Journal of Environmental studies [JES]
An International Journal
https://jesj.journals.ekb.eg/
doi: 10.21608/jesj.2024.330555.1096

Assessment of the Seasonal Effects of Heavy Metals on Kuruma Prawn (*Marsupenaeus japonicus*) and its Impacts on the Ovary and Biochemical Composition

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Citation: *Abd-Ellah, S.M.; El-Sheri, S.S.; Abd-El Latif, H.A.; Nour, O.M. (2025). Assessment of the Seasonal Effects of Heavy Metals on Kuruma Prawn (Marsupenaeus japonicus) and its Impacts on the Ovary and Biochemical Composition. Journal of Environmental Studies, Vol. 37(1): 8-22.*

Article Information
Received 23 Oct. 2024,
Revised 15 Desem. 2024,
Accepted 16 Desem. 2024,
Published online. 1 Mars 2025

Abstract: The kuruma prawn Marsupenaeus japonicus is considered as one of the luxury seafood in Alexandria, Mediterranean Sea, Egypt. The present study is designed to determine the seasonal bioaccumulation levels of Zn, Cu, Fe, Pd, Cd and Co in the whole soft tissues as well as in the ovary of M. japonicus collected from Abu Qir Bay. The kuruma prawn accumulates metals in soft tissues and gonads at concentrations exceeding that of seawater. The order of metals bioaccumulation levels was Zn >Fe >Cu >Pb> Cd>Co. Metals in the whole soft tissues as well as the ovary showed seasonal variations. For all metals, the highest concentrations were detected in summer, while the lowest were in winter. Results revealed that only the bioaccumulation levels of Zn and Fe in kuruma prawn were found to be higher than the permissible limits for human consumption. Moreover, the level of the caloric contents of the soft tissues of M. japonicus was evaluated and seasonal variations were recorded. The seasonal total protein contents in the soft tissues of M. japonicus were arranged as followed: summer > spring > autumn > winter. In contrast, the highest content of total carbohydrates and total lipids was in winter, while their lowest content was in summer. The present study extended also to histological studies of the ovary of M. japonicus at different stages of maturation. Several stages of ovarian cycle of maturation recorded marked histopathological alterations.

Keywords: Marsupenaeus japonicus, prawn, heavy metals, histopathological damage.

1. Introduction

The kuruma prawn M. japonicus mainly inhabits bays and seas of the Indo-West Pacific, which are influenced by warm water currents, and as a Lessepsian migrant has reached the Mediterranean Sea (Galil *et al*., 2002). M. japonicus is commonly known as the kuruma prawn, kuruma shrimp or Japanese tiger prawn and famous commercially as "Gambari azzazi yabani''. It is one of the largest, most consumed and economically important species in Mediterranean countries as luxury seafood items and locally considered as the most common and commercially important prawn in Alexandria, Egypt. Abu-Qir represents important source areas of fisheries in Alexandria (El-Sayed & Moharram, 2007). Abu-Qir Bay is considered as one of the 131 major hot spots which were recorded along the coastline of the Mediterranean Sea (UNEP/WHO, 2003; Zakaria, 2007; Abo-Taleb *et al*., 2017; El-Damhogy *et al*., 2019). Pollution hot spots affect ecosystem, biodiversity, sustainability and human health. The Bay receives several types of pollutants, ranging from trace elements, heavy metals, pesticides, humic acids, and nutrients to domestic wastes, from three main gates: El-Tabia Pumping Station (TPS), Boughaz El-Maddya (the outlet of Lake Edku), and the Rosetta mouth of the River Nile (El- Shanawany, 2010; Khairy *et al*., 2011; Abo-Taleb *et al*., 2017).

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Pollution of the marine environment by heavy metals is an alarming problem worldwide. Pollution of marine environment presents a threat to aquatic organisms with effects such as accumulation in the ecosystem and losses of habitats and biodiversity, acute and chronic toxicity, as well as a threat to human health. Determination of water quality is considered the most important indication to describe the environmental condition of any aquatic ecosystem. Aquatic organisms from microorganisms to large creatures are affected by any change in water quality (Abo-Taleb, 2019). Evaluation of heavy metals in water is one of the most important methods for water quality determination (El-Damhogy *et al*., 2019). In coastal areas, consuming metal-polluted seafood is an indirect pathway to metal exposure. In contrast to the possible health benefits of eating seafood, ingesting metals-contaminated seafood is the main route of human exposure (Hashempour-Baltork *et al*., 2019; Hashempour-Baltork *et al*., 2023). Excessive concentrations of metals in food were associated with the etiology of several diseases, especially renal, hepatic and neurological diseases (Chailapakul *et al*., 2008; Hossain *et al*., 2022; Tahity *et al*., 2022).

The World Health Organization WHO (1995) recommended that heavy metals must be controlled in food sources to assure public safety. Direct consumption of seafood without quality control is more frequent in many African countries due to poverty (Tamele & Loureiro, 2020). Consumers must be aware of the potential risks of consuming seafood contaminated with metals at concentrations higher than the permissible limit.

In aquatic ecosystems, environmental pollutants affect several physiological processes such as metabolism, growth, reproduction, and ultimately survival of the organisms. Reproduction is an essential physiological process for the continued existence of a species generation after generation. Long-term exposure to pollution damages the reproductive organ's tissues, decelerating the reproductive cycle and finally restricting embryogenesis (Maharajan *et al*., 2017). Several studies recorded histopathological changes in the reproductive organs of aquatic animals exposed to environmental pollutants (Jadhav & Sheikh, 2012; Brraich & Jangu, 2015; Raju Subha *et al*., 2016). Histological changes indicate the extent of cellular and tissue damage, serving as an early indicator of pollution hazards (Shaikh *et al*., 2010).

The current study focuses on monitoring the quality of M. japonicus from Abu-Qir Bay for human consumption as safe seafood. This involves determination of the seasonal bioaccumulation levels of Zn, Cu, Fe, Pd, Cd and Co in the whole soft tissues. Additionally, the study aims to assess the potential for oogenesis disruption in M. japonicus due to heavy metal toxicity.

Materials and methods

Ethical approval

The present study was carried out at Abu-Qir, Alexandria, Egypt and accepted according to ethical standards of scientific research.

1.Study area

Abu-Qir Bay has been selected as one of the most important fishery stations in Alexandria. It is a semi-circular basin covering an area of 500 km² in the Eastern Mediterranean Sea, located approximately 35 km northeast of the city of Alexandria, Egypt. This bay is one of the most productive fishing areas in the region (Figure 1).

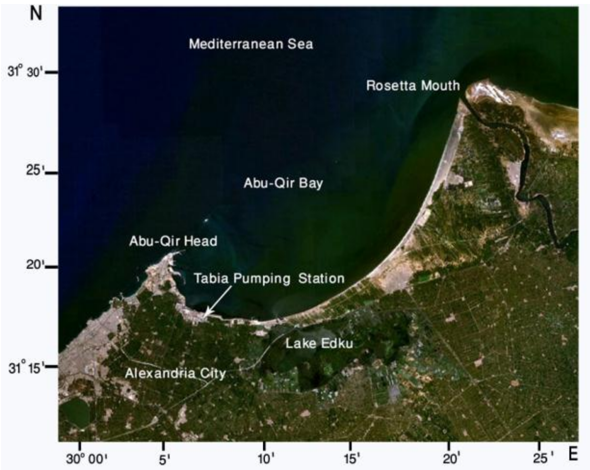

Figure 1: Sample collection area

2. Sampling

Prawn samples were collected from Abu Qir Bay at a depth of about 10 m. M. japonicus was identified according to the species identification sheet provided by FAO (1983). Females M. japonicus were collected seasonally at different stages of maturation and were transported to the laboratory within an hour in an ice box with well-aerated seawater to ensure that they were alive. Seawater samples were collected seasonally during prawn sampling with Van Dorn water sampler with a capacity of 1.2 liters and were transferred to reagent bottles.

3. Metal analysis

Heavy metals Zn, Cu, Fe, Pd, Cd and Co were analyzed seasonally in seawater samples and prawn tissues using Graphite Furnace Atomic Absorption Spectroscopy (Perkin- Elmer 2380) at Central Chemical Laboratory of Faculty of Science, Alexandria University, under the recommended conditions of Bernhard (1976). Seawater samples were collected at the four seasons of the year from the same prawn habitat and were acidified by a measured volume of concentrated nitric acid, filtered through a 0.45 mm micropore membrane filter (Hashmi *et al*., 2002) and metals concentrations were extracted according to Eaton0, 1976. Twelve sexually mature specimens of female M. japonicus, with similar size and weight, were collected seasonally and divided into two groups; each contains six female specimens. In both groups, the exoskeleton was carefully removed. In the first group, the whole soft tissue was analyzed, while in the second group, the females were dissected carefully to isolate the gonads from the remaining soft tissues to detect metal bioaccumulation levels in each tissue separately. The samples taken from both groups were stored in plastic bags at -20 ºC till the time of analysis. Each sample was processed according to the technique of Campbell & Plank (1998). The process involves drying the tissue sample at 80ºC for 24 hours, and then digesting 2 grams of the dried sample in 10 ml of concentrated nitric acid at 60°C for 30 minutes. After that, the tubes were cooled to room temperature and 2 ml of hydrogen peroxide were added and heated until the solution becomes clear, and finally diluted to 100 ml with deionized distilled water. The analytical blanks should be run in the same way as the samples.

4. Biochemical studies

Six mature females were collected in each season (15 - 17.5 cm length) and were washed carefully with distilled water and then drained under folds of filter paper (Adeyeye *et al*., 2008; Dinakaran *et al*., 2009). Prawn samples were beheaded, and the exoskeletons were pelled off (Sriket *et al*., 2007). The whole soft tissues of the six mature females were homogenized seasonally with physiological saline (Dinakaran *et al*., 2009). The ground samples were then stored in polyethylene bags at - 40 °C until the analysis was performed (Valarmathi & Azariah, 2002; Nisa & Sultana, 2010).

