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Original Article

Effect of some heavy metals on the biochemical parameters of the freshwater shrimp, *Macrobrachium nipponense* (De Haan, 1849)

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Abstract: The current study aimed to determine the biochemical parameters, including glucose, total protein, cholesterol, and enzymes of AST and ALT, in freshwater shrimp *Macrobrachium. nipponense* after exposure to different sub-lethal concentrations of heavy metals, including iron, copper, lead, and cadmium. Samples of *M. nipponense* were collected from Shatt Al-Arab, Southern Iraq. The experiment was carried out in a recycling aquaculture system (RAS) consisting of plastic containers, each of 60 liters. Four different treatments were used, each treatment representing three concentrations of Iron (1.5, 2, and 2.5 ppm), Copper (0.25, 0.35, and 9.45 ppm), Lead (0.05, 0.125, and 0.175 ppm), and Cadmium (0.05, 0.1, and 0.17 ppm), in addition to the control group. The results showed a significant decrease in all biochemical parameters compared to the control one. According to the study results, AST and ALT were significantly increased compared to the control. In addition, liver enzymes and biochemical parameters are significantly impacted by heavy metals. This confirms that liver enzymes and biochemical parameters may be biomarkers to monitor heavy metal pollution.

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Introduction

Heavy metals are substances with a high density that are potentially harmful even in small amounts (Bagul et al., 2015). They are a component of the earth's crust, and cannot be transformed or degraded. They are primarily responsible for being dangerous pollutants, and they bioaccumulate in aquatic organisms. Therefore, their presence in the ecosystem is very disturbing (Ideriah et al., 2006). The biota, water, and sediment in aquatic environments serve as metal reservoirs. Heavy metals damage the ecosystem because of their toxicity even at low concentrations and their detrimental effects on human health and other organisms (Gobbi et al., 2020). The majority of enzymatic activities depend on heavy metals, including iron (Fe), magnesium (Mg), copper (Cu), manganese (Mn), zinc (Zn), and calcium (Ca), therefore, the development of an organism depends on these essential heavy metals. These substantial compounds regulate critical processes in the enzymatic system and serve as cofactors. They are required for these processes, and their absence could have negative consequences (Lazorík and Kula, 2015). Some of them, including lead (Pb), cadmium (Cd), mercury (Hg), silver (Ag), and other metals are poisonous at any concentration and are not necessary for the organism to stay alive. These pollutants also pose a threat because they gradually affect living organisms (Gerpe et al., 2019).

Diverse aquatic organisms occasionally process heavy metals in their bodies in various ways. Aquatic invertebrates are more sensitive to contaminants than fish or algae (Baken et al., 2011). Heavy metals can enter aquatic crustaceans' bodies in various ways, including directly through the gills, without effective transport. Aquatic invertebrates can accumulate heavy elements exceptionally because they filter out plankton while feeding (Golovatch et al., 2020). Crustacean biochemical parameters can be used as a biological tool to detect heavy metal contamination in many aquatic environments because heavy metals affect these parameters. Some biochemical parameters decrease when exposed to different levels of heavy metals, while the concentration of liver enzymes

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	Control (0 ppm)	1.5 ppm	2 ppm	2.5 ppm
Glucose (mg/dl)	59.98	56.02	49.37	40.78
Total Protien (mg/dl)	58.83	59.48	56.39	54.27
Cholestrol (mg/dl)	46.75	40.91	36.4	34.84

Figure 1. Glucose, total protein, and cholesterol levels in the hemolymph of *Macrobrachium nipponense* exposed to a different level of Fe.

increases (Al-Taee, 2017; Altaee et al., 2018). This study aimed to assess the biochemical parameters of the freshwater shrimp *Macrobrachium nipponense* following exposure to various sub-lethal concentrations of certain heavy metals, including iron, copper, lead, and cadmium, and to take into account the potential use of heavy metals as biomarkers for monitoring heavy metal pollution. These biochemical parameters included glucose, total protein, cholesterol, and liver enzymes, including AST and ALT.

