Journal of Stress Physiology & Biochemistry, Vol. 19, No. 3, 2023, pp. 187-198 ISSN 1997-0838

REVIEW

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Effects of heavy metals copper and mercury on the biochemical composition and activity pattern of selected enzymes of Molluscs: A Review

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Received May 1, 2023

Heavy metals that enter the aquatic environment pose a serious threat to the biota due to their toxicity. This review reveals that various concentrations of copper and mercury at varying lengths of exposure alter the activity of protein, carbohydrate, lipid, phosphatase, and aminotransferase significantly in the different soft tissues and hemolymph of molluscs, and toxicity is metal, organ, or species specific. The alterations in the levels of biochemical constituents and enzymes suggest adaptations of molluscs to meet high metabolic needs under metal stress. The apparent sensitivity of these biochemical and enzymatic parameters' activities suggests that they have the potential to be promising and reliable biomarkers of water pollution due to copper and mercury and can constitute an important diagnostic tool in toxicological studies. Additionally, the shells of molluscs should be considered contamination biomarkers as they sequester pollutants. A review of the literature also indicates that molluscs can be considered full-time biomonitors as they react to pollutants and can provide useful information on the water quality over time. The "Molluscs Watch" program should be included in environmental surveillance programs to keep watch on the health of the aquatic ecosystem and to protect aquatic biodiversity. This review provides an overview of the effects of heavy metals (copper and mercury) on biochemical constituents such as protein, carbohydrate, lipid, and the activity pattern of enzymes such as acid and alkaline phosphatases, aspartate, and alanine aminotransferases in molluscs.

Key words: Biochemical composition, Biomarker, Biomonitor, Enzymes, Heavy metal stress, Molluscs

Heavy metals that enter the aquatic environment pose a serious threat to the freshwater biota due to their general toxicity, highly persistent nature, tendency to bioconcentrate and bioaccumulate in an organism, and food chain amplification (Coetzee *et al.*, 2002; Ursinyova & Hladikova, 2000; Weis & Weis, 1977). Although copper is an essential trace element required by all life forms, it becomes toxic at high concentrations. Mercury, which has no known metabolic function, is known to be the most toxic of all heavy metals.

The effects of heavy metals on aquatic organisms may manifest at all levels of biological organization. Heavy metals cause severe metabolic, biochemical, physiological, cellular, molecular, and structural impairments in animals. Proteins, carbohydrates, and lipids are the "building blocks of life," providing energy for maintaining the architecture of the tissues in the body. Tissues of all organisms contain enzymes. Phosphatases, which dephosphorylate substances, have a variety of roles in cellular signaling and regulation. Acid phosphatase (ACP), which is associated with the lysosome (Cheng & Rodrick, 1975) and occurs ubiquitously among animal and plant species (Hollander, 1971), plays an important role in mineral metabolism and metal detoxification. Alkaline phosphatase (ALP) is a plasma membrane-bound enzyme (Ciro *et al.*, 1975) involved in membrane transport, glycogen synthesis (Gupta & Rao, 1974), protein synthesis (Pilo *et al.*, 1972), the synthesis of some enzymes (Summer, 1965), and secretory activity (Ibrahim *et al.*, 1974). Aminotransferases such as aspartate amino transferase (AST) and alanine amino transferase (ALT), formerly called glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), that catalyze the process of biological transamination, serve as a major link between protein and carbohydrate metabolism (Lehninger, 1979).

Mollusca, the second-largest metazoan taxon, consist of Aplacophora, Polyplacophora, Monoplacophora, Gastropoda, Cephalopoda, Bivalvia, and Scaphopoda. (Ponder & Lindberg, 2008). It exhibits a wide range of diversity, representing about 50,000 to 1,20,000 living species (C*hapman, 2009*) and 70,000 fossil species (Brusca & Brusca, 2003; Rosenberg, 2014). Molluscs are considered a potential biomonitor for heavy metal contamination in aquatic ecosystems because of their widespread geographical distribution, abundance, availability, sedentary nature, suitable dimensions, size, and weight, ease of handling, collection, identification, and culture, relatively longer life span, high tolerance to environmental changes and persistent toxic chemicals, disease resistance, and high accumulation rates for the metals.

This review provides an overview of the effects of heavy metals (copper and mercury) on biochemical constituents such as protein, carbohydrate, lipid, and the activity pattern of enzymes such as acid and alkaline phosphatases, aspartate, and alanine aminotransferases in molluscs.

EFFECTS OF COPPER AND MERCURY ON THE MOLLUSCS

Effects of copper on biochemical composition of bivalve

Katticaram *et al.* (1995) noticed a decline in total carbohydrate level in the digestive glands of the bivalve *Sunetta scripta* after prolonged exposure to higher concentrations of copper (0.3 to 1 ppm) and significantly higher protein levels at 0.3 ppm and 0.5 ppm copper. Exposure of the European freshwater mussel *Anodonta anatina* to Cu via water (0.3 µmol/L ^{63}Cu) or food (^{63}Cu-loaded algae, equivalent to 0.06 µmol/L) for 24 days results in a decrease in carbohydrate and protein levels in all body compartments (Nugroho & Frank, 2012). Sathyanathan *et al.* (1988) evaluated the effect of sublethal amounts of copper and mercury on the biochemical constituents of the estuarine clam *Villorita cyprinoides* over a range of time and described the changes in glycogen contents and metabolic rate of the tissues of the clam. Satyaparameshwar *et al.* (2006) recorded a decrease in levels of glycogen and a shift in the carbohydrate metabolism from aerobic to anaerobic type in selected tissues of the freshwater mussel *Lamellidens marginalis* due to the sublethal toxicity of copper sulfate. Neuhoff (1983) conducted a study on the synergistic physiological effects of low copper and various oxygen concentrations on the marine bivalve

Macoma balthica and concluded that the glycogen content of the tissues and their dry weight are more affected by a combination of oxygen deficiency and a low copper concentration (2.5 cm^3 O_2 dm^{-3}, 15 µg Cu dm^{-3}). Gills and hepatopancreas of the freshwater mussel, *Lamellidens corrianus*, showed a general trend of depleted levels of glycogen activity at all time periods (24, 72, 120, and 168 h) and in all the exposures to copper (100, 200, and 400 ppb) and mercury (75, 150, and 300 ppb) when compared with the controls (Rajalekshmi & Mohandas, 1993).

