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AGRICULTURAL RUNOFF INDUCED ALTERATION OF WATER QUALITY AND ITS EFFECT ON INHABITING FISH SPECIES IN SELECTED PONDS IN AND AROUND BARGARH TOWN, ODISHA, INDIA

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ABSTRACT

The present study investigates the effect of agricultural runoff on the water quality of a few ponds which are located near paddy fields and are active human use. We further investigated the effect of altered water quality on the inhabiting fish species by evaluating oxidative stress markers and catalase activity in the muscle and liver tissue. Our study showed acidification of pond water, increased nitrate, phosphate, COD, Total Dissolved solids, Total Suspended Solids content, and heavy metals (Pb and Cd) in all the three ponds under study which were found to be above the recommended level for human use. However, the level of Hg in the contaminated ponds was observed to be below the recommended level for human use. One of the ponds under study, i.e. Nua Bandh, was devoid of higher group of vertebrate species like Pisces and exhibited extremely deteriorated water quality. There was increased free radical generation and consequent lipid peroxidation in liver and muscle tissue of fish inhabiting these ponds. The total protein contents in muscle was found to be decreased while it increased in liver tissue compared to fish species inhabiting the control pond indicating elevated detoxification enzyme system and associated proteins in the liver tissue. There was elevated catalase activity in both liver and muscle tissue in the fish species inhabiting contaminated ponds. The present study concludes that the water quality of all the three ponds have been deteriorated due to contamination of agricultural runoff/leachate and are not suitable for direct human use.

KEY WORDS : Agricultural Runoff, Oxidative stress, Water Quality, Ponds

INTRODUCTION

With the commissioning of the Hirakud Dam in the Western part of Odisha on the River Mahanadi, the agricultural activities increased manifold owing to improved irrigation facility and availability of fertile soil. The Bargarh district and its adjoining area became the Rice Bowl of Odisha. The main crop in this area is paddy and the main occupation of the people in this district is farming. However, with adoption of modern agricultural practices like use of agrochemicals, hybrid seeds varieties, pesticide and weedicide to further boost the productivity, the nearby water bodies and rivers are being increasingly getting polluted with leached out/ runoff residues and have been reported to cause adverse health effects on the people directly depending on the water bodies. Agricultural runoff can include pollution from soil erosion, feeding operations, grazing, plowing, animal waste, application of pesticides, irrigation water, and fertilizer. Pollutants from farming include soil particles, pesticides, herbicides, heavy metals, salts, and nutrients such as nitrogen and phosphorus. High levels of nitrates from fertilizers in runoff can contaminate drinking water and cause potentially fatal "blue baby" syndrome in very young infants by disrupting oxygen flow in the blood. Polluted agricultural runoff is the leading source of water pollution in rivers and lakes (wang *et al*., 2019). It

can also trigger algal blooms in coastal waters, and produce "dead zones" in the ocean where there is no oxygen and few fish or wildlife can survive (whittakar *et al*., 2015). Agricultural runoff can create a bad taste and odor in drinking water and contaminate drinking water, well water, and food sources (Ben salem *et al*., 2016; Zhang *et al*., 2017). The pesticides in runoff can accumulate in fish, which can expose people who eat the fish to high levels of these chemicals. Effect of pesticides extend beyond individual organisms and may cause disruption of ecological functioning. Studies show that relentless application of pesticides is one of the major factor effecting biodiversity across the globe.

Fish are the commonly tested aquatic organisms as they are economically important to man and are linked *via* the food chain. Though fishes are non-target aquatic organisms, they are exposed to higher concentration of pesticide residues compared to terrestrial organisms because of the mixing agricultural runoff water. Moreover, in the aquatic environment unlike the terrestrial environment, the body of the organism is bathed by the medium containing the toxicant. Organophosphate pesticides are in wide use throughout the world in agricultural and vector control. They are the most preferred pesticides due to their high effectiveness and low persistence in the environment. These groups of pesticides are neurotoxic to vertebrates by the action of inhibition of acetylcholinesterase (AchE). The wide operation of these pesticides leads to the contamination of inland and coastal waters due to their runoff and results in mass kills of fishes. In the present study, we investigated the effect of agricultural runoff on the water quality of few ponds which are in human use and are located nearby paddy fields. We further investigated the deteriorative effect of altered water quality on the inhabiting fish species by evaluating oxidative stress markers and antioxidant enzyme system like catalase in the muscle and liver tissue.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used in the present experiments were procured from Sisco Research Laboratory (SRL) and Hi Media (India).

