UNIVERSITI PUTRA MALAYSIA

ECOTOXICOLOGY OF HEAVY METALS (Cd, Pb, Zn AND Cu) IN FLAT TREE OYSTERS *ISOGNOMON ALATUS* (GMELIN) FROM SEPANG, MALAYSIA

KATAYONSAED

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By

KATAYON SAED

Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

May 2001

Dedicated to...,

Memory of my father

My mother

My husband

My sisters and brother

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ECOTOXICOLOGY OF HEAVY METALS (Cd, Pb, Zn AND Cu) IN FLAT TREE OYSTER *ISOGNOMON ALATUS* (GMELIN) FROM SEPANG, MALAYSIA

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May 2001

Chairman: Associate Professor Ahmad Ismail, Ph.D.

Faculty: Science and Environmental Studies

The status of heavy metals pollution in the Sepang Besar River and Sepang Kecil River was studied in August 1998. In general heavy metals concentrations in flat tree oyster *I. alatus*, sediments and water from Sepang Besar River were found significantly ($p < 0.05$) higher than those from Sepang Kecil River.

Furthermore field experiments were conducted to study the accumulation and depuration of heavy metals in oysters. The rates of metals accumulations in oysters were found to be 42.70, 1.77, 0.95, 0.84 $\mu g\ g^{-1}\ month^{-1}$ for Zn, Cu, Cd and Pb, respectively. The polluted oysters were transferred to clean area to estimate the depuration rates. Significant reduction of metals concentrations in oyster was observed. About 87%, 83%, 78% and 59% for Cd, Pb, Cu and Zn were detected after six months being transferred to clean area, respectively.

The metals accumulation and depurations patterns in oysters in the laboratory conditions were also investigated. The mean metal concentrations in oysters exposed to metals solution of 100 μg l^{-1} at the end of two weeks were 32.70, 63.19, 35.40, 7.44 μg g^{-1}, which were 44, 3, 8 and 7 times greater than their initial levels for Cd, Zn, Cu and Pb, respectively. The exposed oysters were transferred to clean seawater and the metals depurations were investigated for one week. In general about 78%, 81%, 87% and 88% of the accumulated Zn, Cu, Pb and Cd were depurated from oysters *I. alatus*.

Results from laboratory experiments indicated that there were differences in the mechanisms for the metals accumulation and depurations in various organs of *I. alatus*. In general, in both accumulation and depuration experiments, gill, byssus and mantle play major role whereas muscle, shell and visceral mass have no significant role. Furthermore laboratory experiments were showed that the accumulation rates of metals through contaminated water were higher than the metals accumulation rates through contaminated algae. It is believed that oysters *I. alatus* accumulate heavy metals through contaminated seawater rather than contaminated algae.

The LC_{50} levels of metals for oysters were observed to be 478.63, 436.51, 676.08 and 295.12 μg l^{-1} for Cu, Pb, Zn and Cd, respectively. The toxicity effects of heavy metals on filtration rate of oysters were also studied and the filtration rate of oysters *I. alatus* exposed to 125 and 150 μg l^{-1} of all metals were decreased. In both toxicity experiments the trend of metals toxicity on oysters were found Cd > Pb > Cu > Zn.

In conclusion, the results presented here clearly illustrate oysters *I. alatus* could be a very good heavy metals bioindicator and it would be of great interest if being employed for control of heavy metals pollution in the marine environment and aquaculture practices.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Doktor Falsafah

EKOTOKSIKOLOGI LOGAM BERAT (Cd, Pb, Zn DAN Cu) DALAM TIRAM *ISOGNOMON ALATUS* (GMELIN) DARI SEPANG, MALAYSIA

Oleh

KATAYON SAED

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Kajian status pencemaran logam berat di Sungai Sepang Besar dan Sungai Sepang Kecil telah dijalankan dalam bulan Ogos 1998. Secara umum kepekatan logam berat dalam tiram, enapan dan air dari Sungai Sepang Besar adalah tinggi berbanding Sungai Sepang Kecil.

Kerja lapangan selanjutnya ke atas akumulasi dan depurasi logam berat dalam tiram. Kadar akumulasi logam Zn, Cu, Cd dan Pb adalah masing-masing 42.70, 1.77, 0.95, 0.84 $\mu g\ g^{-1}$ $bulan^{-1}$. Tiram yang tercemar seterusnaya dipindahkan ke kawasan bersih untuk menentukan kadar depurasi. Pada akhir tempoh kajian terdapat penurunan yang bererti pada kandungan logam dalam tiram iaitu 87%, 83%, 78% and 59% masing-masing untuk Cd, Pb, Cu dan Zn.

