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Impact of Paraquat on Acid and Alkaline Phosphatase Activity in Indian Major Carp *Cirrhinus mrigala* Hamilton

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Abstract

Paraquat is a non-selective aquatic herbicide used to control nuisance vegetation in aquatic bodies. It potentially causes fish toxicity. The present investigation was undertaken to evaluate alterations in Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) activity in fingerlings of commercially important Indian Major Carp *Cirrhinus mrigala.* Both ACP and ALP are popular biomarkers of pesticide toxicity- induced aquatic pollution. 96 hours of acute exposure to LC_0 and LC_{50} concentrations and 30 days of chronic exposure to $LC_{1/20th}$ and $LC_{1/10th}$ concentration of paraquat were studied during the present experiment in selected four tissues viz. gill, liver, muscle and brain to interpret its comparative impact at different effective concentrations on selected target tissues of fish. 30 days of chronic exposure to $LC_{1/10th}$ dose of paraquat showed exceptionally highly significant ($p<0.001$) decline in ACP activity of all four tissues among all other treatment groups when compared with the control. The liver tissue of fishes exposed to both the acute concentrations of paraquat shows an exceptionally highly significant ($p<0.001$) decrease in ALP activity among all other treatment groups and tissues when compared with control. From the present study, it can be concluded that paraquat-induced decline in ACP and ALP activity in *Cirrhinus mrigala* can be utilized as a prominent biomarker of aquatic toxicity induced due to pesticides in non-targeted organisms like fish. Paraquat can be potentially considered an aquatic ecosystem hazard leading to severe environmental pollution and eco-toxicity.

1. Introduction

Paraquat Dichloride (1,1'-Dimethyl-4,4'-bipyridinium dichloride) (CAS No = 75-305-73-0) is abroad-spectrum, post-emergent, non-selective, contact herbicide popularly used as terrestrial as well as aquatic weed-killer in various countries across the globe[1,2]. It is highly toxic to humans and even a small teaspoonful dose of its active ingredients may cause death[3], still, it is the second-highest selling herbicide in the world[4] due to its low cost and highly efficient herbicidal properties. Such large-scale use of paraquat dichloride has led to its far-spread residues in both aquatic

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and terrestrial ecosystems through which it ultimately gets into our food chains[5]. Direct application, spray drift, leaching, run-offs and discharge from factories/sewage are the multiple routes by which pesticides enter aquatic environments[6]. The presence of paraquat has already been reported in many water bodies around the world[7]. Study reports also reveal that degradation of paraquat does not easily occur chemically or biologically demonstrating its long-term (around 80%) persistence in river waters[8,9]. In aquatic environments, paraquat is absorbed into sediments and bottom particles and can persist there for a half-life between 2 to 820 years depending on the depth of water, sunlight and other environmental factors[10]. It has been reported that the presence of paraquat is responsible for causing harmful effects on both aquatic as well as terrestrial ecosystems[11–13]. Such harmful effects lead to severe acute and chronic poisoning in aquatic organisms[14,15]**.** Previous studies had demonstrated the harmful effects of paraquat in *Catla catla*[16] and *Labeo rohita*[17]. *Catla catla* is a top feeder while *Labeo rohita* is a column feeder fish in integrated fish farming and both carry high commercial value as Indian Major Carps. But Cheah *et al*., (1998)[18] reported that paraquat applied to surface waters was rapidly absorbed into the bottom sediments. Another experiment conducted to measure herbicidal residue in pond waters and hydrosoil after treating eight ponds with six different types of herbicides concluded that paraquat is relatively less persistent in the water column (8 days) but was highly held by the hydrosoils sediments at the bottom of ponds and persisted in higher concentrations for more than 85 days in it[19]. Thus it was clear that bottom feeder fishes are the most susceptible to being exposed to paraquat residues in the hydrosoils at the bottom of ponds. Therefore a need to conduct a study for finding out the effect of paraquat on a bottom feeder species was noticed to obtain the baseline data. So the test animal chosen for the study is a bottom feeder, commercially important Indian Major Carp *Cirrhinus mrigala*.

