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Effect of arsenic on enzyme activity of a fresh water cat fish, *Mystus vittatus*

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Abstract
The present investigation has been designed to study the effect of sublethal concentrations (10% and 30%) of heavy metal, arsenic on the activity of alkaline and acid phosphatases enzyme in liver and muscles of *Mystus vittatus* after exposure to 30 days. A significant decreased has been observed in alkaline and acid phosphatases level in liver and muscles of arsenic exposed fish *Mystus vittatus*. The result of present study obviously indicates that low concentration of arsenic is toxic to fishes and alters the enzymatic activity in different tissues. With the result the entire metabolic activities of fish becomes disturbed. Thus the arsenic is known to affect the biological potential of aquatic animals.

Keywords: Arsenic, Acid and alkaline phosphatases, Aquatic environment, *Mystus vittatus*.

1. Introduction
The aquatic organisms are susceptible to pollution effect of heavy metal, pesticides as well as industrial effluent. But normally an organism tries to adapt itself to these change by changing their metabolic activities, but at higher concentration these pollutant can cause damage to biochemical system by affecting the organism either organ, cellular level even at molecular level in turn causing changes in biochemical composition (Gijare *et al.*2011) [5].
Arsenic is an important and ubiquitous environmental contaminant and present in different forms. The toxicity of arsenic in animals depends on species, sex, age, exposure dose, duration of exposure, organic or inorganic form, oxidation state etc. In natural water, arsenic is present in both inorganic and organic forms but the inorganic form has been found to be more toxic. The most common source of elevated arsenic concentrations in the environment is attributed to anthropogenic activities. Mining activities have contributed to the contamination of soil and water primarily. However, other anthropogenic activities using arsenic, such as agriculture, forestry, and industry, have also contaminated soil and water at a localized scale (Smith *et al.* 2003) [19].
Arsenic pollution is an environmental problem worldwide due to the large number of contaminated sites that have been identified, particularly in Asia (Mukherjee *et al.* 2004) [14]. Recently the wetlands of neighboring districts of Varanasi were found to be extensively contaminated with arsenic (Kumar and Banerjee, 2016) [11]. Contamination of the aquatic environment by arsenic has increased during recent years primarily due to anthropogenic sources and causes adverse effects in aquatic biota (Horacio *et al.* 2006; Shaw *et al.* 2007) [7, 18]. Thus arsenic is recognized one of the leading toxicants worldwide and is accumulated in different tissues, and the rate of its accumulation is in the order of muscles>liver>gill (Kermi-u Tariang *et al.* 2010) [9]. Arsenic affect the haematological paramerers (Verma and Prakash, 2019) [23] and biochemical parameters such as metabolism of carbohydrate (Verma and Prakash, 2019) [22], lipid and protein (Prakash and Verma, 2019 & 2020) [15, 16] in cat fish, *Mystus vittatus*.
Therefore, it is well recognized that the accumulation of arsenic to the living organism affects metabolic impairment and has been adversely affected to the tissues of aquatic organisms. Human are exposed to arsenic in the environment primarily through the ingestion of contaminated food and water (Cabonell-Barrachina, 2009) [3]. Numerous epidemiological studies have demonstrated that exposure to arsenic is associated with an increased incidence of lung, bladder, and skin cancer, as well as diabetes and cardiovascular disease (Erraguntla *et al.*, 2012) [4].

The biochemical function of animal gets disturbed on exposure to heavy metals. So a better understanding of this mechanism can help us to predict the harmful effect of various toxicants on environment. Hence, the present investigation is aimed to study the effect of sublethal concentrations of heavy metal, arsenic on the Phosphatase enzyme activity in liver and muscles of *Mystus vittatus*.

2. Research method

2.1 Preparation procedures

The healthy *Mystus vittatus* ranging from 7.0-8.0 cm in length and weighting 8.0-9.0 gm were collected from ponds in and around Balrampur and washed with 1% solution of $KMnO_4$ for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. Fish were acclimated to laboratory conditions for 15 days at room temperature. The LC_{50} for arsenic trioxide for 96 hours was calculated using probit method was 3.20 ppm. The LC_{50} values of arsenic for 24, 48, 72 and 96 hours were 4.71, 4.16, 3.68 and 3.25 ppm, respectively. Based on 96 LC_{50}, fish were exposed to sublethal concentrations (10% and 30%) for treated and control period of 10, 20 and 30 days. A control group was maintained in an identical environment. The fish were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants.

2.2 Analysis procedures and data analysis

The fishes were sacrificed from both experimental and control groups on 10^{th}, $20^{th}$and 30^{th} days of exposure periods and subjected to analysis for changes in enzymatic activity. The tissues were homogenized in 0.25 M sucrose solution and centrifuged at 1000x g for 10 minutes. The supernatants were filtered and the filtrates were used for enzyme analysis. Acid and alkaline phosphatases were estimated following the procedure outlined by Tennis Wood *et al.* (1976) [20], a modified method of Bessey *et al.* (1946) [2]. The data obtained from the proximate analysis of all samples were calculated by average value and standard deviation. The statistical significance of difference between control and experimental group was calculated by student's t- test then discussed descriptively.

