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Research on Ecotoxicology and Applications in Singapore: Description of the Sponge Aggregation Assay

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Abstract—Ecotoxicology research has not traditionally been pursued significantly in Singapore. Since 2000, research undertaken by the Ecotoxicology Laboratory, NIE/NTU to assess stress in the tropical marine environment, has focused on the development of ecotoxicological techniques at the cellular and biochemical-levels. Techniques developed include the sponge aggregation assay; green mussel (*Perna viridis*) fertilization and lysosomal integrity assays; sea urchin (*Diadema setosum*) fertilization assay; and various enzyme assays. In this paper, the sponge aggregation assay is detailed, and results of studies on sublethal effects of copper and cadmium on sponges are described. Two-way ANOVA on data from Cu^{2+} and Cd^{2+} exposure tests yielded no significant interaction effects between treatment concentrations and exposure durations. Significant differences in sponge cell re-aggregation were observed between controls and treatments, and between exposure durations. Results showed a decrease in cell re-aggregation with increasing metal concentrations and exposure time. At 48 h exposures, no significant differences were observed between treatments and controls, suggesting that sponges may have the ability to detoxify metals during prolonged exposure. For both metal treatments, EC_{50} (effective concentration at which cells did not re-aggregate) values were computed for 12 h, 24 h and 48 h experiments. Results of field tests are also documented.

Keywords: ecotoxicology testing, cellular bioassay, sponge aggregation, Singapore

INTRODUCTION

Increasing human and industrial activities have resulted in a greater amount of pollutants being discharged into the estuarine and coastal environment (Widdows and Donkin, 1992). Heavy metals are just one of the many pollutants which are found to accumulate in marine organisms as well as in marine sediments at levels higher than the ambient environment (His *et al.*, 1999). Some environmental contaminants are present at levels which may be toxic to the marine organisms and affect the marine ecosystem adversely (Moriarty, 1990). Heavy metals are

one of the common pollutants introduced into the seas, among which mercury, silver and copper are the most toxic. The development of ecotoxicity bioassays is essential to assess the potential toxicity of these contaminants (His *et al*., 1999).

A bioassay has to be sensitive, easy to carry out, scientifically valid and the test organism must be available readily to ensure its practicability (His *et al*., 1999). These underlying principles have been applied since 2000, not only to select test organisms, but also in the development of ecotoxicology techniques at the Ecotoxicology Laboratory, National Institute of Education, Nanyang Technological University, to assess the effects of various heavy metal pollutants in the marine environment.

With regard to local test organisms from Singapore, the tropical green mussel, *Perna viridis* has been used in the development of various ecotoxicology assays. These include a fertilization assay and a mussel haemolymph lysosomal integrity test. In addition, a fertilization assay has been developed using the common sea urchin, *Diadema setosum*. A coastal sponge (*Haliclona* sp.; Order: Halichondrida) has also been the subject of ecotoxicology testing. As it is not possible to document all the tests developed by the Ecotoxicology Laboratory, NIE/NTU, this paper will only describe the sponge aggregation assay in greater detail.

Research on marine sponges has indicated that they have potential to be used as bioindicators of heavy metal pollution (Vogel, 1977). Sponges prove to be potentially better bioindicators compared to other filter-feeders like bivalves and tunicates because they possess no internal organs specifically designed for filtering large volumes of water, of between 100 and 1200 ml $h^{-1}g^{-1}$ sponge (Vogel, 1977; Riisgard *et al*., 1993). In addition, sponges are also unselective filter-feeders that can retain particles, mainly bacteria and ultraplankton, up to 8 μm in diameter (Barthel and Wolfrath, 1989).

Benthic marine sponges inhabiting shallow waters are tolerant to small amounts of copper (Cu^{2+}), but at levels above their threshold concentrations, Cu^{2+} can be highly toxic (Peña *et al*., 1999). It has been established that high levels of Cu^{2+} have a negative effect on marine invertebrates such as bivalves and sponges (Reish *et al*., 1984). Industrialization has put marine sponges at higher risk of heavy metal, pollution through discharge of wastes into rivers in recent years (Gladstone and Dight, 1994; Gladstone, 1996). The increased use of copper-based antifouling paint (because of its effectiveness) as the common tributyltin paint is banned in the shipping industry, has also contributed to the higher Cu^{2+} levels reported (Claisse and Alzieu, 1993). All these activities mean that marine organisms are being exposed to elevated Cu^{2+} and there is a need for monitoring pollution levels.

