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Development of Water Quality Criteria for *Fenneropenaeus indicus* Against Arsenic by Flow-through System in the Central West Coast of India at Goa

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Abstract:

Toxicants as chemical hazard are very common to the surroundings that cause adverse effects on health and environment. The outbreak of toxicity can easily be identified in the aquatic environment and necessary methodology can be applied for water quality criteria development by reducing common sources of toxic elements in the environment. The concern over the use of toxicants causing environmental threat in form of pollution and toxicity risk to non-target organisms has been reported worldwide. Thus, the study has been conducted to estimate the toxic effect of arsenic through acute assay with white prawn. Individuals of larval stage (0.25 ± 0.10g) were acclimated under test conditions (salinity 31.0 ± 1.0%, temperature 29 ± 1.0 ^{o}C, dissolved oxygen 5.25 ± 0.2mg.l^{-1}, pH 8.2 ± 0.5) and exposed to arsenic at 0.15, 0.30, 0.60, 1.20 and 2.40mg.l^{-1} with seawater as control. Tests were run in Flow through system with the above five concentrations in duplicate. Mortality was observed for different time intervals of 24h for next four days, lethal dose concentration (LC_{50}) and 95% of confidence limit was calculated using statistical software such as Probit analysis method. Mean LC_{50} value after 96h of exposure duration was estimated as 0.08mg.l^{-1}. The results of the study were highly helpful in developing water quality criteria for this region.

Keywords: Toxicant, Flow-through system, Acute toxicity, Water quality criteria, Salinity.

1. INTRODUCTION

The experimental model systems and bioassays are presently used in ecological toxicology to supply information for hazard measurement of the chemicals being used and also determine their effects and mode of action (Laine et al., 2015). Acute toxicity tests can easily evaluate the effects of pollutants at different concentrations and can compare the toxicity of different toxicants in a short time (Richardson, 1993). The continuous Flow-through (FT) assay are supposed to give a better approximation of toxicity growing compared to that from static or renewal toxicity tests because they provide a greater control of toxicant concentrations. The change in water quality criteria gets minimized and reduces gathering of waste material in test sample (Rand et al., 1995; Guha et al., 2001, 2001; Welsh et al., 2008). The heavy metals have long been documented as serious pollutants inducing their effects on aquatic fauna. White prawn *Fenneropenaeus indicus* is common in southeast coast

estuaries where it appears to comprise an important food chain link between detritus and the higher carnivores. Arsenic has long been reported both as a poisonous and as a therapeutic agent (Rahman et al., 1998). Million tons of arsenic as pollutant enters our environment annually from various anthropogenic sources (GESAMP, 1986). Arsenic is present in feed additives, pesticides, pharmaceuticals and diverse industrial products such as paints, glass, alloys, electronic components and wood preservatives (Tisler and Zagorc-Koncan, 2002). The most frequently used arsenic compound is arsenic tri-oxide (As_2O_3) which is used for agricultural chemicals and for the synthesis of organic and inorganic compounds. The inorganic form of arsenic such as As(III) and As(V), are the most toxic forms present in food material. In general, seafood's contain high level of arsenic than other heavy metals (Almela et al., 2006).

India has a long coastline of nearly 7000km and many parts of it have rich biodiversity of marine organisms and one such distinct area is Central West Coast of India, Goa. This region has a large number of fishing towns and chemical industries. The waters of the coastal region get easily contaminated by many anthropogenic activities. Although arsenic is one of the chemical contaminants coming out of the industries, there is not information available on content of arsenic in developing water quality criteria in this region.

