Identifying an Ideal Bio-Indicator from Indian Freshwaters to Use in Automated Bio-Monitor

[1] Merin Mathew [2] Anju Theresa Antony [3] Sunny George [4] Ratish Menon [5] Almut Gerhardt
SCMS Water Institute, SCMS School of Engineering and Technology, Karukkutty.
[1] merinjesusan@gmail.com [2] anjuanooja2000@gmail.com
[3] sunnygeorge@scmsgroup.org [4] ratishmenon@scmsgroup.org

Abstract- **The present study has been undertaken to identify the most ideal species that can be used as a bio-indicator in automated bio-monitors. Six different fish species were exposed in a 50% effluent from sewage treatment plant and in open well water (control) for 48 hours. Using an automated bio-monitor named "Multispecies Freshwater Bio-monitor©" developed by Lim Co International, GmbH, Konstanz, Germany was used for continuous monitoring of the behaviour of fish species in both control and effluent. The six fish species used were *Etroplus suratensis, Puntius denisonii, Xiphophorus maculatus, Poecilia reticulata and Oreochromis mossambicus*. All the six species were in juvenile stage and almost same in size (2-3 cm). *O. mossambicus* and *E. suratensis* were identified as the ideal bio-indicators which gives an early warning (stress ventilation, avoidance behaviour followed by reduced movement activity) to the effluent from treatment units. All the other species were observed as more sensitive to effluent.**

Key Words*: *Etroplus suratensis, Puntius denisonii, Xiphophorus maculatus, Poecilia reticulata, Oreochromis mossambicus

I. INTRODUCTION

Pollution of water bodies from various anthropogenic sources is becoming a threat to aquatic life and increasing the difficulty in supply of potable water. Monitoring system is required to evaluate water quality at an early stage of toxicity to aquatic life. At present, control and surveillance of industrial wastewater discharges depend mainly on physicochemical measurements, which, due to the discontinuity of sampling, do not always detect detrimental effluent discharges in time and cannot predict toxicity of complex wastes. Although chemical analytical instruments are mainly being used in water supplies for monitoring water quality, it is unable to effectively detect such toxicants in real time (Gerhardt *et al.*, 2006). Therefore, it is necessary to have novel analytical systems to control water quality.

It is well known that fish are ideal test organism for investigation of behavioural toxicity of chemicals in water (Little and Finger, 1990). Fish exposed to toxic chemicals shows various behavioural alternations, and behavioural change of fish is effective endpoint for evaluation of changes of water quality by toxicants in a short time of period. So in this study, by making use of a Multispecies Freshwater Biomonitor© (MFB) developed by LimCo International, GmbH, Konstanz, Germany the behaviour changes of six different fish species in open well water (control) and 50% concentration of effluent from a sewage treatment plant. The main objective of this study is to find which species will give early warning due to the toxicity of effluent. Some species will be very sensitive to toxicants where some will be highly tolerant, they cannot be considered as a bio-indicator. Organisms which will sustain for a few time period and those can give early warning signals can be considered as bio-indicators, they can be used for continuous monitoring of water quality.

For the automated real-time monitoring using Multispecies Freshwater Biomonitor the test species were placed individually in the sensor chambers where they can move freely in their medium (water, sediment or wet soil). The chambers are open to the medium so any change in water quality will affect the organism in the chamber as same as to the other organisms in the water bodies. By continuous observation on this organism we can predict the health of organism and water quality change at any time. This will help to stop discharging of effluent from various sources and can take necessary precautions from risk causing to the organisms.

II. MATERIALS AND METHODOLOGY

A. Test Species

Five test species (*Etroplus suratensis* - Karimeen, *Puntius denisonii* - Miss Kerala, *Xiphophorus maculatus* - Red Platy, *Poecilia reticulata* - Guppy *and Oreochromis* - Red Tilapia) were collected from Regional Shrimp Hatchery, Azhikode. *Puntius terio* - Poochutty was obtained from the field. All the species were in their juvenile stage. These species were selected because of the

availability of same size and age and it was easy to be cultured.

B. *Multispecies Freshwater Biomonitor©*

Multispecies Freshwater Biomonitor© is an automated, biological early warning system developed by LimCo International, GmbH, Konstanz, Germany; that could be used for continuous online monitoring of water quality in any water bodies. They use the reactions of selected organisms, especially physiological and behavioural reactions to chemical or physical stress as a sensitive alarm system to detect exposure to toxicants within short exposure times and provide data about possible permit violations. MFB continuously detects and monitors effects of mixtures of pollutants, thus allows to detect even unknown substances in due time before pollution irreversibly spreads in the environment or even harms human health. MFB operates under the principle of quadrapole impedance conversion which is described in Gerhardt and Svensson, 1994.

C. *Quality analysis of effluent from Sewage Treatment plant*

The STP in the campus incorporates various treatment units that can be adopted in the treatment of sewage. A process diagram of the MFB system connected to the sewage treatment plant in the campus is shown in fig.1. The quality of wastewater collected from the outlet was tested in the laboratory and different parameters were analysed as prescribed in APHA standard analytical methods. The results of the analysis is tabulated in table 1. The six test species were placed in each chamber and was continuously monitored for 48 hours in both control (open well water) and 50% diluted effluent from STP.

