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Aquatic insects as a biomonitoring and bioindicators for trace metals in the contaminated Al-Mahmoudia Canal, River Nile, Egypt

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ABSTRACT

In the River Nile, water pollution is a fundamentally environmental and public health issues that requires deep consideration. Heavy metals (copper "Cu", zinc "Zn", iron "Fe", cadmium "Cd", manganese "Mn", chromium "Cr", and cobalt "Co") were found in the sediment, water, and three aquatic insects (*Crocothemis* sp., *Diplonychus urinator*, and *Micronecta isis*) at two sites along Al-Mahmoudia canal during a study extending from summer 2019 to spring 2020. In water and sediment, Fe, Mn, and Zn concentrations recorded their highest values, followed by Cu and Cd, whereas Cr concentrations were lower in both seasons. In addition, the highest accumulation seasons were summer and spring, while the lowest accumulation season was winter. In aquatic insects, the highest amounts of Fe, Zn, and Mn were detected, followed by Cu, Cd, Co, and Cr. The lowest bioaccumulation factor (BAF) was recorded for Co, Mn, and Cu, while Fe and Zn showed the greatest. Additionally, the metal pollution index (MPI) value was high in summer and low during winter. In this context, Fe, Mn, and Cu in water and sediment reflected their utmost metal effect on the investigated insects. Furthermore, Mn and Co were more accumulated inside *Crocothemis* sp., while Cu and Cd were more accumulated inside *Diplonychus urinator*, but Zn showed the same accumulation rate inside all insects.

INTRODUCTION

Water is a significant natural resource which is necessary for all activities. Forits importance in the existence of living organisms, water is known as the "elixir of life". The availability of water is dwindling, necessitating the protection of reserves. Agriculture, hydropower, industry, fishing, and recreation are examples of how people use water resources **(Zakaria *et al.*, 2018a, b; El-Naggar *et al.*, 2019)**. Since Egypt is in need of freshwater, study on amphibian creepy crawlies and their application in gauging prosperity and water quality status in Egypt (such as network streams and the Nile) has expanded, but it is still restricted **(Haggag *et al.*, 2018; Zakaria & El-Naggar, 2019; Zakaria *et al.*, 2019)**. Due to their numerous ecological aggravations and open-minded levels, the aquatic insects are useful indications of water qualities **(El-Mehdawy *et al.*,**

2021; Hasaballah *et al.*, 2021). Heavy metals are the most hazardous toxins because they accumulate in water, sediments, and living creatures' tissues due to bio-concentration (absorption from the environment) and bio-magnification (take-up from the food chain) **(Chaphekar, 1991)**. Heavy metals are widely used in industry, and they are a major source of water pollution. Consequently,, it is crucial to determine how harmful they are to aquatic organisms **(Soucek *et al.*, 2011)**. The most typical hotspots for major metals are mineral extraction, volcanic activity and disintegration. Anthropogenic sources of heavy metals include electroplating, purifying, mining, industrial discharge, air pollution, agricultural fertilizer biosolids, pesticide, sludge dumping and etc… The concentrations of heavy metals in aquatic ecosystems can be used to calculate current pollution levels. Moreover, heavy metals are non-biodegradable; they can build up in biological tissue. Increased levels of following metals in various organs of microorganisms are used as a key device or file of metal contamination in an aquatic ecosystem **(Liu *et al.*, 2016; Mona *et al.*, 2019)**. Trace metal contamination is typically seen in aquatic insects. Insects establish crucial linkages in the metal vehicle chain between trophic levels, and hence following metal fixation in insects has an impact on following metal appropriation in the biosphere for synthetic assessments of heavy elements in water do not give information on their bioavailability **(Uluturhan & Kucuksezgin, 2007)**. Heavy metals must be identified as the best indicators of contamination in the Egyptian industrial and agricultural sectors **(Girgin *et al.*, 2010; El-Naggar, 2015)**.

Anthropogenic activities caused harmful impacts on natural ecosystems (**Mona *et al.*, 2019; Shaban *et al.*, 2020; Darweesh *et al.*, 2021**). The increased automobile use in contemporary farming, industrialization and new technology have lead to an increase in heavy metal concentrations in the environment **(Atafar *et al.*, 2010)**. These hazardous elements have become a part of the natural world's biogeochemical cycle **(Lee *et al.*, 2006)**. Insects are frequently utilized as effective bio-indicators of environmental stress **(Davis *et al.*, 2001)**. Butterflies and grasshoppers are among the aquatic insects that are sensitive to environmental changes. Butterflies and grasshoppers are two aquatic insects that are sensitive to changes in the environment. Such insects are good bio-indicators for metal pollution and contamination in the ecosystem **(Chen *et al.*, 2005)**. In the environment, the most susceptible insects to changes are Dragonflies **(Zia *et al.*, 2009)**. They can be found in large numbers in any water body that is not polluted by humans. Since they respond to heavy metal buildup in a sensitive and fast manner, they are recognized as the best ecological indicators in aquatic and terrestrial ecosystems **(Rafi *et al.*, 2009; Cervera *et al.*, 2012)**.

In bio-monitoring programs, aquatic insects are good for detecting organic pollution and heavy metal contamination **(Smolders *et al.*, 2003)**. Chironomids and *Hydropsychid caddisflies* have high metal tolerance, but certain Ephemeroptera taxa have significant metal sensitivity **(Winner *et al.*, 1980; Clements *et al.*, 2000)**. Remarkably, they reflect environmental changes and can provide precise data on habitats and water quality. Thus, aquatic insects are the most widely used organisms in freshwater bio-monitoring of human influences **(Strayer, 2013; Ceneniva-Bastos *et al*., 2017)**. It's vital to identify the more specific components of any metal's bioaccumulation inside an insect's body, which can take a variety of forms depending on the trophic group. Therefore, the amount of metal taken up by insects in water and food is determined by the metal's bioavailable concentration as well as the rate and mechanism by which the metal enters the insect's body **(Souto *et al*., 2019)**.

The aim of the current study was to find heavy metals in the water, sediment, and aquatic insects in Mahmoudia canal and evaluate the use of aquatic insects as a pollution bioindicator.

MATERIALS AND METHODS

2.1. Description of the study area

Al-Mahmoudia canal is located at Northern West side of Egypt (Fig. 1). It receives around 15 Mm^3/day of water from the Rosetta branch in Mahmoudia city, as well as the drainage water from the Zarkon sewer. Alexandria gets its consumed water from the Mahmoudia canal's surface water. Two sites were chosen along Mahmoudia canal for samples collection which was carried out during one year; from spring 2019 till winter 2020. The first site in Mahmoudia city is situated at the canal opening with the Rosetta branch between 31°10'59.24"N - 30°31'40.64"E, while the second site is located in Alexandria city (31°13'11.89"N - 29°59'26.72"E), about 70 km from the first site. The geographical position and altitude of each site were recorded using a Garmin eTrex 30 GPS. The study area map was designed with ArchGis 10.2 software programs. Aquatic insects, water, and sediment samples were taken periodically throughout the daylight at both sites (6.00 am - 10.00 am).