Cadmium (Cd^{2+}) is regarded as a priority pollutant because of its toxicity to both marine organisms and humans. It has also been reported that anthropogenic inputs are the major source of Cd^{2+} contamination (Nriagu, 1980). To assess bioaccumulation and toxicity of Cd^{2+}, it is essential to determine how much of this input remains dissolved in seawater and what chemical forms exist in

sediments and seawater. Cd^{2+} concentration in the pristine marine environment is generally less than 1 ng l^{-1} in seawater, and less than 1 mg kg^{-1} in sediments (Nriagu, 1980). Higher concentrations in seawater, sediments and marine biota can be expected in areas affected by human activities. In general, Cd^{2+} enters the marine environment via atmospheric deposition and through effluent discharges from point sources in near shore areas (Nriagu, 1980). Such activities have contributed to the exposure of heavy metals including Cd^{2+} to marine organisms, and therefore there is a need for pollution monitoring.

Studies have shown that copper, zinc and cadmium accumulated by the sponge, *Halichondria panicea*, is positively correlated with the concentration in the surrounding media (Olesen and Weeks, 1994; Hansen *et al*., 1995). The slow depuration of heavy metals in this species adds to its potential as a bioindicator of heavy metal pollution (Webster *et al*., 2001).

Disassociated sponge cells can form functional aggregates. They first form small primary aggregates of only a few cells before forming secondary aggregates. The secondary aggregates then rearrange themselves to form re-aggregated sponges that have water-channels like before (Müller *et al*., 1977). This natural unique phenomenon of the simple body structured, filter-feeding primitive animal has thus made them useful in water toxicity studies.

Based on the established facts that sponges accumulate certain heavy metals from the surrounding media, and that high levels of metals have a negative impact on them, it is possible that high metal contamination may also affect the ability of sponges to re-aggregate.

In this paper, the effects of elevated Cu^{2+} and Cd^{2+} concentrations on the ability of a sponge, *Haliclona* sp. (Order: Halichondrida), to re-aggregate in laboratory exposures of 12 h, 24 h and 48 h is explored. The paper also documents a preliminary exercise on the transplantation of sponges to various locations around Singapore, to assess the feasibility of using the sponge aggregation test in field deployed experiments.

MATERIALS AND METHODS

Sponge collection and preparation

For each test contaminant, a single large specimen of *Haliclona* sp. (Order: Halichondrida) was collected by hand from the berthing pontoons at Raffles Marina (1°20.53′ N, 103°38.22′ E), located West of Mainland Singapore. One single large sponge was used in each experiment to minimize genetic variability (Webster *et al*., 2001). The sponge was transported to the laboratory within 30 minutes in aerated seawater.

Salinity and pH of seawater from Raffles Marina were measured and recorded using a portable pH meter and Atago refractometer. During the period of collection, pH and salinity ranged from 7.35–8.04 and 27–30‰, respectively. The sponges were then cleaned in filtered sea water (0.45 μm) collected from Raffles Marina to remove associated symbionts, e.g. synaptids.

Laboratory bioassays

In both the Cu^{2+} and Cd^{2+} exposure experiments, five replicates were prepared for each treatment and control. Controls for all treatments contained filtered seawater (0.45 μm) collected from Raffles Marina on the same day as the test sponge specimens. Treatment concentrations were obtained by adding the metal contaminant (Cu^{2+} and Cd^{2+}) to the filtered seawater in appropriate amounts.

Copper (Cu^{2+}) exposure

The reference contaminant used was $CuCl_2 \cdot 2H_2O$. Sponges of approximately 1 g were cut into 3 pieces and subjected to 100 μg l^{-1}, 500 μg l^{-1} and 1700 μg l^{-1} Cu^{2+} concentrations for 12 h.

50 ml beakers were used to contain the sponges for each exposure concentration. During the exposure period, sponges submerged in filtered seawater were not fed but continuously aerated. The samples were removed and the aggregation test carried out after 12 h.

