



UNIVERSITI PUTRA MALAYSIA

**HISTOLOGICAL CHANGES IN THE INTESTINE, MUSCLE OF BROTHIA
COSTULA (*GASTROPODA: PACHYCHILIDAE*)
EXPOSED ALONG THE LANGAT RIVER**

ABDELGALIL OSMAN AWADELSEED

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MUSCLE OF *BROTIA COSTULA* (GASTROPODA: PACHYCHILIDAE)
EXPOSED ALONG THE LANGAT RIVER**

By

ABDELGALIL OSMAN AWADELSEED

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
In Fulfilment of the Requirement for the Degree of Master of Science
January 2002**



Dedicated to

My mother,
Who has inspired me to do this
My father,
Who has taught me how to struggle and persevere
My brothers and sisters
Hatim, Huda, Abdelnasir, Khalid, Zeinab, Gorashi and Amrou
My nephews,
Siddeg and Mohammed
My niece,
Safwa
Your constant encouragement, sacrifice and support
are highly appreciated.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

HISTOLOGICAL CHANGES IN THE INTESTINE, GILL AND FOOT MUSCLE OF *BROTIA COSTULA* (GASTROPODA: PACHYCHILIDAE) EXPOSED TO VARIOUS POINTS ALONG THE LANGAT RIVER

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January 2002

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Faculty: Science and Environmental Studies

Histological changes in the intestine, gills and feet of *Brotia costula* exposed to six stations along the Langat River were studied. Three other parameters such as susceptibility of the snail to river's water that was expressed in rLT₅₀ (relative Lethal Time 50%), water quality of the river and normal ultrastructure of the snail's alimentary canal were also studied.

Snails collected from the clean upper part of Langat River in Selangor were transferred to six stations along the same river. The stations selected were either situated in the areas of agriculture, villages, town or industries. Thirty snail, kept in cages, were left submerged at each station for about four months (from March to July 2000) and sampled weekly for the examination of the histological changes in their intestines, gills and feet.

Results from the light microscopic examinations revealed that erosion and necrosis of intestinal epithelial cells were common in snails exposed to all stations. Hypertrophy and formation of spaces in between epithelial cells were only developed

in snails exposed to particular stations that surrounded by agricultural activities and close to industrial area. The main target of pollutants in gill tissues was the gill filament. The changes have included erosion, hypertrophy and rupture of gill filament. Sites of disruptions in feet of the exposed snails were the epithelial layers, loose connective tissues and striated muscles fibers. The changes were necrosis of striated muscle fibers, disruption of loose connective tissues and loss of epithelial cells. Station 5 that was located under a bridge, close to a small housing project of Jenderam Hilir was the worst among all stations. This was evidenced by the high mortality (rLT₅₀ 9.1 weeks).

The conductivity, TSS (Total Suspended Solid), total phosphate, phosphorus, nitrite and BOD (Biochemical Oxygen Demand), were higher in stations located downstream *i.e.* 4th, 5th, and 6th stations, compared to the first three stations, which were located in the upper part of the river. While DO (Dissolved Oxygen) was higher in the stations located at the upper part of the river. Temperature and pH did show consistency in all stations throughout the study period.

Since this study had demonstrated that some tissues of *B. costula* are sensitive to polluted water it has been concluded that this snail is potentially useful as a biomonitoring material for river pollution.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PERUBAHAN HISTOLOGI DALAM USUS, INSANG DAN OTOT KAKI
BAGI *BROTIA COSTULA* (GASTROPODA: PACHYCHILIDAE) YANG
DIDEDAHKAN SEPANJANG SUNGAI LANGAT**

Oleh

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Januari 2002

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Perubahan histologi dalam usus, insang dan kaki *Brotia costula* yang didedahkan kepada enam stesen sepanjang Sungai Langat telah dikaji. Tiga parameter yang lain turut dikaji iaitu kepekaan siput kepada air sungai yang dijelaskan dalam rLT_{50} (LT_{50} relatif), kualiti air sungai dan ultrastruktur normal kepada salur alimentari siput turut dikaji.

Siput yang dikutip daripada hulu Sungai Langat di Selangor dipindahkan ke berbagai stesen di hilir sungai yang sama. Stesen yang dipilih berada di kawasan pertanian, kampung, bandar dan industri. Siput disimpan dalam sangkar dan dibiarkan tengelam di setiap stesen selama empat bulan (dari Mac hingga Julai 2000) dan disampel setiap minggu bagi mengkaji perubahan histologi pada usus, insang dan kaki.

