Toxicity Evaluation of Effluents from Dye and Dye Intermediate Producing Industries Using Daphnia Bioassay

Y Verma

Citation

Y Verma. *Toxicity Evaluation of Effluents from Dye and Dye Intermediate Producing Industries Using Daphnia Bioassay*. The Internet Journal of Toxicology. 2007 Volume 4 Number 2.

Abstract

Wastewater from seven dye and dye intermediate producing industries and a common effluent treatment plant was assessed to characterize the acute toxicity using Daphnia bioassay. The 24 and 48 h EC50 values and its confidence limits were estimated applying the probit analysis method. The 24 h EC50 values for different dye effluent samples A, B, C, D, E and F were 1.35, 17.32, 17.67, 29.18, 46.49 and 25.11%, and 48 h EC50 values were 1.23, 14.12, 15.52, 24.53, 29.69 and 23.22%, respectively. Result showed linear relationship with high degree of confidence ($0.84< r2 <0.98$) between end point (immobilization) and test concentrations. Effluent of two dye industries 'G' and 'H' had EC50 value >100%. Effluent of industry 'A' exhibited highest toxicity causing 100% immobility at 3.13% effluent concentration. Effluent of industry 'G' and 'H' had shown least toxicity, and was found toxic at the concentration of full strength (100%) causing ≤ 20% and 10% immobility in 24 h. Out of 8 industries effluents tested, 5 showed 100% immobility of daphnia at 50% of effluent concentration, while 1 showed highest toxicity (100% immobility) at a very low concentration (3.13%) of the effluent, and 2 showed lowest toxicity. These results clearly indicate that the bioassay reflects the overall toxicity potential of indusial discharge as it provides the complete response of test organisms to all components of water.

INTRODUCTION

Effluents are very important source of chemicals entering into aquatic ecosystem, deteriorating the water quality and thereby affecting the flora and fauna. There have been increasing trends towards use of biomonitoring to assess the water quality of resources/receiving water bodies [12]. The analysis of a recognized set of water chemistry parameters is the most commonly used method of assessing water quality by pollution control boards/regulatory authorities. However, these parameters provide information about the chemical complexity but not their toxicity. Moreover, study of physico-chemical parameters of effluent does not throw any light on the synergistic or antagonistic effects on biological systems. Thus an adequate monitoring system should assess both the effects and distribution of pollutants [3]. The chemical analysis identifies only the main chemical pollutants available while a bioassay (toxicity test) addresses pollutant bioavailability and response to total effect of actual potential [4].

The traditional acute multi-concentration toxicity tests when applied for the determination of effluent is often recognized as Whole Effluent Toxicity (WET) test. The WET test is widely used in the USA, Canada and European countries, and is an important component of the environmental protection agencies of these countries for detecting and addressing the toxicity of industrial effluents/wastewaters. Determination of acute toxicity of the chemical products is one of the cornerstones of the general hazard assessment of the programs laid down by various environmental protection agencies. However, most of the regulatory agencies and research organizations are using the well-established conventional acute toxicity test with fish and daphnids in which survival/death/immobility is the most frequently monitored end point [567].

Daphnids are routinely used as standard test organism in aquatic toxicity bioassay (acute and chronic) because of their rapid reproduction, sensitivity to chemical environment, and play key ecological role in the aquatic food chain by serving as an intermediate between primary producers and fish [8]. Daphnia magna has been evaluated as a good organism to test effluent toxicity in textile wastewater [910], dye and dyes wastewater [111213].

India is among one of the major producers of dyes and dyes intermediate from Asian region and meets the requirement of the world at large. There are about 900 dyes and dyes intermediate producing industries in India. Most of them are in small-scale unorganized industrial sector, and about five thousand dyes and dyes intermediate are in commercial production. The wastewater generated from dye and dye intermediate industries mainly have intense color having various shades of red, blue green, brown and black through the production of different color containing dyes and usually have high level of COD, BOD, acidity, chlorides, sulphates, phenoloic compounds and various heavy metals viz. copper, cadmium, chromium, lead, manganese, mercury, nickel, zinc etc. [14]. Although it seems logical that dyes should be safer to the environment than neurotoxic insecticides [8], little work has been done to determine the potential impact of dyes on the ecosystem or selected indicator sps. in the laboratory [151116]. Dyes, as they are intensively colored, cause special problems in effluent discharge and even small amount is noticeable. The effect is aesthetically more displeasing rather than hazardous, and can prevent sunlight penetration decreasing photosynthetic activity in aquatic environment. Although, some azo dyes that causes the effluent color have been implicated as being mutagenic/carcinogenic as well as toxic to aquatic life [1718].

