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Effect of bioaccumulation of lead, cadmium, and copper on the protein and lipid content of *Metapenaeus affinis* shrimp muscles in Shatt al-Basra Canal, Iraq

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Abstract

Heavy metal pollution in aquatic environments presents a substantial danger to environmental quality and seafood safety due to the bioaccumulation of trace metals in edible aquatic creatures. Monitoring of commercially important shrimp species provides useful data for the evaluation of metal contamination and its possible effect on human health. There is little information about seasonal and sexual changes of heavy metals bioaccumulation and biochemical composition of the muscle of *Metapenaeus affinis* from southern Iraq. This research analyzed the influence of season and sex on the accumulation of copper (Cu), lead (Pb) and cadmium (Cd) in the abdominal muscle and their relationship with protein and lipid levels in muscle. The season was the dominant factor in the accumulation of heavy metals in *M. affinis*, and sex was the dominant factor in the biochemical composition of the muscle. Concentrations of heavy metals were significantly higher in summer than in winter ($p < 0.001$), and females showed significantly higher muscle protein and lipid contents than males. The strong positive correlations between Cu, Pb and Cd suggest common environmental sources of contamination. Copper concentrations increased from 23.41 ± 2.21 to 28.75 ± 3.39 mg kg^{-1} DW, lead from 1.71 ± 0.20 to 2.37 ± 0.32 mg kg^{-1} DW, and cadmium from 0.280 ± 0.024 to 0.411 ± 0.029 mg kg^{-1} DW from winter to summer. Females showed significantly higher total protein (73.27 ± 1.08%) and lipid (7.21 ± 0.26%) contents than males (72.17 ± 1.05% and 6.81 ± 0.29%, respectively). Pearson's correlation analysis revealed the strongest association between Cu and Cd ($r = 0.637$, $p < 0.001$), whereas only weak positive correlations were observed between heavy metals and muscle protein content. Seasonal environmental conditions strongly regulate heavy metal bioaccumulation in *M. affinis*, whereas the muscle biochemical composition is mainly influenced by physiological processes related to sex. The species is a good bio-indicator for heavy metals pollution and a good source of baseline information for environmental monitoring and seafood safety assessment in Shatt Al-Basrah Canal, Southern Iraq.

Keywords: Bioaccumulation; Heavy metals; *Metapenaeus affinis*; Bioindicator; Shatt Al-Basrah Canal.

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Introduction

Heavy metal pollution has become one of the most serious environmental problems for the aquatic ecosystem all over the world. Compared to many organic pollutants, heavy metals are persistent and non-biodegradable and can accumulate in sediments and aquatic organisms, thus allowing their transfer through aquatic food webs and increasing ecological and human health risks (Alloway, 2013; Briffa *et al*., 2020). Lead (Pb) and cadmium (Cd) are highly toxic non-essential trace metals with no known biological function, whereas copper (Cu) is an essential micronutrient required for several physiological processes but is toxic at elevated concentrations. These metals are classified as priority pollutants in aquatic environmental monitoring because of their persistence and bioaccumulative behavior (Jaishankar *et al*., 2014; Jeong *et al*., 2023). The increasing release of heavy metals into aquatic environments is primarily associated with anthropogenic activities, including industrialization, petroleum production, urban expansion, agricultural runoff, port activities, and municipal wastewater discharge (Ali *et al*., 2019; Aziz *et al*., 2023). These pressures are particularly acute in the southern part of Iraq, where rapid industrial development and widespread oil-related activities have resulted in greater contaminant inputs into estuarine and coastal ecosystems. Previous environmental assessments have indicated high levels of trace metals in the Iraqi aquatic environment, highlighting the need for ongoing surveillance of commercially important aquatic species (United Nations Environment Programme, 2021; World Bank, 2023; Al-Hemidawi *et al*., 2020; Al-Najar *et al*., 2023). Aquatic organisms accumulate heavy metals through direct uptake from water and sediments and through diet. Their accumulation depends on environmental parameters (salinity, temperature, dissolved oxygen, pH, sediment characteristics and metal speciation) and biological parameters (age, sex, body size, metabolic activity and nutritional status). Excessive accumulation of Pb, Cd and Cu disrupts cellular metabolism, induces oxidative stress, damages proteins, lipids, DNA and cellular membranes and ultimately impairs growth, reproduction, immune function and survival. In addition, bioaccumulation and biomagnification of these metals along aquatic food chains make contaminated seafood a potential route of human exposure and a major food safety issue (Briffa *et al*., 2020; Jeong *et al*., 2023; Smriti *et al*., 2023; FAO and WHO, 2011). Marine shrimp are well known as reliable bioindicators of heavy metal pollution due to their benthic lifestyle, close contact with contaminated sediments, and their ability to accumulate trace metals from both water and food. Thus, they are useful indicators for the evaluation of environmental quality and bioavailability of contaminants (Amiard *et al*., 2006; Farag *et al*., 2023; Waqas *et al*., 2024; Banaee *et al*., 2024). M. affinis is one of the economically important penaeid shrimps found throughout the Arabian Gulf and the northwestern Indian Ocean, including the southern waters of Iraq. Because of its commercial importance, rapid growth, bottom feeding habits and direct contact with bottom sediments, it is a suitable sentinel species for monitoring heavy metal contamination in estuarine ecosystems (FAO, 2024; Al-Maliky *et al*., 2019). Shatt Al-Basrah is one of the major man-made waterways in southern Iraq, linking Khor Al-Zubair with the estuary of the Shatt Al-Arab. The canal has considerable ecological and economic importance and is lined with oil terminals, petrochemical industries, commercial ports, agricultural lands and developing urban areas. Therefore, it receives industrial effluents, municipal wastewater, agricultural runoff and discharges from shipping, all of which are contributing to heavy metal contamination of water, sediments and aquatic organisms (United Nations Environment Programme, 2021; World Bank, 2023). The contamination of Iraqi aquatic environments by heavy metals has been the subject of increasing concern, but most previous studies have focused on water, sediments or fish species, with relatively little information available on commercially important crustaceans. In particular, data on concentrations of Pb, Cd and Cu in the edible muscle tissue of *M. affinis* from Shatt Al-Basrah are lacking. Furthermore, recent evidence suggests that population dynamics and reproductive activity of M. affinis are affected by seasonal variation, suggesting that seasonality may affect physiological processes associated with metal accumulation as well (Al-Maliky *et al.*, 2025). To our knowledge, no previous study has simultaneously evaluated the effect of season and sex on bioaccumulation of Pb, Cd and Cu in this species from Shatt Al-Basrah. Therefore, the present study was designed to determine the concentrations of Pb, Cd and Cu in the abdominal muscle of *M. affinis* collected during the winter and summer seasons, compare the metal accumulation between male and female shrimp and investigate the relationships between metal bioaccumulation and muscle protein and lipid contents. The results provide baseline information for environmental monitoring, seafood safety assessment and sustainable management of fisheries resources in southern Iraq and the northwestern Arabian Gulf.

