INTERNATIONAL JOURNAL OF SCIENTIFIC AND UNIVERSITY RESEARCH PUBLICATION

International Journal Of Scientific And University Research Publication

ISSN No **2364/2017**

Listed & Index with

ISSN Directory, Paris

Multi-Subject Journal

Volum : **(3)** | Issue : **211** |

IJSURP
International Journal Research
www.ijsurp.com

Research Paper

ACUTE TOXICITY OF PAPER AND PULP MILL EFFLUENTS TO SOME COMMON INDIAN FRESHWATER FAUNA

Dr. Mimai Chandra Saha || Director of Public Instruction
Govt. of West Bengal
Education Directorate
Bikash Bhavan
Salt Lake
Kolkata-700091
India.

ABSTRACT **Acute toxicity of paper and pulp mill effluents to Cyclops viridis, Clarias batrachus and Branchiura sowerbyi was evaluated in the present study. The 96 h LC50 values of paper and pulp mill effluents for C. viridis, juvenile and adult C. batrachus and B. sowerbyi were 0.62, 2.95, 7.22 and 26.21 mg/liter respectively. C. viridis was most sensitive followed by juvenile and adult C. batrachus and B. sowerbyi. The ethological changes of the organisms were directly proportional to the increasing concentration of paper and pulp mill effluents. The mortality rate also varied significantly with the increasing concentration of paper and pulp mill effluents for both the organisms. The relationship between mortality rate and exposure times was insignificant in all test animals.**

KEYWORDS :Acute toxicity, paper and pulp mill effluents, Cyclops viridis,

INTRODUCTION

The whole paper and pulp mill effluent (PPME) has very high toxic potential and is a major contributor of aquatic pollu- tion (Pathan et al., 2009). The pulping and bleaching processes employed during paper production generate huge amount of wastewaters with high organic content, dark brown colouration, adsorbable organic halide, toxic dyes, bleaching agents, heavy metals, salts, acids and different alkalies. Different derivatives of chlorophenols and chloroguaiacols are present in the effluents which are highly toxic to aquatic life (Zahrim et al., 2007; Dey et al., 2013). Though scanty reports are available on some of the macroinvertebrate communities (Davis, 1973) there are no specific reports on the lethality of whole mill effluents on zooplank- ton and benthic annelids which form an important link in many food chains. The earlier studies on the toxicity of PPME and its ingredients are mostly restricted to fish.The variation in LC50 values of PPME among different fish species was recorded by Nanda et al. (2002). In their study, *Anabas testudineus* was most vulnerable to toxicity of PPME compared to *Channa punctatus* and *Clarias batrachus*. In striped bass, *Morone saxatilis*, 20% of bleached kraft mill effluent caused maximum mortality after 72 h of exposure (Burton et al., 2007). The 96 h LC50 value of prepared concentration of whole mill effluent was found to be 9.5% in *Rasbora daniconius.* At different concentrations of whole mill effluent the fish showed adverse reactions like erratic swimming, convulsions, jumping out of water and vigorous mucus secretion (Pathan et al., 2009). *Labeo rohita* and *Channa punctatus* also showed similar behaviour when they were exposed to paper mill effluents (Srivastava et al., 2007). It was also reported that the crustacean, *Daphnia magna* Straus showed avoidance to pulp mill effluents in a 12 h laboratory test (Rosa et al., 2008). The effects of pulp mill effluent on algae, benthic invertebrates and fish were also recorded by earlier workers (Lowell et al., 2004; Dubé et al., 2008). The purpose of the present study was to determine the sensitivity of different freshwater organisms belonging to diverse niches to find out alternative test species for ecotoxicological studies in the local aquatic ecosystem, to provide further PPME toxicity data for use in ecological risk assessment and to determine the safe disposal level of PPME, especially for Indian local PPME pollution issues. Thus, an attempt was made to assess the acute toxicity of PPME to different trophic level organisms (*Cyclops viridis,* juvenile and adult *Clarias batrachus* and *Branchiura sowerbyi*).