Total protein was determined by the method described by Lowry *et al*. (1951) with bovine albumin (Sigma Chemical Co., St. Louis, MO, USA) as the standard. Total carbohydrate was determined by the method described by Roe (1955). Total lipid was determined Barnes & Blackstock (1973) method.

5. Histological studies of the gonad of female M. japonicus.

For histological study, large numbers of adult females were dissected, and careful removal of their ovaries was done, sections were taken from the middle portion of the ovary. After fixation, the standard methods of dehydration, clearing, and paraffin embedding were followed as described by Horbin (1996). 5µm thick sections were stained with freshly prepared hematoxylin-eosin (HE).

6. Statistical analysis

Data from metal analysis and biochemical studies were statistically analyzed. Analysis of variance (ANOVA) was conducted using SPSS 15.0 software at $p<0.05$. Data was expressed as mean ± SD.

Results

1. Metals analysis

a. Heavy metals in the seawater of Abu-Qir Bay

The concentrations of Zn, Cu, Fe, Pd, Cd and Co in seawater samples collected seasonally from Abu-Qir Bay are presented in Table 1. The results indicated that all the metals reached their highest levels in summer and their lowest levels in winter (Figure 2a). Metal concentrations for all the metals decreased from summer to winter in the following order: summer > autumn > winter, and then increased in the spring. Statistically significant differences (ANOVA, $p\leq 0.01$) were found between seasons for all the metals. Moreover, the concentrations of metals in seawater were in the order: Fe > Zn > Cu > Pd > Cd > Co (Figure 2 b). However, the highest metal concentration was recorded for Fe and the lowest metal concentration was recorded for Co. Metals concentration trends can be summarized in the following order:

Summer : Fe > Zn > Pb > Cu > Cd > Co

Autumn : Fe > Zn > Cu > Pd > Cd > Co

Winter : Fe > Pb > Zn > Cu > Cd > Co

Spring : Fe ≈ Zn > Cu > Pb > Cd > Co

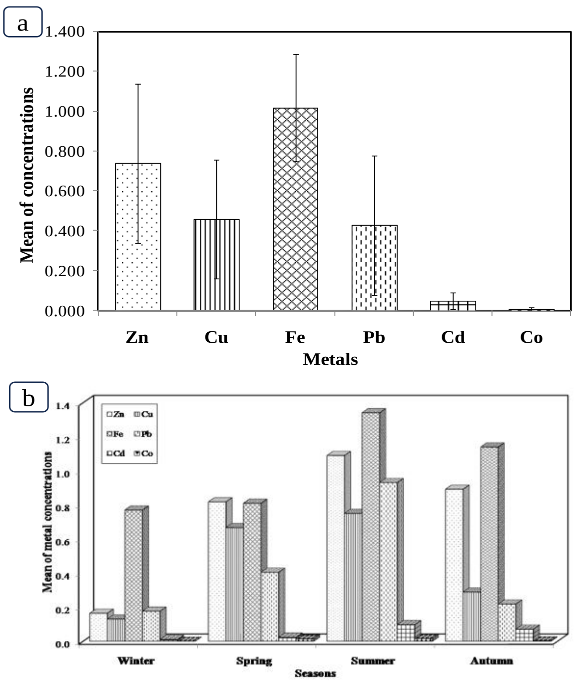

Figure 2. The average concentrations (mg/l) of heavy metals in seawater samples collected from Abu-Qir Bay, (a) their accumulation levels and (b) their seasonal variations

Table 1: Heavy metals concentrations in seawater samples collected seasonally from Abu-Qir Bay.

Metal	Seasons				Average metal concentration	F (p)
	Winter	Spring	Summer	Autumn		
Zn	0.164^a±0.033	0.818^b±0.167	1.090^b±0.218	0.892^b±0.178	0.74 ± 0.40	21.970* (<0.001)
Cu	0.130 ^{a}±0.026	0.667^b±0.133	0.750^b±0.150	0.288 ^{a}±0.058	0.46 ± 0.30	29.419* (<0.001)
Fe	0.770 ^{a}±0.154	0.810^{ac}±0.162	1.341^b±0.268	1.140^c±0.228	1.02 ± 0.27	6.329* (<0.001)
Pb	0.176 ^{a}±0.035	0.405^b±0.081	0.931^c±0.186	0.218^a±0.044	0.43 ± 0.35	40.035* (<0.001)
Cd	0.011 ^{a}±0.002	0.024 ^{a}±0.005	0.097^b±0.019	0.071^c±0.014	0.05 ± 0.04	40.043* (<0.001)
Co	0.00 ^{a} ±0.00	0.013^b±0.003	0.015^b±0.003	0.002 ^{a}±0.001	0.007± 0.008	46.133* (<0.001)

F: F test (ANOVA) Data are represented as mean ± SD

Different superscripts within each row indicate statistically significant differences between groups at $p \leq 0.05$

Values are expressed in mg/l

Table 2: Concentrations of heavy metals (mg kg–^{1}dry weight) in the whole soft tissues of females *M. japonicus* collected seasonally from Abu-Qir Bay.

Metal	Seasons				Average metal concentration	F (p)
	Winter	Spring	Summer	Autumn		
Zn	108.11c± 7.65	122.55 bc ±11.91	144.86a ±10.43	134.72ab ± 24.89	127.56 ± 19.96	6.493 (<0.001*)
Cu	3.24c± 1.21	8.89b± 1.0	17.23a± 4.39	4.99c± 0.42	8.59 ± 5.92	42.417 (<0.001*)
Fe	57.24c± 7.19	66.03c± 4.42	89.26a± 10.99	76.03b ± 4.82	72.14 ± 13.96	21.072 (<0.001*)
Pb	0.10d± 0.01	0.51b± 0.08	0.72a± 0.10	0.27c± 0.06	0.40± 0.25	87.176 (<0.001*)
Cd	0.04a± 0.01	0.21 ^{a} ± 0.33	0.28 ^{a} ± 0.42	0.05 a± 0.05	0.14 ± 0.27	1.187 (<0.001*)
Co	0.00b± 0.00	0.01 b± 0.01	0.06 a ± 0.03	0.00 b± 0.00	0.03 ±0.02	19.127 (<0.001*)

F: F test (ANOVA) Data are expressed as mean ± SD

Different superscripts within each row indicate statistically significant differences between groups at p≤ 0.05

Values are expressed in mg kg−1 dry weight

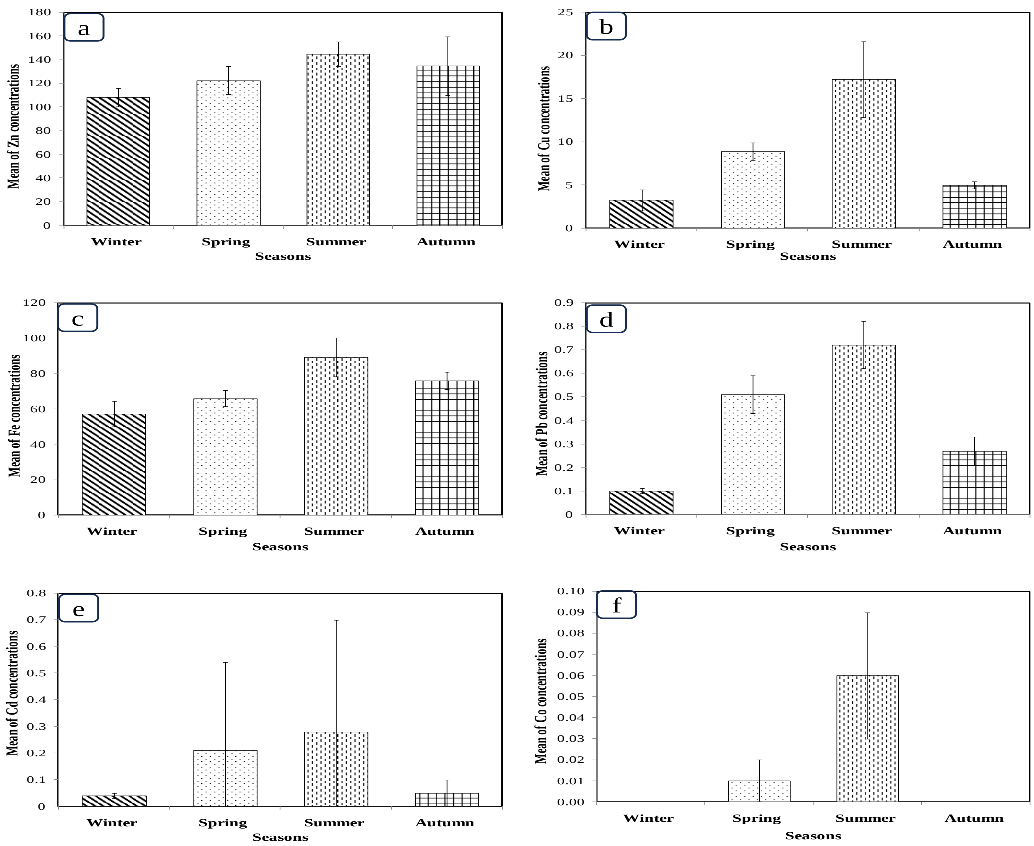

Figure 3. Seasonal variations of heavy metal concentrations in the whole soft tissues of females *M. japonicus*.

