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TOXICOLOGICAL EFFECTS OF INDUSTRIAL EFFLUENT ON THE CHANGES IN WHOLE BODY OXYGEN UPTAKE OF A FRESH WATER FISH, *OREOCHROMIS*

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ABSTRACT

Environmental pollution is mostly due to three interrelated phenomena - the growth of population, the growth of technology and increasing urbanization. One must continue to anticipate increasing density of urban population with all the problems of air, water pollution and waste disposal. The most important thing is the introduction of chemical substances into the environment, which never before existed in nature. Emissions of effluent from various industries into water bodies are having detrimental effects on aquatic species like fish. High percentage of mortality of fish due to the action of the effluent might be due to the pathological changes. The present investigation was designed to study the effect of effluent of a chlor-alkali industry on a fresh water fish, Oreochromis mossambicus, Peters and its toxicological significance. The MAC value of the effluent was found to be 6.41 ml/l for 30 days and to be on the safer side 6.0 ml/l was considered for 28 days of the exposure for sub-lethal toxicological studies. The LC_{10}, LC_{50}, LC_{100} values after 28 days were recorded. It has been observed that all the exposed fishes appeared lethargic after exposure to the effluent. The major clinical symptoms such as inappetance and ataxia appeared immediately after exposure. At higher concentration, the exposed fish showed loss of equilibrium, gradual onset of inactivity, erratic swimming with irregular collision to the inner glass wall of the aquarium were observed. Infection of eyes, exophthalmia and involusions of test fish were observed, when compared to control fish. The whole body oxygen uptake of the control fish was within the range of 0.464 ± 0.036 to 0.468 ± 0.036 mg of O_2 g^{-1} h^{-1} during the entire period of experimentation. However, the whole body oxygen uptake decreased from 0.465 ±. 0.042 to 0.410 ±. 0.036 mg of O_2 g^{-1} h^{-1} after 7 days of exposure, showing a maximum decrease in oxygen uptake by 11.63 % over

the control value. After 7 days of exposure, the whole body oxygen uptake declined significantly to 0.162 ±. 0.024 mg of O_2 g^{-1} h^{-1} after 28 days of exposure, where, a maximum decrease by 65.38% was recorded , over the control value when the effluent exposed fish was transferred to toxicant free tap water medium, no significant recovery was marked rather decrease by 1.21 % and increase by 10.68 % was recorded on 14th and 28th day of recovery, respectively. The correlation coefficient analysis indicated that the control set did not show any correlation with, the exposure period (p = NS). Whereas, in the exposed set a negative significant correlation existed between the exposure period and whole body oxygen uptake (r = -0.976, P≤0.001) with the increase in exposure period, the oxygen uptake significantly declined in exposed fishes.

Keywords: Fish *(Oreochromis mossambicus)*, Chlor-alkali industry, Effluent, Toxic effect, Behaviour, Whole body Oxygen uptake.

INTRODUCTION

To a large extent, pollution is mostly due to three interrelated phenomena - the growth of population, the growth of technology and increasing urbanization. One must continue to anticipate increasing density of urban population with all the problems of air and water pollution and waste disposal. The most important thing is the introduction of chemical substances into the environment, which never before existed in nature. The concept that from the earth it comes, and to the earth it shall return, is no longer valid. Some of the recent discoveries about the metabolic role of some synthetic chemicals are rather alarming.

Werner et al (1978) described the results of experimental work in a specially designed apparatus to study the effects of exposures of gold fish to concentrations of toxicant of 1.8 mg r^{-1} and 0.44 mg $^{-1}$. The fish had been trained before hand to respond to a series of stimuli, and their ability to continue to respond, during toxicant exposure over periods, up to 264 hours was studied. A continuous-flow system was employed to ensure constant concentrations of toxicant in the experimental set up. The lower concentration, 1/25th of the 96 hours LC_{50}, produced definite evidence of behavioral pathology in this period, but even at 264 hours, the fish exposed to the higher concentration were able to perform some complex tasks. Experiments are valuable in demonstrating that effects on behavior were different at different doses, and could occur at concentrations which might otherwise be regarded as too low to have any biological consequences. A number of changes in the biochemistry of fish as a result of exposure to pesticide have been

noted, They found blue gills more sensitive than to other fish, and significant inhibition was produced by a 15 days exposure at 0.001 mg/l^{-1} of several such pesticide, but the more toxic compound azinophos, methyl parathion produced detectable inhibition in 30 days at 0.0001 mg/l^{-1} and the same author (1964) proposed the determination by organophosphorus chemicals.

