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Toxicological perspectives of ammonium phosphate sulphate as environmental impact towards *Pseudetroplus maculatus* validated the element as a strong biological marker

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Abstract

Historically, the environmental resources, especially the river were recognized as limitless and primarily known to harbour plentiful fish species. The frequent and overuse of various pesticides and chemical elements including the fertiliser for agriculture needs may instigate several environmental damage. In this regard, here the author has investigated the possible role of the ammonium phosphate sulphate in *Pseudetroplus maculatus* in context with toxicological and environmental inferences. One-way ANOVA, paired test, cross tab-analysis and Correlation were used to unveil the possible link between the *Pseudetroplus maculatus* and the ammonium phosphate sulphate. All the experiments were conducted using the gill, gut, and muscle of acute and chronic strains of the *Pseudetroplus maculatus*. According to the major findings, the current study has primarily validated the level of various enzymatic activities including Alkaline phosphatase activity and ALT followed by the antioxidant perspectives including LPO and SOD towards the constant exposure of ammonium phosphate sulphate in *Pseudetroplus maculatus*. Furthermore, the biochemical profiling of the *Pseudetroplus maculatus* has also been unveiled in the current study. The results from the current study strongly suggest the selected strains as a validated environmental marker.

Keywords: Fish; ACP; ALP; Enzyme; Ammonium Phosphate Sulphate.

Introduction

The quality of water resources has to be maintained since it directly or indirectly affects human health and ecological balance (Scanlon, Jolly, Sophocleous, & Zhang, 2007). Both natural and man-made activities lead to the destruction and disruption of aquatic resources and its organisms henceforth its vital to conserve natural ecosystems. Climatic alterations, soil erosion, accumulation of pesticides and chemical fertilizers used in the agricultural sector, industrialization along with urbanization were some of the natural and artificial causes of water pollution and degradation of water resources respectively (Giri, 2021). It has been estimated that the chief source of aquatic pollution arises from an immense discharge of nutrients from agricultural sector. As per the previous studies conducted by Food and Agriculture Organization of the United Nations FAO, it has been estimated that nitrate resulting from agricultural sector as the major cause of chemical pollution in aquatic resources worldwide. The accumulation of pollutants in waterbodies adversely affect the quality of drinking water which consequently influence the health of living organisms also became responsible for the ecological imbalance (Madhav et al., 2020). The appearance, effluent capacity of soil geographical characteristic weather, waste materials eliminated from industries, and excrement leakage were some of the elements responsible for the effluence of nitrates from soil. Ammonium is oxidized into nitrites and nitrates in fertilized soil which then join into ground water and surface water resources, disseminated from soil. Over usage of synthetic fertilizers consisting of nitrogenous elements cause both ecological along with health issues to every living being (Davidson et al., 2012). Earlier studies clearly state that about 30% reduction of nitrogen from the soil as an impact of the application of chemical fertilizers, while the improper emission of waste materials from factories, and sewage spilling leads to the accumulation of excessive nitrates in groundwater. Quality of water in aquatic resources can be determined through a comprehensive examination of chemical hazards contained in it thereby essential steps have to be initiated for the protection of water bodies (Brack et al., 2017).

From the previous reports, it is clear that immediate actions must be taken in order to control the concentration of nitrates that reach into fresh water bodies and to save living organisms from the issues caused by eutrophication. The major portion of nitrate discharge into aquatic resources occur by anthropogenic actions. Volatilisation, mineralisation, nitrification and denitrification were some of the processes which responsible for nitrogen release into both soil and ground water (Kendall, Elliott, & Wankel, 2007). Hence, the origin of nitrates discharge can be assessed through the evaluation of microorganisms responsible for this. Water has been considered as the base of life of every ling organism which is essential for survival. The immediate growth and development of urbanisation and industrialisation sectors leads to the excess utilization of aquatic reservoirs caused in its depletion through the immense release of pollutants into nearby rivers consequently disrupting the ecological community along with the decrease in environmental carrying capacity. Gradually aquatic ecological communities of certain urban regions have been severely threatened with great reduction in the quality of water.

The diversity of aquatic ecological community can be estimated through the diversity of fish, zooplankton, phytoplankton, aquatic organisms, benthic macroinvertebrate etc. distributed in its bottom, middle, and surface of water resources (Majumder et al., 2015). The rate of release of annual chemical fertilizer, industrial wastewater, domestic sewage treatment, large-scale agriculture sewage indicates the origin of water pollution. The human population greatly depends on aquatic resources in order to complete the daily routine which consists of purposes like drinking water, other household activities. Along with they depend aquatic organisms like fish as a food item, which has occupied a major portion in most of the human diet (Berezina et al., 2024; Gautam, 2024; Heilpern et al., 2023). Thus, the chemical pollutants in the water adversely affect the quality of fish and consequently, it interrupts the normal rhythm of human life by causing numerous diseases.

