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Seasonal Variations of Some Heavy Metals in Seawater and Soft Tissues of the Bivalve *Brachidontes pharaonis* from the Gulf of Suez, Egypt

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ABSTRACT

Levels of some heavy metal ions were determined in water samples of marine mussels *Brachidontes pharaonis* collected from the shoreline of the National Institute of Oceanography and Fisheries (NIOF), the Gulf of Suez, Egypt, during the period from June 2019 to May 2020. The objective of the present study was to analyze the seasonal concentrations of some heavy metals (Fe, Co, Ni, Cu, Zn, Pb, Mn, and Cd) that have environmental and health interests in the Gulf of Suez using the bivalve *Brachidontes pharaonis* (Fischer, 1876) as a biological monitor. The samples were collected seasonally from the site and divided into three size groups. The analyses for the detection of the selected metal concentrations were carried out by flame atomic absorption spectrophotometer. The results showed that the mean concentrations of heavy metals in water were 0.07, 0.64, 0.71, 4.66, 0.26, 0.08, 12.40, 0.07µg/ L for Cd, Pb, Cu, Zn, Ni, Co, Fe, and Mn, respectively. With regards to assessed metals in the soft tissues, an inverse relationship was found between metal concentration and bivalve size. According to the collected data, the present study area was varied between low to moderate contamination. This change occurred in conjunction with the increase of industrial activities around the study area.

INTRODUCTION

Heavy metals persist for a long time in the ecosystem, making them among the most toxic pollutants in the world. Consequently, researchers in the last decades were directed to determine the concentration and distribution of heavy metals in different environments **(Buzzi *et al.*, 2017)**. Heavy metals are considered as severe pollutants due to their toxicity and persistence in the environment, as they are non-degradable and remain for a long period **(Hyun *et al*., 2006; El Nemr, 2016)**. Therefore, heavy metals could have detrimental effects on the availability and diversity of mussels found naturally in polluted areas **(Chen *et al*., 2010)**.

Over the last years, pollution has increased due to human activities, resulting in higher concentrations of heavy metals in water and organisms, and then eventually back to humans through the food chain **(Chapman &Wang 2001; Buzzi *et al*., 2017)**. **El-Sawy *et al*. (2023)** investigated the impact of land-based activities on the distribution of some heavy metals in water and sediments of the Bitter Lakes, Suez Canal and found that elevated levels of metals were in the industrial regions near the lake.

Hongstrand and Haux (1991) reported that the vital metals that have an essential role in biological systems are iron, copper, zinc, and manganese. Otherwise, mercury, cadmium, and lead were reported as very toxic metals that cause cell death, inefficiency of different organs, low of fertility, and cellular or tissue damage. Hence, they were considered as non-essential metals **(Damek-Proprawa & Sawicka-Kapusta, 2003; Abd El Ghany, 2017)**. In the Protocols of the European Union for risky metals, lead and cadmium have been included **(EC, 2001)**. Therefore, the monitoring of heavy metals is considered as a prerequisite for predictions of contamination rates in different habitats and the accumulation of toxic substances in biota as well as in sediment. This is crucial for assessing environmental risks, as reported by **Kammann *et al*. (2023)**.

It's known that the accumulation of heavy metals in deposit-feeders mostly reflects metal concentration in sediments. On the other hand, the uptake and bioaccumulation of metals in filter-feeder would reflect metal concentration in the water column **(Newman & McIntosh, 1982; Boening *et al*., 1999)**. Bioaccumulation of heavy metals is extremely affected by environmental factors (physical, chemical, and biological). As a result of some biological factors, there were variations in metal bioaccumulation between members of the same bivalve species due to age, individual size, feeding ability, reproductive status, genotype, and phenotype. The physical and chemical factors that affect the bioaccumulation of heavy metals are water temperature, pH, dissolved oxygen, and nutrients **(Martoja *et al.*, 1988; Elder & Collins, 1991)**. It has been reported that, the uptake of heavy metals decreased in case of low pH, low temperature, and plenty of organic components. The decrease in pH and temperature lead to an increasing stress state of animals, resulting in reduced feeding ability and/or decreased mucus secretion in the gills **(Elder & Collins, 1991; Boening *et al*., 1999)**.