The effect is on growth and reproduction as well as other physiological effects has been recorded. One early symptom of acute pesticide toxicity (although not specific to pesticides) is respiratory distress, and a number of investigations of the influence on oxygen consumption have been reported. Entry of toxicants into a fish is largely through the gills (Holden, 1974, Patro,2006). With the onset of symptoms of poisoning, the rate of oxygen consumption increases. Lee (1969) exposed gold fish to methyl parathion and dieldrin and found that while the former tended to depress the respiration rate. Cairns et.al, (1977) examined the chronic effects of dieldrin on the pumpkin seed sunfish (Lepomis gibbosous) and found that an exposure to 0.0017 mg l^{-1} (one-ninth of the 24 hour LC_{50}), for twelve weeks, affected the cruising speed and oxygen consumption of the fish. As these tests were of the static type, and the fish were fed, it is likely that the aqueous concentration was even lower than that intended, but some oral in-take probably occurred.

The present investigation was designed to study the effect of the effluent of a chlor-alkali industry on a fresh water fish, *Oreochromis mossambicus*, Peters, and its toxicological significance. *Oreochromis mossambicus* is a mouth breeder and breeds 2-3 times a year, handy to handle in laboratory conditions, better survival rate in lab. Conditions and bimodal feeding habit and gaseous exchange make the fish as an excellent material for toxicity testing and to study the changes, occurring in physiological parameters. The entire idea of selecting effluent and fish for the present study came to my mind from local complaints lodged by the villagers. The report that change of fish colour from white to black and death of number of fishes both in ponds and the river prompted me to think of effluent poisoning. A field study was undertaken to assess the potentiality of the situation. Choudhury (1992 and 1993) reported the effect of solid waste and the leached chemicals of the solid waste of the chlor-alkali industry on freshwater fishes. The above authors also indicated the acute toxic nature of these toxicants and also opined that the wastes showed similar effect like mercury poisoning on fishes. Panigrahi & Misra (1978, 79, 80) and Panigrahi (1980) studied in details about the effect of mercurial compounds on the behaviour, physiology, haematology, and on the macromolecules of freshwater fishes.

No information was available on the direct effect of the effluent of the industry on freshwater fishes. The effluents of the industry leach and enter into neighbouring ponds and

contaminated the ponds, where this Tilapia fish was mass cultured. Hence, this project was designed for instant information on the issue.

MATERIALS

Selection of the Toxicant:

Effluent of the Chlor-alkali industry.

Selection of the test animal:

Oreochromis mossambicus, Peters

MAITENANCE OF FISHES IN LABORATORY AQUARIUM

Oreochromis mossambicus of medium size (30-35 g) were collected from the local nursery. The fish were allowed to grow in the laboratory reservoirs for acclimatization at least for 15 days before starting the experiment. The fishes were maintained in aquarium of 60 x 58 cm. containing 70 liters of water. Chlorine - free tap water was used in both control and experimental aquarium. The water was changed daily. Living earthworms from garden showing no contamination by pesticides were collected and fed daily to both control and exposed fish initially and slowly the diet was changed to pesticide-free chopped goat liver and then to small slices of boiled eggs during holding and through out the experimental exposure period.. After acclimatization, the fish were washed thoroughly with 1 % dilute potassium permanganate ($KMn0_4$) solution, so as to prevent any infection.. The test solution of the experimental aquarium was changed daily so as to maintain the constancy of the toxicant concentration. The experimental aquarium was washed thoroughly to remove any amount of toxicant adhered to glass surface. Exposed fish were observed daily to record any change in behavior compared to control fish. Test fish, *Oreochromis mossambicus* Peters were collected, acclimatized in the laboratory as described earlier. A graded series of concentrations of the effluent ranging from 1 ml l^{-1} to 20 ml l^{-1} were prepared. 10 healthy fish were exposed to each concentration in 10 litre glass jars. The experiments were conducted in chlorine-free tap water at a room temperature of 28 ± 2°c. The mortality rate of test-fish was studied

following the method described by Patro (2002). Observation on the toxicity of the effluent was made at 24, 48, 72, 96 hours and 28 days after the experimental fishes were first exposed.. Individuals showing no respiratory movements, no opercular movements and no response to a tactile stimulus were recorded as dead, and were immediately removed. The test fish exposed to lower range of the effluent were exposed for a period of five weeks to find out the maximum allowable concentration (MAC), where no mortality was noticed and this was expressed as ml l^{-1}. Different values such as LC_{10}, LC_{50}, and LC_{100} were deduced from graphical interpolation.