A similar experimental set-up was used to test exposure of sponges to Cu^{2+} in 24 h and 48 h time durations. For both the 24 h and 48 h exposures, a Cu^{2+} regime of 100 μg l^{-1}, 500 μg l^{-1} and 1500 μg l^{-1} Cu^{2+} were used.

Cadmium (Cd^{2+}) exposure

The reference contaminant used was $CdCl_2 \cdot 2H_2O$. Sponges of approximately 3 g were cut into 3 pieces and exposed to 0, 100, 500 and 1000 μg l^{-1} Cd^{2+} treatments at durations of 12 h, 24 h and 48 h. For each treatment and controls, 50 ml beakers were used to contain the sponges for the duration of the experiment. Throughout the experiment, the sponges remained submerged in continuously aerated and filtered seawater but were not fed.

Field trial

In addition to the collection site (Raffles Marina, 1°20.53′ N, 103°38.22′ E), two other test sites were selected to conduct a field trial; Lim Chu Kang Fishing Jetty (1°26.78′ N, 103°42.41′ E) and Punggol Marina (1°25.11′ N, 103°54.06′ E). Sponges collected from Raffles Marina were transplanted to these two sites. Nylon nets and threads were used to suspend the sponges in the water column (Barthel and Theede, 1986). Salinity and pH of sea-water from both sites were measured and recorded. In the field exercise, five replicates were deployed at each site, including the source site for the sponges, Raffles Marina.

Sponge cell aggregation

The sponge cell aggregation method used was adapted from that described by Philip (1997). Briefly, approximately 1 g of sponge was cut and rinsed in calcium, magnesium-free seawater (CMFSW, pH 7) for 5 min. The sponge was cut into fine pieces and rinsed with fresh CMFSW for 15 min to obtain a cell

suspension. The resulting cell suspension was pipetted into a test-tube and homogenized using a vortex mixer. The number of cells and aggregations (3 or more cells joined together) in the mixture were then enumerated using a haemocytometer under a compound microscope and recorded.

A 1 ml homogenized cell suspension was transferred to a 1.5 ml siliconized centrifuge tube and 50 μl of 30 μM $CaCl_2$ (final concentration) was added to induce aggregation. The centrifuge tube was agitated for 15 min using a rota-shaker before the numbers of single and aggregations were counted on a haemocytometer again.

The mean proportion of single cells after induced aggregation by $CaCl_2$ was expressed by dividing the number of single cells after induced aggregation by the number of single cells before induced aggregation.

Statistical analyses

Two-way Analysis of Variance (ANOVA) was applied on data to determine significant treatment effects. Data (proportion of single cells) were arcsine transformed prior to statistical analyses to fulfill the requirements of ANOVA. Treatments were regarded as statistically significant at $P < 0.05$. Significant treatment effects were analyzed further using Dunnett's test (Zar, 1999). All tests were carried out using the MINITAB© software. EC_{50} (concentration eliciting 50% cell aggregation) values were then subsequently determined by the use of probit analysis.

RESULTS

Copper exposures

The proportion of single cells after induced cell re-aggregation in the Cu^{2+} treatments ranged from 58.0% to 93.1% (Fig. 1). The greatest range of means obtained was at the 48 h time exposure, ranging from 58.0% to 80.9% single cells. The standard deviations obtained were also larger at the 48 h exposure regime, with the highest at 21.1% for a treatment exposure of 1500 $\mu g\ l^{-1}$ Cu^{2+}. As a result of the narrow range of results obtained, probit values for computation of the EC_{50} values were obtained by expressing the proportion of single cells as proportions of the controls (Fig. 2). Corresponding EC_{50} (effective concentration at which 50% of test samples did not exhibit any cell re-aggregation) obtained for the 12 h, 24 h and 48 h exposures to Cu^{2+} were 467, 307 and 583 $\mu g\ l^{-1}$ Cu^{2+}, respectively.

Two-way ANOVA results on sponge data indicated no significant interaction effects between time and treatment concentrations ($P > 0.05$). Significant treatment effects were observed between the three time exposures and also between treatment concentrations ($P < 0.05$, Table 1).

