



**Marine Ecotoxicology Laboratory
Coastal Systems Research Group, Smart Places
Marine Toxicity Test Results**

Client Details

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Date	12 December 2025
Sample ID	Enviroserv_eThekwini Sample
Order Number	LG000000295365
Reference	CSIR/SPLA/SECO/TOX/Enviroserv/Dec2025/B

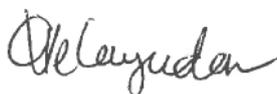
Results

eThekwini Municipality submitted one sample on behalf of Enviroserv Waste Management to the Coastal Systems research group of the CSIR for toxicity testing in December 2025. The toxicity of the sample was determined using the sea urchin fertilisation test with gametes of *Echinometra mathaei*. The test endpoint is the **Minimum Acceptable Toxicant Dilution (MATD)**, which represents the minimum number of times an effluent must be diluted with clean seawater to achieve a fertilisation success in sea urchin gametes that is not statistically significantly different to that for the control (uncontaminated seawater). The results of the toxicity testing are provided in Table 1 below (Data and methods are included in the Appendices).

Table 1. Minimum Acceptable Toxicant Dilution (MATD) of test sample

Sample ID	Sample Date	MATD
Enviroserv_eThekwini Municipality Sample	02/12/2025	13.4

The Minimum Acceptable Toxicant Dilution (MATD) of the sample did not exceed the limit of 200 that is contained in the eThekwini Municipality Sewage Disposal Bylaws (Schedule B). The effluent is thus regarded as ACCEPTABLE for marine discharge.



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Appendix 1: Sea Urchin Toxicity Testing Data

Sample ID	Effluent Concentration (%)	Proportion of Fertilised Eggs			
		Replicate 1	Replicate 2	Replicate 3	Replicate 4
Enviroserv_eThekwini Municipality Sample 02/12/2025	Seawater Control (0%)	0.1000	0.1000	0.9900	0.9900
	0.25	0.1000	0.1000	0.9900	0.9900
	0.2	0.1000	0.1000	0.9900	0.9900
	0.5	0.1000	0.1000	0.9900	0.9900
	1	0.1000	0.1000	0.9900	0.9900
	2	0.1000	0.1000	0.9900	0.9900
	5	0.1000	0.1000	0.9900	0.9900
	10	0.9900	0.9600	0.9800	0.9700
	20	0.3900	0.4000	0.4200	0.4200

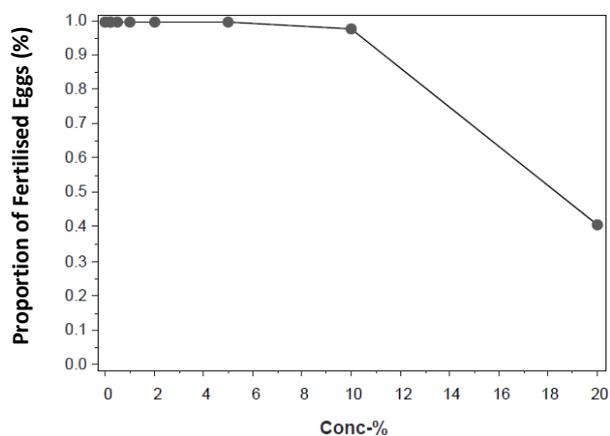


Figure 1. Fertilisation success of sea urchin gametes (%) exposed to dilutions of the sample.

Appendix 2: Reference Toxicant Test Control Charts

Results from the fertilisation reference toxicant tests were within acceptable limits. The control fertilisation is within the range (>95%) considered acceptable by the CSIR.

