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Mucus Secretion Responses to Various Sublethal Copper (II) Concentrations in the Mussel *Perna perna*

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Abstract**—**The purpose of this study was to evaluate the use of mucus production as a biomarker. This was done by exposing the mussel Perna perna to various sublethal concentrations of Cu. Mussels are effective as a bioindicator species as they accumulate Cu in their tissues. Differences in mucus production rates were evaluated at different Cu concentrations. The findings of this study indicate that increasing Cu concentrations had a significant effect on the mucus production rates over a 24 hour exposure. There were also significant differences between the mucus production rates at different Cu concentrations ($p < 0.05$). Thus, mucus is an essential detoxification mechanism.

Keywords**—**Copper, Mucus, Depuration, *Perna perna.*

I. Introduction

HEAVY metals can have significant impacts on the physiological, behavioural and cellular responses of organisms if their concentrations are above the critical threshold [16], [19], [23]. Heavy metals may enter the marine environment in a variety of ways. Erosion, wind and volcanic activity are natural pathways that heavy metals may be introduced into coastal systems from [10]. Heavy metals may be introduced via anthropogenic activities such as dumping of industrial wastewater [9],[10], oil spills [24] and sewage effluent [8]. Hence, there is a need to determine the pollution status of marine systems before negative impacts on the ecosystem become irreversible.

The biomarker approach is the use of quantitative variations in processes within an organism in reaction to the exposure of a foreign substance, while bioindicators are changes that occur at higher levels of organisation such as at the population or ecosystem levels [12]. Mussels are an example of a bioindicator species that can be used in establishing the pollution status of an ecosystem [23]. Mussels are ideal bioindicators as they have the ability to accumulate heavy metals from solution and food particles allowing for the pollution status of the area to be ascertained [10], [15], [23]. Hence, the pollution status of the ecosystem can be remediated before the impacts become irreparable. Being sedentary organisms, mussels are easy and inexpensive to sample hence they can be used extensively in marine pollution impact assessments [6], [10].

Copper is a widespread pollutant in the marine environment [2]. According to [14], copper is a vital metal for bivalve molluscs in low concentrations. Byssus thread formation and metabolism is heavily dependent on low concentrations of copper [14], [20]. However, copper in elevated concentrations can be lethal [14], [15]. Mussels are suitable indicators of high copper concentrations as they may accumulate 3000 times more copper than the surrounding water [22].

Perna perna (brown mussel) is found along the African and South American coasts and in the Gulf of Mexico [1]. *Perna perna* is the most prevalent species of mussel found on the east of South Africa [10]. There has been a steady increase in urban development along the coast of KwaZulu-Natal, South Africa [1]; subsequently, there is a growing need to monitor the pollution status of coastal and estuarine waters. *Perna perna* would be the most suitable bioindicator species since it is indigenous and widespread along the South African coastline [10].

Variations in physiological rates [10], accumulations of heavy metal within tissue [10], [15], [23] and assessing changes in detoxifying secretions [11], [21], [23] in mussels are the most common methods used in determining the pollution status of an area. Mucus secretion by mussels is an example of a detoxifying secretion [11], [21], [23]. Increased production of mucus is evident when high concentrations of heavy metals are present [10], [14], [21], [22], [23]. Hence the evaluation of mucus production rates as a biomarker is necessary. This study aims to determine if differences in mucus production rates between differing copper concentrations are present and if a significant trend exists between increasing copper concentrations and mucus production rates.

II. Materials and Methods

A. Sample Collection and Preparation

Samples of *P. perna* were collected from the rocky shore at Park Rynie beach on the south coast of KwaZulu-Natal (30^o 18’ S; 30^o 44’ E). The byssus threads of the mussels were cut and all epibionts were removed from the shell. Mussels collected from the field were left to acclimate in an aerated re-circulation tank of 1000 litres for three days in a glasshouse before being moved to the exposure tanks. The temperature was not regulated in the re-circulation tank hence individuals were exposed to fluctuations in the ambient temperature. Mussels were left to acclimate further in aerated filtered

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seawater at 24.4ºC within the exposure tanks for 24 hours, prior to the introduction of copper. During the exposure and acclimation within these tanks, individuals were purged. Mussel shell lengths ranged between 32 and 59 mm (mean shell length 44.49 ± 6.01mm). According to the ANOVA test, there was a no significant difference between size measurements for different Cu concentration treatments ($p > 0.05$, $F = 2.479$, degrees of freedom (D.F.) = 21).

