

EFFECT OF CADMIUM TO *STROMBUS CANARIUM* (GASTROPOD: STROMBIDAE)

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Total of 180 specimens of marine gastropod, *Strombus canarium*, collected from southern coast of Peninsular Malaysia were exposed to artificial sea water containing cadmium in the concentration range between 1.0 and 5.0 mg L⁻¹ for 96 hours. The objective of the study is to statistically determine the acute toxicity of cadmium to the gastropod, *Strombus canarium* that has not been reported yet so far. Experiments were carried out under static renewal condition in order to maintain the concentration level of cadmium constant as well as to prevent accumulation of organic pollutants such as excreta of the gastropods and disintegrated dead bodies. The data obtained from the test were subjected to the EPA Probit Analysis Program and the acute toxicity was determined as the median lethal concentration (LC50). Abnormal behaviour such as secretion of mucus in large quantities was observed, especially, at high concentration of cadmium. Neither mortality nor abnormal behaviour was observed in the control group throughout the experiment. As a result, the 96-h LC50 value for *Strombus canarium* was determined to be 1.23 mg L⁻¹. The result indicates that the *Strombus canarium* possesses less tolerance to cadmium ions compared to other gastropods.

Keywords: Acute toxicity test; LC50; cadmium; *Strombus canarium*

Introduction

Metals are often introduced to aquatic systems as a result of both natural causes and human activities associated with urbanization, industrialization and agricultural runoff (Thompson et al., 2005). These pollutants often accumulate in the food chain and cause various toxic effects on marine organisms (Gupta and Singh, 2011). Previous studies have shown that progressive and irreversible accumulation of trace metals can interfere and impair the functions of marine animals, especially when the concentrations exceed certain levels (Storelli & Marcotrigiano, 2005; Nubi et al., 2011). In general, high level of heavy metals in marine sediment and seawater can exert adverse impacts on the spawning and reproduction of many marine species (Glazer and Quintero, 1998).

It is known that accumulation of cadmium in high concentration in gastropods blocks the embryo development and impairs their development growth (Coeurdassier et al., 2003). These adverse effects of cadmium, and other changes in the Malaysian coastal environment due to the anthropogenic factors, could be the cause of disappearance of marine molluscs such as *Strombus* sp., *Perna veridis* and *Anadara granosa*. These gastropods are among the important fishery resources in Malaysia because of their high economic values in the local markets, but they are no longer abundant in the region (Bujang et al., 2006, Ismail, 2006, Cob et al., 2009).

Gastropods are sometimes used as a bio-indicator to assess heavy metal contamination in aquatic ecosystem because of their capability of accumulating heavy metals in high concentration and the relatively limited range of migration. However, the accumulation level in gastropods depends on the amount of heavy metal in the environment, route of uptake, and storage and excretion mechanisms (Walker et al., 2006). It should be noted that the degree of metal accumulation also varies

substantially among the species, and may result in different physiological response and tolerance level. One of the important indices is LC50, but an abnormal behaviour of a marine animal is observed when when it is exposed to a contaminant at a concentration below the lethal level, the animal is more useful as a bio-indicator, because an early detection of environmental deterioration may become possible by continuously observing the marine animals. *Strombus canarium* is such a marine animal that excretes mucus in large quantities when exposed to cadmium even at a non-lethal concentration. And it appeared that the adverse effects of cadmium intensified as the concentration increased.

Heavy metals accumulated in marine animals are a risk to human health through consumption of contaminated seafood (Abdullah et al., 2007). Chongprasith et al. (1999) reported that the concentrations of cadmium in seawater at various locations within ASEAN region exceeded the ASEAN recommended limit for protection of marine aquatic life (0.010 mg L⁻¹). It could be a major public health concern if cadmium accumulates in marine animals, which are consumed as seafood, at a concentration higher than that in the environment. On the other hand, if a marine organism has a low tolerance to cadmium, the marine organism is more susceptible to the contaminant and may perish quickly, thus becomes unsuitable for human consumption before its concentration exceeds the level to be concerned. This study investigates the acute toxic effects of cadmium on *Strombus canarium*, because very limited numbers of studies have been conducted to determine the LC50 of cadmium on *Strombus canarium* even though the molluscs are an important seafood resource.

Methods

Strombus canarium were collected at Tanjung Kupang seagrass bed in Johor Straits in March 2011. 180 specimens of adult *Strombus canarium* with shell length of 48 to 55 mm were selected to ensure uniformity for the test and were kept in moist sediment during transportation to the laboratory. Then the specimens were acclimatized in an aerated tank containing 100 L of artificial seawater and the sediment for two weeks. Instant Ocean synthetic sea salt (Aquarium Systems Inc., Sarrebourg, France) prepared per instruction was used as the artificial seawater. Temperature, salinity, pH and dissolved oxygen (DO) in the acclimatization tank were monitored daily. The observed values are shown in Table 1.