Sampling sites and Sampling procedure

The study area taken for the study is Bargarh, a district situated in the western region of Odisha, in India. The district has an area of 5837 km^2 and a population of 1478833(2011 CENSUS). Bargarh is the administrative headquarters of Bargarh district. The district lies between 21.38^0N and 83.62^0E. The average elevation is 117 meters above the mean sea level.

Water samples were collected from four different ponds in and around the Bargarh Town, Odisha. Out of the four ponds under study, three ponds are located nearer to the paddy crop fields and the water from paddy field can enter to the ponds and the vice versa while one pond is located in the township and agricultural runoff was not entering to the pond. The pond located in the township without any agricultural runoff contamination of water was taken as control in the present study.

Fig. 1. Railway pond, 21°19′48.41"N 83°38′21.68"E, ELEVATION-586ft

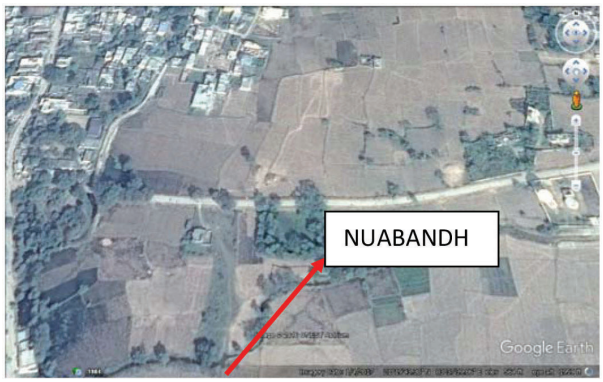

Fig. 2. Nuabandh, 21°19′41.08′′N 83°37′39.70′′E, ELEVATION- 565ft

Experimental Design

The study was performed in two phases. Phase I study was conducted to find out the effect of agricultural runoff on the water quality parameters of the pond ecosystem. In the first phase, water sampling was done in the month of October using water sampling bottles and transported to

Fig. 3. Kumbhar bandh, 21°19′45.66′′N 83°37′59.71′′E, ELEVATION-572ft

Fig. 4. Ruhunía bandh, 21°18′37.67′′N 83°37′53.31′′E, ELEVATION-558ft

Laboratory at Department of Environmental Sciences for analysis of water quality parameters like chemical oxygen demand, Nitrate and Phosphate content, pH, Total Dissolved Solids, Total Suspended Solids. Heavy metals (Pb, Cd and Hg) content in pond water was quantified using Atomic Absorption Spectrometers. Phase II study investigated the effect of agricultural runoff induced altered water quality of pond ecosystem on oxidative stress markers in muscle and liver tissue of fish species. In the second phase, fish species were caught from the three different ponds and biochemical parameters like free radical content, lipid peroxidation, total protein content and catalase activity in the muscle and liver tissue were estimated.

Measurement of pH

The pH meter was standardized against the buffer solutions of known pH values and in the required range. The electrode was kept immersed in the sample taken in a beaker and the pH of the sample was recorded. pH electrode was rinsed thoroughly with every sample before making the measurements.

Measurement of Chemical Oxygen Demand

Chemical Oxygen Demand of the water sample was measured using open reflux titrimetric method. COD was estimated using the following formula:

$$\text{COD (mg/L)} = \frac{(a-b) \times 8 \times N \times 1000}{\textit{volume of sample taken in ml}}$$

Where, a = Volume of FAS consumed in control flask, b = Volume of FAS consumed in experimental flask and N = Strength of FAS

Measurement of Total Dissolved Solid (TDS), Suspended Solid (TSS), Total Solid (TS)

The TDS TSS TS of water sample is determined by gravimetric method. The TSS (Total Suspended Solids) determination is based on the difference of weight of filter paper before and after the filtration and for the TDS (Total Dissolved Solid) determination, the difference of the weights of the beaker before and after by the evaporation is considered. TS (Total Solid) are determined summation of TDS and TSS.

