



UNIVERSITI PUTRA MALAYSIA

***Heavy Metals in Horseshoe Crab Egg
(Tachypleus gigas)***

WAN NUR FATIN SYAFIQAH BT WAN NAWANG

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**This project report is submitted in partial fulfillment of the requirements for
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ABSTRACT

Heavy metals concentrations were evaluated for horseshoe eggs and sediments from two different sites (Sitiawan, Perak and Banting, Selangor). Eggs and sediments samples were digested then heavy metals determined using Inductively Couple Plasma-Mass Spectrometer. Nine elements detected in eggs and sediments were Arsenic (As), Cadmium (Cd), Copper (Cu), Ferum (Fe), Magnesium (Mg), Nickel (Ni), Lead (Pb), Selenium (Se), and Zinc (Zn). The concentration of these elements (ppm) in eggs from Sitiawan ranged from 0 - 0.833 ppm for As, 0.0307 - 0.0553 ppm for Cd, 0.268 - 0.457 ppm for Cu, 0.213 - 0.733 ppm for Fe, 1.773 - 7.443 ppm for Mg, 0.014 - 0.020 ppm for Ni, 0.012 - 0.029 for Pb, 0.016 - 0.024 ppm for Se and 0.309 - 0.565 for Zn. As for Banting, As ranged from 0 - 0.026 ppm, Cd: 0.012 - 0.067 ppm, Cu: 0.032 - 1.282 ppm, Fe: 0.567 - 2.153 ppm, Mg: 5.820 - 30.133 ppm, Ni: 0.036 - 0.048 ppm, Pb: 0.047 - 0.024 ppm, Se: 0.037 - 0.019 ppm, and Zn: 0.416 - 0.437 ppm. There were significant differences ($p < 0.05$) in As, Cd and Cu concentrations before and after 28 days incubation for horseshoe crab eggs from Banting. Only As and Cd differed significantly for eggs from Sitiawan. These results showed that As, Cd and Cu were definitely increased in horseshoe crabs eggs after incubation. However, eggs samples used for each respectively sites were of pooled eggs. It is possible that some of the eggs clumps were of different maternal sources, therefore may have caused the high variations in the metals concentrations. Sediments samples analysis showed that sediments from Sitiawan are highly contaminated with As, Cd, Cu, Fe, Mg, Ni and Pb when compared to Banting. However, Zn was higher Banting, while Se only detected in Banting sediments. This study also showed that autoclaving process does not affect all elements in the sediments except for Mg.

ABSTRAK

Kandungan logam berat ditentukan untuk telur belangkas dan sedimen dari dua lokasi yang berbeza (Sitiawan, Perak dan Banting, Selangor). Sampel telur dan sedimen dicerna dan logam berat ditentukan menggunakan "Inductively Couple Plasma-Mass Spectrometer". Sembilan elemen yang dikesan dalam telur dan sedimen adalah Arsenik (As), Kadmium (Cd), Kuprum (Cu), Ferum (Fe), Magnesium (Mg), Nikel (Ni), Plumbum (Pb), Selenium (Se), dan Zink (Zn). Julat kepekatan elemen ini dalam telur dari Sitiawan adalah dari 0 - 0.833 ppm untuk As, 0.0307 - 0.0553 ppm untuk Cd, 0.268 - 0.457 ppm untuk Cu, 0.213 - 0.733 ppm untuk Fe, 1.773 - 7.443 ppm untuk Mg, 0.014 - 0.020 ppm untuk Ni, 0.012 - 0.029 untuk Pb, 0.016 - 0.024 ppm untuk Se and 0.309 - 0.565 untuk Zn. Sementara untuk Banting, julat As adalah dari 0 - 0.026 ppm, Cd 0.012 - 0.067 ppm, Cu 0.032 - 1.282 ppm, Fe 0.567 - 2.153 ppm, Mg 5.820 - 30.133 ppm, Ni 0.036 - 0.048 ppm, Pb 0.047 - 0.024 ppm, Se 0.037 - 0.019 ppm, dan Zn 0.416 - 0.437 ppm. Terdapat perbezaan ketara ($p < 0.05$) untuk kandungan As, Cd and Cu sebelum dan selepas 28 hari pengeraman untuk telur belangkas dari Banting. Hanya As dan Cd berbeza untuk telur dari Sitiawan. Keputusan ini menunjukkan bahawa As, Cd dan Cu telah meningkat dalam telur belangkas selepas 28 hari pengeraman. Walau bagaimanapun, sampel telur yang telah digunakan dari setiap lokasi adalah sampel yang dikumpulkan. Kemungkinan gumpalan telur tersebut adalah dari sumber induk yang berbeza, justeru menyebabkan variasi yang tinggi dalam kandungan logam. Analisis sampel menunjukkan sedimen dari Sitiawan adalah sangat tercemar dengan As, Cd, Cu, Fe, Mg, Ni dan Pb berbanding dengan Banting. Walau bagaimanapun, kandungan Zn adalah tinggi pada sedimen Banting, sementara Se hanya dikesan dalam sedimen Banting. Kajian ini turut menunjukkan proses pensterilan tidak memberi kesan ke atas semua elemen dalam sedimen kecuali Mg.