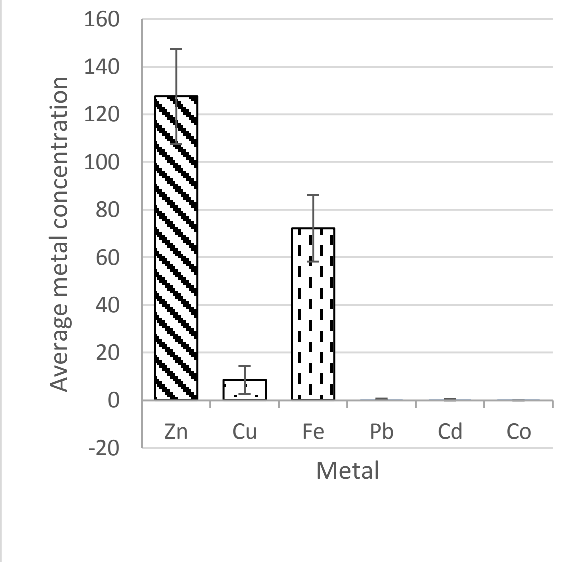

Figure 4: The accumulation levels of metals in the whole soft tissues of female *M.japonicus.*

b. Heavy metals in the whole soft tissues of females *M. japonicus*

The present study recorded the seasonal bioaccumulation levels of Zn, Cu, Fe, Pd, Cd and Co in the whole soft tissues of females *M. japonicus* (Table 2). The recorded results showed seasonal fluctuations in metals bioaccumulation, with the highest levels found in summer while the lowest was recorded in winter (Figure 3). The whole soft tissues of female *M. japonicus* accumulated heavy metals in each season in the following sequence: Zn > Fe > Cu > Pb > Cd > Co (Figure 4). The highest concentration was recorded for Zn (127.56 ±19.96 mg kg^{-1} dry weight; mean ± SD), while the lowest was recorded for Co (0.03). Co was undetectable in the soft tissue of females *M. japonicus* in both winter and autumn. Metals concentration trend during all seasons was in the following order: Zn >Fe > Cu > Pd > Cd > Co.

c. Heavy metals in the ovaries and the remaining soft tissues of M.japonicus.

Ovaries and the remaining soft tissues of females M. japonicus were analyzed seasonally for determination of Zn, Cu, Fe, Pd, Cd and Co (Table 3). The order of metal concentrations for all the studied metals were as follows: the remaining soft tissues > ovaries. Thus, the ovary recorded the least metals concentrations (Figure5). It must be mentioned that Zn and Fe were the highest metals accumulated in the ovaries and the remaining soft tissues of females M. japonicus, followed by Cu,Pb,Cd and Co (Fig. 6) . Seasonal variations in bioaccumulation levels of metals in different tissues of M. japonicus were recorded. Winter revealed the lowest concentrations of metals, while summer recorded the highest concentrations for all metals.

2.Biochemical studies

Females *M. japonicus* were analyzed seasonally to determine the change in the total proteins, lipids, and carbohydrate contents. The present study recorded seasonal variations in total protein, carbohydrates and lipids (Table 4). Protein was found to be the major component followed by carbohydrates then lipids. Its content exhibited seasonal changes where the lowest value was recorded in winter and the highest value was recorded in summer (Figure 7). In contrast, lipids and carbohydrates concentrations recorded the same trend in their seasonal fluctuations, where, the highest contents of total carbohydrates and total lipids were recorded in winter, while their lowest contents were recorded in summer.

3. Histological studies of the gonad of female M. japonicus.

The present study extended also to ovaries at histological level and followed different stages of ovarian maturation to determine the seasonal effect of metals. Oocytes in early stages of oogenesis (*i.e.*, oogonia, meiotic gonial cells, synaptic oocytes, and newly dividing cells) showed no remarkable histopathological changes, while previtellogenic a pronounced feature of several pathological changes oocytes (late perinucleolus oocytes), vitellogenic oocytes, mature oocytes, and the follicle cells revealed a pronounced feature of several pathological changes (plate I). Impaired late perinucleolus oocytes showed marked decrease in their size and contained displaced nuclei. In addition, migration of heterochromatin, pyknosis, and karyokinese nuclei (Plate I, b & c). However, autolytic late perinucleolus oocytes showed invasion of hypertrophic follicle cells indicating phagocytosis and dissociation of oolemma (Plate I, d). Early vitellogenic oocytes showed irregularity, swelling and disruption in the ovarian wall (Plate II, b). Oil globules stage II oocytes showed migration of nucleoli to the periphery of their nuclei forming a complete ring beneath the nuclear membrane (Plate II, d). Oil globules stage I oocytes showed highly degenerated and vacuolated nuclei (Plate II, e). In addition, abnormal union of the ooplasm between two adjacent primary yolk granules oocytes were seen, these oocytes showed a distention margins, degeneration, and numerous phagocytic cells are easily detected with invasion the oolemma of abnormal primary yolk granule oocytes confirming atresia (Plate II, f). The alternation of the vitellogenesis process affected not only the formation but also the morphology of the formed yolk inclusions (plate III). Considerable changes in the secondary yolk granules

Table 3: Concentrations of heavy metals (mg kg−1 dry weight) in the ovaries and the remaining soft tissues of females *M. japonicus* collected seasonally from Abu-Qir Bay.

Metal	Tissue	Seasons				Average metal concentration	F (p)
		Winter	Spring	Summer	Autumn		
Zn	O	15.32^{c}± 3.69	19.42^{cb}± 1.94	27.66 ^{a}± 5.43	21.21^{b}± 2.01	20.90 ± 5.63	12.46 (<0.001*)
	R	92.52^{c}± 3.14	103.17^{b} ±4.79	112.12^{a}± 10.39	109.97^{ab}± 8.15	104.45 ± 10.29	9.013 (<0.001*)
Cu	O	0.21^{b}± 0.06	0.20 ^{b} ±0.09	0.35^{a}±0.04	0.20 ^{b}± 0.05	0.24 ± 0.09	8.179 (<0.001*)
	R	3.23^{c}± 1.20	8.15^{b}± 0.57	15.38^{a}± 0.81	3.91^{c}± 0.69	7.67 ± 5.0	256.821 (<0.001*)
Fe	O	6.59 c± 1.55	10.77^{b}± 2.87	19.78^{a}± 2.21	12.55^{b}± 0.93	12.42 ± 5.22	44.325 (<0.001*)
	R	50.07^{d}± 3.36	56.38^{c}± 3.01	69.83^{a}± 6.96	63.65^{b}± 3.15	59.98 ± 8.68	22.008 (<0.001*)
Pb	O	0.01±0.01^{a}	0.04±0.02^{a}	0.06^{a}± 0.01	0.04 ^{a} 0.03 ±	0.03 ± 0.04	0.844 (<0.001*)
	R	0.01d± 0.01	0.08 ±0.46^{b}	0.67^{a} ± 0.11	0.27^{c} ± 0.02	0.37 ± 0.23	69.788 (<0.001*)
Cd	O	0.00^{b} ± 0.00	0.03^{a}± 0.01	0.03^{a}± 0.01	0.00 ±0.00 ^{b}	0.02± 0.01	33.038 (<0.001*)
	R	0.03^{b}± 0.01	0.05^{b}± 0.01	0.23^{a}± 0.07	0.04^{b}± 0.01	0.09 ± 0.09	46.078 (<0.001*)
Co	O	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.0 ±0.0	-
	R	0.00 ±0.00^{b}	0.01^{b}± 0.00	0.06^{a}± 0.02	0.00 ±0.00^{b}	0.03 ±0.02	64.957 (<0.001*)

Data are expressed as mean ± SD

Different superscripts within each row indicate statistically significant differences between groups at $p \leq 0.05$

Values are expressed in mg kg−1dry weight (O)ovary, (R) remaining soft tissues

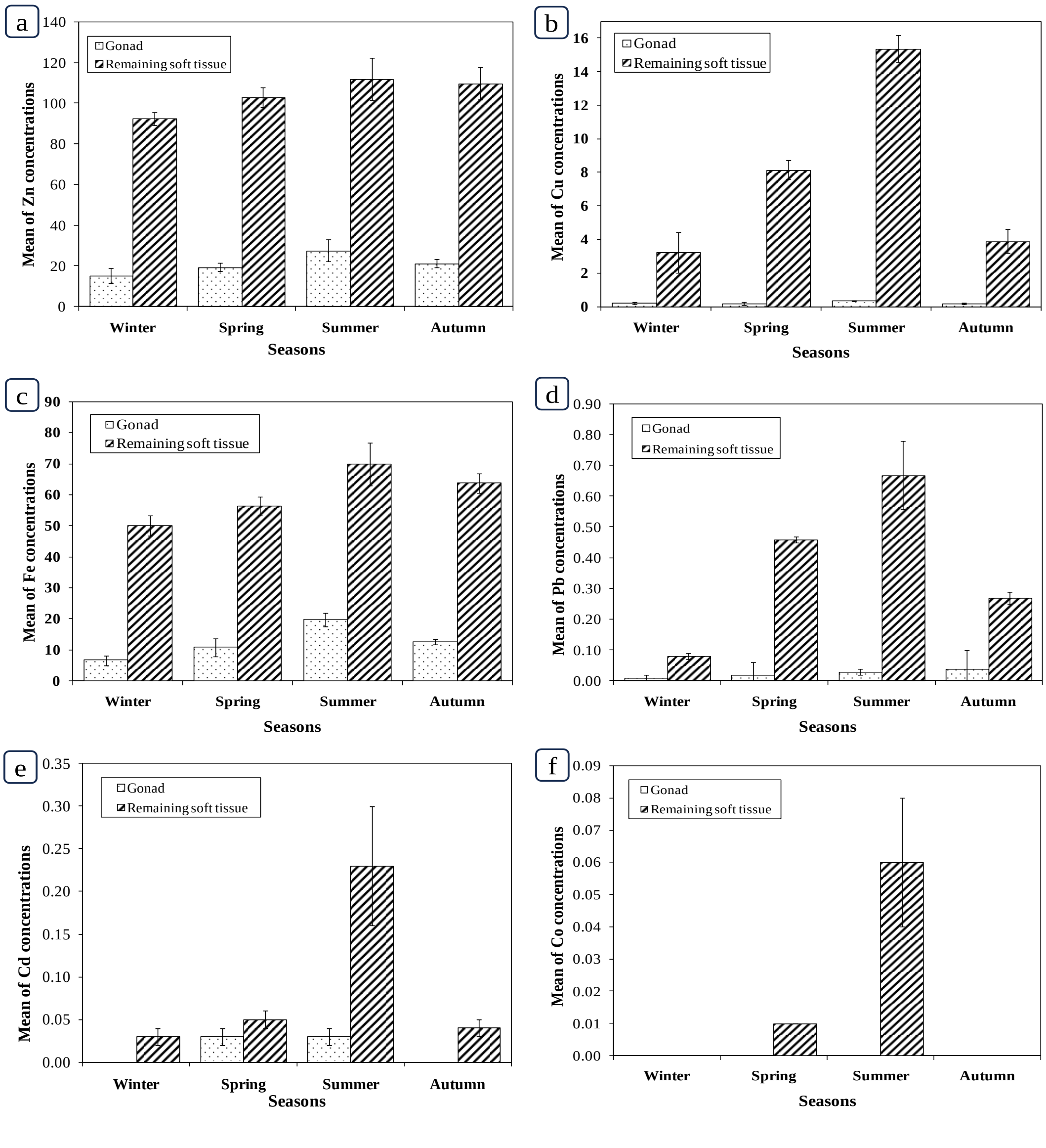

Figure 5: The accumulation levels of metals in the ovaries and the remaining soft tissues of *M. japonicus*

