QUANTIFICATION OF HEAVY METALS IN BIVALVES ALONG NORTHWEST COAST OF SABAH

NOOR DIANI BINTI BAMBANG DWI HARSONO

THIS DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS TO GRADUATE AS A BACHELOR OF SCIENCE WITH HONOURS

MARINE SCIENCE PROGRAMME

FACULTY OF SCIENCE AND NATURAL RESOURCES

UNIVERSITI MALAYSIA SABAH

2016

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: QUANTIFICATION OF HEAVY METALS IN BIVALVES ALONG NORTHWEST COAST OF SABAH.

IJAZAH: SARJANA MUDA SAINS DENGAN KEPUJIAN (SAINS MARIN)

SAYA: NOOR DIANI BT BAMBANG DWI HARSONO SESI PENGAJIAN: 2013 / 2016
(HURUF BESAR)

Mengaku membenarkan tesis *(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

[ ] SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

[ ] TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana Penyelidikan dijalankan)

[/] TIDAK TERHAD

Disahkan oleh:

NORAZLYNNE MOHD. JOHAN @ JACKLYNE
PUSTAKAWAN
UNIVERSITI MALAYSIA SABAH

(TANDATANGAN PENULIS) (TANDATANGAN PUSTAKAWAN)

Alamat tetap: A1-02-13 GOODYEAR, COURT 10 USJ 15, PERSIARAN KEWAJIPAN, 47630 SUBANG JAYA, SELANGOR

ASSOC. PROF DR. JULIAN RANSANGAN
NAMA PENYELIA

Tarikh: 28 JUNE 2016 Tarikh: 28/6/16

Catatan :- * Potong yang tidak berkenaan.
*Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM)

DECLARATION

I declare that this dissertation is the result of my own independent works except for citations like quotations, excerpts, summaries and references, which have been fully acknowledged.

NOOR DIANI BINTI BAMBANG DWI HARSONO

(BS12110816)

27 JUNE 2016

VERIFICATION

Signature

1. SUPERVISOR

(ASSOC. PROF. DR. JULIAN RANSANGAN)

2. DEAN

(PROF. MADYA DR. SAAFIE SALLEH)

ACKNOWLEDGEMENT

All praises and grateful to Allah S.W.T for giving me the opportunity along with good health and strength to be able to complete my Final Year Project. First of all this dissertation will not have been successfully done without the guidance and help of several individuals who one way or another contributed their time and valuable assistance in preparation and completion of this study.

First and foremost I would like to extend my gratitude to my supervisor Assoc. Prof. Dr. Julian Ransangan for his great supervision, guidance, help and patience throughout my study period. Whose sincerity and encouragement I will never forget. The next important person in completing this study is my mentor, Delta Jenetty Denil who always there to help me as I hurdle all the obstacles in completing this study. Not to forget, Mr. Johnny Tan and Dr. Ganesh that had contributed their time to assist me in completing this study.

I would like to extend my gratitude to all laboratory assistances and staff of Borneo Marine Institute for their co-operations and accommodating to all my queries regarding the laboratory. Some of them stayed over their working time and some even came during the weekends just to allow me to use the laboratory. Your contributions are very much appreciated. Of course not to forget my coursemates and housemates for their direct or indirect helps in completing this study.

Last but not least, to my beloved family and close friends that will always be there for me through thick and thin. Although they are not physically present because of the distance, but I am very thankful for the Skype and WhatsApp technologies so I can always stay contact with them whenever I felt demotivated. They are my support system always there to support and encourage me mentally and emotionally. I am so blessed to have them in my life. They are the reasons I got this far today. May Allah S.W.T shower his blessing on these most precious people in my life.