Cirrhinus mrigala is an integral part of integrated fish farming and is reared together with *Catla catla* and *Labeo rohita* extensively across the entire South-Asian continent. According to a 2004 report, in that year, 5,73,627 million tonnes of *Cirrhinus mrigala* fish were annually produced in 2004 through aquaculture contributing back then to around 1.6% of global fish production[20]. Now, these figures may have increased exponentially through the years, but still, a paucity of data was noticed regarding the impact of aquatic weed-killer paraquat on this commercially important non-targeted aquacultural fish species *Cirrhinus mrigala*. Hence the present investigation was designed with the intent to study the toxic effects of paraquat dichloride on *Cirrhinus mrigala* useful in assessing the environmental toxicity and pollution. This data can also be useful to improve the understanding of the use of pesticides and the formulation of policies regarding the management of freshwater resources in the country. Besides *Cirrhinus mrigala* also serves as a grass-root biomarker of a healthy ecosystem useful in the assessment of environmental risks. With this study, the toxicity data of paraquat for all three Indian Major Carp species reared in integrated fish farming (catla, rohu, and mrigal) will be completed. Thus it will help to compare and analyze the differences in behavioral toxicity of paraquat across different water columns and its non-targeted impacts on different column ecosystems. A study conducted by Deivasigamani[21] to evaluate the influence of certain herbicides on the control of water Hyacinth and its impact on fish mortality demonstrated that the herbicide paraquat (Gromoxone) caused the highest mortality percentage of 54.73 in mrigal carp 32 days after spraying. Thus the present study will effectively highlight the alterations in phosphatase activity that actually takes place during such type of mass mortality cases of mrigal carp as distress indicators that lead to economic losses for fish farmers and environmental pollution.

Acid Phosphatases (ACPs) are an important family of lysosomal enzymes that are found in many plant and animal species. They act as a catalyst for facilitating various changes within the cells that are physiologically vital[22]. Thus any alterations in their levels are striking pieces of evidence of physiological disturbances due to damage to internal cells and tissues. Alkaline Phosphatases (ALPs) are plasma-membrane-bound glycoproteins that are widely distributed from prokaryotes to higher eukaryotes[23–28]. Thus any damage to the plasma membrane of cells causes alterations in the ALP levels. Hence alterations in the ACP and ALP activity across the vital organs of fish were selected as markers for monitoring internal cell and tissue damages caused due to the toxicant in the fish.

2. Materials and Methods

2.1 Acquisition and Acclimatization of Experimental Animals

Healthy *Cirrhinus mrigala* fingerlings (weight 3.42 ± 0.49 gm.

and length 4.36 ± 0.73 cm) were procured and transported to the laboratory in oxygen-filled jumbo polythene bags from the Government fish seed production center, Dhom (Wai), District Satara, Maharashtra State, India. At the laboratory, initially, fishes were given a two minutes dip treatment in 0.1% $KmNO_4$ solution for disinfection and then they were transferred to well-aerated aquariums filled with normal tap water for acclimatization. The acclimatization period was 15 days during which natural photoperiod and the normal room temperature was maintained. During this period as well as during the entire experimental procedure, the fishes were fed daily with 'Taiyo Discovery commercial fish food' at a 2% rate of their overall body weight. The water in the aquariums was changed every 24 hours to discard the fecal matter and food debris in the enclosed water system. Water quality parameters were monitored weekly ensuring normal conditions. Any dead or diseased fish were removed immediately from the experiment and only the healthy ones were finally chosen for the study. The selected healthy fish were again acclimatized into 22-liter capacity, well-aerated, open-mouth, transparent plastic containers for 7 days in which they were to be exposed to the toxicant. 24 hours before the test, feeding to the fingerlings was discontinued to minimize the excreta and vomiting in fishes due to toxicant stress.

2.2 Experimental Setup

2.2.1 Acute Toxicity Studies

Initially, acute toxicity studies were conducted to determine the LC_0 and LC_{50} value of paraquat dichloride for *Cirrhinus mrigala* fingerlings by using a static renewal bioassay test as outlined by Sprague (1973)[29] and specified by APHA (1989)[30]. In total 15 transparent cylindrical plastic containers were made ready for this purpose. All containers were facilitated with adequate aeration to maintain the required dissolved oxygen in the water. 20 liters of normal tap water was filled in each container and 10 fish were liberated in each of them. The open mouths of these containers were covered with nylon mesh to prevent fish escape. After completion of every set, the containers were cleaned thoroughly with 5% HNO_3 and dried in sun to remove the trace herbicide adhering to their walls.