Arsenic (As) is a widely occurring environmental contaminant. To assess human exposures to As, public health officials and researchers often conduct biomonitoring. Samples of urine, hair, nails, or blood are collected from potentially exposed people and are analyzed for As compounds and their metabolites (Orloff et al., 2009). Marine shrimp farming is the most imperative aquaculture sector in the world, with 1996 production of 693,000tons worth more than US$ 436.4. Different types of shrimps have contributed greatly to the economy of Southwestern Taiwan (Chiu et al., 2004). Shrimp culture has been making rapid progress in India. Shrimp hatchery production in the country has not kept pace with the growth of industry and the gap between demand and supply has been very large (Laxminarayana et al., 1995). White prawn *P. indicus* is common in southeast coast estuaries where it appears to comprise an important food chain link between detritus and the higher carnivores (Bhattacharya and Sarkar, 2003). The use of prawn as aquatic organism may act as toxicant amplifier making the toxicants available to consumers at dangerously high levels. Food as prawn exposed to arsenic is associated with cancer and noncancer effects in nearly every organ in the body, and evidence is mounting for health effects at lower levels of arsenic exposure than previously thought (Carlin et al., 2015). The study investigates the acute toxicity of arsenic to *P. indicus* using FT bioassay system.

2. MATERIALS AND METHODS

Sample collection: Individuals of larval stage of *Fenneropenaeus indicus* (12.0 ± 2.0mm), (0.2 ± 0.1g) were collected with the help of local fisherman along the coastal region. After collection, animals were brought to the laboratory immediately with aeration and set for acclimation to laboratory condition. The water quality parameters was maintained as temperature, 28^{o} ± 1^{o}C, pH 8.2 ± 0.5, Salinity 31.0 ± 1.0ppt and dissolved oxygen 5.2 ± 1.00mg.l^{-1}. During acclimation, animals were fed with prawn feed at approximately 4% of the body weight per day. The acclimatization process was discontinued a day before the experiment.

Chemical preparation: Arsenic tri-oxide (As_2O_3), was obtained from Sigma Aldrich Chemicals Pvt Ltd. The main stock solutions was prepared by dissolving 1.65g of As_2O_3 in a liter of deionized water and suitable dilution were prepared employing appropriate concentration of test media after Connor and Wilson (1972).

Water quality criteria: The test water was examined for routine water quality assessment (pH, temperature, dissolved oxygen and salinity); ammonia (NH_3); dissolved silicate; inorganic phosphate (PO_4); Nitrite-nitrogen and Nitrate-nitrogen. During the bioassay, the water quality parameters were monitored routinely on daily basis in exposure medium. Samples were collected from siphon by draining the exposure tanks at every 24h intervals in test chambers. The total alkalinity was

considered as the sum of the phenolphthalein and methyl orange alkalinity. Total hardness was considered as the sum total of calcium and magnesium was estimated by Ethylene diamine tetra acetic (EDTA) acid Titrimetric Method (APHA, 1992). Nutrients present in the seawater were measured based on the standard methods after Grasshoff et al. (1999).

Toxicity bioassay: Arsenic toxicity was evaluated in Flow through (FT) toxicity tests for different time periods. Flow-through bioassay (FT) tests were based on the modifications of Connor and Wilson (1972). The bioassay system was used to supply a constant release of seawater and toxicant under test to twenty treatment beakers, each of one liter capacity. All tubing's used were flexible silicone tubing (Tygon, USA) with specific dimensions for flow rate. Sand filtered seawater was stored in fiber glass tank (100lit capacity) and five toxicant gradient concentrations were mixed with seawater in mixing chambers (2lit capacity). Constant flow of mixing of seawater with toxicant in the mixing chamber was maintained by programmed peristaltic pumps (Ismatec, USA). Proportions of mixing were based on the standard methods after Alabaster and Albam (1965). A rapid exchange of toxicant medium in test chamber was maintained by supplying each concentration with toxicant medium at 20ml/min (1.2lit/h) which was monitored as a result in 95% of toxicant medium in each beaker being replaced every 50 minutes. The relation of water flow to animal weight 2-3L/g/day recommended by Sprague (1969) is more with 14.4L/g/day for *Fenneropenaeus indicus* which ensured that the seawater in the test chamber had adequate dissolved oxygen. Prawn loading density was 0.2g/L/day (Table 1).