Materials and Methods

Sample collection: Samples of *M. nipponense* were collected from the Shatt Al-Arab River, Al-Mashab District, North of Basra Governorate, Southern Iraq. The samples were transported directly to the laboratory at the University of Basrah, Pure Science College, Department of Biology. Samples were acclimated in the laboratory for 14 days.

Experiment design: The experiment was performed using the recycling aquaculture system (RAS) consisting of plastic containers, each of 60 liters. Four different treatments were used, each treatment representing three concentrations of Iron (1.5, 2, and 2.5 ppm), Copper (0.25, 0.35, and 9.45 ppm), Lead (0.05, 0.125, and 0.175 ppm), and Cadmium (0.05, 0.1, and 0.17 ppm), in addition to the control treatment (0 heavy elements). Three replicates were used for each treatment. Five shrimp samples of 5 g each were placed in each replicate. The experiments lasted for 21 days. During the experiment, shrimp were fed standard shrimp feed (35% crude protein).

Biochemical parameters: Five shrimp from each treatment were collected after the exposure time of 21 days. Hepatopancreatic glands were used to collect hemolymph using disposable syringes measuring 5 ml. The hemolymph samples were stored at -18ºC. The glucose oxidase method was used to measure the glucose concentrations (Barham and Trinder, 1972). A total protein measurement was made following Doumas et al. (1981). Commercial colorimetric kits (Biodiagnóstica) were used to determine total cholesterol concentrations. Reitman and Frankel (1957) recommended methods were used to measure the enzymes of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). A spectrophotometer was used to read the samples at a wavelength of 550 nm.

Statistical analysis: Data were analyzed using a one-way analysis of variance (ANOVA) test using the Statistical Package for the Social Sciences (SPSS, version 26), and means were tested using the Least Significant Difference (LSD) test. Statistically, significant differences are indicated as $P<0.05$.

Results

Effect of Fe on biochemical parameters and Enzymes (AST and ALT): Figure 1 shows changes in glucose, total protein, and cholesterol levels for *M.*

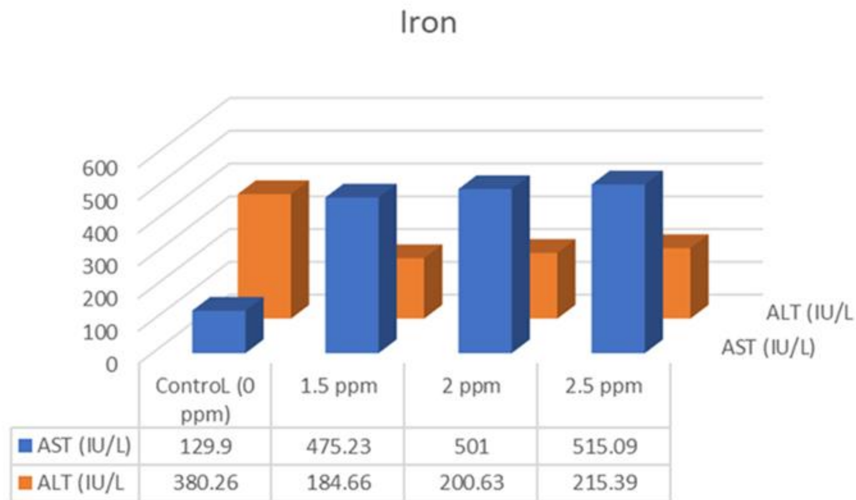


Figure 2. AST and ALT levels in the hemolymph of of *Macrobrachium nipponense* exposed to a different level of Fe.