2.2.2 Exploratory Screening Test

The commercially marketed product of SYNGENTA known as 'Gromoxone' containing a 24% w/w of paraquat dichloride was used as a toxicant in the entire study. To save efforts and time, a screening test was conducted before the definitive test to derive the approximate concentration range of the toxicant likely to be lethal/hazardous to the experimental fish *Cirrhinus mrigala* in their natural habitats[31].

For this purpose fish were exposed to a wide range of toxicant concentrations viz. 70, 80, 90, 100, 110, 120, 130, 140 ppm. At the end of 96 hours, the highest concentration at which all 10 fishes survived and the least concentration at which all 10 fishes were dead, both were considered approximate upper and lower limit concentrations to precisely assess a narrow range of concentrations between them in a definitive test.

2.2.3 Definitive Test

From primary tests, it was observed that beyond 130 ppm all test fishes were dead, while till 80 ppm all test fishes survived. Therefore a narrower range of concentrations that fell between 80 and 130 ppm viz. 80, 85, 90, 95, 100, 105, 110, 115, 120, 125 and 130 were prepared and test fish were introduced into it. All dead fish were removed immediately to avoid cannibalism. Mortalities were recorded for 24, 48, 72 and 96 hours respectively and corrected for a natural response using Abbott's formula[32]. The data so derived was analyzed using Finney's probit analysis[33] to obtain LC_0 and LC_{50} concentrations of paraquat dichloride in response to *Cirrhinus mrigala* fingerlings.

2.3 Acute and Chronic Exposure to Paraquat for Acid and Alkaline Phosphatase Studies

For each of the four sets of experiments viz. acute exposure to Acid Phosphatase activity, acute exposure to Alkaline Phosphatase activity, chronic exposure to Acid Phosphatase activity and chronic exposure to Alkaline Phosphatase activity, different setups were enacted but all four setups had a similar framework design. For each setup, the framework consists of three different cylindrical plastic containers of 22 liter capacity with supported adequate aeration. Each container was filled with 20-liter normal tap water and in each one of them, 10 fish were released. In the first of the three containers, fishes from both acute and chronic groups were not exposed to any toxicant for 96 hours and 30 days respectively. All of them served as control groups for their respective setups. In the second of the three containers, fishes in the acute toxicity group were exposed to LC_0 concentration of toxicant for 96 hours

and in the chronic toxicity group fishes were exposed to 1/20th concentration of LC_{50} dose for 30 days. In the third container among the three, fishes in the acute toxicity group were exposed to LC_{50} concentrations of toxicant for 96 hours and the fishes in the chronic toxicity group were exposed to $LC_{1/10th}$ concentration of the LC_{50} dose for 30 days. After every 24 hours, the toxicant and the water in all the containers were replaced with fresh water and toxicant into it to uphold the optimum concentration during the entire experiment. Dead fish were taken out of containers and disposed of immediately. In the acute exposure group after 96 hours and the chronic exposure group after 30 days live fish from all the containers were sacrificed and their vital organs like gill, liver, muscle and brain were pooled out separately. Acid and Alkaline Phosphatase activity was analyzed separately in the respective groups.