Table. 1. Physico-Chemical Properties of the effluent

Sl. No	Parameter	Data
1	Temperature (^{0}C)	30.3 ± 1.6
2	pH	9.3 ± 0.5
3	Alkalinity (as $CaCO_2$ in mg 1^{-1}	251.6 ± 32.5
4	Hardness (as $CaCO_2$ in mg 1^{-1}	476.1 ± 18.5
5	Chlorinity (in mg 1^{-1})	1719.6 ± 146.8
6	Dissolved oxygen (in mg 1^{-1})	2.3 ± 0.3
7	BOD (in mg 1^{-1})	21.5 ± 1.9
8	COD (in mg 1^{-1})	326.8 ± 8.8
9	Suspended solids (in mg 1^{-1})	188.7 ± 28.2
10	Total nitrogen (in mg 1^{-1})	2.1 ± 0.5
11	Total phosphorus (in mg 1^{-1})	0.18 ± 0.06
12	Total mercury (in mg 1^{-1})	3.64 ± 0.45

Table.2. Showing water quality of control and exposed water of the aquaria in the Laboratory.

Water Quality	Control	Exposed
pH	7.2±0.5	7.8±0.9
Temperature	28±2^{0}C	28±2^{0}C
Illumination	2200±200 lux	2200±200 lux
Total hardness	78.5±9.8 mg1^{-1}	88.6±11.2 mg1^{-1}
Specific conductivity	3.50x100 µmhos	3.91x100 µmhos
Transparency	0.02-0.025	0.045-0.065

(Transparency was measured in terms of optical density at 550 nm taking double glass distilled water as standard).

Table. 3. Toxicity study data;

Exposure period 30 days	
MAC	6.41 ml.l. $^{-50}$
Used concentration	6.0 ml.l. $^{-50}$
LC Value after 30 days of exposure	
LC_{10}	9.22 ml.l. $^{-50}$
LC_{50}	12.36 ml.l. ^{50}
LC_{100}	23.54 ml.l. $^{-50}$

METHODOLOGY

The whole animal oxygen uptake rates of the test and control fish were measured using five wide mouth 2 liters capacity flasks. Each flask containing the test solution and a test fish was hermetically sealed. A reference flask was kept , without fish to check any change of oxygen concentration during the experiments, due to the presence of microorganisms. Any change of oxygen concentration, caused by the microorganism was computed with the final data. After 30 minutes, the dissolved oxygen of all experimental flasks was determined according to the modified Winkler's method (Panigrahi, 1980 and Patro, 2002). The same procedure was repeated for the control fish. The reduction of the dissolved oxygen concentration equals the amount of dissolved oxygen consumed by the fish in 30 minutes. The oxygen uptake was expressed as mg O_2 g^{-1} h^{-1} (Panigrahi, 1980).

The ventilation rates of both exposed and control fish were counted by the number of opercular movements per minute. The fishes were not disturbed or excited. They were allowed to remain undisturbed.

RESULTS

The whole body oxygen uptake of the control fish was within the range of 0.464 ± 0.036 to 0.468 ± 0.036 mg of O_2 g^{-1} h^{-1} during the entire period of experimentation. (Table- 4. and Fig. 1&3). However, the whole body oxygen uptake decreased from 0.465 ±. 0.042 to 0.410 ±. 0.036 mg of O_2 g^{-1} h^{-1} after 7 days of exposure, showing a maximum decrease in oxygen uptake by 11.63% (Table- 4) over the control value. After 7 days of exposure, the whole body oxygen uptake