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LIST OF ABBREVIATIONS / SYMBOLS

As	Arsenic
Cd	Cadmium
Mg	Magnesium
Se	Selenium
Pb	Lead
Hg	Mercury
Cu	copper
Ni	Nickel
Fe	Ferum
Zn	Zinc
ppm	Part per million
HNO ₃	Nitric acid
°C	Degree centigrade
ICP	Inductively Cople Plasma-Mass Spectrometer

CHAPTER 1

INTRODUCTION

Horseshoe crab is significantly important for biomedical research. A kit produced from the horseshoe crab's blue blood can be used to detect human pathogens (Karl, 2004). The high potential of horseshoe crab's blood to be used in biomedical commercialize, the research on it increase rapidly. However, due to human activities towards environment and capture of horseshoe crabs as food, the population of this species is declining rapidly. The horseshoe crabs populations found at spawning and nursery habitats for are getting smaller (Sekiguchi, 1988).

Changes in the coastal environments, pollutes the habitat and horseshoe crabs population may get extinct due to this (Itow *et al.*, 1997). The pollution contributes significant impact on horseshoe crabs. Horseshoe crabs are able to tolerate fluctuating changes in environment with high level of heavy metals (Burger *et al.*, 2002). The high concentrations of heavy metals in coastal will easily enter the horseshoe crab's eggs (Itow *et al.*, 1997). The embryos of horseshoe crabs are very sensitive toward chemical concentration especially heavy metals which can cause malformation and abnormalities on the developing embryos (Botton, 2000). At early stages marine animals eggs, embryo and larvae are very susceptible to pollutions compare to adults.

The horseshoe crabs need to molt to develop and grow. In natural habitat, juvenile of horseshoe crabs will molt several times during the first and second year. Then, it will only molt once in a year. Culture of horseshoe crab juvenile in laboratory, it will molt three to five times in first year and once or twice in annually (Faizul *et al.*, 2011). High level of ammonia, pH, and turbidity will give negative impact on horseshoe crab development and growth.

Living organism need a small amount of heavy metals for good health (Choupagar and Kulkarni, 2011). However, high consumption of heavy metals can lead to health problems. According to Burger *et al.* (2002), horseshoe crab showed better tolerance of heavy metals compared to other marine crustacean. Evident showed that the decline in horseshoe crab population can be due to environmental contamination particularly heavy metal (Kamaruzzaman *et al.*, 2011). Therefore, the main objectives for this experiment are:

1. Determine the effect of autoclavation sand on heavy metals concentration.
2. compare heavy metals concentration in horseshoe crab eggs incubate in autoclaved sand at initial and final stages of embryonic development at Banting, Selangor and Sitiawan, Perak.
3. compare heavy metals concentrations on horseshoe crab eggs at initial and final stages of embryonic development incubated in water.

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