2.4 Final Analysis of Acid and Alkaline Phosphatase Activity

The acid and Alkaline Phosphatase activity in all four tissues and both the acute and chronic groups was estimated by Linhardt and Walter Method (1966)[34]. Initially, to a fresh tissue extract of 100 mg, 1 ml of 0.9% chilled saline solution was added and a homogenate was prepared in a pre-chilled mortar and pestle. This homogenate from Acid Phosphatase sets was centrifuged at 3000 rpm (10,000 g) for 10 minutes at 2-8^{0}C. The supernatants thus obtained contained lysosomal fractions with Acid Phosphatases in them. The homogenate from Alkaline Phosphatase sets was centrifuged at 2500 rpm (8000 g) for 10 minutes at 2-8^{0}C. The supernatants thus obtained contained alkaline phosphatases in them. Further, in both acute and chronic groups the entire supernatant was extracted in a beaker and 0.9% chilled saline solution was added to it to makeup to the 50 ml mark. The solution was mixed immediately and thoroughly. This 50 ml was the final stock solution. Then 0.5 ml of this thoroughly mixed stock sample was taken into 3 (triplicate) different test tubes. Along with them, a separate test tube was taken with 0.5 ml distilled water into it that served as a blank. In the next step, in Acid Phosphatase sets 0.5 ml of 0.1 M citrate buffer (pH- 5.0) and 0.5 ml of 15 mM p-Nitrophenyl Phosphate (pNPP) substrate was added to all the test tubes and in the Alkaline Phosphatase sets 0.5 ml of 0.1 M glycine buffer substrate (pH- 10.4) and 0.5 ml of 15 mM p-Nitrophenyl Phosphate (pNPP) substrate was added to all the test tubes. In addition, all the test tubes were incubated at 37^{0}C for 30 minutes. After incubation 2.5ml of 0.2 N NaOH was added to all the test tubes and again all the test tubes were incubated at room temperature for 15 minutes. Exactly after 15 minutes, the absorbance of the samples against the blank was measured in a spectrophotometer. This value was taken as a first reading. The second, third and fourth readings of the same samples were taken exactly 1, 2 and 3 minutes from the record of 1st reading against the same blank and were marked as 2nd, 3rd and 4th readings respectively. The differences between the absorbance were calculated and finally, the mean of all three absorbances was derived to get an average change in the absorbance unit (A_{410} test).

2.5 Calculations

The enzyme activity in the tissue sample was calculated by using the formula:

$$\text{Enzyme Unit Activity (i mol/Mg tissue protein extract/minute)} = \frac{(\text{A410 nm test - A410nm blank})\ (4.0)\ (\text{df})}{(18.1)\ (30)\ (0.5)}$$

Where,
A410 nm test = test Optical Density (O.D.).
A410 nm blank = Blank O.D.
4.0 = Total Volume of Assay.
df = Dilution Factor =1000 (a concentration of 1 mg/ml is diluted to yield a solution with a concentration of 1 µg/ml).
30 = Conversion Factor = 30 min. to 1 min.
18.1 = milli-molar extension coefficient of p-nitrophenyl phosphate at 410 nm.
0.5 = Volume of Test Sample Used.

Final results in all the groups were expressed in the form of arithmetic mean ± Standard Deviation (SD). The student's t-test was used to derive the level of significance with two-tailed distribution in the two-sample unequal variance (heteroscedastic) method. Time played a very crucial role in this entire experiment for obtaining accurate results, so it was strictly followed that the entire experiment was conducted without any pause or delay between the steps. However, wherever in the experimental protocol the incubation time was specified, exactly at those steps, time pauses were taken with accuracy.

3. Results

The Physico-chemical parameters of the water medium used during the experimental procedure were measured using standard methods described by APHA (1998)[35] as follows: Temperature 28.4^{o}C, pH 6.9-7.3, Dissolved oxygen

5.49-6.15 mg/l, Dissolved Carbon dioxide 15.07 ± 0.32 mg/l, hardness 121.4 ± 1.41 mg/l, phosphates 0.5 ± 0.16 mg/L, nitrates 1.26 ± 0.09 mg/l.

The LC_0 and LC_{50} values derived for the present experiment are 67.32 ppm and 105 ppm respectively with a standard error of 0.0029.

The results of the effect of paraquat dichloride on acid and Alkaline Phosphatase activity in organs like gill, liver, muscle and brain of the test fish *Cirrhinus mrigala* in the control group, LC_0 group and LC_{50} group after acute exposure (96 hours), and in the control group, $LC_{1/20th}$ group, $LC_{1/10th}$ group after chronic exposure (30 days) are illustrated in their respective tables and graphs as follows:

3.1 96 Hours of Acute Exposure to Acid Phosphatase Activity

The results obtained have been summarized in Table 1. The activity recorded in the gill tissue of the control group was 3.81 ± 0.38 µmol/mg tissue protein extract/minute. However, in the LC_0 concentration group fish, gill exhibited 3.37 ± 0.14 µmol/mg tissue protein extract/minute activity while in the LC_{50} concentration group it exhibited 1.56 ± 0.37 µmol/mg tissue protein extract/minute activity.