declined significantly to 0.162 ±. 0.024 mg of O_2 g^{-1} h^{-1} after 28 days of exposure (Fig. 1 &3 and Table- 4), where, a maximum decrease by 65.38 % was recorded (Table 4 and fig 1&3.), over the control value when the effluent exposed fish was transferred to toxicant free tap water medium, no significant recovery was marked rather decrease by 1.21 % and increase by 10.68% was recorded on 14th and 28th day of recovery, respectively (Table- 4 and Fig.2.& 4). The correlation coefficient analysis indicated that the control set did not show any correlation with, the exposure period (p = NS). Whereas, in the exposed set a negative significant correlation existed between the exposure period and whole body oxygen uptake (r = -0.976, P≤0.001) with the increase in exposure period, the oxygen uptake significantly declined in exposed fishes.

Fig. 1. Showing changes in whole body oxygen uptake of control and effluent exposed fish at different days of exposure

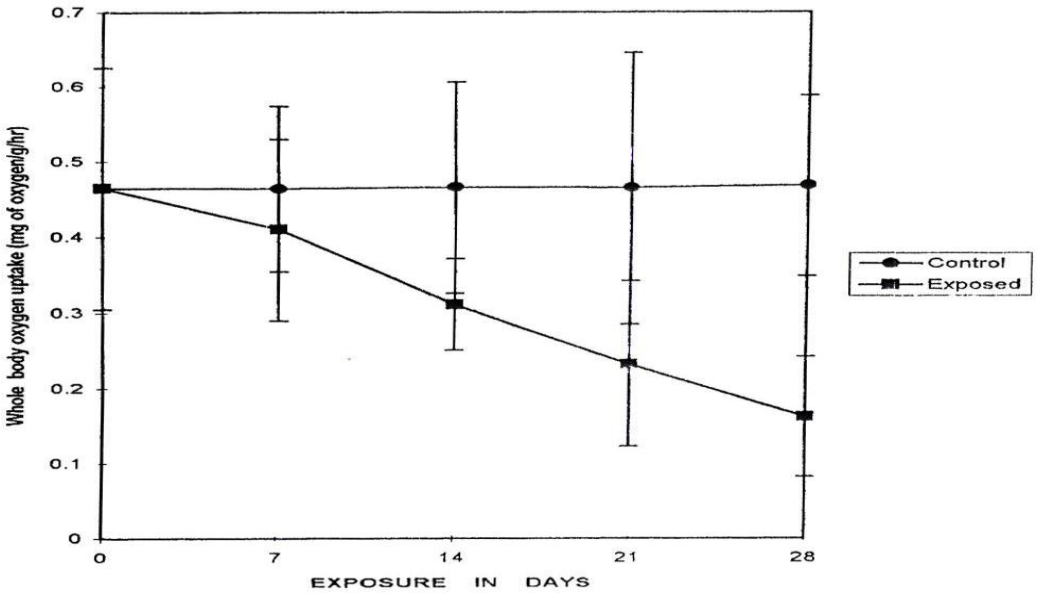

Fig. 2. Showing changes in whole body oxygen uptake of control and effluent exposed fish at different days of recovery

