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HEAVY METAL CONCENTRATIONS OF COPPER AND NICKEL IN PERI-URBAN VEGETABLE AGRO-ECOSYSTEM OF MULTAN, PAKISTAN

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ABSTRACT

Industrialization and urbanization are the major contributors of heavy metal accumulation in soil and vegetables grown under peri-urban agro-ecosystems in Pakistan where farmers usually mix sewage waste water with irrigation water. However, the heavy metal accumulation among different functional groups of insects (i.e. pollinators, pests and predators) largely remains unknown under peri-urban agro-ecosystems. Therefore current study was planned to evaluate the accumulation of copper and nickel heavy metals in insect pollinators, predators and pests on luffa gourd grown in three peri-urban areas of Multan i.e. Soraj Miani,,Vehari Chowk and Rangeelpur. Source of irrigation at Soraj Miani was industrial and household sewage water while it was only household sewage water at other two locations. From each of the location, samples of three insect groups (*Apis dorsata*, *Chrysoperla carnea* and *Spodoptera litura*) were collected along with samples of water, plant, and soil. These samples were analyzed using the atomic absorption spectrophotometer (AAS) in order to quantify the accumulation of copper and nickel heavy metals. It was found that residues of copper and nickel metals were present in samples of soil (0.75 Cu, 0.57 Ni µg/g), water (0.10 Cu, 0.14 Ni µg/g), plants (0.13 Cu, 0.19 Ni µg/g) and insect pest (0.15 Cu, 0.15 Ni µg/g) up to varying extent. Therefore, heavy metals can pose serious threat to ecosystem services of pollination and predation in per-urban agro-ecosystem. Future studies should further investigate the impact of heavy metals on other important pollinators and predators in sewage waste water irrigated agro-ecosystems

Keywords: ecosystem services; insect pests; pollinators; predators; vegetables

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INTRODUCTION

Vegetables are important component for healthy diet since these provides vitamins, minerals, dietary fiber, and phyto-proteins. In daily routine diet, vegetables are strongly recommended for the perfection of good vision, digestive health and also decrease the danger of heart disease, cancer and diabetes (Dias, 2010). Mostly, farmers prefer to grow vegetables in peri-urban areas due to presence of market facility, water scarcity and poverty (Ensink *et al*., 2005; Thebo *et al*., 2014). According to International Water Management Institute (IWMI), 32,500 hectares were directly irrigated with waste water in Pakistan (Ensink *et al*., 2005). Waste water contaminants may cause lethal impact on irrigated plants as well as potential risk to human health due to presence of heavy metals in it (Singh and Kumar, 2006).

The accumulation of heavy metals not only affect the growth of the vegetables but also have negative effects on insects delivering important ecosystem services i.e. pests (Kafel *et al*., 2012; Li *et al*., 2018), predators (Nummelin *et al*., 2007; Hladun *et al*., 2015) and pollinators (Mulder *et al*., 2005; Kosior *et al*., 2007; Moron *et al*., 2012; Hladun *et al*., 2013). In *Helicoverpa armigera*, higher concentrations of Cd, Cu and Zn have been found to affect the food consumption and certain biochemical indices (Baghban *et al*., 2014). Similarly, the higher concentration of cadmium in larval haemolymph of *Spodoptera exigua* was positively correlated with its glutathione oxidation and total anti-oxidant capacity (Kafel *et al*., 2012). The heavy metals (Cd, Cu, Fe, Mn and Zn) have also led to the decline in the populations of a butterfly, *Parnassius apollo* in Finland (Moron *et al*., 2012). Moreover bumble bee decline in Europe has been found to be associated with heavy metal pollution (Kosior *et al*., 2007).

From Punjab, most of the previous studies have reported heavy metals from soil, water and vegetables grown under sewage waste water (Ahmad *et al*., 2014; Randhawa *et al*., 2014; Hamid *et al*., 2016). We know only a single study that reports higher concentration of heavy metals (Cr, Zn, Cd, Cua and Ni) in bodies of different insects i.e. acridid grasshopper (*Oxya hyla*), libellulid dragonfly (*Crocothemis servilia*) and nymphalild butterfly (*Danaus chrysippus*) near industrial area sites as compared to far away sites (Iqra *et al*., 2015).

Keeping in view the dearth of documented work in Punjab, Pakistan; regarding extraction of heavy metals from different insect groups, the current study was planned to evaluate residues of heavy metals (copper and Nickel) from three different insect groups (pest, predator, pollinator) under vegetable agro-ecosystem of Southern Punjab, Pakistan.