Therefore in order to assess the effluent toxicity status of polluting industries traditional acute toxicity test (WET) was applied for dyes and dyes intermediate producing industries using Daphnia magna as a test model. The overall objective of this study was to assess the acute toxicity of industrial effluents of which seven are dyes and dyes intermediate producing industries and one is common effluent treatment plant which receives dyes effluents from more than fifty dyes producing small scale industries. Further, our aim was also to contribute towards the development of a more effective policy of pollution control and effluent regulation using bio-criteria derived from acute WET test results.

MATERIALS AND METHODS

Grab samples of the effluents were collected at the point of outlet discharge from the wastewater treatment plants (WTPs) of seven different dyes and dyes intermediate producing factories (labeled as A, B, C, D, E, G and H) and a common effluent treatment plant (labeled as F) within the plant premises. Factory-A produces cuptohalosyanine complex (CPC crude), factory-B produces azo pigments, auxiliaries and intermediates, factory-C produces synthetic organic dye stuff and pigments dye stuff, auxiliaries and intermediates, azo and diazo reactive dyes, disperse dyes and intermediates, factory-D produces G Salt and R salt, Factory-E produces acid black, red, blue violet, yellow. F-(WTP) Common effluent treatment plant receives wastewater (primarily treated) from >50 dye producing units, factory G - produces reactive dyes, and factory - H produces tartrazine, indigo carmine, allura red. All of the effluent samples of these factories were stored in amber glass bottles (2.5 L) and transported to the laboratory at the earliest in a lightproof ice chest, and stored under refrigeration (at 4 ° C) in darkness until analysis and bioassay. Dissolved oxygen (DO) (WTW OXI-96 oxy-meter), conductivity (TD Scan conductivity meter) and pH (W T W pH - meter) were also measured as soon as samples arrived to the laboratory. Temperature (° C) was recorded at the sampling site.

Daphnia magna Straus used in the experiment were selected from the laboratory stock culture maintained at 25±2 ° C, in 5-litre borosilicate glass beakers and were fed on green algae Selenastrum sps. and yeast powder. The daphnids were reared in photoperiod of 12 h light/12 h dark cycle, with light > 600 Lux (Metcix AVO LM 4 Lux-Meter, France). Reconstituted dilution water (RDW) was used for dilution of effluents to which Daphnia culture was maintained.

To evaluate the aquatic toxicity of dye effluents author performed single species laboratory toxicity test with Daphnia magna, and the 48 h acute toxicity test was conducted after exposing them to various concentrations of the effluent in RDW within 48 h after the effluent sample had arrived in the laboratory. The experimental concentrations were 100, 75.0, 66.6, 50, 33.3, 25, 12.5, 6.25, 3.12, 1.56, 0.8 and 0.4 % of the effluent. Control water and effluent dilutions were prepared with reconstituted dilution water (RDW) with the same formulation used for culture water. Reconstituted water was aerated 12 h before effluent dosage. All the toxicity tests were run at 25±2 ° C. Test chambers were not aerated during experimentation. Twenty-four hours prior to the test adult daphnids (gravid females) were sorted and the young one (neonates) produced from these adults were used in the experiment. Batches of five neonates (≤ 24h) received from gravid females were placed in glass beaker of 100 ml containing 50 ml test volume with two set of control group. Each concentration was replicated a minimum of 4 times. The numbers of immobilized daphnids were recorded after 24 h and 48 h exposure. Daphnids unable to swim for 15 s after gentle stirring were considered as immobile/dead [6]. The test protocol is summarized in Table 1, and details of the methodology is described elsewhere [19]. The experiment was validated with positive control test using reference substance potassium dichromate ($K_2Cr_2O_7$). The 24 h EC_{50} was 0.813 (0.70–1.01) mg/L, after ISO [7].