Although heavy metals occur in marine environments naturally, they sometimes cause dangerous effects on the ecological system when found in sufficient amounts. Living organisms are usually used to determine the quality of the environment where they live, especially bivalves. They can be used as monitors of contamination as they accumulate heavy metals in their tissues. Some of them can accumulate specific heavy metals in their tissues thousands of times higher than the concentration found in water where they live, such as mussels which accumulate cadmium **(Avelar *et al*., 2000; Göksu *et al*., 2005)**.

Bivalves are considered one of the most exposed marine fauna for heavy metals contamination as they can uptake it in different ways (water column, sediment, and food particles). They are sedentary, easily collected, and widely distributed in marine and freshwater. Therefore, they are used for biomonitoring and chemical monitoring in aquatic environments **(Buzzi *et al*., 2017)**. **Saleh *et al*. (2021)** studied the probable use of green mussel as bio-indicator of heavy metal pollution in Indonesia and found that its consumption can increase health risks if a person frequently consume. **Dar *et al*. (2021)** assessed the accumulation of carcinogenic metals (Cu, Ni, Pb and Cd) in the edible tissues of *Paratapes undulatus* and *Ruditapes decussatus* from bivalve fisheries in Lake Timsah and the Great Bitter Lakes, and they found metal concentrations in the two bivalve species are within the permissible safe limits for human consumption except for Pb .

Heavy metals not only accumulate in the soft tissues of bivalves but also in their shells. Different studies proved that mollusks especially bivalves can be good bioindicators of pollution as an accumulation of heavy metals in both shells and soft tissues **(El-Moselhy, 2014)**. **Nour (2020)** evaluated the levels of the metals Fe, Mn, Cu, Zn, Pb, Ni, Cd, and Co in molluscan shells and associated surface sediments from four sites on the Gulf of Aqaba and the Red Sea coasts, Egypt. They reported that these heavy metals could enter the studied ecosystem by terrigenous and anthropogenic sources as a weathering process of the nearby beaches and mountains, harbors, ship movement, landfilling, tourism and industrial activities, ship maintenance, wastewater, and traffic exhaust.

Mytilid mussels are usually utilized in monitoring programs in marine pollution fields in temperate regions of the world. They are considered convenient bio-monitors since they can accumulate heavy metals in higher concentrations than those found in the water column and tolerate intense lethal toxicity levels **(Nicholson & Lam, 2005)**. The detoxication enzymes of mussels are incompetent and only permit small effect on the transformation of pollutants within mussels tissues. Consequently, mussels are considered ideal aquatic biomonitoring organisms used in many pollution monitoring programs in many parts of the world **(Nicholson & Lam, 2005)**.

MATERIALS AND METHODS

I. Study area

The National Institute of Oceanography and Fisheries (NIOF) is located at Suez City on Suez Gulf (Fig. 1). Its coastal zone is greatly affected by water from the new sewage treatment plant of Suez City and the wastes of the vegetable and oil company, steel factory and textile factory **(El-Moselhy *et al*., 2016)**.

Fig. 1. A map of the study area showing the site of collection

II. Sampling

Subsurface water samples were seasonally collected during the period from June 2019 to May 2020 using water sampler, and were kept in acid-washed polyethylene bottles, then acidified and were transferred to the Lab of the National Institute of Oceanography and Fisheries (NIOF), Suez, Egypt for further investigation. Furthermore, specimens of the mussels *Brachidontes pharaonis* were seasonally collected during the same period from the intertidal rocky zone of the shoreline of NIOF (Fig. 1). The individuals of this species were collected during the low tide by hand picking and detached from rocks by forceps, then transferred to NIOF Lab for analysis.

III. Analysis of samples

1. Water

APDC/MIBK (ammonium pyrolidine dithiocarbamate/ methyl isobutyl ketone) solvent extraction was used for pre-concentration of heavy metals in the water samples **(APHA, 1999)**. The heavy metals (Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb, Mn^{2+}, and Cd^{2+}) were measured through the usage of an atomic absorption spectrophotometer (AAS) (Perkin Elmer, Analyst 100), and the results were expressed as µg/ L.