The activity recorded in muscle tissue of the control group was 2.21 ± 0.13 µmol/mg tissue protein extract/minute. However, in the LC_0 concentration group, fish muscle exhibited 1.96 ± 0.09 µmol/mg tissue protein extract/minute activity while in the LC_{50} concentration group it exhibited 1.68 ± 0.12 µmol/mg tissue protein extract/minute activity.

The activity recorded in the liver tissue of the control group was 3.53 ± 0.31 µmol/mg tissue protein extract/minute. However in the LC_0 concentration group fish liver exhibited 2.71 ± 0.13 µmol/mg tissue protein extract/minute activity while in the LC_{50} concentration group it exhibited 0.87 ± 0.33 µmol/mg tissue protein extract/minute activity.

The activity recorded in the brain tissue of the control group was 1.78 ± 0.17 µmol/mg tissue protein extract/minute. However in the LC_0 concentration group fish brain exhibited 1.74 ± 0.15 µmol/mg tissue protein extract/minute activity while in the LC_{50} concentration group it exhibited 0.80 ± 0.16 µmol/mg tissue protein extract/minute activity.

The Acid Phosphatase activity in the LC_0 group showed a significant (t.stat- 4.22, $p<0.05$) decrease in the liver tissue as compared to the control group, while the LC_{50} group showed a highly significant ($p<0.001$) decrease in both gill (t.stat - 7.31) and liver (t.stat - 10.04) tissues and moderately significant ($p<0.01$) decrease in the muscle (t.stat - 4.90) and brain (t.stat - 7.05) tissues as compared to the control group.The post-experimental Acid Phosphatase activity in the four tested tissues was in the order Gill > Liver > Muscle > Brain in the LC_0 group while in the LC_{50} group it was - Muscle > Gill > Liver > Brain.

3.2 30 Days of Chronic Exposure to Acid Phosphatase Activity

The results obtained have been summarized in Table 2. The activity recorded in the gill tissue of the control

Table 1. Effect of paraquat dichloride on the Acid Phosphatase activity in different tissues of the fish *Cirrhinus mrigala* after acute exposure (96 hours)

Groups	Total ACP (µmol / mg tissue protein extract / minute)			
	Gill	Muscle	Liver	Brain
Control Group	3.81±0.38	2.21±0.13	3.53±0.31	1.78±0.17
LC_0	3.37±0.14	1.96±0.09	2.71±0.13 *	1.74±0.15
LC_{50}	1.56±0.37 ***	1.68±0.12 **	0.87±0.33 ***	0.80±0.16 **

(Values expressed as Arithmetic Mean of (n = 3); ±SD),

*= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)

group was 4.26 ± 0.19 µmol/mg tissue protein extract/minute. However, in the $LC_{1/20th}$ concentration group fish, gill exhibited 3.11 ± 0.19 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 2.01 ± 0.37 µmol/mg tissue protein extract/minute activity.

The activity recorded in muscle tissue of the control group was 3.35 ± 0.08 µmol/mg tissue protein extract/minute. However, in the $LC_{1/20th}$ concentration group, fish muscle exhibited 2.04 ± 0.09 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 1.68 ± 0.18 µmol/mg tissue protein extract/minute activity.

The activity recorded in the liver tissue of the control group was 3.64 ± 0.28 µmol/mg tissue protein extract/minute. However, in the $LC_{1/20th}$ concentration group, fish liver exhibited 2.6 ± 0.14 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 1.06 ± 0.11 µmol/mg tissue protein extract/minute activity.

The activity recorded in the brain tissue of the control group was 1.89 ± 0.21 µmol/mg tissue protein extract/minute. However, in the $LC_{1/20th}$ concentration group, the fish brain exhibited 1.71 ± 0.15 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 0.54 ± 0.05 µmol/mg tissue protein extract/minute activity.