Materials And Methods

Study Area

The present study was conducted in Shatt Al-Basrah Canal, Basrah Governorate, southern Iraq (Figure 1). The canal is the main artificial waterway connecting Khor Al-Zubair with the northwestern Arabian Gulf through the Khor Abdullah navigation channel. It is characterized by a subtropical arid climate with marked seasonal variations in water temperature, salinity and freshwater input. The hydrological regime is mainly controlled by the tidal exchange with the Arabian Gulf, limited freshwater discharge and increasing anthropogenic pressures (Al-Asadi *et al.*, 2020; Alhumaidan *et al.*, 2023). The canal is an ecologically and economically important habitat for many commercially important fish and crustacean species, including *M. affinis*. The surrounding area is dominated by intensive industrial, petrochemical, oil-export, port, agricultural and urban activities, which contribute continuous inputs of domestic sewage, industrial effluents, agricultural runoff and shipping-related pollutants into the aquatic environment (Kanani *et al.*, 2025). Recent studies have reported high levels and spatial variation of heavy metals in the water column and bottom sediments, indicating the accumulated effects of these anthropogenic activities and the ecological susceptibility of the canal (Al-Janabi *et al.*, 2019; Al-Asadi *et al.*, 2020; Hamzah, 2024).

Sampling

Sampling was performed by boat in the summer and winter seasons. Specimens of *M. affinis* were collected from several sites over an approximately 22 km stretch of the Shatt Al-Basrah Canal from near the Shatt Al-Basrah Power Station to the downstream section of the canal. The specimens were immediately after collection transported to the laboratory for heavy metals and biochemical analysis.

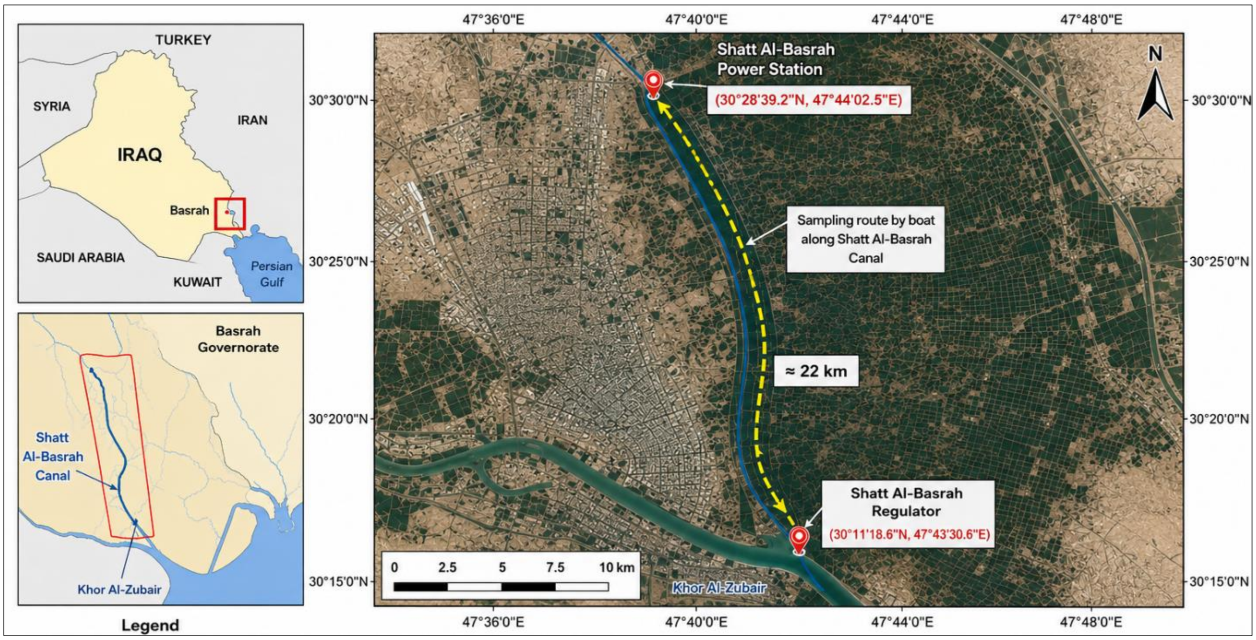

Figure 1: Map of the study area showing the sampling route along Shatt Al-Basrah Canal between the Shatt Al-Basrah Regulator and the Shatt Al-Basrah Power Station, Basrah, Iraq.

Table 1: Geographic coordinates of the start and end sampling locations along the Shatt Al-Basrah Canal, Basrah, Iraq.

Location	Latitude (N)	Longitude (E)
Start of sampling area (Shatt Al-Basrah Power Station)	30°28'39.2"	47°44'02.5"
End of sampling area (Shatt Al-Basrah Regulator)	30°11'18.6"	47°43'30.6"

Sample preparation

Specimens were immediately placed in clean polyethylene bags with crushed ice to maintain the temperature below 4°C during transportation to the laboratory to minimize biochemical deterioration before analyses. The standard procedure was used to dissect each specimen carefully in the laboratory. External morphological features were used to determine the sex of each individual, and the specimens were separated into male and female groups for subsequent comparative analyses. The cephalothorax and internal viscera were removed, but the abdominal muscle was kept for further analysis. The wet muscle tissue was weighed immediately after dissection on a calibrated analytical balance for accuracy of measurement. Samples were then dried in a laboratory drying oven (Binder GmbH, Germany) under controlled conditions to a constant weight. The dried muscle tissues were then analyzed for biochemicals such as total protein, total lipid content determination and heavy metals concentrations quantification.

