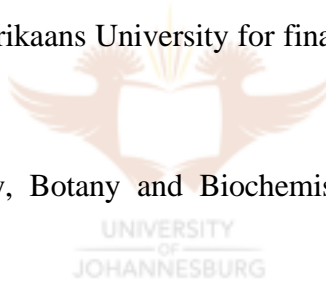


ACKNOWLEDGEMENTS

I extend my profound gratitude to the following persons or institutions:

- My supervisors Dr Dictor Wepener, Dr Bubuya Masola for their valuable knowledge and guidance.
- Laetitia Slabbert, Annette Venter of CSIR, Amina Nel for assistance with biomarker assays and whole effluent toxicity testing.
- WARFSA and Rand Afrikaans University for financial support.
- Department of Zoology, Botany and Biochemistry for assisting with some of required equipment.
- Family and friends for their moral support and above all my creator who made this possible

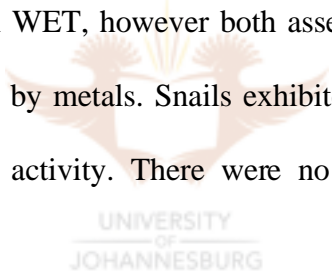


ABSTRACT

The biological integrity of aquatic ecosystems has become threatened by the effects of high nutrient loads, inorganic and organic chemicals. The effect of these xenobiotics needs to be investigated by the application of biotests in whole effluent toxicity testing and biomarkers in active biomonitoring. Whole effluent toxicity (WET) testing determines the toxicity/effect of whole effluent on aquatic organisms. Sub-lethal effects can be determined by analysing the levels of subcellular/physiological indicators/enzymes in aquatic organisms exposed to *in situ* conditions. The procedure used for *in situ* assessments was active biomonitoring (ABM). The aim of this study was to assess the instream health of a known contaminated system (Rietvlei Wetland System, Gauteng, South Africa) using WET and ABM methodologies. Three sites in the Rietvlei Wetland System were selected and sampling was undertaken during high flow (April 2003) and low flow (August 2003) periods. The ABM sampling protocol involved the deployment of aquatic molluscs (*Melanoides tuberculata*) and fish (*Oreochromis mossambicus*) for a period of four weeks at the sites. Following the four week exposure period the organisms were transported back to the laboratory and the following biomarkers were assessed: ethoxyresorufin o-deethylase (EROD), acetylcholine esterase (AChE) and metallothione (MT). Water samples were also collected for WET testing during the low flow period, since this was the only period where mortality was observed in the ABM organisms. Standard WET protocols were used to assess the toxicity of the water from the three sites. These protocols were: 96 h *Poecilia reticulata* lethality test, 48 h *Daphnia pulex* lethality test and 72 h *Selenastrum capricorutum* growth inhibition

test. In addition the same biomarker analyses that were done on the ABM organisms were carried out on WET exposed *D. pulex* and *P. reticulata*.

The WET testing and ABM indicated highest toxicity at Sites 1 and 3. Algal growth inhibition test showed highest stimulation of algal growth at Site 2 and inhibition at Site 3. Sites 1 and 2 showed signs of contamination by organophosphates and carbamates due to elevated AChE and reduced EROD. However there were no significant differences in AChE activity between fish tested in the 96h toxicity test and those used in ABM. Very low AChE activity was recorded in *D. pulex*. Snails also had lower AChE activity when compared to the exposed fish species. Metallothionein content was higher in field-exposed fish than those used in WET, however both assessment protocols indicated that Site 3 was affected the greatest by metals. Snails exhibited higher MT content than fish and *D. pulex* showed no MT activity. There were no significant differences in MT content between the sites.

The logo of the University of Johannesburg is centered on the page. It features a stylized sun with rays at the top, two human figures holding hands in the middle, and the text 'UNIVERSITY OF JOHANNESBURG' at the bottom.

Acetylcholine esterase appears to be a relevant means of investigating biological effects of many neurotoxic contaminants on aquatic habitats and trophic levels. Metallothionein content is a good indicator of toxicity due to heavy metals for active biomonitoring as well as the WET test. Ethoxyresorufin-O-deethylase is a more difficult biomarker to work with since it shows no differences in activity between control and exposed organisms. The EROD assay does not detect very low levels of EROD induction. Acetylcholinesterase and MT are the recommended biomarker assays for the detection of sublethal responses in the WET laboratory toxicity test. The AChE activities and MT exhibit comparable results in both ABM and WET assessment protocols. In future studies

WET testing needs to be complimented with a suite biomarker analyses to determine the type of pollutants in a system and sufficiently describe the pollution status of a system.



LIST OF FIGURES

- Figure 1.** Map of Rietvlei Wetland System indicating sampling sites where ABM exposures were carried out during April 2003 (high flow) and August 2003 (low flow). 40
- Figure 2.** Spatial and temporal biomarker responses of *M. tuberculata* at three sites in the Rietvlei Wetland System following a four week exposure period during high flow (April 2003) and low flow (August 2003) conditions. Data for A: AChE, B: EROD, C: MT are presented as means \pm SE (n=10). Significant differences were regarded as ($p < 0.05$). Sites within flow conditions with similar superscript letters differ significantly from each other. 51
- Figure 3.** Biomarker responses of *O. mossambicus* at three sites in the Rietvlei Wetland System following four weeks exposure during high flow conditions (April 2003). Data for A: AChE, B: EROD, C: MT are presented as means \pm se (n=10). There were no significant differences between site and exposure groups and the laboratory control groups ($p < 0.05$). 52
- Figure 4.** Biomarker responses of *D. pulex* (WET), *M. tuberculata* (ABM), *P. reticulata* (WET) exposed to water from the three sites during the low flow period (August 2003). Data for A: AChE, B: EROD, C: MT are represented as means \pm SE, (n=10). Asterisks indicate biomarker responses in bioindicator organisms that differ significantly from the laboratory control groups ($p < 0.05$). Within sites, bars with different alphabetical superscript differ significantly ($p < 0.05$). 56

LIST OF TABLES

Table 1.	Physico-chemical analysis of water at the three sampling sites in the Rietvlei Wetland System.	49
Table 2.	Percentage mortalities following 48 h <i>D. pulex</i> exposure to 100% and 50% of the raw water from three different sites in the Rietvlei Wetland System.	54
Table 3.	Percentage mortalities following 96 h exposure of <i>P. reticulata</i> to different percentages of raw water from three selected sites in the Rietvlei Wetland System.	54
Table 4.	Percentage change in algal growth relative to the control as measured during 72 h algal growth inhibition test.	55