The Acid Phosphatase activity in the $LC_{1/20th}$ group showed a highly significant ($p<0.001$) decrease in both gills (t.stat - 7.32) and muscle (t.stat - 17.34) tissue and a moderately significantly decrease in the liver (t.stat- 5.39) tissue as compared to control group, while in the $LC_{1/10th}$ group it showed a highly significant ($p<0.001$) decrease in all the four tissues(gill t.stat - 19.39), (muscle t.stat - 13.85), (liver t.stat- 14.55), (brain t.stat - 10.55) as compared to the control group. The post-experimental Acid Phosphatase activity in the four tested tissues was in the order Gill > Liver > Muscle > Brain in the $LC_{1/20th}$ concentration group while in the $LC_{1/10th}$ group it was in the order, Gill > Muscle > Liver > Brain.

3.3 96 Hours of Acute Exposure to Alkaline Phosphatase Activity

The results obtained have been summarized in Table no 3. The activity recorded in the gill tissue of the control group was 4.88 ± 0.14 µmol/mg tissue protein extract/minute. However, in the LC_0 concentration group fish, gill exhibited 3.42 ± 0.38 µmol/mg tissue protein extract/minute activity while in the LC_{50} concentration group it exhibited 3.23 ± 0.27 µmol/mg tissue protein extract/minute activity.

The activity recorded in muscle tissue of the control group was 4.58 ± 0.13 µmol/mg tissue protein extract/minute. However, in the LC_0 concentration group, fish muscle exhibited 4.17 ± 0.09 µmol/mg tissue protein extract/minute activity while in the LC_{50} concentration group it exhibited 3.7 ± 0.2 µmol/mg tissue protein extract/minute activity.

The activity recorded in the liver tissue of the control group was 3.77 ± 0.12 µmol/mg tissue protein extract/minute. However in the LC_0 concentration group fish liver exhibited 2.67 ± 0.06 µmol/mg tissue protein extract/

Table 2. Effect of paraquat dichloride on the Acid Phosphatase activity in different tissues of the fish *Cirrhinus mrigala* after chronic exposure (30 days)

Groups	Total ACP (µmol/mg tissue protein extract/minute)			
	Gill	Muscle	Liver	Brain
Control Group	4.26±0.19	3.35±0.08	3.64±0.28	1.89±0.21
$LC_{1/20th}$	3.11±0.19 ***	2.04±0.09 ***	2.65±0.14 **	1.71±0.15
$LC_{1/10th}$	2.01±0.37 ***	1.68±0.18 ***	1.06±0.11 ***	0.54±0.05 ***

(Values expressed as Arithmetic Mean of (n = 3); ±SD),

*= $p<0.05$ (significant), **= $p<0.01$(moderately significant), ***= $p<0.001$(highly significant)

Table 3. Effect of paraquat dichloride on the Alkaline Phosphatase activity in different tissues of the fish *Cirrhinus mrigala* after acute exposure (96 hours)

Groups	Total ALP (µmol/mg tissue protein extract/minute)			
	Gill	Muscle	Liver	Brain
Control Group	4.88±0.14	4.58±0.13	3.77±0.12	1.11±0.23
LC_0	3.42±0.38 **	4.17±0.09 **	2.67±0.06 ***	0.99±0.08
LC_{50}	3.23±0.27 **	3.7±0.2 **	0.62±0.2 ***	0.62±0.2 *

(Values expressed as Arithmetic Mean of (n = 3); ±SD),

*= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)

minute activity while in the LC_{50} concentration group it exhibited 0.62 ± 0.2 µmol/mg tissue protein extract/minute activity.

The activity recorded in the brain tissue of the control group was 1.11 ± 0.23 µmol/mg tissue protein extract/minute. However in the LC_0 concentration group fish brain exhibited 0.99 ± 0.08 µmol/mg tissue protein extract/minute activity while in the LC_{50} concentration group it exhibited 0.62 ± 0.2 µmol/mg tissue protein extract/minute activity.