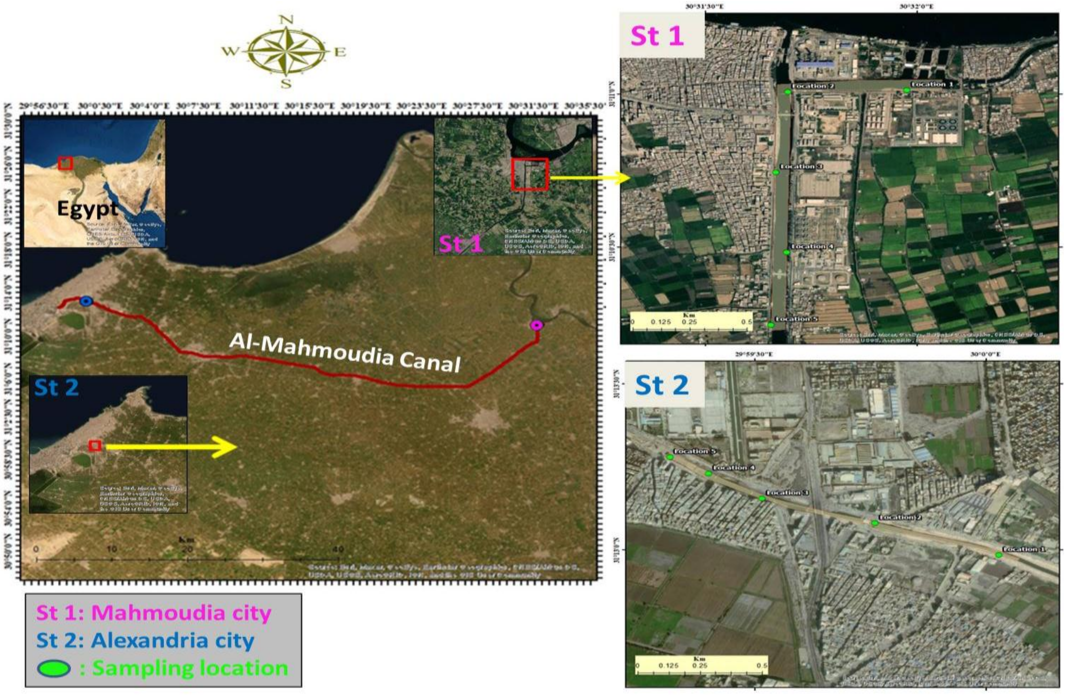

Fig. 1. A map of the study area; St 1: Mahmoudia city and St 2: Alexandria city

2.2. Water sampling and heavy metal assessment

A Van Dorn water sampler with a capacity of 1.2 liters was used to gather water samples from depths greater than 40 cm. After collection, water samples were retained in clean stoppered plastic bottles, and only one liter of each sample was treated with 5 ml of concentrated nitric acid (HNO_3) and stored in a refrigerator at 4°C for analysis **(APHA, 2005)**. In the lab, an amount of 500 mL of the preserved sample was mixed with 20 mL of HNO_3 in a beaker, and the mixture was gently heated and evaporated on a hot plate until the lowest volume was reached before precipitation or until digestion was completed. The digested sample was placed in a volumetric flask with a capacity of 100 mL. The beaker's wall was rinsed with deionized water and then added to the same volumetric flask, bringing the total volume to 100 ml. This flask was cooled, then filled with deionized water, and thoroughly mixed again. The optical density of each sample was measured using an atomic absorption spectrophotometer, and heavy metal concentrations were represented in parts per million (ppm). The measured heavy metal concentrations in water were then compared to the standards of **WHO (2004)**.

2.3. Sediment sampling and heavy metal assessment

Van ven grab was used to collect sediment samples. Stove dried 500 g of sample (70°C) until a consistent weight was gained, then crushed to a homogeneous powder and kept in polythene sacks. **Vercoutere *et al.* (1995)** described the following method for digestion: an amount of 0.5 g of sediment sample was digested for 24 hours at room temperature in a 12 mL mixture of 37% HCl and 65% HNO3 (3:1), then was allowed to set in the digestion flask. On a thermostatically controlled hotplate at 90°C in a smoke cabinet, the suspension was processed to near dryness, and then amounts of 2.5 mL of 37% HCl and 2.5 mL of 30% H_2O_2 were added to finish the digestion. The resulted mixture was reheated before being chilled to room temperature. The suspension was sifted through Whatman filter paper (No. 41) in a volumetric flask, diluted to 50 mL, and stored in polyethylene bottles at 4°C for further study after the flask wall was washed with 10 mL deionized water. The amounts of heavy metals were measured using an atomic absorption spectrophotometer and expressed in parts per million (ppm). Heavy metal concentration levels in sediment were compared to **WHO (2004)** standards.

2.3. Aquatic insect's collection and identification

Sampling techniques of aquatic insects were performed according to the protocol of **Boonsoong *et al*. (2008)** (hand-picked; dip net of 45µm mesh and sweeping net with 45 µm mesh; Kick/sweep method). Aquatic insects were identified using taxonomic keys following the identifications of **Tawfik (2009)** and **Badawy *et al*. (2013)**.

2.4. Preparation of insects for heavy metal analysis

The entire insect was weighed on an electric balance (4 digital) and then frozen for 24 hours at -4°C. To aid tissue solubilization, concentrated nitric acid (0.5-1 ml) was added to each 25 mg tissue, and the tissue was gently heated in the heating block (90°C) and vortexes. The dissolved tissue was then reintroduced to the heating block until the color turned brown, then cooled and digested with 0.1 mL concentrated nitric acid.

Returning the digested tissue to the heating block, the volume was lowered to 0.25-0.5 ml. Subsequently, the digested tissue was cooled and 0.1 ml H2O2 (30%) was added and heated till the volume was reduced to 0.25-0.5 ml. Finally, the digested tissue was diluted to 2 ml in distilled water and examined with an atomic absorption spectrophotometer.

2.5. Spectrophotometric analysis of heavy metals

The heavy metal levels have been determined in the digested water, sediment, and tissues using GBC atomic absorption spectrophotometer (Savanta A) in the National Institute of Oceanography and Fisheries, Egypt. The concentration was calculated in µg/mg dry weight.

2.6. Indices of metal pollution

2.6.1. Bioaccumulation factors of sediment (BSAF)

This forms the ratio of the metal concentration in the organism to the metal concentration in the sediment, and it was computed using the formula below **(Szefer *et al.*, 1999)**.

$$\mathbf{BSAF = C_t/C_s}$$

Where**;** BSAF: bioaccumulation factor concerning sediment, Ct: concentration of the metal in tissue (mg/g tissue), Cs: concentration of the metal in sediment (mg/g)

2.6.2. The metal pollution index (MPI)

MPI was used to compare the total content of heavy metals at the studied sites **(Usero *et al.*, 1996)**.

$$\mathbf{MPI = (Cf1\times Cf2\times Cf3 \ldots\times Cfn)\ 1/n}$$

Where; Cfn: concentration of the metal in the sample (µg/g fresh wt.), n: number of metals.

2.7. Data analysis

The data were analyzed using a variety of mathematical relationships and statistics. With a significance level of $p \leq 0.05$ and $p \leq 0.01$, a multiple correlation analysis was used to determine the relationship between the concentrations of heavy metals in sediment, water and insects. The average heavy metal concentrations in sediment, water, and insects were compared using a T-test to detect the existance of a significant difference. All prior analyses were carried out with the use of many computer programs, including SPSS version 20 (SYSTAT statistical tool), PAST PAleontological STatistics Ver. 3.25, and Excel 2016.

RESULTS

3.1. Heavy metals levels in water & sediment

Table (1) shows that the highest amounts of heavy metals were found in spring, while the lowest was recorded in autumn. However, Fe, Zn, and Mn levels in water were greater in spring, while Fe and Zn levels were lower in autumn, but Co levels were lower in spring. Generally, Mn and Zn were not found in autumn, while Cd was neither found in spring nor in autumn. Furthermore, the majorities of the sediment metals were greater in spring and decreased in winter. Fe and Cu, on the other hand, were greater in spring and summer and lower in winter and autumn. For Fe, Mn and Zn, the data analysis revealed no significant variations among seasons. While, there was a significant difference in Cu, Cd, and Co in the water among seasons.

