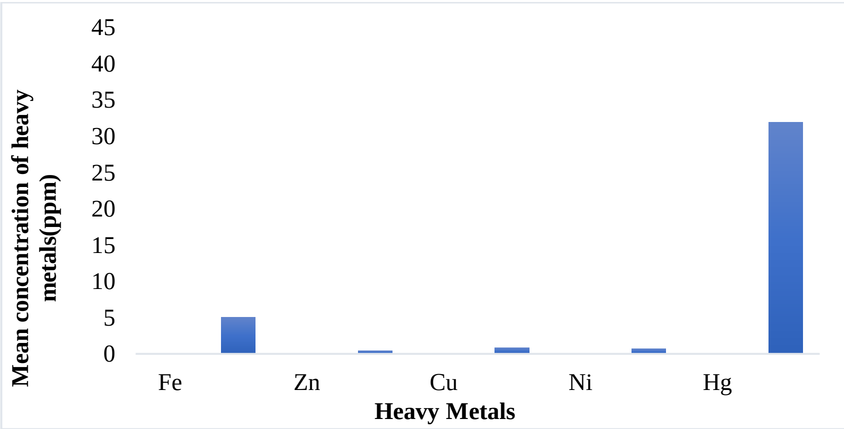

Figure 2: Showing the heavy metals concentration at site 2

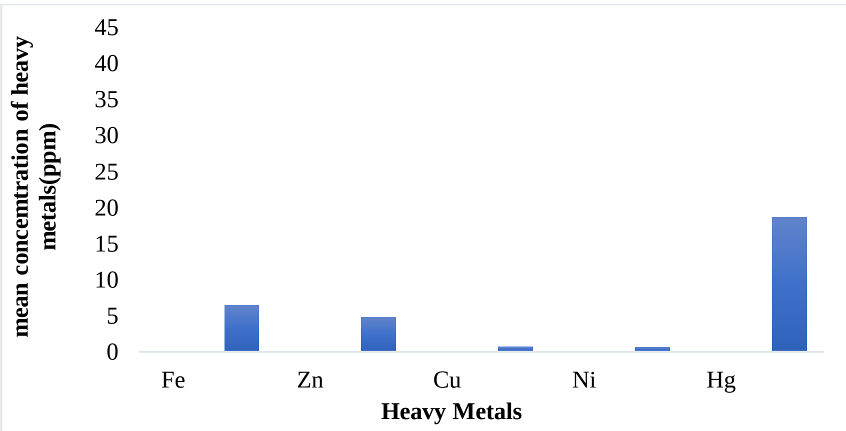

Figure 3: Showing the heavy metals concentration at site 3 (Kalma Chowk)

3.1. Concentration of Ni

Chungi Amar Sidhu had the highest concentration of Ni while the lowest at Kalma Chowk.

Site 2> Site 1> Site 3
Chungi Amar Sidhu> Sadoki > Kalma Chowk
0.6896> 0.616 > 0.61

3.2. Concentration of Hg

Sadoki had the highest concentration of Hg and lowest at Kalma Chowk.

Site 1 > Site 2 > Site 3
Sadoki> Chungi Amar Sidhu > Kalma Chowk
40.5426 > 31.9116 >18.6504

3.3. Concentration of Fe

Sadoki had the highest concentration of Fe whereas the lowest concentrations were observed in samples collected from Chungi Amar Sidhu.

Site 1 > Site 3 > Site 2
Sadoki> Kalma Chowk > Chungi Amar Sidhu
7.44 > 6.44 > 5.06

3.4. Concentration of Cu

Sadoki had the highest concentration of Cu and lowest at Kalma Chowk.

Site 1 > Site 2 > site 3
Sadoki> Chungi Amar Sidhu > Kalma Chowk
2.88 > 0.84 > 0.7

3.5. Concentration of Zn

Kalma Chowk had the highest concentration of Zn and lowest at Chungi Amar Sidhu.

Site 3 > Site 1 > Site 2
Kalma Chowk> Sadoki> Chungi Amar Sidhu
4.87 > 2.54 > 0.44

Data was statistically analyzed using one-way ANOVA with SPSS 15.0 with significant value $p<0.05$.

