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TOXICITY STUDY OF DRINKING WATER IN PILKHUWA (A TOWN IN NATIONAL CAPITAL REGION)

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Toxicity tests are desirable in water quality evaluations because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota. However, the effects of chemical interactions and the influence of complex matrices on toxicity cannot be determined from chemical tests alone. Different species of aquatic organisms are not equally susceptible to the same toxic substances nor are organisms equally susceptible throughout the life cycle. Even previous exposure to toxicants can alter susceptibility. In addition, organisms of the same species can respond differently to the same level of a toxicant from time to time, even when all other variables are held constant.

KEY WORDS: Biota/Chlorosis/*Daphnia Magna*/*Lemna Minor*/Necrosis.

INTRODUCTION

Water quality characteristics of aquatic environments arise from a multitude of physical, chemical and biological interactions. An extensive review work has been done in the field of water pollution due to synthetic dyes. The idea is to make this work well compared with all types of studies that have already been reported in literature [Ratna & Padhi, 2012]. In our earlier study the physical and chemical parameters of various water samples of Pilkhuwa town have been determined quantitatively [Padhi *et al.*, 2013]. Toxicity tests [USEPA, 1991] & [USEPA, 1987] are useful for a variety of purposes that include determining: (a) suitability of environmental conditions for aquatic life, (b) favourable and unfavourable environmental factors such as DO, pH, temperature, salinity or turbidity, (c) effect of environmental factors on waste toxicity, (d) toxicity of wastes to a test species, (e) relative sensibility of aquatic organisms to an effluent or toxicant, (f) amount and type of waste treatment needed to meet water pollution control requirements, (g) effectiveness of water treatment methods, (h) permissible effluents discharge rates, and (i) compliance with water quality standards, effluent requirements, and discharge permits. In such regulatory assessments, use toxicity test data in conjunction with receiving-water and site specific discharge data on volumes, dilution rates, and exposure times and concentrations.

METHODOLOGY

An aquatic toxicity test is a procedure in which the responses of aquatic organisms are used to detect or measure the presence or effect of one or more substances, wastes, or environmental factors, alone or in combination. The procedures [Eaton *et al*., 2005] allow the

measurement of biological responses to known and unknown concentrations of materials in both fresh and waste waters. These toxicity tests are applicable to routine monitoring requirements as well as research needs. Toxicity study refers to bio-analytical techniques applied to organisms at various levels to ascertain the harmful effects of chemicals on them [Blaise *et al*., 1988] & [Slabbert *et al*., 1999]. The assessment of toxicity is done by acute and enrichment toxicity tests [Schowanek, *et al*., 2001].

Traditional acute toxicity tests are performed on bacteria like *E. coli,* fish like *P. reticulate*, algae (*c. vulgaris*) and protozoa (*v. companula*). In these tests the organism is grown in the test water in presence of all the substances essential for growth. The numbers of organisms present in the sample before and after incubation is counted and the concentration (% age volume) of effluent required to affect 50% of the organisms is found [Tonkes *et al*., 1999]. This is the EC_{50} value. The higher this value, the less is the toxicity. Enrichment toxicity tests are done using *enterobacteraerogenes*. This bacterium is present in drinking water. It is grown in a minimal growth medium as a control. If a growth promoting substance is added to the medium, increased colonies are observed. But if a toxic substance is added, a decrease in the number is observed [APHA, 2003]. Phytoplankton duckweed and submerged macrophytes can also be used as test materials for monitoring toxicity [Lee *et al*., 1998] & [Kumar & Prasad, 2004] *Lemna minor*, a duckweed, is widely used by eco-toxicologists. It is widespread and fast growing and is sensitive to many pollutants which are assimilated through the underside of the leaf [Becker *et al.,* 2002]**.**

Daphnia magna is also used for assessment of acute as well as chronic toxicity in wastewaters [Burnham *et al.,* 1981]. This is because of its easy growth and maintenance, simple test procedure and reproducibility as well as high sensitivity of the result [Arsalan-Alaton *et al*., 2004]. The most commonly used and well-studied bioassays used for toxicity evaluation are bioluminescence and respirometric methods. The bioluminescence method most commonly used is Microtox® assay which is based on the naturally occurring luminescent marine bacterium *vibrio fischeri*. Its luminescence is inhibited by toxicants [Ren, 2004]**.** The basis for respirometric tests, on the other hand, is the respiration rate of activated sludge or sludge organisms. This rate can be reduced in presence of toxic substances. This is measured as oxygen uptake [OECD, 1984].

