UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF SELECTED HEAVY METALS BY STABLE ISOTOPE APPROACH OF FOOD WEB ASSEMBLAGE IN INTERTIDAL MANGROVE ECOSYSTEM, SUNGAI PULOH, SELANGOR, MALAYSIA

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

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January 2016

Chair : Prof. Ahmad Ismail, PhD
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The mangroves of Sungai Puloh which supports a great diversity of macro benthic organism and provides social benefits to the local community is becoming a major recipient of the anthropogenic contaminants as a result of industrialization, expansion of population, and urbanization. This study was conducted to evaluate the concentrations, contamination extent, distribution and mobility, and pollution status of heavy metals (Cd, Cu, Ni, Pb, Zn and Fe) in intertidal surface sediment of this mangrove area. The speciation profile of these heavy metals as well as the sediment total organic carbon (TOC) and pH were also determined. This work also elucidated the heavy metal levels in the food web structure and trophic dynamics of macrobenthic organisms in this area. Forty two surface sediment samples were collected from 14 stations representing 4 group sites of possible different anthropogenic sources, and selected macro fauna were randomly collected. The four stage sequential extraction technique (SET) was employed to investigate the heavy metal distribution pattern, while the Risk assessment Criteria Code (RAC) was applied to estimate the metals bioavailability and implication to food chain. And the stable isotope ratio technique was used to determine the trophic positions, dynamics and material flow. The heavy metals concentrations were analyzed using atomic absorption spectrophotometer and the result were presented in µg/g, except for Fe in %. Interim Sediment Quality Guidelines (ISQG) and different contamination indices were employed to determine the quality of surface sediment. The mean values of analyzed metals were below both the ISQG-low and ISQG-high except for Pb concentration that is above ISQG-low, and Zn concentration which is above ISQG-high suggesting Pb and Zn may pose some environmental concern. Pollution Load Index (PLI) indicated deterioration and other indices revealed that the surface sediment is moderately polluted with Cd, Pb, and Zn. Chemical speciation depicted the non-residual fractions of Cd 53%, Ni 51%, and Pb 43.91% to be at the borderline for remobilization. Based on the RAC, Pb and Ni in Sg. Puloh ecosystem pose low risk, while Cd and Zn showed a higher risk to enter into the food chain. The findings of high Pb concentrations in fiddler crabs which are ubiquitous in this study area, and the elevated levels of Pb in catfish which is commercial seafood and a good source of protein to the local community makes Pb the most incriminating contaminant in the study area. With the help of stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ analysis, two distinct food chains and four trophic positions were determined, with catfish occupying the highest trophic level. By using catfish as a model for trophic web system, stable isotope analysis showed that the

carbon source for catfish is discretely from fiddler crabs, complementing the idea that *uca annulipes* found in the stomach content is its staple food. Generally, we concluded that increased anthropogenic activities through industrialization and urbanization impact, and as well lead to heavy metal contamination in Sungai Puloh mangrove estuary through the release of untreated or poorly/ improperly treated effluents. And these heavy metals contamination are evidenced in the food web of Sg. Puloh mangrove. However, the seafood in Sg. Puloh is considered safe for human consumption yet, Pb is considered to be the element of much concern, and therefore should be strictly monitored.

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

MENENTUKAN LOGAM BERAT YANG TERPILIH OLEH PENDEKATAN ISOTOP STABIL BAGI HIMPUNAN JARINGAN MAKANAN DI EKOSISTEM PASANG SURUT BAKAU, SUNGAI PULOH, SELANGOR, MALAYSIA.