Measurement of Phosphate Content

Phosphate content of water sample was determined by Ammonium molybdate method. Phosphate present in sample water reacts with Ammonium molybdate to form Ammonium molybdic acid in acidic medium which reduced by stannous chloride to give blue coloration. The intensity of colour formed is directly proportional to the amount of Phosphate contained. The OD was taken at 690nm.

Measurement of Nitrate content

Nitrate content of water sample was determined by phenol disulphonic acid method. Nitrates react with sulphuric acid producing nitric acid which in dry condition cause nitration of 6th position of 2,4 Phenol disulphonic acid. The resultant product is a nitro phenolic type indicator which in alkaline medium leads to development of intense yellow coloration owing to the formation of sodium 2,4-disulphonic-5-phenolate.The colour intensity is measured spectrophotometrically to quantify Nitrate and was calculated from standard curved prepared.

Measurement of Reactive Oxygen Species in Fish species

Reactive oxygen species mainly hydrogen peroxide

(H_2O_2) and peroxinitrite ($ONOO^+$) in the blood plasma were estimated spectrofluorimetrically using 2,7-dichlorofluorescein-diacetate (DCFHDA) as suggested by Le Bel *et al*. (1990) and modified by Myhre *et al*. (2003). In brief, 1.494 ml of 0.1 M PBS (pH 7.4) was added to 25 ml of the crude homogenate followed by addition of 6 ml of DCFHDA. The sample was then incubated for 15 min at 37 °C in dark and readings were taken at 488 nm excitation and 525 nm emission. The readings were expressed as fluorescent units per mg of protein and converted to percentage by taking normoxic value as 100%.

Measurement of Lipid Peroxidation in muscle and liver tissue of fishes

The lipid peroxidation was determined spectrophotometrically by measuring the Malondialdehyde (MDA) that is produced as an end product. Each molecule of Malondialdehyde reacts with two molecules of thiobarbituric acid (TBA) to form a colored MDA–TBA complex that can be quantified spectrophotometrically at 531 nm. Briefly, to 250 µl of supernatant, 750 µl of 20% TCA and 750 µl of 0.67% TBA were added. The samples were incubated in a water bath at 85 °C for 45 min. The samples were then kept at room temperature followed by centrifugation at 2000 rpm for 5 min. About 200 µl of the supernatant was taken and the absorbance was measured at 531 nm using spectrophotometer. The malondialdehyde formed was estimated using the molar extinction coefficient of MDA–TBA complex i.e., 1.56×10^5 cm^2 $mmol^{-1}$ and the value thus obtained was expressed in µmol/ml of homogenate

Estimation of glycogen in muscle and liver tissue of fishes

The glycogen was estimated by the method of Kemp *et al.,* (1954). 5% homogenates of gill, brain, muscle and 2% homogenates of liver and kidney tissues were prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of trichloroacetic acid (TCA) and boiled for 15 minutes at 100 ^{0}C and then cooled in running water. The solution was made up to 5 ml with TCA to compensate for evaporation and then centrifuged. From this, 2 ml of supernatant was taken into the test tube and 6 ml of concentrated H_2SO_4 was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm in a spectrophotometer (ELICO Model SL171) against a blank. The standard graph was plotted with D-glucose (Analar supplied by B.D.H. Bombay) by the aforesaid method. The glucose obtained was converted to glycogen by the multiplication factor 0.98 (Hawks, 1951) and is expressed as mg of glycogen/gm wet weight of the tissue.

Measurement of Total protein content of liver and muscle tissue of fishes

Total protein content was estimated by the modified method of Lowry *et al.,* (1951). 5% homogenates of gill, muscle and brain and 2% homogenates of liver and kidney were prepared in 5% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded. The suspended protein residue was dissolved in 1 ml of 1N NaOH. From this 0.2 ml of the extract was taken into the test tube and 5 ml of alkaline copper solution (50 ml of 2% Na_2CO_3 and 1ml of 0.5% $CuSO_4$. $5H_2O$ in 1% sodium potassium tartrate) was added. The contents were mixed well and allowed to stand for 10 minutes. To this 0.5 ml of 50% follin phenol reagent (diluted with distilled water in 1:1 ratio) was added. After 30 minutes, the optical density was measured at 540 nm in a spectrophotometer (ELICO Model SL171) against a blank. The standard graph was plotted by the method of Lowry *et al.* (1951) with bovine serum albumin supplied by Sigma chemical Company, U.S.A. The values were expressed as mg/gr wet weight of the tissue.