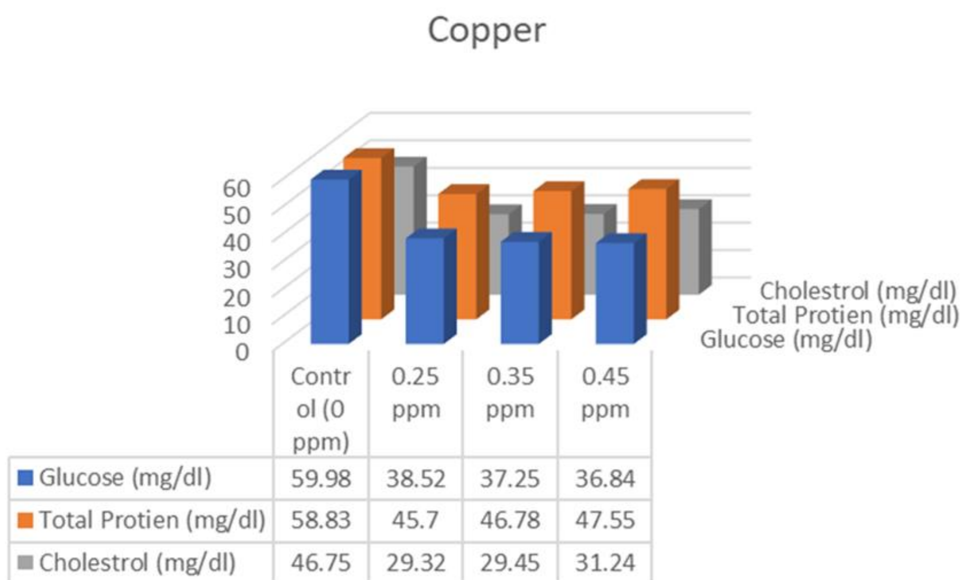
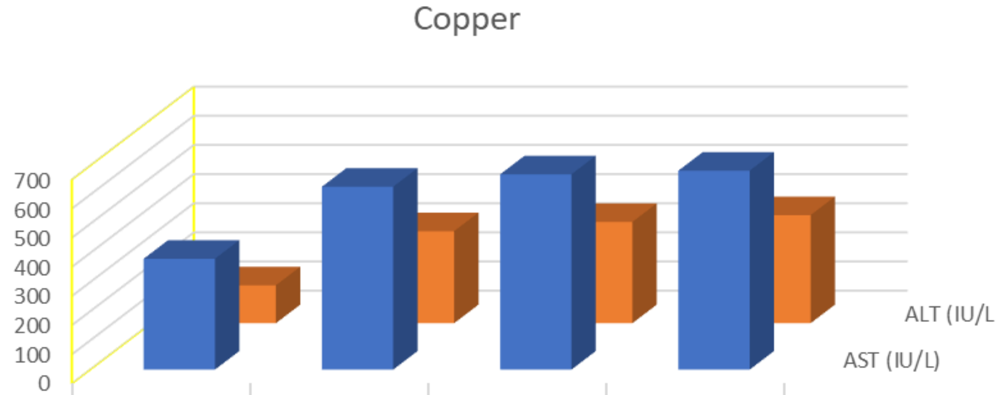


Figure 3. Glucose, total protein, and cholesterol levels in the hemolymph of *Macrobrachium nipponense* exposed to a different level of Cu.