Keputusan daripada kajian mikroskop cahaya menunjukkan lazimnya berlaku hakisan dan nekrosis pada tisu epitelium usus pada siput yang didedahkan di semua stesen. Hipertrofi dan pembentukan ruangan dalam sel epitelium hanya berlaku

dalam siput yang didedahkan di sebahagian stesen yang dikelilingi oleh aktiviti pertanian dan yang berhampiran dengan kawasan industri. Sasaran pencemaran dalam tisu insang ialah di filamen insang. Perubahan ini termasuk hakisan, hipertrofi dan pemecahan dalam tisu insang. Manakala dalam kaki siput pula, gangguan adalah di lapisan epitelium, tisu penghubung longgar dan gentian otot berjalur. Perubahan itu merosakkan dan menekrosiskan gentian otot berjalur, mengganggu tisu penghubung longgar dan kehilangan sel epitelium. Stesen kelima terletak di bawah jambatan, berdekatan dengan projek perumahan Jenderam Hilir adalah yang terburuk di antara semua stesen. Ini diterangkan dengan kadar kematian yang tercepat ($r\text{LT}_{50}$ 9.1 seminggu).

Konduktiviti, TSS (Jumlah Pepejal Terapung), jumlah fosfat, fosforus, nitrit dan BOD (Permintaan Oksigen Biokimia), didapati adalah tinggi pada stesen-stesen di hilir sungai iaitu stesen keempat, kelima dan keenam berbanding dengan tiga stesen yang lain yang terletak di hulu sungai. Sementara itu, DO (terlarut Oksigen) adalah tinggi di stesen yang terletak di hulu sungai. Suhu dan pH adalah konsisten pada semua stesen sepanjang ujikaji dijalankan.

Oleh sebab hasil kajian ini telah menunjukkan bahawa tisu *B. costula* adalah peka terhadap air yang tercemar ianya dapat disimpulkan bahawa siput ini adalah berpotensi untuk digunakan dalam biopemantauan pencemaran air sungai.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institution.

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LIST OF ABBREVIATIONS

BOD	Biochemical Oxygen Demand
DO	Dissolved Oxygen
DOE	Department of Environment
INWQS	Interim National Water Quality Standards
LC ₅₀	Lethal Concentration (50% mortality)
MLD	Million Liter Day
NRC	National Research Council
PE	Post Exposure
rLT ₅₀	relative Lethal Time (50% mortality)
TEM	Transmission Electron Microscope
TSS	Total Suspended Solid
UKM	Universiti Kebangsaan Malaysia
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Fresh water snail, *Brotia costula*, is an aquatic prosobranch, widely distributed in freshwater habitats in Thailand, India, Burma, China, Laos, Cambodia and Indonesia (Brandt, 1974). Malaysia is no exception and the snail is normally found in the upper reach of rivers and considered as a delicacy in certain parts of the country.

The term pollution is now recognized as expressing biological features of the response of environmental systems to contamination (Bayne, 1985). The problem of fresh water pollution is well known to be crucial, particularly for industrialized countries with high population density. An increase in human population together with a rise in living standards cause an increase in the consumption of fresh water and a decrease in the quantity of clean water available. Because both of these phenomena have same basic cause, they combine to create a very dangerous situation in some areas (Mason, 1996).

Owing to fast development of industries and transport in Malaysia and to the development and inundation of land areas, the increase of water pollution can not be avoided. Thus, pollution effect monitoring tools are much needed.



In contaminants impact assessment, there is a growing interest in a different approach, one that examines exposure and effect in aquatic toxicology. This approach uses biological indicators of exposure and effect that can be defined as "measurements of body fluids, cells, or tissues that indicate in biochemical or cellular terms the presence of contaminants or the magnitude of the host response" (NRC, 1987). Many different categories of biological indicators have been introduced to evaluate the different environmental stresses in different organisms. These included biochemical, bioenergetic, histological and histochemical indicators. However, the cellular emphasis in aquatic toxicology is entirely consistent with the bioindicator approach. In addition, cellular studies are likely to lead to the detection of alteration prior to changes at the organism level and since cellular changes are early and sensitive, they may serve as indicator of both exposure and effect (David, 1994).

