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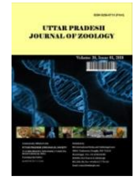

STUDIES ON BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF *Catla catla* (Hamilton, 1822) AND *Clarias magur* (Hamilton, 1822) EXPOSED TO AGRICULTURAL RUNOFF FROM BURDWAN DISTRICT, WEST BENGAL

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AUTHORS' CONTRIBUTIONS

The present work was carried out in collaboration between all authors. Author PC designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author RC managed the analyses of the study. Author BKM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The effluents released from different agricultural fields into the local water bodies leads to pollution of the aquatic environment and it is one of the major concern to the present human civilisation. Pesticides, fertilisers and other components of agricultural runoff may adversely affect the metabolism of aquatic organisms by alternating the enzyme activities. The main aim of the present study is to assess the comparative behavioural and physiological responses of *Catla catla* and *Clarias magur* exposed to agricultural runoff and also evaluating the toxicity of the agricultural runoff of the Burdwan district, West Bengal. The fingerlings were exposed to agricultural runoff of different concentrations (i.e. 0.0%, 10%, 20%, 30%, 40%, 50%) for 72 hours and their behavioural and physiological changes were observed. Both fish species exhibited abnormal behavioural effects such as random surfacing and erratic movement as well as physiological responses such as increased opercular movement, particularly at higher concentration. However, the adverse effects were more pronounced in *Catla* compared to Clarias. The present findings indicate that the agricultural runoff is highly toxic to the living organisms and the results could be used for better agricultural practices and in environmental legislation, respectively.

Keywords: Agricultural runoff; behavioural effects; bioassay; *Catla catla*; *Clarias magur*; Burdwan district.

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1. INTRODUCTION

Green revolution has great impacts on socio-economic movement since its inception from 1960s in USA, Latin America, Asia and other countries of the world. It not only increases crop productivity but also generates employment opportunity and rejuvenates rural economy. However it is achieved at the cost of environmental stability and increased health hazards because, the success of green revolution primarily depends on chemical fertilisers, pesticides and herbicides. A survey showed that pesticide consumption increased to 75,033 tonnes in 1994-95 from a mere 2,330 tonnes at the beginning of the planning period [1]. All these hazardous chemicals are mixed with the nearby water bodies, ponds or lakes as agricultural runoff particularly during rainy season. These chemicals especially the, organo-chlorine and organo-phosphastes are persistent and have long term adverse effects on the environments. Agricultural runoff is water which flows over the agricultural field because of rain, melted snow, or irrigation. As runoff moves, it picks up and carries pollutants like herbicides, pesticides, fertilisers, silt etc. which it can deposit into ponds, lakes, coastal waters. So, agricultural runoff is one of the major problems of present day world. Indiscriminate use of chemical fertilisers and pesticides at different stages of crop production, starting from seed processing to storage of agricultural products, are serious threat to aquatic environment. For pest control biologically active chemicals of pesticides are used which have long lasting effects. Now there is overwhelming evidence that some of these chemicals do pose a potential risk to humans and other life forms and unwanted side effects to the environment [2,3,4]. According to WHO 1990, no segment of the human population is completely protected against exposure to pesticides. It has been reported that around one million people either died or are suffering from chronic diseases due to pesticide poisoning [5].

The toxicity of various agricultural effluents can be measured by means of bioassay and involves different groups of organisms, such as, plankton, algae or fishes. These organisms are exposed to the diluted agricultural runoff for some specific time periods to observe whether there is any adverse effect or not? Therefore, the test helps in assessing the extent of appropriate dilution required for the agricultural effluent before it is discharged into the environment. Thus, the better management of waste water can be done by bioassays [6].

In water quality monitoring fishes are considered as good bioindicator organisms as they can be easily handled in laboratory. In this communication Catla fish (*Catla catla*) and Magur fish (*Clarias magur*) are used as model organisms for evaluating toxicity of agricultural runoff. Moreover, *Catla* is highly palatable fish and preferred for culture due to its high growth rate and taste, and Magur is highly preferred in the diet list of patients having low haemoglobin level and problem in digestion. The purpose of the present study was to evaluate the comparative physiological and behavioural responses of fingerlings of Catla and Magur after exposure to agricultural runoff of various concentrations.

2. MATERIALS AND METHODS

2.1 Method and Place of Agricultural Effluent Collection

Galsi I block (23°20′N 87°42′E / 23.33°N 87.70°E) of Purba Bardhaman district is well known for agricultural practices. The surface runoff of agricultural field of Galsi I block was collected in a 5-litre jar and bought to the laboratory.