Oleh

UDECHUKWU BEDE EMEKA

Januari 2016

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Paya Bakau Sungai Puloh menyokong pelbagai kepelbagaian organisma bentik dan menyediakan keperluan sosial kepada komuniti tempatan telah menjadi penerima utama dalam bahan cemar anthropogenik yang merupakan hasil dari perindustrian, perkembangan populasi dan pembandaran. Kajian ini dijalankan untuk menilai kepekatan, perlanjutan bahan pencemar, taburan dan pergerakkan serta status pencemaran logam berat (Cd, Cu, Ni, Pb, dan Fe) di permukaan pasang surut sedimen yang terdapat di kawasan paya bakau ini. Profil penspesiesan logam berat dan juga jumlah organic karbon sedimen (TOC) dan pH telah dikenalpasti. Kajian ini juga menjelaskan tahap logam berat dalam rantaian makanan dan trofik dinamik organisma makrobentik di kawasan tersebut. Empat puluh dua sampel sedimen permukaan telah dikumpulkan dari 14 stesen mewakili 4 kumpulan kawasan yang keberangkalian adalah daripada sumber anthropogenic yang berbeza dan makrofauna yang terpilih secara rawak telah dikutip. Empat fasa teknik pengekstrakan berjujukan (SET) telah digunapakai dalam mengkaji corak taburan logam berat, sementara Kod Kriteria Penilaian Risiko (RAC) telah di aplikasikan untuk menganggarkan bioketersediaan dan kesannya terhadap rantaian makanan. Teknik nisbah isotope stabil telah digunakan dalam mengenalpasti posisi trofik, dinamik dan aliran bahan. Kepekatan logam berat dianalisa menggunakan Spektrofotometer Serapan Atom (AAS) dan hasil dapatan di bentangkan dalam unit µg/g kecuali Fe iaitu dalam bentuk %. Garis Panduan Kualiti Interim Sedimen (ISQG) dan pelbagai indeks pencemaran telah diguna pakai dalam menentukan kualiti sedimen permukaan. Nilai purata logam yang telah dianalisa adalah berada di tahap bawah kedua-dua indeks ISQG-rendah and ISQG-tinggi kecuali kepekatan Pb iaitu di atas bacaan ISQG-rendah dan kepekatan Zn adalah melebihi bacaan ISQG-tinggi mencadangkan bahawa Pb dan Zn boleh menyebabkan kesan terhadap persekitaran. Indeks beban pencemaran (PLI) menunjukkan kemerosotan dan indekss lain mendedahkan bahawa sedimen permukaan adalah sederhana tercemar dengan Cd, Pb dan Zn. Penspesiesan kimia menggambarkan pecahan bukan sisa Cd 53%, Ni 51%, dan Pb 43.91% berada di atas garsian remobilisasi. Berdasarkan RAC, Pb dan Ni di ekosistem Sungai Puloh terdedah kepada risiko yang rendah manakala, Cd dan Zn menunjukkan risiko yang tinggi terhadap rantaian makanan. Penemuan kepekatan Pb yang tinggi dalam ketam kepiting yang banyak didapati di kawasan kajian dan bacaan Pb yang tinggi di dalam ikan duri yang juga merupakan makanan laut komersial dan juga sumber protein yang baik kepada komuniti tempatan menjadikan Pb

bahan pencemar yang merbahaya di kawasan ini. Dengan menggunakan analisis nisbah $\delta^{13}C$ dan $\delta^{15}N$ isotope stabil, dua rantaian makanan yang berbeza dan empat posisi trofik telah dikenalpasti dan ikan duri tergolong dalam posisi trofik yang tertinggi. Dengan menggunakan ikan duri sebagai model system jaringan trofik, seiring dengan idea "*uca annulipes"* ditemui kandungan perutnya yang merupakan makanan ruji. Secara keseluruhannya, peningkatan aktiviti anthropogenik melalui kesan perindustrian dan pembandaran dan juga merujuk kepada pencemaran logam berat di muara paya bakau Sungai Puloh melalui pelepasan sisa buangan yang tidak diselenggara dan dirawat dengan baik. Malah, pencemaran logam berat ini adalah dibuktikan di dalam jaringan makanan paya bakau Sungai Puloh. Walaubagaimanapun, makanan laut Sungai Puloh masih lagi selamat bagi pengambilan manusia dan Pb dianggap unsur yang mendapat banyak perhatian dan sepatutnya diawasi secara berkala.