Cadmium exposures

At all time exposures, the number of single cells observed to re-aggregate decreased with increasing Cd^{2+} concentrations. The proportion of single cells

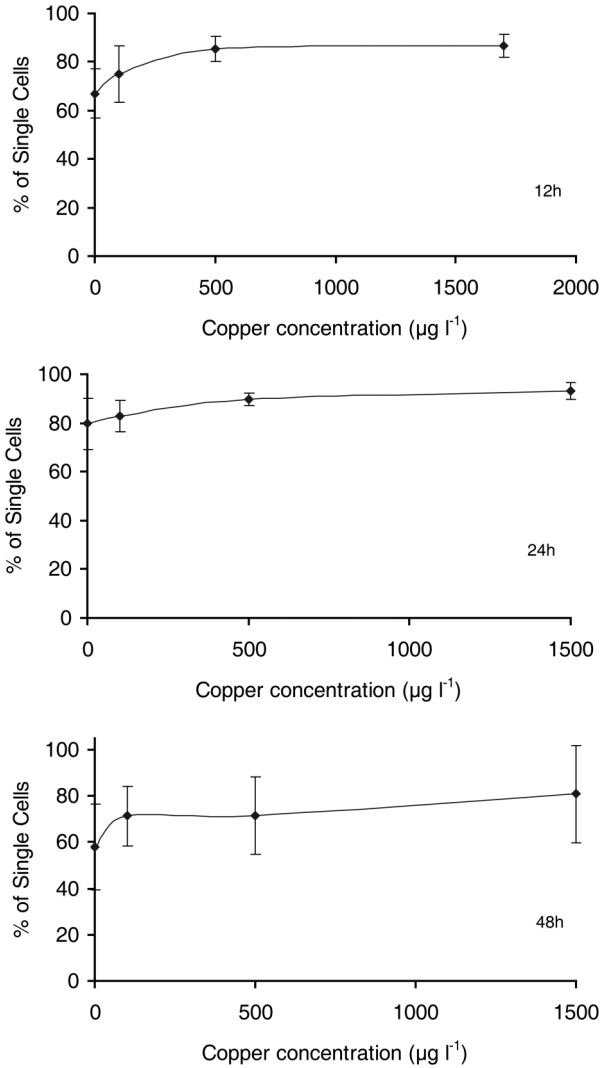

Fig. 1. Percentage of single cells observed in response to $CaCl_2$-induced aggregation after 12 h, 24 h and 48 h exposures at different Cu^{2+} concentrations.

observed in the various Cd^{2+} treatment concentrations ranged from 0.63 to 0.91 (Fig. 3). It was observed that the proportion of single cells was highest at the treatment of 1000 μg l^{-1} Cd^{2+} at all exposure durations, where they were recorded to be higher than 0.9. The mean proportion of single cells obtained was high across all the treatments, at the 48 h exposure duration (Fig. 3).