Thus a large number of toxicity tests using a wide variety of organisms are well documented in literature. The choice of the toxicity test(s) to be used has to be made keeping in mind the possible identity of contaminants. D. T. Sponza [Sponza, 2006] studied the toxicity of textile wastewaters using several toxicity tests and found that besides the dyes themselves, ions like Cr^{6+}, Cd^{2+}, Zn^{2+}, Pb^{2+} also contribute to toxicity of textile effluents. The author found that no single toxicity test can constitute a comprehensive approach to aquatic life protection and a battery of tests based on organisms at various trophic levels should be applied. He also emphasises that in order to gain insight into probable causes of toxicity, a thorough physical and chemical analysis of the effluents should be carried out.

Keeping these factors in mind the concept of toxicity-directed wastewater approach was developed which is based on a combination of fractionation procedures, bioassays and chemical analytical methods [Brack, 2003]. Identification of chemical groups associated with the measured biological result is well documented [Hewitt *et al*., 2005]. A protocol for toxicity identification evaluation consisting of a series of fractionation procedures followed by a bioassay to determine the source of effluent toxicity was proposed by the United States Environment Protection Agency [USEPA, 1988].

Types of Toxicity Tests & Basic Requirements

Toxicity tests are classified according to (a) duration – short-term, intermediate, and/or long-term, (b) method of adding test solutions-static, renewal, or flow-through and (c) purpose- effluent quality monitoring, single compound testing, relative toxicity, relative sensibility, taste or odor, or growth rate, etc. The basic requirements of a toxicity test are (a) an abundant supply of water desired quality, (b) an adequate and effective flowing water system constructed of nonpolluting or absorbing materials, (c) adequate space and well planned holding, culturing, and testing equipment and facilities, (d) an adequate source of healthy experimental organisms and (e) appropriate lighting facilities for plant toxicity tests.

RESULTS AND DISCUSSION

In our study the toxicity level of drinking water was monitored by conducting the following two toxicity tests. 1. *Lemna Minor* and 2. Seed Germination & Seeding Growth Test

1. *LEMNA MINOR* TEST

Lemna minor (also known as common duckweed) is a small flowering aquatic macrophyte (a monocot) widely distributed in quiescent fresh water and estuaries ranging from tropical to temperate zones. It is the most common species of the family Lemnaceae in the United States and many other parts of the world. It is morphologically simple, consisting only of frond and root. The frond size approximately 2 to 4 mm and the root length is up to 50mm. The plant is colonial (up to 8 fronds), multiplies sexually or asexually, and has a growth rate far exceeding those of other flowering plants. Duckweed is a food for waterfowl and small animals, and provides food, shelter and shade for fish and other aquatic organisms. Furthermore, it serves as a habitat for various invertebrates. Common duckweed is an ideal organism for testing aquatic phytotoxicity of herbicides, industrial and municipal wastewaters and other contaminates.

Selecting & Preparing Test Organisms

The organism can be obtained from commercial sources, testing laboratories, or the field. In our study, the organism was obtained from a pond nearby Pilkhuwa town. Then it was first identified and confirmed taxonomically before proceeding for culturing. The new duckweed culture was acclimated to the test environment (for example, lighting, temperature and nutrient conditions) for at least 2 weeks before a test. The growth of the duckweed was observed in a culture vessel (Borosil Glass Tray of size L× W × H = 12 × 8 × 4) containing culture solution. One liter culture solution was prepared by adding 10 mL of each stock nutrient solution A, B, and C (See Table 4.5.6.I) to deionized water. Water depth of at least 40 mm or more was maintained and was exposed constantly to white-fluorescent light (2150 lux to 4300 lux or approximately 30 watt fluorescent bulb) while maintaining the temperature of about 27-30°C. The diluted culture solution (1/4 strength) was added every week.

