

Cytochrome P4501A Detection and Quantification in Relation to different Water quality Parameters of Lake Wular of India

Saima Andleeb^{1*}, Irfan Ahmad¹, Asim Iqbal Bazaz² and Oyas Asimi³

¹Division of Fish Genetics & Biotechnology, Faculty of Fisheries, SKUAST-K (J&K), India.

²Division of Fisheries Resource Management, Faculty of Fisheries, SKUAST-K (J&K), India.

³Division of Fish Nutrition & Biochemistry, Faculty of Fisheries, SKUAST-K, Rangil, Ganderbal (J&K), India.

(Corresponding author: Saima Andleeb*)

(Received 17 September 2022, Accepted 29 October, 2022)

(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Cytochromes P450 (CYP450) enzyme family is a prominent enzyme family that primarily functions in the first phase of xenobiotic detoxification in aquatic species. Stimulation of hepatic CYP1A in fish by specific classes of pollutants serves as an early warning system and the "most sensitive biological response" for determining the levels of environmental contamination. This has impacts on human fish intake as well as the health of aquatic organism. Relationship between Cytochrome P4501A (CYPIA) detected in *Carassius carassius* and water quality parameters like ammonical nitrogen, dissolved oxygen, pH, water temperature of the Wular lake was evaluated. The samples (fishes and water sample) were taken on a monthly basis from the three separate locations of the lake viz., Kulhama, Laherwalpora and Ashtung. Control group was also set up. CYPIA concentration in fishes was detected using Enzyme Linked Immuno Sorbant Assay (ELISA). Using one way analysis of variance, $P < 0.05$ was obtained which indicated that there was a significant difference ($P < 0.05$) in CYPIA levels among the 4 sampling sites viz., Kulhama, Laherwalpora, Ashtung and control group. CYPIA showed significant +ve correlation with ammonical nitrogen ($r = 0.872$, $P < 0.01$); orthophosphate ($r = 0.856$, $P < 0.01$) as well as with pH ($r = 0.690$, $P < 0.01$). However significant -ve correlation was obtained between CYPIA and dissolved oxygen ($r = -0.418$, $P < 0.01$); CYPIA and water temperature ($r = -0.650$, $P < 0.01$). Although there haven't been many studies on the relationships between cytochrome P4501A and water quality, this study discusses how these enzymes (bioindicators) are related to water quality parameters, serving as biomarkers of pollution.

Keywords: Wular lake, CYPIA, Ammonical nitrogen, Orthophosphate, Dissolved oxygen.

INTRODUCTION

With the advancement of civilization, an expanding number of chemicals are being introduced into the environments. These chemicals jeopardize biological species, humans, and our surroundings. Because a significant quantity of chemicals used in industry, urbanization, and agriculture infiltrate marine and other freshwater aquatic habitats, the aquatic environment is particularly vulnerable to the hazardous effects of pollutants (Payne *et al.*, 1987). Due to anthropogenic pressures including intensified overexploitation and expanding watersheds, freshwater habitats are among the most vulnerable ecosystems (Altshuler *et al.*, 2011). Along with these stressors, a variety of toxins brought on by anthropogenic activities have recently been introduced into aquatic habitats. One of these stresses is a chemical. Many hazardous substances are released by pesticides used in agriculture and domestic operations to manage pests, weeds, and plant diseases, reaching and harming various environmental divisions, including aquatic ecosystems (Gunderson *et al.*, 2016). Pollutants that accumulate in the organism initially have molecular and cellular impacts. This might have a detrimental effect on the

organism, causing alterations at the population and community levels in the future. Fish populations in heavily polluted environments frequently exhibit high rates of gross-pathological lesions and neoplasms, which may be linked to higher quantities of hazardous chemicals in the sediments (Payne *et al.*, 1987).

Chemical examinations can quantitatively and precisely quantify a wide variety of pollutants, but it is impossible to effectively evaluate the complex combination of chemical contaminants. Additionally, it obscures how chemical contamination affects the aquatic ecosystem. Utilizing biological markers accomplishes this objective. Generally, there are two types of biomarker responses: those that merely assess exposure to a pollutant and those that measure exposure and the hazardous effects of environmental chemicals (Peakall, 1992; Walker, 1998). The induction of cytochrome P4501A (CYPIA) dependent mixed-function oxidases (MFO) or monooxygenases is the biochemical marker that has been most characterised and used so far (Bucheli and Fent 1995). In fish and other vertebrates, organic contaminants including PCB, PCDD, PCDF, and PAH primarily stimulate liver CYPIA. CYPIA is utilized as a biomarker of exposure to these types of organic pollutants and frequently acts as an early

warning signal of potentially more serious pathologies (Payne *et al.*, 1987). Members of the hemoprotein superfamily, cytochrome P450 (CYP) enzymes participate in the mono-oxygenation processes of a variety of endogenous and foreign substances in mammals and plants (Uno *et al.*, 2012). One of the most widely used pesticides in the world is organophosphate (Kretschmann *et al.*, 2011). Pesticides with organophosphates are reported to inhibit CYP450 enzymes. The detoxifying mode of action triggered by this kind of pesticide entail the emission of sulphur molecules during its stimulation, which produce an oxon analogue to the CYP450 enzyme, resulting in the suppression of CYP450 and subsequently the biomarker (OECD, 2012; Jemec *et al.*, 2007). Taking into account the importance of CYP1A in monitoring the aquatic pollution, the present study was accomplished to assess the environmental contamination.

MATERIALS AND METHODS

The present investigation on *Carassius carassius* (Crucian carp) was carried out at Fish Genetics and Biotechnology Laboratory, Aquatic Environmental Management Laboratory, Faculty of Fisheries, SKUAST Kashmir, Rangil, Ganderbal and Division of Veterinary Microbiology and Immunology, FVSc. & A.H Shuhama, Ganderbal. The study involved the following steps for meeting the set objectives:

A. Experimental Design for Control

The crucian carp fingerlings (n=45) were procured for experimentation from SKUAST-K Shuhama fish farm and transported to the Fish Genetics and Biotechnology laboratory in oxygen filled insulated ice boxes and were acclimatized for 24 hours. The fishes were then reared in separate tubs for a period of 3 months under controlled conditions in polyhouse (Fig. 1). The water source for control group was nearby freshwater stream flowing adjacent to Polyhouse. Water temperature, pH, dissolved oxygen and concentration of ammonical nitrogen and orthophosphate were monitored monthly during the experiment. The fishes were fed with standard diet 2 times a day.