ABSTRACT

Level of heavy metals in marine environment has been intensively studied in recent years as these hazardous substances are very harmful to the organisms and the environment. Generally, heavy metals contaminants in marine environment resulted from anthropogenic discharges that contain harmful substances which flow into the marine ecosystem. In this study, the marine bivalve marsh clams *(Polymesoda expansa)* and green mussels *(Perna viridis)* were collected along the Northwest coast of Sabah particularly in Kota Marudu (Marudu Bay), Kota Belud (Ambong Bay), Tuaran (Salut and Mengkabong rivers) and Sepanggar (Sepanggar Bay) and analyzed for heavy metal elements (As, Cr, Cu, Mn, Ni, Pb and Zn). The study was carried out to measure the concentration of heavy metals in different districts along the coast of Sabah and to compare the concentration of heavy metals in the two bivalves. Bivalves are widely used in many countries as bio-indicator to bio-monitor the contaminants in the marine environment as it can accumulate pollutant such as heavy metals. Bivalves are also very sensitive to contaminants and show good responses to environmental changes. From this study, the highest mean concentration of heavy metals recorded was Zn in marsh clams and green mussels from Tuaran and Sepanggar at 535.849 (μg/ g) and 374.366 (μg/ g), respectively which was exceeded the permissible limit set by Malaysian Regulation (1985) and FAO/WHO (1984) but below the limit set by other countries such as Thailand and Australia. However, when compare with Tolerable upper intake level (TUI) set by Food and Nutrition Board (2001) Zn concentration in marsh clams and green mussels from Tuaran and Sepanggar are safe for human consumption with little precaution. The statistical analysis of Pearson's correlation showed that, the bivalves with longer length and heavier in weight have the tendency to accumulate higher amount of heavy metals.

ABSTRAK

Tahap logam berat di persekitaran marin telah menjadi salah satu focus penyelidikan penting kebelakangan ini. Ini kerana, logam berat amatlah berbahaya kepada organisma dan alam sekitar. Secara umumnya, logam berat dalam persekitaran marin berpunca daripada aktiviti antropogenik yang mengandungi bahan-bahan berbahaya yang mengalir ke dalam ekosistem marin. Dalam kajian ini, lokan *(Polymesoda expansa)* dan kupang *(Perna viridis)* telah diambil dari kawasan kajian sepanjang pantai barat laut Sabah khususnya dari Kota Marudu (Teluk Marudu), Kota Belud (Teluk Ambong), Tuaran (sungai Salut dan Mengkabong) dan Sepanggar (Teluk Sepanggar) bagi menganalisis unsur-unsur logam berat seperti (As, Cr, Cu, Mn, Ni, Pb dan Zn). Kajian ini dilakukan bagi mengukur kepekatan logam berat di daerah yang berlainan di sepanjang pantai barat Sabah dan membandingkan kepekatan logam berat dalam dua jenis spesies dwi-cengkerang. Haiwan dwi-cengkerang digunakan secara meluas di beberapa buah negara sebagai bio-petunjuk untuk bio-memantau bahan-bahan cemar di dalam persekitaran marin kerana ia boleh mengumpul pencemar marin seperti logam berat. Haiwan dwi-cengkerang ini juga sangat sensitif kepada bahan cemar dan menunjukkan respon positif terhadap perubahan persekitaran. Daripada kajian ini, logam berat, Zn dalam lokan dan kupang dari Tuaran dan Sepanggar mempunyai min kepekatan tertinggi iaitu masing-masing 535.849 (µg / g) dan 374.366 (µg / g). Kepekatan logam Zn ini, telah melebihi had yang dibenarkan oleh Penguatkuasaan Malaysia (1985) dan FAO / WHO (1984) tetapi masih di bawah had yang ditetapkan oleh negara-negara lain seperti Thailand dan Australia. Walau bagaimanapun, apabila dibandingkan dengan tahap pengambilan (TUI) yang ditetapkan oleh Lembaga Makanan dan Pemakanan (2001), kepekatan Zn dalam lokan dan kupang dari Tuaran dan Sepanggar adalah selamat untuk dimakan oleh manusia, tetapi perlu mengambil langkah berjaga-jaga. Analisis statistik korelasi Pearson menunjukkan bahawa, semakin panjang dan berat kedua-dua jenis dwi-cengkerang ini, kecenderungan mereka untuk mengakumulasi logam berat adalah tinggi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF FORMULA