Figure 1

Table 1: Summary of test protocol for

Name of the organism	*Daphnia magna*
Age	< 24 hr
Test type	Static (non-renewal type)
Temperature	25 ± 2.0 ºC
Light quality	Cool white fluorescent light >600 Lux
Photoperiod	12 hr
Feeding regime	24 hr before experiment
Dilution water	Reconstituted dilution water (RDW)
Test vessel	100 mL glass beaker
Test solution volume	50 mL
Test specimen / vessel	5 daphnids in each vessel
Test vessel / concentration	Four
Effluent concentrations	100, 75, 50, 25, 12.5, 6.25,3.12, 1.6,0.8,0.4,0 %
Test duration	48 hrs
Observation period	24 and 48 hrs
End point	Immobilization

The acute toxicity data were analyzed when two or more partial immobilizations were observed. The EC_{50} and its 95% confidence limits were calculated by probit analysis method to using SPSS software 6.1. The regression analysis was also performed to determine the correlation between x and y variables for regression equation and r^2 values. r^2 is the correlation coefficient and reflects statistical significance between dependent and independent variables. Toxicity unit (TU) value was calculated using EC_{50} (100/EC_{50}, expressed as volume percentage). Ratio of acute toxicity was calculated by dividing 48 h EC_{50} with 24 h EC_{50}.

RESULTS AND DISCUSSION

The physico-chemical characteristics of the reconstituted dilution water (RDW) used in the experiment showed that water used in this study had pH 7.8, calcium hardness (as CaCO3) 180.0 mg/L, conductivity 0.7 mS. The physico-chemical characteristics of the effluents differed substantially from one another with respect to chemical characteristics, as expected due to a relatively wide spectrum of dyes manufacturing sources. Dissolved oxygen concentration of the effluents ranged from 3.5–5.2 mg/L, the pH ranged from 7.2–8.0 and conductivity from 0.8–1.5 mS and temperature 26.0-31.0 ° C. Acute toxicity test results of 8 dyes industries (24 and 48 h calculated EC_{50} and its 95% confidence limit) are given in Table 2. Effluent-A showed highest toxicity causing 100% mortality at 3.13% effluent concentration and more than 50% mortality in 1.5% effluent concentration. The effluent of industry 'G' showed very low toxicity and was found toxic at the concentration of full strength (100%) causing ≤ 20% mortality in 24 h and ≤ 25% mortality in 48 h. However, the effluent of industry 'H' had lowest toxicity causing only 10% mortality at 100% effluent. Hence, the EC_{50} value could not be derived for both effluents. The 24 and 48 h EC_{50} value for dye industries G and H were always >100, and r^2 =0.56.

The 24 h EC_{50} values and the confidence limit for the effluent of dye industry A, B, C, D, E and F were 1.35 (1.13–1.63), 17.32 (13.94–21.82), 17.67 (14.45–21.61), 29.18 (25.93–33.27), 46.49 (33.43–73.59) and 25.11 (22.55–27.62)%, respectively. The linear regression analysis performed reflected statistical significance between dependent and independent variables (r^2 0.85 – 0 .95). The 48 h EC_{50} values for effluent of dye industry A, B, C, D, E and F were 1.23 (1.02–1.48), 14.12 (11.53– 17.42), 15.52 (12.68–18.86), 24.53(21.48–28.18), 29.69 (26.54–33.55) and 23.22 (20.70–25.94)%, respectively (Table 2).

Figure 2

Table 2: 24 h and 48 h EC50 values, its 95% confidence limits and ratio, and toxicity units (TU) for dyes industrial effluents

Parameters	Dyes and dyes intermediate producing industries							
	A	B	C	D	E	F	G	H
24 h EC_{50}	1.35	17.32	17.67	29.18	46.49	25.11	>100	>100
Confidence Limit (95%)	1.13-1.63	13.9-21.82	14.4-21.61	25.9-33.27	37.4-73.59	22.5-27.61	-	-
48 h EC_{50}	1.23	14.12	15.52	24.53	29.69	23.22	>100	>100
Confidence Limit (95%)	1.02-1.48	11.5-17.42	12.6-18.86	21.4-28.18	26.5-33.54	20.7-25.94	-	-
Ratio of 24-48 h EC_{50}	1.10	1.22	1.13	1.18	1.56	1.10	<1	<1
Toxicity Unit	81.3	7.0	6.5	4.08	3.36	4.3	<1	<1

The linear regression analysis performed reflected statistical significance between dependent and independent variables ($0.84 < r^2$ 0.98). The 24 and 48 h EC_{50} value for the effluent of dye industries G and H were >100%, and r^2 = 0.56, showing least toxic response. The regression equations and correlation coefficients were also worked out. The relationship is a sigmoid curve, but can also be expressed in linear fashion with a high degree of confidence (r^2). The coefficient of determination was as high as r^2 = 0.98. Out of 8 effluents tested 5 showed 100% immobility of daphnia at 50% of the effluent concentration, while 1 showed heights toxicity (100% immobility) at 3.13% concentration of the effluent and remaining two-showed lowest toxicity at full strength (100% effluent concentration).