Heavy metal analysis

Each dried muscle sample (approx. 0.5 g) was accurately weighed and digested with concentrated nitric acid (HNO3) using the standard wet digestion method as described by (AOAC, 2019). The digest was cooled, filtered if necessary and diluted to a final volume of 50 mL with deionized water after complete digestion. The concentrations of Lead (Pb), Copper (Cu) and Cadmium (Cd) were determined using an Atomic Absorption Spectrophotometer (AAS) according to the manufacturer's instructions. Calibration curves were prepared using certified standard solutions, and reagent blanks were included throughout the analytical procedure for quality assurance. Metal concentrations were expressed as mg kg^{-1} dry weight.

Determination of Lipid Content

The total lipid content was determined by the standard method of the Association of Official Analytical Chemists (AOAC, 2019) using a Soxhlet extraction system. Dried muscle tissue (~10 g) was extracted with hexane for 5 h. The extraction flask was dried for 30 min at 80 °C, cooled to room temperature and reweighed to determine the efficiency of the solvent extraction. Total lipid content was calculated gravimetrically from the weight gain of the flask after extraction and expressed as a percentage of the weight of the dry sample by the following equation:

Total lipid (%) = [(Weight of flask after extraction – Weight of flask before extraction) / Sample weight] × 100

Determination of Protein Content

Crude protein was determined by the Kjeldahl method as described by van Dijk (2000) with minor modifications. Each sample was digested with concentrated sulfuric acid in the presence of a potassium sulfate-copper sulfate catalyst mixture at about 5 g. Samples were digested and distilled with 40% sodium hydroxide, and the ammonia released was collected in 20% boric acid containing mixed indicators. The distillate was titrated with standard 0.1 N hydrochloric acid, a reagent blank being run simultaneously. Crude protein content was calculated from nitrogen content using a factor of 6.25 and expressed as a percentage of the sample.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical differences among groups were analyzed using Two-way ANOVA to evaluate the effects of season and sex. Post-hoc comparisons were performed using Duncan's Multiple Range Test, and means with different superscript letters were considered significantly different at $P < 0.05$.

Results

The descriptive statistics (mean ± standard deviation) of dry weight, heavy metal concentrations (Cu, Pb and Cd), total protein and total lipid in male and female M. affinis from Shatt Al-Basrah Canal for summer and winter seasons (n = 15 in each group) are shown in Table 2. The mean dry weight ranged from 4.56 ± 1.19 g in winter males to 5.05 ± 1.17 g in summer males, and the slight differences in dry weight between the groups were not statistically significant, indicating natural variability among individuals. Copper (Cu) concentrations were generally higher during summer than winter in both sexes, with the highest mean value recorded in summer females (29.74 ± 3.49 mg kg^{-1} DW) and the lowest in winter males (22.64 ± 1.88 mg kg^{-1} DW). A similar seasonal trend was observed for lead (Pb), where Summer groups exhibited higher concentrations than Winter groups. Cadmium (Cd) concentrations also showed relatively higher values during summer, particularly in females (0.43 ± 0.06 mg kg^{-1} DW). The total protein content was slightly higher in females than in males, but the values were within a narrow range in the four groups. Likewise, total lipid content was always higher in females than in males, while seasonal variation seemed relatively minor.

Table 2: Mean ± SD of dry weight, heavy metal concentrations, total protein, and total lipid contents in *Metapenaeus affinis* collected from Shatt Al-Basra Canal during Summer and Winter.

Variable	Male (Summer)	Male (Winter)	Female (Summer)	Female (Winter)
Sample size (n)	15	15	15	15
Dry weight (g)	5.05 ± 1.17	4.56 ± 1.19	4.91 ± 0.86	4.68 ± 1.19
Cu (mg kg^{-1} DW)	28.71 ± 3.39	22.64 ± 1.88	29.74 ± 3.49	24.19 ± 2.35
Pb (mg kg^{-1} DW)	2.48 ± 0.45	1.67 ± 0.29	2.27 ± 0.30	1.75 ± 0.41
Cd (mg kg^{-1} DW)	0.39 ± 0.06	0.26 ± 0.06	0.43 ± 0.06	0.29 ± 0.04
Total protein (%)	72.51 ± 0.92	71.84 ± 1.11	73.48 ± 1.24	73.18 ± 1.03
Total lipid (%)	6.82 ± 0.28	6.80 ± 0.31	7.17 ± 0.31	7.13 ± 0.18

The effect of sex and season on heavy metal concentrations, biochemical composition and dry weight of *M. affinis* was tested by using two-way analysis of variance (ANOVA). The results indicated that the season had a significant effect on the accumulation of all the studied heavy metals, whereas sex and the interaction between sex and season had no significant effect. Copper (Cu) concentration was significantly higher in summer (28.75 ± 3.39 mg kg^{-1} DW) than in winter (23.41 ± 2.21 mg kg^{-1} DW) ($p < 0.001$). Similarly, lead (Pb) concentrations were significantly greater during summer (2.37 ± 0.32 mg kg^{-1} DW) compared with winter (1.71 ± 0.20 mg kg^{-1} DW) ($p < 0.001$). Cadmium (Cd) followed the same seasonal trend, with significantly higher concentrations recorded in summer (0.411 ± 0.029 mg kg^{-1} DW) than in winter (0.280 ± 0.024 mg kg^{-1} DW) ($p < 0.001$). In contrast, no significant differences in Cu, Pb, or Cd concentrations were observed between males and females, and no significant sex × season interactions were detected (Tables 3–5). The pattern of biochemical composition was not similar. The percentage of total protein was significantly higher in females (73.27 ± 1.08%) compared to males (72.17 ± 1.05%) ($p < 0.001$), but seasonal variations and the interaction between sex and season were not statistically significant. Likewise, females showed significantly higher total lipid content (7.21 ± 0.26%) compared to males (6.81 ± 0.29%) ($p < 0.001$), with no effect of season or the interaction between season and sex on lipid levels (Tables 6 and 7). There were no significant differences between sexes or seasons in dry weight, and no significant interaction effect was observed ($p > 0.05$). The mean dry weight for all samples was 4.80 ± 1.09 g, indicating that the body mass was similar in the groups sampled (Table 8).

