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Umamaheswari Sepperumal
PG and Research Department of Zoology, Periyar EVR College, Tiruchirappalli- 620 023. Tamil Nadu, India
Email: umadurai73@yahoo.com

Senthilnathan Saminathan
PG and Research Department of Zoology, Periyar EVR College, Tiruchirappalli- 620 023. Tamil Nadu, India
Email: senthilevr@gmail.com

Correspondence:
Umamaheswari Sepperumal
PG and Research Department of Zoology, Periyar EVR College, Tiruchirappalli- 620 023. Tamil Nadu, India
Email: umadurai73@yahoo.com
Tel: +91-9443411627

Modulation of Enzyme activity in *Oreochromis mossambicus* (Tilapia) exposed to Butylbenzylphthalate

Umamaheswari Sepperumal, Senthilnathan Saminathan

ABSTRACT
Wide application of phthalates as plasticizers due to their durability and sustainability has been documented. Phthalates are known to disrupt the enzyme activity of various tissues organs in fishes and rats. The present study focused on the changes elicited by BBP on the enzyme activity (Acid phosphatase, Alkaline phosphatase, Sorbital dehydrogenase, Lactate dehydrogenase) of the various organs (gill, liver and muscle) of *Oreochromis mossambicus*. The statistical analysis of the data reveal that BBP induced significant modulation in enzyme activity of gill, liver and muscle of the fish *Oreochromis mossambicus*. Significant elevation in the ACP, ALP, LDH activity of gill, liver and muscle were observed in BBP exposed fishes. On the other hand, SDH activity of gill and muscle significantly increased, while that of liver significantly declined. This changes in enzyme activity could be due to the mechanism by which the fish overcomes toxic stress.

Keywords: Butylbenzylphthalate, *Oreochromis mossambicus*, ACP, ALP, SDH, LDH

1. Introduction
Phthalate esters have been detected in highest concentration in urban and rural fish eaters than urban and rural vegeterious [23]. Phthalates are esters of phthalic acid (1,2- benzene dicarboxylic acid) that are primarily synthesized using fisher esterification of phthalic anhydride and the corresponding alcohol [27]. The influence of pollutants an enzymatic activity of fish is one of the most important biochemical parameters which are affected under exposure of toxicants. On exposure to a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or distorted. This increase or decrease in enzyme level is a very accurate index for diagnostic of quantity and quality of toxicant [1].
Man-made Xenoestrogens compounds like Diethylphthalate, Diethylhexylphthalate are known to cause alterations in the enzyme activity of various organ in fishes [7, 14, 3, 29, 30]. Sewage fed fisheries is practiced in many Countries including in India with waste water utilisied for the purpose of culturing fishes [13, 8]. Taking these facts in to consideration, the present study was designed to study the effect of sublethal doses of Butylbenzylphthalate on the enzyme activity of gill, liver and muscle of tilapia *Oreochromis mossambicus*

2. Materials and Methods
Butylbenzylphthalate toxicity were assessed using healthy, living specimens of *Oreochromis mossambicus* which were collected from local freshwaters. Prior to experimentation fishes were allowed to acclimate to laboratory conditions for a month. These adult fishes were reared in aquarium tanks for a period of 30 days at standard environmental conditions and used for further experiments. Butylbenzylphthalate (BBP) was purchased from Sigma. St. Louis, USA and was dissolved in acetone to form a stock solution and stored at room temperature. 10 fishes were randomly selected from the stock and exposed to different concentrations of BBP (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm) for 96 hours to determine the median lethal concentration (LC50) of BBP with selection exposure concentration of 5 and 15 ppm for chronic sub-lethal concentration exposure studies. Water was replaced daily with fresh BBP mixed water to maintain constant level of BBP during exposure period. The LC_{50} value for DEP was 50 ppm. For sub-lethal study, $1/5^{th}$ and $1/10^{th}$ of the LC_{50} value were chosen. A control group was maintained simultaneously. All these experiments were performed in triplicates.

2.1 Sample preparation

Tissue homogenate preparation the gill, liver and muscle of the fishes from the exposed and non-exposed groups were dissected carefully and weighed. It was homogenized with chilled sucrose solution (0.25 M) in a glass tube using Teflon coated mechanical tissue homogenizer (MICCRA D-9, Digitronic, Germany). The homogenate was centrifuged at 10000 rpm for 20 min at 4 ^{0}C in a cooling centrifuge machine. The resultant supernatant was removed and stored (- 40 ^{0}C) for use in tissue enzyme assays (Acid phosphatase (ACP), Alkaline phosphatase (ALP), Sorbitol dehydrogenase (SDH), Lactate dehydrogenase (LDH).

2.2 Statistical analysis

Results of the experiment were expressed as mean and standard error of mean of different groups. The differences between the mean values were evaluated by ANOVA (16.0). the values for P<0.001 were considered significant. Accordingly, a statistical software package (SPSS) was used.