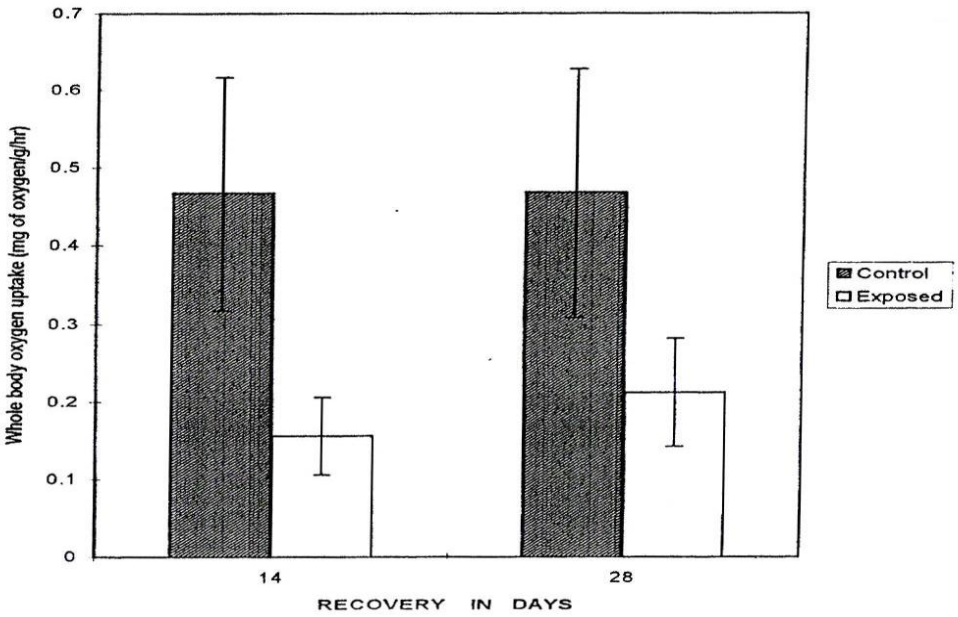

Fig. 3. Showing percent decrease in whole body oxygen uptake of toxicant exposed fishes when compared to control at all exposure periods

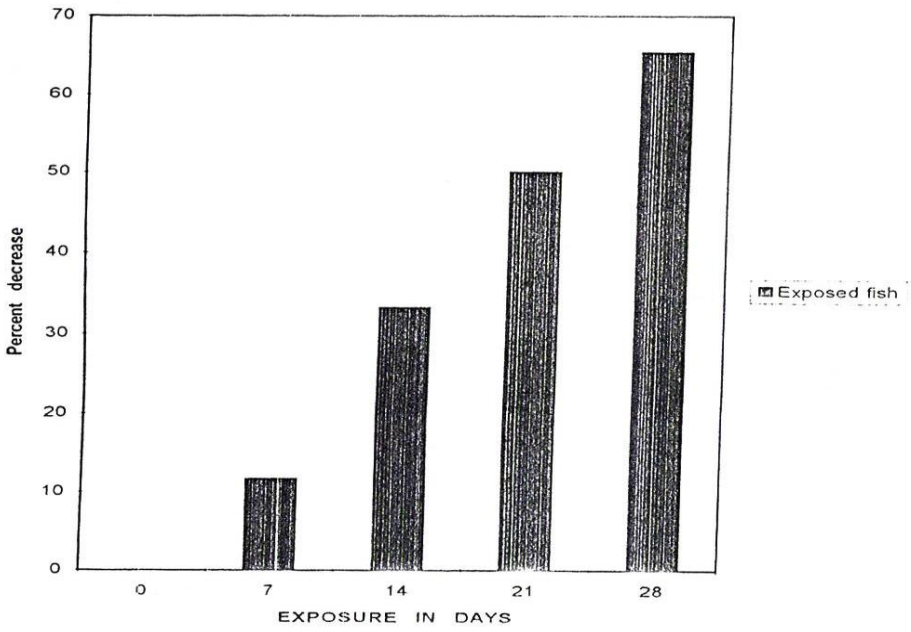

Fig. 4. Showing percent decrease and percent recovery in the effluent exposed fish during recovery periods. .