Table 1. Seasonal variation of heavy metals in water & sediment ($\bar{x}$±SD ppm) in Mahmoudia*

Heavy metal	Water/Sediment	Spring	Summer	Autumn	Winter
Fe	**Water**	0.87 ± 0.25^{a}	0.83 ± 0.28^{a}	0.61 ± 0.24^{a}	0.60 ± 0.28^{a}
	Sediment	13.5 ± 1.4^{b}	11.2 ± 1.3^{b}	07.0 ± 1.8^{a}	07.2 ± 1.7^{a}
Mn	**Water**	0.070 ± 0.02^{a}	0.072 ± 0.20^{a}	--	0.06 ± 0.23^{a}
	Sediment	05.0 ± 1.2^{a}	05.1 ± 1.0^{a}	02.5 ± 0.7^{b}	03.1 ± 0.32^{b}
Zn	**Water**	0.41 ± 0.02^{a}	0.073 ± 0.01^{a}	--	0.06 ± 0.02^{a}
	Sediment	$04.4 \pm 0.7^{a,b}$	04.6 ± 1.2^{b}	$03.0 \pm 0.8^{a,b}$	02.8 ± 0.7^{a}
Cu	**Water**	0.27 ± 0.04^{a}	0.50 ± 0.13^{b}	0.08 ± 0.02^{a}	0.12 ± 0.05^{a}
	Sediment	07.7 ± 1.3^{a}	$05.8 \pm 2.2^{a,c}$	$05.0 \pm 1.2^{c,d}$	$03.6 \pm 0.5^{b,d}$
Cd	**Water**	--	0.02 ± 0.002^{a}	--	0.02 ± 0.003^{a}
	Sediment	03.31 ± 1.2^{a}	03.6 ± 1.0^{a}	01.4 ± 0.3^{b}	01.0 ± 1.3^{b}
Co	**Water**	0.09 ± 0.01^{a}	0.06 ± 0.02^{b}	$0.05 \pm 0.01^{a,b}$	$0.06 \pm 0.01^{a,b}$
	Sediment	02.1 ± 0.4^{a}	03.0 ± 1.1^{b}	01.7 ± 0.7^{a}	02.1 ± 0.2^{a}

*Mean ± SD followed by letter (a): Not significantly different ($P>0.05$), (b): Significantly different ($P<0.05$), (c): Highly significantly different ($P<0.01$), (d): Very highly significantly different ($P<0.001$).--: not detected.

Table (2) shows that the highest amounts of heavy metals were found in the water sampled from Alexandria in spring and summer, but the lowest levels were found in autumn and winter. On the contrary, Mn levels were high in autumn and Co was higher in spring. Heavy metal levels in sediment varied throughout the year, although the highest levels were found in spring and summer, while the lowest levels were found in winter (Table 2). The value of Fe was higher in autumn and lower in the summer. Conversely, the value of Mn was higher in spring and showed its lowest in autumn. The statistical analysis of the data revealed that there were no seasonal significant differences among all heavy metals in water, except for Fe, Zn and Cu.

Table 2. Seasonal variation of heavy metals in water and sediment ($\bar{x}$±SD ppm) in Alexandria*

Heavy metal	Water/Sediment	Spring	Summer	Autumn	Winter
Fe	**Water**	1.0 ± 0.14^{a}	01.4 ± 0.23^{b}	1.0 ± 0.13^{a}	00.87 ± 0.12^{a}
	Sediment	06.21 ± 1.8^{a}	05.40 ± 1.4^{a}	11.27 ± 1.2^{b}	09.50 ± 1.2^{b}
Mn	**Water**	0.14 ± 0.06^{a}	0.19 ± 0.05^{a}	0.40 ± 0.50^{a}	00.30 ± 0.4^{a}
	Sediment	06.40 ± 1.0^{a}	06.19 ± 2.0^{a}	01.90 ± 0.60^{b}	02.5 ± 0.9^{b}
Zn	**Water**	0.45 ± 0.20^{a}	00.64 ± 0.24^{a}	0.10 ± 0.01^{b}	0.074 ± 0.01^{b}
	Sediment	05.20 ± 1.10^{a}	$4.90 \pm 0.10^{a,c}$	02.73 ± 0.5^{b}	$03.60 \pm 0.5^{b,c}$
Cu	**Water**	$0.01 \pm 0.002^{a,b}$	0.08 ± 0.012^{a}	$0.06 \pm 0.01^{a,b}$	0.04 ± 0.03^{b}
	Sediment	$02.80 \pm 0.34^{a,c}$	$04.01 \pm 0.6^{a,b}$	03.50 ± 0.9^{c}	06.1 ± 1.0^{b}
Cd	**Water**	--	0.03 ± 0.01^{a}	--	0.02 ± 0.01^{a}
	Sediment	04.5 ± 0.6^{a}	$03.0 \pm 1.0^{a,b}$	$3.20 \pm 0.61^{a,b}$	02.3 ± 0.50^{b}
Co	**Water**	00.4 ± 0.42^{a}	00.20 ± 0.05^{a}	0.17 ± 0.05^{a}	0.03 ± 0.01^{a}
	Sediment	$01.90 \pm 0.6^{a,b}$	$02.24 \pm 0.3^{a,b}$	02.54 ± 0.53^{a}	01.6 ± 0.51^{b}

*a, b, c and— .See footnote of Table (1).

3.2. Heavy metals accumulation in insects collected from Mahmoudia city

The accumulation of heavy metals in *Crocothemis* sp. was the highest during the summer and spring, and the lowest during the winter (Table 3). Significant variances among metals and seasons were discovered, particularly in the case of Cu. The highest Mn, Zn, and Co concentrations were detected in spring, whereas They showed their lowes in winter. For Mn, an apparent substantial difference was recorded between the examined metals and seasons. The highest concentration of Cd was determined during autumn, while it showed its lowest during winter. Finally, the BSAF showed a fluctuating relation with the seasonal change. Mn showed the highest BSAF, while Cu was the lowest one. Likewise, MPI increased during summer and decreased during winter.

Table 3. Seasonal variation in heavy metals accumulation ($\bar{x}$±SD ppm) and BAFs of *Crocothemis sp.* collected from Mahmoudia city*

Heavy metal	Sp./ Sea.	Spring	Summer	Autumn	Winter	Mean
Fe	**Conc. Level**	229.6±6.1 ^{b}	254.7±7.12 ^{a}	101.2±7.64 ^{c}	95.4±2.53 ^{c}	
	BSAF	17	22.4	14,4	13.3	**170.2**
Mn	**Conc. Level**	104.9±4.41 ^{b}	92.6±3.63 ^{a}	64.21±5.32 ^{c}	51.6±2.30 ^{d}	
	BSAF	21	18.2	25.7	16.6	**78.3**
Zn	**Conc. Level**	37.4±2.32 ^{b}	28.2±2.1 ^{a,c}	30.6±1.4 ^{a}	23.8±1.3 ^{c}	
	BSAF	8.5	6.1	10.2	8.5	**30.0**
Cu	**Conc. Level**	14.6±2.3 ^{b}	20.4±2.1 ^{a}	8.3±1.7 ^{c}	5.2±1.21 ^{d}	
	BSAF	12	3.5	1.7	1.4	**12.1**
Cd	**Conc. Level**	21.6±1.7 ^{b}	74.3±7.12 ^{a}	83.1±3.21 ^{c}	16.5±2.1 ^{b}	
	BSAF	6.5	20.6	59.4	16.5	**48.9**
Cr	**Conc. Level**	9.6±1.5 ^{a}	11.2±1.3 ^{a}	7.4±0.8 ^{a}	3.3±0.72 ^{b}	
	BSAF	----	----	----	---	**7.9**
Co	**Conc. Level**	24±2.4 ^{a}	22.4±1.8 ^{a}	19.3±0.7 ^{a}	11.0±1.4 ^{b}	
	BSAF	11.4	7.5	11.4	5.2	**19.2**
MPI		**35**	**42.6**	**29.5**	**16.7**	**31.0**

*a, b, c, d : See footnote of Table (1), BSAF:Bioaccumulation factor of sediment and MPI: metal pollution index.