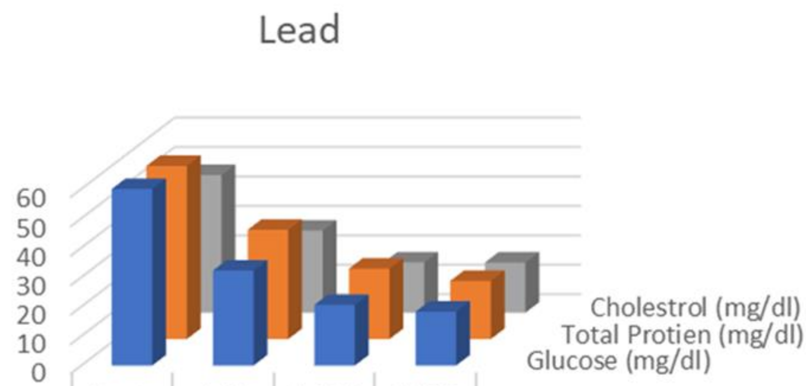
nipponense exposed to a different level of iron. The results showed a significant decrease (P<0.05) in all biochemical parameters. The lowest value was in the treatment of 2.5 ppm (40.78, 54.27, and 34.84 mg/dl) for glucose, protein, and cholesterol, respectively. The results indicated that the AST and ALT enzyme values were higher (P<0.05) than in the control group (Fig. 2). At 2.5 ppm, the AST and ALT enzymes had the highest levels of concentration, reaching 515.09 and 215.39 IU/L, respectively. The results showed an inverse relationship between Fe and all biochemical parameters (r = 0.801, r = 0.605, and r = 0.952), for glucose, total protein, and cholesterol, respectively. The results also showed a positive relationship (r = 0.327, and r = 0.991) between the AST and ALT enzyme and Fe levels. These relationships were statistically significant (P<0.05).

Effect of Cu on biochemical parameters and (AST and ALT) enzymes: Glucose, total protein, and cholesterol for *M. nipponense* exposed to various levels of copper are shown in Figure 3. All biochemical parameters were lower (P<0.05) than the control. The treatment of 0.45 ppm had the lowest values of 36.84, 45.7, and 29.32 mg/dl, for glucose, protein, and cholesterol levels, respectively. The results showed that the AST and ALT enzyme values were higher (P<0.05) than the control group. The highest levels of the AST and ALT enzymes were



	ControL (0 ppm)	0.25 ppm	0.35 ppm	0.45 ppm
AST (IU/L)	380.26	627.34	669.98	682.59
ALT (IU/L	129.9	315.7	347.3	370.62

Figure 4. AST and ALT levels in the hemolymph of *Macrobrachium nipponense* exposed to a different level of Cu.



	Control (0 ppm)	0.05 ppm	0.125 ppm	0.175 ppm
Glucose (mg/dl)	59.98	32.26	20.59	18.31
Total Protien (mg/dl)	58.83	37.13	23.94	19.77
Cholestrol (mg/dl)	46.75	27.89	17.06	17.05

Figure 5. Glucose, total protein, and cholesterol levels in the hemolymph of *Macrobrachium nipponense* exposed to a different level of Pb.

found at 0.45 ppm, reaching 682.59 and 370.62 IU/L, respectively (Fig. 4). The results showed an inverse significant relationship between copper and all biochemical parameters (r = 0.914, r = 0.951, and r = 0.929), for glucose, total protein, and cholesterol, respectively (P<0.05). Also, a positive relationship (r = 0.953, and r = 0.972) between the AST and ALT enzyme and Cu levels was found (P<0.05).

Effect of Pb on biochemical parameters and (AST and ALT) enzymes: Figure 5 displays the biochemical parameters (glucose, total protein, and cholesterol) of *M. nipponense* exposed to various lead concentrations. Compared to the control, glucose, total protein, and cholesterol levels decreased (P<0.05). The lowest value was for the 0.175 ppm treatment (18.31, 19.77, and 17.06 mg/dl, for glucose, protein, and cholesterol, respectively). The findings showed increased enzyme levels for AST and ALT (P<0.05). The AST and ALT enzymes were present at the highest concentrations in the 0.175 ppm level, reaching 1044.98 and 963.38 IU/L, respectively (Fig. 6). The results showed inverse relationships between Pb and biochemical parameters as r = 0.991, r = 0.992, and r = 0.981, for glucose, total protein, and cholesterol, respectively (P<0.05). The statistical analysis shows positive significant relationships (r = 0.953, and r = 0.972) between the levels of AST and ALT enzyme and Pb (P<0.05).