Probits for the computation of EC_{50} values were obtained by converting the

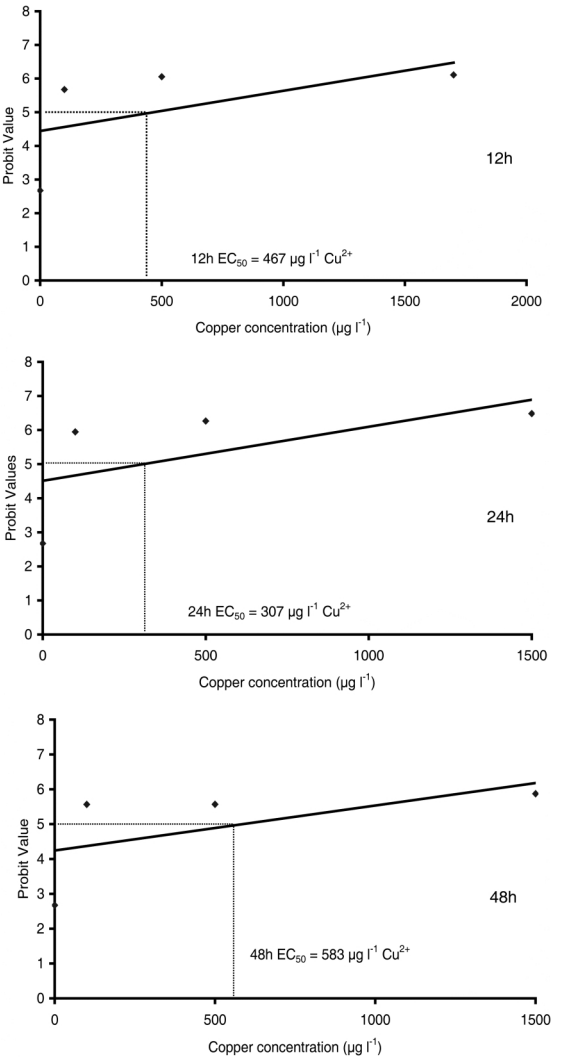

Fig. 2. Probit response curves from 12 h, 24 h and 48 h exposures to Cu^{2+} treatment concentrations.

percentage of single cells to percentage of single cells as proportions of the controls. The observed EC_{50} values for the 12 h, 24 h and 48 h time exposures were determined at 291, 230 and 192 μg l^{-1} Cd^{2+}, respectively (Fig. 4).

Two-way ANOVA on sponge data showed no significant interaction effects between time and Cd^{2+} treatment concentrations ($P > 0.05$). Significant treatment effects were observed between the three time exposures and also between treatment concentrations ($P < 0.05$, Table 2).

Table 1. Results of two-way ANOVA on the variable proportion of single cells crossed with factors: Cu^{2+} concentrations and exposure durations (12 h, 24 h and 48 h), and Dunnett's test results on time and concentrations (C: control; Cu^{2+} concentrations: 100, 500, 1500 μg l^{-1}; n.s. indicates not significant).

Variable	Factors	*P*	Results
Proportion of single cells	Time	0.003	12 < 24 < 48
	Concentration	0.012	C < 100 < 500 < 1500
	Time∗Concentration	0.155	n.s.

Table 2. Results of two-way ANOVA on the variable proportion of single cells crossed with factors: Cd^{2+} concentrations and exposure durations (12 h, 24 h and 48 h), and Dunnett's test results on time and concentrations (C: control; Cd^{2+} concentrations: 100, 500, 1000 μg l^{-1}; n.s. indicates not significant).

Variable	Factors	*P*	Results
Proportion of single cells	Time	0.008	12 < 24 < 48
	Concentration	0.005	C < 100 < 500 < 1000
	Time∗Concentration	0.2	n.s.

Table 3. Results of one-way ANOVA on proportion of single cells in controls and treatments for the different time regimes (12 h, 24 h and 48 h) for Cu^{2+} and Cd^{2+} (C: control; Cu^{2+} concentrations: 100, 500, 1500, 1700 μg l^{-1}; Cd^{2+} concentrations: 100, 500, 1000 μg l^{-1}; n.s. indicates not significant).

Metal	Time (h)	*P*	Results
Cu^{2+}	12	<0.05	C < 100 < 500 < 1700
Cu^{2+}	24	<0.05	C < 100 < 500 < 1500
Cu^{2+}	48	>0.05	n.s.
Cd^{2+}	12	<0.05	C < 100 < 500 < 1000
Cd^{2+}	24	<0.05	C < l00 < 500 < 1000
Cd^{2+}	48	>0.05	n.s.

Duration of contaminant exposures

Further analyses of the sponge aggregation response data applying one-way ANOVA on the data revealed that as exposure time increased, the effects of the metals Cu^{2+} and Cd^{2+} decreased. No significant differences were seen between treatments and controls after exposure at 48 h (Table 3).

Field trial

In the field exercise, the proportion of single cells observed in sponges deployed at the three stations ranged from 65.2% to 67.8%, with standard