B. Experimental Design

Mussels were placed into four exposure tanks with seven litres of filtered seawater which was aerated. The exposure tanks were an example of a static system. Each exposure tank contained seven mussels. A 500 ml stock solution of distilled water and $CuCl_2$ [0.74074 g/l, 350 mg/l Cu^{2+}] was prepared. Each of the four tanks contained a different concentration of Cu (0 µg/l Cu (control), 12.5 µg/l Cu , 25 µg/l Cu and 50 µg/l Cu). 1 ml of stock solution was added to the 50 µg/l Cu concentration. Mussels were exposed for 24 hours.

C. Mucus Collection

Sampling protocol for the extraction of mucus was adapted from [23]. Mussels were taken out of the exposure tanks and placed in filtered seawater for 30 minutes so that may recover from handling. Glass microscope slides were pushed into the mantle cavity of the mussel when the valves of the mussel were opened. Thereafter, microscope slides and mussels were left in pre-weighed glass beakers for 10 minutes. Exposure of mussels to air allows for the collection of mucus [4], [5], [23]. The microscope slide was removed after 10 minutes and mucus washed into the pre-weighed beaker with distilled water. Pre-weighed beakers were placed in an oven at 80ºC for 24 hours. Thereafter, the beakers were weighed and the dry mass of mucus was calculated by the difference of the oven dried mucus and beaker and the dry beaker weight.

D. Tissue Collection

After mucus collection, mussels were frozen for 12 hours to allow for the removal of tissue. Shell lengths were recorded in mm using a calliper. Tissue was scraped off from each mussel using a scalpel into pre-weighed petri dishes. Petri dishes were placed in an oven at 80ºC for 24 hours. Thereafter, the petri dishes were weighed and the dry tissue mass (g) calculated using the formula of the difference of the oven dried petri dish with dry tissue (g) and the dry petri dish weight (g). Furthermore, the mucus production rates were calculated in grams of mucus per gram of dry tissue per minute by the formula of dividing the mucus in grams by the tissue mass and a time of 10 minutes.

E. Statistical Analysis

Statistical tests were performed using SPSS v 15.0 for Windows while Graphpad Prism 5 was used to generate the graphs. A Grubb's test was performed on all data with 1% of all significant outliers being removed (http://www.graphpad.com/quickcalcs/Grubbs1.cfm). A one way ANOVA test with a Bonferroni multi-comparison test was performed on mucus production rates at different Cu concentrations. A simple linear regression was also performed to investigate if a significant trend exists and the magnitude of the relationship for mucus production rates against increasing concentrations of Cu. All assumption tests for normality and equality of variance were satisfied for all statistical tests ($p > 0.05$). The regression assumption test for linearity was also satisfied.

III. Results

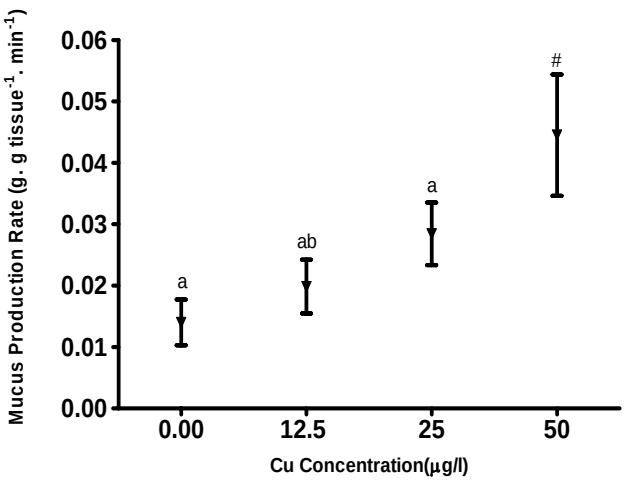

Fig. 1 Mean Mucus production rates with 95% confidence in P. perna at different Cu concentrations (same letters indicate that a significant difference between treatments is present, # indicates that the treatment is significantly different from all other treatments)