LIST OF SYMBOLS

cm	centimeter
°C	degree Celsius
°	Degree
g	gram
µg/g	microgram per gram
µm	micrometer
mm	millimetre
ml	millilitre
km	kilometer
M	mole
%	percentage
±	plus-minus
N	no. of samples
~	approximate
ppm	parts per million
No.	Number
<	Less than

LIST OF ABBREVIATIONS

N	North
E	East
S.E	Standard error
As	arsenic
Cr	chromium
Cu	copper
Mn	manganese
Ni	nickel
Pb	lead
Zn	zinc
HNO_3	Nitric acid
AFS	Atomic Fluorescence Spectrometry
AAS	Atomic Absorption spectrometer
ICP-AES	Inductively Coupled Plasma with Atomic Emission Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma with Mass Spectroscopy
UMS	Universiti Malaysia Sabah
FSSA	Faculty of Science and Natural Resources
BMRI	Borneo Marine Research Institute
ANOVA	Analysis of Variance

SPPS	Statistical Package for Social Science
EC	European Commission
MPHT	Ministry of Public Health Thailand
FDA	Food and Drug Administration of the United States
HKEPD	Hong Kong Environmental Protection Department
NHMRC	Australian Legal Requirements
FAO	Food and Agriculture Organization
WHO	World Health Organization
JECFA	Joint FAO/WHO Expert Committee on Food Addictive
ICES	International Council for the Exploration of the Seas
ABIA	Brazilian Ministry of Health
EOSQC	Egyptian Organization for Standardization and Quality Control
TUI	Tolerable upper intake level
UNEP	United Nation Environment Programme

CHAPTER 1

INTRODUCTION

1.1 General background

Bivalves are essential protein sources in human diet besides meat and poultry products as they provide human with omega-3 that is an unsaturated fat that can diminish cholesterol level and coronary illness, stroke and pre-mature birth (Daviglus *et al.*, 2002; Patterson 2002). Bivalves are rich in protein, calcium, phosphorus, fluorine, and iodine, polyunsaturated and unsaturated fats, and insoluble vitamins which have hypocholesterolic impact against atherosclerosis or cardiovascular diseases (Ismail, 2005; Ikem & Egiebor, 2005). The benefits of bivalves to human are unquestionable.

Despite these benefits, shellfishes could bring about negative effects to health. There has been a rising evidence of heavy metal intoxication that lead to health risk (Jin *et al.*, 2011). Although some metal elements are essential to the human body, others can cause harm to biological system. Numerous marine aquatic organisms such as bivalves can potentially become contaminated by different contaminants due to anthropogenic activities and natural emissions especially activities which involved heavy metals (Langston *et al.*, 1998). These filter feeder species can accumulate high concentrations of heavy metals and often use as the indicator of presence of heavy metals in an aquatic environment (Langston *et al.*, 1998).

Bivalves are known to filter between twenty and one hundred liters of surrounding waters a day. In doing so, they accumulate natural or anthropogenic contaminants. Upon consumption, they present these contaminants to the consumers (Richards, 1988; Lees, 2000 and Robertson, 2007). According to Agusa *et al.* (2005), heavy metals which are accumulated in the bivalve tissues may come from the sediments, water column and some suspended particulate matters. These contaminants in must be evaluated before consumption of bivalves to avoid hazards to human (Usydus *et al.*, 2008).

In recent years, investigation on the benefits and risk of aquatic organisms to human health is growing tremendously (Domingo *et al.*, 2007; Mahaffey, 2004). A few bodies such as the United Nation Environment Programme (UNEP) and Food Agriculture Organization (FAO) have emphasized the requirements to check the substantial metals presence in the nature (UNEP, 1996).