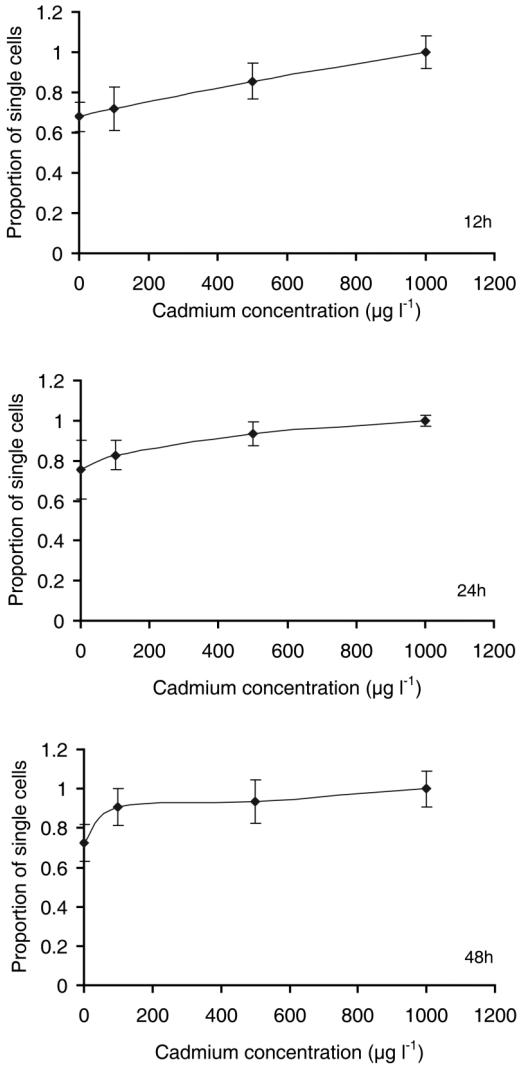

Fig. 3. Proportion of single cells observed in response to $CaCl_2$-induced aggregation after 12 h, 24 h and 48 h exposures at different Cd^{2+} concentrations.

deviations ranging from 5.5% to 6.5% (Table 4). One-way ANOVA performed on cell aggregation data from sponges deployed at the three study sites showed no significant differences in cell aggregation between all sites.

DISCUSSION

The results from the Cu^{2+} and Cd^{2+} exposure experiments indicate that these

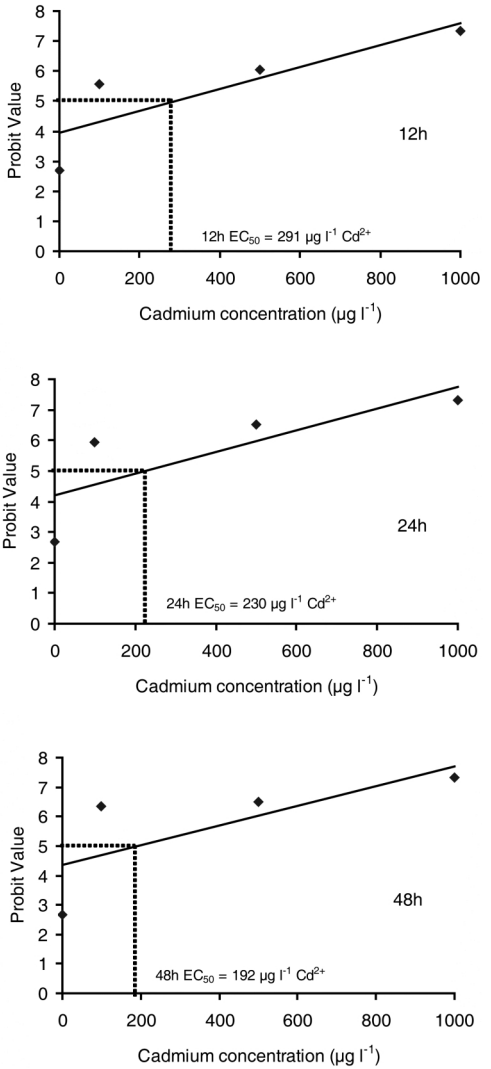

Fig. 4. Probit response curves from 12 h, 24 h and 48 h exposures to Cd^{2+} treatment concentrations.

metals have a negative effect on sponge cell re-aggregation. Results from the 48 h exposure experiments suggest that sponge cells may be able to detoxify the metal contaminants when given a longer exposure time. This is supported by the narrow range of means observed for responses in controls and treatments in the 48 h exposures, and the fact that there were no significant differences between the treatments and controls.

Webster *et al*. (2001) found that Cu^{2+} levels in the sponge cells were lower

Table 4. Proportion of single cells in response to calcium-induced aggregation observed in sponges from Raffles Marina (Controls), Punggol Marina and Lim Chu Kang (data are mean proportion of single cells ± S.D., $n = 5$).

