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*Research Paper*

# Metabolic and antioxidant enzyme activity in hepatopancreas of the adult male freshwater prawn *Macrobrachium malcolmsonii* (H. Milne Edwards) exposed to paravanar river pollutants

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## Abstract

**The hepatopancreas of freshwater prawn *Macrobrachium malcolmsonii* were exposed to paravanar river pollutants, activity of Lactate dehydrogenase (LDH), Succinic dehydrogenase (SDH), Glutamate dehydrogenase (GDH), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione S-transferase (GST) were studied. Freshwater ecosystem are being polluted by a multitude of pollutants from various sources like, industrial effluents, municipal wastes, garbage's, sewage pollutants and agricultural wastes etc. these pollutants contain heavy metals, persistent chemicals and pesticides are carried through food chain, their concentration increasing at each link present a threat to man when we consume the hydrobioants from polluted water. Due to such mixing of pollutants, global fisheries is facing constant decline in prawn stocks. The decreased and increased level of metabolic and antioxidant enzyme were observed, this may probably be attributed, due to the river receiving of effluent sources like untreated industrial, domestic and municipal wastes from the old and new town of Cuddalore District, Tamilnadu. The results are discussed in details.**

**Keywords:** Paravanar river, *M. malcolmsonii*, Hepatopancreas and Enzyme activity.

## Introduction

Aquatic animals inhabiting polluted water ways tend to accumulate toxic chemicals in high concentrations even when the ambient environmental concentrations are low. This is a potentially hazardous situation for the entire food chain. Once a toxic chemical enters into an organism, several biochemical and physiological mechanisms become operational in an attempt to challenge the toxic stress caused by the pollutants. Biological mechanisms that can be studied in organisms include those pertaining to the activity of enzymes involved in oxidative metabolism and hydrolysis. Since enzymes are biological catalysts that enable the most essential metabolic functions to be performed in living cells, disturbances of enzyme functions are most harmful. The enzyme activity in aquatic animals may serve as early indicators of toxicity of pesticides, heavy metals and other pollutants [33, 19, 35]. *Macrobrachium malcolmsonii* (Milne Edwards) is the most common freshwater prawn in India. This species is currently much demand in freshwater aquaculture practices. These prawns are widely distributed in tropical, subtropical and temperature zones and are gaining more and more importance as cultivable species. Over the last two

decades, exploration of modern technology and associated development of chemical industries have resulted in the production and release of vast quantities of manmade chemicals into the environment in liquid, solid and gaseous form. Heavy metals in particular, constitute a serious health hazard to several aquatic biota and ultimately to man. Though some heavy metals which are essential as trace elements, they change to be found as toxicants when exceeding the critical level due to human abuses. The river Paravanar contains effluents from different sources affect the aquatic system by depleting and enhancing different physico-chemical parameters thereby affecting the inhabitants. Various organic and inorganic wastes in industrial and domestic effluents are responsible for pollution. Non-degradable heavy metals are regarded as hazardous to aquatic ecosystems of their environmental persistence and their tendency for bioaccumulation [12]. The crustacean hepatopancreas is assumed to be homologous to the mammalian liver and pancreas [16] and is responsible for major metabolic events, including enzyme secretion, absorption and storage of nutrients, molting of the prawn [9]. Studies on the production of antioxidants against oxidative damage can be conducted by pretreating the animals with antioxidants then subjecting them to oxidative stress induced by oxidants or toxic substances [30].

The impact of pollutants on aquatic organisms is highly dependent on the biochemical nature of the organisms. The interaction between the pollutants and the organisms can be understood properly is the various biochemical changes takes place inside the body of the organisms are known. Freshwater prawns are sensitive to contamination of water and pollutants may significantly damage certain physiological and biochemical process when they enter into the organs of these animals. Alteration in the biochemical values in prawns due to environmental pollution provides an indication to understand the mode of action and type of pollutants.Lactate dehydrogenase, Succinic dehydrogenase and Glutamate dehydrogenase enzymes are several metabolic functions with great physiological significance.