Measurement of heavy metal content of water sample using Atomic Absorption Spectrophotometer

10 ml of water sample is adjusted to alkaline pH and extracted with 5 ml of 0.01% dithizone in chloroform. Chloroform phase is then washed and evaporated to dryness. Dithizone chelated lead is re extracted into 2 ml of 5% HNO_3 and analyzed by Perkin Elmer atomic absorption spectrometer (AAS). This typical extraction protocol results in a 5 fold increase of AAS signals in samples as compared to direct sampling. The linearity of the procedure is excellent at the lead levels between 0 to 40 µg/l. Average recovery of lead in spiked water samples is 97%. Coefficient of variations of the procedure are 4.7% at 10 µg/l, 3.1% at 20 µg/l, and 2.7% at 30 µg/l for within day precision; 10.7% at 10 µg/l, 8.2% at 20 µg/l, and 4.1% at 30 µg/l for day to day precision.

Assessment of Catalase activity of liver and muscle tissue

Catalase activity was assayed following the method of Luck (1974). The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme Catalase. From the decrease in absorbance, the enzyme activity can be calculated. REAGENTS 1. Phosphate buffer: 0.067 M (pH 7.0) 2. Hydrogen peroxide (2 mM) in phosphate buffer A 20% homogenate of the different parts of Fish species were prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay. H_2O_2-phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer (Genesys 10-S, USA). The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

RESULTS

Alteration in the physico-chemical properties of pond water

pH of the water collected from all the three ponds surrounded by agricultural fields was found to be acidic in nature compared to control pond. Out of the three ponds, water of Nua Bandh was found to be highly acidic, i.e. pH 6.42 while water of Kumbhar bandh was found to less acidic compared to water of both Ruhunia and Nua bandh. Water of the entire three ponds under study was found to contain higher level of total suspended solid compared to the control pond. Total Suspended Solids contents of the water of Nua Bandh was found to highest, i.e. 5100 mg/l while water of Ruhunia and Kumbhar Bandh contain TSS level 3400 mg/l and 1800 mg/l respectively. Lowest TSS level was observed in water of Kumbhar Bandh. Water of all the three ponds under study was found to contain higher level of total dissolved solid compared to the control pond. Total Suspended Solids contents of the water of Nua Bandh was found to highest, i.e. 5100 mg/l while water of Ruhunia and Kumbhar Bandh contain TSS level 3400 mg/l and 1800 mg/l respectively. Lowest TSS level was observed in water of Kumbhar Bandh. Nitrate content in the water of all the three ponds was higher in comparison to the control pond. Highest level of Nitrate content was observed in the water of Nua Bandh, i.e.78.21 mg/l. Water of Ruhunia and Kumbhar Bandh was found to contain similar nitrate content, i.e. 70 mg/l and 72 mg/l respectively. Phosphate content in the water of all the three ponds was higher in comparison to the control pond. Highest level of Phosphate content was observed in the water of Nua Bandh, i.e. 7.517 mg/l. Water of Ruhunia and Kumbhar Bandh was found to contain similar Phosphate content, i.e. 6.669 mg/l and 5.928 mg/l respectively.

Highest level of COD was observed in the water of Nua bandh, i.e. 512 mg/l compared to Ruhunia, Kumbhar Bandh and control pond. Water of Ruhunia pond was found to contain higher level of COD value, i.e. 504 mg/l which was higher than the COD value of water of Kumbhar Bandh. Water of Kumbhar Bandh was found to contain lowest COD value, i.e. 348. mg/l though the value was found to be much higher than the COD level of control pond as indicated by Table 1.

Level of heavy metals (Pb, Cd and Hg) in agricultural runoff contaminated pond water

The heavy metals content in the pond water mainly Lead (Pb), Cadmium and Mercury (Hg) was quantified using Atomic Absorption Spectrometry. Higher level of Pb and Cd was noted in the pond water collected from Nua Bandh compared to water from Ruhunia, Kumbhar bandh and control pond.