2.2 Method of Model Fish Collection and Their Acclimatisation

Fingerlings of *Catla catla* and *Clarias magur* were collected from a local pond. They were brought to the laboratory carefully in plastic bags to avoid any injury and disinfected by giving a bath for two minutes in 0.05% $KMnO_4$ Solution. The fishes were transferred to a large glass aquarium of 30 liter capacity. Fishes were kept there for two weeks for acclimatisation to laboratory conditions. During acclimatisation fish were fed daily with 'Tokya' baby pellet, containing fish meal, wheat flour, Vit. A, C, D, E, B_1, B_2, B_6, B_{12} and minerals etc. Dead fishes, whenever located were removed immediately to avoid fouling of the water.

2.3 Experimental Design

After two weeks of acclimatisation fingerlings were starved for 24 hr. prior to the experiment. Fishes of similar size (5 ± 1.0 cm) were sorted out and divided into six groups of ten fish each for both Catla and Magur separately. Each group was placed in an aquarium (3×1×1 ft) containing 10 litres of water. Each set of experiment with six aquariums for Catla and Magur fishes were levelled as C_{00} C_{10}, C_{20}, C_{30}, C_{40}, C_{50} and M_{00}, M_{10}, M_{20}, M_{30}, M_{40}, M_{50} respectively (Table 3). Suffix 00,10, 20, 30, 40, 50 represent 0% (i.e. control), 10%, 20%, 30%, 40% and 50% concentration of agricultural effluent respectively. Both types of fishes were exposed to the agricultural effluents for 72 h. except the control ones. The water in the test aquariums were well aerated and well filtered. During the experiment no food was administered to fishes [7]. The behavioural and physiological changes in both Catla and Magur with different dilutions in agricultural effluents and control ones were examined carefully (Table 2). The whole experiment was executed in triple sets.

3. RESULTS

Fingerlings of both Catla and Magur exhibited abnormal behaviour and physiological responses when exposed to agricultural runoff. These responses were becoming more pronounced along with the increase of concentration of runoff. At first (10%) both the fishes showed irritability, but with the gradual increase of concentration of agricultural effluents, they started to show frequent surfacing, abnormal opercular movement and mucous secretion. Death of the fishes were first registered at 20% concentration level in Catla and at 40% concentration level in Magur. However, abnormal physiological and behavioural responses were more pronounced in Catla. Abnormal physiological and behavioural responses and mortality rates are summarised in Table 3 and Table 2 respectively. Water parameters of all the test aquarium are compiled in Table 1. Table 1 shows that with the increasing concentration of agricultural effluent, total alkalinity, total hardness, phosphorous and nitrates are gradually increasing but pH and DO are gradually decreasing. However, in the test aquarium only phosphorous and nitratres were above the standard value from the very beginning i.e. at 10% concentration level.

Table 1. Showing the physico-chemical parameters of agricultural runoff of different concentration

Concentration of agricultural effluent (%)	pH	D.O (mg/l)	Total Alkalinity (mg/l)	Total Hardness (mg/l)	Phosphorus (mg/l)	Nitrate (mg/l)
Standard value*	7-8.5	3-5	80-100	20-50	0.01-3	0-3
00 (control)	7.6	4.90	90	27	1.8	1.6
10	7.4	4.78	94	35	2.11	1.9
20	7.2	4.56	97	41	5.18	4.1
30	7.1	4.14	102	46	7.21	6.6
40	6.9	3.76	104	49	8.17	7.9
50	6.8	3.54	106	53	9.10	8.4

**Source: http://neospark.com/images/waterqua.pdf and 'A Fish Farmer's Guide to Understanding Water Quality': https://www.extension. purdue.edu/extmedia/as/as-503.html*

Table 2. Showing the behavioural and physiological responses of Catla and Magur with respect to different concentration of agricultural runoff

Concentration of agricultural runoff (%)	Behavioural and physiological responses of Catla	Behavioural and physiological responses of Magur
00 (control)	Normal swimming behaviour and opercular movement (± 60 times/min) were noticed	Normal swimming behaviour and opercular movement (± 32 time/min) were noticed
10	Irritability of the fishes was noticed with almost normal opercular movement	Slight irritability of the fishes noticed with almost normal opercular movement
20	Fishes started frequent surfacing and random opercular movement (±62 times/min)	With irritability fishes started random opercular movement (±35 times/min)
30	Fishes started mucous secretion and showed frequent opercular movement (±65 times/min) with increased frequent surfacing	Fishes showed increased irritability with frequent opercular movement (±35 times/min)
40	Fishes showed abnormal swimming behaviour with frequent surfacing, more frequent opercular movement(±68 times/min) and increased mucous secretion	Fishes started mucous secretion and showed more frequent opercular movement (±38 times/min)
50	Fishes continued to show abnormal swimming behaviour with increased frequent surfacing, random opercular movement (±71 times/min) and profuced mucous secretion	Fishes showed abnormal swimming behaviour with more frequent opercular movement (±39 times/min) and increased mucous secretion