2. Tissue samples

Immediately after the collection process, the specimens were transferred to the laboratory of the NIOF and divided into three size groups (<1.5, 1.5:2, >2cm). Then, the specimens were cleaned from attached sediments and algae by distilled water, shell was carefully removed; only soft tissues were used for digestion process. The preparation of the samples to determine concentration of heavy metals in soft tissue was performed according to the guidelines of **FAO (1976)**. For each size group, composite samples with about 1g from more than one organism were used for metal analysis. Then all composite samples were digested at 100ºC using AR conc. Nitric acid in Teflon digested vessels to complete the digestion process on hot plate. Subsequently, wet digested samples were filtered, diluted with deionized water, measured to the same volume (8ml) and were analyzed for Fe, Co, Ni, Cu, Zn, Pb, Mn and Cd using a flame atomic absorption spectrophotometer (FAAS Perkin Elmer model Analyst 100). The results obtained were expressed in µg/ g wet weight. De-ionized water was used to prepare all solutions and blanks. Moreover, before the analysis, all vessels and glassware were soaked in 10% nitric acid overnight and later rinsed with distilled water.

IV. Bioaccumulation of heavy metals in soft parts

1. Bio-concentration factor (BCF)

Bio-concentration factor (BCF) for mussel ***B. pharaonis*** was calculated using the method of **Qiu (2015)**, as follows:

$$BCF = Cf/Cw$$

Where, Cf (µg /g ww) is the heavy metal contents in the tissues;
Cw (µg /l) heavy metal contents in the water.

According to **USEPA (2010)**, BCF was classified into four groups to measure the potential of bioaccumulation in each individual as follows;
BCF < 0.250, low bio-accumulative;
0.250 < BCF < 1, medium bio-accumulative;
1 < BCF < 5, high bio-accumulative; and
BCF > 5, very high bio-accumulative.

2. Metal pollution index (MPI)

The level of heavy metal contamination in tissues of *B. pharaonis* can indicate the pollution of the surrounding environment **(Soliman *et al.*, 2020)**. Accordingly, metal pollution index (MPI) equation of **Usero *et al*. (2005)** was used to outline the overall metal contents in tissues of *B. pharaonis* as follows:

$$MPI = (M1 \times M2 \times M3 \times \ldots\ldots Mn)^{1/n}$$

Where, Mn is the concentration of each examined heavy metal in tissues for each group of mussels expressed in µg/ g of dry weight; n is the total number of metals determined. The environment can be considered polluted if MPI is above one **(Soliman *et al*., 2020)**.

V. Statistical analysis

The variation of levels of the heavy metals in water and tissue samples was analyzed using ANOVA during different seasons at $P \leq 0.05$.

RESULTS

I. Seasonal fluctuation of the investigated heavy metals in water

The seasonal changes in the estimated heavy metals concentrations (µg/ L) in the water samples from Suez in front of NIOF during the study period are represented in Table (1). The highest concentrations were reported for Fe and Zn at the sampling site in winter and autumn, respectively. However, the concentration of Co was the lowest through the investigated seasons. Meanwhile, the concentrations for the other heavy metals (Cd, Pb, Cu, Ni and Mn) showed irregular different readings along the study period. The metals declined in the following order: Fe> Zn> Cu> Pb>Ni> Co> Cd> Mn. Seasonal investigation of heavy metals in water samples indicated that Fe, Ni, Co, Mn, and Cd peaks were reached in winter, while those of Zn and Cu were observed in autumn, but Pb were reported to peak in the summer season (Fig. 2).

Table 1. Seasonal overall mean of the heavy metals concentrations in the water samples (µg/ L) collected during the study period

Assessed metal (µg/L)	Summer 2019	Autumn 2019	Winter 2020	Spring 2020	Average (µg/L)
Cd	0.11	0.02	0.13	0.02	0.07
Pb	0.97	0.86	0.46	0.28	0.64
Cu	0.56	0.57	1.24	0.46	0.71
Zn	3.25	10.44	3.56	1.37	4.66
Ni	ND	0.15	0.69	0.19	0.26
Co	ND	ND	0.29	0.04	0.08
Fe	18.04	7.38	21.33	2.83	12.40
Mn	0.08	ND	0.12	0.07	0.07