Materials and Methods

Test organisms used in the bioassay were the freshwater *Cyclops viridis* (Class: Maxillopoda, Subclass: Copepoda, Family: Cyclopidae), catfish, *Clarias batrachus* (Order: Siluriformes, Family: Clariidae) and the benthic oligochaete worm, *Branchiura sow-erbyi* (Class: Oligochaeta, Family: Tubificidae). These organisms form important links in many food chains. The test organisms were collected from local unpolluted sources. All test organisms were allowed to acclimate gradually to the test water for a minimum of 48 h.PPME was collected from the main discharge point of Supreme Paper mill in sterile 10 litre plastic containers that were previously cleaned in non ionic detergent, rinsed with tap water and later soaked in 10% HNO_3 for 24 h and finally rinsed with deion-ised water. Immediately after collection it was stored at 4°C in the laboratory for further analysis.Static replacement bioassays with the plankton and worm were conducted in 500 ml glass beakers each containing 300 ml water whereas for fish 15 litre glass aquaria was used each hold- ing 10 litres of unchlorinated tap water (Temperature 26.2 ± 0.5°C, pH 7.2 ± 0.6, Free CO_2 10.1 ± 0.6 mg/l, Dissolved Oxygen 5.2± 0.4 mg/l, Total alkalinity 179 ± 9.8 mg/l as$CaCO_3$, Hardness123 ± 5.9 mg/l as $CaCO_3$). A set of four beakers or aquaria wereexposed to each concentration of PPME. Each set of tests was accompanied by four replicates of control. No feed was given to the test animals 24 h before and during the bioassays. Water chemical analysis and the bioassays were done following the methods outlined in American Public Health Association (2012).The required amount of PPME was weighed and added to the test medium. The test medium was then mixed with a magnetic stirrer. Initially, rough range finding tests were conducted for both the test organisms to determine the dose range at which mortality occurs (data not shown). The selected test concen- trations of PPME were finally used for the determination of 96 h LC50 for the test organisms. Ten plankton (mean length 0.07 ±0.01 mm), ten juvenile fish (mean length 72.4 ± 1.06 mm; mean weight 7.64 ± 0.46 g), ten adult fish (mean length 181.4 ± 1.02 mm; mean weight 115.2 ± 5.04 g) and ten worms (mean length20.5 ± 5.46 mm) were used in each replicate. The number of dead organisms were counted every 24 h and removed immediately from the test medium to avoid any organic decomposition and depletion of dissolved oxygen. A fixed amount of test medium (10%) was replaced every 24 h by freshwater and the desired quantity of PPME was immediately added to water to assure a constant concentration of the toxicant in solution and also to avoid other abiotic factors interfering in the animals' perfor- mance. Similar technique was also followed by earlier workers (Badanthadka & Mehendale, 2005; Mukherjee & Saha, 2012). Cumulative mortality of the test organisms after 96 h was used to estimate LC50 values with 95% confidence limits by a com- puter program (US EPA, 1999). The behavioural changes at each concentration of the toxicant were also observed for the test organisms during the bioassay. Mortality rate at different concentrations and various time of exposure (24, 48, 72, 96 h) were analyzed for correlation using the computer software R version 2.14.0 and (Finney, 1971; Gomez & Gomez, 1984) for testing the significance of variation (p -value)..

Results and Discussion

The lethal concentration of PPME to the different test organisms are summarized in Table 1.

Table 1: Median lethal concentration (LC50) along with 95% confidence limits of PPME to the different test organisms at different hours of exposure (24, 48, 72, 96h)

Test organism		Concentration (mg/l) 24h
Cyclops viridis		2.28 (1.58-2.48)
Clarias batrachus (juvenile)		5.25 (4.69-6.02)
Clarias b atr achus (adult)		10.96 (10.21-11.67)
Branchiura sowerbyi		30.04 (29.54-30.96)

The LC$_{50}$ values indicate that sensitivity to PPME follows the order: *C. viridis*>*C. batrachus* (juvenile)>*C. batrachus* (adult)>*B. sowerbyi*. The mortality rate of all the test organisms showed sig- nificant relationship ($p<0.05$) at different exposure times (24, 48,72 and 96h) for all concentrations of PPME. This is corroborated by the multitrophic study observed with 2,4,6-TCP by Yin et al. (2003).

pendent increase in the rate of swimming, hopping frequency and angular turns and bends (Table 2). All the three types of behaviour reached their peak at 3 mg/l. This was probably due to stress of toxicity. Similar changes in swimming behaviour were observed in *Daphnia magna* Straus exposed to malathion by Rassoulzadegan (2000) and Saler & Saglam (2005). Increased hyperexcitability and decreased tendency of vertically hanging posture was observed in *C. batrachus* with the increase in the dose and time of exposure to PPME (Table 2). Excessive mucus secretion was observed at the higher hours of exposure (48, 72 and 96 h). The primary indication of diseases in fishes is marked by abnormal swimming and orientation pattern (Sindermann,1970). The excess mucus secretion over the body of fish may be due to dysfunction of the pituitary gland under toxic stress (Pandey et al., 1990). Avoidance of stress due to toxicity has resulted in the abnormal behaviour of the exposed fish as they try to adapt a compensatory mechanism to derive more energy (Joshi,2011). The behavioural alterations were probably the signs of toxic effects which were mediated through the disturbed nervous system that controls almost all body functions (Tiwari et al.,2011). The change of orientation and locomotion of the exposed fish was probably due to the disruption of the mechano- and chemoreceptor systems (Gardner, 1975). Impairment of lateral line, olfactory organ and membranous labyrinth necessary for the maintenance of balance may also have resulted in the observed behavioural anomalies of fish (Gardner, 1975). The ethological changes of fish in the present study also correspond with the findings of earlier workers in the fish *Rasbora daniconius.* of *C. viridis* to PPME showed both a dose and time de-time dependent decrease in the rate of movement was found in *Branchiura sowerbyi* exposed to PPME (Table 2). Mucus secretion and wrinkling effect increased with the increase of concen- tration of PPME. Similar observations were also recorded in *B. sowerbyi* exposed to methanol by Kaviraj et al. (2004). The mucus on the body of the worm as well as the microbes present in the mucus may impart high tolerance of the worm to the toxicants (Kaviraj et al., 2004).