Fig. 1. Setup for Control group in Polyhouse SKUAST-K.

B. Selection of sampling sites

For the present study, three sampling sites were selected from Wular lake viz., Site A (Kulhama), Site B (Laherwalpora), Site C (Ashtung). Also Control group

(Site D) was setup in polyhouse of SKUAST-K from December 2019 to February 2020.

C. Collection of Samples

Samples of *Carassius carassius* were collected on monthly basis. The fish samples were collected from the landing centers of the respective areas. A total of 180 samples ranging from 60g to 80g in total body weight were collected from Wular Lake and control group for a period of 3 months. The samples were collected and transported live in sampling box fitted with aerator to the Fish Genetics and Biotechnology Laboratory, Faculty of Fisheries, SKUAST-Kashmir.

D. Preparation of liver homogenates

Liver homogenates were prepared as per the protocol mentioned by the manufacturer (Cusabio Taiwan) on the "Cytochrome ELISA (Enzyme Linked Immunosorbent Assay) detection kit"

E. Principle of the Assay

The assay used the competitive inhibition enzyme immunoassay technique.

F. Detection and quantification of Cytochrome P4501A using ELISA reader

All reagents and samples were prepared as per the instructions given on the "Cytochrome detection kit". The optical density of each well was calculated using ELISA reader (Labsystems, Multiskan) set at 450nm (Fig. 2).



Fig. 2. Dispensing the samples using multichannel pipette.

G. Water chemistry

Samples of surface water were collected from the each sampling site and from experimental control in one litre polyethylene bottles marked distinctly. The sampling was done usually between 11:00am to 2:00 pm. Parameters like water temperature and fixing of Dissolved oxygen were determined at the respective sampling sites and detailed analysis of samples were carried out in AEM Laboratory at Faculty of fisheries, SKUAST- Kashmir using the methods outlined in American Public Health Association (2012). Following parameters were analyzed:

(i) **Water Temperature.** The temperature of water samples were determined by using infrared thermometer (Kusam-Meco). The reading obtained was expressed in °C.

(ii) **Dissolved Oxygen.** Iodometric method was used for determining the concentration of Dissolved oxygen.

The initial fixation of the dissolved oxygen was done on spot by collecting the liquid in the 300 mL BOD bottle, to which 1 ml of each alkali-iodide reagent and manganous sulphate was added.

The D.O. concentration was calculated from the given formula and the results were expressed in mg L⁻¹.

$$\text{Dissolved oxygen (mgL}^{-1}\text{)} = \frac{V_1 \times N \times \text{Eq. Wt}}{V_2} \times 1000$$

V₁ = Volume of sodium thiosulphate used

V₂ = Sample volume taken for titration

N = Normality of sodium thiosulphate

Eq. wt. = Equivalent weight of oxygen

(iii) **pH (Hydrogen ion concentration).** A hand held automated pH meter (Eutech, Singapore) was used to determine the pH of the water samples. Before use, the pH was standardized with buffer solutions (pH 4 and 7).

(iv) **Ammonical Nitrogen.** The concentration of ammonical nitrogen was estimated by phenate method.

(v) **Orthophosphate phosphorous.** The concentration of orthophosphate phosphorous was estimated by Stannous chloride method (molybdenum blue method).

H. Statistical Analysis

The acquired data was statistically evaluated using "PAST-3" software and "Curve expert software". The data was tested to one-way ANOVA (Table 1, Fig. 3) and correlation, with p < 0.005 was regarded statistically significant.

RESULT AND DISCUSSION

A. CYP1A detection and measurement

All of the samples analysed throughout the investigation expressed cytochrome P4501A. The level of CYP in the samples taken from Site A fluctuated from 58.61 pg/l to 118.88 pg/l, with a mean value of

88.74 2.53 pg/l. Samples taken from Site B exhibited CYP concentrations that varied from 97.01 pg/l to 190.11 pg/l, with a mean value of 143.56 3.01 pg/l (Table 1, Fig. 3). Similar to our findings, elevated levels of CYP1A were found in the livers of caged mummichogs, *Fundulus heteroclitus*, exposed to polluted locations near an oil refinery (Loughery *et al.*, 2014). As for Site C, it varied from a low of 71.12pg/l to a high of 125.74pg/l, with a mean value of 98.431.95. However, CYP1A concentrations in control group samples varied from 19.57 pg/l (lowest) to 37.18 pg/l (highest), with a mean value of 28.370.90 pg/l. Numerous studies which have performed the chemical analysis and correlated it with analysis of CYP1A and these studies have showed that concentrations of contaminants such as PAHs and PCBs (measured in fish or in environment) can generally be linked to the induction responses (Van Veld *et al.*, 1990; Goksoyr *et al.*, 1994; Collier *et al.*, 1994). Increase in CYP1A may also be attributed to the presence of organic anthropogenic compounds (polycyclic aromatic hydrocarbons), pesticides, plastics which enters the aquatic environment due to the human activities (Arinc *et al.*, 2000). The results of the current study are consistent with Celander *et al.* (1993), who studied the induction of cytochrome p450 1A1 and conjugating enzymes in rainbow trout (*Oncorhynchus mykiss*) liver when exposed to isosafrole (ISF) or β-naphthoflavone (β -NF). Propiconazole (fungicide) induced the hepatic cytochrome P4501A (CYP1A) in brown trout (*Salmo trutta*), according to Egaas *et al.* (1999). Twelve distinct freshwater fish species from seven different water bodies were examined for CYP1A expression by Williams *et al.* (2021).

Table 1: The dataset provides univariate (descriptive) statistical information about four separate locations.