Toxicity tests were performed in two stages, a preliminary range finding test using Static renewal bioassay (SR) test was conducted to determine the range of five concentrations used in definite test and definite tests were carried out in replicates using Flow through test. Test exposure concentrations of 0.15, 0.30, 0.60, 1.20 and 2.40mg.l^{-1} along with control were kept in replicates on two different occasions. Twenty organisms were exposed to each toxicant concentration (in duplicates) in one liter test chambers. Stock solutions were made up in 5L batches by dissolving the main stock solution in de-ionized water. Control treatment was kept with filtered seawater. Toxicant medium of 20ml.min^{-1} was delivered to each concentration test chamber using programmed peristaltic pump. Routine water quality parameters were monitored every 24hrs in test chambers. The test duration was 96h (4d) to allow estimation of time-dependent lethal concentrations of arsenic. The mortality of the prawn was confirmed by change in white to whitish pink. Mortality was monitored at regular intervals for the first 24h and afterwards till 96h.

Statistical Interpretation: The percent mortality was determined for each treatment in cumulative manner by pooling the mortality data from the replicate exposure tanks. The lethal dose concentration resulting in 50% mortality (LC_{50} values) was estimated using computer aided software program Probit analysis (US-EPA, 1992).

3. RESULTS AND DISCUSSION

Physicochemical characteristics of test water were 5.5 ± 0.7mg.l^{-1} of dissolved oxygen, pH 8.1 ± 0.1, salinity 30 ± 1.0‰ and temperature 28 ± 1°C. The water quality characteristics in detail are listed in Table 1. The acute median lethal concentration (LC_{50}) was calculated for 24h, 48h, 72h and 96h with 95% confidence intervals of arsenic exposed to the white prawn *Fenneropenaeus indicus*. The respective regression equations are shown in Tables 2 and 3. No mortality was observed in the control seawater. Median lethal concentration (LC_{50}) and 95% confidence intervals were not calculable at 24h. Mean (±SD) LC_{50} values at 48,72 and 96h were 0.35 ± 0.01mg.l^{-1}, 0.21 ± 0.01mg.l^{-1} and 0.08 ± 0.12mg.l^{-1} respectively.

Table 1: Water quality parameters of test water sample.

S.No	Parameter	Concentration (Mean ± SD)
1.	Dissolved oxygen (mg.l^{-1})	5.25 ± 0.02
2.	Salinity (‰)	31.0 ± 0.25
3.	Temperature (°C)	28.2 ± 0.50

4.	pH	8.20 ± 0.50
5.	Total alkalinity ($mg.l^{-1}$)	12.0 ± 0.25
6.	Total hardness Calcium hardness ($mg.l^{-1}$)	760.0 ± 0.50
7.	Magnesium hardness ($mg.l^{-1}$)	2385.0 ± 10.0
8.	Ammonia (NH_3) ($\mu g.l^{-1}$)	0.3 ± 0.00
9.	Nitrite – nitrogen ($mmol.l^{-1}$)	1.20 ± 0.11
10.	Nitrate – nitrogen ($\mu mol.l^{-1}$)	12.5 ± 0.40
11.	Inorganic phosphate ($\mu mol.l^{-1}$)	3.70 ± 0.05
12.	Dissolved silicates ($\mu g.l^{-1}$)	6.50 ± 0.50

Table 2: Median lethal concentration (LC_{50}) and respective 95% confidential limit at different time intervals.

Test no	LC_{50} in $mg.l^{-1}$ and (95% CI)			
	24h	**48h**	**72h**	**96h**
1	NC	0.35 (0.24-0.36)	0.21 (0.12-0.22)	0.08 (0.00-0.12)
2	NC	0.32 (0.22-0.32)	0.26 (0.20-0.30)	0.07 (0.01-0.10)

NC- not calculable

Table 3: Mean (± SD) LC_{50} values ($mg.l^{-1}$) and regression equations of arsenic to *P.indicus* at different time interval.