The Alkaline Phosphatase activity in the gill and muscle tissues showed a moderately significant (p<0.01) decrease in both LC_0 (gill t.stat- 6.18) (muscle t.stat - 7.05) and LC_{50} (gill t.stat - 4.44) (muscle t.stat - 6.27) groups while in the liver tissue there was a highly significant (p<0.001) decrease in both the LC_0 (liver t.stat- 13.15) and LC_{50} (liver t.stat- 16.04) groups. In the brain tissue, a significant (p<0.05) decrease was observed in the LC_{50} (brain t.stat- 2.78) group. The post-experimental Alkaline Phosphatase activity in the four tested tissues was in the order Muscle > Gill > Liver > Brain in the LC_0 group while in the LC_{50} group it was in the order, Muscle > Gill > Liver > Brain (Table 3).

3.4 30 Days of Chronic Exposure to Alkaline Phosphatase Activity

The activity recorded in the gill tissue of the control group was 4.9 ± 0.21 µmol/mg tissue protein extract/minute. However, in the $LC_{1/20th}$ concentration group fish,

Table 4. Effect of paraquat dichloride on the Alkaline Phosphatase activity in different tissues of the fish *Cirrhinus mrigala* after chronic exposure (30 days)

Groups	Total ALP (µmol/mg tissue protein extract/minute)			
	Gill	Muscle	Liver	Brain
Control Group	4.9±0.21	4.96±0.25	4.13±0.15	1.65±0.27
$LC_{1/20th}$	4.02±0.1 **	4.11±0.13 **	3.65±0.26	0.73±0.54
$LC_{1/10th}$	3.57±0.28 **	3.16±0.15 ***	2.81±0.13 ***	0.65±0.26 **

(Values expressed as Arithmetic Mean of (n = 3); ±SD),

*= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)

gill exhibited 4.02 ± 0.1 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 3.57 ± 0.28 µmol/mg tissue protein extract/minute activity.

The activity recorded in muscle tissue of the control group was 4.96 ± 0.25 µmol/mg tissue protein extract/minute. However, in the $LC_{1/20th}$ concentration group, fish muscle exhibited 4.11 ± 0.13 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 3.16 ± 0.15 µmol/mg tissue protein extract/minute activity.

The activity recorded in the liver tissue of the control group was 4.13 ± 0.15 µmol/mg tissue protein extract/minute. However in the $LC_{1/20th}$ concentration group, fish liver exhibited 3.65 ± 0.26 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 2.81 ± 0.13 µmol/mg tissue protein extract/minute activity (Table 4).

The activity recorded in the brain tissue of the control group was 1.65 ± 0.27 µmol/mg tissue protein extract/minute. However, in the $LC_{1/20th}$ concentration group, the fish brain exhibited 0.73 ± 0.54 µmol/mg tissue protein extract/minute activity while in the $LC_{1/10th}$ concentration group it exhibited 0.65 ± 0.26 µmol/mg tissue protein extract/minute activity.

The Alkaline Phosphatase activity in the $LC_{1/20th}$ group showed a moderately significant ($p<0.01$) decrease in the gill (t.stat- 6.34) and muscle (t.stat- 5.06) tissue as compared to the control group, while in the $LC_{1/10th}$ group it showed a highly significant ($p<0.001$) decrease in both the muscle (t.stat - 10.50) and the liver (t.stat - 11.47) tissues and moderately significant ($p<0.01$) decrease in the gill (t.stat - 6.42) and brain (t.stat- 4.52) tissues as compared to the control group. The post-experimental Alkaline Phosphatase activity in the four tested tissues was in the order Muscle > Gill > Liver > Brain in the LC_0 group while in the LC_{50} group it was in the order, Gill > Liver > Muscle > Brain.