	Site A	Site B	Site C	Site D
N	45	45	45	45
Min	58.61	97.01	71.12	19.57
Max	118.88	190.11	125.74	37.18
Stand. Dev.	16.97	20.20	13.14	6.04
Median	82.33	119.66	83.71	24.43
Skewness	0.54	1.85	1.52	0.52
Geom. Mean	85.47	120.47	86.07	26.96
Coeff. Var	19.51	16.57	15.89	21.90

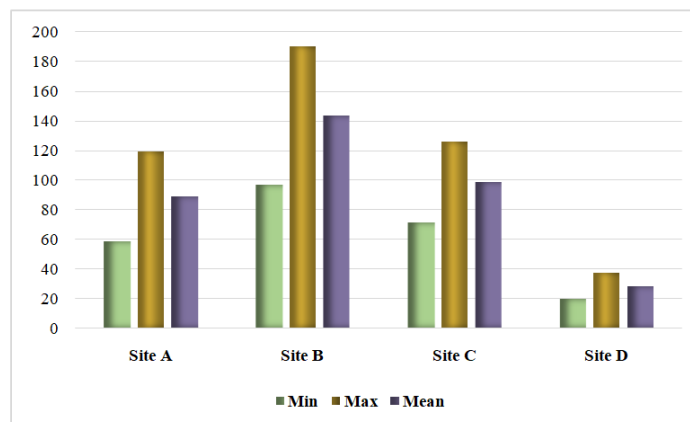


Fig. 3. Minimum, maximum and mean values of CYP1A (pg/ml) level at 4 different sites.

Table 2: Allocation of the mean CYP1A values at the four study locations.

Site	Mean	Std. error	F value	P value
A	88.74	2.53	304.5	<0.05
B	143.56	3.01		
C	98.43	1.95		
D	28.37	0.90		

In the present study, CYP1A showed significant +ve correlation with ammonical nitrogen ($r = 0.872$, $P < 0.01$); orthophosphate ($r = 0.856$, $P < 0.01$) as well as with pH ($r = 0.690$, $P < 0.01$). Perez *et al.* (2002); Fisher *et al.* (2006) also found a statistically significant correlation between CYP1A and Pyrene in Nile Tilapia and CYP1A and PCBs in common carp. After using one way analysis of variance was used, $P < 0.05$ was found, indicating that there was a significant difference ($P < 0.05$) in the levels of CYP1A across the four sample locations, namely Site A, Site B, Site C, and Site D (Table 2).

B. Water Quality Parameters

(i) Ammonical nitrogen. In the present study Site A recorded minimum Ammonical nitrogen concentration of $234.9 \mu\text{g/l}$ in the month of February to a maximum of $275.9 \mu\text{g/l}$ in month of December with a mean value of $252.1 \pm 12.2 \mu\text{g/l}$. For Site B it ranged from a minimum of $333.7 \mu\text{g/l}$ in the month of February to a maximum of $381.5 \mu\text{g/l}$ in month of December with a mean value of $360.8 \pm 14.1 \mu\text{g/l}$, while as, for Site C it ranged from a minimum of $229.3 \mu\text{g/l}$ (February) to a maximum of $275.4 \mu\text{g/l}$ (December) with a mean value of $253.1 \pm 13.3 \mu\text{g/l}$. However, in control group the total Ammonical nitrogen content was recorded in the range of $53 \mu\text{g/l}$ (minimum) in month of February to a $61 \mu\text{g/l}$ (maximum) in the month of December with a mean value of $57 \pm 2.3 \mu\text{g/l}$ (Table 3, Fig. 4).

Table 3: Ammonical nitrogen ($\mu\text{g/L}$) monthly changes at four distinct locations.

Sites	December	January	February	Mean \pm S.E
Site A	275.9	245.5	234.9	252.1 ± 12.2
Site B	381.5	367.4	333.7	360.8 ± 14.1
Site C	275.4	254.8	229.3	253.1 ± 13.3
Site D	61	57	53	57 ± 2.3

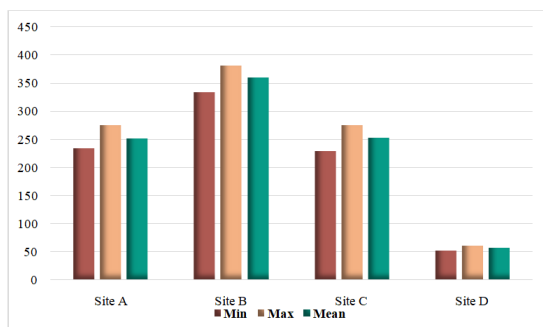


Fig. 4. Minimum, maximum and mean values of ammonical nitrogen ($\mu\text{g/l}$) at 4 different sites.

During the winter months, ammonification and slow rate of nitrification process leads to elevated quantities of ammonical nitrogen. Wetzel (1983); Finlay *et al.* (2007) obtained the similar results during their study. On the other hand, during warmer months the lower ammonical concentration is due to the autotroph's photosynthetic assimilation during their growth in summer (Pandit, 1999). The results of the current research are in consensus with Kayranli *et al.* (2010). They reported high levels of ammonical-nitrogen during winter and attributed it to the relatively low nitrification of ammonical-nitrogen due to low temperatures, which negatively affects the nitrification. (Dar, 2015) obtained the similar results, higher values of ammonia during winter and lower values in summer. Similar findings were also revealed by Ahlgren (1967). This is possibly due to the utilization of NH_4^+N by phytoplankton. (Rasheed, 2019) reported higher values of ammonia during winter could be due to lower metabolic process as development of plant life is very slow and also to leaching from the surrounding terrestrial vegetation due to rains.

(ii) Orthophosphate. In the present study Site A recorded minimum orthophosphate phosphorous concentration of $83.7 \mu\text{g/l}$ in the month of February to a maximum of $105.4 \mu\text{g/l}$ in month of January with a mean value of $92.46 \pm 6.60 \mu\text{g/l}$. For Site B it ranged from a minimum of $81.9 \mu\text{g/l}$ in the month of February to a maximum of $99.7 \mu\text{g/l}$ in month of January with a mean value of $90.93 \pm 5.14 \mu\text{g/l}$, while as, for Site C it ranged from a minimum of $74.1 \mu\text{g/l}$ (February) to a maximum of $101.2 \mu\text{g/l}$ (January) with a mean value of $83.53 \pm 8.84 \mu\text{g/l}$. However, in control group the total orthophosphate content was recorded in the range of $12.4 \mu\text{g/l}$ (minimum) in month of February to a $14.2 \mu\text{g/l}$ (maximum) in the month of January with a mean value of $13.36 \pm 0.5 \mu\text{g/l}$ (Table 4, Fig. 6).