The values of total protein were disturbed in the tissues of brown mussels (*Perna perna)* treated with copper, cadmium, and lead under acute conditions when compared with control mussels (Boudjema *et al.*, 2014). Viarengo (1980) reported a significant reduction in the uptake of amino acids (to about 5–10%) and the rate of protein synthesis (to about 50–70%) of the control value in the gills, digestive gland, and mantle of *Mytilus galloprovincialis* after 7 days of exposure to the sublethal concentrations of metal Cu^{++} (0·08 ppm). Geret *et al.* (2002) observed a decrease in total protein levels in the gills of oysters (*Crassostrea gigas*) and the digestive gland of mussels (*Mytilus edulis*) after 21 days of copper and mercury exposure. The study of Sanders (1994) suggests that expression and accumulation of two major stress proteins, stress 70 and chaperonin 60, were affected in the gills and mantle of blue mussels, *Mytilus edulis*, exposed to a range of Cu concentrations for 7 days. When bivalve sea scallops *Placopecten magellanicus*, in early gametogenesis were exposed in late winter to sublethal levels of copper (10 and 20 µg/l) in a flowing seawater system for eight weeks with sampling at 2-week intervals, gonadal protein concentration dropped in all metal-exposed scallops with time and degree of metal exposure (Gould *et al.*, 1988). Depletion in protein contents in the mantle, foot, gill, gonad, and digestive glands of the freshwater bivalve *Corbicula striatella* after acute and chronic exposure to copper sulfate and mercuric chloride was reported by Mahajan and Zambare (2001). Deshmukh and Lomte (1998) described altered protein activity in the freshwater bivalve, *Parreysia corrugata* under $CuSO_4$ stress. The average protein content of the bivalve *L. marginalis* was decreased in the whole body, foot, hepatopancreas, gills, gonad, and mantle after acute exposures to copper sulfate, mercury chloride, and zinc chloride (Suryawanshi & Deshpande, 2016).

In an experiment, Fokina *et al.* (2013) detected major changes in the gill and digestive gland lipid composition of blue mussels, *Mytilus edulis*, after copper and cadmium exposure. A significant reduction in the activities of glycogen, protein, and lipid was found in the gill, mantle, gonads, and hepatopancreas of mercury chloride-treated *Lamelledis marginalis* (Muniv *et al*., 2020). Sonawane (2015) confirmed that the total lipid content of the whole body, foot, and mantle decreased and digestive glands increased significantly in the bivalve *Lamellidens marginalis* after acute and chronic exposure to copper sulfate.

Miller (1988) assessed the interactive effects of copper exposure at various salinities on the biochemical responses of the ribbed mussel *Geukensia demissa* and pointed out impairments in biochemical functions. Significant differences in biochemical constituents' activity were found when the Asian green mussel *Perna viridis* was exposed to copper, cadmium, lead, and zinc concentrations under a long-term chronic toxicity test (Rajkumar & Milton, 2011).

Effects of mercury on biochemical composition of bivalve

Devi (1996) investigated the effect of mercury on the biochemical composition of the marine fouling dreissinid bivalve *Mytilopsis sallei* and concluded that in time-dependent experiments, carbohydrates were utilized and a decrease in the ratios of glycogen to protein and glycogen to lipid was observed. In concentration-dependent exposure, both carbohydrates and proteins were consumed, and there was also a decrease in the glycogen to lipid ratio, but the glycogen to protein ratio was almost constant at all exposure concentrations. Reddy *et al.* (1986) concluded that the changes in the levels of glycogen in the foot, mantle, and gills of a freshwater mussel, *Parreysia rugosa*, under sublethal concentrations of mercury chloride indicate a decrease in energy supply metabolism through oxidative pathways. Sonawane (2018) noted greater depletion of

average glycogen content in the whole body and digestive gland as compared to the foot and mantle of the bivalve *Lamellidens marginalis* when exposed to $HgCl_2$ under acute and chronic treatment. A significant glycogen decrease was documented by Bhamare *et al.* (2001) in the whole body, followed by the digestive gland, foot, and mantle of *Parreysia favidens* exposed to 3.24 mg/l mercuric chloride for 72 hours as acute treatment and 1.89 mg/l mercuric chloride for 20 days as chronic treatment. Pardeshi and Zambare (2012) found the depletion of glycogen contents in the mantle, foot, gills, gonads, and digestive glands of the bivalve *Parreysia cylindrica* due to acute (0.6 ppm) and chronic (0.12 ppm) doses of mercury chloride.

Ravinder (1991) indicated a decrease in levels of total proteins and an increase in free amino acids and proteolytic activity in the foot, mantle, and gill of the mussel *Parreysia rugosa* under the influence of sublethal concentrations of mercuric chloride (0.5 ppm). Protein contents were decreased in the gills, testis, and digestive glands of bivalves *Lamellidens corrianus* after acute exposure to mercury (0.444 ppm Hg) as compared to those of control bivalves (Gulbhile & Zambare, 2013). Suryawanshi *et al.* (2014) noticed a significant decrease in protein content in the mantle, adductor muscle, gonad, siphon, gills, hepatopancreas, and mantle of oysters, *Crassostrea cattuckensis*, under 0.01 ppm and 0.04 ppm mercury exposure for 15 days.

Effects of copper on the biochemical composition of gastropod

Ramalingam and Indra (2002) stated a change in the levels of total carbohydrates and glycogen in the body mass muscle, foot muscle, digestive gland, and hemolymph of the giant African snail *Achatina fulica* under the sublethal toxicity of $CuSO_4$. Kulkarni and Vyawahare (1987) discovered that the sublethal dose of copper sulfate during the reproductive period decreased the glycogen content of the hepatopancreas and foot significantly in land slugs, *Laevicaulis alte*. The metal $CuSO_4$ altered the total glycogen content of the foot, digestive gland, mantle, and whole body of the snail *T. tuberculata* significantly after acute (5.45 ppm) and chronic (1.14 ppm) treatment, indicating altered carbohydrate metabolism (Mule & Lomte, 1993). Rao and Jayashree (1990) showed a marked decrease in glucose, glycogen, total lipids, and total protein levels in the foot, mantle, and digestive gland of the adult *Bellamya dissimilis* snail exposed to 96-hour LC_{50} concentrations of copper sulfate.