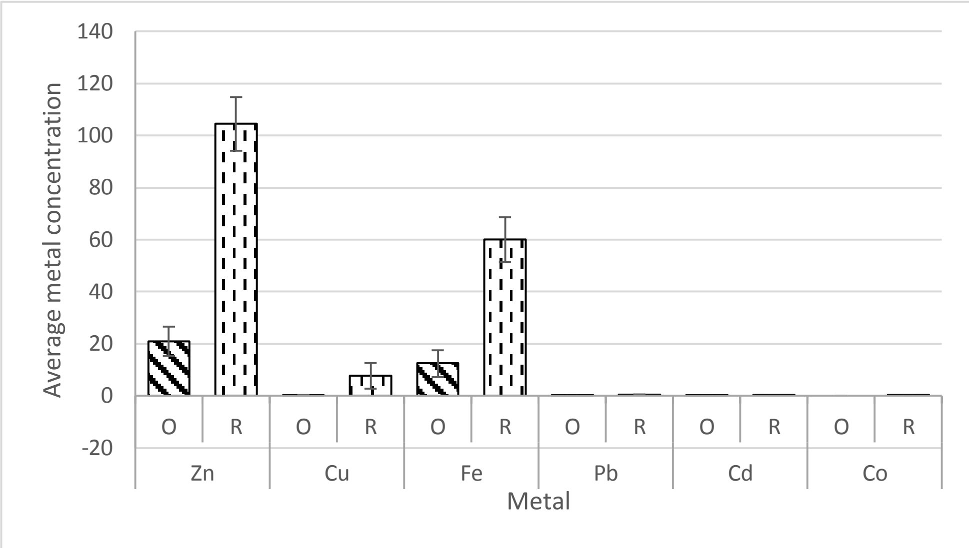

Figure 6: The seasonal accumulation levels of metals in the ovary (O) and the remaining soft tissues (R) of females *M. japonicus.*

Table 4: Seasonal fluctuations in the proximate chemical compositions (mg g-1 wet weight) in the soft tissue of females M. japonicus collected from Abu-Qir Bay.

Chemical compositions	Seasons				F (p)
	Winter	Spring	Summer	Autumn	
Total protein	261.91[a]± 1.79	275.28[b]± 1.89	307.54[c]± 4.97	269.36[d]± 0.73	151.404* (<0.001)
Total lipids	3.27 [a] ± 0.03	3.14 [a] ± 0.12	2.45[b] ± 0.13	2.74[c] ± 0.13	34.808* (<0.001)
Total carbohydrate	64.58[a] ± 5.97	45.93[b]± 2.30	42.23[b] ± 0.32	56.91[c] ± 2.03	27.854* (<0.001)

F: F test (ANOVA) Data are expressed in Mean ± SD

Different superscripts within each row indicate statistically significant differences between groups at $p \leq 0.05$

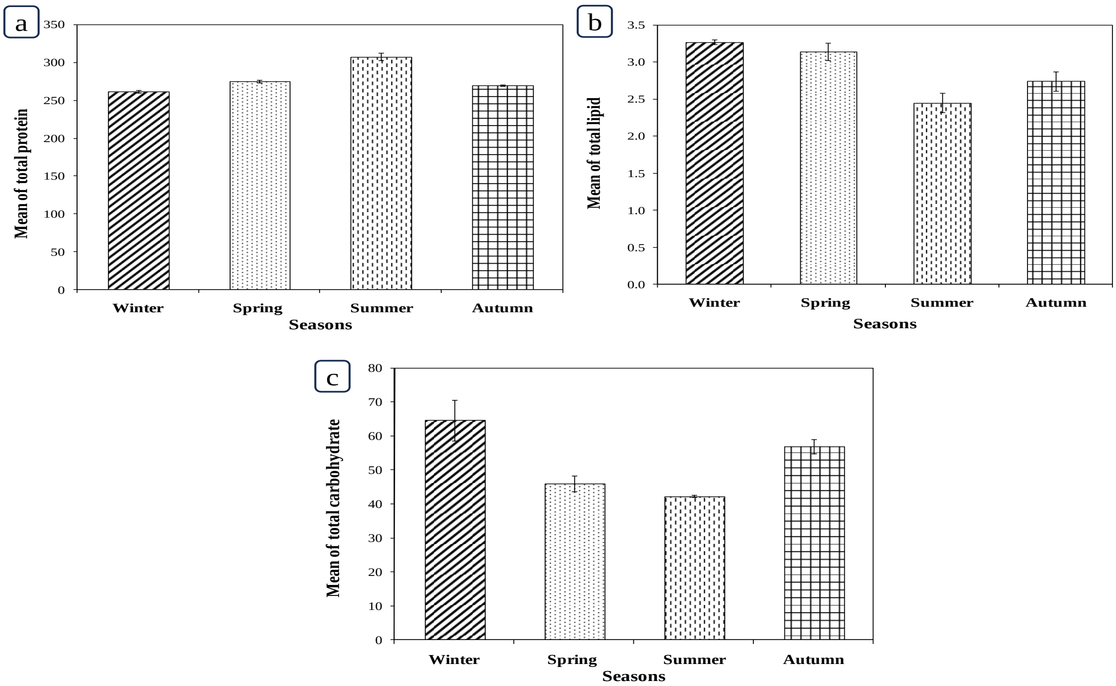

Figure 7: Seasonal variations of **a)** total protein content, **b)** total lipid content and **c)** total carbohydrate content in females *M. japonicus.*

oocyte were observed in abnormally developing ovary. Its ooplasm was packed with small abnormal yolk bodies which could prevent its development. Also, the nucleus showed a lytic nucleoplasm (Plate III, b).

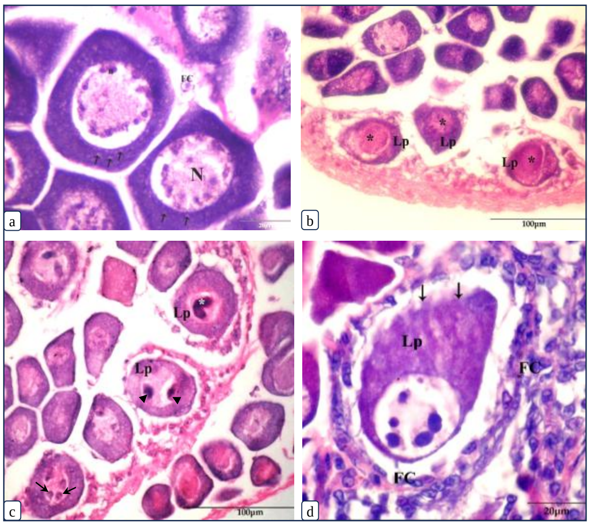

Plate I: Transverse sections of the ovary at stage I of maturity (Immature stage). a) late preinucleolus oocytes with strongly basophilic cytoplasm loaded with numerous basophilic vesicles (arrows), the nucleus (N) had numerous basophilic nucleoli (n), follicle cells (FC) with rounded shaped. b) late perinucleolus oocytes (Lp) with abnormal nuclei (asterisk). c) late perinucleolus oocytes, pyknosis (white asterisk), karyokinesis (arrow head), migration of heterochromatins (arrows). d) anomalous late perinucelous oocyte (Lp) with dissociated oolemma, and numerous hypertrophied phagocytic follicle cell (FC) ensheathed the oocyte.

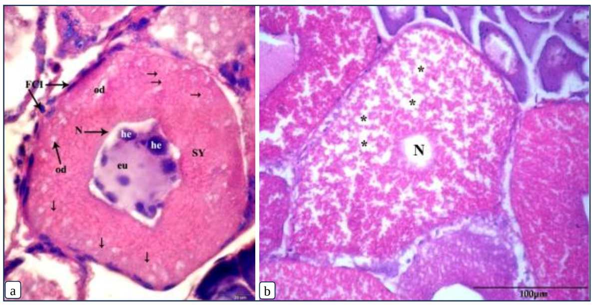

Plate III: Transverse sections of the ovary at stage III of maturity (mid developing ovary). a) secondary yolk granules oocyte (SY) showing its polyhedric shape, surrounding with flat and strongly basophilic type 1 follicle cells (FCI), acidophilic ooplasm; and the faintly fluidy nucleus (N). Micropinocytosis vesicles (arrows) filled with minute yolk granules, euchromatin (eu), heterochromatin (he), oil droplets (od). b) secondary yolk granules oocyte with degenerated nucleus (N) and abnormal distribution of yolky materials (asterisks).