Table 2: Impact of paper and pulp mill effluent (PPME) on behaviour of different test organisms (SR: swimming rate; HF: hop- ping frequency; ATB: angular turns and bends; M: movement; MS: mucus secretion; WE: wrinkling effect; HE: hyperexcitability; VHP: vertically hanging posture; –: none; +: mild; ++: moderate; +++: strong) at various concentrations during different hours of exposure

Dose (mg/l)	24h	48h	72h	96h
Behaviour of *Cyclops viridis*				
SR	HF	ATB	SR	HF
0.0	+	+	+	+
0.7	+	+	+	+
1.0	++	+	++	++
3.0	++	++	++	++
Behaviour of *Clarias batrachus*				

	HE	VHP	MS	HE
0.0	-	+++	-	-
4.0	-	+++	-	-
6.0	+	++	-	+
10.0	+	+	-	++
12.0	+	+	-	++
Behaviour of *Branchiura sowerbyi*				
	M	MS	WE	M
0.0	+++	+	-	+++
10.0	+++	+	-	+++
20.0	+++	+	+	++
30.0	+	+++	+++	+
40.0	+	+++	+++	+

The present results on median lethal concentration (LC50) indi- cate that PPME is highly toxic to aquatic organisms (Table 1). The food chain and community function in the aquatic environment are adversely affected by PPME even at low concentration as indicated by the high sensitivity of plankton to PPME. The benchmark levels of PPME at both the regional and national scale can be established from the LC50 values. However, the useof only PPME for acute toxicity study is not sufficient. Besides, potential risk from various ingredients emanating from paper and pulp mills should be considered to get a more complete picture in terms of toxicity.

CONCLUSION

We are extremely thankful to the Head, Postgraduate Department of Zoology and the Principal of Bid- hannagar College and Barasat Government College for allowing us to carry out this research work.