In a study carried out by Brown *et al.* (2004), significant effects were measured in the total protein and lysosomal stability of the hemolymph of the common limpet snail *Patella vulgata* and the blue mussel *Mytilus edulis* at a copper concentration of 6.1 µg/L. The high protein content change was found in the digestive gland, followed by the foot and mantle, in the freshwater snail *Thiara tuberculata* exposed to 5.45 and 1.14 ppm of copper sulfate as acute and chronic treatments, respectively (Mule & Lomte, 1995). Kumar *et al.* (2011) determined that the snail *L. acuminata* fed with sub-lethal doses, i.e., 20% and 60% of the 24 h and 96 h LC_{50} of different molluscicides inside snail attractant pellets, caused a significant decrease in proteins and free amino acids in the ovotestis of the snail. Rawi *et al.* (2011), when exposed the snail *B. alexandrina* to sublethal doses (LC_{10}) of $CuSO_4$ and plant extract, observed a reduction in the total protein and total lipid contents in the snail's hemolymph. A significant decrease in soluble proteins, along with an insignificant decrease in structural proteins and a significant increase in free amino acids and protease activity, was observed by Johnson (1990) in the hepatopancreas and foot of the snail *Pila globosa* exposed to a lethal concentration of copper.

Wang *et al.* (2009) learned that the crude lipid in the carcass of juvenile abalone, *Haliotis discus,* decreased with increasing dietary copper and was significantly lower in abalone fed dietary Cu ≥ 26.84 mg/kg.

Effects of mercury on the biochemical composition of gastropod

According to Mahajan (2005), the heavy metal mercury induced an alteration in the protein content of the gastropod *Bellamya bengalensis*. In the study conducted by Lomte and Sontakke (1992) on snails *Thiara lineata*, during a 24-hour exposure to mercuric chloride (2 ppm), the foot, mantle, and digestive gland showed an increase in protein content and during 48-, 72-, and 96-hour exposures, a considerable decrease in

protein was observed in the foot, while an increase in protein was noticed in the mantle and digestive gland. Patil *et al.* (2011) noted that a sublethal concentration of mercuric chloride (0.1 ppm) showed a significant decrease in the level of glycogen, an increase in the level of protein, and an insignificant decline in the level of lipids in the Asian snail *Indoplanorbis exustus.*

Mule and Lomte (1993) evaluated the effect of mercury chloride on the total lipid content of *Thiara tuberculata* and depicted that in chronic treatment the lipid contents of the foot and the mantle were increased while those of the digestive gland were decreased, and in acute treatment the lipid contents of the digestive gland and foot were decreased while those of the mantle were increased.

Effects of copper and mercury on the activity of phosphatase and aminotransferases of bivalve

Suresh and Mohandas (1990a) stated a high level of acid phosphatase in the hemolymph of the bivalve *Sunetta scripta* exposed to 1 ppm copper and a lower value at higher concentrations. According to Jing *et al.* (2007), copper treatment affected the activities of an immune enzyme (ACP) in the mantle of *Pinctada fucata*. In research by Anthony and Patel (2000), exposure of the tropical arcid blood clam *Anadara granosa* to sublethal concentrations of Cu at different salinities inhibited the activities of the acid phosphatase. Rajalakshmi and Mohandas (2005) demonstrated variation in ACP activity in the gills and hepatopancreas of the mussel *Lamellidens corrianus* exposed to different concentrations of copper for 24, 120, and 168 h. A reduction in the latency of lysosomal enzymes and changes in the composition of copper-binding proteins were detected in the digestive gland cells of *Mytilus edulis* exposed to increased sublethal copper concentrations (Harrison, 1983). While studying the effects of copper (0.05 µM and 0.5 µM) on the activity of an enzyme involved in immune defense (ACP) and a metal-sensitive enzyme (ALP) in the gills and digestive gland of the pear oyster, *Pinctada fucata*, at 12, 24, 48, and 72 h of exposure, Jing *et al*. (2006) observed the altered activities of phosphatase by copper. Research by Sharma *et al.* (2006) on the green mussel *Perna viridis* exposed to 25 pg/l of copper and mercury (sampling on days 0, 7, and 14) depicted higher ACP activity in the digestive gland cells of treated mussels. The results of the study carried out by Sharma and Thomas (2007) revealed that the sublethal concentration (25 µg/l) of copper and mercury at 24 h, 72 h, the 7th day, and the 14th day can induce remarkably higher acid phosphatase activity (hyper synthesis) in the hemolymph of green mussels (*Perna viridis*), but on continued exposure, the ACP activity shows a considerable decline. Regoli and Principato (1995) carried out analyses on the digestive gland and gills of mussels (*Mytilus galloprovincialis*) exposed to copper under field and laboratory conditions and concluded that, compared to control mussels, mussels transplanted into the polluted environment and mussels exposed to copper under laboratory conditions showed significant variations in the alkaline phosphatase enzyme. The mercury showed the inhibitory effects on acid and alkaline phosphatase activities in various tissues (digestive gland, gills, foot, siphon, and mantle) analyzed in the clam *Scrobicularia plana* (Mazorra *et al.,* 2002).

Ravinder (1991) recorded a rise in the levels of amino acid metabolizing enzymes (AST and ALT) in the foot, mantle, and gills of a freshwater mussel, *Parreysia rugosa*, under the influence of sublethal concentrations of mercuric chloride (0.5 ppm). Kulkarni and Kulkarni (1987) indicated elevated levels of transaminase in the gills and mantle of the clam *Katelisia opima* when exposed to mercury.

Effects of copper and mercury on the activity of phosphatase & aminotransferases of gastropod

In a study by Ramalingam and Indra (2002), the change in phosphatase activity in the body mass muscle, foot muscle, digestive gland, and hemolymph of *Achatina fulica* under sublethal toxicity of copper sulfate was recorded. Li *et al.* (2009) examined the toxicity of copper (1.35-4.20 mg/L) on the activities of the metabolic enzymes of the marine gastropod *Onchidium struma* after a 1-week exposure and detected an increase, then a decrease, and, finally, a slight increase in acid and alkaline phosphatase activity in the hepatopancreas, while aspartate and alanine transferases were activated by lower concentrations of

Cu and inhibited at higher concentrations in the hepatopancreas and muscle. Suresh *et al.* (1993) experimented on the effects of copper (0.010, 0.015, and 0.020 ppm) on the activity of phosphatases and transaminases in the hemolymph of the snails *Indoplanorbis exustus*, *Lymnaea acuminata,* and *F. rufescens* at 2, 6, 12, 24, and 48 h post-exposure and concluded that copper can cause hyper synthesis of lysosomal enzymes and, to a certain extent, can inhibit the activity of both enzymes.