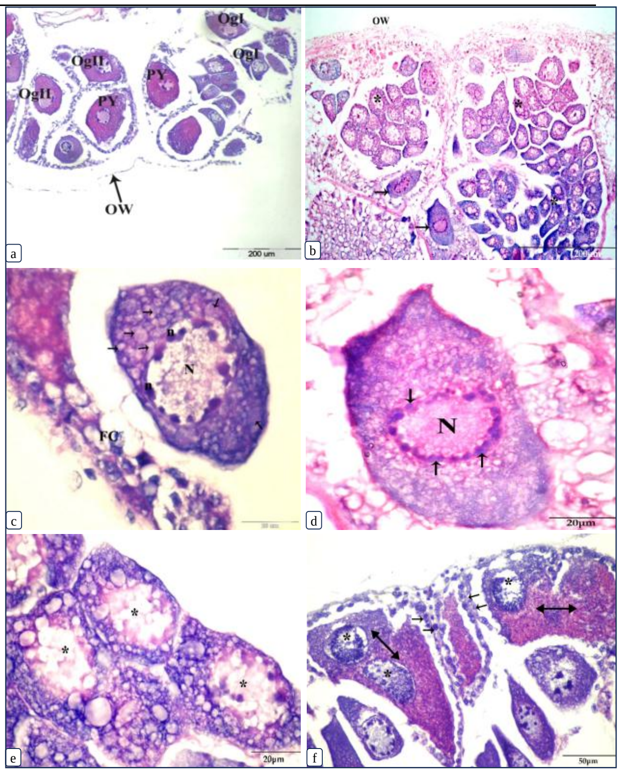

Plate II: Transverse sections of the ovary at stage II of maturity (Early developing stage). **a)** oil globule I oocyte (OgI), the oil globule II oocytes (OgII), the primary yolk granule oocytes (PY), thin ovarian wall (OW). **b)** showing irregularity and swelling of anomalous ovarian wall (OW), abnormal oil globule I oocytes (asterisk), and oil globule II oocytes (arrows). **c)** oil globules I oocyte at the end of its development demonstrating that the ooplasm became slightly eosinophilic, enlarged lipid droplets (arrows), follicle cells (FC), strongly basophilic nucleus (N) with numerous periphery basophilic nucleoli (n). **d)** abnormal oil globule II oocytes showing migration of the nucleoli in the nucleus (N) forming a circle beneath the nuclear membrane (arrows). **e)** abnormal oil globule I oocytes demonstrating vacuolated and degenerated nuclei (asterisks). **f)** abnormal union of two primary yolk granule oocytes (union arrows), degenerated nuclei (asterisk), phagocytic cells invading lytic primary yolk granules oocyte (arrows).

Ovaries exhibited mass destruction of late vitellogenic oocytes and abnormal union of ooplasm of two adjacent tertiary yolk granule oocytes (Plate IV, a). In addition, a group of anomalous tertiary yolk granule oocytes were observed forming a complex of united abnormal cells which had no distinctive investment (Plate IV, b). Stage VI of maturity recorded obvious pathological changes where numerous mature oocytes failed to complete their developments where the outer surrounding membrane became undulating and the cortical rods appeared separated from it (Plate V, b) or forming a complete sheath around the abnormal mature oocyte (Plate V, c). Moreover, some mature oocytes showed aggregation of the peripheral cortical rods to be in the central of the mature oocyte (Plate V, d). In addition, hyperplasia and hypertrophy of follicle cells were detected in defected mature ovaries (Plate V, e).

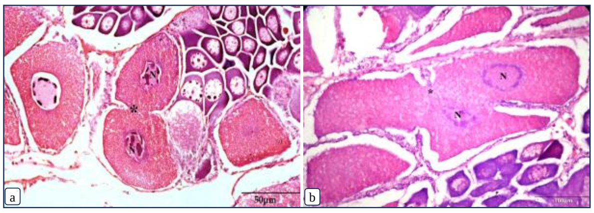

Plate IV: Transverse sections of the ovary at stage IV of maturity (late developing stage). a & b demonstrating abnormal union of tertiary yolk granule oocytes(asterisk) with abnormal nuclei (N).

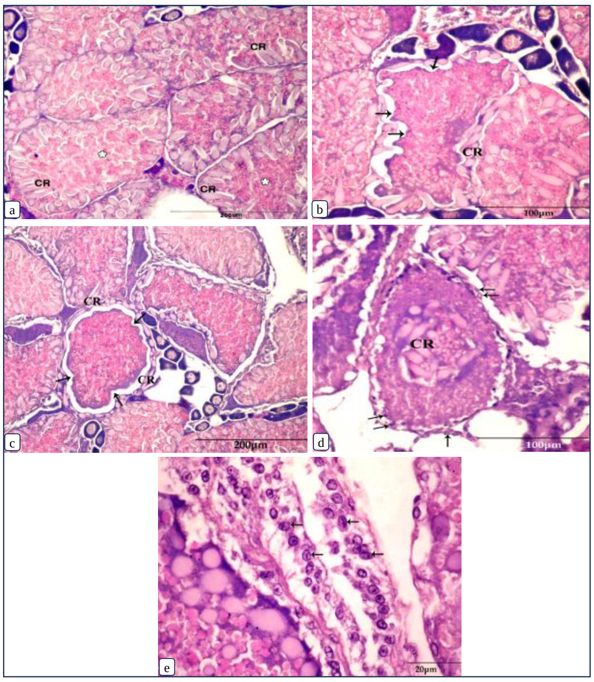

Plate V: Transverse sections of the ovary at stage VI of maturity (mature stage) .a) magnified mature ova showing numbers of yolk granules (white asterisks) in a faintly basophilic matrix , cortical rods (CR) at the periphery of the oocyte. b) degenerated mature oocyte with irregular cell membrane (arrows), separation of cortical rods (CR) from the oolemma. c) degenerated mature oocyte with irregular cell membrane (arrows), separation of cortical rods (CR) from the oolemma forming a complete ring around the oocyte. d) abnormal mature oocyte encircling with phagocytic cells (arrows), aggregation of cortical rods (CR) in the center of the oocyte. e) numerous hypertrophied follicle cells (arrows).

Discussion

1. Heavy metals in the seawater of Abu-Qir Bay

In the present study the concentrations of Zn, Cu, Fe, Pd, Cd and Co in seawater samples collected seasonally from Abu- Qir Bay showed their maximum values in summer and their minimum values in winter. Several previous studies recorded seasonal variations in metal concentrations in aquatic ecosystems. Abd-Ellah *et al.* (2020) recorded the highest concentrations of metals in seawater samples collected from the Eastern Harbor, Alexandria, Egypt during summer, while the lowest concentrations were during winter. Duysak & Uğurlu (2020) reported that seawater collected from Iskenderun Bay, Turkey, recorded the highest concentrations for all metals in summer, while the lowest concentrations were in winter. In contrast, El-Damhogy *et al*. (2019) recorded seasonal variations of heavy metals in seawater samples collected from Abu-Qir Bay, with the highest concentrations were recorded during summer and the lowest concentrations in spring. Ali *et al.* (2016) suggested that in the dry season, low rainfall leads to lower water flow and as a result, higher concentrations of heavy metals. According to El-Serafy *et al*. (2003a) and El-Ewa *et al.* (2007) the summer peak for most of the measured metals may be due to the decomposition of sediments of the bottom layer and organic matter under the effect of high temperature releasing heavy metals to the overlying water. According to Maanan (2008), the seasonal variations of metal accumulation may be attributed to physicochemical parameters such as temperature, pH and dissolved oxygen. The elevated temperature in summer decreases the amount of free oxygen (El– Haddad, 2005). Peng *et al.* (2011) reported that in summer the elevated temperature and the decrease in dissolved oxygen may increase the concentrations of dissolved metals. Gaber *et al.* (2017) suggested that winter represents the season when there is a decrease in temperature and pH of water, favoring the mobilization of metals.

The present results of heavy metals concentrations in seawater samples were compared with the maximum permissible concentrations applied for fishery purposes by WHO, 2008 (Table 5). It was apparent that the concentrations of Zn and Cu were below the permissible levels. On the other hand, the concentrations of Fe, Pb, Cd and Co exceeded the permissible levels.

Table 5: Concentrations of heavy metals (mg/L) in seawater samples collected from Abu Qir Bay and maximum permissible concentrations (MPC) of metals according to WHO (2008).

Metal (mg/L)	Zn	Cu	Fe	Pb	Cd	Co
MPC	3.0	2	0.3	0.01	0.003	0.005
Present study	0.74	0.46	1.02	0.43	0.05	0.007

(MPC) Maximum permissible concentrations of metals

2.Heavy metals in the whole soft tissues of females M. japonicus

The present study showed seasonal fluctuations of metals bioaccumulation levels in the whole soft tissues of females *M. japonicus*. Metals levels were significantly higher in summer compared with other seasons. This seasonal variability may result from changes in the availability of metals in Abu- Qir Bay where *M. japonicus* was collected. The seawater collected from Abu Qir recorded their maximum values for all metals in summer and their minimum values in winter. The chemical composition of the seawater can affect metal bioaccumulation by shrimps (Madhusudana *et al*., 2020). Several explanations for seasonal variations of heavy metals have been postulated. Seasonal variations observed in the heavy metal load may be due to feeding rate changes (Farkas *et al*., 2003). Seasonal fluctuations of metals can be associated with changes in the run-off of metal particles to the sea caused by heavy precipitation, food supply, and phytoplankton productivity (Ferreira *et al*., 2005). Phytoplankton abundance means an increase in prawn nutritional status, leading to higher levels of metal bioaccumulation.

Metals concentration trend during all seasons was in the following order: Zn >Fe > Cu > Pd > Cd > Co. The relatively low bioaccumulation levels of Pb, Cd and Co in crustaceans may be attributed to the well-developed systems for excreting toxic metals (Pourang *et al*., 2004). The bioaccumulation levels of Zn and Fe are higher than other metals and appear to be the most abundant metals in the whole soft tissues of *M. japonicus* followed by Cu.

Lee *et al*. (2017) recorded that Fe, Cu and Zn are the most abundant elements in different parts of *Penaeus vannamei* whereas Cd and Pb are the least abundant elements detected. Cogun *et al*., (2005) reported that in crustaceans the higher levels of Zn and Cu compared to Pb and Cd may be due to the role of these metals in enzymatic and respiratory processes. Zn is an essential element that is required for several normal physiological functions as it plays a role as a cofactor in more than 200 metalloenzymes and also a component of several enzymes regulating the metabolism of carbohydrates, lipid, proteins and nucleic acid (Wu *et al*., 2008). Additionally, Zn has a low excretion rate and tends to accumulate inside the cell (Bryan & Langston, 1992). On the other hand, Fe plays a vital role in growth, development, and maintenance of aquatic organisms (Frenet & Alliot, 1985). Everaarts & Nieuwenhuize (1995) suggested that the elevated Cu concentrations in crustaceans may be because it serves as an oxygen carrier in their blood same as haemoglobin in the blood of vertebrates.