Kajian corak akumulasi dan depurasi logam tiram juga dijalankan di dalam makmal di mana tiram didedahkan kepada larutan logam 100 $\mu g\ l^{-1}$ selama 2 minggu. Min kepekatan logam dalam tiram pada akhir minggu kedua adalah 32.70, 63.19, 35.40 dan 7.44 $\mu g\ l^{-1}$, iaitu 44, 3, 8 dan 7 kali ganda lebih besar berbanding kepekatan awal logam Cd, Zn, Cu

dan Pb. Tiram yang telah didedahkan kepada larutan logam dipindahkan ke dalam air laut bersih kemudian kajian depurasi logam dijalankan selama satu minggu. Di akhir tempoh kajian, lebih kurang 78%, 81%, 87% dan 88% daripada logam Zn, Cu, Pb dan Cd yang terakumulasi mengalami depurasi dari tiram *I. alatus*.

Keputusan menunjukkan terdapat perbezaan mekanisma akumulasi dan depurasi logam dalam organ-organ tertentu pada tiram *I. alatus*. Dalam kajian akumulasi, didapati kadar pengambilan logam berat adalah tinggi pada insang, mantel dan byssus. Bagaimapun, tidak terdapat kesan bererti dalam akumulasi logam pada otot, cengkerang dan jirim visceral. Dalam tempoh satu minggu kajian depurasi, didapti tiada kesan bererti ke atas otot, cengkerang dan jirim visceral manakala byssus dan mantel tiram *I. alatus* mengalami depurasi secara bererti.

Kajian dalam makmal telah menunjkkan kadar akumulasi logam dalam air tercemar juga adalah lebih tinggi berbanding alga tercemar dan dipercayai bahawa akumulasi logam berat dalam tiram *I. alatus* adalah melalui air laut yang tercemar.

Paras LC_{50} bagai Cu, Pb, Zn dan Cd adalah masing-masing 478.63, 436.51, 676.08 dan 295.12 $\mu g\ l^{-1}$ untuk tiram. Kajian kesan toksik logam berat ke atas kadar penapisan tiram menunjukkan bahawa kadar penapisan menurun apabila tiram *I. alatus* didedahkan kepada 125 dan 150 $\mu g\ l^{-1}$ bagi semua jenis logam. Corak toksisiti logam berat ke atas tiram bagi kedua-dua kajian adalah Cd>Pb>Cu>Zn.

Kesimpulannya *I. alatus* adalah penunjuk biologi yang sangat baik dan amat berguna dalam pengurusan logam berat bagi persekitaran marin dan juga untuk kegunaan sector akuakultur.

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I certify that an Examination Committee met on 3rd May 2001 to conduct the final examination of Katayon Saed on her Doctor of Philosophy thesis entitled "Ecotoxicology of Heavy Metals (Cd, Pb, Zn and Cu) in Flat Tree Oysters *Isognomon alatus* (GMELIN) from Sepang, Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

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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 institutions.

Katayon Saed

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LIST OF ABBREVIATIONS

A.A.S.	Atomic Absorption Spectrophotometer
DOE	Department Of Environment
D.O.	Dissolved Oxygen
JICA	Japan International Cooperation Agency
TBT	Tributyltin
LC_{50}	Lethal concentrations resulting 50% mortality of test organism
MPL	Maximum Permissible Level
MT	Metallithionein
$mg\ l^{-1}$	Milligram per liter
$\mu g\ l^{-1}$	Microgram per liter
$mg\ g^{-1}$	Milligram per gram
ppm	Part per million
ppb	Part per billion

CHAPTER I

INTRODUCTION

Marine pollution is defined as an introduction of substances by man into the marine environment resulting harmful effects to aquatic resources and health hazards to human. Heavy metals are among the most intensely studied pollutants in marine environment and estuarine because of above threshold availability and their long lasting toxicological effects to organisms living in that environment. Heavy metals ultimately accumulate in variety of marine organisms including fish and shellfish, which eventually accumulate as it moves higher in the food chain and eventually effecting human health.

The west coast of Peninsular Malaysia is densely populated and industrialized area, of which the Straits of Malacca is experiencing the discharge of pollutant from land based sources (Ismail et al., 1993). Land based sources have been identified as the main sources of the major pollution problems such as heavy metals in Malaysian waters (Ahmad, 1997). Of the total quantity of waste generated in Malaysia 11.79% contained heavy metals (DOE, 1998). In 1998, four major activities in Malaysia, namely manufacturing, agro-based industry (raw natural rubber and crude palm oil), domestic sewage and animal husbandry (pig rearing) were identified as chief source of water pollution and pig slurry accounting 16.7% of those sources (DOE, 1998). These pollutants were discharged directly or indirectly through rivers, into the Strait of Malacca.