Table (4) shows metal deposited in *Diplonychus urinator* (Dufour), reaching its highest concentration levels throughout summer and spring, with the exception of Cd, which peaked in autumn. The lowest Cr value was found in fall, while the lowest Cu value was monitored in spring. These findings revealed considerable changes across metals and seasons, particularly for Fe and Mn. In addition, Cd and Fe had the greatest BSAF values, whereas Cu and Co showed the lowest. In summer and spring, the MPI levels increased, whereas in winter, a decrease was noted.

The highest concentration of heavy metals accumulated in *Micronecta isis* (Horvath) was detected during spring and summer, while the lowest was detected during winter (Table 5). These results showed significant differences between metals and seasons, except for Fe. In addition, the BSAF demonstrated a seasonal variation relationship. Zn showed the highest BSAF. Fe, on the other hand, had the smallest BSAF. Furthermore, MPI values were higher in summer and lower in the winter.

Table 4. Seasonal variation in heavy metals accumulation (x̅±SD ppm) and BAFs of *Diplonychus urinator* (Dufour) collected from Mahmoudia city*

Heavy metal	Sp./Sea.	Spring	Summer	Autumn	Winter	Mean
Fe	Conc. level	212.3±5.1 ^{b}	171.4±4.0 ^{c}	188.2±4.1 ^{d}	170.2±5.$^{4\ a}$	
	BSAF	49.3	22	29	62	**313.5**
Mn	Conc. level	56.5±4.1 ^{b}	53.5±3.1 ^{a}	50.7±3.1 ^{c}	32.5±3.4 ^{d}	
	BSAF	12.6	10.6	17.5	21.8	**58.6**
Zn	Conc. level	28.2±1.6 ^{b}	23.2±2.1 ^{a}	21.1±1.8 ^{a}	9.2±1.7 ^{c}	
	BSAF	9.1	5.7	11.6	3.8	**21.2**
Cu	Conc. level	10.3±1.9 ^{b}	14.5±1.7 ^{a}	11.6±2.2 ^{b}	12.9±2.2 ^{c}	
	BSAF	1.3	2.3	4.7	3.2	**13.3**
Cd	Conc. level	30.5±27.2 ^{b}	56.6±2.4 ^{a}	65.1±2.0 ^{a,b}	35.0±2.7 ^{a,b}	
	BSAF	9.5	18	68.3	22	**51.8**
Cr	Conc. level	2.1±0.6 ^{a}	2.6±0.5 ^{a}	1.9±0.7 ^{a}	2.2±1.0 ^{a}	
	BSAF	---	---	---	---	**2.1**
Co	Conc. level	5.1±1.6 ^{b}	3.2±1.1 ^{a}	2.9±1.5 ^{a}	2.2±1.2 ^{a}	
	BSAF	3.2	1.1	1.8	1.4	**4.5**
	MPI	**23.7**	**24.8**	**21.3**	**16.5**	**21.6**

*a, b, c, d : See footnote of Table (1); Conc., BSAF and MPI. : See footnote of Table (3)

Table 5. Seasonal variation in heavy metals accumulation (x̅±SD ppm) and BAFs of *Micronecta isis* (Horvath) collected from Mahmoudia city*

Heavy metal	Sp./Sea.	Spring	Summer	Autumn	Winter	Mean
Fe	Conc. Level	8.6±1.7 ^{a}	10.2±1.5 ^{a}	9.3±1.8 ^{a}	7.8±1.3 ^{a}	**9**
	BSAF	1.5	1.7	0.8	0.9	
Mn	Conc. Level	13.1±1.6 ^{a}	12.4±1.3 ^{a,b}	9.0±1.4 ^{b}	4.5±0.7 ^{c}	**9.8**
	BSAF	2.5	2.2	3.8	1.8	
Zn	Conc. level	20.0±1.9 ^{b}	14.8±1.7 ^{a,c}	18.0±2.1 ^{a,b}	12.0±1.3 ^{c}	**16.2**
	BSAF	7	4.3	10.5	5.6	
Cu	Conc. level	6.9±1.5 ^{a}	8.7±1.5 ^{a}	3.2±1.2 ^{b}	2.3±1.2 ^{b}	**5.3**
	BSAF	0.9	1.4	1	0.9	
Cd	Conc. level	6.1±1.0 ^{b}	4.2±1.2^{a,b}	3.1±1.1 ^{a}	2.6±1.0 ^{a}	**4**
	BSAF	1.5	0.9	2.4	1.7	
Cr	Conc. level	2.1±0.8 ^{a,b}	4.5±1.1 ^{a}	2.2±0.6 ^{a,b}	1.3±0.6 ^{b}	**2.5**
	BSAF	---	---	---	---	
Co	Conc. level	10.3±1.3 ^{b}	8.03±1.5 ^{a}	6.6±2.1 ^{c}	2.9±1.2 ^{c}	**7**
	BSAF	5.6	2.3	2.5	2.2	
	MPI	**7.9**	**8.2**	**5.8**	**3.7**	**6.4**

*a, b, c, d : See footnote of Table (1); Conc., BSAF and MPI. : See footnote of Table (3)

3.3. Heavy metal accumulation in insects collected from Alexandria city

Heavy metal concentrations in *Crocothemis* sp. were highest in spring, while a drop was observed in winter, with the exception of Fe and Cd, which were greater in summer but declined in winter (Table 6). With respect to Cd, the current findings revealed considerable disparities between metals and seasons. The maximum values of Zn, Cu, Cr, and Co were recorded in spring, while in winter, the minimum values were reported. For Cu and Cr, there were obvious and significant variances across metals and seasons. The BSAF displayed an inconstant relationship when the seasons changed. Fe showed the highest BSAF, whereas Cu had the lowest BSAF. The MPI was high in summer and low in winter.