3. Result

Table1: Changes in the Acid Phosphatase of the various tissues of *Oreochromis mossambicus* exposed to Butyl benzyl Phthalate

BBP Treatment	Gill (µmol/mg protein)	Liver (µmol/mg protein)	Muscle (µmol/mg protein)
Control	0.576±0.031^c	3.150±0.017^c	7.260±0.025^c
5-ppm	1.290±0.056^b	5.300±0.047^b	11.400±0.25^b
15-ppm	3.213±0.020^a	7.203±0.594^a	13.180±0.07^a
F-value	1.199E3	34.635	434.266
P-value	0.000	0.001	0.000

***Significant at P<0.001. In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT).

Table 2: Changes in the alkaline phosphatase of the various tissues of *Oreochromis mossambicus* exposed to Butyl benzyl Phthalate

BBP Treatment	Gill (µmol/mg protein)	Liver (µmol/mg protein)	Muscle (µmol/mg protein)
Control	15.426±0.049^c	21.583±0.023^c	17.183±0.017^c
5-ppm	25.266±0.029^b	31.213±0.020^b	18.146±0.034^b
15-ppm	31.270±0.047^a	41.576±0.020^a	21.173±0.026^a
F-value	3.434E4	2.195E5	5.911E3
P-value	0.000	0.000	0.000

***Significant at P<0.001. In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT).

Table 3: Changes in the Sorbital Dehydrogenase of the various tissues of *Oreochromis Mossambicus* exposed to Butyl benzyl Phthalate

BBP Treatment	Gill (U/mg protein)	Liver (U/mg protein)	Muscle (U/mg protein)
Control	17.306±0.049^c	15.243±0.049^b	49.343±0.040^c
5-ppm	18.213±0.053^b	17.436±0.529^a	51.476±0.078^b
15-ppm	21.820±1.438^a	13.123±0.014^c	62.213±0.035^a
F-value	8.243	49.235	1.727E4
P-value	0.19	0.000	0.000

***Significant at P<0.001. In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT).

Table 4: Changes in the Lactate Dehydrogenase of the various tissues of *Oreochromis mossambicus* exposed to Butyl benzyl Phthalate

BBP Treatment	Gill (U/mg protein)	Liver (U/mg protein)	Muscle (U/mg protein)
Control	16.473±0.073^c	28.586±0.026^c	32.383±0.017^c
5-ppm	17.193±0.008^b	32.183±0.017^b	37.200±0.026^b
15-ppm	19.326±0.046^a	38.290±0.028^a	39.226±0.049^a
F-value	865.351	3.962E4	1.062E4
P-value	0.000	0.000	0.000

***Significant at P<0.001. In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT).

Chronic exposure of Zebrafish *Danio rerio* to Butylbenzyl phthalate and the resultant ACP activity of gill, liver and muscle are presented in table 1. Gill ACP activity significantly (F=1.99E3, P<0.001) elevated on exposure of Zebrafish to Butylbenzyl phthalate when compared to BBP unexposed ones (0.576 ± 0.031µmol/mg protein). As the concentration of BBP increased, the ACP activity of the gill also increased 5 ppm: 1.29±0.056 µmol 0/mg protein; 15 ppm: 3.213±0.20 µmol/mg protein. Similarly, liver ACP activity also significantly F=34.635, P<0.001 increased on exposure of Zebrafish *Danio rerio* to BBP when compared to the unexposed ones 3.150±0.017 µmol/mg protein. BBP at 5 ppm and 15ppm registered. ACP gill activity of 5.300±0.047 µmol/mg protein and 7.203±0.594 µmol/mg protein, respectively. Muscle also exhibited similar pattern of ACP activity in BBP exposed Zebrafish. BBP elicited significant F=434.266 P<0.001 elevation in (P<0.001) muscle ACP activity 5 ppm: 11.400±0.25 µmol/mg protein; 10 ppm: 13.180±0.07 µmol/mg protein when compared to the DEHP unexposed ones 7.260±0.025 µmol/mg protein. Thus, a dose dependent relationship between the concentration of BBP and ACP activity of Gill, Liver and muscle was evident.