The distribution of orthophosphate revealed substantial fluctuation, with average values in winter being higher while average values in summer are lower. Orthophosphate reaches lakes through home wastewater and agricultural runoff, which explains why eutrophication has intensified (Vyas *et al.*, 2006). The production of an insoluble calcium-phosphate complex is the cause of the low orthophosphate-phosphorous concentration in waters. This phenomenon eliminates dissolved organic debris through absorption and serves as a scavenger of certain inorganic nutrients (Wetzel and Otsuki 1974).

(iii) Dissolved Oxygen. In the present study Site A recorded minimum Dissolved oxygen concentration of 9.2mg/l in the month of February to a maximum of 10.8mg/l in month of December with a mean value of $10.03 \pm 0.46 \text{mg/l}$. For Site B it ranged from a minimum of 8.5mg/l in the month of February to a maximum of 9.2mg/l in month of December with a mean value of $8.86 \pm 0.20 \text{mg/l}$, while as, for Site C it ranged from a minimum of 9.2mg/l (February) to a maximum of 10.7mg/l (December) with a mean value of $10.03 \pm 0.44 \text{mg/l}$. However, in control group the total Dissolved oxygen concentration was recorded in the range of 9.7mg/l (minimum) in month of February to 10mg/l (maximum) in the month of December with a

mean value of 9.86 ± 0.08 mg/l (Table 5; Fig. 8). The findings of the current analysis showed that Wular lake had adequate dissolved oxygen with a mean concentration ranging from 8.86 ± 0.20 mg/L to 10.03 ± 0.46 mg/L. Slightly lower levels of dissolved oxygen at Site B could be due to the combined effect of the increased rate of decomposition of organic matter, increased amount of organic matter coming through agricultural runoff and sewage requiring oxygen for decomposition, (Lewis, 2000; Okbah and El-Gohary 2002). The higher concentration of dissolved oxygen at site A and Site C is due to luxuriant growth of submerged macrophytes which act as main sources of aeration for the lake, thereby enhancing light penetration and hence photosynthesis (Kumar and Pandit 2007; Srivastava *et al.*, 2008).

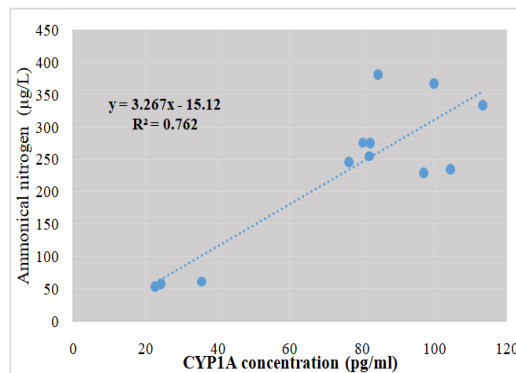


Fig. 5. Scatter plot showing relationship between CYP1A and ammonical nitrogen.

Table 4: Orthophosphate phosphorous ($\mu\text{g/L}$) monthly changes at four distinct locations.

Sites	December	January	February	Mean \pm S.E
Site A	88.3	105.4	83.7	92.46 \pm 6.60
Site B	91.2	99.7	81.9	90.93 \pm 5.14
Site C	75.3	101.2	74.1	83.53 \pm 8.84
Site D	13.5	14.2	12.4	13.36 \pm 0.52

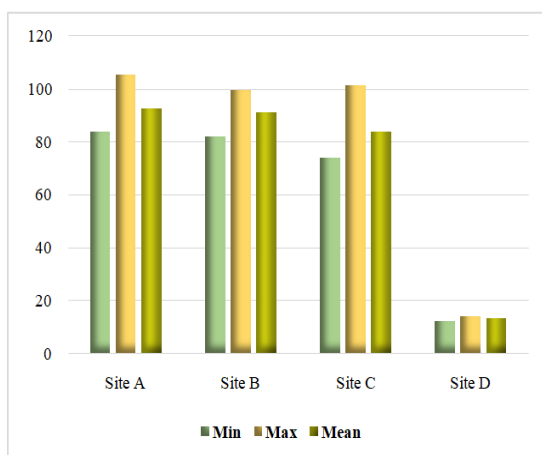


Fig. 6. Minimum, maximum and mean values of Orthophosphate phosphorous ($\mu\text{g/l}$) at 4 different sites.

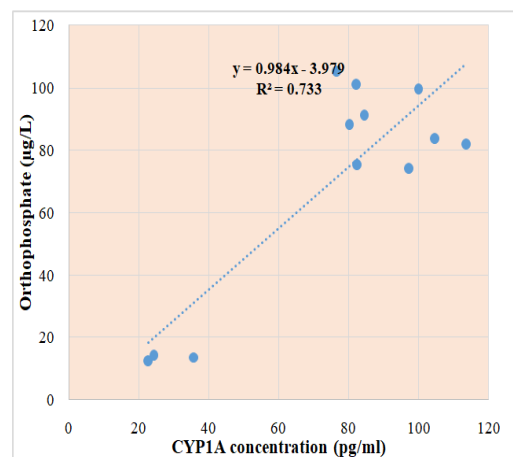


Fig. 7. Scatter plot showing relationship between CYP1A and orthophosphate phosphorous.

Table 5: Monthly variations in Dissolved oxygen (mg/L) at 4 different sites.