MATERIALS AND METHODS

Study Area: Three locations were selected in the vegetable grown peri-urban areas of Multan (South Punjab), based on the nature of sewage water. Location 1 (SorajMiani: 30.2212° N, 71.4322° E) is linked with Sikandari drain, location 2 (Vehari Chowk: 30.1736° N, 71.5090° E) is connected with Nu-Bahar canal while location 3 (Rangeelpur: 30.1520° N, 71.4574° E) is connected with Wali Muhammad drain. Nature of sewage water at location 1 is Tanning industrial waste and at location 2 and 3, it is household waste. At each of the three locations, a single luffa gourd field of one acre (grown under sewage waste water) was selected.

Soil, Water and Plant Sampling: The samples of soil, water and plant (leaf, flower) were collected from all the three study sites. The samples were brought to MNS University of Agriculture, Multan, Pakistan for later quantification of heavy metals.

Heavy metal analysis: The samples of adult *Spodoptera litura* L (pest), *Chrysoperla carnea* L (predator) and *Apis dorsata* L (pollinator) were collected from each of the three locations by using the sweep nets and properly labeled for each location. Insects samples were preserved in freezer (-80°C) for further analysis at MNS University of Agriculture, Multan, Pakistan.

Before the digestion, samples were first dried at 60°C and stored at room temperature. The samples were then dried at 105°C whole night before the chemical analysis. In the test tube, 0.5 g of dry sample was mixed with 5 ml $HN0_3$ and subsequently heated at 50°C for two hours. After this, 5 ml H_2O_2 was added and heated at six hours and samples were filtered and diluted with 25ml water (Nummelin *et al*., 2007).

For digestion of soil and plant samples, each sample was mixed with 15 ml triacid mixture and then heated at 100°C until the transparent solution was obtained. For heavy metals (Copper and Nickel) analysis in soil, plant, water and insects, flame atomic absorption spectrophotometer was used (Varian FAAS-240) at the Central Lab, MNS University of Agriculture, Multan.

Statistical analysis: Data regarding concentration of each of heavy metal (Cu, Ni) in different insect groups (pest, predator and pollinator), soil, water and plant was statistically analyzed by using one-way ANOVA. Means were compared using Tukey's test at alpha 0.05. Pearson correlation was used to see the relationship among the soil, water, plant and insects. All the analysis was performed on computer software XLSTAT (XLSTAT, 2018).

RESULTS

Copper Concentration: There was a significant difference in concentration of copper in samples of *A. dorsata* ($F = 91.93$, d.f = 2, $p < 0.000$), *Chrysoperla carnea* ($F = 82.59$, d.f = 2, $p < 0.000$) and *Spodoptera litura* ($F = 159.24$, d.f = 2, $p < 0.000$) at all the three study sites. Concentration of copper was found to be the highest in all the three insect groups at location 1 while it was the lowest at location 2 (Table 1).

Analysis of variance showed significant differences among the three locations in terms of copper concentration found in water ($F = 32.68$, d.f = 2, $p < 0.001$), soil ($F = 241.25$, d.f = 2, $p < 0.000$) and plant ($F = 30.97$, d.f = 2, $p < 0.001$). The highest concentration of copper (for each of soil, water and plant) was found at location no.1 while it was the lowest at location 2 (Table 2).

Pearson correlation matrix revealed significant positive relationship among all the four variables i.e. insect, water, soil and plant. The highest correlation value (0.957) was obtained for water-insect interaction followed by plant-insect interaction (0.899) while it was lowest for soil-insect interaction (0.525) (Table 3).

Nickel Concentration: There was also a significant difference in concentration of nickel in samples of *A. dorsata* ($F = 87.59$, d.f = 2, $p < 0.000$), *Chrysoperla carnea* ($F = 99.86$, d.f = 2, $p < 0.000$) and *Spodoptera litura* ($F = 601.92$, d.f = 2, $p < 0.000$) at all the three study sites. Concentration of nickel was found to be the highest in all the three insect groups at location 1 while it was the lowest at location 2 (Table 4).

Ni accumulation was significantly different for all the three locations in terms of water ($F = 38.41$, d.f = 2, $p < 0.000$), soil ($F = 251.72$, d.f = 2, $p < 0.000$) and plant ($F = 107.89$, d.f = 2, $p < 0.000$). The highest Ni concentration was observed at location 1 while it was the lowest at location 2 (Table 5).

All the four variables (plant, soil, water and insect) were positively correlated for Ni concentration. The highest correlation value (0.950) was obtained for plant-insect interaction while lowest of it was recorded for water-insect interaction (0.797) (Table 6).

There was a significant difference in concentration of each of Cu ($F = 415.22$, d.f = 3, $p < 0.000$) and Ni ($F = 161.29$, d.f = 3, $p < 0.000$) metal for all the four variables. The highest accumulation was found in soil while the lowest in insect (Table 7).