The toxic materials contained in synthetic chemicals gradually affect aquatic ecosystem in a negative way and sometimes kill the fish together with other aquatic creatures (Thakur & Sharma, 2024). It also adversely affects the non-targeted organisms affecting the ecological imbalance. Agriculture an important sector which plays a great role in economy have to be seriously managed in order to prevent the consequences resulting through the large-scale application of synthetic chemicals, as aquatic populations like fish species have found a significant part in providing various essential nutrients required for the good state of human beings. Several varieties of fish species were consumed by humans as food but the enhanced use of toxic chemicals reduced its population size. Though many studies have been conducted in fish varieties regarding the impact of chemicals, not many more studies were organised focusing on the impact of ammonium phosphate sulphate chemical in *Pseudetroplus maculatus* fish species particularly. Thus, through the current study risk factors faced by *Pseudetroplus maculatus* in the use of chemical fertilizer can be determined. Thereby the fish population can be conserved along with the ecological community. In order to estimate the impacts caused by the use of chemical ammonium phosphate sulphate both biochemical and enzymatic assays were conducted, the study also concentrated on the aftereffects of these chemicals in aquatic resources and the organisms living in them.

Materials and Methods

The *Pseudetroplus maculatus* were collected from the Bharathapuzha River of various location and (10°37'29.5"N, 76°35'57.1"E, 10°38'47.9"N, 76°34'11.7"E, 10°36'49"N, 76°36'32.1"E) using cast net and gill net transported to the lab in the aerated plastic bags and proceeded for the treatment with potassium permanganate solution for 1 minute to remove subcutaneous adherents. Fishes were acclimated in a 50L cement tank filled with well water, 75% of the water is renewed daily. During acclimation of fishes were proceeded towards a photoperiod of 12 hrs of light and 12 hours of darkness.

Enzymatic activities towards the Ammonium Phosphate Sulphate

ALP (Alkaline phosphatase activity (ALP)

For the enzymatic estimation, the muscle, gut and gill tissues of the *Pseudetroplus maculatus* were dissected, weighed and homogenised using Homogenizer - Invitrogen™. The protocol recommended by (Chu et al., 2019) was used for the determination of ALP activity. The final reading was measured at 405 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific).

ACP (Acid phosphatase activity) and ALT (Alanine Aminotransferases (ALT)

To measure the Acid phosphatase activity and Alanine Aminotransferases activity in the *Pseudetroplus maculatus*, the muscle, gut, and gill tissues were dissected carefully. The dissected, weighed and homogenised tissue samples of *Pseudetroplus maculatus* were proceeded for the methods recommended by (KS & Mary, 2009). The tissues were then homogenized on ice using the Invitrogen™ Homogenizer to ensure complete cell disruption and release of intracellular contents. To balance the enzyme stability, the dissected and weighed samples were then treated with Tris buffer having a pH of 8. The formed mixture was then proceeded for centrifugation at speed of 10,000 rpm at 4°C for 10 minutes and it allowed the separation of various soluble elements from debris. After the incubation with specific substrate including p-nitrophenyl phosphate, sodium hydroxide (NaOH) was added to break further hydrolysis and consequent unnecessary reactions. The final reading was measured at 405 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific). The ALT (Alanine Aminotransferases (ALT) was determined according to the method suggested by (Bergmeyer, Bowes Jr, Hörder, & Moss, 1976). The final reading was measured at 340 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific). Triplicates were maintained for all the executed experiments to eliminate any chance of errors.

Biochemical Profiling

The biochemical composition of the *Pseudetroplus maculatus* was analysed using Carbohydrate Estimation, Protein, and Glucose estimation in the following manner.

Carbohydrate Estimation

Reagent Preparation

For the preparation of Anthrone reagent, to 1L of H_2So_4 2g of anthrone Was mixed and used as fresh. The glucose stock solution was prepared by adding 200µg of glucose in 1ML distilled water and sufficient quantity was prepared. The experiment were carried out in the following manner: The glucose stock solution was supplied in series of test tubes and diluted up to 1ml of distilled water. To each of the test tube 5ml of anthrone reagent was added and vortexed. The test tubes were proceeded for cooling and after that, they were covered and incubated at incubated at 90°C for 17 minutes. Then it kept at room temperature and allowed to cool. The absorbance was measured at 620 nm. All the experiments were executed with triplicates and control was maintained.