Table 1. Physico-chemical properties of water sample in selected ponds in Bargarh Town

Water quality parameters	Railway Pond (Control)	Ruhunia Bandh	Nua Bandh	Kumbhar Bandh
pH	7.1± 0.3	6.6± 0.6	6.4± 0.2	6.7± 0.3
TSS (mg/l)	980± 82	3420± 169	5100± 177	1800± 55
TDS (mg/l)	900± 61	4960± 172	5120± 188	2600± 151
Nitrate (mg/l)	18± 1.3	70± 11	77± 16	70± 13
Phosphate (mg/l)	0.2± 0.03	6.7± 0.8	7.5± 0.6	5.9± 0.7
COD (mg/l)	130± 0.3	500± 0.3	510± 0.3	340± 0.3

Water from Ruhunia and Nua bandh was observed to possess high level of Pb and Cd compared to control pond as shown in Fig.1. However, no difference was found in the level of mercury among the all pond under study and the level of Hg was below the toxic level.

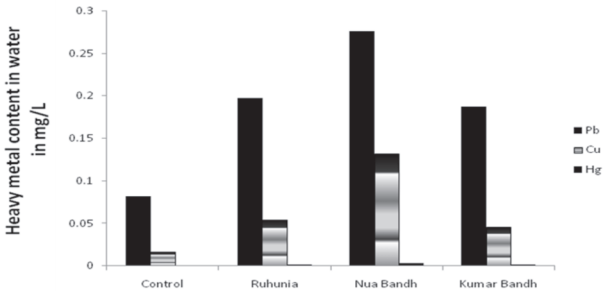

Fig. 1. Showing the level of heavy metals (Hg, Cd and Pb) in the water of control, Ruhunia, Nua bandh and Kumbhar Bandh

Level of Reactive Oxygen Species (ROS) in Liver and muscle tissue of fishes residing in ponds contaminated with agricultural runoff

The level of reactive oxygen species generated in the muscle tissue of the fish species collected from the Ruhunia and Kumbhar bandh was found to significantly higher compare to the muscle tissue of the fish species of control pond. Higher level of ROS in muscle tissue of fish species collected from Kumbhar bandh was noted compared to that of Ruhunia though the difference was not statistically significant. On the other hand, the level ROS in liver tissue was found to be significantly higher in the fish species collected from Ruhunia and Kumbhar bandh compared to that of control pond. However, higher level of ROS was observed in liver tissue of the fish species collected from Ruhunia compared to that of Kumbhar Bandh as shown in Fig. 2.

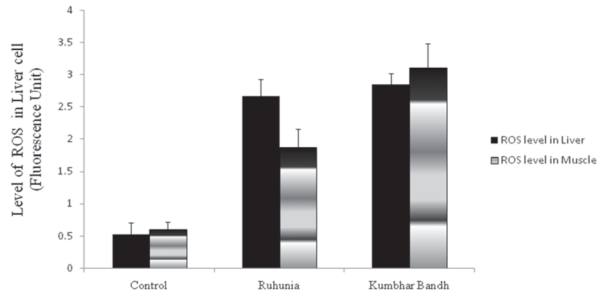

Fig. 2. Showing level of Reactive Oxygen Species (Fluorescence Unit) in liver and muscle tissue of fish species of control, Ruhunia and Kumbhar bandh.

Level of Lipid Peroxidation (MDA) in Liver and muscle tissue of fishes residing in ponds contaminated with agricultural runoff

The level of lipid peroxidation in liver tissue as estimated by the MDA level assay showed significantly elevated lipid peroxidation in liver and muscle tissue of fish species collected from Ruhunia and Kumbhar bandh compared to the fish species of control pond. MDA level was found to be higher in liver and muscle tissue of fish species of Ruhunia compared to that of Kumbhar bandh as shown in Fig. 3.