Table. 1. Mean ± STDEV of copper concentration (μg/g) found in the body of different insect groups.

Location	Location Name	Swage water source	*Apis dorsata*	*Chrysoperla carnea*	*Spodoptera litura*
1	Soraj Miani	Industrial water + Household water	0.12 ± 0.01 a	0.15 ± 0.02 a	0.13 ± 0.01 a
2	Vehari Chowk	Household water	0.07 ± 0.01 c	0.06 ± 0.01 c	0.07 ± 0.01 c
3	Rangeelpur	Household water	0.11 ± 0.01 b	0.08 ± 0.01 b	0.11 ± 0.01 b

Table 2. Mean ± STDEV of copper concentration (μg/g) found in the water, soil and plant.

Location	Location Name	Water source	Water	Soil	Plant
1	Soraj Miani	Sewage water + Industrial water	0.10 ± 0.01 a	0.75 ± 0.02 a	0.13 ± 0.02 a
2	Vehari chok	Sewage water	0.06 ± 0.01 c	0.65 ± 0.02 b	0.07 ± 0.01 c
3	Rangeel pur	Sewage water	0.08 ± 0.01 b	0.55 ± 0.01 c	0.09 ± 0.01 b

Table 3. Correlation matrix among the different sampling variables of copper concentration.

Variables	Insect	Water	Soil
Insect			
Water	0.957 (0.001)		
Soil	0.525 (0.147)	0.599 (0.088)	
Plant	0.899 (0.001)	0.956 (0.001)	0.732 (0.025)

Table 4. Mean ± STDEV of Nickel (μg/g) found in the body of different insect groups.

Sr. no	Location Name	Water source	*Apis dorsata*	*Chrysoperla carnea*	*Spodoptera litura*
1	Soraj Miani	Sewage water + Industrial water	0.12 ± 0.01 a	0.15 ± 0.01 a	0.14 ± 0.01 a
2	Vehari chok	Sewage water	0.08 ± 0.01 b	0.09 ± 0.01 c	0.05 ± 0.01 c
3	Rangeel pur	Sewage water	0.07 ± 0.01 c	0.11 ± 0.01 b	0.07 ± 0.01 b

Table 5. Mean ± STDEV of Nickel (μg/g) found in the water, Soil, plant.

Sr.no	Location Name	Water source	Water	Soil	Plant
1	Soraj Miani	Sewage water + Industrial water	0.14 ± 0.01 a	0.57 ± 0.02 a	0.19 ± 0.01 a
2	Vehari chok	Sewage water	0.10 ± 0.01 c	0.46 ± 0.01 b	0.11 ± 0.01 c
3	Rangeel pur	Sewage water	0.12 ± 0.01 b	0.36 ± 0.01 c	0.15 ± 0.01 b

Table 6. Pearson's correlation matrix among the different sampling variables of Nickel concentration.

Variables	Insect	Water	Soil
Water	0.834 (0.005)		
Soil	0.797 (0.010)	0.356 (0.347)	
Plant	0.950 (0.001)	0.940 (0.001)	0.595 (0.091)

Correlation is significant at P value < 0.05. (P value given in parenthesis)

Table 7. Mean ± STDEV of Copper and Nickel (μg/g) found in the Soil, Plant, water, insect

Sr. no	Category	Copper	Nickel
1	Soil	0.64 ± 0.09 a	0.47 ± 0.08 a
2	Plant	0.09 ± 0..03 b	0.15 ± 0.04 b
3	Water	0.07 ± 0.03 b	0.12 ± 0.04 b
4	1nsect	0.10 ± 0.04 b	0.10 ± 0.03 c

DISCUSSION

In the preset study, copper and nickel metals were found in *A. dorsata* that has been reported as the most effective pollinator of cucurbit crops in the study area (Saeed *et al*., 2012; Ali *et al*., 2014). Perugini *et al*., (2011) also reported the accumulation of heavy metals (Hg, Cr, Cd, and Pb) in honey bees (*Apis mellifera*) from several sampling sites around central Italy including both polluted and wildlife areas. Kosior *et al*., (2007) reported that accumulation of heavy metals in environment and soil has threatened 80% populations of honey bees in Europe. Moreover, heavy metal pollution is also being reported as an important reason of colony collapse disorder in honey bees (Ellis *et al*., 2003). The widespread decline of the butterfly pollinator (*Parnassius apollo*) in Finland has also been associated with heavy metal (Cu) intoxication (Nieminen *et al*., 2001).