In toxicological experiments, the time of exposure has large effect on biological response. The general rule of thumb is that the longer the exposure time lesser the EC_{50} value and greater is the toxicity. In our study too, results showed similar pattern having lesser 48 h EC_{50} value than 24 h EC_{50} (Table 2). The ratio of EC_{50} (24 h to 48 h) was found in between 1.1–1.2 except for one sample (1.5), which showed the delayed acute toxic response (Table 2). Kim et al. [20] reported the acute toxicity ratio (24 h to 48 h) for hexavalent chromium 1.2. Our results are in accordance with the above report.

The classification of effluent [2122] based on general criteria (Table 3) showed that out of 8 effluent tested one was moderately acutely toxic (48 h EC_{50} >1.2%), 5 were minor acutely toxic (48 h EC_{50} >14.12 –29.69%) and the remaining 2 were not acutely toxic (48 h EC_{50} >100%). Researchers [31423] used the toxicity classification based on Toxicity Unit to classify the effluents toxicity. Based on this classification two effluent sample (G and H) had TU < 1 and remaining 6 were having TU >1.

Figure 3

Table 3: General criteria of toxicity classification and relationship between LC, LD and EC and toxicity rating

LD50 (mg/kg)	LC_{50} (mg/L)	EC_{50}% of effluent	Toxicity rating
>5000	>100	> 100%	Relatively not acutely toxic
500-5000	10-100	10-100%	Minor acutely toxic
50-500	1-10	1-10%	Moderately acutely toxic
<50	<1	< 1%	Very acutely toxic

Despite the recent developments in chronic toxicity test, traditional acute toxicity (WET) test are still widely used as standard eco-toxicological procedure by environmental agencies/toxicology laboratories to assess the potential impact on ecological system [24]. Hence the acute toxicity test was conducted exposing the Daphnia magna to treated wastewater of eight dye producing industries. The acute multi-concentration toxicity test yielded a range of response values as a function of effluent concentration, which causes some difficulty in assessing toxicity for effluent classification. However, assessment of industrial effluent by means of acute toxicity test seems to be sufficient for preliminary classification of effluent whether toxic or non toxic. Some authors have already used this idea to assess effluent in terms of their acute toxicity. If the sample whose 48 h EC_{50} > 100% or show null or poor response of the test organism in a bioassay could be classified as non-toxic [2122].

Eco-toxicity is the conservation of living communities in receiving aquatic ecosystems, it seems reasonable that those effluents having >1 TU be considered as Toxic, since this would imply that full-strength effluent would adversely affect 50% or more of the exposed organisms. Accordingly, samples having a 48-h EC_{50} > 100% or which show null or poor response of the test organisms in the bioassays could be classified as Non-Toxic. In accordance with this criterion, only two effluent samples (G and H) in the present study were classified as Non-Toxic. Criteria to classify effluents into toxicity categories (from Low Toxic to Very Toxic) are more difficult. Calleja et al. [25] have proposed for leachates assessed with Daphnia magna acute toxicity tests the category of Low Toxic when 100 > EC_{50} > 75%, Toxic for 25 < EC_{50} < 75%, and Very Toxic for EC_{50} < 25%. Based on these criteria, it is shown that among the 8 industrial effluents 2 had 48-h 100 >EC_{50} > 75%, 1 had 25< EC_{50} 75%, 5 had EC_{50} < 25%. Thus they can be considered as being Very Toxic. In toxicity units, Very Toxic effluents have >4 TU, only 2 samples (G and H) could be considered to be Low Toxic (TU 1) following the guidelines by Calleja et al. [25]. Remaining 6 samples are considered to be Toxic >4 TU (25 < EC_{50} < 75%), i.e. A, B, C, D, E and F.

