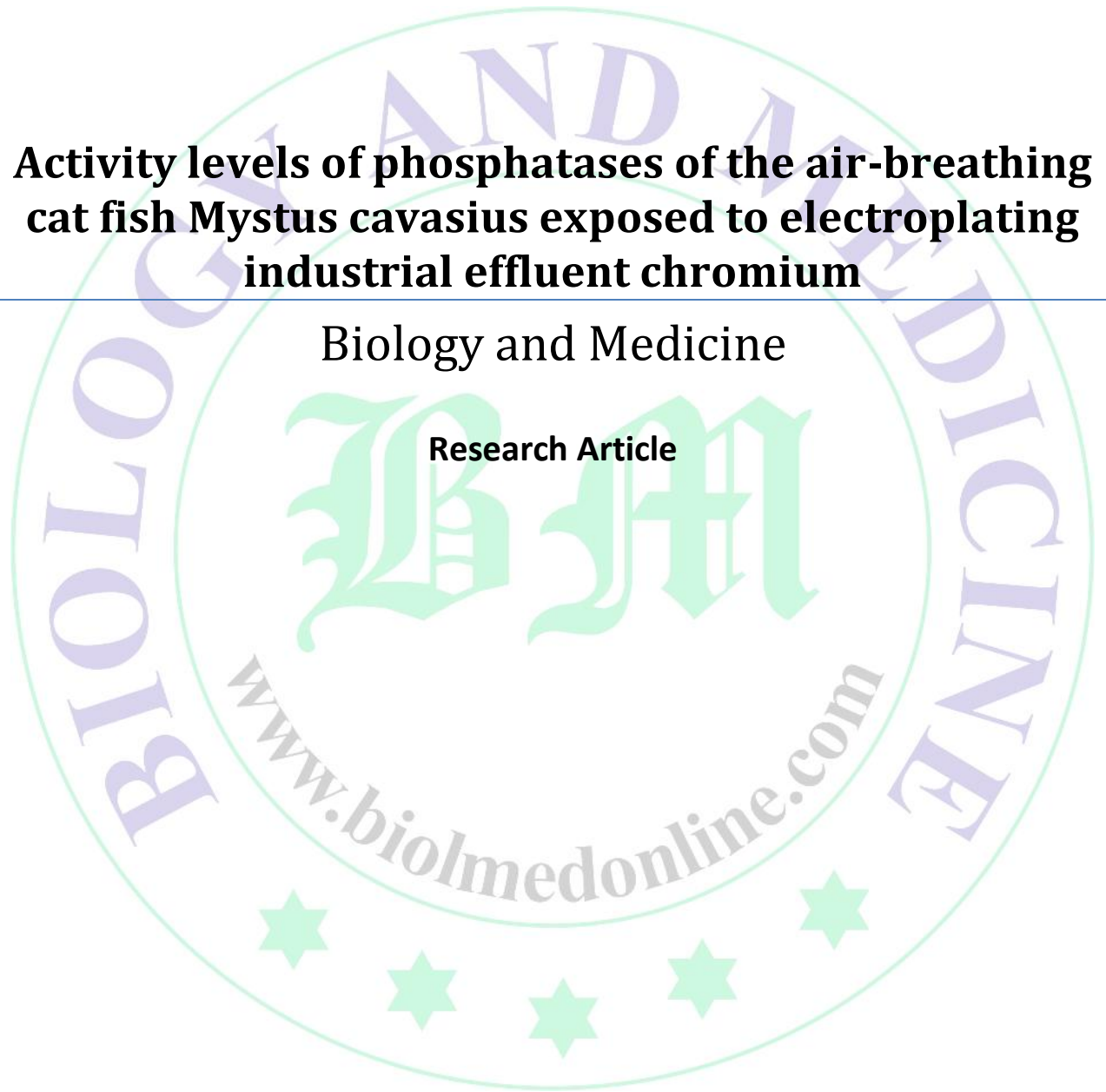


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Activity levels of phosphatases of the air-breathing catfish *Mystus cavasius* exposed to electroplating industrial effluent chromium

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Abstract

In India chromium is widely used in all electroplating industries. Chromium in electroplating industrial effluent has been shown to inhibit many enzymes at different segments of metabolism. Chromium especially inhibits pyruvate oxidases system and phosphatases. The present study was undertaken to evaluate the effect of effluent chromium on phosphatases on exposure to sub-lethal concentration (0.25%) for a period of 24, 48, and 72 h and 15 d. Activity levels of acid phosphatase and alkaline phosphatase decreased significantly in the gill and air bladder tissues of experimental catfish, *Mystus cavasius*, when compared with that of controls.