Glucose Estimation and Protein estimation

The methods suggested by (Zhang et al., 2023) was used for the estimation of glucose in the selected dissected tissues. The protein estimation was carried out using the Lowry´S Method (Waterborg, 2009).

Statistical analysis

All the statistical analysis such as ANOVA, Cross Tab Analysis, Paired Test and Correlation were performed using the Origin Pro 2021 and SPSS version 27.0.0.

Results and Discussion

Studies based on the enzymatic and biochemical profiling of fishes are vital for unveiling the adaptive responses of various enzymes in specific tissues including muscle, gut, and gills. Such studies can offer novel insights concerning how the various environmental impacts the function and allowing as health indicator. The current study has enclosed the various inferences in this regard and the present study has been primarily focused on the comparative analysis of various enzymatic activities (Figs. 1-4) including ALP (Alkaline phosphatase activity), ACP (Alkaline phosphatase activity), ALT followed by the antioxidant inferences such as LPO and SOD. Furthermore, the biochemical perspectives of the gill, gut and muscle of the collected specimen was unveiled through carbohydrate, protein and glucose analysis. Furthermore, the inferences concerning the initial and final length and weight of the study specimen according to the compound exposure have also been analysed in the current study. Among the various enzymes analysed in the current study, The Alanine aminotransferase (ALT) was previously known to possess a significant role in the deamination and synthesis of chemical elements (Dave & Kadeppagari, 2019; Sookoian & Pirola, 2015) such as amino acids during the various circumstances including stress, which need high demand of energy for the specific organism. The current study has verified the prominent influence of ACP and ALP activity in this regard with strong statistical significance ($F=65.869$; $P < 0.05$) as mentioned in the Table 1.

Table 1 Enzymatic activities towards the constant exposure

Enzymatic activities	df	Mean Square	F	Sig.	
ΔA/min	Between Groups	8	.205	79.465	.000

	Within Groups	36	.003		
	Total	44			
ALP Activity	Between Groups	8	1517030.578	65.869	.000
	Within Groups	35	23030.996		
	Total	8	.205	79.465	.000
ΔA/min	Between Groups	8	.013	4.322	.001
	Within Groups	36	.003		
	Total	44			
ACP Activity	Between Groups	8	30421.708	.814	.595
	Within Groups	36	37380.850		
	Total	44			
ΔA/min	Between Groups	8	.120	9.542	.000
	Within Groups	36	.013		
	Total	44			
ALT Activity	Between Groups	8	364252.017	9.535	.000
	Within Groups	36	38200.233		
	Total	44			