Table 1: Water conditions in acclimatization tank

| Parameter | Characteristic |
|-----------------------|----------------------------|
| Temperature | 25-29 °C |
| Salinity | 25-30 ppt |
| pH | 7.0-8.0 |
| Dissolved Oxygen (DO) | 5.0-6.0 mg L ⁻¹ |

The acute toxicity test was carried out under static renewal condition, which followed the procedures described by ASTM E729-96 (2007) with some modifications. A standard solution of Cd (NO₃)₂ (1000 mg L⁻¹ as cadmium, Merck, Germany) was used to prepare the test seawater. The test concentrations were pre-determined using range-finding test and prepared by adding calculated amount of the cadmium solution to 20 L of artificial seawater. A set of five tanks, each tank containing ten *Strombus* specimens, were prepared with different concentrations of cadmium at 1.0, 2.0, 3.0, 4.0 and 5.0 mg L⁻¹. The exposure period was 96 hours. A control tank was also prepared with ten *Strombus* specimens in 20 L of artificial

saltwater without any cadmium. The test seawater and control seawater were renewed every 24 hours.

The *Strombus* specimens were not fed during the test. Temperature, salinity, pH and DO were monitored continuously in the test tanks. Mortality rate in each test tank was recorded at 24-hour interval and dead *Strombus* were removed immediately. The experiments were conducted in triplicate for each test concentration. The EPA Probit Analysis Program (version 1.5, US EPA) was used to determine the median lethal concentration (LC50) value at 96 hours with 95% confidence limit.

Results and Discussion

During the test period, the environmental conditions in the tanks were regulated at the predetermined values (Table 1). The monitored fluctuations of temperature, salinity, pH and DO, were within the ranges between 5.5 and 6.0 mg L⁻¹, 29.0 and 29.3 ppt, 7.8 and 8.0 and 24.5-26.1 °C, respectively.

The results of cadmium toxicity test and the statistical analysis for *Strombus canarium* are summarized in Tables 2a and 2b, respectively. As shown in Table 2b, the 96-h LC50 value of cadmium for adult *Strombus canarium* was determined to be 1.23 mg L⁻¹. No mortality was observed in the control group throughout the experiment indicating the *Strombus* specimens subjected to the test were free from stress and therefore the accuracy of the data was ensured (ASTM, 2007). Moreover, high mortality rate was recorded as the increasing of cadmium concentration as shown in Figure 1. A total of 100% mortality rate was recorded at the concentrations of 3.0, 4.0 and 5.0 mg L⁻¹ after 96 hours of exposure.

Table 2a: Results of the 96-hour toxicity test

| Concentration of Cd (mg L ⁻¹) | Number of gastropods exposed | Number of gastropods died | Mortality rate |
|---|------------------------------|---------------------------|----------------|
| 0.0 | 10 | 0 | 0.0 |
| 1.0 | 10 | 4 | 0.4 |
| 2.0 | 10 | 7 | 0.7 |
| 3.0 | 10 | 10 | 1.0 |
| 4.0 | 10 | 10 | 1.0 |
| 5.0 | 10 | 10 | 1.0 |

Table 2b: Results of lethal concentration of Cd determined by the EPA Probit Analysis Program

| Point | Exposure Concentration | 95% confidence Limits | |
|-----------------|------------------------|-----------------------|--------------|
| | | Lower | Upper |
| LC 1.00 | 0.374 | 0.058 | 0.663 |
| LC 5.00 | 0.531 | 0.124 | 0.842 |
| LC 10.00 | 0.639 | 0.186 | 0.959 |
| LC 15.00 | 0.725 | 0.244 | 1.050 |
| LC 50.00 | 1.233 | 0.726 | 1.620 |
| LC 85.00 | 2.099 | 1.597 | 3.383 |
| LC 90.00 | 2.380 | 1.805 | 4.292 |
| LC 95.00 | 2.867 | 2.116 | 6.245 |
| LC 99.00 | 4.066 | 2.753 | 13.071 |

Theoretical Spontaneous Response Rate (control) = 0.000 (mg L⁻¹)