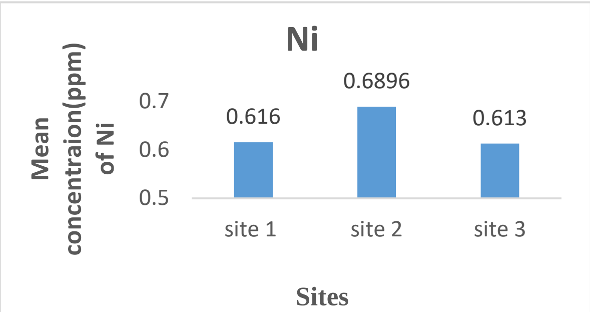

Figure 4: Ni concentration at each site

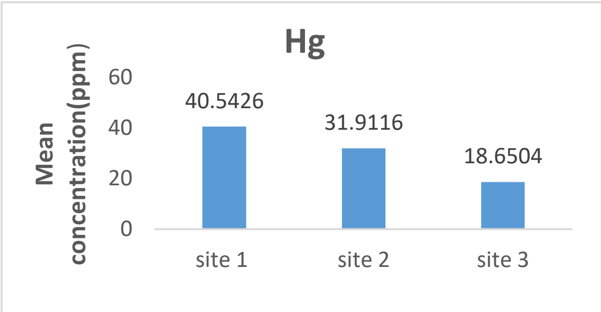

Figure 5: Hg concentration at each site

Figure 6: Fe concentration at each site

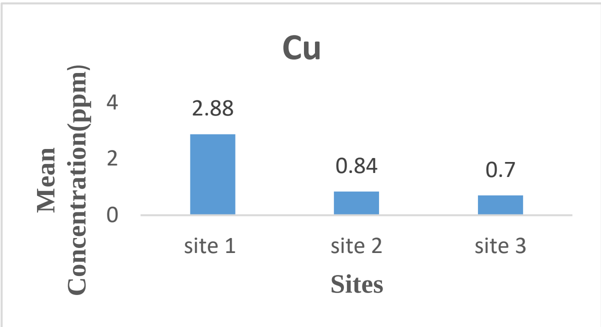

Figure 7: Cu concentration at each site

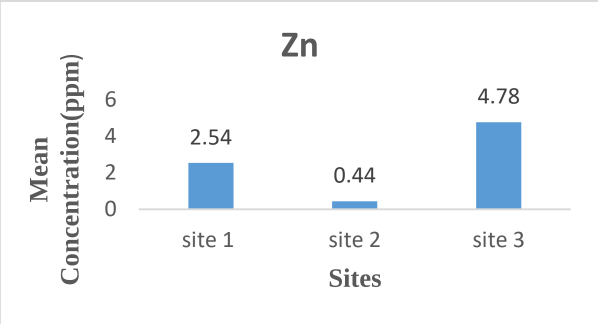

Figure 8: Zn concentration at each site

3.6. Duncan's Test

These are the following considerations of Duncan's test for Ni, Zn, Hg, Cu and Fe. Duncan's test showed that iron (Fe) and Mercury (Hg) show significance while Nickle (Ni), Zinc (Zn) and Copper (Cu) showed non-significance. These results showed remarkable site based variation in metal concentration and their bioaccumulation in mosquitoes.

Table 4: Duncan's test showing the level of significance

	Duncan's Test				
No.	**Heavy metals**	**Sites**			**Sig.**
		Site 1	**Site 2**	**Site 3**	
1.	Zn	2.5400	0.4400	4.7800	0.253
2.	Cu	2.8800	0.8400	0.7000	0.165
3.	Hg	18.65040	31.91160	40.54260	1.000
4.	Ni	0.61600	0.68960	0.61300	0.579
5.	Fe	7.4400	3.3320	6.4400	0.071

4. Discussion

Heavy metals are naturally occurring elements which are important for the earth's crust but excessive accumulation of heavy metals leads to the environmental pollution that drastically affects the living creatures or even causes physiological changes in them. Most environmental contamination and human exposure results from anthropogenic activities such as industries, vehicle exhaust, mining, tanneries, excessive use of pesticides etc.