Table 6. Seasonal variation in heavy metal accumulation (x̄±SD ppm) and BAFs of *Crocothemis* sp. collected from Alexandria city*

Heavy metal	Sp./Sea.	Spring	Summer	Autumn	Winter	Mean
Fe	**Conc. level**	207.4±9.1 ^{a}	273.8±16.23 ^{b}	201.8±12.5 ^{b}	110.8±11.62 ^{c}	**198.5**
	BSAF	33.4	50.7	17.9	11.7	
Mn	**Conc. level**	72.1±6.71 ^{a}	61.4±6.1 ^{b}	69.6±9.3 ^{a,b}	46.1±2.5 ^{c}	**62.3**
	BSAF	11.3	9.9	36.6	18.4	
Zn	**Conc. level**	27.7±2.7 ^{a}	22.8±2.21 ^{b}	21.5±1.6 ^{c}	19.6±1.7 ^{c}	**22.9**
	BSAF	5.3	4.7	8	5.4	
Cu	**Conc. level**	12.5±2.5 ^{a}	10.1±1.91 ^{b}	7.2±1.3 ^{c}	2.3±0.7 ^{d}	**8**
	BSAF	4.5	2.6	2.1	0.4	
Cd	**Conc. level**	21.6±3.74 ^{a}	62.9±6.63 ^{b}	50.1±5.04 ^{c}	16.7±2.6 ^{d}	**37.8**
	BSAF	4.8	20.9	15.7	7.3	
Cr	**Conc. level**	10.4±0.8 ^{a}	7.7±1.2 ^{b}	3.6±1.3 ^{c}	2.21±0.5 ^{d}	**6**
	BSAF	---	---	---	---	
Co	**Conc. level**	23.3±2.2 ^{a}	21.8±2.41 ^{a}	11.7±2.1 ^{b}	4.6±1.5 ^{c}	**15.4**
	BSAF	12.3	9.7	4.6	2.9	
MPI		**30.9**	**32.8**	**24**	**12.2**	**25**

* a, b, c, d : See footnote of Table (1); Conc., BSAF and MPI. : See footnote of Table (3)

In Table (7), the highest concentrations of heavy metals accumulated in *Diplonychus urinator* (Dufour) were discovered throughout spring and summer, whereas Cu and Cd were higher in winter and autumn, respectively. Significant disparities between metals and seasons were found in the current findings, particularly in the case of Fe and Cd. The differences between metals and seasons are profound. In addition, the BSAF exhibited a seasonal fluctuating relationship. The highest BSAF was found in Fe, while the lowest was found in Co. MPI values were higher in summer and lower in winter.

The highest levels of heavy metals were found in *Micronecta isis* during the summer and spring, and they decreased in winter, with the exception of Cr, which decreased in autumn (Table 8). These findings revealed considerable changes in metals and seasons, particularly for Mn and Zn. According to seasonal variations, the BSAF showed a variable relationship. The maximum BSAF was detected in Zn, whereas the lowest was recorded in Mn. During summer, the MPI increased, while a decrease was witnessed during winter.

Table 7. Seasonal variation in heavy metals accumulation ($\bar{x}$±SD ppm) and BAFs of *Diplonychus urinator* (Dufour) collected from Alexandria city*

Heavy metal	Sp./Sea.	Spring	Summer	Autumn	Winter	Mean
Fe	**Conc. level**	201.6±11.34 ^{a}	150.3±4.8 ^{b}	182.1±10.1 ^{c}	97.6±7.9 ^{d}	**157.9**
	BSAF	32.5	27.8	16.2	10.3	
Mn	**Conc. level**	45.4±4.2 ^{a}	40.7±3.4 ^{b}	23.6±3.5 ^{c}	21.03±4.4 ^{c}	**32.7**
	BSAF	7.1	6.6	12.4	8.4	
Zn	**Conc. level**	26.7±1.9 ^{a}	21.1±2.1 ^{b}	22.2±2.3 ^{a}	10.4±1.9 ^{c}	**20.1**
	BSAF	5.1	4.3	8.1	2.9	
Cu	**Conc. level**	8.7±1.2 ^{a}	11.6±1.43 ^{b}	12.5±1.7 ^{a}	15.1±1.6 ^{c}	**12.0**
	BSAF	3.1	2.9	3.6	2.5	
Cd	**Conc. level**	36.4±2.8 ^{a}	51.4±4.4 ^{b}	61.3±2.9 ^{c}	30.2±2.8 ^{d}	**44.8**
	BSAF	8.1	17.1	19.2	13.1	
Cr	**Conc. level**	7.2±0.98 ^{a}	10.6±0.9 ^{b}	5.6±1.04 ^{b,c}	3.7±1.6 ^{c}	**6.8**
	BSAF	---	---	---	---	
Co	**Conc. level**	2.2±0.53 ^{a}	3.2±1.1 ^{b}	3.0±0.8 ^{a,b}	2.2±0.6 ^{b}	**2.7**
	BSAF	1.2	1.4	1.2	1.4	
MPI		**19.9**	**22.1**	**19.9**	**13.4**	**18.8**

* a, b, c, d : See footnote of Table (1); Conc., BSAF and MPI. : See footnote of Table (3)

Table 8. Seasonal variation in heavy metals accumulation ($\bar{x}$±SD ppm) and BAFs of *Micronecta isis* (Horvath) collected from Alexandria city*

Heavy metal	Sp./Sea.	Spring	Summer	Autumn	Winter	Mean
Fe	**Conc. level**	15.17±1.6 ^{a}	18.2±1.6 ^{a}	12.9±3.2 ^{b}	16.7±1.5 ^{a}	15.7
	BSAF	2.4	3.4	0.3	1.8	
Mn	**Conc. level**	21.5±1.9 ^{a}	16.8±1.9 ^{b}	8.4± 0.8 ^{c}	2.3±0.7 ^{d}	12.3
	BSAF	3.4	2.7	4.4	0.1	
Zn	**Conc. level**	27.3±2.4 ^{a}	11.7±2.1 ^{b}	22±2.4 ^{c}	15±2.1 ^{d}	19.0
	BSAF	5.3	2.4	8.1	4.2	
Cu	**Conc. level**	12.4±1.0 ^{a}	16.7±3.1 ^{b}	3.34±1.1 ^{c}	1.9±0.8 ^{c}	8.6
	BSAF	4.4	4.2	1.0	0.3	
Cd	**Conc. level**	1.23±0.53 ^{a}	2.2±1.12 ^{b}	1.5±0.6 ^{b}	1.70±0.4 ^{a,b}	1.7
	BSAF	0.3	0.7	0.5	0.7	
Cr	**Conc. level**	4.2±1.1 ^{a}	5.4±1.4 ^{a}	1.7±0.4 ^{b}	2.9±0.6 ^{b}	3.4
	BSAF	---	---	---	---	
Co	**Conc. level**	8.8±1.71 ^{a}	15.4±2.4 ^{b}	5.5±1.4 ^{b,c}	2.3±0.9 ^{c}	8.0
	BSAF	4.6	6.9	2.2	1.4	
MPI		**9.1**	**10.1**	**5.3**	**3.8**	**7.1**

* a, b, c, d : See footnote of Table (1); Conc., BSAF and MPI. : See footnote of Table (3)

3.4. Insects as heavy metal bioindicators

3.4.1. The correlation between Insects and heavy metals

For both the Mahmoudia and Alexandria sites, the correlation between the investigated insects and heavy metals explained that Fe concentration in water had a positively direct effect on the Fe accumulation in each *Crocothemis* sp. and *Micronecta isis* (r= 0.747 and 0.801, respectively, "$p < 0.05$"). Likewise, Mn concentration in water was inversely correlated with the Mn accumulation in *Diplonychus urinator* (r=-0.798, "p

< 0.05"), while its concentration in sediment had a strongly direct effect on the Mn accumulation in *Micronecta isis* (r=-0.871, "p<0.01"). Moreover, the Cu concentration in water was only positively correlated with the Cu accumulation in *Crocothemis* sp. (r=-0.795, "p<0.05"). On the other hand, all other measured heavy metals weren't affected by their concentration in all the investigated insects (Fig. 2).