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as a fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory committee were as follows:

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LIST OF ABBREVIATIONS

BSAF	Biota sediment accumulation factor
CEC	Cation exchange capacity
CRM	Certified Reference Material
CF	Contamination factor
CAM	Crassulacean acid metabolism
DIC	dissolved inorganic carbon
DDW	Double distilled water
EFLE	Easily freely leachable and exchangeable
ERL	Effective low range
ERM	Effective median range
EF	Enrichment factor
I_{geo}	Geoaccumulation Index
IRMS	Isotope Ratio Mass Spectrometer
MINT	Malaysia Nuclear Agency
OC	Organochlorines
PLI	Pollution Load Index PLI
PAHs	Polycyclic aromatic hydrocarbons
RI	Potential ecological risk index
E_i	Potential risk of individual heavy metal
PEL	Probable effect level
RAC	Risk assessment Code
SET	Sequential extraction technique
$PbEt_4$	Tetraethyl lead
TEL	Threshold effect level
TOC	Total organic carbon
T_i	Toxic response factor for a given substance
TTF	Trophic Transfer Factor
TBT	Trybutyltin
USDA	United States Department of Agriculture

CHAPTER 1

INTRODUCTION

Elemental contamination in coastal areas arising from rapid urbanization and industrialization has become an increasing worldwide concern, because this ecosystem receives anthropogenic and industrial wastes. These wastes which are bio-accumulative, toxic and persistent include heavy metals. Heavy metals are introduced to the aquatic environment and accumulate in sediments by several pathways via natural and anthropogenic processes (Akoto et al. 2008). These processes include disposal of liquid effluents, runoffs and leachates, surface soil erosion, bioturbation, dredging, water drainage, atmospheric deposition, and indiscriminate use of heavy metal-bearing fertilizer and pesticides in agricultural fields (Soares et al. 1999; Yang and Rose 2005; Nouri et al. 2008). One of the most affected ecosystems in coastal areas is the mangrove ecosystem.

Mangrove ecosystem is important for wild life, fisheries and aquacultural initiatives. This unique ecosystem has been known as nursery areas for juvenile fishes, crustaceans and marine life; they also provide refuge to numerous macrobenthic organisms from their predators during low tide. The mangrove ecosystem as ecotones between terrestrial/ freshwater and marine environment (Vane et al. 2009) makes it a good trap zone against sea and land debris. The mangrove ecosystem is one of the world's major productive ecosystems because they are key ecological habitats that link terrestrial and marine environments (Vane et al. 2009). Mangroves are unique systems that have the potentials to stabilize shore lines, trap sediments, and improve shore line protection (Bosire et al. 2008). The intertidal mud flats of this ecosystem sustain a good diversity of marine organisms including catfish, mudskippers, gastropods, crabs, barnacles, rodong shell, mullet, mussels monitor lizard, migratory shorebirds and aquaculture products (principally fish and prawn) which are commonly consumed in South-East Asia (Bayen et al. 2005; Hashim et al. 2010).

However, today's mangrove ecosystem has been subjected to intense and continuous chemical anthropogenic inputs resulting from increased urbanization and industrialization. Among the major chemical contaminants from anthropogenic inputs are heavy metals (MacFarlane, 2002). The surface sediments of mangrove ecosystem have become significantly polluted with heavy metals, especially in coastal regions near urban areas and harbors (Loring and Rantala 1992). A greater percentage of these heavy metals, which are released into aquatic systems, are quickly taken up by plankton (Sanders and Riedle, 1998; Wang and Guo, 2000) and are adsorbed to suspended particulate matter, which eventually settles down and becomes incorporated into bottom sediments. Heavy metals can be mobilized from sediment if there is a change in physicochemical properties such as acidification, redox potential, or organic ligand concentrations (Kelderman et al. 2000; Yuan et al. 2004) and consequently result in trophic transfer to detritivores and other higher level trophic organisms in the food web. These bioaccumulative elements may