Glutamate dehydrogenase, a mitochondrial enzyme, catalysis the oxidative deamination of glutamate providing α-ketaglutarate to the Krebs cycle. This enzyme is having several metabolic functions with great physiological significance. GDH in extra-hepatic tissues could be utilized for channeling of ammonia released during proteolysis for its detoxification into urea in the liver. Hence, the activities of GDH are considered as sensitive indicators of stress [17]. Oxyradical are capable of inducing oxidative tissue damage, lipid peroxidation, nucleic acid damage, enzyme inactivation and protein degradation[6]. In order to prevent damage to cellular components, numerous enzymatic antioxidant defenses act to scavenge oxyradicals including superoxide dismutase, catalase and glutathione S- transferase. These biotransformation and antioxidant enzymes have been extensively studied in fishes [15, 1] while fewer studies have been done with invertebrates [22, 23, 24].

The activity of glutathione S-transferase (GST), which is involved in the detoxification of pesticides, was first studied to determine the response of *Macrobrachium malcolmsonii* to the toxic effects of endosulfan. Exposure to endosulfan is known to impair growth potential of this species by causing reductions in major biochemical constituents, such as total protein, carbohydrate, glycogen, free sugars, and lipid ultimately impairing general metabolism [29]. The investigation of enzymatic properties is an essential mission to study the physiological adaptations expressed by organism in response to different environmental conditions. The pollution of aquatic breeding environment, such as the shift of acidity, alkalinity, heavy metal ions [26] and organic solvents, affects the enzyme activity, growth and survival of the animal. To clarify whether the passage from the embryonic to adult life in freshwater prawn *Macrobrachium malcolmsonii* is associated with changes of detoxifying enzymes SOD, CAT, and GST particularly, the cytosolic fraction prepared from different stages of development such as egg, embryo, larvae and adult tissues of *Macrobrachium malcolmsonii* were studied by [1].

## Materials and Methods

### Description of study area

The Paravanar River originating from Virudhachalam Taluk in Cuddalore District, Tamilnadu, India. It is tributary of Gadilum River which originates from the foot of the hills of North eastern part of the Shervarayan hills and runs along for a distance of 250 kms, joins with adjoining Paravanar estuarine

otherwise called Uppanar estuary and finally discharging into the Bay of Bengal. In this river, Station-I is the unpolluted region at the village Alappakam. Station-II is the less polluted region at the village Poondiankuppam. This is less polluted when compared to Station-III, due to the absence of direct discharge of effluents. The Station-III is more polluted region at the village Sonaganchavadi, and it is received the untreated drainage of municipal and domestic sewage from the cuddalore old and new towns and the wastes from the coconut husk retting grounds are discharged regularly. Agricultural wastes also enter into this area through small drainage channels from the nearby agricultural lands. In addition, effluents from SIPCOT (State Industrial Promotion Corporation of Tamilnadu) industrial complex are discharged into this river which is major pollutant agents of this river. The distance between Stations-I to Station-II is 2 kms and Station-II to Station-III is 4 kms. The depth of the river is more than 5 meters (Figure 1).

Physico-chemical parameters of the water collected from Station-I, II and Station-III of the Paravanar river were studied. Among the various parameters, temperature was high during summer in both stations, at Station-III, the temperature was higher than at Station-I and II this may be due to the industrial effluents. Statistical analysis revealed that there are significant differences between the three stations. When compare two stations, the pH, dissolved oxygen, salinity, alkalinity, Biological Oxygen Demand, Chemical Oxygen Demand, total dissolved solids, calcium, nitrite, phosphorus and ammonia content were found to be higher at Station-II and III. Statistical analysis revealed that there are more significant differences between the three stations. The accumulation of heavy metals, mercury, cadmium, copper and lead in the water, sediment and tissues were analyzed. The heavy metal concentrations were increased in water, sediment and the tissues of prawn at Station-II and III than at Station-I.