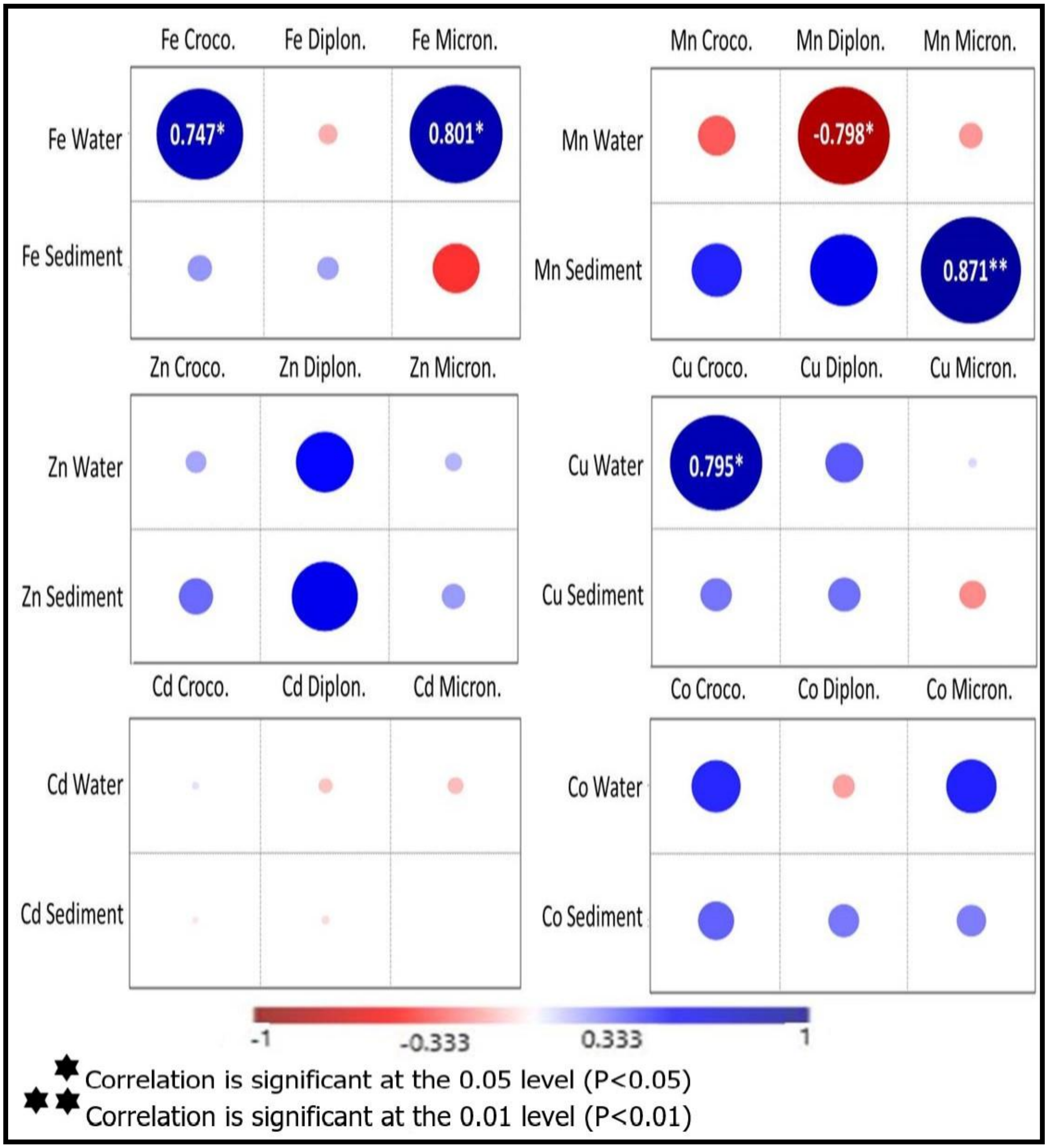

Fig. 2. Correlation plot showing the relationship between heavy metals' concentrations in water and sediment with their accumulations in investigated insects

3.4.2. The heavy metal accumulation inside insects

Undoubtedly, heavy metals are accumulated inside insects like any other animals (Fig. 3), and this accumulation varies from one insect to another. It is clear from Fig (3) that the Fe accumulation was semi-equal in both *Crocothemis* sp. and *Diplonychus urinator*, while its accumulation inside *Micronecta isis* was very poor. In addition, it was highly accumulated inside *Crocothemis* sp. during summer in both Alexandria and El-Mahmodia cites, but in *Diplonychus urinator,* Fe metal was more highly accumulated in El-Mahmodia than in Alexandria. The Cd metal had the same accumulation pattern equal to Fe.

Astoundingly, *Crocothemis* sp. has high ability to accumulate Mn and Co metals inside its body, but *Micronecta isis* and *Diplonychus urinator* do not. Additionally, the Mn and Co accumulation inside *Crocothemis* sp. was higher at El-Mahmodia during spring and summer than in Alexandria. On the other hand, Zn was identically accumulated inside the three investigated insects, and it was distributed equally between the two surveyed sites and during the whole study period. Concerning the Cu metal, *Diplonychus urinator* had a high ability to accumulate the metal inside the body than other insects.

DISCUSSION

Total heavy metal concentrations in aquatic environments can reflect current pollution levels since heavy metals are non-degradable and can accumulate in living tissues **(Liu *et al*., 2016)**. Based on the present findings, the heavy metals in water can be classified in order as follows: Fe > Cu > Zn > Co > Mn > Cd in Mahmudiyah, Fe > Zn > Mn > Co > Cu > Cd in Alexandria. Moreover, Cr was not detected in samples from both areas.

Iron (Fe) has established itself as the highest metal in these areas; it is a copious and vital element in the earth's crust and unmatched by any other heavy metal **(El-Naggar *et al*., 2009)**. Its concentration levels were slightly higher in Alexandria city during summer, albeit being within **WHO (2004)** approved limits. This increase could be attributed to anthropogenic organic matter, which can concentrate in the surface coating and stabilize metals through complexation due to its surfactant and correlation qualities. Even though Zn is a minor component, excessive amounts can be harmful to one's health, and its toxicity to humans is well-known **(Clark *et al*., 1981)**. Zn is one of the most toxic and readily available metals, and it contributes to environmental contamination when exposed to the air and washed into huge bodies of water. In the present study, Zn concentrations were below **WHO (2004)** authorised limits, and notably, they were in the lower range, validating modern sewage. Cadmium (Cd) is an extremely toxic heavy metal that is considered non-essential to life. Even though it was missing for several seasons in both locations, it was identified at the WHO's upper permissible threshold **(WHO, 2004)**.

Cd’s insatiable need to be adsorbed on suspended materials could explain its disappearance. Manganese (Mn) is an abundant trace element in the lithosphere **(Schmidt *et al*., 2018)**. It was higher in Alexandria than in Mahmudiyah, particularly in fall and winter. This could be attributed to agricultural discharges, household and industrial waste inputs, reducing the water level in the stream, and thus raising anthropogenic organic matter concentrations **(Osman, 2012)**. The amount of Co in this study was slightly greater than the WHO’s recommended level **(WHO, 2004)**.

