Impact of Mercury Containing Industrial Effluent on the Changes in Clinical Symptoms, the Brain Somatic Index and Hepato Somatic Index of fresh water fish, *Oreochromis mossambicus*, Peters.

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ABSTRACT

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. Autopsy studies revealed that the liver and brain of exposed fish were congested, pale and tender. The liver somatic index and brain somatic index decreased (r = -0.989, p :S. 0.001 and r = -0.934, p :s 0.001) with the increase in exposure period, when compared to control fish (r = 0.897, p :S. 0.05 and r = 0.946, p :S. 0.0 1). The percent inhibition of BSI and LSI increased in exposed fish with the increase in exposure period. The control fish remained clinically healthy throughout the period of experiment indicate the percent decrease in BSI & LSI in exposed fish when compared to control fish. The percent decrease of BSI, increased. With the increase in exposure period except on 28th day of exposure and a maximum decrease of 14.46 % was recorded on 21 set day of exposure. In case of LSI, a similar trend was observed but here the index decreased maximum by 32.24 %, when compared to control fish, after 28 days of exposure. When the fish was transferred to toxicant free medium, no recovery was marked, rather percent decrease increased up to 27.81 % was recorded in BSI, whereas, further depletion in LSI was marked. A maximum of 44.3 % decrease on 14th day & 39.4 % decrease on 28th day of recovery was marked. The two ways analysis of variance ratio test based on the data related to BSI and LSI of control and exposed fish. No significant difference between rows and columns was observed, BSI data indicate the existence of significant difference between rows, LSI data and non-significant difference between columns, When the exposed fish were transferred to toxicant-free tap water, no significant recovery in their activity was observed.

Key Words: *Fish (Oreochromis mossambicus), Chlor-alkali industry, Effluent, Toxic effect, Behaviour, BSI & LSI*

INTRODUCTION

Environmental pollution is the unfavorable alteration of our surrounding through direct or indirect effects on changes in energy pattern, radiation levels, chemical and physical constitution and abundance of organisms. Mount (1962) described the result of endrin poisoning in blunt nose minnows, as indicative of disorders of the central nervous system, commencing with rapid, jerky movements of the body and fins, an increase in ventilation rate, and insensitivity to external stimuli. The fish then moved to the surface, swimming slowly and sometimes backwards, and convulsions followed. These symptoms enhanced until equilibrium was lost and the fish often barrel-rolled or spiraled at intervals before respiration eventually ceased.

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 our 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 us 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 Oreochromis mossambicus was mass cultured. Hence, this project was designed for instant information on the issue.

MA TERIALS & METHODOLOGY

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.$^{-50}$

BEHAVIORAL STUDIES

Long term sub-lethal effects resulting from chronic exposure of organisms to lower levels of toxicants probably have the most important effect on the aquatic organisms. Effluent in the aquatic environment was recognised 2-3 decades back as a serious problem. A good number of workers dealt this problem to find out the impact of different types of effluents on aquatic flora and fauna. Panigrahi (1980) reported the behavioral changes observed in inorganic mercury exposed fresh water fishes. Panigrahy (1984) reported that fishes showed a change in behavior when exposed to Emisan-6 (MEMC). Macleod and Passah (1973) reported the behavioral changes induced by mercury in Rainbow trout. Larson and

Lewander (1973) reported metabolic effects of starvation in the eel, Anguilla Anguilla L., Gibilin and Massaro (1973) observed behavioral changes induced by methyl mercury in Rainbow trout Stone et al. (1977) reported changes in body weight, kidney and liver weight in Japanese quail induced by lead. Bhatia et. al. (1973) reported behavioral changes induced by dieldrin in albino rats.

During the acute toxicity studies fish were observed for behavioral changes influenced by the effluent containing mercury collected from a chIor-alkali industry. Experimental fishes were sacrificed; brain and liver were dissected out carefully and washed thoroughly with distilled water. The sacrificed fishes were weighed. Brain and liver were separated and weighed carefully in a single pan electric balance and the different somatic indices were calculated. The individual changes in body weight of both control and experimental fish were noted at 7-day interval and the percent change in body weight was computed.

OBSERVATION

All the exposed fish appeared lethargic after exposure to the effiuent. The major clinical symptoms such as inappetance and ataxia appeared immediately after exposure. At higher concentration of the effluent of the industry, the exposed fish showed erratic movements. The other signs of toxicity such as loss of equilibrium, gradual onset of inactivity, erratic swimming with irregular collision to the inner glass walls of the aquarium were observed.

