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Influence of Textile Dyeing and Printing Industry Effluent on ATPases in Liver, Brain, and Muscle of Mudskipper, *Periophthalmus dipes*

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Textile dyeing & printing industries use a variety of chemical compounds, a major portion of which occurs in the effluent. The effluent is reported to be complex in nature, rich in dissolved and suspended solids, various organic compounds, moderate heavy metal content and very high pH (Kundu et al. 1989). Effects of various industrial pollutants on some physiological parameters in fish has been reported earlier (Jana and Sahana 1988). Geetha et al. (1992) reported an inversely relationship between survival of a freshwater carp and increasing concentration of a dye factory effluent. Dyeing and printing industry effluents are known to be potent inhibitors of various enzymes including the membrane bound ATPases (Kundu et al. 1992). The membrane bound ATPase system is responsible for, among others, movements of ions accross membrane. The experimental animal of the present study Periophthalmus dipes, a euryhaline teleost inhabiting the coastal mudflats, is an important constituent of the coastal food chain and is occasional consumed by the local fisherfolks. Therefore, in the present study an attempt has been made to assess the dose and duration dependent toxicity of dyeing and printing industry effluent on a few ion dependent ATPases in liver, brain and muscular tissues of P. dipes.

MATERIALS AND METHODS

The experimental animals of the present study P. dipes collected from Gulf of Kutch, with the help of local fishermen. Fishes were acclimated to the laboratory conditions for about a week in cement troughs containing coastal mud and seawater. During this period fishes were provided ad libitum food and about 26°C temperature. However, fishes were not fed during various experiments. Textile dyeing & printing

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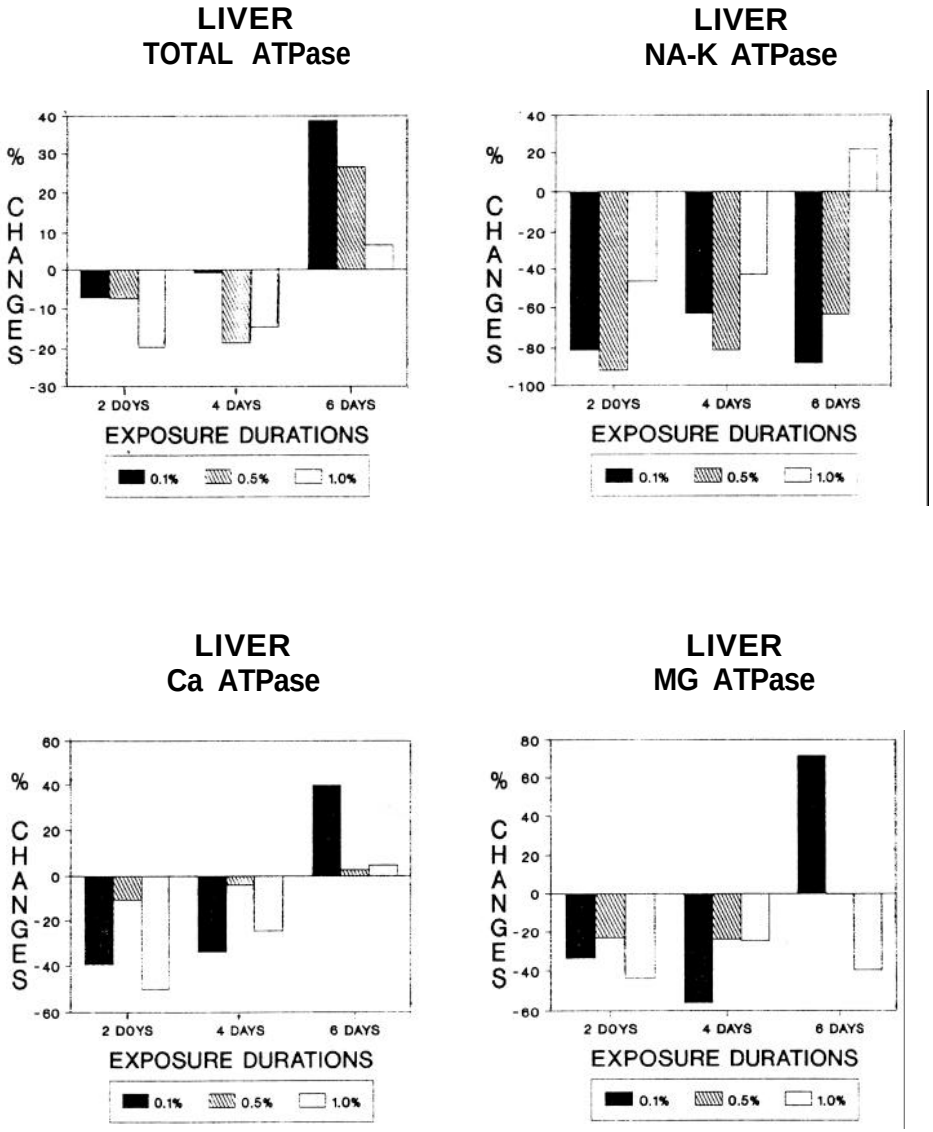