Table 3: Combined Effects of Sex and Season on Cu Concentration (mg kg^{-1} DW)

Sex	Summer	Winter	Mean ± SD
Male	28.71	22.64	25.67 ± 4.11^{a}
Female	28.80	24.19	26.50 ± 3.73^{a}
Mean ± SD	28.75 ± 3.39^{a}	23.41 ± 2.21^{b}	26.08 ± 3.91

LSD (0.05) = 1.48, Sex: NS ($p = 0.270$), Season: *** ($p < 0.001$), Sex × Season: NS ($p = 0.330$)

Table 4: Combined Effects of Sex and Season on Pb Concentration (mg kg^{-1} DW)

Sex	Summer	Winter	Mean ± SD
Male	2.48	1.67	2.07 ± 0.55^{a}
Female	2.27	1.75	2.01 ± 0.44^{a}
Mean ± SD	2.37 ± 0.32^{a}	1.71 ± 0.20^{b}	2.04 ± 0.50

LSD (0.05) ≈ 0.19, Sex: NS, Season: ***, Sex × Season: NS

Table 5: Combined Effects of Sex and Season on Cd Concentration (mg kg^{-1} DW)

Sex	Summer	Winter	Mean ± SD
Male	0.390	0.263	0.327 ± 0.088[a]
Female	0.432	0.297	0.364 ± 0.085[a]
Mean ± SD	0.411 ± 0.029[a]	0.280 ± 0.024[b]	0.346 ± 0.088

LSD (0.05) ≈ 0.029, Sex: NS ($p = 0.097$) , Season: *** ($p < 0.001$), Sex × Season: NS

Table 6: Combined Effects of Sex and Season on Total Protein (%).

Sex	Summer	Winter	Mean ± SD
Male	72.51	71.84	72.17 ± 1.05[b]
Female	73.35	73.18	73.27 ± 1.08[a]
Mean ± SD	72.93 ± 0.60[a]	72.51 ± 0.95[a]	72.72 ± 1.15

LSD (0.05) ≈ 0.55, Sex: *** ($p < 0.001$) , Season: NS ($p = 0.130$) , Sex × Season: NS ($p = 0.360$)

Table 7: Combined Effects of Sex and Season on Total Lipid (%).

Sex	Summer	Winter	Mean ± SD
Male	6.82	6.80	6.81 ± 0.29[b]
Female	7.24	7.18	7.21 ± 0.26[a]
Mean ± SD	7.03 ± 0.28[a]	6.99 ± 0.28[a]	7.01 ± 0.29

LSD (0.05) ≈ 0.15, Sex: *** ($p < 0.001$), Season: NS ($p = 0.600$), Sex × Season: NS ($p = 0.823$)

Table 8: Combined Effects of Sex and Season on Dry Weight (g).

Sex	Summer	Winter	Mean ± SD
Male	5.05	4.56	4.80 ± 1.21[a]
Female	4.91	4.68	4.79 ± 0.98[a]
Mean ± SD	4.98 ± 0.98[a]	4.62 ± 1.18[a]	4.80 ± 1.09

LSD (0.05): NS, Sex: NS ($p = 0.972$) , Season: NS ($p = 0.210$) , Sex × Season: NS ($p = 0.657$)

Results of Pearson's correlation analysis among dry weight, heavy metal concentrations (Cu, Pb and Cd), total protein and total lipid are presented in Tables 9 and 10. The Pearson correlation matrix for dry weight, heavy metal concentrations (Cu, Pb, and Cd), total protein, and total lipid in male *M. affinis* is given in Table 9. No significant correlations between dry weight and any of the measured variables were observed ($p > 0.05$). Heavy metals showed significant positive correlations, for instance, Cu with Pb ($r = 0.466$, p =

0.009), Cu with Cd (r = 0.676, p < 0.001) and Pb with Cd (r = 0.544, p = 0.002). Total protein showed a significant positive correlation with Pb (r = 0.571, p = 0.001) and a marginal positive correlation with Cd (r = 0.361, p = 0.050), but no correlation with Cu (p = 0.146). Total lipid was not significantly correlated with any of the variables measured (p > 0.05), and total protein was not significantly related to total lipid (r = 0.001, p = 0.996). Table 10 shows the correlation analysis of female M. affinis with the Pearson method. Similarly to males, the dry weight was not significantly correlated with heavy metals or biochemical parameters (p > 0.05). Significant positive correlations were found between Cu and Pb (r=0.399, p=0.029), Cu and Cd (r=0.584, p=0.001) and Pb and Cd (r=0.522, p=0.003). However, total protein and total lipid did not show a significant correlation with heavy metal concentrations in females (p > 0.05). In addition, no significant correlation was found between total protein and total lipid (r = −0.012, p = 0.948). Correlation analysis revealed positive correlations for Cu, Pb and Cd in both sexes. Biochemical parameters, particularly total lipid, showed a weak correlation with the bioaccumulation of heavy metals. Total protein and Pb showed a significant association only in males.

Table 9: Pearson Correlation Matrix Among Variables in Male *Metapenaeus affinis* (N= 30).