Sites	December	January	February	Mean \pm S.E
Site A	10.8	10.1	9.2	10.03 \pm 0.46
Site B	9.2	8.9	8.5	8.86 \pm 0.20
Site C	10.7	10.2	9.2	10.03 \pm 0.44
Site D	10	9.9	9.7	9.86 \pm 0.08

(iv) **Water temperature.** The site A recorded water temperature ranged from a minimum of 5.5°C in the month of December to a maximum of 8.5°C in the month of February with mean value of $6.9 \pm 0.86^\circ\text{C}$. For site B it ranged from a minimum of 5.4°C in the month of December to a maximum of 8.3°C in the month of February with mean value of $6.7 \pm 0.84^\circ\text{C}$ while as, for Site C it ranged from a minimum of 5.7°C in the month of December to a maximum of 7.2°C in the month of February with mean value of $6.3 \pm 0.45^\circ\text{C}$. In control it ranged from a minimum of 5.2°C in the month of December to a maximum of 12.5°C in the

month of February with mean value of $8.8 \pm 2.10^\circ\text{C}$ (Table 6, Fig. 11).

In the present investigation, water temperature of the lake showed monthly increase, minimum (average) in the month of December ($5.45 \pm 0.1^\circ\text{C}$) followed by January ($7.02 \pm 0.5^\circ\text{C}$) and highest in the month of February ($9.12 \pm 1.1^\circ\text{C}$). Increase in water temperature is attributed to the increase in solar radiation due to comparatively longer day length. Similarly, a gradual reduction in solar radiation may explain fall in temperature during winter months and again it begins to increase during warmer months. Fig. 9 depicts

comparative CYPIA level (in fishes) and orthophosphate, ammonical nitrogen, dissolved oxygen (in water) collected from respective sites.

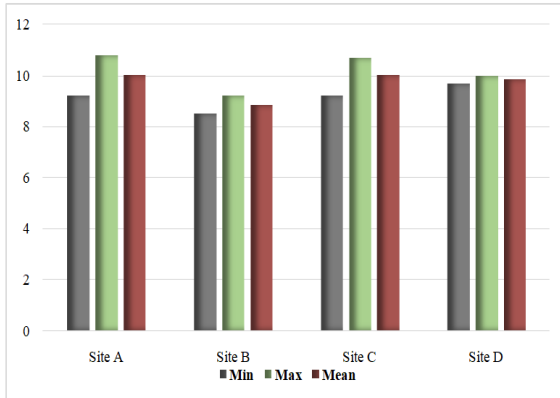


Fig. 8. Minimum, maximum and mean values of Dissolved oxygen at 4 different sites.

Munawar (1970) reported a direct relation between water temperature and bright sunshine. Bartram and Balance (1996) reported this variation may be related to many environmental factors such as elevation, current velocity, water depth, degree of shading, bottom materials exposed to direct sunlight, vegetation cover, wind speed and evaporation. Toma (2000); Rasheed (2008) found the similar results. Dar (2015) reported that the meteorological conditions such as trade winds, sunshine durations and absorption of the solar radiation by the shallow lake water body might be responsible for the monthly variations.

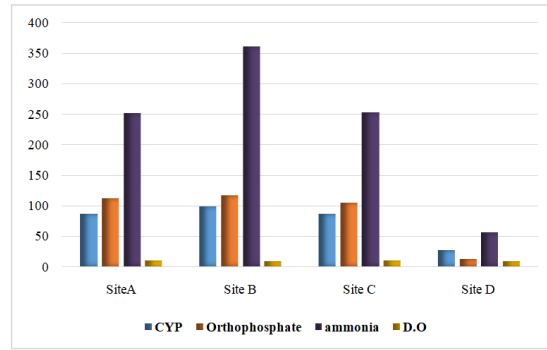


Fig. 9. Comparative CYPIA level (in fishes) and orthophosphate, ammonical nitrogen, dissolved oxygen (in water) collected from respective sites.

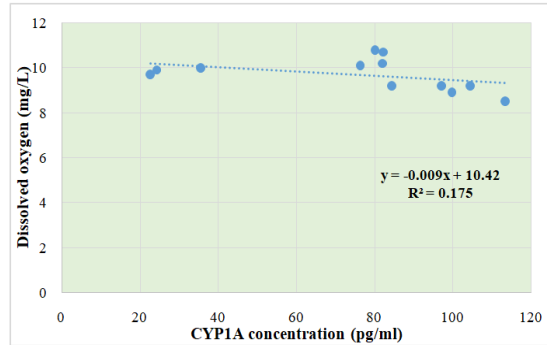


Fig. 10. Scatter plot showing relationship between CYPIA and dissolved oxygen.

Table 6: Monthly variation in water temperature (°C) at different sites.

Sites	December	January	February	Mean ±S.E
Site A	5.5	6.9	8.5	6.9 ±0.86
Site B	5.4	6.5	8.3	6.7 ±0.84
Site C	5.7	6	7.2	6.3 ±0.45
Site D	5.2	8.7	12.5	8.8 ±2.10
Mean ±S.E	5.45 ±0.1	7.02 ±0.5	9.12 ±1.1	-

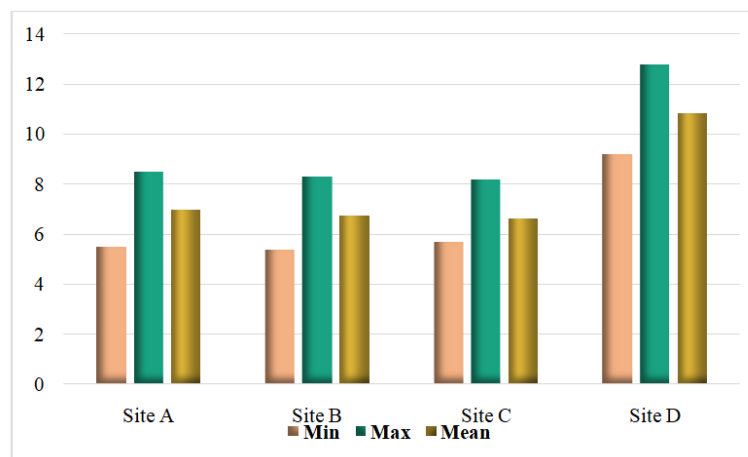


Fig. 11. Minimum, maximum and mean values of temperature at 4 different sites.