Masola *et al.* (2003) mentioned that the activities of AST rose by up to 4.7-fold in the homogenate and the mitochondrial and cytosolic fractions with increasing concentrations of copper; these activities, however, fell at copper concentrations of approximately 1 mg/L, which coincided with the massive deaths of the snails *Helisoma duryi* and *Lymnaea natalensis*. Ragab (2003) presented a marked increase or inhibition in the activity of either AST or ALT in the digestive gland of the three forms of snails, Egyptian *Biomphalaria glabrata,* by $CuSO_4$. Rawi *et al.* (2011) showed that copper sulfate affected the activity of transaminase enzymes in the hemolymph of the snail *B. alexandrina*.

Effects of copper and mercury on the activity of phosphatase & aminotransferases of cephalopod

Copper (2.3 µg/L) stimulated acid phosphatase activity at the end of embryonic development in the eggs of the cuttlefish, *Sepia officinalis* (Lacoue-Labarthe, 2010).

DISCUSSION

This review reveals that various concentrations of copper and mercury at varying lengths of exposure alter the levels of protein, carbohydrate, lipid, phosphatase, and aminotransferase significantly in the different soft tissues and hemolymph of molluscs. Cu and Hg have adverse effects on the biochemical composition and enzymes of molluscs, and their toxicity is metal-, organ-, or species-specific.

The presence of heavy metals is associated with oxidative stress conditions (Semedo *et al.*, 2012) that cause a lowering of the nutritional reserves of molluscs via the degradation of biomolecules like carbohydrates, lipids, and proteins to meet the increased metabolic needs, which get increased above normal levels. Though carbohydrates serve as a readily available energy source during stress, their depletion can be correlated to the increased consumption of carbohydrates because of the anoxia, hypoxia, or anaerobic metabolism caused in order to encounter the heavy metal stress in the environment (Dezwan & Zandee, 1972; Javed & Usmani, 2015). Since after carbohydrate, lipids are utilized to overcome the heavy metal stress conditions, this leads to a reduction in the level of total lipid in the organs of molluscs due to inhibition of lipid synthesis, increased lipase activity, or mobilization of stored lipid by β oxidation (Demir *et al.*, 2005; Jha, 1991). Few studies reported an increase in lipid content, which might be due to increased lipogenesis, inhibition of lipase, diminished lipolysis, diminished transport of lipid, or a shift in carbohydrate and protein metabolism to lipid synthesis (Coley & Jensen, 1973; Swami *et al.*, 1983). The decrease in protein level in the tissues of metal-exposed molluscs might be due to the enhancement of proteolysis, decrease in anabolism of protein, denaturation of protein, or enzyme inhibition (Pottinger *et al.*, 2002; Sastry & Gupta, 1978; Waykar & Lomte, 2001). Elevated protein levels in the digestive gland indicate the synthesis of metal-binding proteins (stress proteins) and metallothionein (MT) proteins and their importance in the detoxification of toxic metals.

The inhibition of phosphatase activity is related to the breakdown of glycogen to meet the energy demand under stress, a decrease in the transphosphorylation rate, or the uncoupling of oxidative phosphorylation (Kaviraj & Gupta, 2014). The development of osmotic gradients in the lysosome due to the accumulation of toxicants results in the swelling of the lysosome, leading to increased lysosomal lability, which could result in the leakage of ACP into the hemolymph, leading to a decrease in ACP (Chvapil *et al*., 1972; Verity & Reith, 1967). The increase observed in aminotransferase activities might be attributed to a compensatory mechanism attempting to provide energy to drive an impaired metabolism (Knox & Greenguard, 1965). Metals may interfere with substrate binding or bind to the active sites of enzymes, altering enzyme activity

(Ulmer, 1970). Metals are found to inhibit, stimulate, or influence the rate of action of certain enzymes by inactivation, activation, and uncoupling reactions (Suresh & Mohandas, 1990a; Tallendini *et al.*, 1986).

Lysosomes and the cell membrane are the first targets of xenobiotic, as lysosomes are concerned with the disintegration of foreign bodies and metal detoxification, and the cell membrane functions as the first line of defense against xenobiotic encounters. Acid phosphatase is designated as an index of the lysosomal system (De Duve *et al*., 1955; Edwards & James, 1987). Lysosomal biomarkers are early warning signals of the biological effects caused by environmental pollutants. Aminotransferases are useful indicators of metabolic alterations in molluscs (Cheng *et al*., 1980). Changes in enzyme activity levels are of great diagnostic value.

The apparent sensitivity of these biochemical and enzymatic parameters' activities suggests that they have the potential to be promising and reliable biomarkers of water pollution due to copper and mercury and can constitute an important diagnostic tool in toxicological studies. Additionally, the shells of molluscs should be considered a contamination biomarker as they sequester pollutants (Elder & Collins, 1991; Koide *et al.*, 1982; Protasowicki, 2008). A review of the literature also reveals that molluscs can be considered a full-time biomonitor as they react to pollutants and can provide useful information on water quality over time. The "Molluscs Watch" program should be included in environmental surveillance programs to keep watch on the health of the aquatic ecosystem and to protect aquatic biodiversity.

The present review is expected to open up further detailed research in the field of molluscan toxicology, especially on metal-induced pollution aspects in molluscan biochemistry and physiology. It can provide information on the status, trends, and risks facing the aquatic ecosystem. It will be very useful in the fight against pollution by supporting environmental policies, laws, standards, and control methods. It can inform the choice of action to restore ecosystem structures, functions, and services effectively and efficiently in those ecosystems that are already affected by pollution and make important contributions to protecting food resources in aquaculture around the world.

CONCLUSION

Various concentrations of copper and mercury at varying lengths of exposure alter the levels of protein, carbohydrate, lipid, phosphatase, and aminotransferase significantly in the different soft tissues and hemolymph of molluscs. The apparent sensitivity of these biochemical and enzymatic parameters' activities suggests that they have the potential to be promising and reliable biomarkers of water pollution. Molluscs can be considered full-time biomonitors as they react to pollutants and can provide useful information on water quality over time.