The bioaccumulation levels of Zn, Fe, Cu, Pb, Cd and Co in the whole soft tissues of *M. japonicus* were compared with permissible limits for human consumption of prawn set by FAO (1983), Seafood Standards (2003), WHO (2005) and TFC (Elnabris *et al*.,2013) (Table, 6). There is no prescribed maximum concentration guideline for Co. The bioaccumulation levels of Cu, Pb and Cd were found to be below the permissible limit for human consumption recommended by FAO (1983), Seafood Standards (2003), WHO (2005) and TFC (Elnabris *et al*., 2013). On the other hand, the bioaccumulation levels of Zn and Fe were higher than the permissible limits. Zn concentration was four times higher than the permissible limits set by FAO (1983) and WHO (2005) and nearly twice the permissible limits set by TFC (Elnabris *et al*., 2013). Although Zn is essential for several biological processes involved in growth and development (FAO/WHO, 2002), too much Zn is also harmful to human health, and it largely remains more toxic than Pb (His *et al*, 1999). Cogun *et al*., (2005) reported that Zn may become toxic when it exceeds a specific threshold. Fe concentration was much higher than the permissible limit set by WHO (2005) for human consumption. Fe concentration in body must be tightly regulated because excessive amounts can lead to tissue damage (Abbaspour *et al*., 2014).

Table 6: Maximum Permissible Limit (MPL) of heavy metals for human consumption (mg kg−1) according to international standards.

Organization	Metal MPL						Reference
	Zn	Fe	Cu	Pb	Cd	Co	
FAO	30	-	30	0.5	0.5	-	FAO (1983)
Seafood Standards	-	-	20	0.5	0.5	-	Seafood Standards (2003)
WHO	30	0.5	30	2	0.5	-	WHO (2005)
TFC	50	-	20	0.5	0.5	-	Elnabris *et al.* (2013)
Present study	127.5	72.1	8.59	0.4	0.14	0.02	

MPC, maximum permissible limit
TFC, Turkish Food Codex

3. Heavy metals in the ovaries and the soft tissues of *M. japonicus*

The results obtained from the analysis of the ovaries and remaining soft tissues of females *M. japonicus* showed that, the order of metal concentrations for all the studied metals were as follows: the remaining soft tissues > ovary. Thus, the ovary had the lowest metal concentration, with Zn and Fe being the most accumulated metals. Essential metals (Zn, Fe, Cu, Co) have established biological functions and improved feed utilization and the growth of several aquatic species (Akter *et al*., 2021; Rohani *et al*., 2021). Zn was found to be the most concentrated metal in the ovaries, highlighting its importance in reproduction of crustaceans (Frenet & Alliot, 1985; Song *et al*., 2017). Jeckel *et al*., (1996) suggested that Zn may play a role in stabilizing storage proteins during embryogenesis and early larval development. Fe is actively involved in physiological processes such as oxygen transportation, cellular respiratory activities, and lipid peroxidation (Taslima *et al*., 2022). The accumulation of Fe in ovaries and its transfer to eggs could be explained by its role in oxidative metabolism, sustaining metabolic processes during oocyte growth and embryogenesis (Mendez *et a*l., 2001).

4. Biochemical studies

A deep understanding of the mode of action of heavy metals requires more knowledge about energy precursors such as carbohydrates, proteins and lipids. Biochemical profiles are often utilized as indicators of stress (Safahieh *et al*., 2010 , Heydarnejad, *et al*., 2013 , Fazio *et al*., 2022).

Seasonal fluctuations in the levels of total protein, carbohydrates and lipids in the whole soft tissues of female *M. japonicus* were recorded. Protein was found to be the major component followed by carbohydrates and lipids. In conclusion, females *M. japonicus* when exposed to elevated heavy metals (Zn, Fe, Cu, Pd, Cd and Co) in summer, the levels of carbohydrates and lipids decreased while the protein content increased.Protein is one of the most important biochemical components and plays a crucial role in biochemical reactions and metabolic pathways (Suryavanshi *et al*., 2009). Organisms respond to pollutants by developing the necessary potential to counteract that stress (Kharat *et al*., 2009a; b). Susan *et al*. (2010) suggested that the toxicant stress might have stimulated protein synthesis for enzymic detoxification. Therefore, an assessment of the total protein content in tissues could be used as a diagnostic tool to determine the physiological status of an organism (Prasath & Arivoli, 2008) .In crustaceans changes in lipid content seem to be a common stress response (Chinni & Yallaoragada, 2002 ; Samyappan *et al*., 2007; Ghonim, 2012) to meet the energy demands. A reduction of carbohydrate content was reported by several investigators in crustaceans under toxicant stress (Chinni & Yallaoragada, 2002; Castiglioni *et al*., 2007; Samyappan *et al*., 2007; Kalyanaraman & Kumaar, 2009). According to Shakir *et al*., 2013; 2014, carbohydrates are degradable molecules that are affected by stress conditions and the decrease in carbohydrate levels indicates the direct effects of pollutants. Garg *et al*. (2009), reported a significant reduction in carbohydrates due to their rapid utilization to meet energy demands under the stress of pollutants.

5. Histological studies of the gonads of female *M. japonicus*

Histopathological examinations of the *M. japonicus* ovaries revealed manifest pathological changes. In the present investigation, different signs of ovarian stress were detected at various developmental stages. There was a disruption in the ovarian wall at stage II of maturity, mass destruction of late vitellogenic oocytes, vacuolization and degeneration, hyperplasia and hypertrophy of follicle cells. In addition, the outer surrounding membrane of numerous mature oocytes became undulating, and the cortical rods appeared separated, forming a complete sheath around the abnormal mature oocyte. Several histopathological changes were recorded in the ovary and may be attributed to the effects of heavy metals. Mazrouh & Mahmoud (2009) recorded that the gonads of *Oreochromis niloticus* exposed to higher concentrations of pollutants showed gonadal abnormalities in the form of deformed oocytes.

Research on decapod crustaceans showed inhibitory effects of heavy metals on gonads (Reddy *et al*., 1997). Several previous studies recorded histological abnormalities in ovaries of crustaceans exposed to pollutants.

Kharat, *e. al*. (2011) recorded vacuolization and alteration in the shape of the ovary, destruction of the epithelial layer, disorganization of the nucleus and degenerating oocytes with disintegrated nuclei in freshwater prawn, *Macrobrachium kistensis* exposed to tributyltin chloride. Dode *et al*. (2012) observed damage to the ovarian structure of freshwater prawn, *M. kistensis* exposed to cuprous oxide ranging from destruction of the epithelial layer, vacuolization at the periphery, rupturing of follicular epithelium, shrinkage in ooplasmic material, disorganization of the nucleus to degradation of oocytes. Suresh *et al*. (2015) recorded damage to ovarian structure,including vacuolization, destruction of the epithelial layer, rupturing of the follicular epithelium, degeneration of oocytes and shrinkage of oocytes of crab (*Uca annulipes*) exposed to cadmium. Maharajan *et al*. (2017) recorded rupturing of oocyte membrane, disturbances in the supporting connective tissue and vacuolization of peripheral oocytes after acute and chronic exposure to profenofos in mangrove crab, *Perisesarma bidens*. Degenerative and necrotic changes were observed in some oocytes, while most oocytes exhibited proliferation in the granulosa. Additionally, there was adhesion in the cellular coat of some oocytes, as well as separation of the follicular layers from the oocytes. These findings were recorded in the ovaries of several aquatic species exposed to pollutants (Shobikhuliatul *et al*., 2013; Ambani, 2015; Azab *et al*., 2019).

In the present study, abnormal union between adjacent primary yolk granule oocytes were recorded. Moreover, tertiary yolk granules oocytes were seen to from a complex structure of united abnormal oocytes, this could be a specific type of reflected action against metal pollution, and it could be a restricted response of some species like *M. japonicus*. Al-Kandari (2009) recorded an abnormal union between early and late vitellogenic oocytes in the crab *Charybdis hellerii* collected from heavily polluted area. Malformation of yolk inclusions was noticed in secondary vitellogenic oocytes, which might be attributed to the distortion of the vitellogenesis process because of metal exposure. This result agrees with that noticed by Sehgal & Saxena (1986) in adult fish *Lebistes reticulatus* after long exposure to zinc. In the current study, the irregularity of oolemma was observed in some abnormal mature oocytes. The adverse alternations in the egg membrane may result in reduction of gas diffusion, and changes in osmoregulatory capacity. Rosenthal & Alerice (1976) suggested that such eggs may not be fertile, or the progeny developed from such eggs may be abnormal. In the present study, mass destruction of late vitellogenic oocytes and active atresia of mature ova was noticeable in ovary of *M. japonicus*. Oocytes atresia can be employed as a histopathological marker for metals. in addition, hyperplasia and hypertrophied follicle cells were clearly observed in abnormal ovaries which were not regarding as common occurrence in normal ovaries. Oocytes atresia is a pathological symptom reported in various studies concerned with marine pollution (Kogan *et al*., 2000, Deshmukh & Kulkarni, 2005; Olfat & El-Greisy, 2007; Al-Kandari,2009; Brraich & Jangu, 2015). In many instances persistent pollutions are suspected to be cause of impaired reproduction (Hammerschmidt et al., 2002; Al-Kandari, 2009; Ghonim, 2012). Prolonged exposure to the pollutant causes considerable damage to the tissues of reproductive organs, delaying the reproductive cycle and restricting egg development (Khalaf-Allah & Shehata, 2011; Ciftci *et al*., 2015; Maharajan *et al*. ,2017).

Conclusion.