Biological systems function at many levels in a hierarchy of organized states, from the cell to the community, the impact of pollutants on an organism is realized as perturbations at different level of functional complexity (Bayne, 1985; Moore, 1985). Marine and estuarine environments receive a variety of contaminant chemical inputs. Consequently, there are many ways in which structure and/ or function of organelles and cells can be disturbed by these contaminants. Slater (1978) has conveniently classified these into four main categories:

1. Depletion or stimulation of metabolites or co-enzymes. This may be serious enough to produce a morphological-evident lesion in the cell.
2. Inhibition or stimulation of enzymes and other specific proteins. The result may be a disturbance of metabolic integration leading to damage to cellular function.

3. Activation of xenobiotic elements (toxic environmental chemical) to a more toxic molecular species.
4. Membrane disturbance. Many toxic substances or their metabolites result in cell injury by reacting primarily with biological membranes.

Although more sensitive and practical techniques have become available for environmental monitoring, most of the monitoring approaches are long-term and involve a massive volume of parameters requiring huge logistic support and budget. In this long-term process, the situation either radically changes or goes beyond control by the time a mitigation plan is implemented. Furthermore, environmental bioassays involve the use of biological systems to predict or assess the impact of pollutants on ecosystem. Generally, the acute toxicity (LC_{50}) bioassay has been employed as a major technique (more than 90% of the toxicity test) for assessing pollution impacts (Maltby and Calow, 1989). However, it is now being criticized for misleading the determination of toxic level (Smith and Oseid, 1975) and also fails to maximize predictive capabilities of biological and ecological relevance (Adams, 1990).

1.2 Statement of the Problem

In this study, a particular attention was paid on histological changes, which has some primary advantages:

- It has particular relevance to field assessments.
- Many organ systems can be evaluated at leisure following collection.
- It has precision with which lesions of specific cells types can be identified.
- It allows the determination of the sex and reproductive status of the animals.
- Changes can be assessed in animals that are too small to dissect for biochemical purpose as stated by David and Darrel (1990).

Some organisms that live within a lake are, in many cases, sensitive to changing environmental conditions and thus may be used as indicator organisms because there is a general equilibrium between a biotic community and the environment (Karmer, 1988).

Studies of the impact of pollution at cellular and subcellular levels in molluscs have been reported in Europe, USA, and Asia. Most of these studies have concentrated on histological changes in intestines, gills, and foot muscles. Only few studies have been conducted in the fields, as most of them have done under laboratory conditions (Hans *et al.*, 1992; Marigomez *et al.*, 1996; Tripp *et al.*, 1984; Weinstein, 1996; Auffret, 1988; Jonnalagadda and Rao, 1996; Syasina *et al.*, 1996; Maleeya *et al.*, 1998).

1.3 Significance of the Study

Molluscs are widely recognized as biological indicator species where its presence indicate particular features of the environment that are otherwise difficult to determine (Harman, 1974). Aquatic snails are particularly useful indicators because they are easily recognized even by non-specialist, abundant in their preferred habitats, possess a relatively long life span, comparatively sessile *i.e.* do not migrate away from stressed environment and more importantly they are responsive to changes when exposed to pollutants. The suitability of molluscs for monitoring has resulted in the development of procedures for assessing the impact of environmental stresses.

In Malaysia, no studies have been conducted using histological changes in gastropods to determine the impact of pollutants on molluscs. Thus, this study has come out to validate the use of the fresh water gastropod, *Brotia costula*, as a freshwater pollution indicator in Malaysia.

The water quality of the Langat River, which receives wastewater from some densely populated areas and a considerable number of industries, is of particular concern for the regulatory authorities since the river is a major source of public water supply. The results of the present study however, can give an idea about the degree of pollution in the Langat River, which in turn could be use to develop criteria and standards as mean for the protection and enhancement of water quality of the river.

1.4 Objectives of the Study

The objectives of this study therefor, are

1. To study the normal histological structure of the alimentary canal, gill and foot muscle of *B. costula*.
2. To investigate the histological changes that may occur in the intestine, gill, and foot muscle of *B. costula* due to exposure to several points along the Langat River.
3. To study the susceptibility of *B. costula* to various degree of pollution along the Langat River.
4. To study the normal ultrastructure of the alimentary canal of *B. costula*.