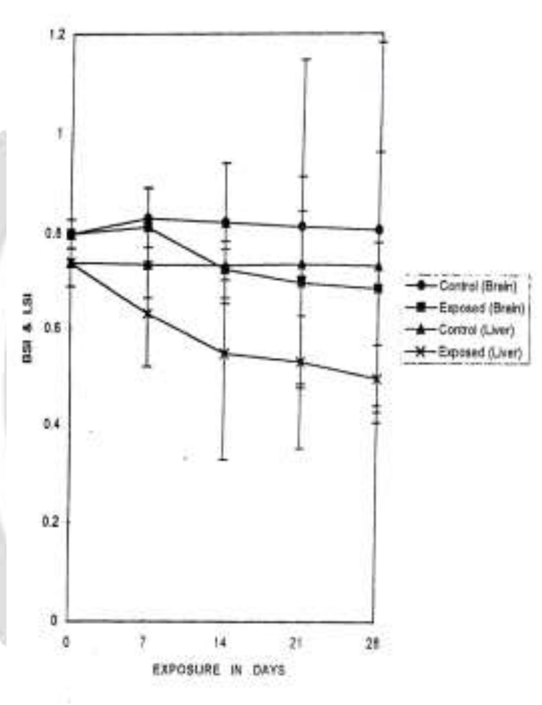
Infection of eyes, exophthalmia and involusions of test fish were observed. Identical symptoms were reported by Panigrahi and Misra (1978) Panigrahi (1980) and Panigrahy (1984). Exposed fish could not regain their pre-exposed activity after transferring the exposed fish to effluent free water in recovery studies.

RESULTS

Autopsy studies revealed that the liver and brain of exposed fish were congested, pale and tender. The liver somatic index and brain somatic index decreased (r = -0.989, p :S. 0.001 and r = -0.934, p :s 0.001) with the increase in exposure period (Fig. 2.3 and Tab.- 4), when compared to control fish (r = 0.897, p :S. 0.05 and r = 0.946, p :S. 0.0 1).

The percent inhibition of BSI and LSI increased in exposed fish with the increase in exposure period (Tab-.4). The control fish remained clinically healthy throughout the period of experiment Table 5 indicate the percent decrease in BSI & LSI in exposed fish when compared to control fish. The percent decrease of BSI, increased. With the increase in exposure period except on 28th day of exposure and a maximum decrease of 14.46 % was recorded on 21 set day of exposure. In case of LSI, a similar trend was observed but here the index decreased maximum by 32.24 %, when compared to control fish, after 28 days of exposure (Table - 5 and Fig. 4). When the fish was transferred to toxicant free medium, no recovery was marked, rather percent decrease increased up to 27.81 % was recorded in BSI, whereas, further depletion in LSI was marked. A maximum of 44.3 % decrease on 14th day & 39.4 % decrease on 28th day of recovery was marked. Table II indicate the two ways analysis of variance ratio test based on the data of Table-4 related to BSI and LSI of control and exposed fish. No significant difference between rows and columns was observed in Table - 4, BSI data (Table - II). Table - III indicate the existence of significant difference between rows of Table - 4, LSI data and non-significant difference between columns of the table - 4, LSI data (Table - III). When the exposed fish were transferred to toxicant-free tap water, no significant recovery in their activity was observed.

Fig. 2 . Showing changes in BSI and HIS / LSI in control and effluent exposed fish at different days of exposer.



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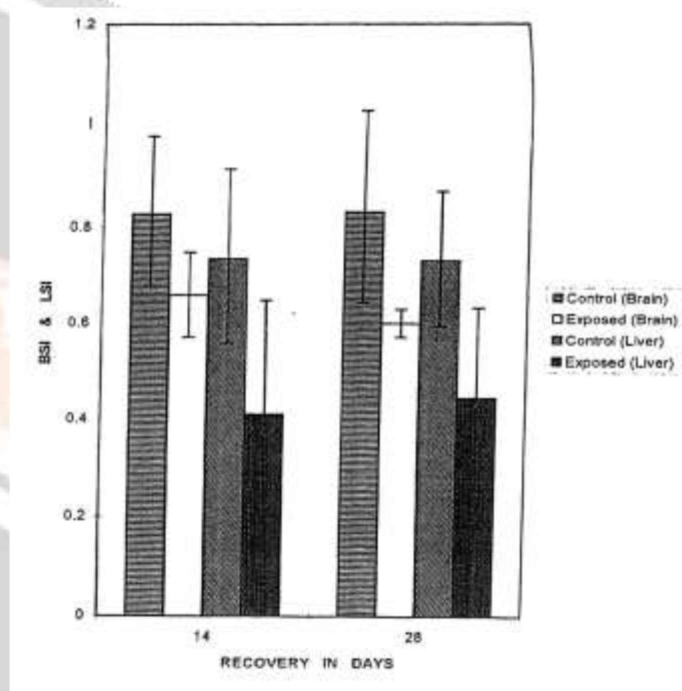