Time point (h)	LC_{50} $mg.l^{-1}$	R^2
24	NC	0.565
48	0.33 ± 0.05	0.255
72	0.23 ± 0.05	0.762
96	0.07 ± 0.04	0.487

Acute toxicity tests can detect the toxic damage of pollutants in a short period. They also make it easy to compare the degree of toxicity among different pollutants and the relative sensitivities of animals to the same pollutant (Buikema et al., 1982). Arsenic has a difficult marine biogeochemistry that has major implications for its toxicity to marine organisms and their consumers, including humans. In aquatic reservoir, arsenic can form stable compounds in different oxidation states under different redox conditions (Soto et al., 1993, Neff, 1997). The mean LC_{50} of 0.08$mg.l^{-1}$ in the present study at 96h indicated that arsenic is highly toxic at very low concentration. In other crustaceans, the reported 96h LC_{50} values of arsenic were 0.25 $mg.l^{-1}$ in *Cancer magister* crab under toxicity assay, 1.75 to 7.22$mg.l^{-1}$ in different life stages of tiger prawn under static test, 1.74$mg.l^{-1}$ in mysid *Mysidopsis bahia* under FT test and 15.5$mg.l^{-1}$ in *Scylla serrata* under static test. Thus the 96h LC_{50} values of arsenic found in the present study is very high LC_{50} compared to the values found for various crustaceans reported.

Thus, it is evident that the difference in the toxicity values may be due to biological variables and the chemical formulation. Arsenic was found to be significantly inductive to white prawn by inducing its anti-oxidant enzymes which would be crucial in the detoxification of oxyradicals to non reactive molecules (Vieira et al., 2008). So, it can be concluded that the larval stages of *Fenneropenaeus indicus* (0.20 ± 0.10g; 15.0 ± 5.0mm) and the data obtained from the present study will be useful in deriving water quality criteria with respect to specific chemical and the toxic nature of the species. Further investigations are highly mandatory to be performed for other species of marine world, with maximum emphasis on bivalves and fishes living in the mud, to ensure that they are safe for consumption.

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REFERENCES

1. Almela, C., Clemente, M.J., Vélez, D. and Montoro, R. (2006). Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. *Food Chem. Toxicol.* 44: 1901.
2. Abulude, F.O., Fapohunda, O.O. and Awanlemhen, B.E. (2006). Determination of some heavy metals in *Procambaris clakii*, *Palaenon* sp., *Macrobrachium vollenhovenii* and *Penaeus notalis* from the Coastal Water of Ondo State, Nigeria. *J. Anim. Vet. Adv.* 5 (1): 38–41.
3. Alabaster, J.S. and Abram, F.S.H. (1965). Development and use of a direct method of evaluating toxicity to fish. In: *Advances in water pollution research,* Proc. 2nd Int. Conf., Tokyo, 1964, (ed. O. Jaag), Pergamon Press, Oxford. 1: 41–54.
4. A.P.H.A. (1992). Standard methods for the examination of water and wastewater, 18th Ed. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
5. Bhattacharya, A.K. and Sarkar, S.K. (2003). Impact of overexploitation of shellfish: Northeastern coast of India. *Ambio.* 32: 70-76.
6. Buikema, A.L., Niedertehner, R.R. and Cairns, J. (1982). Biological monitoring Part IV-toxicity testing. *Water Res*. 16: 239–262.
7. Carlin, D.J., Naujokas, M.F., Bradham, K.D., et al. (2016). Arsenic and environmental health: State of the science and future research opportunities. *Env. Hlth. Perspect*. 124: 890.
8. Chiu, H.F., Ho, S.C. and Yang, C.Y. (2004). Lung cancer mortality reduction after installation of tap-water supply system in an arseniasis-endemic area in Southwestern Taiwan. *Lung Cancer* 46: 265.
9. Connor, P.M. and Wilson, K.W. (1972) A Continuous flow apparatus for assessing the toxicity. *J. Exp. Marine Biol. Ecol*. 9: 209–215.
10. European, C.B. (2006) European chemicals bureau: Toxicology and chemical substances, testing methods (Annex V). 9p http://ecb.jrc.it/DOCUMENTS/Testing-Methods/ANNEXV/ C01web 1992.
11. G.E.S.A.M.P. (IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution) (1986). Review of potentially harmful substances– Arsenic, Mercury and Selenium. *Reports and Studies* 28: 73.
12. Grasshoff K, Ehrhardt M and Kremling K (Eds.). Methods of Sea Water Analysis, 3rd ed., pp. 75–89, 1999; 159–196, VCH, Wainheins, Germany.
13. Guha Mazumder, D.N., De, B.K., Santra, A., et al. (2001). Randomized placebo-controlled trial of 2,3-dimercapto-1-propanesulfonate (DMPS) in therapy of chronic arsenicosis due to drinking arsenic-contaminated water. *J Toxicol Clin Toxicol* 39: 665.
14. Laine, J.E., Bailey, K.A., Rubio-Andrade, M. et al. (2015). Maternal arsenic exposure, arsenic methylation efficiency, and birth outcomes in the Biomarkers of Exposure to Arsenic (BEAR) pregnancy cohort in Mexico. *Env. Hlth. Perspect*. 123: 186.
15. Laxminarayana, A., Pillai, S.M., Surendran, K.K. and Sasidharan, C.S. (1995). Backyard hatchery technology for the white prawn, *Fenneropenaeus indicus*. *CIBA Bull.* 8: 1–13.
16. Magos, L. (1990). Marine health hazards of anthropogenic and natural origin. In: UNEP: Technical annexes to the report on the state of the marine environment. *UNEP Reg Seas Rep Stud* 114/2: 447–507.
17. Neff, J.M. (1997). Review: Ecotoxicology of arsenic in the marine environment. *Env. Toxicol. Chem.* 16: 917–927.
18. Orloff, K., Mistry, K. and Metcalf, S. (2009). Biomonitoring for environmental exposures to arsenic. *J. Toxicol. Env. Hlth. B Crit. Rev*. 12: 509.
19. Phongdara, A., Chotigeat, W., Chandumpai, A., Tanthana, C. and Duangtong, P. (1999). Identification of *Penaeus merguiensis* and *Fenneropenaeus indicus* by RAPD-PCR derived DNA Markers. *Sci. Asia* 25: 143–151.
20. Rand, G.M., Wells, P.G. and McCarty, L.S. (1995). Introduction to aquatic toxicology. In: Rand, G.M. (Ed.), Fundamentals of Aquatic toxicology: Effects, Environmental Fate, and Risk Assessment, second ed. Taylor & Francis, Washington, DC, pp.3–67.