Toxicity Test Procedure & Results

The samples collected are mainly from ground water hence the microbial population is low. Therefore static toxicity method was adopted because the test solutions were stable due to low microbial population. The duckweed nutrient solution was prepared as per Table 4.5.6.I. In this test both dilution water and control water were identical to duckweed nutrient solution. Approximately 20 mL of test solution was prepared by adding 25 mg of potassium chromate, 1.5 mL of each nutrient solution (A, B & C) and the requisite amount of sample water. Each test solution was taken in a 60 × 15 mm glass petri dish and was marked with site

identification number. The duckweed specimens from stock cultures that have been grown under the same conditions were selected. 20 duckweed fronds were placed in each petri dish containing the test solution and covered. Each test solution was illuminated with continuous cool-white fluorescent light at water surface and incubated at 27-30°C. The test duration of each was approximately 96 hours.

Table I. Duckweed Nutrient Solution

Solution	Stock solution concentration (g/L)	Element	Final concentration (mg/L)
A. $NaNO_3$	2.55	N	4.2
$NaHCO_3$	1.50	Na	11.0
K_2HPO_4	0.104	C	2.14
		K	0.469
		P	0.186
B. $CaCl_2.2H_2O$	0.441	Ca	1.20
$MgCl_2$	0.570	Mg	2.90
$FeCl_3$	0.0096	Fe	0.033
$Na_2EDTA.2H_2O$	0.03		
$MnCl_2$	0.0264	Mn	0.115
C. $MgSO_4.7H_2O$	1.47	S	1.91
H_3BO_3	0.0186	B	0.0325
$Na_2MoO_4.2H_2O$	0.726	Mo	0.0028
$ZnCl_2$	0.327	Zn	0.0016
$CoCl_2$	0.078	Co	0.0004
$CuCl_2$	0.0009	Cu	0.000004

Note: To prepare for duckweed nutrient solution, add 1mL of each stock solution to 100 mL deionized water.

Table II: Growth of duckweed specimens (*lemna minor*) in test solutions

Site	No. of *lemna minor* units placed	Status after 96 hours		
		Live	Dead	*C/N/CB/RD
Control	20	16	01	03
S-01	20	10	07	03
S-02	20	12	06	02
S-03	20	10	07	03
S-04	20	10	06	04
S-05	20	10	05	05
S-06	20	12	07	01
S-07	20	12	06	02
S-08	20	10	06	04
S-09	20	10	05	05

S-10	20	09	07	04
S-11	20	11	07	02
S-12	20	10	06	04
S-13	20	10	07	03
S-14	20	10	06	04
S-15	20	10	04	06
S-16	20	08	07	05
S-17	20	10	06	04
S-18	20	11	05	04
Fresh	20	15	02	03

*__C__ = Chlorosis **N** = Necrosis **CB** = Colony Break-up **RD** = Root Destruction

The growth rate of duckweed plants was observed under a lighted magnifying glass for symptoms, including chlorosis (loss of pigment/yellowing), necrosis (localized dead tissue), colony break up and root destruction. Table II shows the exact picture of duckweed plants in each petri dish containing the test solution after a stipulated time period. A comparison of affected fronds with duckweed specimens in the control has been made to ascertain the extent of toxicity.

Table II shows that number of live duck weed plants (*lemna minor* units) in the water samples collected from Site-10 and Site-16 is least as compared to other sites. However, the ratio of live and dead units in water samples collected from Site-01, Site-03, Site-04, Site-05, Site-08, Site-09, Site-13, Site-14, Site-15 & Site-17 is 50:50. This clearly indicates that the level of toxicity in water is relatively high in the case of Site-10 and Site-16 whereas this is moderate in the case of other sites.

2. SEED GERMINATION & SEEDING GROWTH TEST

In order to ascertain the level of toxicity in drinking water seed germination and seeding growth test may be conducted. This test is being carried out by selecting a suitable method and test species depending upon the availability, cost, similarity of test species to species of interest, consistent performance and high germination percentage. In this study the test species being used as black gram (*kalachana*) and the method used as static method because the tests are not highly volatile and degradable. The seed germination and initial root growth was done using deionized fresh water which is shown in Table III.