The adult prawn, *Macrobrachium malcolmsonii* were collected from the three Stations of the Paravanar river. The prawn was dissected in the field itself to collect the hepatopancreas to find out the impact of Paravanar river pollutants on metabolic and antioxidant enzyme activity.

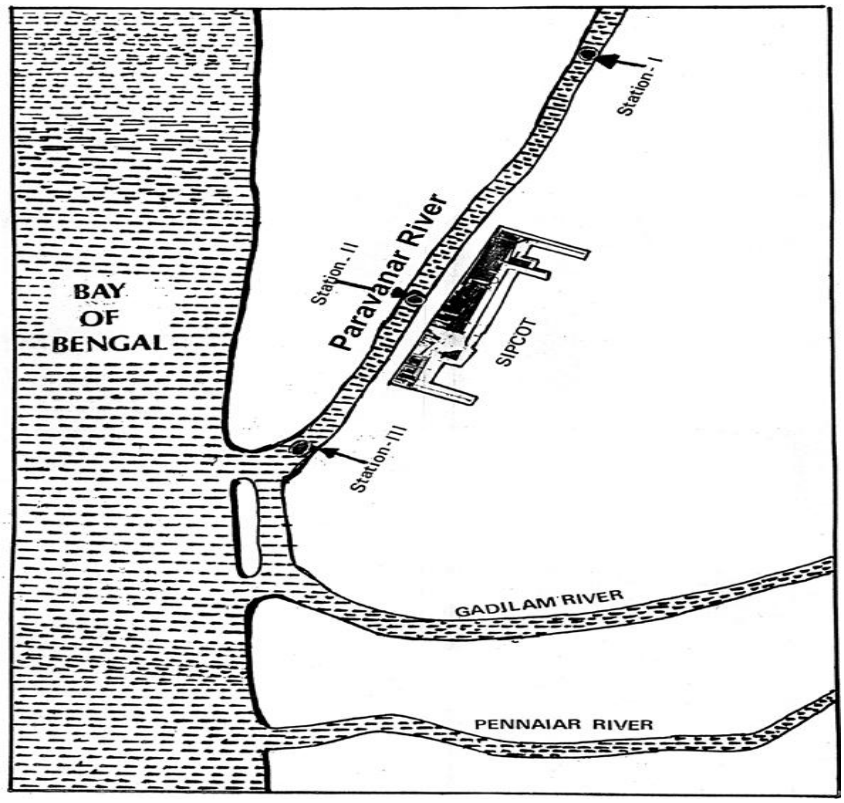


**Figure: 1 Map showing the study area of the Paravanar river**

**Enzyme assay**

**Lactate dehydrogenase (LDH)**

Lactate dehydrogenase (L- Lactate; NAD + Oxireductase). The enzyme LDH was assayed by the method of King [21]. The incupation mixture was contained 1.0 ml of buffered substrate solution and 0.1 ml of enzyme. The tubes wre kept in an incubator at 37°c for 15 minutes. Exactly after 15 minutes, 0.2 ml of $NAD^+$ was added to 'test' and mixed and left in the incubator for further 15 minutes. After adding $NAD^+$, 1.0 ml of the dinitrophenyl hydrazine reagent was added to each tube (test and blank) and 0.2 ml of $NAD^+$ to blank, mixed and left in the incubator for further 15 minutes and then added 7.0 ml of 0.4N sodium hydroxide. The color was read at 429 nm. A standard curve with sodium pyruvate (0.1-1.0M) solution was also obtained for calibration. The enzymes activities were expressed of µ moles of pyruvate liberated per mg protein per minute.