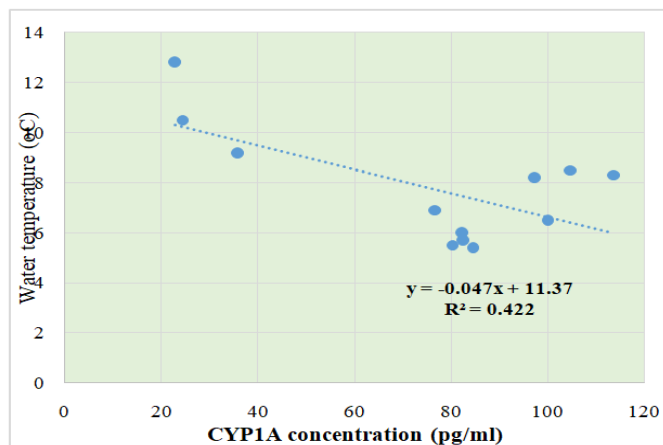


Fig. 12. Scatter plot showing relationship between CYP1A and water temperature.

(v) pH. The minimum pH of 7.3 was recorded from site A in the month of December and a maximum of 7.5 was recorded in the month of February with average pH of 7.4 ± 0.05 . Site B recorded a minimum pH of 7.6 in the month of December and a maximum pH of 7.8 in the month of February. The average pH of Site B was recorded as 7.7 ± 0.05 . Similarly, Site C recorded a minimum pH of 7.4 in the month of December and a maximum pH of 7.8 in the month of February and the average pH of 7.6 ± 0.11 was recorded from the site C. In control group the minimum pH of 7.2 was recorded in the month of December and a maximum pH of 7.4 in the month of February with the mean pH of 7.3 ± 0.05 (Table 7, Fig. 13). The pH recorded in the present study was in neutral to alkaline range suggesting that the lakes were well buffered throughout the study period. Lower pH during winter is because of the increased decomposition of organic matter under least water depth (Reimer *et al.*, 2008). The pH variations recorded during the present study fall within the recommended range for the support of aquatic life (Kamran *et al.*,

2003). Similar trend was followed in the study conducted by Agarkar and Garode (2000), they attributed the lowest pH values, to lower rates of photosynthesis, increasing CO_2 levels. Conversely, the increased pH in the summer season may be associated with the decrease in free CO_2 as a result of photosynthesis (Wetzel, 1975). Similar trend in pH (lower during winter and high during summer) was reported by Raut *et al.* (2015).

Dar (2015) reported that the Wular lake has good water quality with the pH range of 7.1-8.5. Hassan *et al.* (2015) while studying the Influence of land use/land cover on the water chemistry of Wular Lake in Kashmir Himalaya (India) reported the neutral to alkaline pH throughout the study period. The findings of the present study are in close proximity with the findings of Naik *et al.* (2015), who reported lower values of pH during winter months. The changes in the rate of photosynthesis and decomposition seemed to cause pH fluctuations, and this was consistent with the conclusion drawn by Zutshi and Khan (1988).

Table 7: Monthly variation in pH at 4 different sites.

Sites	December	January	February	Mean \pm S.E
Site A	7.3	7.4	7.5	7.4 ± 0.05
Site B	7.6	7.7	7.8	7.7 ± 0.05
Site C	7.4	7.6	7.8	7.6 ± 0.11
Site D	7.2	7.4	7.3	7.3 ± 0.05

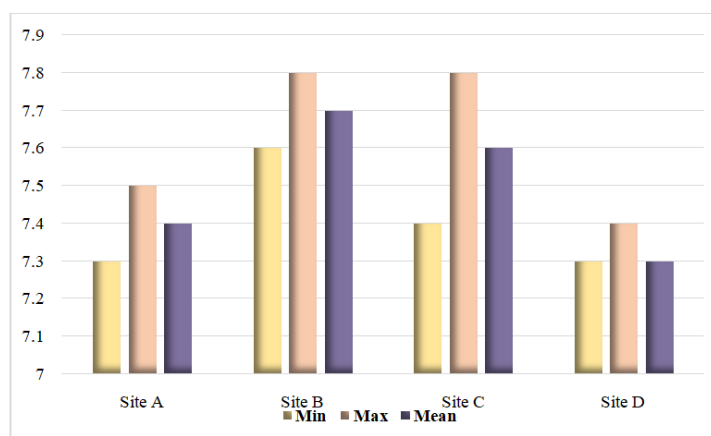


Fig. 13. Minimum, maximum and mean values of pH at 4 different sites.

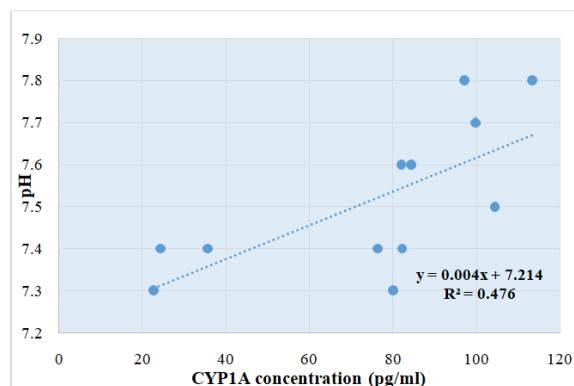


Fig. 14. Scatter plot showing relationship between CYPIA and pH.

(vi) **Correlation between different parameters.** The 4 sampling sites viz., Site A, Site B and Site C and Site D. CYPIA showed significant +ve correlation with ammonical nitrogen ($r = 0.872$, $P < 0.01$); orthophosphate ($r = 0.856$, $P < 0.01$) as well as with pH ($r = 0.690$, $P < 0.01$) (Table 8, Fig. 5, 7 & 14).

However significant -ve correlation was obtained between CYPIA and dissolved oxygen ($r = -0.418$, $P < 0.01$); CYPIA and water temperature ($r = -0.650$, $P < 0.01$) (Table 9, Fig. 10 & 12)

Table 8: Correlation between CYPIA and ammonical nitrate, orthophosphate and pH.

	Ammonical Nitrate	Orthophosphate	pH
CYPIA	0.872*	0.856*	0.690*

*= significant at 0.01 (1%) level of significance

Table 9: Correlation of CYPIA with dissolved oxygen and water temperature.