It is said that less prepared food are healthier but somehow, it does not comply to all food products especially bivalves, which could bring risk to human health if eaten raw or half cooked (Murchie *et al.*, 2005). However, bivalves are normally negligibly prepared and eaten raw or half cooked in many societies (Romalde *et al.*, 1994; Lees, 2000 and Murchie *et al.*, 2005). Consumption of bivalves which are contaminated with high level of heavy metals can lead to a positive risk of cancer (Gagnon *et al.*, 2004).

The increase in human population in certain areas and the expansion of industrial activities intensify the exposure of bivalves to heavy metals contaminants (Lee *et al.*, 1999; Lees, 2000 & Brands *et al.*, 2005). Bivalves inhabit estuarine areas and coastal waters are easily exposed to these pollutants (Burkhardt & Calci, 2000; Croci *et al.*, 2002).

According to Hemroth *et al.* (2012), the uncontrolled runoff from the rainfall events, septic tank leakages, agricultural and industrial discharges, are all flow into the coastal waters and bring along the contaminants which might poses health risk both to human and marine organisms.

Such data are easily available and well established in other parts of the world where monitoring activities are actively and regularly taking place. However, this is not the case in Sabah, Malaysia. Hence, this study evaluates the heavy metals contamination in bivalves along the northwest coast of Sabah.

1.2 Problem Statement

Coastal zones are important areas for both fishery resources and development (Castro *et al.*, 1999; Usero *et al.*, 2005). In many developing countries, many of these coastal zones are utilized for industrialization and agricultural activities. Such activities, if not monitored properly, may contribute to the heavy metals contamination in the coastal waters and cause harm to many aquatic organisms (Yu *et al.*, 2011; Muhammad *et al.*, 2011). Similar development is also seen along the northwest coast of Sabah which could potentially affect the marine organisms such as bivalves in the area. Consequently, it elevates the health risk to humans who consume them.

1.3 Objectives

The objectives of this study are:

1) To measure the heavy metals concentration of bivalves, Marsh Clam *(Polymesoda expansa)* and Green Mussel *(Perna viridis)* along the coastal areas particularly Kota Marudu, Kota Belud, Tuaran and Sepanggar.

2) To compare the heavy metals concentrations in Marsh Clam *(Polymesoda expansa)* and Green Mussel *(Perna viridis)* collected from the sampling sites.

1.4 Hypotheses

Bivalves from coastal waters of Tuaran district are expected to contain high levels of heavy metals contamination than Kota Belud, Kota Marudu and Sepanggar.

1.5 Significance of study

This study fills in the knowledge gap about the status of heavy metals contamination in commercial bivalves particularly green mussels (*Perna viridis*) and marsh clams (*Polymedosa expansa*) collected from coastal areas on the northwest coast of Sabah, particularly in Kota Marudu, Kota Belud, Tuaran and Sepanggar

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of Bivalves

Phylum Mollusca is among the most abundance marine invertebrate groups. Invertebrates that fall under phylum Mollusca such as class Bivalvia are unsegmented, consists of hard external calcium carbonate divided into two halves shell surrounding the internal organs. The two halves are hinged and have a very powerful adductor muscles that draw them tightly together to protect the soft body tissues (Reese *et al.*, 2008). A large muscular foot usually use for locomotion, securing food and also for anchorage purposes.

According to Morrissey & Sumich (2012), marine bivalve includes clams, oysters, mussels and scallops are all aquatic invertebrates and can easily be found from intertidal areas to below 5000 m water depth. Bivalves lack of radula but they consist of gills. The gills are covered with cilia. Cilia help bivalves to circulate water for exchange of gas, filter and sorting out the tiny food particles that trapped around mucus that secreted over the gills surfaces.

Bivalves are specialized to feed on suspended particles such as bacteria, phytoplankton, detrital particles found in the sediments or in water column (Navarro & Thompsan 1997). Some bivalves are burrowers such as clams but most bivalves attach on to rocks, corals or other suitable surfaces (Castro & Michael, 2005).