The results of low exposure concentration range are suitable for determining the no effect concentration (NOEC). The NOEC is the concentration at which a substance does not cause significant effect in comparison with control sample. It has an important ecological implication because it indicates the concentration above which adverse effect can be expressed. The use of ECx values ($EC_{10/20}$) for responses <50% has often been chosen in effluent assessment, since these values are usually closer to the acute no-effect concentrations (NOEC). The observed acute no effect concentrations were 0.4, 6.25, 6.25, 12.25, 16.67, 12.25, 66.67 and 75% for effluents A, B, C, D, E, F, G and H, respectively. The EC_{10} values derived by probit analysis showed closer to acute NOEC. The EC_{10} values were 0.75, 7.39, 8.59, 14.24, 19.89 and 15.65 for effluent A, B, C, D, E and F.

Toxicological criteria, such as ECx, acute or chronic NOEC, which are used as parameters for effluent toxicity assessment, should be regarded as ‘hypothetical' doses that will not cause adverse effects in the communities of the receiving aquatic systems, since the real environmental effects are subject to abiotic and biotic modifying factors which cannot be precisely predicted [4]. However, assessment of industrial effluents by means of acute toxicity tests seems to be sufficient for a preliminary classification of effluents. Some authors have already used this idea to assess effluents in terms of their acute toxicity. Thus, samples whose 48-h EC_{50} >100% or which show null or poor response of the test-organisms in the bioassays could be classified as Non-Toxic [2526]. However, when the EC_{50} >100%, there can be a risk of long-term toxicity which should be evaluated by means of chronic toxicity tests. Therefore, it is important that effluent assessment involves initial screening based on acute tests followed by chronic bioassays. However, NOEC values are dependent on the dilution factor and NOEC is not a statistical estimation like ECx, which implies that the use of limits for this parameter is not appropriate. Chapman [4] has argued against the use of NOECs in regulations and they consider the ECx to be a more consistent and reliable parameter, less subjected to inter and intra laboratory variability. It is not the purpose of this paper to discuss possible alternatives to the classic toxicological parameters, but it is interesting to note that this is currently a subject of debate in the scientific community.

A study conducted by Lanciotti et al [14] showed that textile wastewaters besides containing dyes have considerable amount of metals viz. chromium, copper, lead and zinc leading to toxicity to aquatic organism. Only 3% of the samples showed considerable acute toxicity (EC_{50} < 100; TU.1) to Daphnia magna. The EC_{50} of paper mill wastewater containing dyes had value between 50-100%, and EC_{50} were >25% [27]. In another study Tonkes et al. [22] tested the effluent of 10 chemical industry using traditional acute toxicity test with Daphnina magna showed that out of 10 only 6 had acute toxicity < 100% and 4 had >100. These studies also showed that dyes effluents had acute toxic properties, and therefore should be discharged into the aquatic environment after proper treatment. Finally it is concluded that effluents should always be subjected to acute multi-concentration test, yielding ECx values, which are useful for effluent characterization and ranking in the scale of toxicity classes from non-toxic to very toxic either in v/v% value or by using Toxicity Unit (TU). Result of this study showed that it is not possible to speculate on the mode of the toxic action due to difficulties in characterization of the chemical content of the effluent. Therefore, effluent composition should also be characterized. From the ecotoxicological view point single species toxicity provides limited data, hence a battery of test sps. should be included representing different tropic levels. Studies on wastewater effluents indicated that toxicity test have a viable role to play in water quality monitoring. Our results are intended to be basis for further environmental risk assessment.

ACKNOWLEDGEMENTS

The author is thankful to Dr. S. K. Dave, Officer-In Charge, and Ex. Director of the Institute, and Ex. HOD of the laboratory for the encouragement. Author is also thankful to all the technical staff for their help render during the study.