Variable	Dry weight	Cu	Pb	Cd	Total protein	Total lipid
Dry weight	1.000					
Cu	r = 0.036, p = 0.852	1.000				
Pb	r = 0.029, p = 0.880	r = 0.466, p = 0.009**	1.000			
Cd	r = 0.027, p = 0.888	r = 0.676, p < 0.001**	r = 0.544, p = 0.002**	1.000		
Total protein	r = -0.025, p = 0.895	r = 0.272, p = 0.146	r = 0.571, p = 0.001**	r = 0.361, p = 0.050*	1.000	
Total lipid	r = -0.245, p = 0.193	r = 0.265, p = 0.158	r = 0.031, p = 0.870	r = 0.316, p = 0.089	r = 0.001, p = 0.996	1.000

Note: Values represent Pearson correlation coefficients (r). * Correlation is significant at the 0.05 level (two-tailed). ** Correlation is significant at the 0.01 level (two-tailed). Sample size (N = 30; males: 15 in summer and 15 in winter).

Table 10: Pearson Correlation Matrix Among Variables in Female *Metapenaeus affinis* (N = 30)

Variable	Dry weight	Cu	Pb	Cd	Total protein	Total lipid
Dry weight	1.000					
Cu	r = -0.072, p = 0.704	1.000				
Pb	r = 0.295, p = 0.114	r = 0.399, p = 0.029*	1.000			
Cd	r = -0.002, p = 0.993	r = 0.584, p = 0.001**	r = 0.522, p = 0.003**	1.000		
Total protein	r = 0.009, p = 0.963	r = 0.213, p = 0.259	r = 0.146, p = 0.441	r = 0.079, p = 0.676	1.000	
Total lipid	r = 0.250, p = 0.184	r = 0.064, p = 0.738	r = 0.191, p = 0.311	r = 0.114, p = 0.549	r = -0.012, p = 0.948	1.000

Note: Values represent Pearson correlation coefficients (r). * Correlation is significant at the 0.05 level (two-tailed). ** Correlation is significant at the 0.01 level (two-tailed). Sample size (N = 30; males: 15 in summer and 15 in winter).

The Pearson correlation analysis revealed several significant relationships among heavy metal concentrations and biochemical parameters in the combined samples of male and female *M. affinis* (N= 60) (Table 11). Dry weight showed no significant correlation with any of the measured variables ($p > 0.05$). Copper (Cu) concentration exhibited a significant positive correlation with lead (Pb) ($r= 0.427$, $p= 0.001$) and cadmium (Cd) ($r = 0.637$, $p < 0.001$). In addition, Cu was positively correlated with total protein content ($r = 0.263$, $p = 0.042$), whereas its correlation with total lipid content was not statistically significant ($r = 0.203$, $p = 0.121$). Lead (Pb) concentration was positively correlated with cadmium (Cd) ($r = 0.505$, $p < 0.001$) and total protein content ($r = 0.306$, $p = 0.018$), but showed no significant association with total lipid content ($r = 0.043$, $p = 0.746$). Cadmium (Cd) demonstrated significant positive correlations with total protein ($r = 0.290$, $p = 0.024$) and total lipid ($r = 0.303$, $p = 0.019$). Furthermore, total protein and total lipid contents were positively correlated ($r = 0.270$, $p = 0.037$). Overall, significant positive correlations were observed among the three heavy metals, particularly between Cu and Cd, while dry weight remained independent of metal accumulation and biochemical composition.

Table 11: Pearson Correlation Matrix among Variables in Male and Female *Metapenaeus affinis* (N = 60).

Variable	Dry Weight (g)	Cu (mg kg⁻¹ DW)	Pb (mg kg⁻¹ DW)	Cd (mg kg⁻¹ DW)	Total Protein (%)	Total Lipid (%)
Dry Weight (g)	1.000					
Cu (mg kg⁻¹ DW)	-0.011 (0.936)	1.000				
Pb (mg kg⁻¹ DW)	0.133 (0.312)	0.427 (0.001**)	1.000			
Cd (mg kg⁻¹ DW)	0.013 (0.922)	0.637 (<0.001**)	0.505 (<0.001**)	1.000		
Total Protein (%)	-0.011 (0.936)	*0.263 (0.042*)*	*0.306 (0.018*)*	*0.290 (0.024*)*	1.000	
Total Lipid (%)	-0.032 (0.808)	0.203 (0.121)	0.043 (0.746)	0.303 (0.019*)	0.270 (0.037*)	1.000

Notes: Values are presented as Pearson's correlation coefficient (*r*). $p < 0.05$. ** $p < 0.01$. Sample size (N) = 60 (Females: 30 in summer + 30 in winter)