2.2 Feeding behavior of Bivalves

There are two types of feeding behavior in bivalves which are the suspension feeders and deposit feeders. The deposit or facultative suspension feeders can be observed from their morphology of siphon (Yonge, 1949).

According to Navarro *et al.* (2008), the suspension feeder bivalves obtain food such as the suspended particulate particles from the water column. On the other hand, the deposit feeders feed on particulate organic material deposited on the sediments. Navarro & Thompsan (1997) state that, this food sources for deposit feeders are produced by the benthic primary productivity and organic particles. For examples, phytoplankton, bio-deposits or detritus particles that are settling from the water column.

The suspension and deposited feeders will have different levels of limitation of food availability and there will be competition for food among bivalve species. For example, deposited feeders bivalves, the food sources are limited compared to suspension feeders (Levinton, 1972). The limited food sources on the sediments for deposited feeders and unlimited food availability from the water column for suspension feeders would be the factors of the different types of feeding behavior in bivalves (Cadée, 1984; Hummel, 1985 and 'Olafsson, 1986).

Some bivalves would change their feeding behavior from deposit feeder to suspension feeder depending on the limitation of food availability in a particular area (Cadée, 1984; Hummel, 1985 and 'Olafsson, 1986).

2.3 Accumulation of heavy metals in Bivalves

The most crucial source of heavy metals bioaccumulation in bivalve molluscs is from suspended particles such as from the water column, in the case of deposited feeders heavy metals are obtained from sediments (Bryan, 1976).

According to Phillips (1976), the ability of bivalves to accumulate heavy metals in the organs such as digestive gland and gills (Jocelyn & Jean, 2009) in its soft tissue from the marine environment is well known and has become a focus in research since half a decade ago. Metals that are known as metallic chemical elements that contain high level of density and are very toxic and poisonous even in low quantity is referred to heavy metals (Jin *et al.*, 2011). Arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd) and chromium (Cr) are very toxic even at low concentration (Govind & Madhuri, 2014). Some essential elements such as manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn) and molybdenum (Mo) if in high concentrations are also known as heavy metals (Khaled, 2004).

According to Turkmen *et al.* (2008), metals for example iron (Fe), copper (Cu), zinc (Zn) and manganese (Mg), are important metals in biological system to maintain the human body metabolism but very toxic if consumed at higher concentration. Other metals for example mercury (Hg), lead (Pb) and cadmium (Cd) are harmful to biological system even in small quantity.

Similar to the essential metals, the non-essentials metals once taken up by bivalves will also be accumulated in their tissues (Jakimska *et al.,* 2011). Aquatic organisms such as bivalves from phylum Mollusca accumulate both essential and non-essential metals during respiration, feeding or via absorption onto their body surface and are stored in various parts in their tissues (Denton & Burdon-Jones, 1981; Vigh *et al.*, 1996).

The toxic heavy metals do not impact the environment and organisms immediately the effects usually become apparent after a few years (Danis, 2006). The process of heavy metals accumulation in the soft tissues and organs of bivalves are species-dependent and related to the metabolism and detoxification mechanisms in the species. As a consequence, we may find that variety of species from same environment to have different levels of heavy metal concentration accumulated in their tissues and organs (Ritterhoff & Zauke, 2003; Kahle & Zauke, 2003 and Prowe, 2006).

REFERENCES

Abdallah, M. A. M. & Abdallah, A. M. A. 2008. Biomonitoring study of heavy metals in biota and sediments in the South Eastern Coast of Mediterranean Sea, Egypt. *Environmental Monitoring and Assessment,* **146**: 139-145.

ABIA (Associac˜ao Brasileira das Ind´ustrias de Alimentac˜ao). 1991. *Atos do Minist ˆerio da Sa ´ude: Comp ˆendio da Legislac˜ao de Alimentos.*

Agency for Toxic Substance and Disease Registry (ATSDR), 2003. Toxicological Profile for Arsenic. US Department of Health and Humans Services, Public Health Human Services, Centers for Diseases Control, Atlanta.