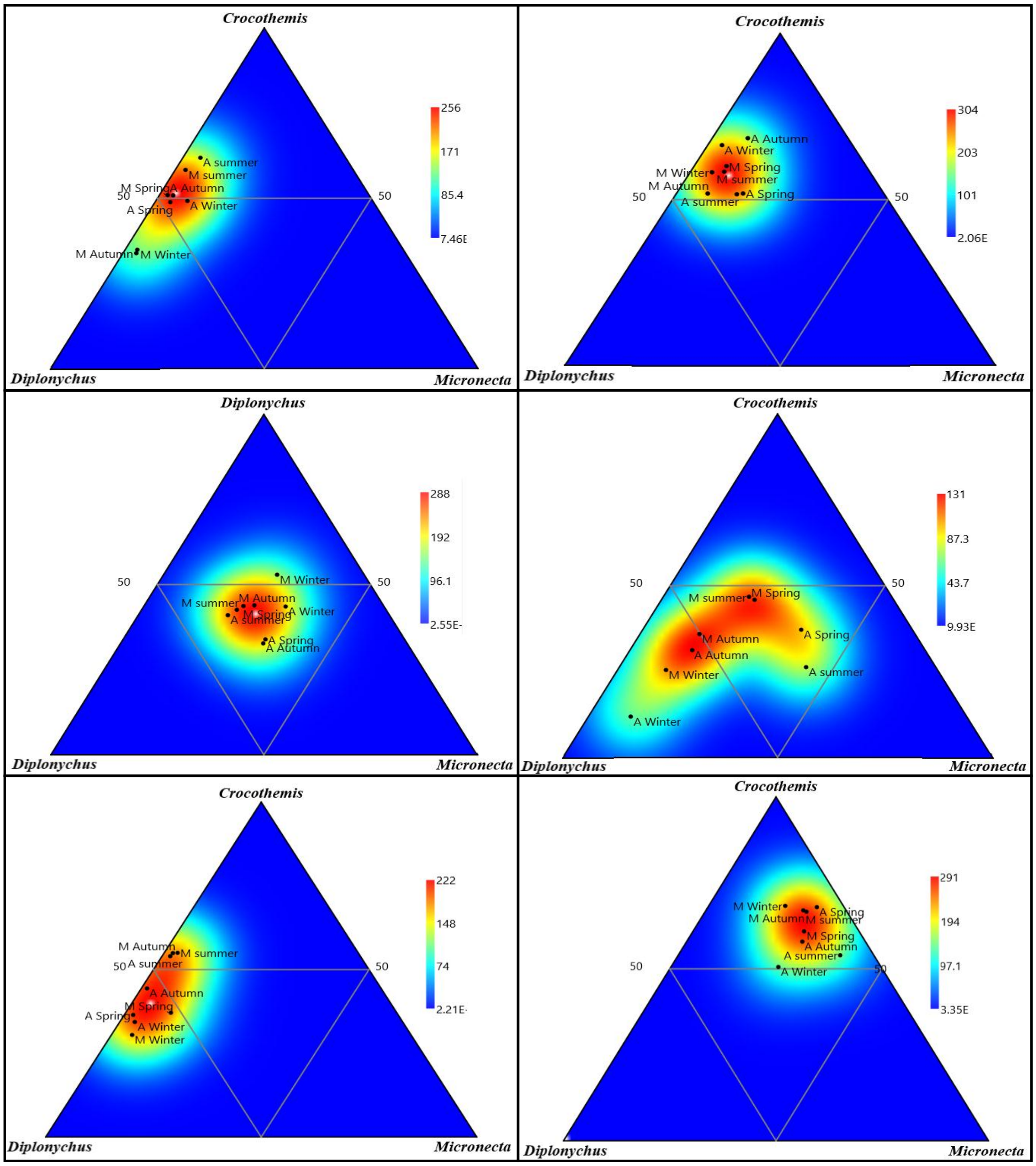

Fig. 3. Ternary plot showing the variation of heavy metal accumulation between investigated insects, M= El-Mahmodia and A=Alexandria

Metal levels can fluctuate due to changes in the volume of sewage effluents, agricultural drainage water and industrial pollutants **(Bakhoum & Abdallah, 2002)**. According to **Ali and Abdel-Satar (2005)**, the release of heavy metals from the sediment into the overlying water during hot seasons is driven by both high temperatures and a fermentation process produced by organic matter decomposition. Meanwhile, **Ormerod (1995)** stressed the importance of raising the temperature in order to increase heavy metal concentrations in water.

Sediment quality can be used to evaluate the level of contamination in the water column since sediments act as pollution sinks. The chemical composition of the sediment has an impact on the abundance and dispersion of invertebrates **(Aderinola *et al*., 2012)**. It's not surprising that the number of important metals discovered in the residue was larger than that found in water, confirming the hypothesis of **Eja *et al*. (2003)**. The current heavy metals in sediment investigation revealed the following accumulation order from high to low concentrations: Fe > Cu > Mn > Zn > Cu > Cd > Co in Alexandria and Fe > Cu > Mn > Zn > Cu > Cd > Co in Mahmoudia. Cr was also undetectable in samples of both areas. Iron (Fe) is the highest heavy metal in water analysis since it is a plentiful and necessary element for life **(El-Naggar *et al*., 2009)**. During the hotter seasons, Fe concentrations in both sites marginally increased, although they remained below the lower range of WHO permissible ranges **(WHO, 2004)**. During the hot seasons, however, a slight increase was recorded in the Mn concentrations in both study sites. Furthermore, **Osman (2012)** stated that the enrichment of this element in a particulate matter is a result of household and industrial waste inputs resulted in low dissolved oxygen content and H2S formation by bacterial activity, which is consistent with the present recorded rise in Mn level.

The amounts of Zn and Cu in sediment samples collected at the current study sites were lower than the WHO's acceptable limit **(2004)**. The levels of zinc and copper in sediment samples taken at the current study sites were below the WHO's permitted guideline **(2004)**. Heavy metal leaking into the soil's deeper layers and groundwater could be attributed to the lower Zn content **(Bream *et al*., 2019)**. Furthermore, iron behaviour could be linked to the lowest amount of Zn in one location during the fall and its absence in another. This season's increase in Fe supported the notion that the drop in zinc concentration was due to the adsorption on Fe (OH)3 sedimentation **(Martinez *et al*., 2000)**. On the other hand, **Hong-yun *et al*. (2005)** predicted the occurrence of copper in sediment as a result of quarrying, mining, sewage sludge, and fertiliser application, including the use of copper-containing fungicides, among other human activities that occurred in widespread soil pollution with Cu, which was not found in this study in both regions, excluding this reason for contamination. In contrast to their concentrations in water, cobalt was detected in lower quantities in the sampling sediments when compared to **WHO (2004)** values. This could be attributed to the silt releasing Co into the water. For its widespread occurrence, metal contamination in aquatic systems has won increased attention. Some heavy metals can form toxic metallic combinations in organisms, which can then be bioaccumulated and amplified in the food chain, putting human health at risk **(Zhou *et al*., 2008)**.

Cadmium (Cd) levels in the sampling sediments acquired from surviving studies exceeded **WHO (2004)** values in some seasons, particularly, the hot ones. Anthropogenic cadmium sources are more important than natural cadmium emissions, and they are responsible for the ubiquitous occurrence of the metal in sediment. The application of phosphatic fertilizers, plant-mediated Cd cycling from lower depths to the surface, and air deposition all contributed to the enhanced Cd levels **(ATSDR, 2008)**. Metals in trace concentrations may play a crucial role in aquatic creatures' metabolic life processes, while large levels have deadly effects on aquatic biota, particularly, when exposed for long periods of time **(Diop *et al*., 2015)**.

In the current investigation, the levels of heavy metals found in the entire bodies of different insects demonstrated a rather wide range for each metal tested. Furthermore, at different seasons, Fe, Mn, and Zn showed the highest concentrations in both streams, but Cr and Co recorded the lowest. **Tayel *et al*. (2008)** reported that, a rise in total dissolved metals in the Nile water and sediments was linked to an increase in heavy metal accumulation in fish. Furthermore, increased metal concentrations in different fish species are owing to higher degrees of exposure to these elements through water and sediments **(Bahnasawy & Khidr, 2011)**.