Discussion

The present study showed significant seasonal variation in the accumulation of Cu, Pb and Cd in the muscle tissue of *M. affinis*, with significantly higher concentrations during summer than winter. Sex had no significant effect on metal accumulation. The results indicate that the seasonal environmental conditions are the main influencing factor for bioaccumulation of heavy metals in M. affinis in the Shatt Al-Basrah Canal. Similar seasonal patterns of increased levels of heavy metals during summer were also reported in several marine and estuarine crustaceans, where high temperature, increased evaporation, decreased freshwater inflow and high salinity during summer increased the mobility, solubility and bioavailability of heavy metals, which in turn increased the uptake of heavy metals by aquatic organisms. Moreover, higher temperatures increase the rate of metabolism, feeding, respiration and ion exchange across the gills, all of which increase metal uptake and accumulation in tissues (Dietrich and Ayers, 2021; Ramos-Miras *et al.*, 2023). The metal build-up in the Shatt Al-Basrah Canal during summer could be attributed to a greater extent to the increased industrial and oil activities in the vicinity of the canal, coupled with the reduced freshwater influx that results in a reduced dilution capacity and thus an increase in the concentration and bioavailability of dissolved metals in the aquatic environment. These environmental and anthropogenic factors in combination likely contributed to the significantly higher metal concentrations measured during the summer season (kanani *et al*., 2025). The seasonal variation of metal accumulation might be due to the combined effect of natural and anthropogenic factors in the Shatt Al-Basrah Canal. Naturally, the very high summer temperatures, reduced freshwater inflow, increased evaporation, and enhanced seawater intrusion increase salinity and reduce the canal's dilution capacity, increasing the bioavailability of dissolved and sediment-associated metals. The canal is exposed to intense industrial, petrochemical, oil-related, agricultural and urban activities, anthropogenically, which constantly add metal-containing effluents to the aquatic environment. Summer low-flow conditions lead to higher concentrations of these contaminants, resulting in a greater uptake of metals by benthic organisms such as M. affinis. These interacting natural and anthropogenic factors probably result in significantly higher concentrations of Cu, Pb and Cd recorded during the summer season (Biswas *et al*., 2021; Ra *et al*., 2023). The dominance of copper over lead and cadmium (Cu > Pb > Cd) determined in the present investigation corresponds to the physiological characteristics of crustaceans. Copper is an essential trace element involved in many vital biological processes such as mitochondrial respiration, antioxidant defence, enzymatic reactions and oxygen transport by the respiratory pigment hemocyanin. Therefore, crustaceans have specific mechanisms for the uptake and regulation of Cu, resulting in generally higher concentrations in tissues than non-essential metals such as Pb and Cd. However, the ability of these regulatory mechanisms to prevent Cu accumulation inside the cell and oxidative stress (production of reactive oxygen species) can be overwhelmed by excessive environmental exposure (Keshavarzifard *et al*., 2021; Dietrich and Ayers, 2021). The relatively high concentrations of Cu in *M. affinis* in the Shatt Al-Basrah Canal are likely due not only to its physiological importance but also to continuous anthropogenic inputs. The canal is affected by intense industrial, petrochemical and oil activities, in addition to discharges from municipal and agricultural sources that are important sources of contamination of the aquatic environment with copper. Such continuous inputs might have contributed to the high Cu concentrations obtained in shrimp muscle tissues (Comber *et al*., 2023). Cu was detected at much higher concentrations than Pb and Cd, but both showed the same seasonal pattern, indicating common environmental controls over their accumulation. When compared to copper, Pb and Cd are not known to play a physiological role and can adversely affect cellular metabolism at low concentrations by interfering with calcium-dependent pathways, inhibiting enzyme activity, inducing oxidative stress and impairing immune and reproductive functions. Therefore, the higher concentrations in summer reflect increased environmental availability rather than physiological regulation. The higher accumulation of Pb and Cd in the Shatt Al-Basrah Canal during summer could be due to the remobilization of sediment-bound metals at high salinity and temperature conditions, in addition to constant inputs from oil-related activities, agricultural runoff, and other anthropogenic activities around the canal. These processes are likely to increase the bioavailability of Pb and Cd and facilitate their uptake by benthic organisms such as *M. affinis* . Similar observations have been reported for penaeid shrimp inhabiting estuarine and coastal environments impacted by industrial and agricultural activities, where seasonal increases of Pb and Cd are largely associated with decreased freshwater dilution, sediment remobilization and increased anthropogenic inputs (Liu *et al*., 2023; Ra *et al*., 2023). In general, the concentrations found in this study are comparable to those reported by other studies for Metapenaeus affinis and other penaeid shrimp from the Arabian Gulf and adjacent regions, although absolute concentrations vary among studies due to differences in environmental quality, sediment properties, seasonal conditions, analytical methods, and the extent of local anthropogenic inputs. *M. affinis* is an effective biomonitor species in recent studies of Iraqi waters due to its close association with bottom sediments and ability to accumulate contaminants while maintaining relatively stable biochemical characteristics (Hassan *et al*., 2024; Mohamed *et al*., 2025). However, previous studies have mainly focused on reporting contaminant concentrations or assessing the species as a bioindicator, without comprehensively investigating the combined effect of biological factors (sex) and seasonal variation on metal bioaccumulation. Thus, the present study is the first integrated assessment of effects of sex and season on the accumulation of Cu, Pb and Cd in M. affinis from the Shatt Al-Basrah Canal, providing baseline data unavailable previously for this ecologically and commercially important species in southern Iraq (Wang, 2022; Ramos-Miras *et al*., 2023). These results provide an important baseline for future environmental monitoring programs and contribute to the advancement of knowledge on the ecological processes that drive heavy metal bioaccumulation in the northwestern Arabian Gulf. The present study also found that there was no statistical difference in the concentration of heavy metals between males and females. Females had slightly higher mean concentrations of Cu and Cd, but these differences were not enough to result in significant sex-related variation. In several crustacean species, similar results have been found, suggesting that sex is seldom a determinant for heavy metal accumulation when both sexes occur in the same habitat and are exposed to similar environmental conditions. Generally, reproductive condition, feeding activity, molt stage, age and seasonal physiological changes exert greater influence than sex itself (Ramos-Miras *et al*., 2023; Hassan *et al*., 2024). The slightly elevated concentrations observed in females may nevertheless reflect increased nutritional requirements during ovarian maturation and vitellogenesis, processes that increase food consumption and metabolic activity, thereby slightly enhancing contaminant uptake. However, because these physiological differences were relatively small, seasonal environmental exposure remained the dominant factor controlling tissue metal concentrations in the present investigation. The two-way ANOVA demonstrated that season exerted a highly significant effect on the accumulation of Cu, Pb, and Cd, whereas neither sex nor the interaction between sex and season significantly influenced metal concentrations. These findings indicate that environmental factors associated with seasonal fluctuations play a much greater role in determining heavy metal accumulation than intrinsic biological and physiological differences between males and females (Asare *et al*.,2025). Recent studies on marine crustaceans inhabiting estuarine ecosystems have consistently demonstrated that seasonal environmental variability is the principal factor controlling heavy metal bioaccumulation. Fluctuations in water quality, salinity, suspended particulate matter, and sediment geochemistry account for a greater proportion of the variability in tissue metal concentrations than biological characteristics such as sex or body size (Banaee *et al*., 2024; Gray *et al*., 2025). Seasonal variations in metal speciation, adsorption–desorption processes and sediment–water exchange affect the bioavailability of metals for uptake by benthic organisms. The present results suggest that the seasonal changes in salinity and water quality in Shatt Al-Basrah Canal are more important than the physiological differences between the two sexes, indicating that environmental conditions are the main factors driving metal accumulation in M. affinis. The lack of a significant season × sex interaction supports this