	Dissolved oxygen	Water Temperature
CYPIA	-0.418*	-0.650*

*= significant at 0.01 (1%) level of significance

CONCLUSION

The need to identify and assess the implications of pollution, often small concentrations of increasingly complicated mixtures, prompted the development of molecular markers or biomarkers. Utilizing monitoring programmes, it is possible to assess the rates of environmental deterioration and risk associated with it. Because of its sensitivity to extremely low concentrations of pollutants that may otherwise not be identified by any other lab procedure, cytochrome P450 enzymes (CYPs) have been demonstrated to be the best susceptible biomarker for water contamination detection till date. The present study thus demonstrated the relationship of CYPIA with various water parameters. It was found that CYPIA showed significant positive relation with ammonical nitrogen, orthophosphate and pH. While as, significant negative relationship was obtained between CYPIA, dissolved oxygen and water temperature. In eutrophic environments, fish at the top of the food chain and top consumers are prone to accumulate contaminants from their surroundings, and the resulting high CYP levels may be utilized as a possible signal of stress in fish.

FUTURE SCOPE

Further examination of this study could contribute a significant addition to the fundamental understanding of fish adaptability and the harmful consequences of numerous xenobiotics found in water bodies. The impact of pollutants on the expression of CYPIA in

freshwater fish can be further investigated. Procedures involving the detection of this gene for the assessment of water pollution can be validated by direct correlations between levels of CYPIA expression inside the fish and pollution levels in the water.

Conflict of Interest. None.

REFERENCES

- Agarkar, S. V. and Garode, A. M. (2000). Evaluation of physico-chemical and microbiological parameters of Vyazadi reservoir water. *Indian Hydrobiol.*, 3, 3-5.
- Ahlgren, I. (1967). Limnological studies of Lakes Norrviken, a eutrophicated Sewedish lake. Water chemistry and nutrient budget. *Schweizerische Zeitschrift fur Hydrologie*, 29, 53-90.
- Altschuler, I., Demiri, B., Xu, S., Constantin, A., Yan, N. D. and Cristescu, M. E. (2011). An Integrated Multi-Disciplinary Approach for Studying Multiple Stressors in Freshwater Ecosystems: Daphnia as a Model Organism. *Integr. Comp. Biol.*, 51, 623-633.
- APHA (2012). Standard Methods for the examination of Water and Waste water. APHA, AWWA, WEF. Washington, D.C.
- Arinc, E., Sen, A. and Bozcaarmutlu, A. (2000). Cytochrome P4501A and associated mixed function oxidase induction in fish as a biomarker for toxic carcinogenic pollutants in the aquatic environment. *Pure Applied Chemistry*, 72(6), 985-994.
- Bartram, J. and Balance, R. (1996). Water Quality Monitoring (a practical guide to design and implementation of freshwater quality studies and monitoring). In: E and FN Spon, an imprint of Chapman and Hall. London. U K, 383 pp.