Figure 1. Percent changes of the activity, over their respective controls (control values are taken as zero percent), of four ATPases in the liver of P. dipes exposed to various concentrations of dyeing and printing industry effluent for three exposure durations.

industry effluent were collected directly from the main effluent discharge duct from a number of factories and pooled together. The physico-chemical nature of the effluent was reported earlier (Kundu et al. 1989). Bioassay experiments were carried out for the estimation of LC_{50} (96h) for this species and found to be 1.72%. On the basis of this LC_{50} value three sublethal concentrations (0.1%, 0.5% & 1.0%) of dyeing & printing industry effluent were prepared by diluting with normal seawater (salinity 35.47‰). At least 30 fishes were put in glass troughs containing these effluent concentrations and each treatment was exposed for three time durations (2,4 & 6 days) . Along with the treatments control groups were also maintained separately. The experimental media were renewed every 12 h. After the scheduled exposure animals were sacrificed and whole liver, whole brain and a cube of myotomal white muscle from the middle portion of the body were quickly dissected out and placed in prechilled buffered sucrose solution (pH 7.1). Tissues from all dissected animals exposed for a particular concentration and duration were pooled together and 200 mg of each tissue was taken for the tissue preparation and enzyme assays as described earlier (Lakshmi et al.1990). The activities of Total-ATPase, Na^{+},K^{+}-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase were estimated in the present study and expressed as umol Pi mg $Protein^{-1}h^{-1}$. All experiments were repeated at least thrice and a two level nested ANOVA was performed to estimate the statistical significance treated animals over the controls (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION

Result of the present investigation shows a general inhibition of almost all the enzymes studied in three tissues. However, in a few cases significant stimulations were also observed. In liver, varying degrees of inhibition were observed in all four enzymes studied (Fig 1). However, in most of the cases significant stimulation was observed in the lowest concentrations (0.1%) exposed for longest time (6 d). In brain tissue, significant inhibition was observed in the activity of most of the enzymes (Fig 2). In this case significant stimulation was also observed in lowest concentration exposed for longest time. In case of muscle, total and Na^{+},K^{+}-ATPase was observed in highest dose (Fig 3). The other two enzymes showed inhibition in higher dose and stimulation in lower dose. In liver a general trend of inhibition was observed but, in few cases stimulation was also observed in higher exposure durations. It is possible that toxic organic compounds and heavy metals present in the media may reach to liver via gill, digestive tract or skin