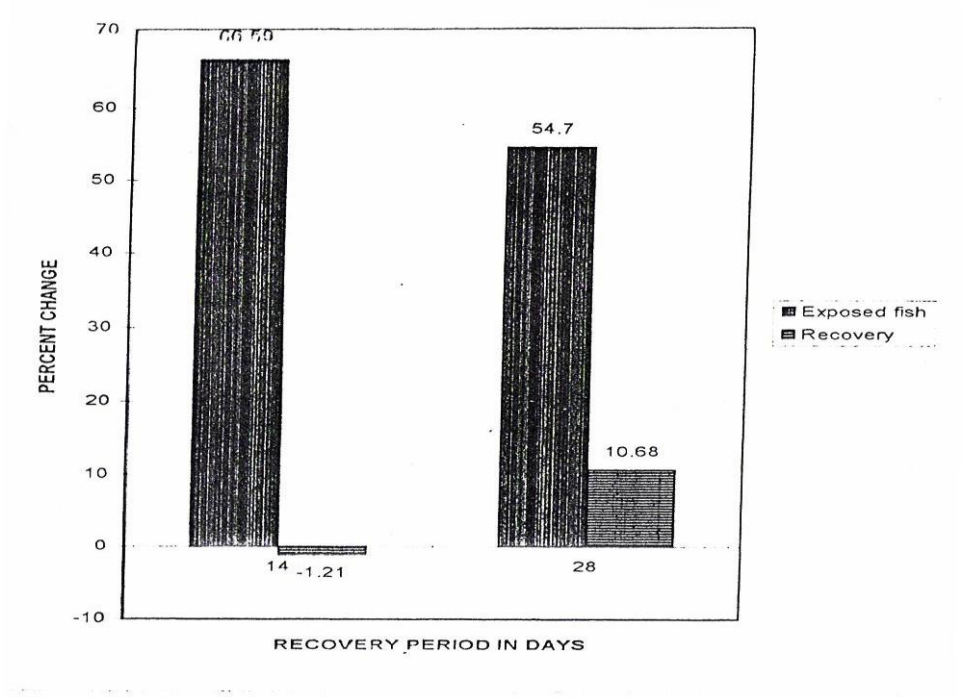

Table. 4 : Showing changes in whole body oxygen uptake (mg of O_2 g^{-1} h^{-1}) in control and effluent exposed fish at different days of exposure and recovery under laboratory controlled conditions. Data calculated from the mean of the samples ± standard deviation. Percent change in whole body oxygen uptake in exposed fish, when compared to control value, was also shown percent recovery was calculated basing on the values of 28th day of exposure. [p= Levels of significance]{'+' indicate increase; '-' indicate decrease}

	EXPOSURE PERIOD IN DAYS					RECOVERY PERIOD IN DAYS	
Status	0	7	14	21	28	14	28
CONTROL	0.465±0.042	0.464±0.036	0.466±0.034	0.465±0.028	0.468±0.036	0.467±0.032	0.468±0.041
EXPOSED	0.465±0.042 NS	0.410±0.036 NS	0.311±0.026 P≤0.05	0.232±0.018 P≤0.01	0.162±0.024 P≤0.001	0.156±0.031 P≤0.001	0.212±0.028 P≤0.01
Percent change, when compared to control. (%)	--	-11.63	-33.26	-50.10	-65.38	-66.59	-54.70
Percent recovery (%)	--	--	--	--	--	-1.21	+10.68

DISCUSSION

Chlor-alkali plants are widely accepted as a potential industrial emission of mercury (Flewelling, 1971). This dragged our attention to the environmental problem caused due to build up of mercury at various zones of the Rushikulya river mouth to which the effluents of a chlor-alkali factory are released. A high level of mercury concentration was noticed in water and sediment. The fishes collected from the river and estuary indicated very high level of mercury in brain, liver and muscle samples of the fish. The effect of the effluent of the factory and the leached chemicals of the solid waste dump, on the estuarine fish was studied and reported. Estuarine fish was collected but these fishes could not survive longer (less than 15 minutes). Hence, it was not possible to study the effect of these chemicals on the whole body respiration or on ventilation rate. Hence, the present experimental study was designed to study the effect of the effluent of the chlor-alkali industry on a freshwater fish. Importance was given to respiratory physiology.

Panigrahi and Misra (1978, 79, 80); Panigrahi (1980) reported the effects of inorganic mercury compound on freshwater fishes. Gouda et al (1981) reported that due to breathing difficulties, the fish were seen with extended operacular flap in fish, *Anabas scandans* when exposed to Sevin and Lindex. Similar observation was not marked in this study. Loss of appetite, ataxia, decrease in body weight, loss of moisture content and decrease in somatic index were some other important behavioural changes marked in this study. Autopsy studies showed a pale, tender and congested liver and brain of the exposed fish. The growth rate of the exposed fish also declined with the increase in the exposure period, whereas, the control fish remained clinically healthy. Small fish have a relatively higher metabolic (oxygen consumption) rate than the large ones (Calabrese et. al, 1975). Respiratory distress is a symptom of acute toxicity (Holden, 1974). Most of the mercurial compounds, mercury based pesticides, mercury contained wastes and effluents, & pesticides affect metabolic processes. The respiration rate in brain, muscle, liver and gill tissue depleted with the increase in exposure period. Liver was affected earlier than brain and gill. Panigrahy (1984) reported drastic depletion in respiratory metabolism in MEMC exposed fishes. The present results showed an inverse correlation between the exposure period and the tissue slice respiration of liver, brain, muscle and gill.