Table 3. Showing the percentage of mortality of Catla & Magur due to exposure of agricultural runoff of various concentration

No. of fish exposed	Concentration of agricultural runoff (%)	Catla							Magur						
		Aquarium	No of dead fish			Average No. of dead fish	Average No. of survived fish	Percentage mortality	Aquarium	No of dead fish			Average No. of dead fish	Average No. of survived fish	Percentage mortality
			1st Set	2nd Set	3rd Set					1st Set	2nd Set	3rd Set			
10	00	C_{00}	0	0	1	0*	10	00	M_{00}	0	0	0	0	10	0
10	10	C_{10}	0	0	0	0	10	00	M_{10}	0	0	0	0	10	0
10	20	C_{20}	1	0	2	1	9	10	M_{20}	1	0	0	0*	10	0
10	30	C_{30}	1	3	2	2	8	20	M_{30}	0	0	0	0	10	0
10	40	C_{40}	3	4	4	4*	6	40	M_{40}	1	2	1	1*	9	10
10	50	C_{50}	6	4	5	5	5	50	M_{50}	3	2	2	2*	8	20

** Rounded off*

4. DISCUSSION

Abnormal swimming behaviour and other behavioural and physiological responses of fishes in the present experiment indirectly proves the toxic effect of Agricultural run-off on aquatic organisms. However, toxic effect is less pronounced in Magur as it is adopted to live in environmentally stress condition. Magur is an air breathing fish, inhabitant of aquatic bodies characterised by turbid water and low dissolved oxygen levels. The behavioural changes in fishes are due to change in its physiological process. To manifest the biological responses i.e, behavioural as well as physiological changes in fishes the physical, chemical and biological components of the environment play an important role. Many factors are responsible for toxicity of particular pollutants such as animal weight [8], developmental stages [9], period of exposure and temperature, pH, hardness of water and dissolved content of the medium [10,11].

During the experiment when the catla and magur were exposed to agricultural runoff water, they were stressed and showed abnormal swimming behaviour. Blocking of gills by the clay particles might be one reason for this type of abnormal behaviour. In control group of both Catla and Magur fishes normal swimming behaviour were noticed. Earlier similar results were reported by Anderson and Weber [12], where guppies were exposed to dieldrin. Irritability and abnormal swimming behaviour are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and neuromuscular junction [13]. The mucous secretion is shown to have direct relationship with concentration of pollutant in water [14]. Excessive mucous secretion was not observed in control fishes. The increased mucus secretion after the waste water exposure is probably an adaptive response to counter the irritating effect of the agricultural runoff water on the body surface and mucous membrane. In control fish the opercular movements were normal in comparison to both the experimental fishes. In both the fishes the rate of opercular movement increased with increase in effluent concentration, but more rapidly in Catla than Magur. Bull and Mc Inerney [15] also observed similar results in Coho salmon treated with fenitrothion. The fish mortality due to agricultural runoff water exposure depends upon its sensitivity to the various pollutants present in the runoff water. In addition to this, fishes can have their gills blocked by the clay particles of the agricultural runoff and this can result in death. However, few mortalities are might be due to other than agricultural runoff as evidenced by the mortality in control group.

4.1 Agricultural Runoff and Its Impact on Aquatic Organisms

4.1.1 Pesticides and herbicides

The increase in the usage of pesticides and harbicides, particularly insecticides, has mounted considerably in recent years. The careless use of pesticides by farmers are contaminating foodstuffs and posing a health hazard. Modern pesticides are primarily of the organic type, they are either chlorinated hydrocarbons or DDT. These new insecticide pollution causing more terrible effect on other living beings. Chlorinated hydrocarbon insecticides, now among the world’s most widely distributed chemicals. According to Hurley et al. [16], organochlorine compounds could pollute the tissues of virtually every life form on the earth, the air, the lakes and the oceans, the fishes that live in them and the birds that feed on the fishes. The endocrine disruptors, are known to elicit their adverse effects by mimicking or antagonising natural hormones in the body and it has been postulated that their long-term, low-dose exposure is increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer [16,17,18].