ref_str

1. **American Public Health Association** (APHA) (2012). Standard methods for the examination of water and wastewater. E.W. Rice, R.B. Baird A.D. Eaton, & L.S. Clesceri (Eds.), American Public Health Association, American Water Works Association, Water Environment Federation: Washing-ton DC.
2. **Badanthadka, M.** & Mehendale, M.H. (2005). Chlorophenols. pp. 567-568. In: Encyclopedia of Toxicology-1.
3. **(Ed. Wexler, P.) Academic Press,** Elsevier. Burton, D.T., Hall, L.W., Klauda, R. J. & Margrey, S. L. (2007). Effects of treated bleached kraft mill effluent on eggs and prolarvae of striped bass (Morone saxatilis). Journal of American Water Resources As- sociation, 19(6), 869-878.
4. **Davis, R.M.** (1973). Benthic macroinvertebrate and fish populations in Maryland streams influenced by acid mine drainage. Contr. No. 528. Natural Resourc- es Institute, Univ. of Maryland.
5. **Dey, S., Choudhury,** M.D. & Das, S. (2013). A review on toxicity of paper mill effluent on fish. Bulletin of Environment, Pharmacology and Life Sciences,2(3), 17-23.
6. **Dubé M.G.,** Munkittrick, K.R. & Hewitt, L.M. (2008). Case study: Pulp and paper mill impacts. In: The Toxicology of Fishes, (Eds. Di Giulio, R.T. and Hinton, D.E.) CRC Press, Taylor and Francis Group, pp. 935-936.
7. **Finney, D.J.** (1971) Probit analysis. London: Cambridge University Press.
8. **Gardner, G.R.** (1975). Chemically induced lesions in estuarine or marine teleosts. In: The Pathology of fishes, (Eds. Ribelin, W.E. and Migaki, G.M.). Wisconsin Press, Madison, pp. 657-693.
9. **Gomez, K.A**. & Gomez, A.A. (1984). Statistical procedures for agricultural research, 2nd Ed. New York: Wiley.
10. **Joshi, P.S.** (2011). Impact of zinc sulphate on behavioural responses in the freshwater fish Clarias batrachus (Linn.). Online Interna- tional Interdisciplinary Research Journal, 1(2), 76-82.
11. **Kaviraj, A.,** Bhunia, F. & Saha, N.C. (2004). Toxicity of Methanol to Fish, Crustacean, Oligochaete Worm and Aquatic Ecosystem. International Journal of Toxicology, 23, 55-63.
12. **Lowell, R.B.,** Munkittrick, K.R., Culp, J.M. McMaster, M.E. & Grapentine, L.C. (2004). National response patterns of fish to pulp and paper mill effluent exposure: reproductive disruption in combination with nutrient enrichment and other effect. In: Pulp and paper mill effluent environmental fate and effects, (Eds. Bor- ton, D.L., Hall, T.J., Fisher, R.P. and Thomas, J.F.). DesTech Publ., Lancaster, PA, pp. 147-155.
13. **Mukherjee, D.** & Saha, N.C. (2012). Acute toxicity of 2, 4, 6-Trichlorophenol to copepod, Cy- clops viridis and oligochaete worm, Branchiura sowerbyi. Environment and Ecology, 30(3C), 1165-1170.
14. **Nanda, P., Panigrahi, S.**, Nanda. B. & Behera, B.K. (2002). Toxicity of paper mill effluent to fishes. Environment and Ecology, 20(2), 496-498.
15. **Pandey, A., Kumar, G.K**. & Munshi, J.S.D. (1990). Integumentary chromatophores and mucus glands of fish as indicator of heavy metal pollution. Journal of Freshwater Biology 2, 117-121.
16. **Pathan, T.S.,** Sonawane, D.L. & Khillare, Y.K. (2009). Toxicity and behavioural changes in freshwater fish Rasbora dani- conius exposed to paper mill effluent. Journal of Biotechnology Research International, 2(4), 263-266.
17. **Rassoulzadegan, M.** (2000). Malathion (Organic Fosforlu İnsektisit)' un Daphnia magna Straus, 1820 (Crustacea, Cladocera) Üzerindeki Toksik Etkilerinin Araştırılması, Ege Ü. Fen Bil. Ens Yüksek Lisana Tezi, 38s.
18. **Rosa, R.,** Moreira-Santos M. & Lopes, I. (2008). De- velopment and sensitivity of a 12-h laboratory test with Daphnia magna Straus based on avoidance of pulp mill effluents. Bulletin of Environmental Contamination and Toxicology,81(5), 464-9.
19. **Saler, S.** & Saglam, N. (2005). Acute toxicity of malathion on Daphnia magna Straus, 1820. Journal of Biological Sciences, 5(3), 297-299.

20. **Sindermann, C.J.** (1970). Principal diseases of fish and shellfish. Academic Press, London/New York, p. 369.
21. **Srivastava, S.,** Prabhakar, S., Singh, P. & Srivastava, B.C. (2007). Toxicity and behaviour of the fish Labeo rohita and Channa punctatus exposed to pulp paper mill effluent. Journal of Ecotoxicology and Environmental Monitoring, 17(3), 241-244.
22. ,**Tiwari, M.Nagpure, N.S.**, Saksena, D.N., Kumar, R., Singh, S.P., Kushwaha, B. & Lakra, W.S. (2011). Evaluation of acute toxicity levels and ethological responses under heavy metal cadmium exposure in freshwater teleost, Channa punctatus (Bloch). International Journal of Aquatic Science, 2(1), 36-47.
23. **US EPA.** (1999). Probit program version 1.5. Ecological Monitoring Research Division, Environmental Monitor- ing Systems Laboratory, US Environmental Protection Agency, Cincinnati, Ohio 45268. http://www.epa.gov/nerleerd/stat2.htm. Accessed 14 Jan 2012.
24. **Yin, D., Hu, S., Jin, H.** & Yu, L. (2003). Deriving freshwater quality criteria for 2, 4, 6-trichlorophenol for protection of aquatic life in China. Chemosphere, 52, 67-73.
25. **Zahrim, A.Y., Gilbert, M.L.** & Janaun, J. (2007). Treatment of pulp and paper mill effluent using Photo-fenton's process. Journal of Applied Sciences, 7(15), 2164-2167.

IJSURP Publishing Academy

International Journal Of Scientific And University Research Publication

Multi-Subject Journal

Editor.

International Journal Of Scientific And University Research Publication

+965 99549511

+90 5374545296

+961 03236496

+44 (0)203 197 6676

www.ijsurp.com