

Chapter 3

Effluent Toxicity

3.1. Introduction

As discussed in Chapter 2, the potential ecological risk posed by the discharge of an effluent into a marine receiving environment can be estimated by comparing concentrations of constituents in the effluent to water quality guidelines or other standards derived to be protective of ecosystem health. Simple mass balance modelling can be used to estimate effluent constituent concentrations in a marine receiving environment after initial dilution and these can be compared to water quality guidelines or other standards derived to be protective of ecosystem health to identify the potential for adverse ecological effects. However, both approaches have limitations. First, water quality guidelines are specific to individual constituents, yet effluent is a complex mixture of physical and chemical constituents present in variable concentrations. Water quality guidelines fail to consider possible antagonistic, additive or synergistic effects between effluent constituents. Second, water quality guidelines can only be used to estimate the risk posed by effluent constituents for which guidelines have been derived. However, of the 84 000 or so synthetic chemicals that are estimated to be used daily in domestic, commercial and industrial applications (Schnoor, 2014) only a very small proportion (estimated at <1%) have associated toxicology information and even fewer an associated water quality guideline. Third, it is impossible to analyse for all chemicals in effluent. The risk of unknown constituents in an effluent cannot, therefore, be estimated.

It is in this context that whole effluent toxicity testing assumes an important role. Whole effluent toxicity testing (sometimes called direct toxicity testing) measures the toxicity of an effluent (or any other liquid) regardless of its physical and chemical properties and takes into account antagonistic, additive and synergistic toxic effects of the

potentially thousands of constituents in an effluent. Toxicity testing puts chemical data into perspective by acting as a measure of bioavailability. This is important since the presence of a chemical in an environmental compartment (*e.g.* water, sediment) does not mean it is in a bioavailable form (*i.e.* in a form that can cross biological membranes). Only if it is in a bioavailable form can a chemical exert a biological effect (adverse or beneficial). Metals bound in the crystal lattice of sediment grains, for example, are in a non-bioavailable form, while metals dissolved in the water column are generally in a bioavailable form. To further complicate matters, because a chemical is in a bioavailable form does not mean it will exert an effect. This can only occur if there is exposure, that is, if an organism comes into contact with the chemical. Toxicity testing takes these complexities into account.

This chapter discusses findings of the toxicity testing of effluent collected from the Green Point, Camps Bay and Hout Bay wastewater works in 2016.

3.2. Materials and Methods

3.2.1. Fieldwork

Effluent was collected from the Green Point, Camps Bay and Hout Bay wastewater works by the Scientific Services Branch of the City of Cape Town at approximately two month intervals over an eight month period in 2016. The samples were held on ice until return to the laboratory, where they were refrigerated at 4°C. The samples were transferred overnight on ice to the CSIR toxicology laboratory in Durban, where they were refrigerated until testing within four days of collection.

3.2.2. Laboratory Analyses

Adult sea urchins (*Echinometra mathaei* and *Tripneustes gratilla*) were collected at Vetch's Pier in Durban and maintained at ambient temperature

in natural seawater in large, flow-through tanks in the laboratory. Gametes (sperm and eggs) were obtained for testing by inducing sea urchins to spawn. This was achieved by injecting 2 - 4 ml of 0.5 molar potassium chloride (KCl) solution into the coelomic cavity of each test organism. Gametes from males and females were collected separately. Females were inverted over glass beakers filled with seawater and eggs were allowed to settle. Sperm was collected 'dry' in pasteur pipettes. Sea urchins that provided relatively few gametes were excluded from consideration for testing.

The quality of eggs and sperm was evaluated prior to testing by adding diluted sperm from each male to eggs from each female in 20 ml of seawater in vials. After ten minutes the eggs were examined under a microscope for the presence of a fertilisation membrane. Combinations of eggs and sperm that did not produce at least 90% fertilisation success were excluded from consideration for testing.

Sperm was activated by exposure to seawater. One hundred microliter aliquots of sperm suspension were then transferred to control (seawater) and effluent dilution treatments. Effluent dilutions were prepared by the addition of relevant amounts of clean seawater to effluent, with four replicates for each treatment. After ten minutes of sperm exposure, 1 ml of egg suspension was added and left for a further ten minutes. The test was then terminated by adding 100 µl of formalin. Fertilisation success was determined by microscopic examination of an aliquot of the egg suspension from each replicate. Sea urchin gamete sensitivity was assessed using two reference toxicants (positive controls), namely formaldehyde, and copper, following a similar procedure to that outlined above.