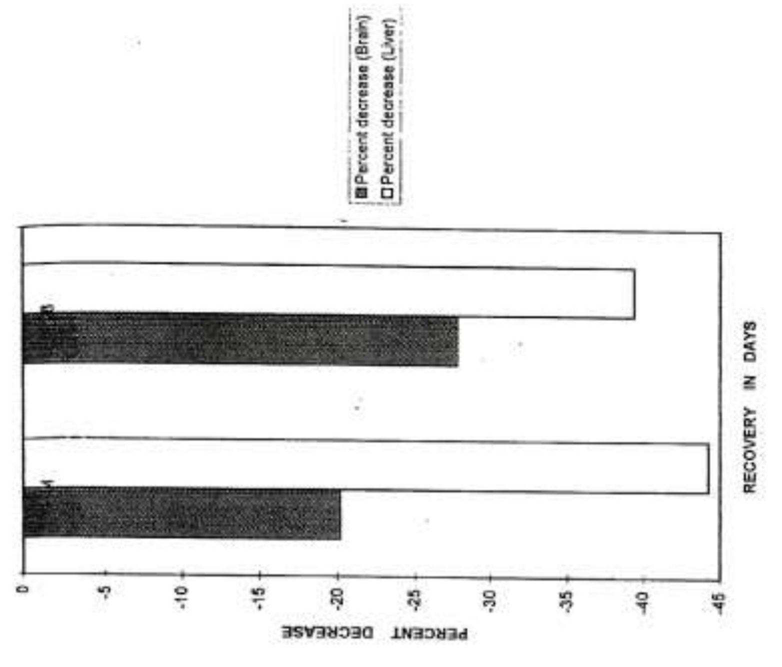

Fig.. 3 . Showing percent decrease in BSI & HIS / LSI in Effluent exposed fish at different days of recovery.

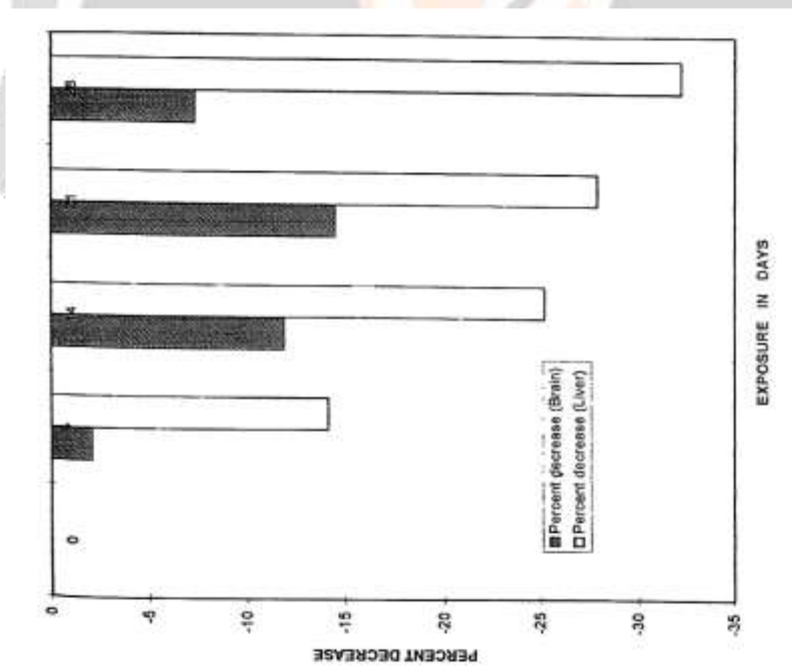

Fig...4. Showing percent decrease in BSI & HIS/ LSI in Effluent exposed fish at different days of

Table- 4 : Changes brain somatic index and liver somatic index in control and exposed fish at different days of exposure and recovery. Data calculated fom the mean of the samples.