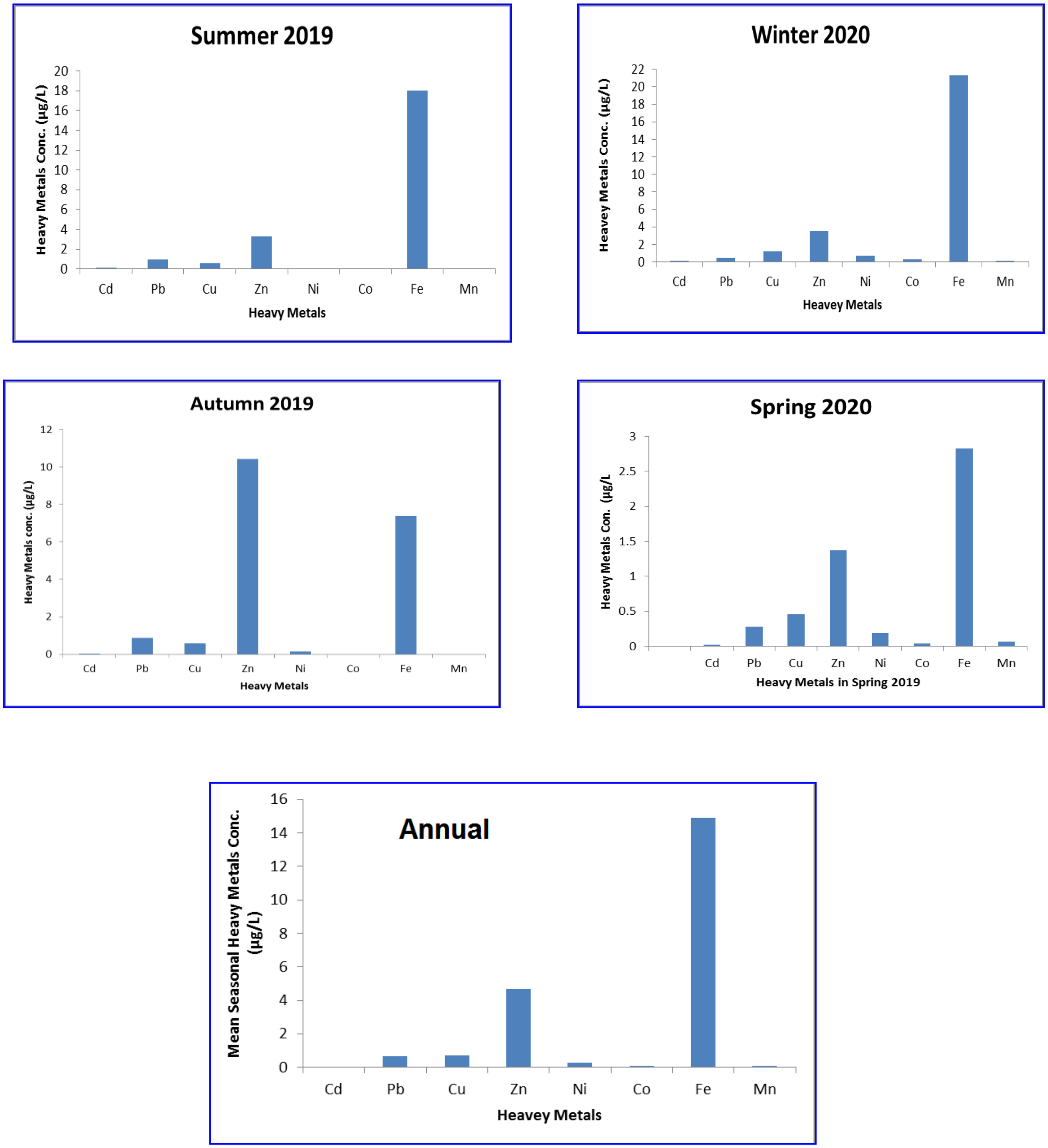

Fig. 2. Seasonal and annual concentrations of the investigated heavy metals in water in front of NIOF on Suez Gulf

II. Seasonal fluctuation of the investigated heavy metals in water

Table (2) showed the mean concentrations of the heavy metals in the soft tissues of the bivalve *B. pharaonis* (μg/ g wet wt.) collected from Suez during the study period. The results indicate that individuals of the bivalve *B. pharaonis* can accumulate high concentrations of heavy metals in their bodies. Generally, the maximum levels were determined in winter. Individual of size group 1 achieved the highest concentrations of all heavy metals for most of the year with mean concentrations of 265.06± 52.11, 4.22± 0.40, 3.13± 0.05, 2.80± 0.21, 2.02± 0.06, 1.26± 0.07 and 0.47± 0.07 for the metals iron, copper, manganese, lead, nickel, cobalt and cadmium, except zinc which is the only metal in this size group that recorded the lowest concentration in all seasons during study period.