The present study recorded seasonal variations in the concentrations of Zn, Cd, Cu, Pb, Fe and Co in seawater and the whole soft tissues of kuruma prawn *M. japonicus* collected from Abu-Qir Bay and confirmed the sensitivity of crustaceans to heavy metal pollution.The bioaccumulation levels of Zn and Fe in *M. japonicus* were above the permissible limits. Accordingly, the kuruma prawn, collected from Abu-Qir Bay should be consumed in moderation. Biochemical studies showed seasonal variations in the level of the caloric contents of the soft tissues of *M. japonicus*. Assessment of the total protein content in tissues could be used as a diagnostic tool for stress. Moreover, the results of the present study indicate that the gonadal tissue of the female *M. japonicus* is sensitive enough to accurately reflect environmental pollution caused by metals. Finally, it was concluded that histological abnormalities in ovaries due to pollutants exposure may decline reproductive activity and indirectly reduce the regenerative capacity of *M. japonicus*. Accordingly, the prawn *M. japonicus* can be used as a bioindicator for evaluating metal pollution in the marine environment. On the other hand, the gametotoxic effects of metals could be indicated by histopathological changes in the ovarian tissues of the prawn *M. japonicus*.

Metal analysis, biochemical studies and histopathological examination are valuable tools for assessing the effects of various types of environmental pollutants on aquatic animals. It can be concluded that heavy metal analysis should be carried out as frequently as possible in edible parts of aquatic organisms and consumers must be warned about the potential health risks associated with the consumption of the metal-polluted seafood. Ingesting metals-contaminated seafood is a major threat to public health.

References

Abd-Ellah, S. M.; El-Sherif, S.; El-Morshedy, R. (2020). Seasonal effects of heavy metals on the date mussel *Lithophaga lithophaga* (Mollusca:Bivalvia) at Eastern harbor, Alexandria, Egypt .Swed. *J. BioSci. Res.*, 1(1): 62 - 77.

Abo-Taleb, H.A.; Shaban, W.M.; Hellal, A.M.; Aboul Ezz, S.M.; Sharaf, M.B. (2017). Assessing the ecological status of Edku Lake by using Rotifera as bio-indicators. *Al-Azhar Bulletin of Science,* 9:235-249.

Abo-Taleb, H.A. (2019). Importance of plankton to fish community. (Book Chapter). In: Biological research in aquatic science. Yusuf Bozkurt, editor.: Intechopen. London, UK. http://dx.doi.org/10.5772/intechopen.85769

Abbaspour, N.; Hurrell, R.; Kelishadi, R. (2014). Review on iron and its importance for human health. *J Res Med Sci*.,19:164-174.

Adeyeye, E.I; Adubiaro, H.I.; Olufemi, J.; Awodola, O.J. (2008). Comparability of chemical composition and functional properties of shell and flesh of Penaeus notabilis. Pakistan. *J. Nutr.*, 7 (6): 741-747.

Ahdy, H.H.H.; Abdallah, A.M.A.; Tyel, F.M. (2007). Assessment of heavy metals and non-essential content of some edible and soft tissues. *Egypt. J. Aquat. Res.*, 33(1): 85-97.

Azab, A.M.; Aly-Eldeen, M.A.; Khalaf-Allah, H.M.M.; El-Battal, M.M.A. (2019). Effect of heavy metals on the ovary of *Tilapia zillii* in some canals of Nile Delta area, Egypt. *Egyptian Journal of Aquatic Biology & Fisheries*, 23(3): 329 –345.

Akter, S.; Jahan, N.; Rohani, M.F.; Akter, Y.; Shahjahan, M. (2021). Chromium supplementation in diet enhances growth and feed utilization of striped satfish (*Pangasianodon hypophthalmus*), *Biol. Trace Elem. Res*., 4811–4819.

Ali, M.M.; Ali, M.L.; Islam, M.S.; Rahman, M.Z. (2016). Preliminary assessment of heavy metals in water and sediment of Karnaphuli River, Bangladesh. *Environ. Nanotechnol. Monit. Manag.*, 5: 27–35.

Ambani, M.M. (2015). Effects of reproductive biology on heavy metal pollution on the histopathological structure of gonads in India., 3 (2): 223 -227.

Brraich, O.S.; Jangu, S. (2015). Some aspects of reproductive biology on effect of heavy metal pollution on the histopathological structure of gonads in *Labeo rohita* (Hamilton- Buchanan) from Harike wetland. *India. International Journal of Fisheries and Aquaculture*, 7(2): 9-14.

Bryan, G.W.; Langston, W.I. (1992). Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: A review. *Environmental Pollution*, 76(2): 89-131.

Chailapakul, O.; Korsrisakul, S.; Siangproh, W.; Grudpan, K. (2008). Fast and simultaneous detection of heavy metals using a simple and reliable microchip-electrochemistry route: An alternative approach to food analysis. *Talanta*, 74:83–689. doi:10.1016/j.talanta.2007.06.034

Ciftci, N.; Ay, O.; Karayakar, F.; Cicik, B.; Erdem, C. (2015). Effects of zinc and cadmium on condition factor, hepatosomatic and gonadosomatic index of *Oreochromis niloticus*. *Fresenius Environmental Bulletin*, 24 (11): 1-4.

Cogun, H.; Yuzereroglu, T.A.; Kargin, F.; Firat, O. (2005). Seasonal variation and tissue distribution of metals in shrimp and fish species from the Yumurtalik coast of Iskenderum Gulf, Mediterranean. *Bulletin of Environmental Contamination and Toxicology*, 75:707–715. doi: 10.1007/s00128-005-0809-6

Deshmukh, S.; Kulkarni, K.M. (2005). Effect of cadmium chloride on gonads of the fish *Channa orientalis* (Sch.). *Indian J. Environ. Ecoplan*., 10:239-245.

Dinakaran, G.K.; Soundarapandian, P.; Saunak, K.C. (2009). Proximate Composition of Edible Palaemonid Prawn Macrobrachium idea (Heller, 1862). *Curr. Res.J. Biol. Sci*., 1(3): 78-82.

Dode, C.R.; Chourpagar, A.R.; Dongre, S.B.; Nagabhushanam, R. (2012). Histological alteration in ovary of the freshwater prawn, *Macrobrachium kistensis* exposed to cuprous oxide. *Elixir Aquaculture* ,53: 12086-12089

Duysak, Ö.; Uğurlu, E. (2020). Trace Metal Concentrations in the Seston of the Gulf of İskenderun (Turkey, North- Eastern Mediterranean). *Thalassas*, 36: 125–132.

El-Damhogy, K.A.; Abo-Taleb, H.A.; Ahmed, H.O.; Aly-Eldeen; Abdel-Aal, M.M. (2019). The relationship between the concentrations of some heavy metals in the water of Abu-Qir Bay and within the tissues of the blue crab *Portunus pelagicus* . *Egyptian Journal of Aquatic Biology & Fisheries*, 23(3): 347 –359

El-Haddad, E.S. (2005). Physicochemical studies for domestic water for Ismailia canal from El-Mazalat to Anshas. *M.Sc Thesis Faculty of Science, Al Azhar University, Cairo Egypt*.

Elnabris, K.J.; Muzyed, S.K.; El-Ashgar , N.M. (2013). Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza strip (Palestine). *J. Assoc. Arab. Univ. Basic. Appli. Sci*., 13(1):44–51

Elewa, A.A.; Saad, E.A.; Shehata, M.B.; Ghallab, M.H. (2007). Studies on the effect of drain effluents on the water quality of Lake Manzala, *Egyptian Journal of Aquatic Biology and Fisheries*. 11(2): 65-78.

El-Serafy, S.S.; EI-Gamal, M.M.; El-Sayed, D.S. (2003a). Seasonal variations of trace metals levels in water and the limpet *Patella caerulea* of Alexandria Coast, EGYPT. Egypt. *J. Aquat. Bio. & Fish*., 7(4):283-312.

Everaarts, J.M.; Nieuwenhuize, J. (1995). Heavy metals in surface sediment and epibenthic macroinvertebrates from the coastal zone and Continental Slope of Kenya. *Mar. Pollut. Bull*., 31: 281-290.

FAO (1983). (Food and Agriculture Organization), Compilation of legal limits for hazardous substances in fish and fishery products. *FAO Fishery Circular*, 464: 5–100. Retrieved from http://www.fao.org/docrep/014/q5114e/q5114e.pdf

FAO/WHO (1992). Codex Alimentarius Commission, Standard Program Codex Committee on Food Additives and Contaminants, 24th Session, Hague.

Fazio, F.; Habib, S.S.; Naz, S.; Hashmi, M.A.H.;

Saoca, C.; Ullah, M. (2022). Cadmium sublethal concentration effect on growth, haematological and biochemical parameters of *Mystus seenghala* (Sykes, 1839). *Biol. Trace Elem. Res*., 200: 2432–2438. https://doi.org/10.1007/s12011-021-02852-6

Frenet, M.; Alliot, A. (1985). Comparative bioaccumulation of metals in *Palaemonetez ravianz* in polluted and non-polluted environ- ments. *Mar Environ Res.*, 17: 19−44.

Gaber, H.S.; Mohamed, Y.M.; EL-Kashif, M.M.; AL-Afify, A.D.G. (2017). Assessment of some heavy metals and histological alterations in gills and muscles of *Oreochromis niloticus* from Lake Nasser, *Egypt. J. Egypt. Acad. Soc. Environ. Develop*., 18 (1):193- 207.

Galil, B.; Frogila, C.; Noel, P. (2002). Atlas of exotic species in the Mediterranean. Volume 2. Crustaceans: decapoda and stomatopods. In: *Briand F (ed), CIESM, Monaco*, 192 pp

Garg, S.; Gupta, R..K.; Jain, K.L. (2009). Sublethal effects of heavy metals on biochemical composition and their recovery in Indian major carps. *J. Hazard. Mat.* ,163:1369–1384

Hashempour-Baltork, F.; Hosseini, H.; Houshiarrad, A.; Esmaeili, M. (2019). Contamination of foods with arsenic and mercury in Iran: a comprehensive review. *Environ. Sci. Pollut. Res*., 26 (25): 25399–25413.