3. Results and discussion

The level of alkaline and acid phosphatases content in the different tissues of control and arsenic treated fish, *Mystus vittatus*, are presented in Table 1 and 2. The both alkaline and acid phosphatases level were high in muscles in comparison to liver in all experimental groups of fish. The maximum decrease of alkaline phosphatase level was observed in the tissues of fish exposed to 30% sublethal concentration of arsenic reared for 30 days 78.29% in muscles and 61.64% in liver. The acid phosphatase was highest in liver in comparison to muscles was observed in all experimental group of fish. The maximum decrease of acid phosphatase level was observed in the tissues of fish exposed to 30% sublethal concentration of arsenic reared for 30 days was 72.67% in liver and 62.55% in muscles tissues. The decrease in alkaline and acid phosphatases levels in the tissues was statistically significant (p<0.05) in both sublethal concentrations at all the exposure periods.

Table 1: Effects of sublethal concentrations of arsenic on alkaline phosphatase level (µg Oleic acid mg/hr) in liver and muscles of *Mystus vittatus* at different period of exposure(N=6).

Tissues	Group	Exposure periods in days			F. value
		10	20	30	
Liver	Control	4.39±0.33	4.35±0.34	4.38±0.32	0.037^{NS}
	10%	3.51±0.41 (-20.04%)	3.11±0.35 (-28.50%)	2.41±0.31 (-44.97%)	213.22*
	30%	3.15±0.32 (-28.24%)	2.35±0.33 (-45.97%)	1.68±0.32 (-61.64%)	365.37*
Muscles	Control	2.29±0.38	2.33±0.35	2.35±0.37	0.024^{NS}
	10%	1.69±0.34 (-26.20%)	1.06±0.32 (-54.50%)	0.81±0.33 (-65.53%)	223.15*
	30%	1.17±0.39 (-48.90%)	0.82±0.29 (-64.80%)	0.51±0.33 (-78.29%)	463.16*

NS= Non Significant; *=Significant at 5% level of F test (p<0.05)

Table 2: Effects of sublethal concentrations of arsenic on acid phosphatase (µg Oleic acid mg/hr) content in liver and muscles of *Mystus vittatus* at different period of exposure(N=6).

Tissues	Group	Exposure periods in days			F. value
		10	20	30	
Liver	Control	3.32±0.26	3.36±0.43	3.33±0.37	0.771^{NS}
	10%	1.71±0.23 (-48.49%)	1.51±0.38 (-55.05%)	1.27±0.31 (-61.86%)	77.31*
	30%	1.27±0.32 (-61.74%)	1.01±0.31 (-69.94%)	0.91±0.33 (-72.67%)	75.22*
Muscles	Control	2.17±0.31	2.22±0.28	2.19±0.019	0.315^{NS}
	10%	1.89±0.32 (-12.90%)	1.44±0.33 (-35.13%)	1.19±0.35 (-45.66%)	0.51*
	30%	1.33±0.34 (-38.70%)	1.03± 0.32 (-53.60%)	0.82±0.44 (-62.55%)	0.47*

NS= Non Significant; *=Significant at 5% level of F test (p<0.05)

Enzyme acid and alkaline phosphatases are membrane-bound lysosomal enzymes and the sensitive biomarkers in toxicological study as they provide early information regarding potentially hazardous changes in aquatic organisms inhibited in contaminated water. These enzymes synthesized in the liver catalyze the hydrolysis of monophosphate esters and their activities usually find a relation to cellular damage. Both enzymes are concerned with the biosynthesis of fibrous proteins (Johnson and Mc Minn, 1958) [8] and mucopolysaccharides (Kroon, 1952) [10], or they may serve as regulators of intracellular phosphatase concentration (Gutman, 1959) [6].

Alkaline phosphatase is a multifunctional enzyme that acts as transphosphorylase at alkaline medium and also plays an important role in membrane transport activities and mineralization of the skeleton (Bernt *et al.*, 2001 and Lan *et al.*, 1995) [1, 12]. Acid phosphatase is a hydrolyzing enzyme and acts as a good indicator of stress conditions in biological

systems (Verma *et al.*, 1980) [24].

In the present study significant decrease in acid and alkaline phosphatases level in both tissues might be associated with the direct action of the arsenic on the enzyme system and impairment of lysosomal metabolism in liver and muscles (Sanisa *et al.*, 1982) [17]. Alkaline phosphatases are capable of inactivating phosphorylase enzyme necessary for glycogen breakdown (Martin *et al.*, 1973) [13]. The decrease in alkaline phosphatase activity in liver and muscles probably facilitates the increased activity of phosphorylase enzyme and subsequent breakdown of glycogen for energy release during arsenic stress condition. Verma and Prakash (2019) [22] reported that glycogen content in liver and muscles tissue of arsenic exposed *Mystus vittatus* was decreased significantly to fulfill the demand of excess energy needed to cope with stress under arsenic exposure. Hence, the reduction in alkaline phosphatase enzyme activity in liver and muscles facilitate the breakdown of glycogen resulting in tissue lactic acidosis (Venkateswaran and Ramasamy, 1987) [21]. This acidosis development might be responsible for the inhibition of alkaline phosphatase in liver and muscles of *Mystus vittatus* which in turn help to breakdown of glycogen to meet the energy demand in stress condition.

Conclusion

Arsenic enters in aquatic ecosystem from both natural and anthropogenic sources. The present study obviously indicates that low concentration of arsenic is toxic to fishes and alters the enzymatic activity in different tissues. With the result the entire metabolic activity of fish becomes disturbed. Since fishes are one of the important food sources of man, the manmade pollution will return to him through the food they eat. Hence the use of arsenic should be reduced to an extent that our future generations should be protected from the deleterious effects of arsenic. Understanding the toxicological effects of arsenic in aquatic environment is important to mitigate its deleterious effects on aquatic health, particularly in fish and the organisms including human who consumes fish. Therefore, the information obtained may be useful for management and monitoring of heavy metals contamination in aquatic environment.

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