**Succinic dehydrogenase (SDH)**

The enzyme SDH was assayed by Bernath and Singer method [3]. To the main compartment of each of 5 Warberg vessels, 0.5 ml of phosphate buffer, enzyme (4 to 12 units) and sufficient water were added, so that the final volume of each vessel contained 3 ml. In case of mitochondria, the main compartment also received 0.03 to 0.06 ml of $CaCl_2$ and Tris buffer was substituted for phosphate. The side arm of each vessel received 0.3 ml of succinate, followed by the dye. The volume of dye varied in each vessel in order to permit extrapolation to infinite dye concentration. The amounts recommended were 0.2, 0.1, 0.07, 0.05 and 0.04 ml. Cyanide (0.3 ml) was added at least and each vessel was immediately placed on its manometer, and the stopcock was closed in order to prevent escape of HCN. After 7 minutes of temperature equilibration at 38°c, the contents at the side arms were tripped, and $O_2$ uptake was recorded in the interval from 2 to 7 minutes after tripping. Activity was calculated from double reciprocal plots of activity against dye concentration. One unit of enzyme is that amount which causes the uptake 1 µl of $O_2$ per minute for the above conditions. Specific activity was expressed as units per mg of protein.

**Glutamate dehydrogenase (GDH)**

L- Glutamate, $NAD^+$ oxidoreductase deamination. The enzyme activity was assayed by the method of Stretcker [32]. The gills, testis, vas deferens, hepatopancreas and androgenic gland of the prawn were homogenized in 10 volumes of cold acetone and centrifuged. After washing, the precipitate with cold acetone, the residue was dried in a stream of dry nitrogen gas at room temperature. The powder from tissues was extracted with 20 volume of 0.1 mM phosphate buffer (pH 7.4). The reaction mixture in a final volume of 3.0 ml contained (final concentrations) 90 nM phosphate buffer (pH 6.4) , 33 mM potassium glutamate, 0.33 mM $NAD^+$ and 0.1 ml of enzyme extract, the reaction was started by adding enzyme extract to the reaction mixture equilibrated at 30°C. The change in extinction per minute at 340 nm was recorded in Bausch and lamp Spectronic – 20 uv visible spectrophotometer. The values were expressed as µ moles NAD+ reduced / min / mg / protein.

**Superoxide dismutase (SOD) (EC 1.15.11)**

Superoxide dismutase was assayed by the kakkar method [20]. 0.5 ml of the hemolysate was diluted with 0.5 ml of water. To this, 2.5 ml of ethanol and 1.5 ml of chloroform were added. All reagents used, were kept at 4°C. The mixture was shaken for one minute at and 4°C and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of phenozine methosulphate, 0.3 of NBT and 0.5 ml enzyme preparation. The reaction was started by the addition of 0.2 ml of NADH. After incubation at 30° C for 90 seconds, the reaction mixture was stirred and shaken with 4.0 ml of n-butanal, the mixture was allowed to stand for 10 minutes, centrifuged and the color intensity of the chromogen in the butanal layer was measured at 560 nm. The system devoid of enzyme served as control. 1 unit of activity was taken as the enzyme reaction which gave 50 % inhibition of NBT reduction / mg hemoglobin.

**Catalase (CAT) (EC 1.11.1.6)**

Catalase was assayed calorimetrically by the Sinha method [31].
To 0.9 ml of phosphate buffer, 0.1 ml of hemolysate and 0.4 ml of $H_2O_2$ were added. After delete 90 seconds, 2.0 ml of dichromate acetic acid mixture was added. A control containing was also processed at test. The tubes were kept in boiling water bath for 10 minutes and the color developed was read at 620 nm. Standards in the range of 2-10 µ mol. were taken and processed as the test values were expressed as µ moles of $H_2O_2$ consumed per minute per mg hemoglobin.

**Glutathione S-trnaferase (GST)**

Activity of GST was measured in tissue homogenate by following the increase in Absorbance at 340 nm in Spectronic-20 (Bausch and Lomb) using 1-chloro-2, 4-dinitrobenzene as a substrate.