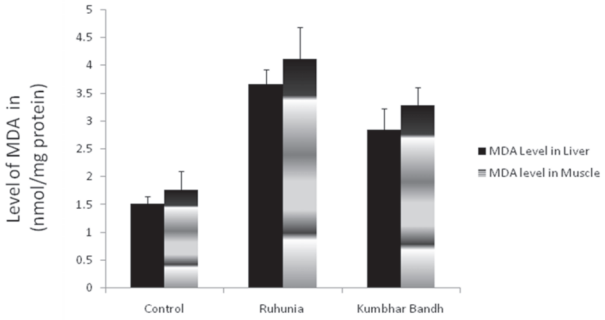

Fig. 3. Showing level of Malonaldehyde (MDA) (nmol / mg of protein) in the liver and muscle tissue of fish species of control, Ruhunia and Kumbhar bandh.

Level of Total Protein content in Liver and muscle tissue of fishes residing in ponds contaminated with agricultural runoff

The level of total protein in the muscle tissue of the fish species collected from the Ruhunia and Kumbhar bandh was found to significantly lower compared to the muscle tissue of the fish species of control pond. Higher level of total protein in muscle tissue of fish species collected from Kumbhar bandh was noted compared to that of Ruhunia though the difference was not statistically significant. On the other hand, the total protein level of liver tissue was found to be significantly higher in the fish species collected from Ruhunia and Kumbhar bandh compared to that of control pond. However, higher level of total protein was observed in liver tissue of the fish species collected from Ruhunia compared to that of Kumbhar Bandh as shown in Fig. 4.

Changes in Catalase activity in liver and muscle tissue of fishes residing in ponds contaminated with agricultural runoff

The catalase in the muscle tissue of the fish species collected from the Ruhunia and Kumbhar bandh was found to significantly lower compared to the

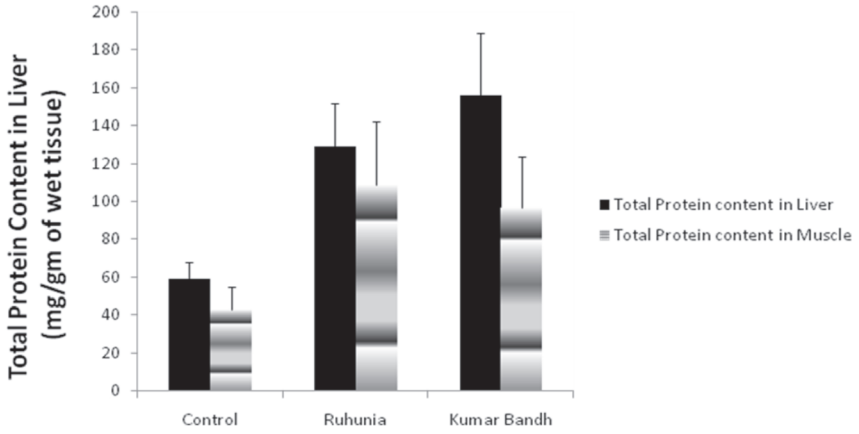

Fig. 4. Showing level of total protein content in the liver and muscle tissue of fish species of control, Ruhunia and Kumbhar bandh.

muscle tissue of the fish species of control pond. Higher catalase activity in muscle tissue of fish species collected from Kumbhar bandh was observed compared to that of Ruhunia though the difference was not statistically significant. On the other hand, the catalse activity of liver tissue was found to be significantly lower in the fish species collected from Ruhunia and Kumbhar bandh compared to that of control pond. However, higher catalse activity was observed in liver tissue of the fish species collected from Ruhunia compared to that of Kumbhar Bandh as shown in Fig. 5.

DISCUSSION

The present study demonstrates acidification of the pond water following contamination with agricultural runoff which may be because of increased Nitrate and Phosphate content of organic and/or synthetic chemical fertilizers induced eutrophication of pond water. Increased algal bloom as a result of eutrophication of water in lentic system and the consequent acidification of water have been well established (Noges *et al*., 2011; Rahul *et al*., 2013). Water quality parameters like level of Total Dissolved Salts and Total Suspended Salts in the pond water of Nua bandh, Kumbhar bandh and Ruhunia was found to be higher compared to control pond and much higher than the recommended level. Water of Nua bandh have been observed to possess highest level of TDS and TSS which could be because it is located downhill of the paddy field and more likely to receive higher quantity of agricultural runoff. Our finding further find support from increased level of Phosphate and nitrate in the water of Nua bandh. Nua bandh was further found to contain higher value of chemical oxygen demand among all the three ponds under study and is having highest level of heavy metal like Pb and Cu in its water. Its water doses not support