CONFLICT OF INTERESTS

The authors declare that they have no potential conflicts of interest.

REFERENCES

Anthony K. and Patel B. (2000). Combined Effect of Salinity and Cadmium in the Presence of Other Metals (Zn & Cu) and GSH on Lysosomal Responses in an Arcid Blood Clam Anadara granosa. *Indian J. Fish*., 47(4), 311-319.

Bhamare P.R., Desai A.E. and Deoray B.M. (2001). Effect of mercuric chloride on glycogen content of the freshwater mussel parreysia favidens. *Pollut. Res.,* 20(1), 13-15.

Boudjema K., Kourdali S., Bounakous N., Meknachi A. and Badis A. (2014). Catalase Activity in Brown Mussels (Perna perna) Under Acute Cadmium, Lead, and Copper Exposure and Depuration Tests. *J. Mar. Biol.*, 1-9. https://doi.org/10.1155/2014/830657

Brown R.J., Galloway T.S., Lowe D., Browne M.A., Dissanayake A., Jones M.B. and Depledge M.H. (2004). Differential Sensitivity of Three Marine Invertebrates to Copper Assessed Using Multiple Biomarkers. *Aquat. Toxicol.,* 66(3), 267-278. https://doi.org/10. 1016/j.aquatox.2003.10.001

Brusca R.C. and Brusca G.J. (2003). Invertebrates (2nd ed.). Sinauer Associates, 702.

Chapman A.D. (2009). Numbers of Living Species in Australia and the World *(2nd ed.). Canberra:*

Australian Biological Resources Study.

Cheng T.C. and Rodrick G.E. (1975). Lysosomal and other enzymes in the hemolymph of Crassostrea virginica and Mercenaria mercenaria. Comp. Biochem. Physiol., 52B, 443-447.

Cheng T.C., Rodrick G.E., Moran S. and Sodeman W. A. (1980). Notes on Theba pisana and quantitative determinations of lysosomal enzymes and transaminases from the head-foot of this gastropod. *J. Invert. Pathol.*, 36, 1-5. https://doi.org/10.1016/0022-2011(80)9 0126-3

Chvapil M., Ryan J.N and Zukoski C.F. (1972). The effect of zinc and other metals on the stability of lysosomes. *Proc. Soc. Exp. Biol. Med.*, 140, 642-646. DOI:10.3181/00379727-140-36521

Ciro B., Giovanni C., Gaetano I. and Francesco C. (1975). Purification and some properties of an alkaline phosphatase from beef brain. *Comp. Biochem. Physiol. Part B,* 50(1), 203-206. https://doi.org/10.1016/0305-0491(75)90323-5

Coetzee L., Du Preez H.H. and Van Vuren J.H.J. (2002). Metal concentrations in Clarias gariepinus and Labeo umbratus from the Olifants and Klein Olifants River, Mpumalanga, South Africa: Zinc, copper, manganese, lead, chromium, nickel, aluminium and iron. *Water SA*, 28(4), 433-448. DOI:10.4314/wsa.v28i4.4917

Coley R.M. and Jensen R.S. (1973). Pesticides and lipid metabolism in the rats. *Lipid*, 8, 43.

De Duve C., Pressman B.C., Gianetto R., Wattaux R. and Applemans F. (1955). Tissue fractionation studies. 6. Intracellular distribution pattern of enzymes in rat-liver tissue. *Biochem. J.,* 60, 604-617.

Demir T.A., Akar T. and Akyuz F. (2005). Nickel and cadmium concentration in plasma and Na/K ATPase activities in erythrocytes membranes of people exposed to cement dust emissions. *Environ. Monit. Assess.,* 104, 437-444.

Deshmukh M. and Lomte V.S. (1998). Effect of heavy metal ($CuSO_4$) on protein activity of freshwater bivalve, Parreysia corrugata. *Environ. Ecol.* 16(3), 704-708.

Devi V.U. (1996). Changes in Oxygen Consumption and Biochemical Composition of the Marine Fouling Dreissinid Bivalve Mytilopsis sallei (Recluz) Exposed to Mercury. *Ecotoxicol. Environ. Saf.*, 33(1), 168-174. https://doi.org/10.1006/eesa.1996.0022

Dezwaan A. and Zandee D. I. (1972). The utilisation of Glycogen and accumulation of some intermediates during anaerobiosis in Mylilus edulis L. *Comp. Biochem. Physiol.,* B 43(1), 47-54. https://doi.org/10.1016/0305-0491(72)90200-3

Edwards J.H. and James H.C. (1987). Acid phosphatases of the mosquito Culex tarsalis coquillett. *Comp. Biochem. Physiol.,* 87(4), 773-782.

Elder J.F. and Collins J.J. (1991). Freshwater Molluscs as Indicators of Bioavailability and Toxicity of Metals in Surface-Water Systems. *Rev. Environ. Contam. Toxicol.,* 122, 37-79.

Fokina N.N., Ruokolainen T.R., Nemova N.N. and Bakhmet I.N. (2013). Changes of Blue Mussels Mytilus edulis L. Lipid Composition Under Cadmium and Copper Toxic Effect. *Biol. Trace Elem. Res.,* 154(2), 217-225.

Geret, F., Jouan, A., Turpin, V., Bebianno, M.J. and Cosson, R.P. (2002). Influence of Metal Exposure on Metallothionein Synthesis and Lipid Peroxidation in Two Bivalve Mollusks: The Oyster (Crassostrea gigas) and the Mussel (Mytilus edulis). *Aquat. Living Resour.,* 15(1): 61-66.

Gould E., Thompson R.J., Buckley L.J., Rusanowsky D. and Sennefelder G.R. (1988). Uptake and Effects of Copper and Cadmium in the Gonad of the Scallop Placopecten magellanicus: Concurrent Metal Exposure. *Mar. Biol.*, 97(2),217-223.

Gulbhile S.D. and Zambare S.P. (2013) Curative role of caffeine on mercury induced alterations of protein levels in the Lamellidens corrianus (Lea). *Int. J. of Devel. Res.,* 3(6), 1-4.

Gupta V. and Rao G. (1974). Histological studies on the chroidpleves of Goat embryos. *Acta Histochem.,* 49, 60-63.