	Raffles Marina	Punggol Marina	Lim Chu Kang
Mean	62.7%	67.8%	65.2%
S.D.	5.7%	5.5%	6.5%

than that of the surrounding media. They suggested that that the lower Cu^{2+} concentrations in sponge tissues could be due detoxification mechanisms in sponges that bind the highly toxic form of Cu^{2+} to less toxic complexes. A multixenobiotic resistance pump as a cellular defence mechanism has also been described by Müller *et al*. (1996) in the sponge *Suberites domuncula* and by Kurelec and Pivcevic (1992) in the sponge *Tethya aurantium*. Other marine organisms such as fish, bivalves and snails also have been found to have such multixenobiotic mechanisms (Kurelec, 1992; Kurelec *et al*., 1992; Livingstone, 1994; Smital and Kurelec, 1998). The "multixenobiotic resistant pump" has been described as a mechanism whereby sponge cells accumulate toxins in some cells and these cells undergo apoptosis (programmed cell death) to eliminate the potentially harmful materials (Batel *et al*., 1993; Wagner *et al*., 1998). The toxicity to the whole sponge is thus minimized in this way. The results obtained from this study, as well as other published research suggest that sponge cells have the ability to overcome toxicity by such detoxifying mechanisms, given a period of time.

Webster *et al*. (2001) reported high mortality in the sponge *Rhopaloeides odorabile* when exposed to 223 μg l^{-1} Cu^{2+} for 48 hours. Hansen *et al*. (1995) however, reported no mortality of the sponge *Halichondria panicea* exposed to 1000 μg l^{-1} Cu^{2+} for 14 days. Our results here suggest that some sponge species are more able to tolerate heavy metals, and a detoxification mechanism may be in place.

The environmental conditions the sponges were subjected to before they were collected could also have been a factor for the tolerance. The sponges were obtained from the berthing area for boats at Raffles Marina, which is a recreational private boating facility. The environment in which the sponges were growing may originally have been contaminated. The sponges may thus have been pre-conditioned to survive in polluted water and hence the resultant narrow range of results observed.

The results from the field studies indicated that there were no significant differences in the sponge aggregation responses between all the study sites. It may have been that all transplant locations were similar in environmental quality, and hence the inconclusive results. However, the results of the transplantation experiment illustrate that the sponge aggregation assay is feasible in field deployed environmental studies.

It is evident that the sponge aggregation assay is a potentially viable cellular

bioassay that has an application in the field. This is one of a few tests that are currently being investigated further for application in ecotoxicology testing by the Ecotoxicology Laboratory, National Institute of Education, Nanyang Technological University.

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REFERENCES

Barthel, D. and H. Theede (1986): A new method for the culture of marine sponges and its application for experimental studies. *Ophelia*, **25**, 75–82.

Barthel, D. and B. Wolfrath (1989): Tissue sloughing in the sponge *Halichondria panicea* : a fouling organism prevents being fouled. *Oecologia*, **78**, 357–360.

Batel, R., N. Bihari., B. Rinkevich, J. Dapper, H. Shäcke, H. C. Schräder and W. E. G. Müller (1993): Modulation of organotin-induced apoptosis by water pollutant methyl mercury in a human lymphoblastoid tumor cell line and a marine sponge. *Mar. Ecol. Prog. Ser.*, **93**, 245–251.

Claisse, D. and C. Alzieu (1993): Copper contamination as a result of antifouling paint regulations? *Mar. Pollut. Bull.*, **26**, 395–397.

Gladstone, W. (1996): Trace metals in sediments, indicator organisms and traditional seafoods of the Torres Strait. Report series 5a, Great Barrier Reef Marine Park Authority, Townville, Australia.

Gladstone, W. and I. J. Dight (1994): Torres Strait baseline study. *Mar. Pollut. Bull.*, **29**, 121–125.

Hansen, I. V., J. M. Weeks and H. Depledge (1995): Accumulation of copper, zinc and chromium by the marine sponge *Halichondria panicea* Pallas and implications for biomonitoring. *Mar. Pollut. Bull.*, **31**, 133–138.

His, E., R. Beiras and M. N. L. Seaman (1999): The assessment of marine pollution-bioassays with bivalve embryos and larvae. *Adv. Mar. Biol.*, **37**, 1–178.