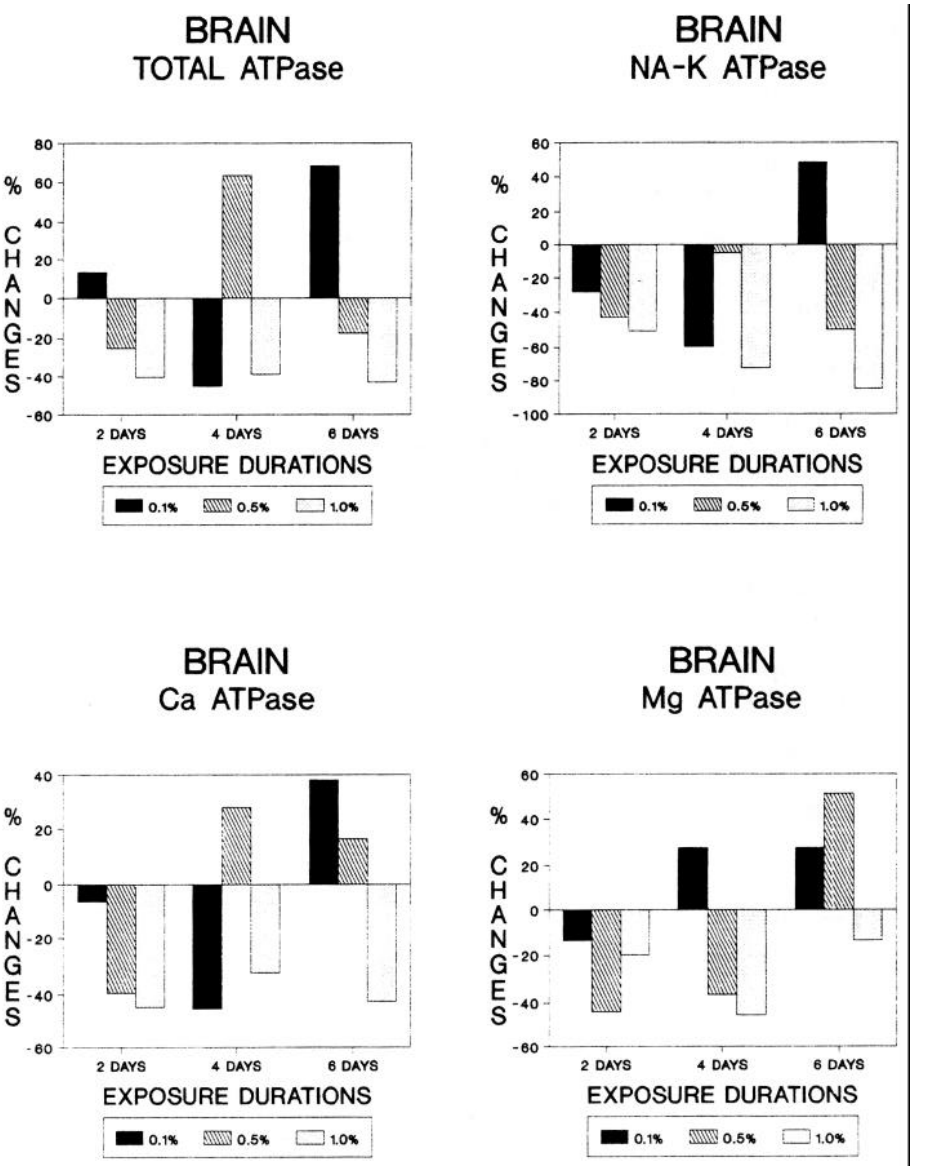

Figure 2. Percent changes of the activity, over their respective controls (control values are taken as zero percent), of four ATPases in the brain of P. dipes exposed to various concentrations of dyeing and printing industry effluent for three exposure durations.

Table 1. Results of two way nested ANOVA between different treatments and exposure durations. Calculated "F" values are given in the Table. "Degrees of Freedom" (df) are (a) among doses = [3,8] and (b) among durations within doses = [8,24]. Critical values of F for these df are also given.

TISSUE		ENZYMES			
		Total ATPase	Na-K ATPase	Ca ATPase	Mg ATPase
LIVER	Among doses	0.63	12.75	0.53	0.56
	Among durations within doses	7.68	52.80	27.07	84.66
BRAIN	Among doses	1.28	2.83	1.40	1.26
	Among durations within doses	627.49	369.30	84.46	1.68
MUSCLE	Among doses	0.66	4.89	0.77	2.80
	Among durations within doses	291.51	164.05	47.89	14.80

Critical values of "F" at various df :

Probability	F [3,8]	F [8,24]
P = 0.05	4.07	2.36
P = 0.01	7.59	3.36
P = 0.001	15.83	4.99

(Kundu et al. 1992). It is possible that the toxic organic compounds and heavy metals, like Cr, present in the effluent, reacted with the membrane bound ATPases and brought about an alteration in the active transport mechanism (Lakshmi et al. 1990). The shrinkage of liver, after exposure to effluent is also a strong sign of its being affected by the effluent.

The ATPase system has long been identified as a target for toxic compounds and the inhibition of the enzyme caused by heavy metals or toxic organic compounds present in the effluent could alter the cellular membrane configuration by binding to the phospholipid portion of the membrane, altering its active site