The sublethal concentration of pesticide present in any aquatic environment is might not be responsible for rapid mortality directly but the metabolism, normal behaviour of the organism may be affected. The toxic stress on an organism can be assessed by some important physiological parameters such as its respiratory potential or oxygen consumption. The effect of pollutant or toxicants on aquatic organisms is more pronounced on their respiration as they have their outer body surface and respiratory organs such as gills almost entirely exposed to the water, which may contain toxicants. From the agricultural effluents the pesticides may enter into the body of the fish through their gills mainly and the rate of oxygen consumption increases as a result. These destroy a number of valuable aquatic food organisms by destroying their larval stages. These depress photosynthetic activity of phytoplanktons. Recently it is found that pesticides inhibit photosynthesis in algae. Now there is a danger to the life of diatoms which produce half of the earth’s oxygen and form the main food base oceanic life.

4.1.2 Fertilisers

Modern agricultural techniques require the use of millions of tons of artificial fertilisers to improve the fertility of land and obtain a better yield of crop. Only a fraction of the nitrogen-based fertilisers is converted to produce and other plant matter. The remainder accumulates in the soil or lost as runoff. Excess of nitrates and phosphate used as fertilisers seep into ground water or is carried into ponds or lakes. This excess nutrient causes eutrophication and "dead zones" that kill aquatic life. The nutrients, especially nitrates, in fertilisers can cause problems for natural habitats and for human health. Nitrates on entering the intestine are converted into nitrites by intestinal bacteria. These nitrites are entering blood stream. Haemoglobin has stronger affinity for nitrites than oxygen and, therefore, infants suffer from acute lack of oxygen (methemoglobinema or blue baby syndrome).

4.1.3 Soil particles and sedimentation

The most prevalent source of agricultural water pollution is soil that is washed off fields. Rain water carries soil particles (sediment) and dumps them into nearby lakes or streams causing turbidity of the aquatic bodies. According to Bhatnagar et al. [19] turbidity range from 30-80 cm is good for fish health; 15-40 cm is good for intensive culture system and < 12 cm causes stress. Higher turbidity has several negative effects. It can cause increasing of temperature and DO stratification in ponds. Sedimentation can decrease the transport capacity of ditches, streams, rivers, and navigation channels. It can also limit the amount of light penetrating the water, which affects aquatic biota by slowing photosynthetic processes which in turn can lower the production of dissolved oxygen. The resulting turbidity from sedimentation can interfere with feeding habits of fishes, affecting population dynamics. It can cause clogging of gills or direct injury to tissues of aquatic organisms. It also prevents the development of egg and larva of aquatic organisms. It can also be an indicator of the higher concentration of bacteria, nutrients and pollutants in the water.

5. CONCLUDING REMARK

Agricultural runoff is the leading source of water pollution in rivers and lakes. Contamination occurs not only due to current use of agrochemicals but also due to leaching of persistent ingredients from soil. Agrochemical contamination of surface water in a particular region depends on several factors, such as closeness of crop fields to surface water, characteristics of surrounding fields (distance to water bodies from the agricultural field, slope of the field, soil and grassland), and climate conditions (Wind, temperature, precipitation and humidity). Most of the water bodies, particularly in developing world, are dumping ground for the common. Hardly, there is any measure to control the water qualities. In addition to domestic use, water bodies are loaded with sewage, industrial and agricultural runoff, no matter whether it is used for recreation or aquaculture. The pollutants containing effluents are released into the water bodies from industrial and agricultural areas and most of them being highly persistent, in terms of both space and time, their levels fast reach to life threatening marks [20,21]. In India, problem is more grave due to lack of proper policy implementation and illiteracy.

Indiscriminate use of pesticides and herbicides should be discouraged. In order to check the pest population, different alternative methods should be adopted. Now it is being insisted that fruits and vegetables be organically grown, i.e. without chemicals. Pesticides should be sparingly used, only when absolutely necessary. Chemicals like DDT and its derivatives (Aldrin, Dieldrin, Endrin, Lindane, Hepatachlor and Chlordane) should be avoided altogether. Only such compounds should be used which can be easily reduced to harmless compounds. The scientific attention has diverted more and more to biological methods for pest control. IPM, organic farming or GMO might be the alternative choice to reduce overreliance on agrochemicals and for better agricultural management practices.

DISCLAIMER

In India, including West Bengal, there is no legal ban on selling of Magur fish in local market. So it is expected that Magus fish can be considered as model organism for scientific experiment if required.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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