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Comparative Toxicity of an Effluent from a Chlor-Alkali Industry and $HgCl_2$

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Release of waste waters from industries severely affect aquatic flora and fauna. Effluents from industries involved in the production of pesticides, or from industries utilizing toxic substances in their production processes are of particular concern. One such industry, the chlor-alkali industry, utilizes mercury as a cathode in an electrolytic process. Though no mercury is being utilized in the production process, losses are inevitable during washings (Skei 1978). Release of mercury into the aquatic environment has received much international attention following the tragedies in Japan in the 1950s and the ornithological disaster in Sweden in the 1960s. Since then, attempts have been made to evaluate the toxicity of effluents in a number of ways. One of the most important ways is to determine the minimum concentration of industrial effluents that kill fish in a particular period. Only with the help of such bioassay experiments can the toxicity of any pollutant in an effluent be established (Sprague 1973), though its toxic effect on aquatic systems also depends on the latter's quantity, volume and flow of the water, as well as the nature of the bottom sediment. Still, more valuable is the evaluation of the LC_{50}, i.e. the concentration of the substance which kills 50% of the organisms. Long-term exposure experiments of Mount & Stephan (1967), Herbert et al. (1965), Edwards & Brown (1967) and Warren (1971) support the view of the development of application factors which are no doubt important, but the LC_{50} values are also quick and useful for asserting harmful effects of a pollutant.

The present study reports the toxicity assessment of the effluent from a chlor-alkali factory, Jayshree Chemicals Ltd., Ganjam, Orissa (30 kms away from the town of Berhampur) The reported production capacity of the plant is 50 tons of NaOH per day (Satpathy 1984). The effluent discharged, 50,000 l per day, contains mercury ranging up to 1.5 mg/l (Shaw et al. 1988), much more than the permissible limit of 0.01 mg/l set by the Central Board for the Prevention and Control of Water

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Pollution, India. The effluent enters the Rushikulya River Estuary and finally the Bay of Bengal. This paper was also designed to determine whether the mercury present in the effluent was toxic or whether the toxicity involved some other parameters of the effluent as well.

MATERIALS AND METHODS

Effluent from the chlor-alkali plant was collected in ten 40 l plastic containers. Ten such samples were taken at 15 day intervals. The containers were brought to the laboratory immediately and the effluents were transferred to a large plastic container. The parameters to be analyzed were measured within 2 days. pH was determined by a digital pH meter (ELICO, India). Alkalinity, hardness, chloride, DO (dissolved oxygen), IDOD (immediate dissolved oxygen demand), BOD (biological oxygen demand), COD (chemical oxygen demand), total phosphorus, total nitrogen and the reactive silicate were determined following the "Analytical Methods Manual" (EC 1979). For mercury determination, ECIL Analytical Method (1981) was followed.

Two freshwater fish, Anabas scandens and Tilapia mossambica, were selected for the toxicity study. A.scandens has an air breathing system (bimodal gaseous exchange), while T.mossambica does not. However, both species are known for their resistance to disease and can easily be maintained under laboratory conditions. The fish, 10-12 cm (same age group), were collected from the nursery of the Fisheries Department of Berhampur, Ganjam (Orissa). They were treated with $KMnO_4$ (5 mg/l) to kill external parasites (Curtis et al. 1979). After the treatment, the fish were transferred to large aquaria and allowed to acclimatize for a minimum period of 15 days. Since the experiment was conducted for over 4 months, the fish were collected in batches and acclimatized for 15 days. They were fed chopped goat liver, the feeding being suspended two days before the experiment.

Toxicity experiments were performed in cylindrical glass jars of about 30 l capacity. During the experiments, the jars were wrapped with a wet thick cloth to maintain the temperature at 30 ± 2°C. For each experiment, four sets of jars were taken. Two sets were aerated to maintain the dissolved oxygen level around 6.5 ± 1 mg/l and the other two were not. Out of four, two sets (one aerated and one non-aerated) were for A.scandens and the other two for T.mossambica. The number of jars in each set were 11 (1 control + 10 experimental) in case of the effluent treatment and 9(1 control + 8 experimental) in case of the $HgCl_2$ treatment. The test concentrations, either with $HgCl_2$ or effluent, were made using tap water as diluent. Simple tap water served as the control medium. Ten fish were transferred to each jar. Dissolved oxygen levels of each container were determined at 24, 48 and 72 hours. Dead fish were also counted and removed at these time intervals. A stock

solution of $HgCl_2$ was used to obtain mercury concentrations of 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 and 1.8 mg/l. The experiment was repeated 10 times and the mean value of mortality then calculated. For the effluent tests, the percentage dilutions were 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%. Effluent experiments were conducted immediately after the collection of the effluent. The mean mortality values of the results of 10 tests were determined and presented as a single study.