A significant ANOVA test ($p < 0.005$, $F = 19.679$, $N = 83$) indicates that there was a difference between the mucus production rates at different Cu concentrations. According to Fig. 1, the highest mucus production rate, 0.044 ± 0.0217 g.g $tissue^{-1}.min^{-1}$, occurred at the 50 µg/l Cu while the lowest mucus production rate was measured at 0.014 ± 0.0082 g.g $tissue^{-1}.min^{-1}$ in the control treatment (0 µg/l Cu). From the Bonferroni multi-comparison test, there was significant difference between the mucus production rate at 50 µg/l Cu treatment and all other Cu concentrations ($p < 0.005$). There was also a significant difference between the mucus production rate at the 0 µg/l Cu treatment and the 25 µg/l Cu treatment ($p < 0.05$). However, there was no statistically significant difference between both the 12.5 µg/l Cu and 25 µg/l Cu treatments and the 0 µg/l Cu and 12.5 µg/l Cu treatments ($p > 0.05$). The Bartlett's test for equal variances indicates that there was a significant difference between the variances between treatments ($p < 0.05$).

Fig. 2 illustrates the relationship between Cu concentration and mucus production rates. From the regression analysis, there is a significant increasing trend between mucus production rates and Cu concentrations ($p < 0.005$, $F = 60.130$, D.F. = 82). The magnitude of the model indicates that for every 1 unit in Cu concentration (µg/l), there is a 0.001 g.g

tissue^{-1}.min^{-1} increase in the mucus production rate. According to the R^2 value of 0.426, 42.6% of the variation in the mucus production rate is explained by the increasing Cu concentration.

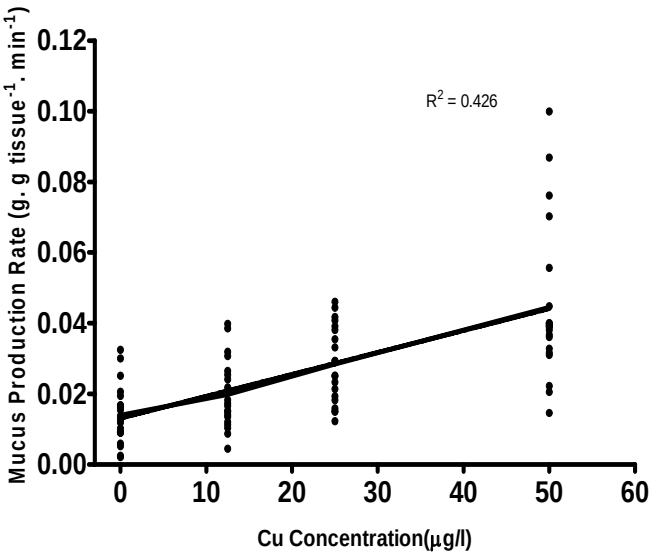

Fig. 2 Mucus production rates when exposed to various concentrations of Cu over 24 hours

IV. Discussion

Mucus can be described as a complex carbohydrate-sulphate [22] which is essential for maintaining homeostasis in all marine molluscs [23]. Mucus is vital in feeding from lining feeding apparatus [7], [23] to selection of particles from solution [7]. It is also integral for locomotion in limpets [3]. In biomonitoring, few studies have been conducted on the role of mucus depuration against environmental pollutants. Mucus is an essential detoxification mechanism [11], [21], [23].