Keywords: Electroplating industry; chromium; acid phosphatase; alkaline phosphatase; gill; air bladder; *Mystus cavasius*.

Introduction

In India chromium is widely used in all electroplating industries. Potassium dichromate is an oxidizing agent in chrome tanning industries. Vast quantities of effluents containing chromium are dumped in inland water bodies. The chromium that is used in chrome tanneries is a potential pollutant to Indian freshwater bodies used for fish culture and public health. Natural water receives chromium from anthropogenic sources such as industrial effluents derived from the production of corrosion inhibitors and pigments (Galvin, 1996), which then becomes a pollutant to aquatic ecosystem and thus harmful to aquatic organisms (Srivastava and Singh, 1981). Chromium (IV) appears to pass readily through the gill membrane but accumulates rapidly in various tissues including brain, gall bladder, gastro-intestinal tract, intestine, kidney, opercular bone, spleen, and stomach (Buhler *et al.*, 1977; Van der Putte *et al.*, 1981) at higher levels than in gills (Holdway, 1988). Changes in the enzyme activity of aquatic animals are used as an index for metal toxicity (Sastry and Sunitha, 1983; Lan *et al.*, 1993a,b, 1995). Enzyme assay has recently emerged as an important diagnostic tool in the field of environmental toxicology (Baskaran, 1991). Changes in the co-enzyme patterns can provide valuable clues to the affected organ, tissue, cell, or subcellular compartment (Folmar, 1993). Acid

phosphatase (ACP; EC 3.1.3.2) is hydrolytic in nature and helps in the autolysis of the cell after death (Novikoff, 1961). It could be used as an indicator for studying cell mortality due to intoxication. Alkaline phosphatase (ALP; EC 3.1.3.1) is also responsible for transphosphorylation. Therefore, an attempt has been made in the present study to understand the activity levels of these phosphatases in the gill and air bladder tissues of fresh air-breathing catfish, *Mystus cavasius*.

Materials and Methods

Healthy adult *M. cavasius* (14–20 gm weight and 12–18 cm length) were collected and acclimated to the laboratory conditions with softened tap water under the following conditions: Ca, 0.725 mm; Mg, 0.135 mm; pH 7.1 ± 0.4; D.O., 7.4 ± 0.2 mg/l. Water was checked daily for NH₃, nitrite, and nitrate and replaced every 2 d for half of the volume. Water was filtered with a trickling filter and biological kits were used for NH₃, nitrite, and nitrate measurements to ensure that their levels never exceeded 0.1, 1, and 20 mg/l, respectively. The LC₅₀ value was determined by Finney (1971) method. One-third of the sub-lethal concentration of chromium (0.25%) was used on experimental fishes for 24, 48, and 72 h and 15 d. After exposure, the control and experimental fishes were sacrificed and the tissues were isolated and kept in cold. For enzyme assays, the tissues were homogenized

in 0.25 M cold sucrose solution using Yarco homogenizer and centrifuged at 10,000 rpm at a temperature below 8°C. The supernatants were used for enzyme assays. ACP and ALP were assayed using Wootton's method (1964) with *p*-nitrophenyl phosphate as substrate. The specific activity was expressed as microgram of *p*-nitrophenyl formed per minute per milligram protein. The assay was carried out with the help of a UV spectrophotometer at 420 nm. All data were statistically analyzed using the methods described by Zar (1984).