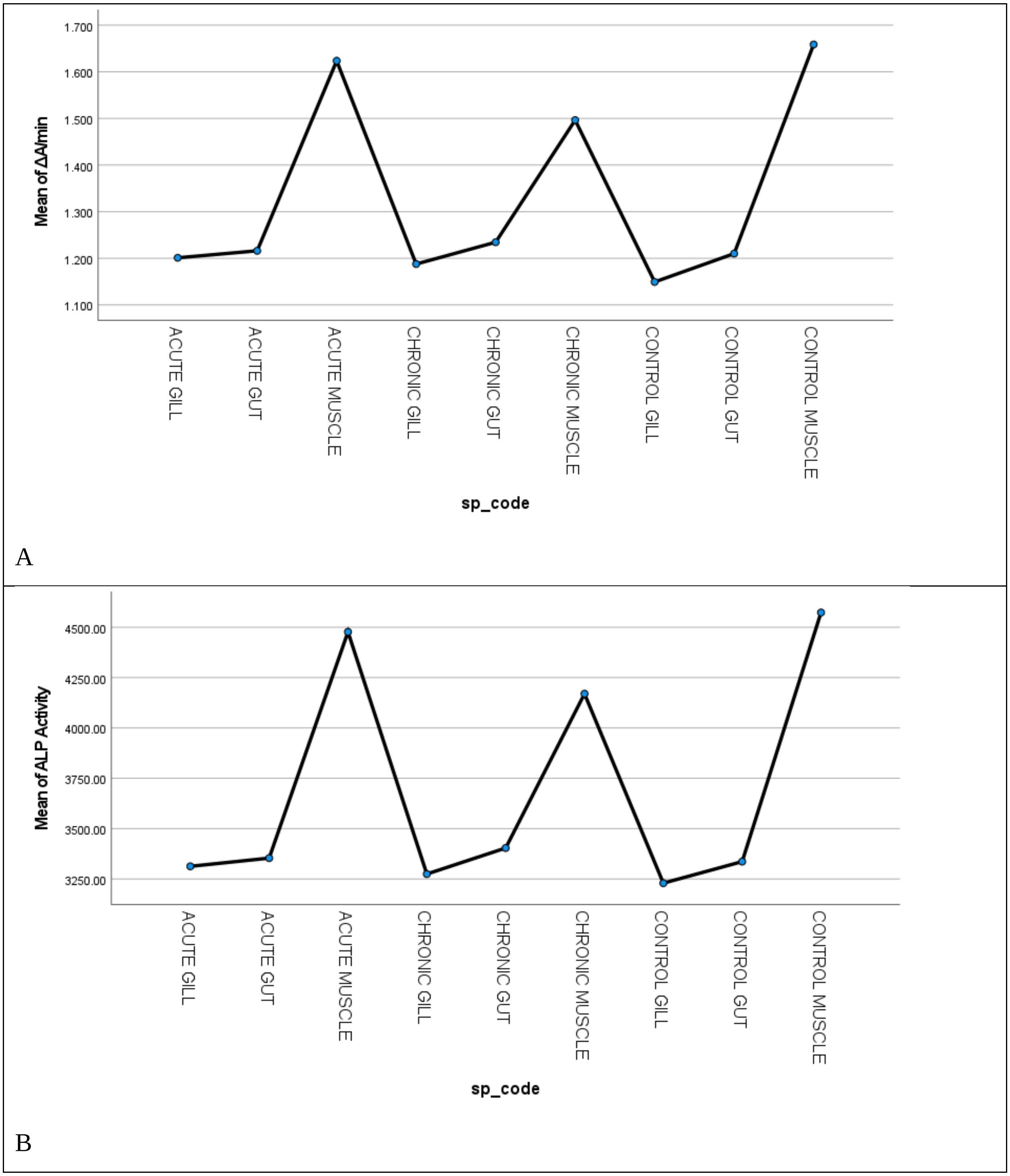
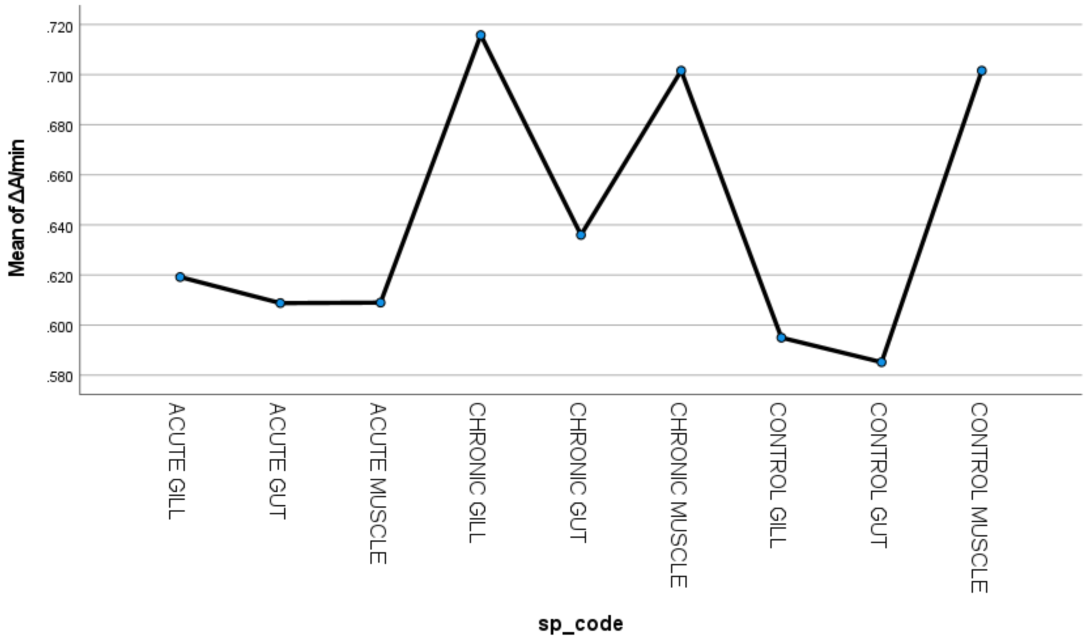