interpretation, with males and females responding similarly to seasonal environmental variation. It implies that the physiological mechanisms of metal uptake, transport, detoxification and storage are largely similar in both sexes under the environmental conditions of the study area. Similar observations were reported on penaeid shrimp and other decapod crustaceans, where the effect of environmental exposure is generally more pronounced on metal bioaccumulation than sex-specific physiological differences, except at advanced reproductive stages or when pronounced differences in feeding behavior and habitat use exist (Banaee *et al*., 2024; Gray *et al*., 2025). These results further confirm the hypothesis that the heavy metal accumulation in *M. affinis* is mainly driven by environmental pollution and not by sex-related physiology. There was no significant effect of seasonal variation, unlike the total protein and lipid contents, which were significantly higher in females than in males. These differences are likely related to the higher energetic costs of female reproduction. Ovarian maturation in crustaceans demands large amounts of structural proteins and lipid reserves for the support of vitellogenin synthesis, oocyte development, membrane formation and embryonic growth. The main energy reserve carried to developing oocytes is lipids, and proteins are involved in tissue growth, enzyme synthesis and reproductive metabolism (Taslima *et al*., 2022). Females thus tended to have higher concentrations of muscle protein and lipids than males, even under relatively constant environmental conditions. Similar results have been described for several penaeid shrimp species, where females had higher biochemical reserves because of ovarian maturation and reproductive investment and not due to differences in environmental contamination (Liang *et al*., 2022). This result indicates that the levels of Cu, Pb and Cd recorded were lower than the limit that can cause detectable biochemical alteration in the muscle tissues of *M. affinis*. In the Shatt Al-Basrah Canal, although the current levels of metal accumulation have not reached critical concentrations, continuous and long-term environmental exposure may result in cumulative adverse effects on the biochemical health, physiological performance and vital functions of shrimp. Thus, the lack of significant alterations in muscle protein and lipid content should not be considered as evidence of a lack of biological effect, but rather as an indication that the current levels of exposure are still within the physiological capability of the species to regulate and detoxify. Similar findings have been reported in crustaceans, where physiologically relevant concentrations of metals induced defence and detoxification mechanisms with no significant loss of muscle protein and lipid reserves. Major biochemical impairment was generally seen only after long-term exposure or experimentally increased metal concentrations (Ra *et al*., 2023; Altofan *et al*., 2023). Likewise, Liu *et al*. (2024) concluded that substantial alterations in protein synthesis and lipid metabolism generally occur only under chronic or high-level metal exposure capable of inducing oxidative stress and mitochondrial dysfunction. Comparable conclusions were also reported by Abdul-Sahib and Ajeel (2005) and Al-Silawy *et al*. (2025), who found that seasonal environmental fluctuations alone were insufficient to significantly modify the biochemical composition of penaeid shrimp when contamination levels remained within moderate ranges. To summarize, these results are compatible with the interpretation that the relatively constant protein and lipid levels measured in the current study reflect efficient physiological regulation in the current environmental conditions, despite the measurable accumulation of metals. The Pearson correlation analysis showed high positive correlations between Cu, Pb and Cd in males, females and in the pooled dataset. In particular, Cu was most correlated with Cd, and there were also significant positive correlations between Cu and Pb, and Pb and Cd. Such correlations suggest that these three metals may be derived from the same environmental sources and may be bioavailable at the same time for accumulation. Similar correlation patterns have been widely reported in estuarine and coastal ecosystems, where significant positive inter-metal correlations indicate common anthropogenic sources and similar geochemical behavior. In addition, multivariate statistical methods such as Pearson correlation, principal component analysis (PCA) and cluster analysis (CA) have been consistently demonstrated that Cu, Pb and Cd are commonly associated with industrial effluents, petroleum related activities, municipal wastewater, agricultural runoff and shipping operations in estuarine environments (Zhang *et al*., 2015; Li *et al*., 2023; Liu *et al*., 2023; Liang *et al*., 2025). Likewise, a recent investigation in Guanghai Bay, South China Sea, showed that Pearson correlation analysis combined with PCA effectively identified common anthropogenic sources controlling the distribution of Cu, Pb, and Cd in coastal sediments, supporting the interpretation that these metals often exhibit synchronized accumulation due to shared pollution sources rather than independent environmental processes (Jin *et al*., 2025). Interesting was the lack of significant correlations between dry weight and the investigated metals. Thus, body size was not a major factor influencing metal accumulation within the relatively narrow size range of specimens examined. Similar observations have been made for many populations of penaeid shrimp, where environmental exposure, habitat quality and feeding ecology have more effect on tissue metal concentrations than morphometric characteristics. The absence of size dependence also indicates that the sampled shrimp were exposed to rather homogeneous environmental conditions and experienced similar exposure histories, thus minimizing inter-individual variability induced by growth (Arisekar *et al*., 2022; Kumar *et al*., 2024). Protein content was weakly but significantly correlated positively with Cu, Pb and Cd in the combined dataset, while a significant correlation with Pb was found only in males. Although these correlations were relatively modest, they are probably more representative of adaptive physiological responses to moderate environmental stress than to direct toxic effects. The stimulation of the synthesis of metallothioneins, heat shock proteins, and antioxidant enzymes following exposure to environmentally relevant concentrations of heavy metals has been shown to increase metal sequestration and detoxification and the maintenance of cellular homeostasis rather than a depletion of tissue proteins (Jeong *et al.*, 2023; Banaee *et al.*, 2024). In the Shatt Al-Basrah Canal, these associations could suggest that M. affinis has the capacity to activate adaptive defence mechanisms in response to chronic environmental pollution, maintaining relatively stable muscle protein levels in the face of the accumulation of Cu, Pb, and Cd. This interpretation agrees with studies showing that sublethal metal exposure activates antioxidant and detoxification pathways with little change in muscle biochemical composition in aquatic invertebrates and crustaceans (Jeong *et al.*, 2023; Waqas *et al.*, 2024). Furthermore, metallothioneins are well known as sensitive biomarkers of exposure to heavy metals, since their expression is rapidly enhanced after exposure to metals, allowing intracellular metal binding and protection of tissues against oxidative damage (Yang *et al.*, 2024). Overall, these data suggest that the relatively stable protein content observed in the present study is indicative of effective physiological compensation and cellular defence and not indicative of a lack of biological responses to metal exposure. However, because the correlations found in the present study were relatively weak and inconsistent between sexes, intrinsic biological factors such as nutritional status, metabolic activity, growth and reproductive condition are likely to have had a greater influence on muscle protein composition than metal exposure alone (Banaee *et al.*, 2024; Waqas *et al.*, 2024). The lipid content was poorly related to the heavy metals, with only a weak positive association between Cd and total lipid in the pooled data set. This finding is in agreement with previous reports that muscle lipids are generally less sensitive than biochemical parameters measured in metabolically active tissues to moderate heavy metal exposure. The main site of heavy metal accumulation and intracellular sequestration in decapod crustaceans is the hepatopancreas, which is the main organ for nutrient storage, intermediary metabolism and detoxification (Ahearn *et al.*, 2004; Ariano *et al.*, 2021). Many studies have demonstrated that trace metals preferentially accumulate in the hepatopancreas, while muscle tissue generally exhibits much lower concentrations and undergoes relatively minor biochemical changes at environmentally relevant exposure levels (Mahmudiono *et al.*, 2024; Waqas *et al.*, 2026). The weak correlations observed between heavy metals and lipid content in muscle in the present study are therefore consistent with the tissue-specific distribution and toxicokinetic behavior of metals in decapod crustaceans, where detoxification processes are primarily focused on the hepatopancreas rather than on edible muscle tissue (Ahearn *et al.*, 2004).