21. Rahman, M., Tondel, M., Ahmad, S.A. and Axelson, O. (1998). Diabetes mellitus associated with arsenic exposure in Bangladesh. *Am. J. Epidemiol*. 148: 198.
22. Rand, G.M. and Petrocelli, S.R. (1985). Fundamentals of aquatic Toxicology: Methods and applications. Washington: Hemisphere Pub. Corp.
23. Richardson, M.L. (1993). Epilogue. In: Ecotoxicology monitoring (Ed. Richardson ML) New York VCH Verlagsgesellschaft. 335–343.
24. Soto, E.G., Rodriguez, D.P., Rodriguez, E.A., Mahia, P.L., Fernandez, E. and Zubieta, A.C. (1993). Inorganic As (III) and As (V) quantification in marine organisms. *Marine Pollut. Bull.* 26: 335–338.
25. Sprague, J.B. (1969). Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Res*. 3: 793–821.
26. Tisler, T. and Zagorc-Koncan, J. (2002). Acute and Chronic Toxicity of Arsenic to Some Aquatic Organisms. *Bull. Env. Cont. Toxicol*. 69: 421–429.
27. U.S. E.P.A. (1992). EPA Probit analysis program, version 1.5. Ecol Monit Res Div EMSL Cincinnati, OH.
28. Viera, L.R., Sousa, A., Frasco, M.F., Lima, I., Margado, F. and Guilhermino, L. (2008). Effects of benzopyrene, anthracene and a fuel oil on biomarkers of the common goby *Pomatoschistus microps*. *Sci. Totl. Env*. 395: 87–100.
29. Welsh, P.G., Lipton, J., Mebane, C.A. and Marr, J.C.A. (2008). Influence of flow-through and renewal exposures on the toxicity of copper to rainbow trout. *Ecotoxicol. Env. Safety* 69: 199–208.