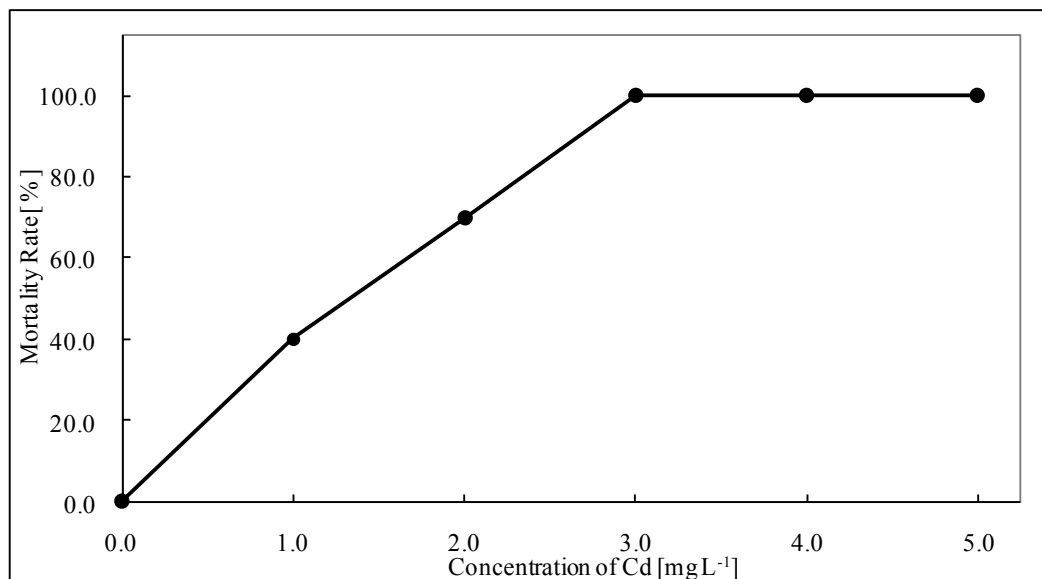


Figure 1: Percentage of dead *Strombus canarium* in relation to different concentrations of cadmium

Shuhaimi-Othman et al. (2010) reported that the sensitivities and effects of heavy metals substantially varied from species to species. Some of the LC50 values reported for other gastropods are summarized in Table 3 for comparison. As shown in Table 3, the LC50 value of cadmium for *S. canarium* determined in this study is lower than those of other gastropods such as the scavenging gastropod, *Nassarius festivus*, which 96-h LC50 value was reported to be 1.52 mg L⁻¹ (Cheung et al., 2002). Lakshmi and Rao, (2002) reported much higher LC50 value for the intertidal gastropod, *Turbo intercostalis*, which is 6.18 mg L⁻¹. Moloukhia and Sleem (2011) found that the 96-h LC50 value of cadmium for two gastropods, *Caelatura companyoi* and *Cleopatra bulimoides*, were even greater at 8.0 and 7.5 mg L⁻¹, respectively. Therefore, *S. canarium* is considered to possess a high sensitivity to cadmium at lower concentration range than other gastropods. The LC50 values of cadmium on various gastropods vary in a wide range as Shuhaimi-Othman et al. (2010) reported.

Table 3: Comparison of 96-hour LC50 values of Cd among *S. canarium* and other gastropods

| Gastropod species | LC50 value (mg L ⁻¹) | Reference |
|-----------------------------|----------------------------------|-----------------------------|
| <i>Turbo intercostalis</i> | 6.18 | Lakshmi and Rao, 2002 |
| <i>Nassarius festivus</i> | 1.52 | Cheung et al., 2002 |
| <i>Nucella lapillus</i> | 2.64 | Mangas-Ramirez et al., 2007 |
| <i>Monodonta lineate</i> | 2.44 | Mangas-Ramirez et al., 2007 |
| <i>Caelatura companyoi</i> | 8.0 | Moloukhia and Sleem, 2011 |
| <i>Cleopatra bulimoides</i> | 7.5 | Moloukhia and Sleem, 2011 |
| <i>Strombus canarium</i> | 1.23 | This study |

During the course of the exposure tests, *S. canarium* showed abnormal behaviour depending upon the concentration of cadmium. In the control group, their behaviour remained unchanged and normal where no fatality was observed within 96 hours. *S. canarium* in other experimental tanks added with different concentration of

cadmium secreted large amounts of mucus, especially, at higher concentrations during the first 24 hours of exposure, even though there was no death observed in the period. After 48 hours of exposure, the specimens moved slowly and mucus secretion increased significantly. Similar observation was made and reported by Volland et al. (2011) with *Strombus pugilis*. Baker (2002) speculated that a gastropod secreted mucus to prevent direct contact between toxic compound and its digestive tract to reduce the toxic effect of the compound to itself. At 4.0 and 5.0 mg L⁻¹ of cadmium concentrations, all the specimens gradually slowed down in their movements before they died. Whereas, at 1.0, 2.0 and 3.0 mg L⁻¹ of cadmium concentrations, the specimens' eyestalk tentacles, which normally extend forward, appeared very weak and withdrawn into their shells. During the period between 72 and 96 hours from the start, the motions of all the living specimens became extremely slow and the opercula were retreated into their shells with continuous mucus secretion.

Conclusion

The value of 96-h LC50 for *Strombus canarium* exposed at various concentrations of cadmium was determined to be 1.23 mg L⁻¹. The EPA Probit Analysis Program was employed to evaluate the data on toxicity response of the gastropods to cadmium under the controlled experimental conditions. No mortality was observed in the control group, which indicated no stress had occurred to the gastropods, except for artificially added cadmium, throughout the test. During the exposure test, *Strombus canarium* secreted mucus in large quantities, and the secretion appeared to increase as the cadmium concentration increased. Result of LC50 obtained in this study was lower than that of other gastropods, which implies that cadmium is significantly toxic to *S. canarium*. Since *S. canarium* is an important seafood resource in the coastal regions, a further study is necessary to investigate in detail the accumulation and excretion of ingested cadmium and other heavy metals by the gastropods.

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