4. Discussion

The vital role of Acid Phosphatases (ACPs) in the number of metabolic activities like water sorption, proliferation, ionic conduction, excretion and transformations marks its importance in life processes[36]. On the other hand, Alkaline Phosphatases are also important markers to measure the functionality of cell membrane[37]. It is also a useful indicator of hepatic function[38,39]. The overall activities of both enzymes are very important biomarkers for vital cellular function. Paraquat undergoes redox cycling inside the cell[40] which gives rise to all types of Reactive Oxygen Species (ROS). Excess of such ROS is responsible for excessive cellular oxidation stress[41–43] causing damage to vital cellular components. In both acute and chronic exposure phenomenal decrease in acid and Alkaline Phosphatase activity was observed in all four vital tissues indicating severe internal cellular and tissue damage due to the action of ROS generated by paraquat in the fish body. Acid Phosphatases are lysosomal enzymes that function during the cellular degradation process. Ample studies reporting damage to the lysosomal membrane due to pesticide toxicity can be found[44–46]. Such damage results in the loss of lysosomal membrane integrity. Damage to the lysosomal membrane causes the free flow of Acid Phosphatases (ACPs) into the cytoplasm resulting in their decreased activity[47]. Reports of paraquat causing lysosomal membrane damage have already been recorded[48]. A study supporting the results of this experiment states that paraquat causes a decrease in the release of lysosomal enzymes like Acid Phosphatases and β- glucronidase[49]. Another study supporting inhibition of lysosomal activity by paraquat in both acute and chronic exposure is also present[50]. A study by Kuang-Chang[51] demonstrated positive relation between serum tartrate-resistant ACP and body weight in human male adolescents.Thus decrease in ACP levels may be correlated with a decrease in fish weight, which can cause economic losses to fish farmers. Rashalwar and Ilyas (1983)[52] demonstrated a similar decrease in Acid Phosphatase activity in the liver tissue of freshwater fish *Nemachelius denisonii* due to Basalin (herbicide) intoxication. Similarly Yuhang *et al*., (2017)[53] too demonstrated a similar decrease in ACP activity in Chinese Mitten crab *Eriocheir sinensis* on exposure to the popular herbicide glyphosate.

Alkaline phosphatases are glycoproteins bound to the plasma membrane[54,55]. The plasma membrane functions as the primary barrier for toxicant[56]. Thus any injury to the plasma-membrane results in altered Alkaline Phosphatase (ALP) activity[57]. Besides reports of ALP involvement in glycogen metabolism[58], active transport[59], synthesis of some enzymes[60] and secretory activity[61] state that any alterations in ALP affect the healthy growth of fish inculcating financial losses to fish farmers. As Alkaline Phosphatase activity plays an important role in protein

synthesis, a reduction in its activity may cause a reduction in protein levels[62]. Thus reduced protein level directly corresponds to fish weight loss causing economic losses. A study indicating a relationship between weight loss and a decrease in serum ALP levels was observed in several different species of cetaceans[63]. Thus ALP may prove to be a reliable indicator of metabolic states. ALP can also be considered a simple and useful indicator to detect malnutrition[64]. Another study of reduction in weight marked by a reduction in ALP activity in human beings is also available[65].Thus reduced ALP activity after exposure to a toxicant in the present experiment may be indicators of malnutrition and weight loss in the fish that leads to economic losses for fish farmers.

Studies showing the paraquat-induced loss in weight of fish are available[50]. Another study related to chronic exposure of paraquat to *O. niloticus* showed a significant ($p<0.05$) decrease in its growth rate[66]. Similar results of a decrease in Alkaline Phosphatase (ALP) activity due to toxicity of paraquat dichloride in freshwater fish *Catla catla* was demonstrated by Bashini *et al*., (2019)[16]. Bashini and Senthilkumaar (2018)[17] also demonstrated a decrease in the ALP activities in freshwater fish *Labeo rohita* due to the action of the toxicant herbicide paraquat dichloride. Besides paraquat can also induce DNA damage and have mutagenic potential in fish[67].

5. Conclusion

The present study concluded remarkable damage caused by paraquat dichloride to vital tissues (gill, liver, muscle and brain), with reference to ACP and ALP activity. In commercially important Indian Major Carp (IMC) *Cirrhinus mrigala*, demonstrating hazards linked with acute as well as prolonged exposure to paraquat as a toxic pollutant. Enzyme activity disruption in the gill, liver, muscle and brain tissues of the fish as a result of oxidative stress is responsible for tissue damage in the fish due to the action of toxicant pesticides. Thus present study contributes baseline data on paraquat toxicity in *Cirrhinus mrigala* and provides useful insights into its eco-toxicological effects on biota and humans even when exposed to very low concentrations of it. Further, this data also represents the behavioral toxicity of paraquat in non-targeted organisms of the benthic aquatic ecosystem. It can be effectively useful to compare and analyze the behavioral differences of paraquat toxicity across different water columns and its impacts on non-targeted organisms.

6. References

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