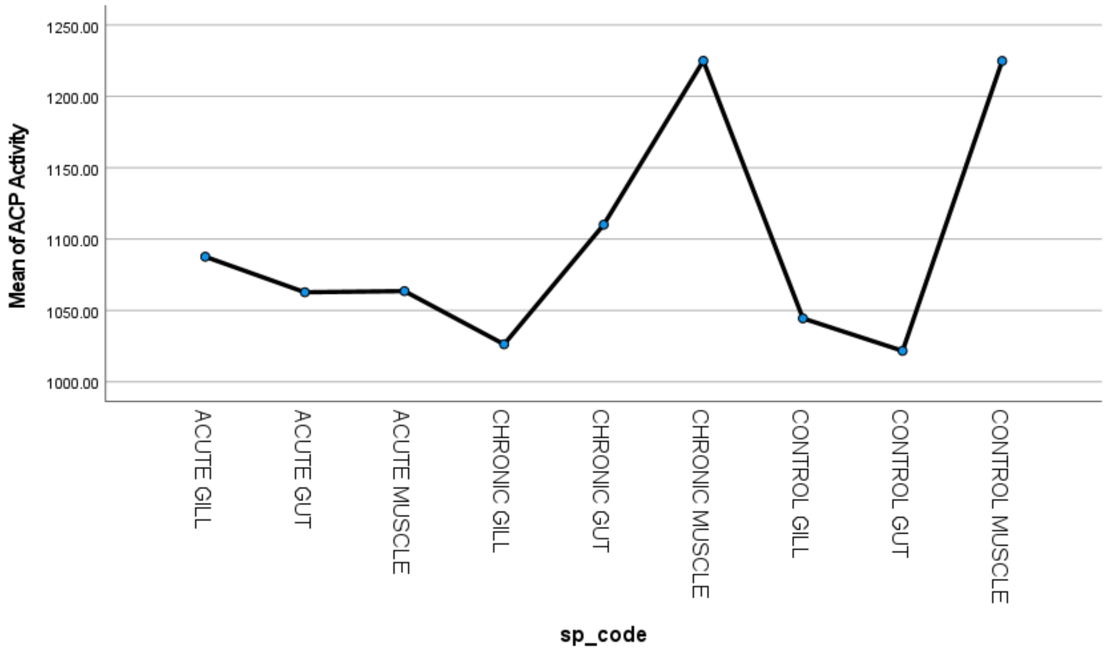