Prior to studies by [21] it was doubted whether there was an actual increase in the mucus production rate when exposed to metals. However the present study and the study by [23] confirm the hypothesis that mucus is fundamental in its role in depuration and indeed increases at higher Cu concentrations during short term exposures (24 hours). The straining of particles from solution is performed by the gills [7]. Mucus may be seen as the first "line of defence" against foreign matter as it may be found across the gills which are constantly in contact with the water [11], [23]. It was found that high concentrations Cu ions would be accumulated in the mucus preventing Cu from building up in the tissues [10], [15], [23]. Hence mucus would be lost back to the water and Cu concentrations in the tissue would be minimal [11], [23]. From the data, higher Cu concentrations result in higher mucus production rates for short term exposures.

Copper is a vital metal in mussels in low concentrations for byssus thread formation and metabolism [14], [20]. Hence mucus production rates not differing in the 0 µg/l Cu concentrations and 12.5 µg/l Cu concentrations are expected. However, higher concentrations of Cu are toxic [14,15] and subsequently there is a significant difference between mucus production rates in the 0 µg/l Cu and 25 µg/l Cu concentrations as well as between the 0 µg/l Cu and 50 µg/l Cu. According to [11, pg. 103], "Mucus prevents uptake of Cu by binding the positively charged cations onto the active site of mucus-glycoproteins." Hence mucus prevents Cu ions from building up in the tissue of mussels [10], [15], [23].

[23] and [10] attribute the increase in mucus production rates at increasing Cu concentrations to an increase in the number of mucus glands. However, too high Cu concentrations within tissues are responsible for loss of functionality of mucus glands [23]. Hence it would be expected that the mucus production rate would decrease at higher concentrations. An increasing mucus production rate at higher Cu concentrations could be attributed to the time of exposure. A 24 hour exposure could cause less accumulation of Cu within tissues than the long term study over three months performed by [23]. This is confirmed by the decrease in mucus production rates in the 2nd and 3rd month of exposure by [23] where Cu accumulation in tissue would be much higher, damaging mucus glands. *P. perna* has the ability to depurate Cu from its tissues at a fast rate [10]. Mussels were placed in filtered seawater for 30 minutes to recover from handling before mucus production rates were measured; this could contribute to increasing mucus production rate as Cu would not have sufficient time to damage mucus glands and subsequently decrease mucus production rates.

Perna perna is quite sensitive to high Cu concentrations; it has a 96 hour LC_{50} of 250 µg/l Cu [13]. Some species of mussel such as *A. trapesialis* have a higher 96 hour LC_{50} of 2000 µg/l Cu [13]. At the 50 µg/l Cu concentration, it was observed that spawning occurred. Hence, it can be seen that even at this sublethal concentration, mussels are under stress. The Bartlett's test indicates there was significant difference in the variance between different Cu concentrations. This indicates that mussel individuals may have different reactions to high Cu concentrations. Similiar individual variation was also observed in cardiac responses [14] and in filtration rates [1].

According to [3], concentrations of metals within mucus may be related to the size of a particular organism. The ANOVA test illustrates that there was no significant difference between the sizes of mussels specimens between treatments hence mussel size was ruled out as a confounding factor. Subsequently, size cannot be taken into account as a parameter that could affect the increasing mucus production rate. Mucus collection was performed after mussels had been fed, which could cause an increase in mucus production rates as mucus is used in particle selection during feeding [7], [11].

The use of mucus production as a biomarker can be recommended [10]. It is used in detoxification [11], [21], [23] and the accumulation of Cu within tissues could be considerable showing a significant increasing relationship with increasing Cu concentration [10], [21], [22], [23]. However, extraneous factors such as temperature, salinity and

water depth need to be taken into consideration [17],[18], [23] as well as the loss of mucus to surrounding water [11],[23]. Feeding could also have an impact on the mucus production rates [7], [11].

In conclusion, it is clear that mucus production rates in mussels show the same increasing relationship during short term exposures ranging from 24 hour to 60 day exposures [23]. However, long term exposures actually cause a decrease in the mucus production rates and these factors need to be taken into account if mucus production rates are to be used as an effective biomarker.

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