References

1. Mwinyihija M, Meharg A, Dawson J, Strachan NJC, Killham K. An ecotoxicological approach to assessing the impact of tanning industry effluent on river health. Arch Environ Hlth 2006; 50:316-24
2. Rodriguez P, Martinez-Madrid M, Cid A. Ecotoxicological assessment of effluents in the Basque country (Northern Spain) by acute and chronic toxicity tests using Daphnia magna Straus. Ecotoxicol 2006; 15:559-72
3. Sponza D. Incorporation of toxicity test in to the Turkish industrial discharge monitoring. Arch Environ Contam Toxicol 2002; 43:186-97
4. Chapman PM. Whole effluent toxicity testing-usefulness, level of protection, and risk assessment. Environ Toxicol Chem 2000; 19:3-13
5. US EPA. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. United States Environmental Protection Agency, Washington DC, 1993 EPA/600/4-90/027F
6. OECD. Daphnia sps. Acute immobilisation test and reproduction test. In: Guidelines for the testing of chemicals 202. 1993 OECD, Paris, pp 5-16
7. ISO. Water quality-determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea)-acute toxicity test. International Standard ISO, 6341, 3rd ed. 1996, Geneva, Switzerland
8. Hanazato T. Pesticide effects on freshwater zooplankton: an ecological perspective. Environ Poll 2001; 112: 1-10
9. Villegas-Navarro A, Ramiez MY, Salvador MS, Gallardo, JM Determination of wastewater LC50 of the different process stages of the textile industry. Ecotox Environ safety 2001; 48: 56-61
10. Meric S, Selcuk H, Belgiorno V. Acute toxicity removal in textile finishing wastewater by Fenton's oxidation, ozone and coagulation-flocculation processes. Water Res 2005; 39:1147-153
11. Verma Y, Ruparelia SG, Hargan MC, Venkaiah K, Kulkarni PK. Acute toxicity of azo-dyes to waterflea, Daphnia magna. Indian J Environ Protec 1993; 13: 804-07
12. Meric S, Kaptan D, Olmanz T. Color and COD removal from wastewater containing reactive black 5 using fenton's oxidation process. Chemos 2004; 54:435-41
13. Bae JS, Freeman HS. Aquatic toxicity evaluation of new direct dyes to Daphnia magna. Dyes Pigments 2007; 73:81-85
14. Lanciotti E, Galli S, Limberti A, Givannelli L. Ecotoxicological evaluation of wastewater treatment plant effluent discharge: A case study in Parto (Italy) Ann Ig, 2004; 16: 549-58
15. Bae JS, Freeman HS, Kim SD. Influence of new azo dyes to the aquatic ecosystem. Fibers Polymers 2006; 7:30-35
16. Walthall WK, Stark JK. The acute and chronic toxicity

of two xanthene dyes, fluorescein sodium salt and phloxine B, to Daphnia pulex. Environ Poll 1999; 104: 207-15
17. Gottlib A, Shaw C, Smith C, Wheatle A, Forsyth S. Toxicity of textile reactive azo dyes after hydrolysis and decolorisation. J Biotec 2003; 101: 49-56
18. Mathur N, Bhavnagar P, Nagar P, Bijarnia PK. Mutagenicity assessment of effluents from textile/dye industries of Sanganer, Jaipur (India): A case study. Ecotoxicol Environ Safety 2005; 61:105-13
19. Verma Y, Ruparelia SG, Hargan MC, Kulkarni PK. Toxicity of tannery effluent to waterflea (Daphnia magna). J Ecotox Environ Monit 2003; 13: 111-15
20. Kim SD, Park SK, Gu MB. Toxicity of hexavalent chromium to Daphnia magna influence of reduction by ferrous ion. J Hazard Material 2002; A 93: 155-64
21. Canton JW. Catch-up operation on old pesticides: An Integration. 1991 RIVM-Report No. 678 80 1002
22. Tonkes M, deGraaf PJF, Graansma J. Assessment of complex industrial effluents in the Netherland using a whole effluent toxicity (WET) approach. Water Sci Tech 1999; 39: 55-61
23. Movahedian H, Bina B, Asghari GH. Toxicity evaluation of wastewater treatment plant effluents using Daphnia magna. Iran J Environ Hlth Sci Eng 2005; 2:1-4
24. Callow P. The three criteria of ecotoxicology. Func. Ecol 1992; 6: 617-619
25. Calleja A, Baldasano JM, Mulet A. Toxicity analysis leachates from hazardous wastes via microtox and Daphnia magna. Tox Assess 1986; 1:73-83
26. Fisher DJ, Knott MH, Turley BS, Yonkos LT, Ziegler GP. Acute and chronic toxicity of industrial and municipal effluents in Maryland, US. Water Environ Res 1998; 70:101-07
27. Slabert JL, Venter EA. Biological assay for aquatic toxicity testing. Water Sci Technol 1999; 39: 367-373

Author Information

Yogendra Verma
National Institute of Occupational Health