Fig. 1 ALP activity

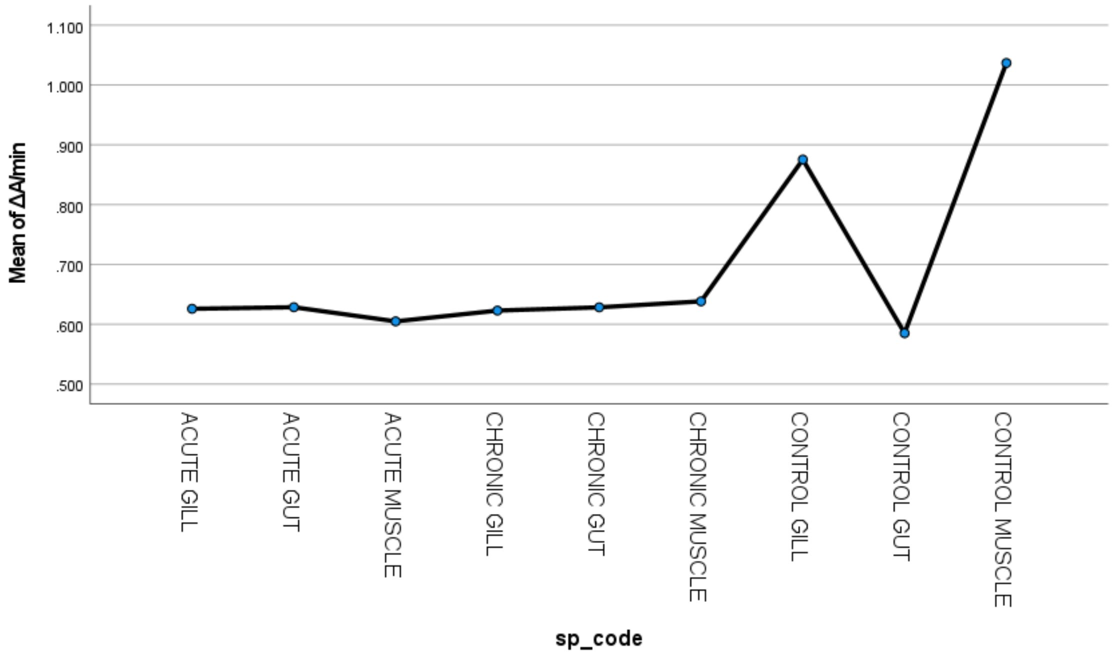


A

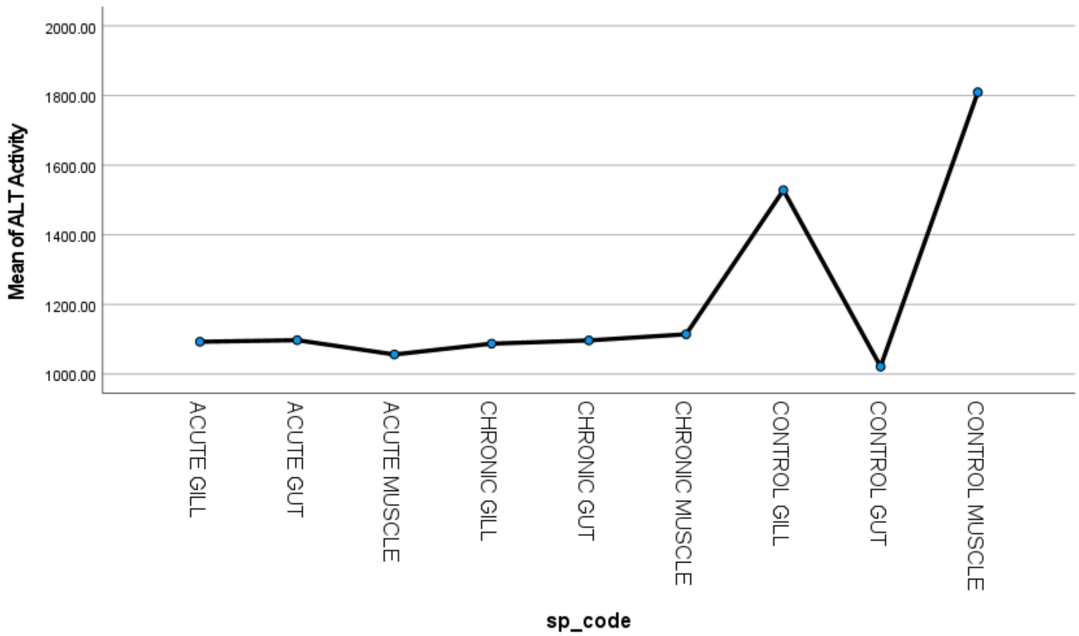


B

Fig. 2 ACP activity



A



B

Fig. 3 ALT Activity

The reported ALP activity in various analysed tissues of the current study may probably allied with the tissue damage which was instigated by the ammonium phosphate sulphate or any abnormal processes linked with the TCA cycle or Krebs' cycle. Any such damages or abnormal circumstances allied with the TCA cycle or Krebs' cycle may result in the subsequent leakage of the specific enzyme into the bloodstream (Dahl, Johansen, Nilsson, & Lefevre, 2021). The aforesaid perspective in response to the stress instigated by cypermethrin was verified by (Jee, Masroor, & Kang, 2005). Furthermore, as reported by the (Begum, 2004), the enhanced ALT activity in *Clarias batrachus* may directly represent the need for high energy during the stress conditions instigated by the toxic element carbofuran, which was previously known to possess a significant role in the agricultural sector. Similar to their findings, the current investigation has reported the enhanced ALT activity in the selected fish species. Similar kind of perspectives was also reported by (Al-Attar, 2005; Samanta, Pal, Mukherjee, Senapati, & Ghosh, 2015) and their conclusive remarks validated that the enhanced activity of ALP may probably be due to the cellular damage in the analysed tissues of the fishes, which need much more energy to combat the circumstances allied with the stress instigated by the particular toxic elements.

In order to unveil the impact of various chemicals towards aquatic organisms including the fishes, certain studies have used the applicability of enzymatic activity analysis. For instance, an investigation by (Shah & Parveen, 2022) assessed the strong impact of certain toxic elements, especially the commonly used pesticides that are uninterruptedly entering the water body and found that the gills can be used as biomarkers for environmental contamination. In addition to this the following studies are also reported similar findings; (Connon, Geist, & Werner, 2012; Fent, 2001; Livingstone, 1993; Valavanidis, Vlahogianni, Dassenakis, & Scoullos, 2006). The results from the current study have also possessed the influence of LPO, which may probably associated with the target toxic compound in the selected fish. Precisely, the present study has ruled out the possible link between the LPO levels (Table 2) and the stress probably instigated by the toxic element, as verified by the (Nakano et al., 2014).