Methods described by Finney (1971) for LC_{50} and Feducial limit calculations were used. The percentage mortality was converted to probit of kill and plotted against the log of concentrations, and LC_{50} values for 24, 48 and 72 hours of treatment were determined. Patterns of change in the DO levels in the non-aerated aquaria were also plotted. Difference 't' test (Misra and Misra 1983) was performed to determine the significance of the difference in the DO levels of the exposure media with increasisng concentrations of the toxicants. Difference 't' test was also done to determine whether any significant differences in the LC_{50} values were obtained under aerated and non-aerated conditions.

RESULTS AND DISCUSSIONS

Results of the analysis of the physico-chemical parameters in effluent samples are presented in Table 1. Characteristics which impart a toxic nature to effluent are COD, BOD, mercury, alkalinity and pH. Values of all parameters were found at high levels. Mean BOD and COD values were as high as 68.9 ± 17.84 mg/l and 448.1 ± 119.71 mg/l, respectively. Standard deviations indicate large fluctuations in the different batches of effluent. The mercury level in the effluent was also alarming. The total mercury level was 0.68 ± 0.46 mg/l, out of which 0.15 ± 0.18 mg/l was present in soluble form. Thus, the effluent contained much less mercury in soluble form. However, the soluble mercury levels were also much more than the safety limit of 0.01 mg/l set by the Central Board for the Prevention and Control of Water Pollution, India. The pH (10.31 ± 0.98) and the alkalinity (620.56 ± 384.63 mg/l) values were also very high . Though hardness and chloride levels were higher than levels which might affect freshwater fish, the values were insignificant in comparison to those in the aquatic system (Estuary) in which the effluent is discharged.

The effluent was found to be highly toxic; 90% of A.scandens and T.mossambica died at 60% and 50% of the effluent concentration, respectively. At concentrations of 80 to 100% effluent, both species of fish died within 5-10 minutes. Before death, they showed quick, almost vibratory opercular movements and signs of distress and moved about quickly. With the $HgCl_2$ treatment, however, such type movements were not observed at 1.5 mg/l, T.mossambica, and 1.6 mg/l, A.scandens, where 100% mortality occurred. Instead, fish showed impaired swimming ability, paralysis and ataxia.

Table 1. Analysis of physico-chemical parameters.

Parameters	Mean values of the effluent samples analyzed ± standard deviation
Color	Mostly whitish, sometime reddish
Odor	Mostly with the odor of chlorine (pungent)
pH	10.31 ± 0.98
Suspended solids, mg/l	598.59 ± 287.88
Alkalinity as $CaCO_3$, mg/l	620.56 ± 384.63
Hardness, mg/l	646.35 ± 309.24
Chloride, mg/l	2515.14 ± 1920.37
Total phosphorus, mg/l	0.10 ± 0.01
Total nitrogen, mg/l	1.96 ± 0.17
Reactive silicate, mg/l	124.04 ± 23.34
Total mercury, mg/l	0.68 ± 0.46
Mercury in the filtered effluent (soluble fraction), mg/l	0.15 ± 0.18
Dissolved oxygen (DO), mg/l	3.1 ± 0.55
BOD, mg/l	68.90 ± 17.84
COD, mg/l	448.10 ± 119.71
COD (filtered effluent), mg/l	149.50 ± 112.46
IDOD, mg/l	15.29 ± 6.02

Difference 't' test revealed that with $HgCl_2$ treatment, differences in the LC_{50} values for aerated conditions were insignificant for both A.scandens and T.mossambica (Table 2). With the effluent treatment, the same was significant for both species; the difference was more pronounced for T.mossambica ($p \leq 0.01$) than for A.scandens ($p \leq 0.05$). With $HgCl_2$, there was no significant effect of non-aeration in either of the species. The effect of non-aeration was only pronounced in case of the effluent treatment; the effect was more severe on T.mossambica than A.scandens.