Harrison F.L., Lam J.R. and Berger R. (1983). Sublethal

Responses of Mytilus edulis to Increased Dissolved Copper. *Sci. Total Environ.,* 28, 141-158. https://doi.org/10.1016/S0 048-9697(83)80014-X

Hollander, V.P. (1971). Acid phosphatase, In: P. D. Boyer (ed) The Enzymes, 3rd ed., Vol. 4, Acad. Press, New York, USA.

Ibrahim A. M., Higazi M. G. and Demian E. S. (1974). Histochemical localization of alkaline phosphatase activity in the alimentary tract of the snail Marisa cornuarieties (L.). *Bull. Zool. Soc., of Egyp.*, 26, 94-105.

Javed M. and Usmani N. (2015). Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish Channa punctatus inhabiting river polluted by Thermal Power Plant effluent. *Saudi J. Biol. Sci.,* 22, 237–242. http://dx.doi.org/10.1016/j.sjbs.2014.09.0 21.

Jha B. S. (1991). Alteration in the protein and lipid content of intestine, liver and gonads in the lead exposed freshwater fish Channa punctatus (Bloch). *J. Environ. Ecoplan.* 2(3), 281-284.

Jing G., Li Y., Xie L. and Zhang R. (2006). Metal Accumulation and Enzyme Activities in Gills and Digestive Gland of Pearl Oyster (Pinctada fucata) Exposed to Copper. *Comp. Biochem. Physiol. Part C: Toxicol.& Pharmacol.,* 144(2), 184-190. https://doi.org/10.101 6/j.cbpc.2006.08.005

Jing G., Li Y., Xie L. and Zhang R. (2007). Different Effects of Pb^{2+} and Cu^{2+} on Immune and Antioxidant Enzyme Activities in the Mantle of Pinctada fucata. *Environ. Toxicol. Pharmacol.,* 24(2), 122-128. https://doi.org/10.1016/j.etap.2007. 04.002

Johnson A. (1990). Lethal and sublethal effects of copper on some aspects of protein metabolism of the freshwater snail Pila globosa (Swainson). Ph.D. Thesis, Sri Krishnadevaraya University, Anantapur, India.

Katticaram C. M., Mohammed K. Y. and Joseph P. S. (1995). Copper induced alterations in total carbohydrate and protein levels in bivalve, Sunetta scripta (bivalvia). *Indian J. of Mar. Sci.,* 24(3), 171-174.

Kaviraj A. and Gupta A. (2014). Biomarkers of Type II Synthetic Pyrethroid Pesticides in Freshwater Fish. *Biomed. Res. Int.,* 1-7. http://dx.doi.org/10.1155/20 14/928063

Knox W. E. and Greenguard O. (1965). An introduction to enzyme physiology; Advan. Enzyme. Regul. (G Weber Ed.). Pergamon Press, New York, USA, 3, 247-248.

Koide M., Lee D.S., Goldberg E.D. (1982). Metal and transuranic records in mussel shells, byssal threads and tissues. *Estuar. Coast. Shelf. Sci.*, 15(6), 679-695. https ://doi.org/10. 10 16/0272-7714(82)90079-8

Kulkarni A.B. and Vyawahare P.M. (1987). Changes in Glycogen Level in Certain Tissues of Pest Land Slug Laevicaulis alte Exposed to Carbaryl and Copper Sulfate During Reproductive Period. *Environ. Ecol.,* 5(3), 601-603.

Kulkarni B.G. and Kulkarni R.G. (1987). Effect of mercury exposure in enzymes in the calm, Katelysla oplma (Gmelin). *Indian. J. Mar. Scl.*, 16, 265-266.

Kumar P., Singh V. K. and Singh D. K. (2011). Bait formulations of molluscicides and their effects on biochemical changes in the ovotestis of snail Lymnaea acuminata (Mollusca; Gastropoda: Lymnaeidae). *Rev. Inst. Med. Trop. Sao Paulo.*, 53(5), 271-275. DOI:10.1590/s0036-46652011000500006

Lacoue-Labarthe T., Le Bihan E., Borg D., Koueta N. and Bustamante P. (2010). Acid Phosphatase and Cathepsin Activity in Cuttlefish (Sepia officinalis) Eggs: The Effects of Ag, Cd, and Cu Exposure. *ICES J. Mar. Sci.* 67(7), 1517-1523. https://doi.org/10.1093/ic esjms/fsq044

Lehninger A.L. (1979). Biochemistry. Worth Publishers, New York, USA, 629.

Li X.B., Hou X.L., Mao Q., Zhao Y.L., Cheng Y.X. and Wang Q. (2009). Toxic Effects of Copper on Antioxidative and Metabolic Enzymes of the Marine Gastropod, Onchidium struma. *Arch. Environ. Contam. Toxicol.,* 56(4), 776-784. DOI:10.1007/s00244-009-92 9 0-2

Lomte V.S. and Sontakke Y.B. (1992). Effect of Mercuric Chloride on Protein content of Thiara lineata. *Environ. Ecol.,* 10(3), 734-735.

Mahajan A.Y. and Zambare S.P. (2001). Ascorbate effect on copper sulfate and mercuric chloride induced alterations of protein level in freshwater bivalve, Corbicla Striatella. *Asian J. microbial. Biotech. Envio. Sci.,* 3(1-2), 95-100.

Mahajan P. R. (2005). Effect of Caffeine (1, 3, 7-Trimethaylxanthine) on certain heavy metal induced physiological alteration in the fresh water Gastropod Bellamya (viviparous) bengalensis. Ph. D. Thesis, North Maharashtra University, Jalagon (M.S.) India.

Masola B., Chibi M., Naik Y.S., Kandare E. and Zaranyika M.F. (2003). Activities of Glutamate Dehydrogenase and Aspartate and Alanine Aminotransferases in Freshwater Snails Helisoma duryi and Lymnaea natalensis Exposed to Copper. *Biomarkers*, 8(1), 33-42. https://doi.org/10.1080/13547500210166757

Mazorra M. T., Rubio J. A. and Blasco J. (2002). Acid and alkaline phosphatase activities in the clam Scrobicularia plana: kinetic characteristics and effects of heavy metals. *Comp. Biochem. and Physiol.,* 131B, 241-249. https://doi.org/10.1016/S1096-4959(01)00502-4

Miller III E.R. (1988). Physiological, Exfoliative Cytological, Histological, and Biochemical Responses of Geukensia demissa (Mollusca) Exposed to Dissolved Copper at Various Salinities. Ph.D. Thesis, Univ. of Connecticut, CN:252.