Table III: Seed Germination and Seeding Growth Test Conditions

Test Variable	Condition or Value in Test
Test species	Black Gram (*kalachana*)
Pretreatment	20 min, hypochlorite solution (3.33g OCl^-/L)
Test type	Static
Temperature	27˚C
Light quality	Light
Test vessel	100 mL culture dish
Test solution	8 mL/vessel
Specimens	15/vessel
Replicates	Single
Control solution	Standard water

Test duration	120 hours (5 days)
Indicators	Seed germination, root/shoot elongation, root/shoot dry biomass and abnormal appearance

Test Procedures and Results

The water samples were taken in the pre-marked test vessels (6'' diameter petri dish) keeping in view of the conditions mentioned in Table 4.5.6 III. Seeds were arranged in such a manner that they should not contact each other or sides of the culture dish. The seeds were treated with sodium hypochlorite (3.33 g/L) solution in order to avoid any interference of fungi or microorganisms with seed germination. Then the seeds were rinsed at least 4-5 times with deionized water before placing in the test vessel. Each germinated seed was counted whose radicle touches a length of 5 mm or longer. All root and shoot elongation of each germinated seed in each dish were recorded in Table 4.5.6 IV. The length of each primary root from the transition point of the hypocotyl to the tip of the root was measured. Abnormal appearance such as discoloration, stunted growth, and chlorosis in some seeds has also been observed. The sample toxicity in percent inhibition (% I) relative to the control can be expressed by the formula, % I = 100 (C-T)/C, where C and T are mean seed germination percentages in control water and test solutions, respectively, if seed germination is used as the test indicator. If root/shoot elongation is used as the test indicator, C and T are root length (in mm) in control and test solutions.

Table IV: Seed Germination and Seeding Growth in Test Samples

Site	S.G. (%)	$I_{S.G.}$ (%)	R/S E (%)	$I_{R/S\ E}$(%)
Control	**80.00**	**00.00**	**100.0**	**00.00**
S-01	13.33	83.34	35.48	64.52
S-02	46.66	41.67	64.52	35.48
S-03	40.00	50.00	58.06	51.94
S-04	60.00	25.00	90.32	9.680
S-05	46.66	41.67	61.29	38.71
S-06	53.33	33.33	74.19	25.81
S-07	46.66	41.67	64.52	35.48
S-08	40.00	50.00	54.84	45.16
S-09	53.33	33.33	77.42	22.58
S-10	13.33	83.34	35.48	64.52
S-11	33.33	58.34	48.38	51.62
S-12	40.00	50.00	58.06	41.94
S-13	26.67	66.66	38.71	61.29
S-14	46.66	41.67	64.52	35.48
S-15	53.33	33.33	74.19	25.81
S-16	20.00	75.00	35.48	64.52
S-17	40.00	50.00	54.84	45.16
S-18	33.33	58.34	48.38	51.62
Fresh	**73.33**	**8.337**	**91.61**	**8.390**

S.G. (%) = Percentage of Seed Germination, $\mathbf{I_{S.G.}}$ **(%)** =Percentage of Seed Germination Inhibition, **R/S E (%)** = Percentage of Root/Shoot Elongation, $\mathbf{I_{R/S\ E}}$**(%)** = Percentage of Root/Shoot Elongation Inhibition

The data shown in Table IV reveal that the percentage of seed germination and the percentage of root or shoot elongation in the case of water samples of Site-01, Site-10, Site-13 & Site-16 are extremely low whereas the percentage of seed germination inhibition and the percentage of root elongation inhibition of these samples are high. However, the percentage of

seed germination and the percentage of root elongation as well as their inhibitions percentage are relatively moderate. This may be inferred that the toxicity level of water samples from these sites, such as, Site-01, Site-10, Site-13 & Site-16 is relatively high. This result is in agreement with the result of our earlier toxicity test that is *lemna minor* test.

Conclusions

The quality of drinking water cannot be evaluated only by measuring the physical and chemical parameters. However, the measurement of BOD and COD offers a good indication of the organic pollution of water. But these procedures alone are not sufficient to get information about the potential harmful effects of chemicals. The toxic effects of other unknown and undetermined substances in drinking water and complex wastewaters can be estimated only through aquatic toxicity tests. The assessment of toxicity is done by acute and enrichment toxicity tests. Hence in our study the level of toxicity has been ascertained by conducting the *lemna minor* and seed germination & seeding growth tests. Our results reveal that the toxicity level of drinking water of sites S-10 and S-16 is relatively high.