Conclusion

Seasonal variation was the most important factor affecting the accumulation of heavy metals in the muscle tissue of *Metapenaeus affinis*. The concentrations of Cu, Pb and Cd were significantly higher in summer. There was no significant effect of sex on metal accumulation, suggesting that environmental conditions are more important than biological differences. Accumulated metal was dominated by copper, and positive correlations between metals suggest common anthropogenic sources. Differences in protein and lipid content between males and females were more related to reproductive physiology than to metal exposure. To summarize, M. affinis was identified as a suitable biomonitor for heavy metal pollution, and continuous environmental monitoring is essential for the conservation of the ecosystem and for food safety in Shatt Al-Basrah Canal.

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مركز علوم البحار، جامعة البصرة، العراق

تأثير التراكم الحيوي للرصاص والكادميوم والنحاس في محتوى البروتين والدهون في عضلات روبيان *Metapenaeus affinis* في قناة شط البصرة، العراق

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المستخلص

يمثل تلوث البيئات المائية بالمعادن الثقيلة خطراً كبيراً على جودة البيئة وسلامة الأغذية البحرية، وذلك بسبب التراكم الحيوي للعناصر النزرة في الكائنات المائية الصالحة للاستهلاك. ويُعد رصد الأنواع التجارية المهمة من الروبيان وسيلة فعالة لتقييم مستويات التلوث بالمعادن وآثارها المحتملة على صحة الإنسان. وعلى الرغم من أهمية روبيان *Metapenaeus affinis* في جنوب العراق، لا تزال المعلومات المتعلقة بالتغيرات الموسمية والجنسية في تراكم المعادن الثقيلة والتركيب الكيميائي الحيوي لعضلاته محدودة. هدفت هذه الدراسة إلى تقييم تأثير الموسم والجنس في تراكم النحاس Cu والرصاص Pb والكادميوم Cd في العضلات، ودراسة العلاقة بين تراكيز هذه المعادن ومحتوى البروتين والدهون في العضلات. أظهرت النتائج أن الموسم كان العامل الرئيس المؤثر في تراكم المعادن الثقيلة في *M. affinis*، في حين كان الجنس العامل الرئيس المؤثر في التركيب الكيميائي الحيوي للعضلات. سُجلت تراكيز المعادن الثقيلة مستويات أعلى بصورة معنوية خلال فصل الصيف مقارنة بفصل الشتاء ($p < 0.001$)، كما أظهرت الإناث محتوى أعلى معنوياً من البروتين والدهون في العضلات مقارنة بالذكور. وأشارت الارتباطات الإيجابية القوية بين النحاس والرصاص والكادميوم إلى احتمال وجود مصادر بيئية مشتركة لهذه الملوثات. ارتفعت تراكيز النحاس من 23.41 ± 2.21 إلى 28.75 ± 3.39 ملغم/كغم وزن جاف، والرصاص من 1.71 ± 0.20 إلى 2.37 ± 0.32 ملغم/كغم وزن جاف، والكادميوم من 0.280 ± 0.024 إلى 0.411 ± 0.029 ملغم/كغم وزن جاف من الشتاء إلى الصيف. كما سجلت الإناث محتوى أعلى معنوياً من البروتين الكلي (73.27 ± 1.08%) والدهون الكلية (7.21 ± 0.26%) مقارنة بالذكور (72.17 ± 1.05% و6.81 ± 0.29% على التوالي). وأظهر تحليل ارتباط بيرسون أن أقوى علاقة كانت بين النحاس والكادميوم($r = 0.637$، $p < 0.001$)، في حين لوحظت ارتباطات إيجابية ضعيفة فقط بين تراكيز المعادن الثقيلة ومحتوى البروتين في العضلات. وتشير النتائج إلى أن الظروف البيئية الموسمية تؤدي دوراً أساسياً في تنظيم التراكم الحيوي للمعادن الثقيلة في *M. affinis*، بينما يتأثر التركيب الكيميائي الحيوي للعضلات بصورة رئيسة بالعمليات الفسيولوجية المرتبطة بالجنس. وتؤكد الدراسة أن هذا النوع يُعد مؤشراً حيوياً مناسباً لرصد تلوث المعادن الثقيلة، كما يوفر بيانات أساسية مهمة لدعم برامج المراقبة البيئية وتقييم سلامة الأغذية البحرية في قناة شط البصرة، جنوب العراق.

الكلمات المفتاحية: التراكم الحيوي، المعادن الثقيلة، *Metapenaeus affinis*، المؤشر الحيوي، قناة شط.