In recovery studies, it was observed that the system could show lower percentage of recovery. In most of the cases, no recovery was marked, when the exposed fish was transferred to toxicant free medium. If at all here and there, in few parameters, partial recovery was

recorded. This we cannot under any circumstances, at1ribute to recovery studies only, where excretion of the toxicant is theoretically expected. As a consequence of excretion, the system might have tried to adapt or acclimatized. In most of cases, it was observed that the exposed fish after prolonged exposure i.e. more than 28 days (the period of exposure) died at a later time. This indicated that once the fish is exposed to a toxicant for a prolonged period, the toxicant no doubt damages most of the vital systems, and metabolic systems, which induces aging. Sahu (1998) observed many parameters to decrease significantly up to 14 days of exposure and after that with the increase in exposure period the exposed Values showed increment. But these values could not reach to the pre-test levels of control values. He also opined that 100 % recovery was not possible in recovery studies, though the toxicant has short half life period, where the effects were more drastic like cholinesterase activity and pigment dispersion. This may force the exposed system to age at the earliest and die early, which we call as premature death induced by the toxicant. Patro, 2007.

Cromer (1962) noticed increase in inhibition of oxygen uptake and CO_2 production in brain slice metabolism. The results reported here are in agreement with the findings of above authors. Many authors agreed that at higher concentration of the toxicant, the respiration rate declined significantly. They also opined that at lower concentrations of the toxicant, the toxicant acts as stimulation. This is probably valid for mercurial compounds, where the mercurial compound initially increased the respiration rate, then after prolonged exposure, the respiration rate declined significantly. We also agree and support the same line of thought. But we feel, probably this might be a probability, at very low concentration of the toxicant and that must be much less, when compared to the MAC dose. It was observed in the present investigation that ventilation rate increased significantly in the exposed fishes, when compared to control fishes Panigrahi (1980), Panigrahy (1984), Samant (1989) and Sahu (1989) Patro (2002) showed higher ventilation rate of the fish, when exposed to a toxicant. But we feel, the higher ventilation rate cannot be attributable to higher respiration rate. It was observed that though the ventilation rate increased, during the same period, respiration rate decreased significantly. The probable cause of increased ventilation rate might be due to the fact; the fish might be engulfing and pumping a lot of water through the gills, due to deficiency of oxygen in the body. The deficiency might due less absorption of dissolved oxygen, might be due to ineffective functioning of the gills. Probably, a cover layer acted as a barrier between the gill filaments and water and this layer was

formed might be due to the pesticide. The real mechanism of formation of a layer or coating is yet to be worked out in details to reach to a logical conclusion.

CONCLUSION

The effluent of the industry without proper treatment is discharged into Rushikulya River, which joins the estuary at a small distance. Very recently, the industry instead of discharging the effluent into the river, now, they are storing the effluents in small tanks, nearer to the industry. It was observed that due to storage, higher amount of effluent is now soaking and leaching to the peripheral areas. All the ponds nearer to the soaking pits, get the leached effluent in addition to the leaching are carried by run off water and enters into ponds and aquatic bodies. In ponds, the fishes come in contact with the leached chemicals of the effluent and solid waste and absorb the killer chemical (i.e. mercury) and finally suffer because of the killer chemical.

Hence, the effluent should be diluted for possible use as a stimulant ill the crop fields. But we should not forget the bio-concentration of mercury and biomagnifications of mercury in a food chain, jn the ecosystem, which can be hazardous and an incident similar to Minamata Bay incidence or the incidence at Niigata prefecture may not be ruled out. Hence, proper care should be taken, while handling the issue at early phases. Protection and preservation of the environment is more important then short term benefits. Environmental protection and control laws should be modified as per need and strict rules should be adopted and stringent punishment should be imposed on the polluter for a better and purer environment for the future generations. Hence, all care should be taken to treat the effluent properly before discharge into the environment. The escaping mercury should be recycled hundred per cent, to ensure safety. Careful handling and disposal of the waste is an important factor in Environmental Management. We must keep in our mind the bio-concentration and bio-magnification factor operation in natural ecosystems, while handling killer chemicals and discharging the liquid wastes into the environment, in a mass scale. Because, these killer chemicals enter into human body though the food chain and ultimately MAN suffers.

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