In all treatments, the LC_{50} levels for A.scandens were always higher than for T.mossambica (Table 2). The fact that both species differed in their response to the test treatments is determined from the significant differences ($p \leq 0.05$) obtained in their mortality rates even under aerated conditions. The difference in their response to non-aerated conditions can be explained if we take into account the changes in the dissolved oxygen content of the treated exposure medium (Fig.1). With the $HgCl_2$ treatment, there was no significant change in the dissolved oxygen level in the non-aerated tests, and, thus, only insignificant differences in the LC_{50} values for aerated and non-aerated conditions were marked. In contrast to the $HgCl_2$ treatment, changes in the dissolved oxygen levels with increasing concentrations of the effluent were significantly different. A sudden depletion in the oxygen level occurred at the 10 to 20% levels of effluent treatment and remained more or less constant up to the 80% level of treatment. The pattern of depletion remained the

Table 2. LC_{50} values for effluent and $HgCl_2$ tests on T.mossambica and A.scandens under different oxygen conditions and time intervals. Values in parentheses indicate 95% Feducial limits around the LC_{50} values. The table also indicates the results of difference 't' analysis for LC_{50} values under both aerated and non-aerated conditions.

Time interval in hrs.	LC_{50} values for A.scandens				LC_{50} values for T.mossambica			
	$HgCl_2$ (mg/l)		Effluent (%)		$HgCl_2$ (mg/l)		Effluent (%)	
	A	NA	A	NA	A	NA	A	NA
24	1.38 (1.33-1.44)	1.39 (1.33-1.44)	39.36 (34.04-45.50)	33.88 (29.65-38.73)	1.38 (1.26-1.36)	1.29 (1.23-1.35)	31.26 (26.92-36.31)	19.72 (16.07-29.91)
48	1.34 (1.29-1.39)	1.33 (1.28-1.38)	37.15 (31.77-43.45)	30.55 (26.06-35.81)	1.26 (1.22-1.31)	1.23 (1.18-1.28)	26.00 (21.63-31.26)	16.03 (12.97-19.82)
72	1.30 (1.25-1.35)	1.30 (1.24-1.35)	35.89 (30.55-42.17)	27.86 (23.66-32.18)	1.20 (1.15-1.25)	1.19 (1.15-1.24)	23.44 (18.79-29.24)	13.80 (10.86-17.54)
Presumable harmless conc.	0.38	0.37	9.93	7.45	0.35	0.34	5.40	3.18

Values of the difference 't' test analysis:

AsHgA vs AsHgNA	= 0.00 (N S)	AsEA vs AsENA	= 9.03 ($p \leq 0.05$)
THgA vs THgNA	= 3.46 (N S)	TEA vs TENA	= 17.72 ($p \leq 0.01$)
AsHgA vs THgA	= 9.45 ($p \leq 0.05$)	AsHgNA vs THgNA	= 31.00 ($p \leq 0.01$)
AsEA vs TEA	= 8.20 ($p \leq 0.05$)	AsENA vs TENA	= 101.99 ($p \leq 0.001$)

As = A.scandens, T = T.mossambica, E = Effluent, Hg = $HgCl_2$, N S = Not Significant, A = Aerated condition, NA = Non-aerated condition, p = Level of significance

same even with increasing time, although the dissolved oxygen levels declined further. This depletion in dissolved oxygen levels may have resulted in increased mortality of T.mossambica, when compared to A.scandens, under non-aerated test conditions since T.mossambica is not an air-breather as is A.scandens. The involvement of oxygen depletion in the effluent treatment, due perhaps to the high BOD and COD content of the effluent, in decreasing the LC_{50} values is also evident from the fact that the significance level of the differences in the LC_{50} values for A.scandens and T.mossambica increased from $p \leq 0.05$ under aerated conditions to $p \leq 0.001$ under non-aerated conditions. With $HgCl_2$ treatment, however, the increase in the significance level of the difference was only from $p \leq 0.05$ under aerated conditions to $p \leq 0.01$ under non-aerated conditions. Another point to note is that under non-aerated conditions 50% death of T.mossambica occurred between 10 and 20% effluent treatment, which is also the point of maximum oxygen depletion. This indicated that at least 50% of the death was due to oxygen