Parameter	EXPOSURE PERIOD IN DAYS						Recovery in days	
		0	7	14	21	28	14	28
BSI	Control	0.799	0.832	0.824	0.816	0.811	0.828	0.834
	Exposed	0.799d	0.814c	0.726c	0.698b	0.687b	0.661b	0.602a;
LSI	Control	0.739	0.736	0.734	0.738	0.73	0.738	0.736
	Exposed	0.739d	0.632c	0.549b	0.532b	0.498a	0.411a	0.446a

(a = $P \leq 0.001$; b = $P \leq 0.01$; c = $P \leq 0.05$ and d= P- Not significant)

Table- 5 : Percent change in BSI and LSI in exposed fish, when compared to control. Data calculated from mean of the samples.

Parameter	EXPOSURE IN DAYS					RECOVERY IN DAYS	
	0	7	14	21	28	14	28
BSI	0.0	-2.16	-11.89	-14.46	-7.27	-20.16	-27.81
LSI	0.0	-14.13	-25.2	-27.91	-32.24	-44.3-0	-39.40

.DISCUSSION

The rapid absorption of the effluent along with mercury through the gill, skin and gastro-intestinal tract of fish is well evident (Panigrahi, 1980). The toxicity of effluent becomes more apparent in a very shorter period in aquatic animals. Panigrahi (1980) documented the detail behavioral changes in relation to inorganic-mercury intoxication. Panigrahy (1984) reported the impact of MEMC on Anabas scandens, a fresh water fish and noted a substantial change in behavior of the test fish. Although he has observed swelling of eyes and consequent blindness. Such type of symptom was not observed in Nuvan exposed fish, probably due to absence of mercury as a base in the pesticide. Higgins (1974) and Macleod and Passah (1973) reported that loss of appetite, loss of weight , nervousness, dizziness, loss of equilibrium, erratic swimming and gradual onset of inactivity were the sub clinical effects of inorganic mercury intoxication. Panigrahy (1984) also reported similar symptoms relating to mercury based fungicide. Neurological damage in inorganomercury intoxicated fish relating to behavioral studies was also reported (Panigrahi 1980), and he opined that the damage was caused only by inorganic mercury.

The observed depression in active metabolism in effluent exposed fish were indicative of damage to nervous tissues, inhibition of enzymes or a vital system was totally in agreement with Panigrahi (1980) and Panigrahy (1984). Patro (2006) Fry (1957) demonstrated the depression in active metabolism directly reduced "scope for activity". This in turn may result in decreased growth, impaired swimming ability with erratic out burst at times. Loss in weight due to starvation was noted by Larsen and Lewander (1973). But the loss due to starvation was very less. Patro (2016), Panigrahi and Misra (1978, 1980) reported the loss of body weight due to mercury intoxication and confirmed that this loss in body weight was only due to mercury stress. In addition, expose fish maintained their feeding habit only after a short span of time. Hence the weight loss cannot be correlated with starvation but can only be related with mercury based effluent intoxication. Where as, the control fish showed increase in weight. The differences observed between control fish and exposed fish in terms of body weight was only due to the administration of mercury based effluent in the exposed aquarium.

Patro (2009), Borg et al. (1970) reported inappetance, muscular weakness, ataxia and loss of body weight as the main clinical symptoms of mercury poisoning. On autopsy, he found muscular atrophy, which can be correlated with the weight loss in the goshawks. Hanko et. al. (1970) reported loss of appetite, weakness of the extremities, excitation in the animal and loss of body weight due to mercury poisoning in chickens. A decline in liver somatic index and brain somatic index in exposed fish could be correlated with the degeneration of cells and decrease in other macromolecular variables caused due to the effect of mercurial compounds present in the effluent. The observed changes are in agreement with Panigrahi (1980), and Panigrahi and Misra (1978, 1979, and 1980). The same authors opined and confirmed that the observed changes were solely due to the mercurial compound. Hence, here the observed changes were only due to mercury present in the effluent. Concisely, at this stage it is not possible to establish the cause of loss in body weight and decrease in liver and brain somatic index, except a generalized comment on the behavior of the exposed fish toxicant stress that the observed symptoms were most probably be due to mercury intoxication. Bhatia et al. (1973) reported that change in behaviour in test fish had some relationship with insecticide intoxication. Panigrahi (1980) reported significant change in the behaviour of the exposed fish in mercurial toxicity, when compared to control fish. Panigrahi (1984) also reported identical findings like Panigrahi (1980) and also opined in a similar interpretation. Rath and Misra (1980) reported the change in behavioral activity of fish exposed to dichlorvos and confirmed the idea that the change in activity was related to toxicant intoxication, which is totally agreeable with our observation in this study.

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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