Pollinators are declining across the world due to various reasons (Moron *et al*., 2012) and heavy metals pollution is among the major factors for decline of pollinators through harmful intoxication (Beyrem *et al*., 2007; Piola and Johnston, 2008). Pollinator decline will have serious threats to the socio-economics of various agriculture countries (Kremen *et al*., 2002; Klein *et al*., 2007; Potts *et al*., 2010).

Besides pollinators, copper and nickel residues were also extracted from the bodies of predator (*Chrysoperla carnea*). Heavy metal pollution is among the important factors for the decline of natural enemies around the world (Nummelin *et al*., 2007; Gardiner and Hardwood, 2017). Previously, negative effect of copper has been reported on the predation activity of nymphs and adult of the earwig, *Forficula auricularia* (Malagnoux *et al*., 2015). Biological control agents provide important ecosystem service of managing harmful insect pests on the crops and it is one of the most economical method for managing insect pests (Schmidt *et al*., 2003).

In our study, the highest concentration of Cu and Ni -in each of three insect groups- was found at location 1 where the source of waste water was the industry as compared to location 2 and 3 where it was the household. Heavy metals flow from the industries is the main source of soil contamination since it contains high convergence of substantial metals (including cadmium, copper, lead and nickel) (Wauna and Okieimen, 2011). These components may cause poisonous impact on plants and subsequent bio-magnification poses a potential threat to human wellbeing (Sawidis *et al*., 2001; Mapanda *et al*., 2005). Plants soak up the heavy metals from their roots which are grown in polluted lands irrigated with industrial, municipal and domestic waste water. Through adsorption and filtration, plants absorb these heavy metals from their roots and transfer it to their different parts (i.e. leaves, flowers, stem, fruit and grains) that induce the chemical changes in plants (Jacob, *et al*., 2018; DalCorso *et al*., 2019,).

In the present study, the concentrations of Cu and Ni metals were found to be higher in soil as compared to water, plant and insects in all the three study locations. When sewage water is applied to the crops, water starts leaching down into the soil and metals get accumulated in higher amount at the bottom of soil (Keller *et al*., 2002). Some other studies have also reported higher quantity of heavy metals (copper, zinc and cadmium) in the soil than in mushroom and tobacco plants (Tuzen, 2003; Evangelou *et al*., 2007). Larger amount of metal in soil could also be due to use of different quantity and quality of sewage and sludge applied to field for the better development of vegetables crops (Mclaren *et al*., 2004).

Person correlation matrix showed highly positive correlation between plants and insects for accumulation of both copper and nickel metals. A previous study also elucidated that greater copper accumulation in plants leads to higher extraction from insects (Nummelin *et al*., 2007). Plants uptake heavy metals from soils through ionic exchange, redox reaction and precipitation dissolution (Peer *et al*., 2005). Different plant parts contain different heavy metals quantities; the highest quantities have been reported in leaves, flowers and roots while lowest in fruits and seeds (Almehdi *et al*., 2019). That is why, in our study, concentration of both copper and nickel was found higher in *Chrysoperla carnea* (0.15 Cu,0.15 Ni µg/g) followed by *Spodoptera litura* (0.13 Cu,0.14 Ni µg/g) and *A. dorsata* (0.12 Cu, 0.12 Ni µg/g). Previous studies have reported higher concentrations of Cu and Ni in soil (17.9 Cu,13.0 Ni µg/g), water (5.9 Cu,4.0 Ni µg/g), plants (35.98 Cu,15.93 Ni µg/g) and insects bodies (1.49 Cu,0.88 Ni µg/g) (Balistrieri *et al*., 2007; da Silva *et al*., 2020). The normal body burden of Cu in insect is <5 µg/g, while minimum lethal concentration for Ni in insects is 230 µg/g (Cheruiyot *et al.,* 2013, Di *et al*., 2016). Although, the results of our study indicate lower levels of heavy metals in insects as compared to other studies but still these heavy metal residues have deleterious effects on behavior and physiology of insects especially disturbing their cellular functions (Skorbiłowicz *et al.,* 2018; Monchanin *et al.,* 2021)

In conclusion, it was found that residues of copper and Ni metals were present in samples of soil, water, plant and insect pest up to varying extent. Therefore, these heavy metals pose serious threats to ecosystem services of pollination and predation in our per-urban vegetable ecosystem that may lead to severe reduction in vegetable yield. Future studies should further investigate the impact of heavy metals on other important pollinators and predators present in vegetables grown under sewage waste water.

Authors' contributions: All authors contributed to the study conception and design. Material preparation and data collection was done by Hamza Afzal and Mudssar Ali while data analysis was performed by Mudssar Ali and Asif Sajjad. The first draft of the manuscript was written by Hamza Afzal while all the authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest: All authors declare that they have no conflict of interest

Ethical approval: The study involves work with insects and does not require ethical approval.

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