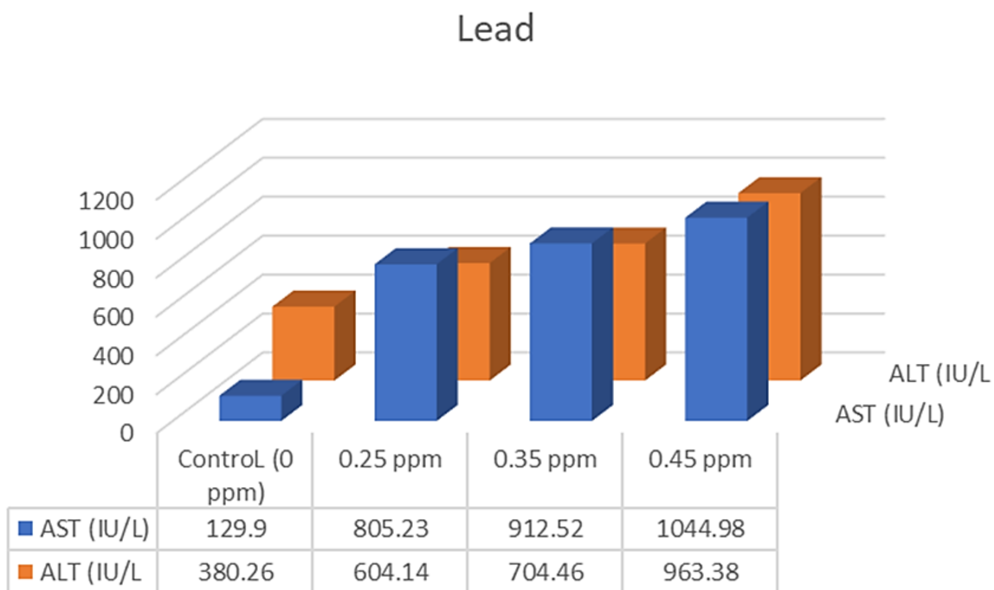


Figure 6. AST and ALT levels in the hemolymph of *Macrobrachium nipponense* exposed to a different Pb level.

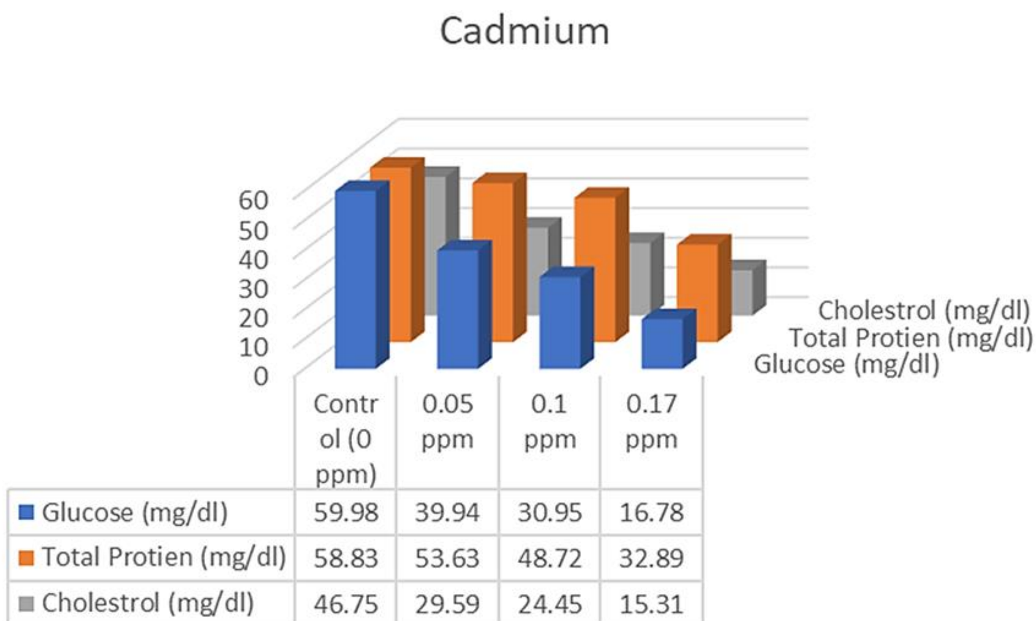


Figure 7. Glucose, total protein, and cholesterol levels in the hemolymph of *Macrobrachium nipponense* exposed to a different level of Cd.