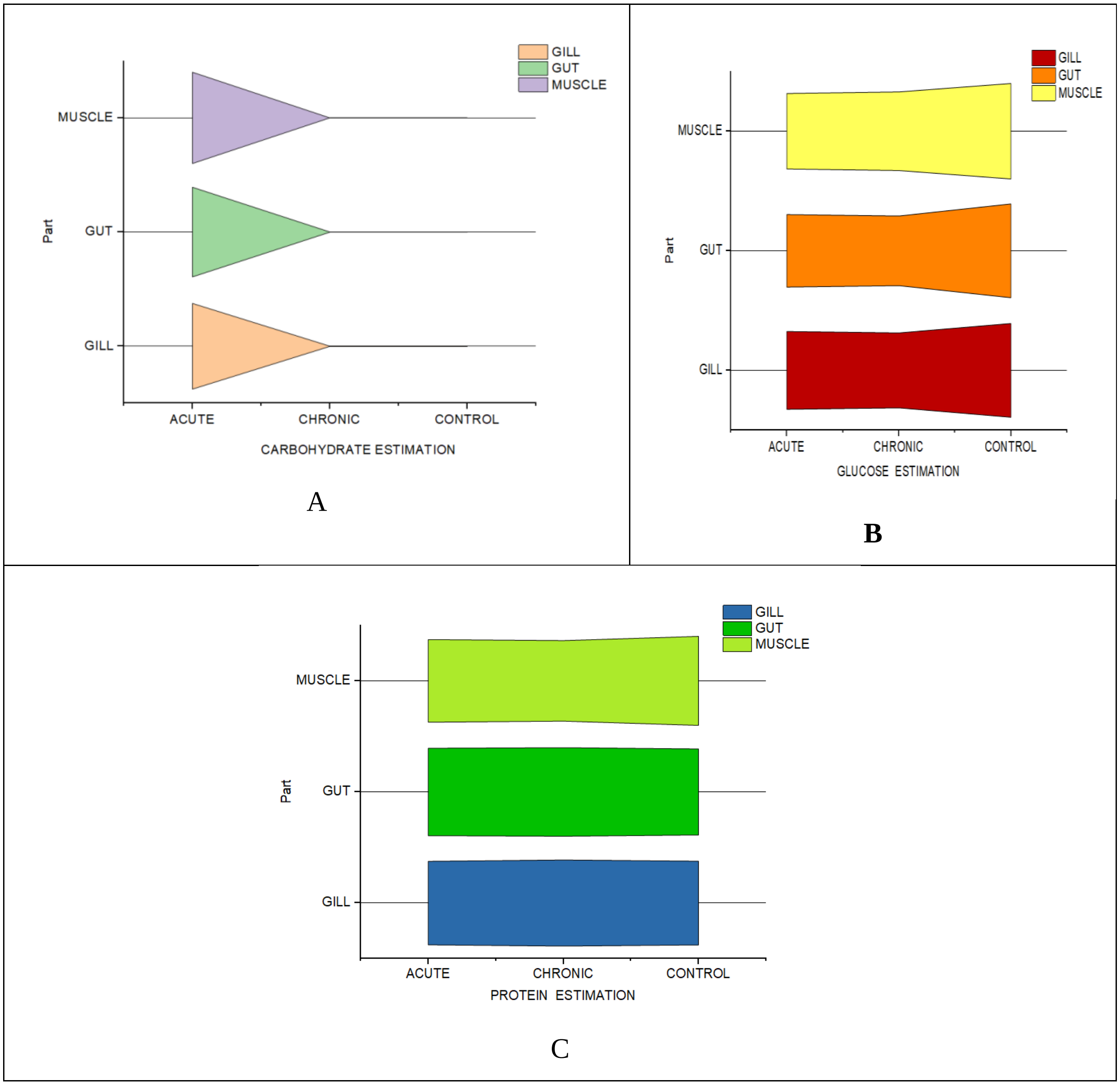


Fig. 4 Biochemical profiling. A. Carbohydrate B. Glucose C. Protein

Table 2 LPO and the exposure revealed through Cross tab

Directional Measures				
Sample	Value	Asymptotic Stand	Approximate T^b	Approximate

					ard Error[a]		Significance
ACUTE GILL	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
ACUTE GUT	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
ACUTE MUSCLE	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
CHRONIC GILL		Lambda	Symmetric	1.000	.000	4.472	.000

	Nominal by Nominal		Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
CHRONIC GUT	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
CHRONIC MUSCLE	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
CONTROL GILL	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000

CONTROL GUT	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
CONTROL MUSCLE	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			LPO Dependent	1.000	.000	4.472	.000
Total	Nominal by Nominal	Lambda	Symmetric	.544	.040	10.160	.000
			Conc Dependent	.972	.027	12.550	.000
			LPO Dependent	.186	.059	3.119	.002
a. Not assuming the null hypothesis.							
b. Using the asymptotic standard error assuming the null hypothesis.							
c. Based on chi-square approximation							