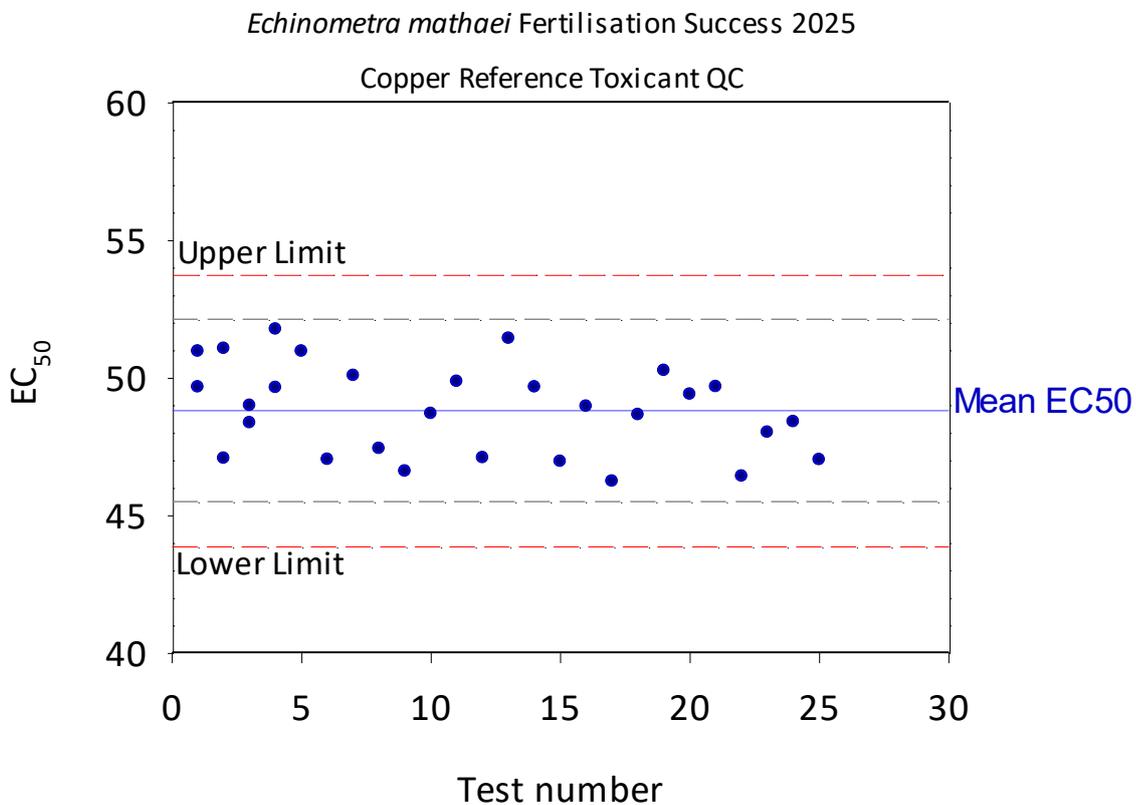


Figure 2. Copper reference toxicant control chart for the sea urchin fertilisation test.

Appendix 3: Methods

Laboratory Analyses

The toxicity of the samples was tested using the acute sea urchin fertilisation test based on methods defined by the USEPA (1995). Adult sea urchins were collected from Vetch's Pier in Durban and maintained at ambient temperature in seawater in large flow-through tanks in the laboratory. Gametes (sperm and eggs) were obtained by inducing sea urchins to spawn, by injecting 1 - 2 ml of a 0.5 molar potassium chloride solution into their coelomic cavity. 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 before testing by adding diluted sperm from each male to eggs from each female in 20 ml of seawater in vials. Eggs were examined under a microscope for the presence of a fertilization membrane. Combinations of eggs and sperm that did not produce at least 90% fertilisation success or showed abnormal membrane development were excluded from consideration for testing. Sperm was activated by exposure to seawater and 100 µl aliquots of the suspension were then transferred to each of four replicate vials for the control ('clean' seawater), test samples and a positive control (copper). The positive control provided a sensitivity reference for the sea urchins used. After ten minutes of sperm exposure, 1 ml of egg suspension was added and left for ten minutes. The test was then terminated by adding 100 µl of 37% formaldehyde to each test vial. The control and each test sample comprised four replicates. Fertilisation success was determined by the microscopic examination of the egg suspension from each replicate.

Table 1: Summary of test conditions and test acceptability criteria for sea urchin acute toxicity tests with effluents and receiving waters.

Summary of Toxicity Test	
Test	Acute Sea Urchin Fertilisation Test
Test Type	Static, non-renewal
Test Species	<i>E. mathaei</i> or <i>T. gratilla</i>
Control / Dilution water	Uncontaminated filtered natural seawater
Salinity	35 ± 1°C
Temperature	23 °C
Photoperiod	Ambient laboratory illumination
Test endpoint	Fertilisation of eggs
Acceptability criteria	> 95% fertilisation success in the control
No. of replicates	4

Analysis of Data

The Minimum Acceptable Toxicant Dilution (MATD) represents the minimum number of times an effluent must be diluted with clean seawater to achieve a fertilisation success in sea urchin gametes that is not statistically significantly different to that for the control treatment. The Minimum Acceptable Toxicant Dilution was determined using Comprehensive Environmental Toxicity Information System software (CETIS V1.9.6.3; Tidepool Scientific Software, USA), by applying the linear interpolation method.

The effective concentrations eliciting a response in 50% of the gametes or larvae (EC₅₀) exposed to copper concentrations in reference toxicant tests were also calculated using CETIS software.