3.2.3. Data Analysis

Toxicity testing over the period in question necessitated the use of two urchin species due to differences in breeding season (*E. mathaei* was used in April and *T. gratilla* at other times). These species exhibit different sensitivities to copper, which is used as a reference toxicant for quality assurance purposes, and probably also have different sensitivities to effluent constituents. This

complicates the direct comparison of effluent toxicity between surveys when gametes from the different species were used. In addition to inter-species differences in sensitivity there are intra-species differences in sensitivity. Since effluent from each wastewater works was tested at the same time differences in effluent toxicity between the works were compared directly using the Minimum Acceptable Toxicant Dilution. This represents the minimum number of times the effluent needed to be diluted with clean seawater for sea urchin gametes to achieve a fertilisation success statistically similar to that in the control treatment. The Minimum Acceptable Toxicant Dilution was determined by modelling the dose-response relationship using the logistic regression model (Barnes *et al.*, 2003):

$$\text{Response} = \text{Plateau} \div (1 + \exp(-\text{slope} \times (\log \text{Rate} - \log \text{EC}_{50})))$$

The fitted parameters were used to define the Minimum Acceptable Toxicant Dilution at the point where the predicted fertilisation success (response) was the mean minus twice the standard deviation for fertilisation success in the control treatment.

3.3. Results and Discussion

The Minimum Acceptable Toxicant Dilution for effluent collected from the Green Point, Camps Bay and Hout Bay wastewater works is provided in Figure 3.1. Apart from the April 2016 survey the Minimum Acceptable Toxicant Dilution for Green Point and Hout Bay effluents was fairly consistent, at about 52 - 62 and 29 - 32 respectively. In other words, these effluents required a dilution, on average, of about 57 and 30 times to render it non-toxic, but at times (one survey each) a dilution as high as 137 for Green Point effluent and as low as 13 for Hout Bay effluent. The Minimum Acceptable Toxicant Dilution for Camps Bay effluent was more variable, providing a Minimum Acceptable Toxicant Dilution of 60 - 248. The Minimum Acceptable Toxicant Dilution for this effluent was thus typically somewhat higher than for Green Point effluent, which in turn was typically far higher than for Hout Bay effluent. If the effluent samples are taken as representing the range in variability for effluent discharged through the outfalls then from a toxicity

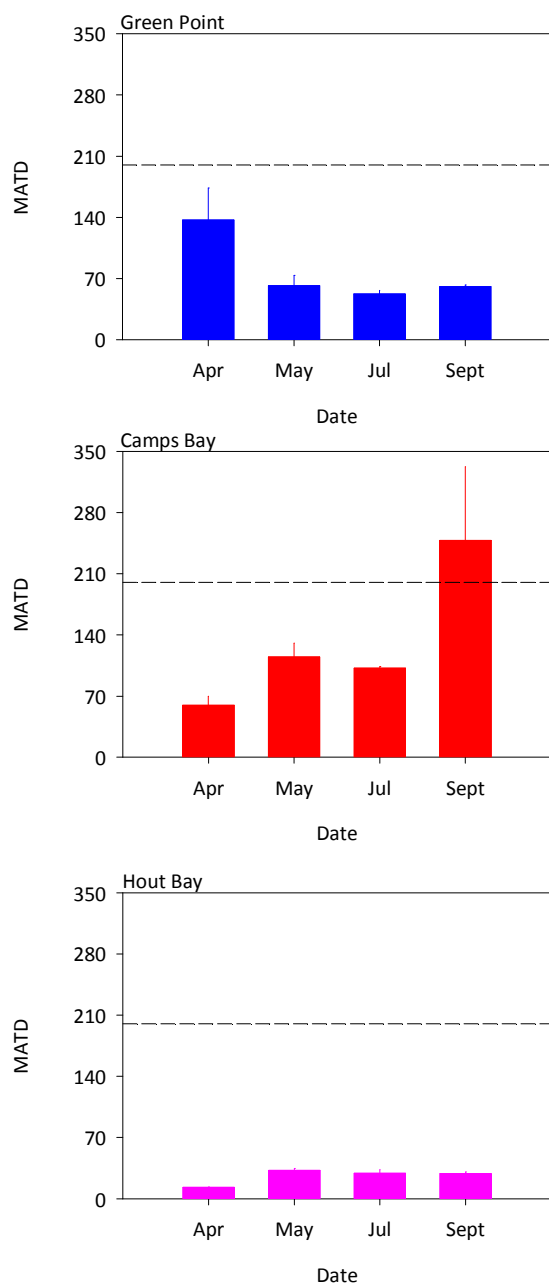


Figure 3.1. Minimum Acceptable Toxicant Dilution (MATD) for four effluent samples collected from the Green Point, Camps Bay and Hout Bay wastewater works in 2016. The horizontal dashed line represents a Minimum Acceptable Toxicant Dilution of 200.

perspective Camps Bay effluent poses the highest and the Hout Bay effluent the lowest acutely toxic risk to organisms in the marine receiving environments.