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

The reaction mixture contained 1.0 ml of phosphate buffer, 0.1 ml of CDNB, 1.0 ml of homogenate and 0.7 ml of distilled water. The reaction mixture was preincubated at 37$^o$C for 5 minutes and then the reaction was started by the addition of 0.1 ml of reduced glutathione. Reaction mixture without the enzyme was used as blanks.

$$\text{Activity} = \frac{OD \times 3 \times 100}{9.6 \times 5 \times protein\ in\ mg}$$

9.6 are the difference in the millimolor extinction coefficient between CDNB-GSH conjugate and CDNB. Values were expressed as µ moles of CDNB-GSH conjugate formed / min / mg protein for tissues.

The enzyme Lactate dehydrogenase (LDH) (L- Lactate ; NAD + Oxireductase) was assayed by the method of King [21]. The enzyme Succinic dehydrogenase (SDH) was assayed by Bernath and Singer (1962) method. Glutamate dehydrogenase (GDH) (L- Glutamate, $NAD^+$ oxidoreductase deamination) was assayed by the method of Stretcker (1965). Superoxide dismutase (SOD) was assayed by the method of Kakker [20]. Catalase (CAT) was assayed calorimetrically by the method of Sinha (1972). Activity of Glutathione S-transferase (GST) was measured in tissue homogenate by following the increase in absorbance at 340 nm in Spectronic-20 (Bausch and Lomb) using 1-chloro-2, 4-dinitrobenzene as a substrate.

## Results and Discussion

The Lactate dehydrogenase activity ranges in hepatopancreas of the prawn collected from Station-I was 0.540 µ moles/mg/protein. The prawn collected from Station-II and III, the LDH activity were found to be about 0.735 and 0.182 µ moles/mg/protein, respectively (Table 1 and Figure 2). The LDH activity was decreased at Station-III than the hepatopancreas collected from Station-I and Station-II. The level of LDH in the hepatopancreas of test prawns were found to be higher than the concentration in the same tissues of the prawn collected from Station-I. Further it has been observed that the differences noted in concentration of LDH in different groups of test prawns were statistically significant in comparison to respective hepatopancreas collected from unpolluted region (Table1 and Figure 2). This enzyme LDH disrupts the normal functioning of the cell protein and inhibits the actions of the enzymes, ultimately impairing various biochemical and physiological mechanisms (18, 36). In the present study, pollutants manifested stress responses in *M. malcolmsonii*, which were reflected in various biochemical alterations.
The Succinic dehydrogenase levels in hepatopancreas of the prawn collected from Station-I was 0.016 µ moles/mg/protein. The prawn collected from Station-II and III, the SDH activity were found to be about 0.008 and 0.002 µ moles/mg/protein, respectively (Table 1 and Figure 2). The SDH activity was decreased at Station-III than Station-I and Station-II. The Succinic dehydrogenase enzymes are several metabolic functions with great physiological significance. The level of SDH activity in hepatopancreas of test prawn of more polluted and less polluted were found to be lower than unpolluted region. In addition, the hepatopancreas of the test prawn collected from more polluted region at Station-III were found to be

lower than that in the same tissues of unpolluted region. The differences noted in the concentration of SDH in different groups of test prawn were statistically significant to respective prawn collected from Station-II (Table 1 and Figure 2).

The Glutamate dehydrogenase level in hepatopancreas of the prawn collected from Station-I was 0.724 µ moles/mg/protein. The prawn collected from Station-II and III, the GDH activity were found to be about 1.630 and 0.525 µ moles/mg/protein, respectively (Table 1 and Figure 2). The GDH activity was decreased at Station-III than the hepatopancreas of the prawn collected from Station-I and Station-II. The GDH enzyme activities in hepatopancreas of test prawn collected at all the Stations were found to be lowered when compare to prawn collected from Station-I whereas, the enzyme activity in the tissues of the test prawn collected from Station-III exhibited very less activity than Station-I and at Station-II. Glutamate dehydrogenase is also known to play crucial role in ammonia metabolism and is known to be affected by a variety of effectors [13]. After several metabolic functions with great physiological significance and known to be closely associated with the detoxification mechanisms of tissues. GDH in extra-hepatic tissues could be utilized for channeling of ammonia released during proteolysis for its detoxification into urea in the liver. Enhancement in Glutamate dehydrogenase activity in the tissues provided ketoglutarate and reduced neucleotides, which may fulfill the energy requirements during toxicity manifestations [8].