Effect of Cadmium on biochemical parameters and (AST and ALT) enzymes: Biochemical parameter levels were lower than the control (P<0.05) for all treatments (Fig. 7). The lowest value for glucose, protein, and cholesterol was for the 0.17 ppm treatment (16.78, 32.89, and 15.31 mg/dl, respectively). The results revealed increases in AST and ALT enzyme levels compared to the control group (P<0.05). The highest levels of the AST and ALT enzymes, 1927.34 and 1704.64 IU/L, respectively, were found in the 0.17 ppm level (Fig. 8). The results showed inverse relationships between cadmium and all biochemical parameters as r = 0.984, r = 0.895, and r = 0.996, for glucose, total protein, and cholesterol, respectively(P<0.05). The results also showed positive relationships (r = 0.963, and r = 0.947) between the AST and ALT enzyme and cadmium levels (P<0.05).

Discussions

Heavy metals are metals or metalloids with high atomic weights and specific densities, and they are

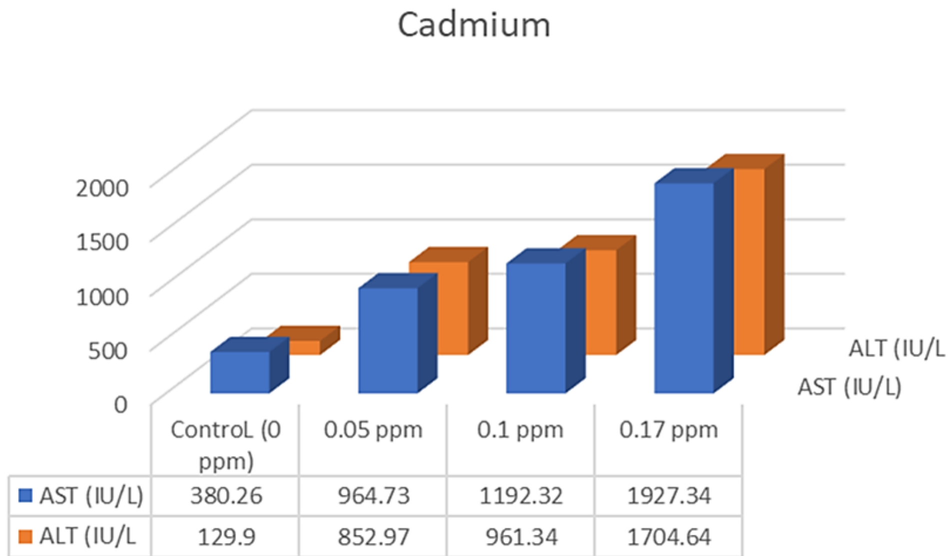


Figure 8. AST and ALT levels in the hemolymph of *Macrobrachium nipponense* exposed to a different level of Cd.

frequently considered toxic even at very low concentrations (Duruibe et al., 2007). Geogenic or human-made sources are the primary causes of heavy metal contamination in the aquatic environment (Bennett et al., 2001; Das et al., 2004). Because of their propensity to dissolve easily and quickly in water and be subsequently ingested by the living organisms that inhabit the medium and their inability to biodegrade, heavy metals pose a serious threat to the environment (Golovanova et al., 1999). Most heavy metals are necessary for invertebrate life at low concentrations because they help in developing bones, shells, and muscles. However, at high concentrations, they can cause serious physiological changes that can even result in death. When these substances are consumed, they not only pose a risk to the health of invertebrates but also to humans because most of the changes they cause are not immediately apparent (Piotrowski and Coleman, 1980; Thomas and Merian, 1991).