Kurelec, B. (1992): The multixenobiotic resistance mechanism in aquatic organisms. *Crit. Rev. Toxicol.*, **22**, 23–43.

Kurelec, B. and B. Pivcevic (1992): The multidrug resistance mechanism in the marine sponge *Tethya aurantium*. *Mar. Environ. Res.*, **34**, 249–253.

Kurelec, B., S. Krca, B. Pivcevic, D. Ugarkovic, M. Bachmann, G. Imsiecke and W. E. G. Müller (1992): Expression of P-glycoprotein gene in marine sponges. Identification and characterization of the 125-kDa drug-binding glycoprotein. *Carcinogenesis*, **13**, 69–76.

Livingstone, D. R. (1994): Recent developments in marine invertebrate organic xenobiotic metabolism. *Toxicol. Ecotoxicol. News*, **1**, 88–95.

Moriarty, F. (1990): *Ecotoxicology. The Study of Pollutants in Ecosystems*. Academic Press, London, 289 pp.

Müller, W. E. G., J. Arendes, B. Kurelec, R. K. Zahn and I. Müller (1977): Species-specific aggregation factor in sponges. Sialyltransferase associated with aggregation factor. *J. Biol. Chem.*, **271**, 23558–23565.

Müller, W. E. G., R. Steffen, B. Rinkevich, V. Matranga and B. Kurelec (1996): The multixenobiotic resistance mechanism in the marine sponge *Suberites domuncula*: its potential applicability for the evaluation of environmental pollution by toxic compounds. *Mar. Biol.*, **125**, 165–170.

Nriagu, J. O. (1980): Production, uses and properties of cadmium. p. 36–75. In *Cadmium in the Environment*, ed. by J. O. Nriagu, John Wiley & Sons, New York.

Olesen, T. M. and J. M. Weeks (1994): Accumulation of Cd by the marine sponge *Halichondria panicea* Pallas: effects upon filtration rate and its relevance for biomonitoring. *Bull. Environ. Contam. Toxicol.*, **52**, 722–728.

Peña, M. M. O., L. Jaekwon and D. J. Thiele (1999): A delicate balance: homeostatic control of copper uptake and distribution. *J. Nutr*., **129**, 1251–1260.

Philip, R. B. (1997): Effects of pH and oxidant stressors (hydrogen peroxide and bleach) on calcium-induced aggregation of cells of the marine sponge *Microciona prolifera*. *Comp. Biochem. Physiol*., **118**, 347–351.

Reish, D. J., P. S. Oshida, F. G. Wilkes, A. J. Mearns, T. C. Ginn and R. S. Carr (1984): Effects on saltwater organisms. *J. Water Pollut. Cont. Fed*., **56**, 759–774.

Riisgard, H. U., S. Thomassen, H. Jakobsen, J. M. Weeks and P. S. Larsen (1993): Suspension-feeding in marine sponges *Halichondria panicea* and *Haliclona urceolus*—effects of temperature on filtration-rate and energy cost of pumping. *Mar. Ecol. Prog. Ser*., **96**, 177–188.

Smital, T. and B. Kurelec (1998): The chemosensitizers of multixenobiotic mechanism in aquatic invertebrates: A new class of pollutants. *Mutat. Res*., **399**, 43–53.

Vogal, S. (1977): Current induced flow through living sponges in nature. *Proc. Natl. Acad. Sci. USA*., **74**, 2069–2071.

Wagner, C., R. Steffen, C. Koziol, R. Betal, M. Lacorn and H. Steinhart (1998): Apoptosis in marine sponges: a marine biomarker for environmental stress (cadmium and bacteria). *Mar. Biol*., **131**, 411–421.

Webster, N. S., R. I. Webb, M. J. Ridd, R. S. Hill and A. P. Negri (2001): The effects of copper on the microbial community of a coral reef sponge. *Environ. Microbiol*., **3**, 19–31.

Widdows, J. and P. Donkin (1992): Mussels and environmental contaminants: Bioaccumulation and physiological aspects. p. 383–423. In *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture. Developments in Aquaculture and Fisheries Science*, No. 25, ed. by E. Gosling, Elsevier, London.

Zar, J. H. (1999): *Biostatistical Analysis*. 4th ed., Prentice-Hall International, New Jersey.

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