The antioxidant Superoxide dismutase activity ranges in hepatopancreas of the prawn collected from Station-I was 30.15 µ moles/mg/protein. The prawn collected from Station-II and III, the SOD activity of hepatopancreas were found to be about 30.22 and 10.23 µ moles/mg/protein, respectively (Table1 and Figure 2). The SOD activity was decreased at Station-III than the hepatopancreas of prawn collected from Station-I and Station-II. SOD activity has been reported to be present in many shrimp species and play an important role in immunity [28]. The antioxidant SOD converts this microbiocidal metabolite superoxide anion into oxygen and hydrogen peroxide that passes freely through membranes. SOD activity correlated closely with immune stimulation, disease, and healthy status of the prawn [11, 27, 34]. Thus, antioxidant enzymes could also be used as a biomarker for the detection of reactive oxygen species in freshwater prawn, *M. malcolmsonii.*

The Catalase activity ranges in hepatopancreas of the prawn collected from Station-I was 45.30 µ moles/mg/protein. The hepatopancreas of prawn collected from Station-II and III, the catalase level were found to be about 38.20 and 32.32 µ moles/mg/protein, respectively. The enhanced activity of CAT suggests that CAT is mainly involved in the removal of $H_2O_2$ that was generated by superoxide dismutase. Hence the elevated trend of CAT was similar to superoxide dismutase pattern and this finding can be corroborated with the observations of [25] who measured an enhanced SOD and CAT activity in *Limanda* after 80 days of exposure to PAH contaminated sediment. The CAT activity in hepatopancreas was decreased at Station-III than Station-I and Station-II (Table 1 and Figure 2)

**Table 1: LDH, SDH, GDH, SOD, CAT and GST activity in the hepatopancreas of the adult male prawn *Macrobrachium malcolmsonii* collected from Station-I, II and III**

| Tissue | Enzymes | Station-I Un polluted region | Station-II Less polluted region | Station –III More polluted region | $t_1$ | $t_2$ | $t_3$ |
|---|---|---|---|---|---|---|---|
| Hepatopancreas | LDH | 0.540 ± 0.125 | 0.735 ± 0.135 | 0.182 ± 0.075 | 1.83 | 4.25* | 6.20* |
| | SDH | 0.016 ± 0.004 | 0.008 ± 0.001 | 0.002 ± 0.001 | 3.36* | 5.88* | 7.34* |
| | GDH | 0.724 ± 0.058 | 0.630 ± 0.042 | 0.525 ± 0.050 | 2.27 | 4.50* | 2.78 |
| | SOD | 30.15 ± 0.80 | 30.22 ± 0.60 | 10.23 ± 0.52 | 0.12 | 36.16* | 43.60* |
| | CAT | 45.30 ± 0.75 | 38.20 ± 0.65 | 32.32 ± 0.55 | 12.39* | 24.17* | 11.96* |
| | GST | 1.160 ± 0.040 | 2.175 ± 0.62 | 3.125 ± 0.75 | 2.82 | 4.53* | 1.69 |

Values are mean ± SE of six different estimations

All values are expressed in µ moles of NADH oxidized/mg/protein/minutes

* indicates that significant at 0.05% level

$t_1$ ='t' value of station I and II, $t_2$ ='t' value of station I and III, $t_3$ ='t' value of station II and III

The Glutathione S-transferase level in hepatopancreas of the prawn collected from Station-I 1.160 µ moles/mg/protein. The prawn collected from Station-II and III, the GST activity were found to be about 2.175 and 3.125 µ moles/mg/protein, respectively (Table 1 and Figure 2). The GST activity was increased at Station-III than the prawn collected from Station-I and Station-II. The concentration of GST in the hepatopancreas of prawn collected from less and more polluted region were found to be higher than that in the same tissues of unpolluted region. The differences were most significant and pronounced in the prawn that had been found in more polluted region. The increased levels of GST activities in the prawn were statistically significant compared with respective unpolluted and less polluted regions (Table 1 and Figure 2).