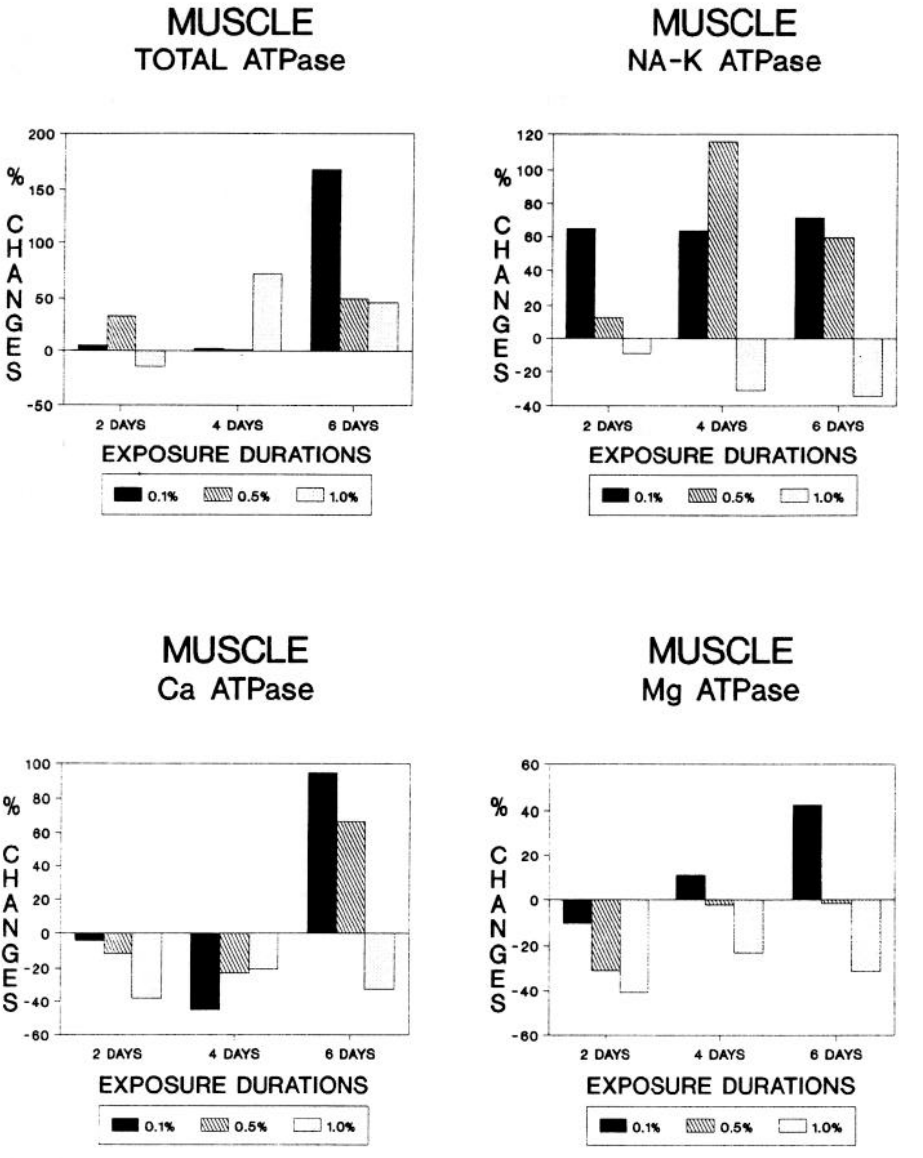

Figure 3. Percent changes of the activity, over their respective controls (control values are taken as zero percent), of four ATPases in the muscle of P. dipes exposed to various concentrations of dyeing and printing industry effluent for three exposure durations.

(Wells et al. 1979). The progressive inhibition in the activities of ATPases may indicate an acute disturbance in the liver (Mehra and Kanwar 1986). Owing to the importance of Na^+,K^+-ATPase in the osmoregulation it is possible that inhibition of this enzyme by heavy metals may cause alterations in the ion transport process in cell membrane (Gilles 1979).

The brain of effluent-intoxicated fishes showed progressive inhibition in the activity of Na^+,K^+-ATPase. The inhibition of this enzyme indicates a blockade of active transportation of Na^+ and K^+ ions. This reduction hampers the K^+ influx and Na^+ efflux from the cell (Nishikawa and Shimizu 1989). Ca^{++} and Mg^{++} ions are vital for the proper functioning of many physiological processes such as release of different chemical neurotransmitters (Wilson 1979). Thus the inhibition of Ca^{++}-ATPase and Mg^{++}–ATPase indicate the reduction of Ca^{++} and Mg^{++} which might cause delay in transmission at the neural junctions. However, the exact neurotoxicity is not clearly understood at this stage.

In the case of muscle most of the enzymes were found to be stimulated in lower concentrations. Inhibition of Na^+-k^+ ATPase activity, brought about a blockade of Na^+-K^+ pump may disrupt the normal distribution of ions (Kundu et al. 1992). The inhibition of Ca^{++}, Mg^{++} ATPases reduced the uptake and transport of Ca^{++} and Mg^{++} which are responsible for the contractility of muscle and storage and transport of Ca^{++} is affected.

In all three tissues, as suggested by the statistical analysis (Table 1), the effluent caused a predominantly exposure duration dependent effects. Toxic material can reach these organs only through blood. It is possible that the toxic substances present in blood affected the ATPase system of the epithelial tissues of the concerned organs causing severe physiological disturbances (Kundu et al. 1995). However, fishes exposed to long term low doses sometimes showed significant stimulation of the enzymatic activity in the organs studied which may be a form of adaptation to the toxicants.

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