Table 3 SOD and the exposure revealed through Cross tab

Directional Measures							
Sample				Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
ACUTE GILL	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000
ACUTE GUT	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000
ACUTE MUSCLE	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000

CHRONIC GILL	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000
CHRONIC GUT	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000
qCHRONIC MUSCLE	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000
CONTROL GILL	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000

			SOD Dependent	1.000	.000	4.472	.000
CONTROL GUT	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000
		Goodman and Kruskal tau	Conc Dependent	1.000	.000		.453c
			SOD Dependent	1.000	.000		.453c
CONTROL MUSCLE	Nominal by Nominal	Lambda	Symmetric	1.000	.000	4.472	.000
			Conc Dependent	1.000	.000	4.472	.000
			SOD Dependent	1.000	.000	4.472	.000
Total	Nominal by Nominal	Lambda	Symmetric	.564	.037	12.214	.000
			Conc Dependent	1.000	.000	13.416	.000

			SOD Dependent	.190	.061	3.119	.002
a. Not assuming the null hypothesis.							
b. Using the asymptotic standard error assuming the null hypothesis.							
c. Based on chi-square approximation							

According to the findings reported by (Mila-Kierzenkowska et al., 2005), it was perceived that the highest SOD activity (Table 3) have directly related to the constant exposure towards the particular element. Similar to their results and the reported findings of the current study, (Oruc, Sevgiler, & Uner, 2004) reported that the prominent range of SOD activity in gill as reported in the current study can be used as biomarkers in various circumstances for pollution monitoring. In addition to the reported results, the current study also assessed the biochemical composition and found that prominent range of carbohydrates and sugar in the selected tissues of various chronic and acute strains of gills, gut and muscle tissues. Similar inferences were reported by (Parihar, Javeri, Hemnani, Dubey, & Prakash, 1997). The aforesaid inferences were previously verified by (Kandyliari et al., 2020). According to the conclusive remarks, here the author has also analysed the influence of toxic elements towards the length and weight of the studied fish and found that there may be the possibility that there exists a possible link between the length and weight of the fish according to the constant chemical exposure. In agreement with our findings, the following studies are also reported similar observations which justified our perspectives and findings; (Brian et al., 2005; Brian et al., 2007; Vosylienė & Kazlauskienė, 1999).

Table 4 Influence towards the length and weight revealed through Paired Samples Test (A) and Correlation (B)

A.

	Paired Differences				t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			

					Lower	Upper			
Pair 1 Chronic	I LENGTH (cm) - F WEIGHT (g)	2.2831	.6765	.0881	2.1068	2.4593	25.923	58	.000
Pair 2 Acute	I LENGTH (cm) - F WEIGHT (g)	2.2811	.012	.0041	2.1876	2.3821	23.418	48	.826

B. Paired Samples Correlations

		Correlation	Sig.
Pair 1 Chronic	I LENGTH (cm) & F WEIGHT (g)	.713	.000
Pair 2 Acute	I LENGTH (cm) & F WEIGHT (g)	1.837	.889

Conclusion

The rapid decline of the population diversity of various fishes over the globe reminds the scientific community to search for possible reasons. Many of the previous studies have primarily directed towards the impact of various pesticides towards aquatic organisms. In this regard, here the author has verified the possible role of ammonium phosphate sulphate in *Pseudetroplus maculatus* with special reference to the ALP (Alkaline phosphatase activity), ACP (Alkaline phosphatase activity), ALT, LPO and SOD using way ANOVA, paired test, Cross Tab analysis and correlation. Amid many enzymes determined, The Alanine aminotransferase (ALT) was previously reported to have a significant role in the deamination and synthesis of chemical elements. This study has strongly verified the prominent influence of determined enzymes with strong statistical significance ($F=65.869$; $P < 0.05$). The influence of LPO with possible relation with the target toxic compound in the selected fish has also been analysed in this study. Furthermore, this study also determined the biochemical composition and found that a prominent range of carbohydrates and sugar in the chronic and acute strains of gills, gut and muscle tissues. According to the conclusive remarks, the findings of the current study strongly suggest the selected strains as a validated environmental marker and the biochemical profiling with special reference to the Carbohydrate, Protein and Glucose depicts its significant role in recent research.

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DECLARATIONS

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not required

Availability of data and material

The data analysed during the present investigation are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors contribution

Conceptualization, Project administration, Data curation, Supervision, Validation, Writing - review & editing: Sasikala G

Investigation, Methodology, Resources, Software, Roles/Writing - original draft, Formal analysis, and Visualization: Veena V

Writing - review & editing: Selvaraju Raja

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