Mule M.B. and Lomte V.S. (1993). Effects of Copper Sulfate on Glycogen Metabolism in Freshwater Snail Thiara tuberculata. *Comp. Physiol. Ecol.,* 18(3), 110-113.

Mule M.B. and Lomte V.S. (1993). Effect of Mercuric Chloride on the Body Lipids of the Gastropod, Thiara tuberculata. *Comp. Physiol. Ecol.*18(2), 65-68.

Mule M.B. and Lomte V.S. (1993). Effect of Molluscicide Copper Sulfate on Lipid Content of the Gastropod Thiara tuberculata. *Environ. Ecol*., 11(3), 570-573.

Mule M.B. and Lomte V.S. (1995). Copper Sulfate Induced Alterations of Protein in Freshwater Gastropod Thiara tuberculata. *J. Ecobiol.,* 7(3), 177-180.

Muniv Y.S., Supanekar S.P. and Khan A.M. (2020). Biochemical studies on fresh water bivalves exposed to the mercury chloride. *Asian J. Adv. Res.*, 4(2), 11-17.

Neuhoff H.G. (1983). Synergistic Physiological Effects of Low Copper and Various Oxygen Concentrations on Macoma balthica. *Mar. Biol.,* 77, 39-48.

Nugroho A.P. and Frank H. (2012). Effects of Copper Exposure on Calcium, Carbohydrate, and Protein Levels in the Freshwater Mussel Anodonta anatina. *Toxicol. Environ. Chem.,* 94(1), 99-108. https://doi.org/10.1080/02772248.2011.63 0910

Pardeshi A. and Zambare S.P. (2012). Effect of L-Ascorbic Acid on the Glycogen Contents during Mercury Intoxication in the Freshwater Bivalve, Parreysia cylindrica. *J. Adv. Lab. Res. Biol.,* 3(3), 190-194.

Patil H. Z, Mahale P. N. and Kulkarni A. B. (2011). Alterations in the biochemical content in the foot of a freshwater snail, *Indoplanorbis exustus* exposed to heavy metals. *J. Exp. Sci.,* 2(3), 12-14.

Pilo B., Asnani M. V. and Shah R. V. (1972). Studies on wound healing and repair in pigeon. III: Histochemical studies on acid and alkaline phosphatase activity during the process. *J. Animal. Morpho. Physiol.,* 19, 205-212.

Ponder W.F., Lindberg D.R. (2008). Phylogeny and Evolution of the Mollusca. Berkeley, CA: University of California Press, 481.

Pottinger T. G., Carrick T. R., Yeomans W. E. (2002). The three-spined stickleback as an environmental sentinel: effects of stressors on whole-body physiological indices. *J. Fish. Biol.*, 61, 207-229. DOI:10.1111/j.1095-8649.2002.tb01747.x

Protasowicki M., Dural M., Jaremek, J. (2008). Trace metals in the shells of blue mussels (Mytilus edulis) from the Poland coast of Baltic sea. *Environ. Monit. Assess.,* 141(1-3), 329-337. DOI:10.1007/s10661-007-9899-4

Ragab F.M.A., El Khayat H.M.M., Mostafa B.B. and Gawish F.A. (2003). Difference in the Susceptibility to Certain Molluscicides and Schistosoma mansoni Infection of Three Forms Egyptian Biomphalaria glabrata. *J. Egypt. Soc. Parasitol.,* 33(3), 743-760.

Rajalekshmi P. and Mohandas A. (1993). Effect of Heavy Metals on Tissue Glycogen Levels in the Freshwater Mussel, Lamellidens corrianus (Lea). *Sci. Total Environ. Suppl.,* 617-630. https://doi.org/10.1016/S0048-9697(05)80064-6

Rajalakshmi S. and Mohandas A. (2005). Copper-Induced Changes in Tissue Enzyme Activity in a Freshwater Mussel. *Ecotoxicol. Environ. Saf.,* 62(1), 140-143. https://doi.org/10.1016/j.ecoenv.2005.01.003

Rajkumar J.S.I. and Milton M.C.J. (2011). Biochemical Changes Induced by Cadmium, Copper, Lead and Zinc Exposure to Perna viridis Under Long term Toxicity Test. *Int. J. Pharma. Bio. Sci.,* 2(3), 50-59.

Ramalingam K. and Indra D. (2002). Copper Sulfate ($CuSO_4$) Toxicity on Tissue Phosphatases Activity and Carbohydrates Turnover in Achatina fulica. *J. Environ. Biol.*, 23(2), 181-188.

Rao P.M. and Jayashree N. (1990). Copper sulfate and Zinc sulfate induce biochemical changes in the fresh water snail Bellamya dissimilis (Muller). Abstracts *11th Ann. Sess. Acad. Environ. Bio.*, Aurangabad, India.

Ravinder R.T. (1991). Effect of Mercuric Chloride on Protein Metabolism of a Freshwater Mussel, Parreysia rugosa (Gmelin). *J. Ecotoxicol. Environ. Monit.,* 1(3), 230-233.

Rawi S.M., Al-Hazmi M. and Al-Nassr F.S. (2011). Comparative Study of the Molluscicidal Activity of Some Plant Extracts on the Snail Vector of Schistosoma mansoni, Biomphalaria alexandrina. *Int. J. Zool. Res.,* 7(2), 169-189. DOI:10.3923/ijzr.2011.169.189

Reddy T.R., Kumar N.V. and Chari N. (1986). Effect of Mercuric Chloride on Carbohydrate Metabolism of Mantle, Foot and Gill of a Fresh Water Mussel, Parreysia rugosa (Gmelin). *J. Environ. Biol.,* 7(4), 225-229.

Regoli F. and Principato G. (1995). Glutathione, Glutathione-Dependent and Antioxidant Enzymes in Mussel, Mytilus galloprovincialis, Exposed to Metals Under Field and Laboratory Conditions: Implications for the Use of Biochemical Biomarkers. *Aquat. Toxicol.,* 31(2), 143-164. https://doi.org/10.1016/0166-445X(9 4)00064-W

Rosenberg G. (2014). A new critical estimate of named species-level diversity of the recent mollusca. *Am. Malacol. Bull.,* 32 (2), 308–322.