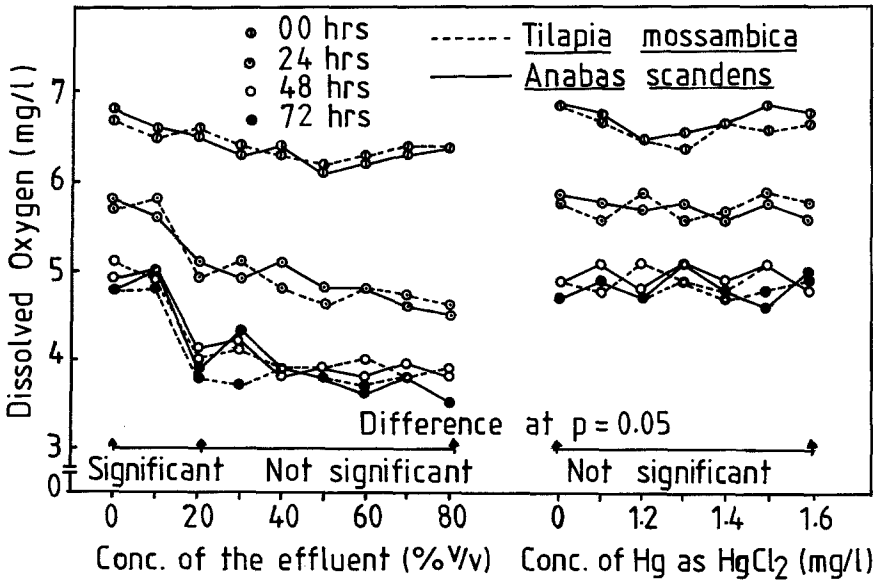

Figure 1. Changes in the dissolved oxygen pattern of the exposure medium with increasing concentrations of effluent and mercuric chloride, and the time interval.

deficiency and the rest due to the toxicity of the effluent. Correlation analysis revealed that (the calculation was done from the original values before taking the mean) the correlation was only significant for fish mortality vs COD and BOD ($p \leq 0.01$), but not for the other parameters. Since the input of organic matter to the aquatic system greatly reduces dissolved oxygen levels and since most fish are not air breathers, BOD and COD level of the effluents must be given proper attention.

In this study it appeared that mercury present in the effluent did not play any vital role in determining the acute lethal effect of the effluent. The lowest concentration of mercury (in the form of $HgCl_2$) in our test resulting in at least 50% death was 1.2 mg/l, a concentration much higher than the effluent's mercury content. Symptoms like respiratory distress and increased opercular movement, which are the signs of oxygen depletion in the exposure medium (Lal et al. 1984), and the non-existence of any correlation between fish mortality and the mercury concentration further support this fact. However, this does not imply that the level of mercury in the effluent is safe for disposal. Mercury is well known for its toxic properties and its potential of accumulation in the aquatic environment, only later to be converted to much toxic methylated form by micro-organisms (Jernelov 1970).

It is clear form the present study that the effluent studied is not fit for disposal into any waterbody as it will be hazardous to aquatic life. BOD and COD values were higher than the maximum specified limit of 30 mg/l (BOD) and 250 mg/l

(COD) fixed by The Indian Standard Institute. Hart et al. (1945) have proposed the following formula for the safe disposal of toxicants by translating the LC_{50} values into a presumable harmless concentration.

$$C = (48 \text{ hr } LC_{50} \times A)/S^2$$

where C = presumable harmless concentration;
A = Application factor (0.3);
S = 24 hr LC_{50}/48 hr LC_{50}

Presumable concentrations calculated on this basis (Table 2) revealed that the effluent must be diluted at least 20 times before it can be released to an aquatic system or, at the least, it must attain such dilution with the receiving water. BOD and COD levels in the estuarine water about 1 km downstream from the mixing zone were found to vary from 20 to 40 mg/l and 80 to 150 mg/l, respectively, throughout the period of analysis. These values indicate that the effluent was not getting sufficiently diluted with the estuarine water. A study of total fish productivity in the estuary would probably provide a better understanding of the effect of the effluent. However, dead fish sometimes found floating in the estuary may very well be indicative of the extent of effluent pollution in this system.

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