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References

1. Ratna & Padhi, B. S., Pollution due to synthetic dyes : Toxicity & Carcinogenicity Studies and Remediation. *International Journal of Environmental Sciences*, **3** (3), 940-955 (2012).
2. Padhi, B. S., Sharma, Kamini and Tomer, S. S., Study of Quality of Drinking Water in Pilkhuwa (A Town in National Capital Region). *Acta Ciencia Indica*.
3. U.S. Environmental Protection Agency, 1991. Technical support document for water quality-based control.EPA-505/2-90-001 (PB91-127415), off. Water, U.S. Environmental Protection Agency, Washington, D.C.
4. U.S. Environmental Protection Agency, 1987. Permit Writer's Guide to Quality-Based Permitting for Toxic Pollution. Off. Water, U.S. Environmental Protection Agency, Washington, D.C.
5. Book: Standard Methods for the Examination of Water and Wastewater, 2005, 21st Edition (Centennial Edition) Edited by Andrew D. Eaton, L. S. Clesceri, E. W. Rice and A. E. Greenberg.
6. Blaise, C., Sergey, G., Wells, P., Bermingham, N., van Coillie, N., Biological testing-development, application and trends in Canadian environmental protection laboratories. *J. Tox. Assess*., **3**, 385-406 (1988).
7. Slabbert, J. L., Venter, E. A., Biological assay for aquatic toxicity testing. *Water Sci. Technol*., **39**, 367-373 (1999).
8. Schowanek, D., Fox, K., Holt, M., Schroeder, F., GREAT-ER: A new tool for management and risk assessment of chemicals in river basin. *Water Sci. Technol*., **43**, 179-185 (2001).
9. Tonkes, M., deGraf, J.J., Graqansma, J., Assessment of complex industrial effluents in the Netherlands using a whole effluent toxicity approach. *Water Sci.Technol*., **39**, 55-61 (1999).
10. *APHA, Standard Methods for Water and Wastewater Analysis, 21st edition*, Washington DC, USA, (2003).
11. Lee, C. L., Wang, T. C., Hsu, C. H., Chiou, A. A., Heavy metal sorption by aquatic plants in Taiwan. *Bull. Environ. Contam. Toxicol*., **61**, 497-504 (1998).

12. Kumar, G.P., Prasad M.N.V., Cadmium toxicity to ceratophyllumdemersum L. morphological symptoms, membrane damage and ion leakage. *Bull. Environ. Contam. Toxicol*., **72**, 1038-1045 (2004).
13. Becker, A.M., Heise, S., Ahlf, W., Effects of phenanthrene on Lemna minor in a sediment water system and the impacts of UV-B. *Ecotoxicology*, **11**, 343-348 (2002).
14. Burnham, L.N., Melvin, W.W., Buchan, R.M., Acute toxicity of an in citu shale oil process waste water and its major components to Daphnia magna. *Bull. Environ. Contam. Toxicol*., **27**, 338-343 (1981).
15. Arsalan-Alaton, I., Gurses, F., Fenton-like and photo-fenton-like oxidation of procaine penicillin G formulation effluent. *J. Photochem. Photobiol. A : Chem*., **165**, 65-75 (2004).
16. Ren, S., Assessing waste water toxicity to activated sludge: Recent research and developments. *Environ. Int*., **30**, 1151-1164 (2004).
17. Organisation for Economic Cooperatrion and Development (OECD) Activated Sludge Respiration Inhibition Test, OECD Method, **209**, OECD, Paris, France (1984).
18. Sponza, D.T., Toxicity studies in a chemical dye production industry in Turkey. *J. Haz. Materials A*, **138**, 438-447 (2006).
19. Brack, W., Effect directed analysis: A promising tool for the identification of organic toxicants in complex mixtures? *Anal-Bioanal. Chem*., **377**, 397-407 (2003).
20. Hewitt, D.M., Marvin, C.H., Analytical methods in environmental effects directed investigations of effluents. *Mut. Res*., **589**, 208-232 (2005).
21. USEPA, *Methods for Aquatic Toxicity Identification Evaluation*, Duluth, MN (1988).

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