The bioaccumulation factor of sediment (BSAF) showed maximum levels of Fe, Mn, and Zn and the lowest levels of Cu and Co overall seasons, with a few exceptions, especially during the hot seasons (spring and summer). The aquatic insects have a high proclivity to concentrate heavy metals throughout their bodies; a result which is similar to the finding of **Eja *et al*. (2003)** for aquatic invertebrates. Meanwhile, the metal pollution index (MPI), according to **Mokhtar *et al.* (2009)**, gives a delegated image of the natural status of any ecological impacts on the aquatic ecosystem.

It was noticed that, the elevated MPI values in both locations during hot seasons and their declines during cold seasons (fall, winter) could be connected to the increased metabolism of aquatic invertebrates **(Bahnasawy & Khidr, 2011)**. The whole-body metal concentrations in insects were larger and more accurate than those from individual organs **(Bream *et al*., 1995)**. In the current study, Fe, Mn, and Zn were the most prevalent metals in water, sediment, and insects from both sites. This synchronisation of data is supported by the findings of **Shakweer (1998)**, who discovered that the concentration of trace metals in several organs of aquatic invertebrates predicts the degree of water pollution in aquatic environments.

In the present study, *Crocothemis* sp. and *Diplonychus urinator* stored iron and manganese in the two locations, followed by *Micronecta isis* nymphs. *Crocothemis* sp. exhibited the highest MPI as well, especially during the hot months. Furthermore, *Crocothemis* sp. and *Diplonychus urinator* had the highest BSAF of lead throughout summer. Heavy metal concentrations in aquatic insects change substantially over time, probably due to alterations in the water and sediments where these insects crawl, and due to their ecological demands, metabolism and feeding habits **(Mansour & Sidky, 2002)**.

As a result, metal bioaccumulation in insects can be utilized to assess metal pollution in aquatic ecosystems **(Tawari-Fufeyin & Ekaye, 2007)**. Furthermore, the current study revealed that the biggest levels of deposited metals in aquatic insects occur in the immature stage rather than the adult stage, which could be owing to their slow growth, which allows metals to accumulate in large concentrations. According to **Livonen *et al*. (1992)**, the highest amounts of deposited metals in aquatic insects were reported during hotter seasons, which could be attributed to increased warmth and acidity, which would lead to increased food intake from the surrounding environment. The number of heavy metals accumulated in a group of aquatic insects was related to their pace of growth. The ability of the measured essential components to act as an activator of a variety of enzymes found in aquatic invertebrates could account for their much greater BSAF. These findings mirrored those of numerous writers, including **Uluturhan and Kucuksezgin (2007)** and **Jayaprakash *et al*. (2015)**, who showed that the greatest BSAF values of Mn, Co, Cr, Fe, Cu, and Zn were found in fish gills and livers. In the meantime, an increase in MPI in insects could be due to the accumulation of inactive metabolic organs that could be used for long-term storage or release. According to **Omar *et al*. (2014)**, metabolically active tissues have a significant tendency to concentrate the most metals in their tissues, validating this notion. Despite their low metabolic activity, aquatic invertebrates have the highest fat content in tissues with a low susceptibility to combine with metals, that may be related to the smallest MPI **(Uluturhan and Kucuksezgin, 2007)**.

The current findings showed that aquatic insects can be used as a heavy metal bio-reagent. Iron (Fe) is the most abundant trace metal inside every animal since it is abundant and essential for oxygen transport in respiration **(Dojlido and Best, 1993)**. *Crocothemis* sp. and *Micronecta isis* can be used to detect iron concentrations in water, according to recent research, because the iron concentration in their bodies is significantly related to the concentration of iron in the water. In all species, iron plays a key role in a variety of metabolic processes, and an iron deficiency can have a serious impact on a variety of physiological systems **(Wieser *et al*., 2013)**. Iron bio-indicators can be found in insects such as the Gerridae and Chironomidae **(Nummelin *et al*., 2007)**. **Bream *et al*. (2017)** discovered that iron is the most prevalent metal in *Crocothemis* sp., confirming the current findings.

Similarly, the dragonfly (*Crocothemis* sp.) has a high rate of Cu buildup inside its body. According to our findings, Cu was absorbed in large amounts by water, with a positive relationship between Cu concentration in the water and *Crocothemis* bodies. Copper is, without a doubt, necessary for the formation and growth of aquatic insects **(Kabata-Pendias and Pendias, 2001)**. According to **Lavilla *et al*. (2010)**, copper was found in the inner portions of dragonfly larvae. Cu can also be reabsorbed from the water before molting **(Keteles and Fleeger, 2001)**. The dragonfly (*Crocothemis* sp.) has a high rate of Cu accumulation in its body as well. According to our data, Cu was absorbed in substantial amounts from water, with a positive link between Cu concentration in the water and *Crocothemis* bodies. Copper is unquestionably important for aquatic insect development and growth **(Kabata-Pendias and Pendias, 2001)**. According to **Lavilla *et al.* (2010)**, copper was discovered in the inner parts of dragonfly larvae. Also, **Keteles and Fleeger (2001)** stated that Cu can also be reabsorbed from the water before molting.

Micronecta was shown to have a substantial positive correlation with the Mn level in the sand, whereas *Diplonychus urinator* had a negative correlation with Mn. Cu, Zn, and Mn, which were detected in high quantities in the sediments, were found to be highly bio-accumulated in the insects **(Addo-Bediako and Malakane, 2020)**. The quantities of components in the aquatic insect were similarly found to be 5 to 10 times higher than in the sediment. **Santoro *et al.* (2009)** find bioaccumulation of heavy metals in a range of trophic groups of benthic macro-invertebrates. According to the study by **Bream *et al.* (2017)**, *Micronecta* and *Crocothemis* sp. retained the most lead, manganese, and cadmium. In contrast, the quantities of other metals investigated in insect bodies, such as Co, Cd, and Zn, did not appear to be related to their concentrations in water and sediment. These heavy metals may be accumulating in aquatic insects from sources other than water or sediment, such as their dietary sources. In a study of metal bioavailability in the food chain, **Besser *et al.* (2001)** reported considerable zinc concentrations in benthic fauna when compared to other metals. Minerals such as Cu, Fe, Mg, Mn, and Zn can also be obtained by aquatic insects via sediment and food **(Corbi and Froehlich, 2010)**.

Finally, trace elements can be found in both aquatic and terrestrial systems, and they build up in plants and animals. They also make their way into people via the food chain **(Varol and Sen, 2012)**. Because dragonflies eat a lot of other insects and accumulate a lot of metals from the aquatic environment, they had a greater heavy metal load than the other insects investigated. Dragonflies are excellent ecological markers for wetland and river quality because they reflect the state of both aquatic and terrestrial systems **(Haro, 2014; Pryke *et al.*, 2015)**.

CONCLUSION

In the present study, the utilisation of aquatic insects as biomonitors and bioindicators for metal pollution is effectively working in assessing the quality of water. The use of aquatic insects as biomonitors and bioindicators for metal contamination is helpful in monitoring the quality of water in the current study. Regular chemical analysis is generally recognised to be only useful for short-term quantification of contaminants, whereas aquatic insects are employed to determine metal pollution in streams and rivers over a longer period of time. Furthermore, the concentration of heavy metals in water and sediment is reflected in the accumulation of heavy metals in aquatic insects. As a result, insects could be employed as heavy metal contamination biomarkers.

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