On the other hand, the determined values for size group 3 showed the lowest levels of heavy metals with mean concentrations of 55.79± 13.05, 1.87± 0.18, 1.14± 0.05, 1.04± 0.14, 0.81± 0.21, 0.54± 0.18 and 0.19± 0.06 for the metals iron, copper, lead, manganese, nickle, cobalt, and cadmium, respectively.

Seasonal readings of heavy metals had indicate that Fe, Mn, Cu, Zn and Cd peaks were reached in winter, while those of Ni, Co and Pb were observed in autumn (Table 2 & Fig. 3). Seasonal concentrations of heavy metals in soft tissues (μg/ g) of *B. pharaonis* showed that values of iron (Fe) and zinc (Zn) were dominant, while those of cadmium (Cd) were the lowest.

Table 1. Average concentrations of the heavy metals in the soft tissues of the bivalve *Brachidontes pharaonis* (µg/ g wet wt.) collected from Suez during the study period

Bivalve size	Estimated metal	Summer		Autumn		Winter		Spring	
		Mean	std.D	Mean	std.D	Mean	std.D	Mean	std.D
Group 1	Cd	0.36	0.06	0.43	0.02	0.47	0.07	0.46	0.02
	Pb	1.78	0.47	2.80	0.21	2.41	0.44	1.61	0.07
	Cu	2.46	0.38	3.84	0.34	4.22	0.40	2.32	0.13
	Zn	15.98	2.12	21.58	3.25	25.15	3.74	13.75	0.19
	Ni	1.66	0.28	2.02	0.06	1.95	0.29	1.97	0.005
	Co	0.92	0.24	1.26	0.07	1.22	0.18	1.09	0.002
	Fe	101.36	4.30	131.55	20.72	256.05	52.11	143.50	8.98
	Mn	1.83	0.49	3.02	0.25	3.13	0.05	2.23	0.17
Group 2	Cd	0.22	0.03	0.30	0.03	0.37	0.12	0.24	0.01
	Pb	1.12	0.23	1.67	0.07	2.01	0.48	1.09	0.07
	Cu	1.51	0.23	3.91	0.58	4.34	1.96	1.97	0.11
	Zn	14.05	3.15	23.33	0.69	30.55	14.97	13.82	0.24
	Ni	1.01	0.14	1.30	0.09	1.64	0.59	1.09	0.002
	Co	0.53	0.11	0.80	0.03	0.98	0.33	0.60	0.001
	Fe	67.97	16.90	98.61	0.81	181.85	135.02	64.21	8.47
	Mn	1.04	0.18	1.70	0.04	2.55	1.17	1.61	0.19
Group 3	Cd	0.29	0.02	0.19	0.06	0.27	0.02	0.30	0.01
	Pb	1.36	0.18	1.19	0.54	1.44	0.19	1.14	0.05
	Cu	1.87	0.18	2.40	1.57	3.90	0.24	2.19	0.14
	Zn	27.01	2.66	22.55	13.48	34.65	5.02	20.13	1.44
	Ni	1.21	0.04	0.81	0.21	1.11	0.02	1.21	0.03
	Co	0.71	0.04	0.54	0.18	0.70	0.03	0.80	0.05
	Fe	70.48	11.62	55.79	13.05	75.40	13.05	68.45	4.04
	Mn	1.15	0.08	1.04	0.14	1.64	0.14	1.35	0.13

G1: individuals with size group (<1.5cm).

G2: individuals with size group (1.5cm).

G3: individuals with size group (>2cm).