Results

The ACP activity in the gill and air bladder tissues of the experimental fishes differed significantly from that of the control. A significant decrease in the activity was observed in the gill and air bladder tissues during all the exposure periods. The decrease in ACP was found to be maximum in the gill (-8.28%) and air bladder (-17.49%) at 15 d exposure period. ALP activity decreased significantly in the gill (-12.90%) at 72 h and in the air bladder (-18.58%) at 15 d exposure period. The results are summarized in Tables 1 and 2.

Table 1: ACP contents of the gill and air bladder of *M. cavasius* after exposure to chromium-rich effluent (0.25%) ($\mu\text{m phenol/mg protein/h}$).

Tissues	Control	24 h	48 h	72 h	15 d
Gill	175.40 \pm 3.88	171.62 \pm 4.17	166.92 \pm 3.88**	160.88 \pm 3.94***	169.74 \pm 5.81**
% Change		-2.16	-4.83	-8.28	-3.23
Air bladder	160.18 \pm 4.06	152.08 \pm 3.26*	146.90 \pm 3.00**	140.19 \pm 2.20**	132.17 \pm 1.90***
% Change		-5.06	-3.30	-12.48	-17.49

Notes: Each value is the mean of six individual observations, \pm indicate S.E. The signs + or - indicate percentage of increase or decrease over control. Values are significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Other = N.S.

Table 2: ALP contents of the gill and air bladder of *M. cavasius* after exposure to chromium-rich (0.25%) effluent ($\mu\text{m phenol/mg protein/h}$).

Tissues	Control	24 h	48 h	72 h	15 d
Gill	186.21 \pm 4.00	172.15 \pm 5.81*	166.50 \pm 6.30**	160.11 \pm 4.83***	162.19 \pm 5.16**
% Change		-7.55	-10.58	-14.01	-12.90
Air bladder	172.18 \pm 3.16	161.50 \pm 4.25**	156.17 \pm 3.96**	148.15 \pm 4.46**	140.19 \pm 3.24***
% Change		-5.20	-9.30	-13.96	-18.58

See Table 1 notes

Discussion

The present study aims to understand the alterations in phosphatases activities at different time intervals in the tissues like gill and air bladder of freshwater air-breathing catfish, *M. cavasius*, on exposure to electroplating industrial effluent chromium toxicity. ACP (EC 3.1.3.2) and ALP (EC 3.1.3.1.) catalyze the hydrolysis of various phosphate-containing compounds and act as transphosphorylases at acid and alkaline pH, respectively. ACP acts as marker enzyme for the detection of lysosomes in cell fractions and can be altered by the presence of

xenobiotics (Cajaraville *et al.*, 2000), whereas ALP is intrinsic plasma membrane enzyme found on the membrane of almost all animal cells. The activity of both enzymes has been studied in several organisms and the influence of heavy metals has been reported (Blasco *et al.*, 1993). These enzymatic activities are involved in variety of metabolic processes, such as molecule permeability, growth and cell differentiation, and steroidogenesis (Ram and Sathayanesan, 1985). ACP and ALP are mainly involved in the catabolic and autophagic processes in the cells. Their decreased

synthesis or increased rate of degradation targets the lysosomal disruption. They may also impair the endoplasmic reticular secretions in the cells. Salts of lead, copper, mercury, beryllium, cadmium, and silver have a detrimental effect on liver enzymes in the killifish (Jackim *et al.*, 1970). Alterations in ACP activity in the kidney and ovary of teleost fish, *Channa punctatus*, after mercuric chloride intoxication was reported by Shastry and Aggarwal (1979). Galdhar *et al.* (1978) reported inhibition of this enzyme's activity in rats due to various insecticides. Decrease in the activity of this enzyme in the liver and intestine of *C. punctatus* due to exposure to diazinon has been noticed (Sastry and Malik, 1981). Plant poisons are known to inhibit the secretion of this enzyme in air-breathing fish *Anguilla bengalensis* (Mallikaraj, 2004).

Conclusion

The present study clearly indicates that introduction of small amounts of many relatively toxic heavy metal cations via the effluents into an aquatic environment causes multiple changes in the internal dynamics of the enzyme system of freshwater fishes even at sub-lethal levels.

Conflict of Interests

Authors have no conflicting interests.

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