- Bucheli, T. D. and Fent, K. (1995). *Critical Reviews in Environmental Science and Technology*, 25, 201–268.
- Celander, M., Leaver, M. J., George, S. G. and Forlin, L. (1993). Induction of cytochrome P450 1A1 and conjugating enzymes in rainbow trout (*Oncorhynchus mykiss*) liver: A time course study. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 106(2), 343–349.
- Collier T. K., Anulacion, B. F., Stein, J.E., Goksoyr, A. and Varanasi, U. (1994). A field evaluation of cytochrome P450 1A as a biomarker of contaminant exposure in three species of the catfish. *Environ. Toxicol. Chem.*, in press.
- Dar, N. A. (2015). Distribution, production and biochemical status of dominant macrophytes in Wular Lake, A Ramsar site in Kashmir Himalaya. Ph.D Thesis submitted to P.G department of Environmental Sciences, Faculty of Physical and Material Science, University of Kashmir Srinagar, 190006, J&K.
- Egaas, E., Sandvik, M., Fjeld, E., Kallqvist, T., Goksoyr, A., and Svensen, A. (1999). Some effects of the fungicide propiconazole on cytochrome P450 and glutathione S-transferase in brown trout (*Salmo trutta*). *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 122(3), 337–344.
- Finlay, J. C., Sterner, R. W. and Kumar, S. (2007). Isotopic evidence for in-lake production of accumulating nitrate in Lake Superior. *Ecol. Appl.*, 17(8), 2323–2332
- Fisher, M. A., Mehne, C., Means, J. C. and Ide, C. F. (2006). Induction of CYP1A mRNA in Carp (*Cyprinus carpio*) From the Kalamazoo River Polychlorinated Biphenyl-Contaminated Superfund Site and in a Laboratory Study. *Archives of Environmental Contamination and Toxicology*, 50: 14-22.
- Goksoyr, A., Beyer, J., Husoy, A. M., Larsen, H.E., Westheim, K., Wilhelmsen, S. and Klungsoyr, J. (1994). Accumulation and effects of aromatic and chlorinated hydrocarbons in juvenile Atlantic cod (*Gadus morhua*) caged in a polluted fjord (Sorfjorden, Norway). *Aquat. Toxicol.*, 29: 21-35.
- Gunderson, M. P., Pickett, M. A., Martin, J. T., Hulse, E. J., Smith, S. S., Smith, L. A., Campbell, R. M., Lowers, R. H., Boggs, A. S. P. and Guillette, L. J. (2016). Variations in Hepatic Biomarkers in American Alligators (*Alligator mississippiensis*) from Three Sites in Florida, USA. *Chemosphere*, 155, 180–187.
- Hassan, Z., Shah, J. A., Kant, T. A., and Pandit, A. K. (2015). Influence of land use/land cover on the water chemistry of Wular Lake in Kashmir Himalaya (India). *Ecological Processes*, 4, 9.
- Jemec, A., Drobne, D., Tisler, T., Trebse, P., Ros, M. and Sepcic, K. (2007). The Applicability of Acetylcholinesterase and Glutathione S-Transferase in Daphnia Magna Toxicity Test. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*, 144, 303–309.
- Kamran, T. M., Abdus, S., Muhammed, L. and Tasveer, Z. (2003). Study of the seasonal variations in the physicochemical and biological aspects of Indus River Pakistan. *Pakistan Journal of Biological Sciences*, 6 (21), 1795–1801.
- Kayranli, B., Scholz, M., Mustafa, A. and Hedmark, A. A. (2010). Carbon storage and fluxes within freshwater wetlands: A critical review. *Wetlands*, 1, 111-124.
- Kretschmann, A., Ashauer, R., Preuss, T. G., Spaak, P., Escher, B. I. and Hollender, J. (2011). Toxicokinetic Model Describing Bioconcentration and Biotransformation of Diazinon in Daphnia Magna. *Environ. Sci. Technol.*, 45, 4995–5002.
- Kumar, R. and Pandit, A. K. (2007). Physico-chemical features of water in Hokersar wetland reserve in Kashmir Himalaya. *Poll. Res.*, 26(4), 649-654.
- Lewis, W. M. (2000). Basis for the protection and management of tropical lakes. *Lakes Reserv. Res. Manage.*, 5, 35-48.
- Loughery, J. R., Arciszewski, T. J., Kidd, K. A., Mercer, A., Hewitt, L. M., MacLatchy, D. L. and Munkittrick, K. R (2014). Understanding the Chronic Impacts of Oil Refinery Wastewater Requires Consideration of Sediment Contributions to Toxicity. *Arch Environ Contam Toxicol.*, 66, 19–31.
- Munawar, M. (1970). Limnological studies on freshwater ponds at Hyderabad, India. The distribution of unicellular and colonial phytoplankton in the polluted and the unpolluted environments. *Hydrobiologia*, 36, 105-128.
- Naik, G., Rashid, M. and Balkhi, M. H. (2015). Changes in Physico-chemical Parameters at different Sites of Manasbal Lake of Kashmir, India. *Fish Aquac J.*, 6, 148.
- OECD. (2012). Test No. 211: Daphnia Magna Reproduction Test; Organisation for Economic Co-Operation and Development: Paris, France.
- Okbah, M. A. and El-Gohary, S. E. (2002). Physical and chemical characteristics of Lake Edku water, Egypt. *Mediterranean Marine Science*, 3(2), 27-39.
- Pandit, A. K. (1999). Trophic structure of plankton community in some typical wetlands of Kashmir, India. In: *Limnological Research in India (SR Mishra)* Daya Publishing House. Delhi, pp: 190-224.
- Payne, J. F., Fancey, L. L., Rahimtula, A. D. and Porter, E. L. (1987). *Comp. Biochem. Physiol.* 86C, 233–24
- Peakall, D.B.(1992). *Animal Biomarkers as Pollution Indicators*, Chapman & Hall, London/New York.
- Perez, O., Gold-Bouchot, G., Ortega, A., Lopez, T. and Albores, A. (2002). Effect of pyrene on hepatic Cytochrome P4501A (CYP1A) expression in Nile Tilapia, *Oreochromis niloticus*. *Archives of Environmental Contamination and Toxicology*, 42, 477-485.
- Rasheed, R. O. (2008). Evaluation of heavy metals and polyaromatic hydrocarbons in water, fish and sediments within debendikhan reservoir. Ph.D. Thesis. University of Sulaimania, Iraq.
- Rasheed, S. (2019). Detection of cytochrome P450 in fish- A potential biomarker of aquatic environmental contamination. M.FSc thesis submitted to Faculty of Fisheries SKUAST-K.
- Raut, K. S., Shinde, S. E., Pathan, T. S. and Sonawane, D. L. (2015). Seasonal variations in physico-chemical characteristics of Ravivar Peth Lake at Ambajogai District. Beed Marathwada Region, India. *Journal of Research in Biology*, 4, 258-262
- Reimer, A., Landmann, G. and Kempe, S. (2009). Eastern Anatolia, Hydrochemistry and History.
- Srivastava, J., Gupta, A. and Chandra, H. (2008). Managing water quality with aquatic macrophytes. *Rev. Environ. Sci. Biotechnol.*, 7, 255–266.
- Toma, J. J. (2000). Limnological study of Dokan lake, Kurdistan region of Iraq. M.Sc. Thesis, University of Salahaddin, Arbil.
- Uno, T., Ishizuka, M. and Itakura, T. (2012). Cytochrome p450 (CYP) in Fish. *Environmental toxicology and pharmacology*, 34, 1–13.
- Van Veld, P. A., Westbrook, D. J., Woodin, B. R., Hale, R. C. Smith., C. L. Huggett, R. J. and Stegeman, J. J. (1990). Induced cytochrome P-450 in intestine and liver of spot 95 (*Leiostomus xanthurus*) from a polycyclic

- aromatic hydrocarbon contaminated environment. *Aquatic Toxicol.*, 17, 119-132.
- Vyas, A., Mishra, D. D., Bajapai, A., Dixit, S. and Verma, N. (2006). Environment impact of idol immersion activity lakes of Bhopal, India. *Asian Journal of Experimental Science*, 20(2), 289–296.
- Walker, D. C. H. (1998). *Ecotox. Environ. Safety*, 40, 65–70
- Wetzel, R.G., Otsuki, A. (1974). Allochthonous organic carbon of a marl lake. *Arch Hydro Biol* 73:31–56
- Wetzel, R. G. (1975). Primary Production and River Ecology. In: 2nd Ed. Blackwell Scientific Publication, Oxford.
- Wetzel, R.G. (1983). *Limnology*. In: 2nd ed. Michigan State University C.B.S.College, Philadelphia, New York, Chicago.
- Williams, R., Taggart, T., Ganger, K., Koetsier, T., Johnson, S. and Dinchman, A. (2021). CYP1A expression in freshwater fish of western New York as an indicator of pollution levels. *Ecology and Evolution*, 12, e8526.
- Zutshi, D. P. and Khan, M. A. (1988). Eutrophic gradient in the Dal lake, Kashmir. *Indian J. Env.Health*, 30, 4.

How to cite this article: Saima Andleeb, Irfan Ahmad, Asim Iqbal Bazaz and Oyas Asimi (2022). Cytochrome P4501A Detection and Quantification in Relation to different Water quality Parameters of Lake Wular of India. *Biological Forum – An International Journal*, 14(4): 866-875.