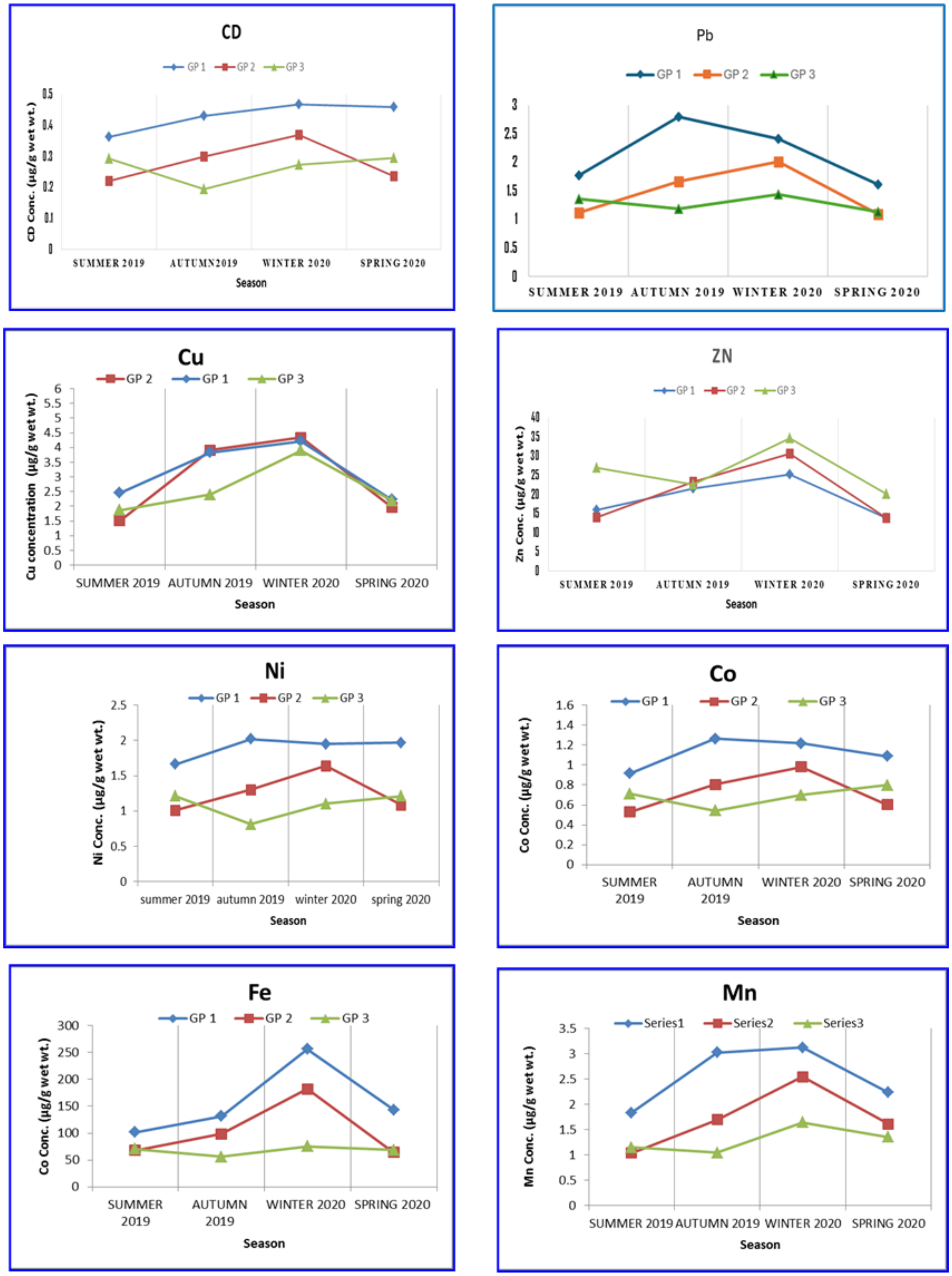

Fig. Error! No text of specified style in document.. Seasonal and annual concentrations of the investigated heavy metals in water in front of NIOF on Suez Gulf different size groups of the bivalve *Brachidontes pharaonis* collected from NIOF, Suez Gulf during different seasons

III. Bioaccumulation of heavy metals in tissues

1. Bio-concentration factor (BCF)

Data in Table (3) indicate that Zn exhibited the highest BCF value (1124.25) in the tissues of mussel *B. pharaonis* during autumn, while the lowest values were recorded in summer (168.26). Similarly, the highest BCF values for the heavy metals Pb, Cu, Ni, Co, Fe and Mn were in autumn and the lowest ones were in summer. However, Fe had the lowest seasonal BCF value throughout different seasons. Generally, all the investigated heavy metals had very high bio-accumulative levels (BCF> 5) in all seasons, except for Cd that recorded high bio-accumulative levels in summer and winter seasons (Table 3).