Hashempour-Baltork, F.; Jannat, B.; Tajdar-Oranj, B.; Aminzare, M.; Sahebi, H.; Alizadeh, A.M.; Hosseini, H. (2023). A comprehensive systematic review and health risk assessment of potentially toxic element intakes via fish consumption in Iran. *Ecotoxicol. Environ. Saf*., 249: 114349. doi: 10.1016/j.ecoenv.2022.114349. Epub 2022 Dec 9. PMID: 36508819.

Hashmi, M.I.; Mustafab, S.; Tariqa., S.A. (2002): Heavy metal concnetrations in water and tiger prawn, Penaues monodon, from grow-out farms in Sabah, North Borneo. *Food Chem*.,79:151-156.

Heidarieh, M.; Maragheh M.G.; Shamami, M. A; Behgar, M.; Ziaei1, F.; Akbari, Z. (2013). Evaluate of heavy metal concentration in shrimp (*Penaeus semisulcatus*) and crab (*Portunus pelagicus*) with INAA method," *Springer Plus*, 2: 72. https://doi.org/10.1186/2193-1801-2-72

Heydarnejad, M.S.; Khosravian-Hemamai, M.; Nematollahi, A. (2013). Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*), *Ir. Vet. J* , 66 : 1–7.

Hossain, M.B.; Bhuiyan, N.Z.; Kasem, A.; Hossain, M.K.; Sultana, S.; Nur, A.-A.U.; Yu, J.; Albeshr, M.F.; Arai, T.,(2022).Heavy Metals in Four Marine Fish and Shrimp Species from a Subtropical Coastal Area: *Accumulation and Consumer Health Risk Assessment. Biology*, 11: 1780. https://doi.org/10.3390/ biology11121780

Jadhav, T.J.; Shaikh, J.D. (2012). Histopathological changes in the ovary of freshwater crab, *Barytelphusa cunicularis* exposed to Endosulphan. *Int. J. Sci*., 1(2): 139-140

Jeckel, U.H.; Roth, R.R.; Ricci,L. (1996). Patterns of trace metal dis- tribution in tissues of Pleoticuz muellevi (Crustacea: Decapoda: Solenoceridae). *Mar Biol*., 125: 297−306

Khalaf Allah, H.M.M.; Shehata, S.M.A. (2011). Report on state of atresia in the ovaries of some Mediterranean Sea fishes from the Egyptian coast, *Al-Azhar Bull. Sci*., 22(1) :1 – 12

Kharat, P.S.; Shejule, K.B.; Ghobale, B.C. (2011). Histopathological changes in ovary of freshwater prawn, *Macrobrachium kistensis* exposed to TBTCL/. *World Journal of Zoology*, 6(3): 296-300.

Korkmaz, C.; Ay, Ö.; Çolakfakıoğlu1, C.; Erdem, C. (2019). Heavy Metal Levels in some Edible Crustacean and Mollusk Species Marketed in Mersin: *An International Journal of Marine Sciences*, 35: 65–71.

Maanan, M. (2008). Heavy metal concentrations in marine mollusks from the Moroccan coastal region. *Environ. Pollut.*, 153:176–183.

Maharajan, A.; Ganapiriya, V.; Shanmugavel, K. (2017). Histology Biomarkers in Muscle and Ovary of Mangrove Crab, *Perisesarma bidens* Exposed to Profenofos. *International Journal of Biomedical Science and Engineering*, 5(1): 1-8

Mazrouch, M.M.; Mahmoud, H.H. (2009). Some aspect of reproductive biology with emphasis on the effect of pollution on the histopathological structure of gonads in *Oreochromis niloticus* from Rosetta Branch, Nile River, Egypt. *World J. Fish. Mar. Sci.*, 1(3):190-198.

Mendez, L.; Racotta , I.S.; Acosta, B.; Rodriguez-Jaramillo, C. (2001).Mineral concentration in tissues during ovarian development ofthewhiteshrimp *Penaeusvannamei*[Decapoda:Penaeidae] *Marine Biology*, 138: 687−692.

Madhusudana, P. P.;Uma , K. N. and Pandey, D. (2020). Heavy metals occurrence in the tissues of marine prawn *Penaeus monodon* (Fabricius1798) and water along the coastline of Tamil (Chennai). Asian Journal of Advances in Research, 3(2): 23-28,

Nisa, K.; Sultana, R. (2010). Variation in the proximate composition of shrimp, Fenneropenaeus penicillatus at different stages of maturity. Am-Eur. *J. Sci.Res*., 5 (4): 277-282.

Olfat, M.N.; El-Greisy, Z.A. (2007). Comparative impact of different waste sources on the reproductive parameters and histology of gonads, liver and pituitary gland of *Signus rivulatus. J. Appl. Sci. Res*., 3:236- 244.

Peng, S.H.; Hung, J.H.; Hwang, J.S. (2011). Bioaccumulation of trace metals in the submarine hydrothermal vent crab *Xenograpsus testudinatus* off Kueishan Island, *Taiwan. Mar. Pollut. Bull*., 63(5-12): 396-401.

Pourang, N.; Dennis, J.H.; Ghourchian, H. (2004). Tissue Distribution and Redistribution of Trace Elements in Shrimp Species with the Emphasis on the Roles of Metallothionein. *Ecotoxicology*,13: 519–533. doi:10.1023/B:ECTX.0000037189.80 775.9c

Raju Subha; Vasanthi, L.A.; Dhanasekar, K.; Munuswamy, N. (2016). Heavy metal accumulation and its impact on structural and biochemical changes in the lobster *Panulirus homarus homarus* (Linnaeus, 1758). *J. Mar. Biol. Ass. India*, 58 (1): 21- 28.

Rohani, M.F.; Bristy, A.A.; Hasan, J.; Hossain, K.; Shahjahan, M. (2021). Dietary zinc in association with vitamin E promotes growth performance of Nile tilapia. *Biol. Trace Elem. Res*., 9: 4150-4159.

Seafood Standards, (2003). http://www.Okyanusambari .com/yonet/y-suurunleri.pdf. (In Turkish).

Shakir, H.A.; Chaudhry, A.S.; Qazi, J.I. (2013). Impact of anthropogenic activities on physico-chemical parameters of water and mineral uptake in *Catla catla* from river Ravi, Pakistan. *Environ. Monit. Assess*., 185: 2842–3833.

Shakir, H.A.; Qazi, J.I.; Chaudhry, A.S. (2014). Examining muscles of *Cirrhinus mrigala* for biochemical parameters as a bio-indicator of water pollution by municipal and industrial effluents into River Ravi. *Pakistan In. Aquat. Res*., 6:221–228

Shaikh, F.I.; Ustad, I.R.; Ansari, N.T. (2010). Effect of heavy metal on the ovary of freshwater crab, *Barytelphusa cunicularis* (Westwood). *The Bioscan*., 5(2): 335-338.

Shobikhuliatul, J.J.; Andayani, S.; Couteau, J.; Risjani, Y.; Minier, C. (2013). Some aspect of reproductive biology on the effect of pollution on the histopathology of gonads in *Puntius javanicus* from Mas river, Surabaya, Indonesia. *J. Biol. Life Sci.*, 4(2):191-205.

Song, Z.X.; Jiang, W.D.; Liu, Y.; Wu, P.; Jiang, J.; Zhou, X.Q.; Kuang, S.Y.; Tang, L.; Tang, W.N.; Zhang, Y.A.; Feng, L. (2017). Dietary zinc deficiency reduced growth performance, intestinal immune and physical barrier functions related to NF-κB, TOR, Nrf2, JNK and MLCK signaling pathway of young grass carp (Ctenopharyngodon idella). *Fish Shellfish Immunol*., 66: 497–523.

Stewart, I.; Tan, J.; R.; Kenyon, R.; Kiermeier, A. ;Malhi, N.; Sehmbi, A.; McLeod, C. (2014). 2012-13 Survey of Australian Wild-Caught Prawns for Analysis of Cadmium and Selenium, Project No. 2009/787, *SARDI Food Safety and Innovation*.

Sriket, P.; Benjakul, S.; Visessanguan, W.; Kijroongrojana, K. (2007). Comparative studies on the effect of the freeze thawing process on the physicochemical properties and microstructures of black tiger shrimp (Penaeus monodon) and white shrimp (Penaeus vannamei) muscle. *Food Chem*., 104: 113–121.

Suresh,V.; Palraj, A.; Deecaraman, M. (2015). Histopathological changes in the ovary of the brackish water fiddler crab (*Uca annulipes*) exposed to cadmium. *journal of global biosciences*, 4(8): 3037-3043.

Susan, T.A.; Sobha, K.; Veeraiah, K.; Tilak, K.S. (2010). Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade. *J. Toxicol. Environ. Health. Sci*., 2(5):53–62.

Tahity, T.; Islam, M.R.U.; Bhuiyan, N.Z.; Choudhury, T.R.; Yu, J.; Noman, M.A.; Hosen, M.M.; Quraishi, S.B.; Paray, B.A.; Arai, T. (2022). Heavy Metals Accumulation in Tissues of Wild and Farmed Barramundi from the Northern Bay of Bengal Coast, and Its Estimated Human Health Risks. *Toxics*, 10: 410.

Taslima, K.; Al-Emran, M.; Rahman, M.S.; Hasan, J.; Ferdous, Z.; Rohani, M.F.; Shahjahan, M. (2022). Impacts of heavy metals on early development, growth and reproduction of fish – A review *Toxicology Reports*, 9 :858–868.

Valarmathi, J.; Azariah, F. (2002). Biochemical changes in the tissue of crab Seama quadratum (Decapoda) exposed to CuCl2. *Indian J. Mar. Sci*., 31: 246-248.

Wei Peng Lee, Carolyn Payus, Siti Aishah Mohd Ali, and Leong Wan Vun, (2017). Selected Heavy Metals in *Penaeus vannamei* (White Prawn) in Aquaculture Pond near Likas Lagoon, Sabah,Malaysia. *International Journal of Environmental Science and Development*, 8(7): 530-533.

World Health Organization (2005). Background document for development of WHO guidelines for nickel in drinking-water quality and fish. WHO/Sde/Wsh/05.08/55 English only.

World Health Organisation (2008). Guidelines for Drinking Water Quality. World Health Organization; Geneva, Switzerland. [Google Scholar]