Agusa, T., Kunito, T., Yasunaga, G., Iwata, H., Subramanian, A., Ismail, A. & Tanabe, S. 2005. Concentrations of trace elements in marine fish and its risk assessment in Malaysia. *Marine Pollution Bulletin,* **51** (8-12): 896-911.

Akberali, H.B, & Davenport, J. 1981. The responses of the bivalve *Scrobicularia plana* (da costa) to gradual salinity changes. *Journal of Experimental Marine Biology and Ecology,* **53**: 251-259.

Alina, M., Azrina, A., Yunus, M. A. S., Mohd, Z. S., Mohd, I. E. H. & Muhammad, R. R. 2012. Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. *International Food Research Journal,* **19** (1): 135-140.

Alexander, G. & Young, D. 1976. Trace metals in Southern California mussels. *Marine Pollution Bulletin,* **7**: 7-9.

APHA (American Public Health Association). 1992. Standard Methods for the examination of waters and wastewaters. 16th edition. Washington, DC.

ATSDR (Agency for Toxic Substances and Disease Registry) 2007. Toxicological profile for arsenic. In: U.S. Department of Health and Human Services. Edition 2007.

Balaji, M. & Satyanarayana, R. K. 1999. Size dependent bioaccumulation of heavy metals by *Mystilopsis sallei* (Recluz) at Visakhapatnam harbour. *Indian Journal of Experimental Biology*, **38**: 405-407.

Beldi, H., Gimbert, F. S., Maas, R., Scheifler, & Soltani, N. 2006. Seasonal variations of Cd,Cu, Pb and Zn in the ediblemollusc *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba, Algeria. *African Journal of Agricultural Research*, **1** (4): 85–90.

Boening, D. W. 1999. An evaluation of bivalves as bio-monitors of heavy metals pollution in marine waters. *Environmental Monitoring and Assessment*, **55**: 459–470.

Brands, D. A., Inman, A. E., Gerba, C. P., Mare, C. J., Billington, S. J. & Saif, L. A. 2005. Prevalence of Salmonella spp. in oysters in the United States. *Applied and Environmental Microbiology*, **71** (2).

Brar, N. K., Waggoner, C., Reyes, J. A., Fairy, R. & Kelley, K. M. 2009. Evidence for thyroid endocrine disruption in wild fish in San Francisco Bay, California, USA. Relationships to contaminant exposures. *Aquatic Toxicology*, **10**: 1016.

Bryan, G. W. 1976. *Some aspects of heavy metal tolerance in aquatic organisms. In effect of pollutants on aquatic organism.* A.P.M Lockwood Ed. Cambridge University Press, London and New York.

Burkhardt, W. & Cald, K. R. 2000. Selective accumulation may account for shellfish associated viral illness. *Applied and Environmental Microbiology*, **66** (4).

Cadée, G. C. 1984. 'Opportunistic feeding', a serious pitfall in trophic structure analysis of (Paleo) faunas. *Lethaia*, **17**: 289-292.

Castro, H., Aguilera, P. A., Martinez, J. L. & Carrique, E. L. 1999. Differentiation of clams from fishing areas an approximation to coastal quality assessment. *Environmental Monitoring and Assessment*, **54**: 229–237.

Castro, P. H. & Michael E. 2005. *Marine Biology*. 5th Ed. McGraw Hill, USA, pp 108-120.

Croci, L., Suffredini, E., Cozzi, L., & Toti, L. 2002. Effects of depuration of molluscs experimentally contaminated with Escherichia coli, Vibrio cholerae O1 and Vibrio parahaemolyticus. *Journal of Applied Microbiology*, **92** (3).

Copat, C., Giovani, A., Maria, F., Caterina, L., Roberto, F., Salvatore, S. & Margherita, F. 2013. Heavy metals concentration in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. *Food and Chemical Toxicology*, **53**: 33-37.