ALP activity of Gill, Liver and Muscle also elevated similar response in the BBP exposed Zebrafish *Danio rerio.* Control groups recorded gill ALP activity of (15.426±0.049 µmol/mg protein) which was found to be significantly F=3.434E4, P<0.001 lower than BBP treated fishes 5 ppm: 25.266±0.029 µmol/mg protein; 15 ppm: 31.270±0.047 µmol/mg protein (Table-2). In liver, ALP activity significantly elevated F=2.195E5, P<0.001 in Zebrafish on exposure to BBP. BBP at 5ppm and 10 ppm registered liver ALP activity of 31.213±0.20 µmol/mg protein and 41.576±0.020 µmol/mg protein, respectively, whereas control registered ALP activity of 21.583±0.023 µmol/mg protein. The data displayed table-2 reveals elevated muscle ALP activity of Zebrafish *Danio rerio* exposed to BBP when compared to the control 17.183±0.017 µmol/mg protein, BBP exposed fish recorded muscle ALP activity of 18.146±0.034 µmol/mg protein and 21.173±0.026 µmol/mg proteinat 5ppm and 15ppm, respectively. As in the case of gill ACP activity, muscle ALP also elicited dose dependent relationship between the concentration of BBP and enzyme activity. As the concentration of BBP increased, gill, liver and Muscle ALP and ACP activity also significantly increased.

SDH activity of gill and muscle significantly elevated on exposure of the Zebrafish to BBP(Table-3) In comparison to the control 17.306±0.049 U/mg protein BBP treated Zebrafish exhibited significant F=8.243, P<0.19 elevation in gill SDH activity 5ppm:18.213±0.053;U/mg protein 15 ppm:21.820 ±1.438 U/mg protein. Muscle tissue also elecited similar response with regard to SDH activity. BBP at 5 ppm and 15 ppm recorded SDH activity of 51.476±0.078 U/mg protein and 62.213±0.035U/mg protein, respectively. On the other hand control group registered muscle SDH activity of 49.343±0.040U/mg protein. Thus. muscle SDH significantly elevated (F=1.727E4, P<0.001). In BBP exposed fish *Danio rerio* (Table-3), On contrary, liver SDH activity significantly declined (F=49.235, P<0.001) on exposure of the Zebrafish to BBP.BBP unexposed Zebrafish Liver SDH activity of 15.243±0.049 U/mg protein, which was found to the significantly lower than BBP exposed fishes 5 ppm: 17.436±0.529 U/mg protein. In addition, BBP at 15ppm

recorded SDH activity of 13.123±0.014 U/mg protein which was found to be significantly lower than BBP unexposed ones.
BBP induced significant elevation in the LDH activity of gill 865.351 U/mg protein, P<0.001, liver 3.962E4 U/mg protein, P<0.001 and muscle (1.062E4 U/mg protein, P<0.001) in Zebrafish *Danio rerio* when compared to the control. BBP at 5ppm and 15ppm registered gill LDH activity of 17.193±0.008 U/mg protein and 19.326±0.046 U/mg protein, respectively. On the other hand control group registered gill LDH activity of 16.473±0.073 U/mg protein. Liver LDH activity of BBP exposed fishes were found to the 32.183±0.17 U/mg protein (5 ppm) and 38.290±0.028 U/mg protein (15ppm) which was significantly higher than BBP unexposed ones 28.556±0.026 U/mg protein. Significant elevation in the muscle LDH activity was noticed in the BBP treated fishes (5ppm and 15 ppm: 37.200±0.026 U/mg protein and 39.226±0.049 U/mg protein, respectively, when compared to the control 32.383±0.017 U/mg protein.
The results of this study indicate fluctuations in the enzyme activity (ACP, ALP, SDH, LDH) of gill, liver and muscle tissue of Zebrafish *Danio rerio* on exposure to sublethal dosages of BBP. Thus this alteration in the enzyme activities could be due to protective mechanism to overcome the toxic stress caused by BBP in Zebrafish *Danio rerio*.