Effect of heavy metals on biochemical parameters

Glucose: One of the most crucial nutrients for shrimp's health is glucose. Adding glucose to the diet significantly increased shrimp growth, survival rates, and feed efficiency. This study's results showed that the hemolymph glucose concentration was decreased in all heavy metals treatments. These results agree with studies by Molnar and Fong (2012) on *Penaeus indicus* when exposed to high Fe concentrations. To fulfill the demand for energy under stressful conditions, the enzyme phosphorylated glycogen activity was enhanced, resulting in an increase in glycogen breakdown and is the reason for the decrease in glucose levels. Norwood et al. (2003) confirmed a decrease in the level of glucose in *Penaeus semisulcatus* exposed to high concentrations of Cu. They showed that the decrease in glucose concentration was attributed to the toxicity of Cu when it was used in high concentrations, which may affect the production of sugars through the inhibition of carbohydrates. Balakrishnan et al. (2012) pointed out that the glucose concentration in the hemolymph of *M. niponense* shrimp had decreased after 14 days of exposure to two different doses of Pb. This was attributed to the fact that, during acute lead exposure, carbohydrates were mobilized to varying degrees as a compensatory metabolism in response to energy stress. As a result of the pollutant's impact on the gluconeogenesis process, the lead-treated group in the current study displayed lower glucose levels than the control group. Bhavani and Sujatha (2014) examined the hemolymph of the crustacean *Portunus pelagicus*, they discovered that exposure to cadmium chloride and mercuric chloride increased the level of glucose. According to Lofts and Tipping (2011), the hepatopancreatic gland and shrimp muscles of

Macrobrachium spp., were directly affected by the use of different concentrations of heavy metals. In addition, the current study found a significant inverse relationship between glucose and the concentrations of Fe, Cu, Pb, and Cd in the treated groups. This finding agrees with a study by Al-Taee (2017, 2018) that observed a negative relationship between glucose and most heavy metals found in the mud crab *Chiroenetes boulengeri.*

Total protein: The body produces proteins in many different parts, and the total protein is crucial for the health and function of cells and tissues. The level of total protein in shrimp is an essential tool in monitoring their physiological conditions when they adapt to different environmental conditions (Vanhaecke and Persoone, 1981). The total protein is one of the important indicators for pollution in the waterbodies (Matozzo et al., 2011). The current study shows a decrease in the total protein concentration in the hemolymph of shrimp exposed to heavy metal treatments, and this finding is consistent with Gerpe (2019), who indicated a decrease in the concentration of total protein in the hemolymph of the *Penaeus marguensis* exposed to different concentrations of Cu. our findings are in agreement with those of Al-Taee (2017, 2018), which showed that the total protein concentration is decreased in the hemolymph of river crab *C. boulengeri* exposed to different heavy metals and showed that the total protein was inversely associated with most of the heavy metals. Thomas et al. (2001) indicated that the biochemical components of an organism vary according to the nature and concentration of the heavy metals, as well as the organism's size, temperature, availability of food, stage of maturity, and other factors.

Cholesterol: All cells and crustacean hemolymph contain cholesterol. The development and survival of shrimp and other crustaceans depend on dietary cholesterol because they cannot synthesize it. Cholesterol levels show a dramatic drop in correlation with the occurrence of pollution (Dawood, 2018). The study results showed a decrease in the concentration of cholesterol in the hemolymph of all heavy metal treatments, which is consistent with the study of Ostrovsky (2021), which showed a significant decrease in the concentration of cholesterol in the hemolymph of *Penaeus kerathurus*, when exposed to Cu. Waldock (2020) pointed out that fatty acids serve as the primary building block for cholesterol, consequently, an increase in Pb concentration inhibits the reaction, disrupting the production of fatty acids, which will decrease cholesterol in *Lobester homupus* hemolymph. The current results agreed with the findings of Matozzo (2011), which found that *Carcinus aesturii* crabs collected from heavy metal-contaminated areas had hemolymph with lower lipids.