produce toxic effects on various biological levels (cell – tissue – organ – organism – system), e.g. by disturbing the permeability in the cell membrane, the formation of hormones, or the structure and the formation of regulating proteins. The toxic effect could lead to limited biodiversity, reduction in food intake and impaired growth of mangrove organisms. In addition, the accumulation of heavy metals in sediments can produce harmful effects on the biota living in them (Harikumur and Nasir, 2010; Joksimovic et al. 2011). The determination of source and material flow of these contaminants especially heavy metals in the mangrove food web system has been elusive until the advent of stable isotope analysis of consumer tissue which has provided a valuable tool to assist in the interpretation of levels of contaminants in food webs (Kidd, 1998). Animals are related isotopically due to isotopic diet-tissue discriminating factor. This is primarily due to stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) measurement on consumers indicate both food sources and trophic information of an individual. In particular $\delta^{13}C$ and $\delta^{15}N$ have been used by some researchers to examine trophic dynamics in the food web system (Wada et al. 1993; Lajitha and Michener, 1994; Larsson et al. 2007; Whitteveen et al. 2011).

Malaysia being one the fast developing countries in the world is threatened with intense anthropogenic activities, and therefore increased chemical foot print as a result of increasing population, industrialization and urbanization is expected in her coastal areas. Sungai Puloh mangrove ecosystem which supports a variety of marine and terrestrial life for economic purposes is situated in the state of Selangor in West coast of Peninsular Malaysia. As a result of its location this mangrove accumulate materials discharged from the nearby sea (Straits of Malacca) during tides, solid wastes from the nearby communities, and heavy metal contaminants through boating and agricultural activities, effluents and metallic anthropogenic wastes disposed from nearby light industries, and domestic works. In order to protect and conserve this fragile ecosystem, a complete understanding of mangrove environment and evaluation of anthropogenic inputs of various heavy metals is essential. This understanding could be achieved through the examination and study of sediment quality to reveal the pollutant variations, degradations and cycles, and their chronic effects on water pollution, since sediment quality has been recognized as an important indicator of water pollution, owing to the fact that they act as pool for contaminants (Santos et al. 2003). It is also of paramount importance to have detailed information on the potential availability of metals to biota under various environmental conditions, and trophic transfer mechanism. Since bioavailability and biological toxicity critically depends upon the chemical form in which a metal is available in the sediment, it is essential to determine and monitor metal speciation in surface sediments (Davutluoglu et al. 2010). There are no studies assessing anthropogenic influences on sediment chemical composition, bioavailability and trophic positions in the food web of intertidal Sungai Puloh mangrove which is one of the major productive mangrove ecosystems with great biodiversity in west Peninsula Malaysia.

Therefore there is an imminent need to identify the main anthropogenic sources of heavy metals, material flow and trophic positions of macrobenthic organisms in this intertidal mangrove area, and determine the most threatening contaminant element to the benthic organisms. The scope of the research is to determine the concentrations,

sources, pollution status, and biolavailability of Cd, Cu, Pb, Ni, Zn, and Fe, in surface sediments, and the biological samples. Also to characterize the trophic positions of these biological samples viz: mudskipper- *Periophthalmodon schlosseri*, catfish - *Osteogeniosus militaris*, gastropoda – *Telescopium telescopium; Nerita nerita*, bivalvia - *Polymesoda coaxans*, crustacea - *Penaus monodon*, crab - *Uca annulipes*, *Dotilla myctiroides*, zooplanktons and phytoplanktons in the food web structure of Sungai Puloh intertidal mangrove ecosystem.

1.1 Aims and Objectives

1. To investigate the distribution and concentration levels of selected heavy metals and stable isotopes in intertidal surface sediment and selected flora and fauna in Sungai Puloh mangrove ecosystem.

2. To identify the sources of selected heavy metals found in Sungai Puloh mangrove ecosystem.

3. To characterize the trophic levels of selected flora and fauna in Sungai Puloh mangrove ecosystem by using stable isotopes.

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