Over the years pig farmers have indiscriminately discharging untreated pig slurry into public waterways, rivers and low land areas. Sometimes the rivers are so polluted that no animals are able to live in its (Kaun, 1993). This situation is not uncommon throughout the major pig producing areas in the country (Babjee et al., 1983). Teoh et al. (1988) also reported that indiscriminate discharge of untreated waste from pig farms caused serious water pollution in Malaysia.

Previous studies on heavy metals accumulation in sediments and molluscs from Peninsular Malaysia indicated that samples from estuaries receiving effluent from pig farms contained high levels of heavy metals concentrations (Nather Khan and Lim, 1991; Ismail et al., 1993; Ismail and Ramli, 1997). Arzul and Maguer (1990) also reported that pig farms contributed to high levels of heavy metals to the environment, which could cause water and sediment pollution. Heavy metals such as Zn, Cu and Cd are the most important pollutants caused by pig slurry (Bernal et al., 1992).

It has been estimated that the 99% of the pig farms in the country were located in the states of Negeri Sembilan, Selangor, Perak, Johore and Malacca, with the majority of big farms in Negeri Sembilan (DOE, 1998). Sepang Besar River is the river bordering Negeri Sembilan and Selangor, which is highly polluted by waste discharged from pig farm activities. According to the Department Of Environment document, Sepang Besar River is one of the most polluted rivers in Malaysia since 1987 until 1998 (DOE, 1993; 1998). Previous study in this river indicated that heavy metals (Cu, Pb, Cd and Zn) in gastropods and sediments, which were close to pig farm effluent were relatively higher than those collected from the river mouth (Ismail and Ramli, 1997).

Metals such as Cu, Pb, Cd and Zn were given special attention in environmental monitoring studies especially in biological samples because bioaccumulation of these metals can caused potentially toxic effects to the organism and to humans who consume the seafood (Phillips and Rainbow, 1992; Kennish, 1996). Aquaculture activities such as fish, prawn, crab, mussels and cockles farming are mainly carried out in the coastal areas especially those along the west coast of Peninsular Malaysia, where developments had been focused (Thomas, 1988; Gopinath and Shariff, 2000). On the other hand seafood products supply about 75% of the protein requirements of the population in Malaysia. Average Malaysian populations consume about 100 g day^{-1} $person^{-1}$ of seafood products (Sarmani and Majid, 1994). Therefore serious consideration should be taken in terms of metals pollution. As we know, molluscs such as oysters are becoming popular for the seafood trade in restaurants and tourist resorts. Therefore, in view of the growing economic importance of the molluscs industry to many countries of the region and the potential health risks from a variety of contaminants, it is appropriate that greater emphasis should be placed on bivalves to ensure that such products do not present a health hazard and at the same time affect the market demand and economic. With the above situation in mind, baseline information on heavy metals levels should be gathered to compare with the pollution from future developments.

It is known that marine molluscs accumulate heavy metals in their tissues to concentrations that are above ambient levels in the environment. Oysters are one of the marine molluscs that have been used extensively as bioindicators of metals pollution in estuaries and marine systems. The exceptional bioaccumulation capacity of oysters

makes them very suitable species for biomonitoring (Mo and Neilson, 1993). They can accumulate metals in their tissues in proportion to the degree of environmental contamination better than other bivalves (Lytle and Lytle, 1982). Although monitoring programs have achieved considerable success, a more complete understanding of the rates of metals accumulation and depuration in these organisms will improve monitoring efficiency and unambiguous interpretation of monitoring data (Abbe et al., 2000). A better knowledge of accumulation and depuration kinetics in field conditions might contribute to the understanding of oysters' capacity for different metals and provide useful information for interpreting metal levels in natural oysters populations. In order to predict the transport of metals through the marine ecosystem and to interpret the significance of metal burden in oysters and how certain metals in the ambient water are reflected in the their body burden, it is necessary to have information on the accumulation and depuration rate of metals (Riisgard et al., 1987; Phillips, 1994).

In pollution monitoring studies, bioindicator species may be transplanted from locations where natural populations are found to new locations of interest where natural populations are absent. Martincic et al. (1992) suggested that the transplanted bivalves are more suitable for monitoring of heavy metals than indigenous bivalve populations, because of biological similarities and better reproducibility of the results. Widdows et al. (1980-81) have observed that the transplantation of mussels or similar bivalves to various areas may provide a useful approach to marine environmental monitoring. By using transplanted organisms, it is possible to measure the actual presence of the metals (Regoli and Orlando, 1994).