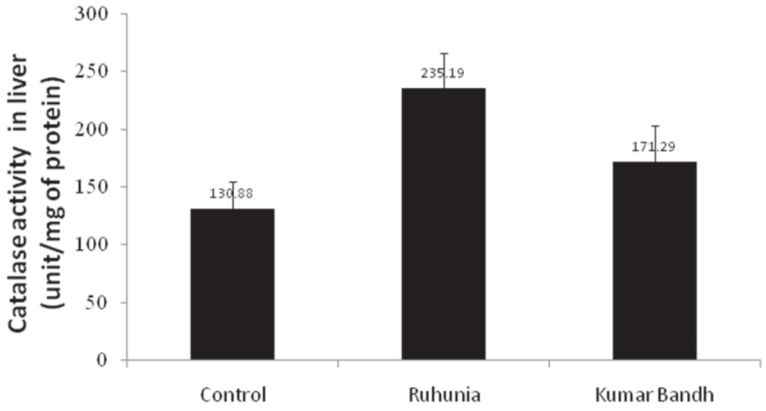

Fig. 5. Showing level of Catalase activity in the muscle and liver tissue of fish species of control, Ruhunia, Kumbhar bandh.

higher vertebrate like Pisces which further indicate that Nua bandh is completely eutrophied and most unsuitable among all the three ponds for human use. However, level of mercury was found to be below the acceptable limit and hence mercury is not a concern in the water bodies nearby paddy field.

Comparison of the water quality of Kumbhar Bandh and Ruhunia indicate that water of Kumbhar bandh is of poor quality having higher acidic pH, TDS, TSS, Phosphate, Nitrate, Chemical Oxygen Demand and heavy metals (Pb and Cd). Fish inhabiting Kumbhar bandh and Ruhunia showed higher level of oxidative stress markers like Reactive Oxygen species in Liver and Muscle tissues of fish species compared to that of the control pond indicating the induction of oxidative damage to these vital organs as a result of toxic effect of agricultural runoff on living system. Excessive generation of ROS could be a result of compromised anti-oxidant defence system which chelate/ prevent ROS accumulation in these organs. In line with the above finding, elevated lipid peroxidation was observed in both Liver as well as Muscle tissue of fish species inhabiting the Kumbhar bandh and Ruhunia which could be a result of free radicals induced damage to cellular membrane leading to increased cellular death in the liver and muscle.

Further, Higher level of lipid peroxidation was observed in liver and muscle tissue of fish inhabiting Ruhunia compared to Kumbhar bandh though the free radical level was observed to higher in Liver and muscle tissue of fish inhabiting Kumbhar bandh. This result indicates the higher efficacy of free radical scavenging system in Liver and Muscle tissue of fish inhabiting in pond water of Ruhunia that neutralize the damaging effect of excessive free radical generated due to agricultural runoff. To assess the status of free radical scavenging system, we assayed the activity of the prominent free scavenging enzyme catalase in both liver and muscle tissue of fish species inhabiting in Kumbhar bandh and Ruhunia. Supporting our previous finding, the catalase activity was found to be elevated in both muscle and liver tissues of fish species inhabiting pond water of Ruhunia compared to that of Kumbhar bandh indicating augmented free radical scavenging and protection from consequent peroxidation of lipid membrane of cells of these vital organs (Abhijith *et al*., 2016; Alttas *et al*., 2010).

The total protein content in muscle and liver tissue of fish species inhabiting Ruhunia and Kumbhar bandh was found to be decreased compared to that of control pond. The reduction of protein content was higher in muscle tissue compared to the liver tissue in fishes agricultural runoff polluted ponds could be because of enhanced protections from augmented free radical scavenging system and detoxification enzyme system in liver tissue compared to that of muscle tissue (Kumari *et al.,* 2014; Hussain *et al.,* 2015).

CONCLUSION

The present study concludes that agricultural runoff degrades the water quality in all three ponds under study and impedes the survivability of fish species. Further, the quality is not suitable for direct human use without treatment. Hence, care should be taken while such contaminated water is being used for any purpose.

Conflict of Interest

The authors declare no conflict interest exists.

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