Table Error! No text of specified style in document.**2.** Seasonal and annual variations of values of bio-concentration factor (BCF) for mussel *B. pharaonis* from NIOF on Suez Gulf

Heavy metal	**BCF**				
	Summer 2019	**Autumn 2019**	**Winter 2020**	**Spring 2020**	**Annual**
Cd	**3.81**	**22.36**	**3.38**	**18.53**	**6.10**
Pb	**12.55**	**94.29**	**14.60**	**53.41**	**22.48**
Cu	**17.22**	**169.04**	**30.99**	**88.83**	**39.90**
Zn	**168.26**	**1124.25**	**224.74**	**662.50**	**300.73**
Ni	**12.20**	**78.23**	**10.60**	**58.95**	**40.00**
Co	**6.37**	**43.44**	**7.19**	**34.51**	**11.62**
Fe	**4.76**	**12.67**	**7.76**	**37.43**	**9.10**
Mn	**11.88**	**96.02**	**18.20**	**72.19**	**25.55**

2. Metal pollution index (MPI)

The estimated seasonal and annual MPI values of the heavy metals Pb, Cu, Zn, Ni, Co, Fe and Mn in the tissues of *B. pharaonis* were found to be higher than one across the investigated seasons, as shown in Table (4). However, the seasonal and annual MPI values for Cd were less than one during the four seasons.

Table 3. Seasonal and annual variations in values of metal pollution index (MPI) for mussel *B. pharaonis* from NIOF, the Suez Gulf

Heavy metal	MPI				
	Summer 2019	Autumn 2019	Winter 2020	Spring 2020	Annual
Cd	0.29	0.30	0.78	0.32	0.32
Pb	1.39	1.76	1.90	1.27	1.59
Cu	1.89	3.26	4.09	2.11	2.86
Zn	17.71	21.78	28.86	5.22	21.02
Ni	1.29	1.52	1.37	1.37	1.39
Co	1.05	1.66	2.51	1.58	1.70
Fe	75.24	85.83	144.53	82.02	98.59
Mn	1.30	1.74	2.34	1.69	1.78

DISCUSSION

The anthropogenic activities have negative impacts on the Red Sea coast of Egypt, where it suffer from several contamination sources from industrial activities, oil production, different land-based activities, shipping, mining, and tourism activities **(Saad *et al.*, 2023)**. In the present study the highest concentrations of the heavy metals Fe and Zn in water samples from Suez in front of NIOF were recorded in autumn and winter, respectively, while the concentration of Co was the lowest during the four seasons. On the other hand, the levels of the heavy metals (Cd, Pb, Cu, Ni and Mn) showed variations along the four seasons. They arranged in an descending order as follows: Fe> Zn> Cu> Pb> Ni> Co> Cd> Mn.

Saad *et al.* (2023) reported that the levels of Pb, Cd, Cu, Zn, Co, Ni, Mn, and Fe in seawater samples collected from the Red Sea proper, Gulf of Suez and Aqaba Gulf varied according to regions and seasons. The highest values of all Pb, Cu, Zn, Co, Ni, and Fe were recorded in summer, while those of Cd and Mn were in winter. Moreover, they added that high levels of the non-essential toxic elements (Pb, Cd and Ni) were recorded in seawater of the Suez Gulf, where there are industrial, petroleum, harbor and shipping activities. On the other hand, the high concentrations of the essential elements (Cu, Zn, Co, Mn and Fe) were found in the Gulf of Aqaba and the Red Sea proper, where there are sources of pollution.

According to **USEPA (2010)**, it was obvious from the current estimated seasonal and annual values of the bioaccumulation factor (BCF) of the investigated heavy metals Pb, Cu, Ni, Co, Fe and Mn that they had very high bio-accumulative levels in the tissues of mussel *B. pharaonis* annually and in the four seasons (BCF> 5), except for Cd which recorded high bio-accumulative levels (BCF< 5). This indicates the high ability of *B.*

pharaonis to accumulate the investigated heavy metals irrespective to their values in sea water.

In the current study the annual and seasonal values of MPI of the heavy metals Pb, Cu, Zn, Ni, Co, Fe and Mn in tissues of *B. pharaonis* were above one, indicating that the environment at the investigated site in Gulf of Suez is considered polluted with these heavy metals **(Soliman *et al*., 2020)**. However, the MPI values for Cd in the same site were less than one annually, as well as in the four seasons, which indicates the absence of pollution of the environment with this heavy metal.

CONCLUSION

As a result of the continuous increase in human activities, including iron and steel industry, power plants, petroleum refining, and shipping activities in the study area, it is necessary to perform a periodical monitoring evaluation for heavy metal concentration to discover early environmental changes and overcome the biological effects related to them.

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