Thus, to determine the heavy metals pollution level, heavy metal analysis for Fe, Cu, Zn, Hg, Ni was carried out in current study by using mosquitoes as a bio-indicator, collected from different sites in Lahore (Sadoki, Chungi Amar Sidhu and Kalma Chowk). Among all the metals Hg is a highly toxic metal. These sites and specifically Lahore was selected because with the rapid urbanization, deforestation, there is also an increase in the anthropogenic activities such as industrialization, use of auto-mobiles, batteries vehicle exhaust, pesticides and chemical use which are beneficial in one way but not eco-friendly as they accumulate heavy metals in the environment.

Descriptive statistics show that mercury and iron concentrations were significant for these sites and all the sites showed the highest level for Mercury (Hg), however, the lowest levels were discovered for Nickel (Ni). The *p*-value for mercury (Hg) and Iron (Fe) were 0.055 and 0.00 respectively which shows significance while the *p*-value for Nickel (Ni), Zinc (Zn) and Copper (Cu) were 0.797, 0.473, 0.260 respectively, showing non-significance.

In current study, significant Fe was discovered at all analysed site. Similar results were reported in a study conducted to analyse the impact of heavy metals (Cd, Cr, Cu, Fe, Pb, Mn and Zn) on the presence of *Anopheles gambiae*, *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles funestus* larvae in Kisumu and Malindi, Kenya. In aquatic larval habitats significant concentrations of Mn and Fe discovered (Mireji *et al*., 2007). Nummelin *et al*. (2007) conducted a study on heavy metal concentration of various predatory insects close to a steel factory. Metal concentration was found to be higher in insects close to the manufactory.

Azam *et al*. (2015) studied the accumulation and contamination of heavy metals (i.e, Cd, Cr, Cu, Ni, and Zn) in industrial areas of district Gujrat, Punjab. In insect species, Cd accumulation was highest followed by Cu, Cr, Zn, and Ni at $p < 0.05$ while mercury (Hg) level was found highest among all heavy metals. Metal concentrations in insects were significantly higher close to the industries and nallahs which concur results in present research work.

Nasirian *et al*. (2014) evaluated the quantity of some trace metals of mosquito larvae in Shadegan international Wetland from Iran. They analysed mosquitoes for As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, and Zn. Results confirmed that besides As and Hg, the waterbed sediment and *Cu. subochrea* larvae were polluted with all trace metals. These results are contradictory to present work since all the mosquitoes samples collected from different sites possessed the heavy metals contamination.

A study was undertaken in Lagos, Nigeria to review the *Anopheles* breeding in contaminated water bodies during a malaria upward thrust due to rapid industrial enterprise and developmental activities. Atomic Absorption Spectro-photometry was used to investigate the level of heavy metals (Zn, Co, Cu, Pb, Mn, Fe, Hg and Ni) pollution. The degree of Fe, Cu and Pb was thrice at polluted water bodies than the natural breeding sites of *An. Gambiae*. Results from following study are similar to their findings which also concluded that the level of heavy metals was higher at the polluted site (Awolola *et al*., 2007).

5. Conclusion

Present study showed that toxic heavy metals especially mercury and iron concentrations were found in significant levels at all analysed sites. Although the lowest levels were discovered for Nickel, metals such as Mercury and Iron were also detected. Current study thus advocates a need for proper measures to be taken to lessen increasing environmental pollution by strictly implementing pollution control laws and enforcing proper disposal of industrial effluents in industrial zones.

6. Acknowledgments

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7. Author's Contribution

All authors contributed equally towards this manuscript. Prof Dr. Farkhanda Manzoor compiled results and proof read the final manuscript. Dr. Saffora Riaz wrote the manuscripts and performed the statistical analysis of data. Fariha Akram performed lab work and collected data.

8. Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

9. Novelty Statement

The present study highlights the heavy metals pollution due to development of industries and tanneries in and around Lahore city. The extent of threat to insect and human lives in industrial area of Lahore as a result of increasing metal concentration of Fe, Cu, Zn, Hg, Ni is also presented. It also brings forward scope of different insects to be used as tool to study environment quality and conditions.

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