4. Discussion

The pattern of enzyme activity evinced in this study reflects that BBP induces changes in the enzyme activity of various tissues, resultantly could cause metabolic changes in the fishes *Danio rerio*. Elevated levels of ACP activity of gill, liver and muscle in the BBP exposed Zebrafish *Danio rerio* gains partial support from this findings of Barse *et al*.[3] who have reported increased ACP activity in the muscle tissue of *Cyprinus carpio* exposed to 4-tert-butylphenol (1.38 and 2-3mgL^{-1}). In addition, they have also noticed that at 0.68 mg L^{-1} dose of 4-tert-butylphenol, ALP activity declined which is contrary to the present findings. On contrary to the present result, Barse *et al*. [2] have observed decreased muscle ACP activity in *Cyprinus carpio* exposed to diethyl phthalate 20 ppm dosage 28 days exposure. Increased ACP only was evident in clams exposed to contaminated sites with pollutants such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals Tay*et al*. [27]. In our previous findings we have registered decline in gill. liver and muscle ACP activity of Zebrafish *Danio rerio* on exposure to DEHP Barse *et al.* [3]. This observation disagrees with the present result Furthermore, we have also evinced. Elevated liver ACP activity in DEHP exposed Zebrafish *Danio rerio* which coincides with the increased liver activity of BBP exposed Zebrafish evinced in this study. It has been shown that the liver in the prime location for removing xenobiotics and biocides in fishes Roy. [21]. Similarly, Valavanidis *et al*. [32] have noted significant elevation in gill ACP and AKP(Alkaline phosphate) activity in *Oreochromis mossambicus* exposed to RPR-V (2-butenoic acid-3- diethoxy phosphinothionyl ethyl ester).
On contravery to the elevated liver ACP and ALP only observed in the present investigation, Venkateswara Rao [32] detected significant decrease is liver ACP and AKP activity of Tilapia (*Oreochromis mossambicus*). They have also suggested that the elevation of Alkaline phosphatase in due to increase in the lysosomal mobilization and cell necrosis due to pesticide toxicity.
The present findings partially coincides with the reports of Alireza Safahieh *et al*. [1] who have observed considerable decline in serum ALP activities and significant increase in ACP activities of *Yellow fin Sea bream* (*Acanthopagrus latus*) on exposure to mercury (10 µg to 80 µg)
The present findings lies in parallel to the observations of Karan*et al*. [16] who have found increased levels of serum ACP on exposure of Carp *Cyprinus carpio* to copper sulphate for 14 days. It was shown by Poleksic *et al*. [19] that ACP activity elevates on exposure *of Cyprinus carpio* to the herbicides Trifluralin.
Our results correlates with Nte *et al*. [18] who have evinced significant increase in ALP and ACP activity of gill and muscle of *Sarotherodon malanotheron* exposed to RIVOC industrial effluents for 14 days On the other hand, they have noticed significant decline in liver ACP activity when compared to the control. This report is not in good accord with the present results. Our results are in agreement with that of Ghorpade *et al*. [7] who have observed significant increase in liver and muscle ALP level in DEP treated fish *Cirrhina mrigala* and have attributed it to increased lysosomal activity in the liver and muscle. The present observation agrees with that of Barse*et al*. [2] who have reported increase in muscle ALP in *Cyprinus carpio* in response to DEP treatment.
The present observation not in good accord to the observation of Barse *et al*. [3] who have demonstrated significant decline (P<0.05) in alkaline phosphate in the muscle of 4-tert butyl phenol fish *Cyprinus carpio.* Increase in the levels of ALP and AST has been shown to reflect liver damage, while rise in the ALP level may be indicative of renal and liver damage Bhattacharya *et al*. [5] Gill*et al*. [9] Guluzar Atli *et al*. [10] have generalized that ALP activity in the liver, intestine, and serum of *Oreochromis niloticus* increased following metal exposure. In contraction to the present findings, Bernet *et al*. [4] showed that there was a decrease in serum ALP activity in *Salmo trutta* exposed to effluent form the sewage treatment compared to tap water. Reduction in ALP activity was observed with increasing concentration of nitrite 1, 2, 4, 8 and 10.4 mg L^{-1} in serum and brain, as well as in gill of *Cirrhina mrigala*, *Catla catla* and *Labeo rohita.* While reduced ACP activity was observed in the gill of *Cirrhina mrigala*, C*atla catla* and *Labeo rohita* Das*et al*. [6].These observation are contradictory to the present results.
Elevation in SDH activity of gill and muscle of Zebrafish *Danio rerio* exposed to n- butylbezylphthalate observed in this study coincides with that of Ghorpade *et al*. [7] who have evinced increased SDH activity of muscle of *Cirrhina mrigala* and have attributed it to the involvement of mitochondria in DEP toxicity. Further they have also noticed in significant increase in liver activity which disagrees with the present observation.
Organophosphate induced reductions in the activities of LDH,MDH,SDH, cytochrome oxidase, glucose 6 phosphatase, acid phosphatase and acetylcholinesterase (AChE) in various tissues of fishes have been reported by several investigators Rao *et al*. [20]. Sastry *et al*. [25] Kabeer-Ahammadsahib *et al*. [15] Natarajan [17], Inbaraj *et al*. [12] Tripathi *et al*. [28] Samuel *et al*. [23] Valavanidis *et al*. [31] Our results partially agrees with Sastry *et al*. [24] who have found inhibition in the activity of LDH in liver, kidney, intestine, brain, gill and skeletal muscle of snake headed fish *Channa punctatus* exposed chronically to

quinolphos. In addition they have detected elevated SDH activity in the intestine and decreased SDH activity in the liver, kidney, gill, skeletal muscle, ovary and testis) and have attributed it to impairment of lactate utilisation aerobically by these tissues on exposure of *Channa punctatus* to quinolphos.
The present study is in good accord with the findings of Hung-Hung Sung *et al*. [11].Who have noticed significant elevation in ACP level in haemocyte of green neon shrimp *Neocaridina denticulate.*

5. Conclusion
The enzyme assay of various tissues of BBP exposed Tilapia *Oreochromis mossambicus* reveal that BBP has altered the enzyme activity of gill, liver and muscle tissues.

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