Table 1 : Quality of Effluent of STP

Parameter	Outlet of STP	Standard (Environmental (Protection) Rules, 1986)
pH	6.9	5.5 - 9.0
Temperature (°C)	27.6	NA
COD (mg/l)	183	NA
BOD_5 at 20°C (mg/l)	92.5	100
Iron (mg/l)	0.9	NA
Nitrate (mg/l)	0.47	NA

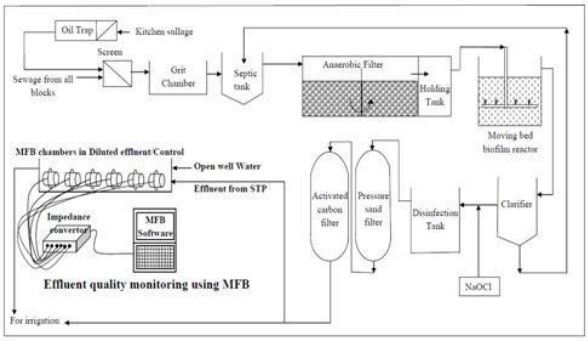

Figure 1: Test using waste water from STP of SCMS campus

D. *Statistical Analysis*

The raw data on movement activity of all six fish species in both the control and 50% effluent were statistically analysed and plotted using Sigma Plot 13.0 (Systat Inc.). Normally distributed data of behaviour measurements of species were analysed with Friedman Repeated Measures Analysis of Variance on Ranks followed by Normality Test (Shapiro-Wilk): Failed ($P < 0.050$) or Tukey test for non-parametric data. There is a statistically significant difference ($P =< 0.001$) in the median values among the treatment groups (between all species in control and the same species exposed in 50% effluent) were found.

III. RESULTS AND DISCUSSIONS

E. suratensis showed early warning through high avoidance behaviour (> 30% occurrence of 0 – 2Hz) compared to control and increased stress ventilation (2.5 – 5 Hz). This species showed an avoidance behaviour for almost 16 hours. At 50% effluent concentration early warning was obtained by detecting reduced movement activity (< 25% occurrence of 0 – 2Hz) in *S. denisonii, P. reticulata*. Increased stress ventilation (2.5 – 5 Hz) was shown by *X. maculatus, O. mossambicus* and *E. suratensis. P. terio* has shown avoidance behaviour. 100 % mortality was observed visually for all six species. *S. denisonni* and *P. terio* survived only for the first 3 hours of the experiment, *X. maculatus* survived for 4 hours and *P. reticulata* for 6 hours. *O. mossambicus* survived for 25 hours and *E. suratensis* survived for 37 hours. All these observations indicates that the effluent concentration above 50% will be harmful to aquatic life. Percentage of movement activity of six fish species in both control and effluent dilution is statistically analysed and plotted in figure 2.

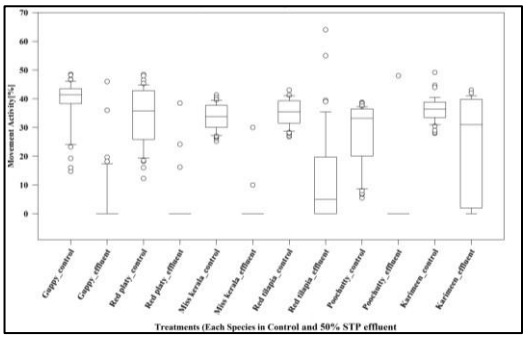

Figure 2 : Movement Activity of all species in control and 50% effluent from SSET STP

The main stress causing factors to fish species in effluent were expected as iron, nitrate, reduction in availability of external dissolved oxygen and other unidentified chemicals present in effluent. Gills are the main site of water contamination and toxicity (Arabi 2004). The metal ion complexes act as a potential cell toxicant, which leads to disturbances in cell functions and cause cell death (Dalzell and Macfarlane 1999). The effluent from SSET STP has an iron concentration of 0.9 - 1.2 ppm. During the continuous aeration in the flow through chamber the iron in wastewater gets precipitated as ferric hydroxide, this may caused the physical clogging of gills and gill damage.

The concentration of nitrate in SSET STP effluent was 0.47 - 2 ppm. Maximum level of nitrate was recommended as 2.0 mg NO_3-N/l for protecting the most sensitive freshwater species (Camargo et al. 2005). Therefore, nitrate in the effluent may also caused stress to fish life. The DO in diluted effluent was 5ppm, were COD and BOD value of effluent from STP is 183 and 93ppm respectively, so even continuous aeration is given in the chamber there will be a reduction in external availability of dissolved oxygen in effluent dilution than in control which was an another reason for fish mortality. Using MFB it was observed that at a 50% effluent concentration the fishes given early warning to stress conditions and obtained 100% mortality. These findings using MFB could be helpful for early environmental warning system and for monitoring fish health.

IV. CONCLUSION

It can be concluded that, *O. mossambicus* and *E. suratensis* at their juvenile stage can be used as an indicator organism for giving an early warning to the toxic discharge of effluents from treatment units. From earlier studies, it is observed that adult *P. reticulata* can be used as a bio indicator. However, it is majorly used as an ornamental fish. Since *O. mossambicus* and *E. suratensis* have more economical value, it is more suitable as bio indicator in Indian conditions. *S. denisonii* and *X. maculatus* are highly sensitive to toxicity so it is not suitable as a bio indicator. *P. terio* is a very common fish found in all freshwater bodies in India. However, it is highly sensitive to toxicity which shows that wastewater at this concentration is harmful for aquatic organisms.

By using Multispecies Freshwater Biomonitor (with indicator organisms from Indian conditions) the continuous online monitoring of effluent discharge from wastewater treatment plants can be done. While getting the early warning from indicator species we can stop the further discharge into water bodies and we can retreat the effluent until it become harmless to aquatic life. By this, we can ensure the decrease in degradation of water quality of water bodies and make sure the safety of aquatic life and human health. So, it will be a great remedy to further pollution of water sources.

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