Sanders B.M., Martin L.S., Howe S.R., Nelson W.G., Hegre E.S. and Phelps D.K. Tissue-Specific Differences in Accumulation of Stress Proteins in Mytilus edulis Exposed to a Range of Copper Concentrations (1994). *Toxicol. Appl. Pharmacol.,* 125(2), 206-213. https://doi.org/10.1006/taap.1994.1066

Sastry K.V and Gupta P.K. (1978). In vitro inhibition of digestive enzymes by heavy metals and their reversal by chelating agent: Part I. Mercuric chloride intoxication. *Bull. Environ. Contam. Toxicol.*, 20(6), 729-35. DOI:10.1007/BF01683593.

Sathyanathan B., Nair S.M., Chacko J. and Nambisan P.N.K. (1988). Sublethal Effects of Copper and Mercury on Some Biochemical Constituents of the Estuarine Clam Villorita cyprinoides var. cochinensis (Hanley). *Bull. Environ. Contam. Toxicol.,* 40(4), 510-516. https://doi.org/10.1007/BF01688374

Satyaparameshwar K., Reddy T.R. and Kumar N.V. (2006). Study of Carbohydrate Metabolism in Selected Tissues of Freshwater Mussel, Lamellidens marginalis Under Copper Sulfate Toxicity. *J. Environ. Biol.*, 27(1), 39-41.

Semedo M., Armanda Reis-Henriques M., Rey-Salgueiro L., Oliveira M., Cristina Delerue Matos, C., Morais, S. Ferreira, M.(2012).Metal accumulation and oxidative stress biomarkers in octopus (Otopus vulgaris) from Northwest Atlantic. *Sci. Total Environ.,* 433, 230-237. https://doi.org/10.1016/j.scitotenv.2012.06.058

Sharma S.R.K., Asokan P.K. and Sahoo A.K. (2006). Effect of Sub-lethal Level of Copper and Mercury

on Digestive Gland Cells and Lysosomal Enzyme Activity in the Green Mussel (Perna viridis L.). *J. Mar. Biol. Assoc. India.,* 48(2), 245-248.

Sharma S.R.K. and Thomas S. (2007). Hemolymph Acid Phosphatase Activity in the Green Mussel (Perna viridis Linnaeus,1758) Exposed to Copper and Mercury. *J. Mar. Biol. Assoc. India*, 49(2), 226-229.

Sonawane S.M. (2015). Effect of molluscicide copper sulfate on the lipid content of the bivalve, Lamellidens marginalis *Biosci. Biotech. Res. Comm.*, 8(1), 74-77.

Sonawane S.M. (2018). Effect of Heavy Metals $Hgcl_2$ and $Cdcl_2$ on Glycogen Activity of Bivalve Lamellidens Marginalis. *IOSR J. Pharm.,* 8(8), 28-35.

Sumner A. T. (1969). The distribution of some hydrolytic enzymes in the cells of the digestive gland of certain lamellibranchs and gastropods. *J. Zool. London,* 158, 277-291.

Suresh K. and Mohandas A. (1990a). Hemolymph acid phosphatase activity pattern in copper-stressed bivalves. *J. Invert. Pathol*., 55(1), 118-125. https://doi. org/10.1016/0022-2011(90)90041-4

Suresh P.G., Reju M.K. and Mohandas A. (1993). Hemolymph Phosphatase Activity Levels in Two Fresh-Water Gastropods Exposed to Copper. *Sci. Total Environ.* 134(2), 1265-1277. https://doi.org/10.1016/S0048-9697(05)80132-9.

Suryawanshi G. D., Kurhe, A. R. and Rodriguez, M. A. (2014). Mercury Exposure Produce Changes in Protein Content in Different Body Parts of Oyster Crassostrea Cattuckensis (Newton and Smith). *J. Environ. Sci. Comp. Sci. Eng. Tech.,* 1, 65-71.

Suryawanshi G. D. and Deshpande P. A. (2016) Changes in protein content in different body parts of bivalve Lamellidens marginalis due to heavy metals. *Trends Fish. Res.,* 5(3), 25-29.

Swami K.S., Rao J.K.S., Reddy S.K., Murthy L.C., Chetty C.S. and Indira S.K. (1983). The Possible metabolic diversion adapted by the fresh water mussels to control the toxic metabolic effects of selected pesticides. *Indian J. Comp. Anim. Physiol.*, 1, 95-103.

Tallendini L., Cassini A., Favero N. and Albergoni V. (1986). Regulation and subcellular distribution of copper in the freshwater molluscs Anodonta cygnea (L) and Unio elongatulus (Pf). *Comp. Biochem. Physiol.,* 84C(1), 43-49.

Ulmer D. D. (1970). Effects of metals on protein structure: In: Maniloff J., Coleman J. R., Miller M. W. (ed.) Effects of metals on cells, subcellular elements and macromolecules. Charles C. Thomas Publishers, Springfield, 11-35.

Ursinyova M., Hladikava V. (2000). Cadmium in the environment of central Europe. Trace Metals in the Environment 4, 87-107. https://doi.org/10.1016/S0927-5215 (00)80006-5

Verity M.A. and Reith A. (1967). Effect of mercurial compounds on structure-linked latency of lysosomal hydrolases. *J. Biochem.*105(2), 685-690. DOI:10.1042/bj1050685

Viarengo A., Pertica M., Mancinelli G., Capelli R. and Orunesu M. (1980). Effects of Copper on the Uptake of Amino Acids, on Protein Synthesis and on ATP Content in Different Tissues of Mytilus galloprovincialis Lam. *Mar. Environ. Res.,* 4(2), 145-152. https://doi.org/10.1016/0141-1136(80)90052-5

Wang W., Mai K., Zhang W., Ai Q., Yao C., Li H. and Liufu Z. (2009). Effects of Dietary Copper on Survival, Growth and Immune Response of Juvenile Abalone, Haliotis discus hannai Ino. *Aquac.*, 297(1-4), 122-127. https://doi.org/10.1016/j.aquaculture.2009. 09.006

Waykar B. and Lomte V. S. (2001). Total protein alteration in different tissues of fresh water bivalve, Parreysia cylindrica after cypermethrin exposure. *Ecol. Env. Cons*., 7(4), 465-469.

Weis J.S. and Weis P. (1977a). Effect of heavy metals on embryonic development of the Killifish, Fundulus heteroclitus. *J. Fish Biol.,* 11, 49-54.