The Minimum Acceptable Toxicant Dilution for Green Point, Camps Bay and Hout Bay effluents are within the range for effluent discharged into the marine environment through deepwater outfalls off Durban, and for effluent discharged through one outfall off Richards Bay (CSIR, unpublished data).

For example, the median (range) Minimum Acceptable Toxicant Dilution for effluent discharged through the Central Works and Southern Works outfalls off Durban since 2009 is 82 (10 - 5990) and 58 (13 - 259) respectively (note the upper limit of the range for Southern Works effluent is a clear outlier - if ignored the upper limit is 690). However, effluent from one industry is discharged through a dedicated outfall off Richards Bay has a far higher Minimum Acceptable Toxicant Dilution (in the thousands).

As stated above, the minimum initial dilution for the Green Point, Camps Bay and Hout Bay outfalls is uncertain. To estimate the potential for the exceedance of water quality targets after initial dilution in Chapter 2, minimum initial dilutions of 20 and 200 were considered. The Minimum Acceptable Toxicant Dilution for Green Point and Camps Bay effluents clearly exceeds 20, and for Hout Bay effluent was on average slightly higher than 20. As stated above, the minimum initial dilution of effluent discharged at depths >20 m under calm conditions is usually about 200 and >1 000 in current flows >0.2 m.s⁻¹ (DWAF, 2004). If the minimum initial dilution for the Green Point, Camps Bay and Hout Bay outfalls is assumed to be 200 considering the diffuser sections are in water exceeding 20 m depth (see Table 1.1) then effluent discharged through the outfalls should be diluted sufficiently to render it not acutely toxic to marine organisms that are similarly or less sensitive to toxicants than sea urchin gametes after initial dilution with the exception of Camps Bay effluent. In the latter case the required dilution to render it non-toxic exceeds the minimum initial dilution about 25% of the time if the toxicity testing provides a reasonable approximation of effluent toxicity outside the periods of testing. The testing of four effluent samples is, however, inadequate for understanding the toxicological characteristics of an effluent, especially if it displays a high variability in toxicity over time.

The above discussion does not consider confidence intervals associated with the Minimum Acceptable Toxicant Dilutions. The upper 95% confidence interval for the effluents was generally not inordinately higher than the Minimum Acceptable Toxicant Dilution and does not alter the above

conclusions. However, the upper confidence limit for one Green Point effluent sample was 173.7 and for one Camps Bay effluent was 332.3. In other words, the Minimum Acceptable Toxicant Dilution could theoretically be this high (and conversely lower than the Minimum Acceptable Toxicant Dilution if the lower 95% confidence limit is considered).

3.4. Conclusions

Toxicity testing of effluent collected from the Green Point, Camps Bay and Hout Bay wastewater works on four occasions over an approximately eight month period in 2016 revealed Minimum Acceptable Toxicant Dilutions of 52 - 137, 59 - 248, and 13 - 32 respectively. If it is assumed the minimum initial dilution for each outfall under calm conditions is 200 then it is unlikely effluent constituents will be acutely toxic to marine organisms similarly or less sensitive to toxicants than sea urchin gametes after initial dilution apart from Camps Bay effluent on one occasion. As discussed in Chapter 4, this is borne out by the results of toxicity testing of surface seawater samples collected in the marine receiving environment when effluent was clearly visible at the sea surface. However, since the minimum initial dilution for the outfalls is uncertain this conclusion

must be confirmed by the measurement of other indicators of water quality near the outfall diffuser sections and supported by numerical modelling and tracer dye studies on effluent dispersion and dilution.

Whole effluent toxicity testing has limitations. It does not protect human health through exposure to chemicals accumulated in the tissue of fish and shellfish. It also assesses the immediate toxicity of chemicals in effluent but not the persistence and long-term cumulative effects of chemicals. For example, chemicals can accumulate to toxic concentrations in sediment over time. Effluent also differs in its composition over the short-term and it may be difficult to obtain a sample that is fully representative of this variability.

Only four effluent samples from each wastewater works were tested and the results should not be taken as indicative of the variability in the toxicity of effluent discharged through the outfalls. It is recommended, therefore, that the toxicity of effluent from the Green Point, Camps Bay and Hout Bay wastewater works be tested for toxicity once a month for 12 months, using effluent collected for physical and chemical characterisation. Based on the results a decision can be made on the frequency of testing required over the long-term.