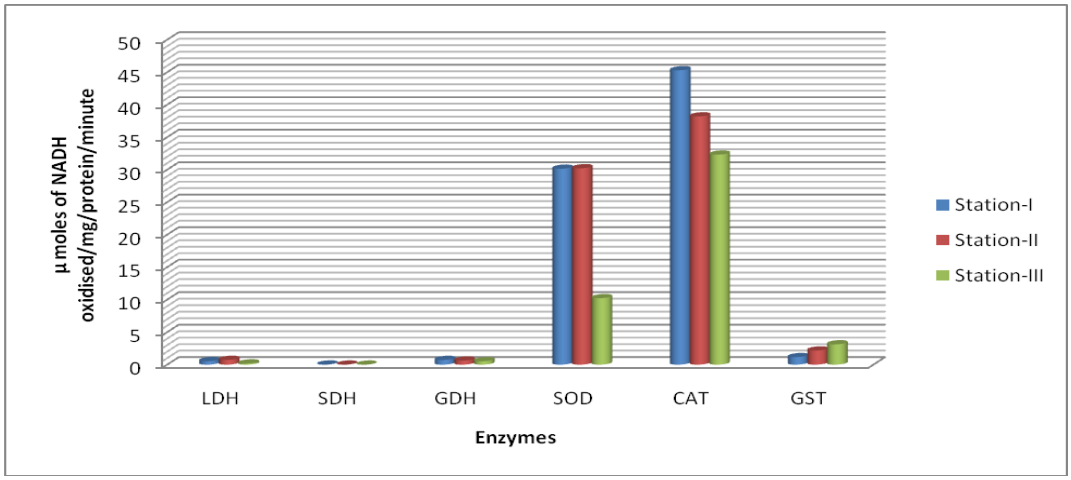


**Figure 2: Showing LDH, SDH, GDH, SOD, CAT, and GST activity in the hepatopancreas of adult male prawn *Macrobrachium malcolmsonii* collected from Station-I, II and III**

Glutathione S-transferase is a family of intracellular multifunctional dimeric protein, plays a major role in the intracellular transport of endogenous compounds, metabolizes various electropphilic xenobiotics, ligand transport and thus protects cells against toxic effects [15, 37]. In aquatic organisms, it is an important component of the detoxification system. Glutathione S-transferase activity has been detected in gills and hepatopancreas of *Macrobrachium malcolmsonii* exposed to pesticides [4, 5]. It has wide substrate specificities. Glutathione (GSH, L-γ-glutamyl-cysteinyl-glycine) is a substrate in the GSH S-transferase system and the availability of GSH can be a major factor in the metabolism of xenobiotics by this enzymic system. It is capable of chelating and detoxifying metals as soon as they enter the cell [7]. It also forms a substrate for GSH peroxidase, an enzyme capable of both removing hydrogen peroxide ($H_2O_2$) from the cells and repairing peroxidatively damaged membranes [10, 14].

## Conclusion

To conclude, exposure of *M malcolmsonii* to various pollutants severely affects various physiological mechanisms, and this is reflected in alterations in concentrations of various biochemical constituents. The elevated GST level suggests that there was an activation of a mechanism to detoxify xenobiotics. However, the toxic effects of xenobiotics were not fully neutralized, and there was an evidence of protein denaturation, disturbances of cellular metabolic activities, and impairment in neural transmission. Therefore, pollution of the aquatic environment by certain pesticides and heavy metals would adversely affect the biology of economically important animals, such as prawns. Since *M. malcolmsonii* significantly exhibits several biochemical stress responses to pollutants this species of prawn can be taken as a biological indicator of pesticide, heavy metal and other pollution in the freshwater environment.

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