According to the results of the current study, there is a significant negative relationship between the concentration of Cu and the cholesterol levels in the hemolymph of the treated groups. This is consistent with the study of Al-Taee (2017, 2018), which explored an inverse relationship between the cholesterol levels in the hemolymph of river crabs *C. boulengeri*. The shrimp *Litopenaeus stylirostris* uses its reserve of cholesterol, which it needs to manage resistance in the concentration of Fe, Cu, Pb, and Cd, which results in a decrease in its concentration in the hemolymph, as shown by Alyuruk and Avas (2013). Our findings are in agreement with Paul et al. (2014), who established that exposure to high Cu concentrations causes a significant drop in cholesterol concentration in *Penaeus kerathurus* hemolymph.

Effect of heavy metals on AST and ALT enzymes: Aminotransferase enzymes like AST and ALT are present in many body tissues. One of the liver functions tests (LFTs) used to monitor liver damage is the estimation of ALT and AST in plasma or serum (Choudhury et al., 2009). Changing the level of enzymes is one of the primary methods for determining the toxicity of pollutants in aquatic systems (Molnar and Fang, 2012). One of the contaminants-sensitive enzymes is ALT, and its level is used as a biomarker of liver health (Mobley, 2018). The results of the current study are consistent with those of Biney and Ameyibor (1992), who found that exposure to high Fe concentrations increased the level of stress enzymes in *Penaeus margnisis*. The results of the current study also showed an increase in ALT of the hemolymph in all heavy metal treatments. According to Svensson et al. (2005), the toxicity of some heavy metals and their effects on cellular components may be the reason behind the alteration in the levels of ALT. Consistent with Al-Taee (2017, 2018), who found a positive correlation between the concentration of ALT and the concentration of heavy metals in the river crab, *C. boulengeri*, our results revealed a significant positive correlation between the concentration of ALT and the heavy metals (Fe, Cu, Pb, and Cd) in all treatments. Mobley (2018) found that the level of the ALT enzyme increased in the hemolymph of *Penaeus californiesis* exposed to high Cu concentrations, Guastavino et al. (199) pointed out that copper does not increase the level of stress enzymes in the shrimp even when exposed to a high concentration.

AST is known as serum glutamic-oxaloacetic transaminase, or SGOT. When liver cells are damaged, AST leaks into the bloodstream and the level of AST in the blood rises (Almo et al., 1994). The results of the current work showed an increase in the concentration of AST in the hemolymph of all heavy metal treatments. Our results also showed a significant positive relationship between the concentration of AST enzyme and the Fe, Cu, Pb, and Cd concentrations in the hemolymph of the treated group. This is consistent with the findings of Al-Taee (2017, 2018), which indicated a positive correlation between the level of AST enzyme and the concentration of iron in the hemolymph in river crab *C. boulengeri*. Intamat et al. (2017) found that *Penaeus marguensis* had more AST enzyme in its hemolymph as a result of exposure to lead acetate. The current study's results agreed with those of Mitra (2010), which showed that *P. marguensis* shrimp exposed to semi-lethal concentrations of Pb experienced an increase in the level of AST enzyme in the hemolymph. According to Geisen et al. (2019), extreme oxidative stress inhibits the activity of antioxidant enzymes, which are essential for preventing oxidative stress caused by either the metabolism itself or by pollutants.

Conclusion

This study showed the potential impacts of heavy metals (Fe, Cu, Pb, and Cd) on the biochemical parameters (Glucose. Total protein, and cholesterol), and liver enzymes (ALT and AST) of *M. nipponense* exposed to different sublethal concentrations. The hemolymph glucose. Total protein and cholesterol levels were decreased in all heavy metals exposed treatments, while the liver enzyme levels were increased in all heavy metals exposed treatments. An inverse relationship between studied heavy metals and biochemical parameters and a positive relationship between studied heavy metals and liver enzymes (ALT and AST) were found. Therefore, we can conclude that liver enzymes and biochemical parameters are biomarkers to monitor heavy metal contamination in shrimp *M. nipponense*.

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