Corner, E. D. S, & Sparrow, B, W. 1959. The mode of action of a toxic agent. Observation on poisoning of certain crustaceans by copper and mercury. *Marine biological association of the United Kingdom*, **57**: 63-74.

Danis, B., Wantier, P., Flammang, R., Perneth, P. H., Chambost-Manciet, Y., Coteur, G., Warnau, M. & Dubois, P. H. 1979. Bioaccumulation and effects of PCBs and heavy metals in sea stars (*Asterias rubens*, L.) from the North Sea: a small scale perspective. Science Total Environment, **356**: 275.

Daviglus, M., Sheeshka, J. & Murkin, E. 2002. Health benefits from eating fish. *Comments Toxicology*, **8:** 345–374.

De Marco, S. G., Botte, S. E. & Marcovecchio, J. E. 2006. Mercury distribution in abiotic and biological compartments within several estuarine systems from Argentina: 1980–2005 period. *Chemosphere* **65**: 213–223.

Denton, G. R. W. & Burdon-Jones, C. 1981. Influence of temperature and salinity on the uptake, distribution and depuration of mercury, cadmium and lead by the black-lip oyster *Saccostrea echinata*. *Marine Biology*, **64**:317-326.

Department of Health Malaysia, 2004. Regulation relating to maximum levels for heavy metals in foodstuffs. Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972).

Department of Fisheries Malaysia, 2003. *Fish inspection and Quality Control Division.* At: http://www.dof.gov.my/31;jsessionid=C8EF39C47BAE0FB3EAB8E1FE29ADC291. Accessed on December 9, 2015.

Department of Fisheries Malaysia, 2010. *Fish inspection and Quality Control Division.* At: http://www.dof.gov.my/31;jsessionid=C8EF39C47BAE0FB3EAB8E1FE29ADC291. Accessed on December 11, 2015.

Domingo, J. L., Bocio, A., Falco´, G. & Llobet, J. M. 2007. Benefits and risks of fish consumption. Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Toxicology,* **230** (2–3): 219–226.

Draghici C. & Chirila E., 2015. *Heavy Metals Determination in Environmental and Biological Sample.* At: http://www.researchgate.net/publication/225988843. Accessed on November 10, 2015.

Ebeed, A., Saleh, K. M., Sadek, Safaa, H & Ghorbal. 2014. Bio-estimation of selected heavy metals in shellfish and their surrounding environment media. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering,* **8** (11).

EC (European Commission). 2001. Commission Regulation (EC) No. 466/2001 of 8 March 2001. *Official Journal of European Communities,* **1**.

EC (European Commission). 2006. Setting maximum levels of certain contaminants in foodstuff. *Journal of European Union Legislation,* **65**: 5–24.

Edward, F. B., Yap, C. K., Ismail, A. & Tan, S. G. 2009. Interspecific variation of heavy metal concentrations in the different parts of tropical intertidal bivalves. *Water, Air, and Soil Pollution,* **196**(1–4): 297–309.

EOSQC (Egyptian Organization for Standardization and Quality Control). 1993. Maximum level for heavy metals contamination in food. *Environmental Sciences,* **2360.**

FAO/WHO. 1984. List of maximum levels recommended for contaminants by the Joint FAO/ WHO Codex Alimentarius Commission. Second Series. CAC/FAL, Rome **3**: 1–8.

FAO/WHO. 2004. List of maximum levels recommended for contaminants by the Joint FAO/WHO Codex Alimentarius Commission.

FAO (Food and Agriculture Organization). 1996. Report (No. 22) on the ninth steering Committee Meeting, Gaborone, Botswana. 13-16 February 1996.

Faruk, M. H., Sinnin, H., & Rezaur, R. 2015. Review on the risk assessment of heavy metals in Malaysian clams. *The Scientific World Journal*, **7**.

FDA (Food and Drug Administration). 1976. Guidelines for cadmium, lead, chromium and mercury in Seafoods, California.

FDA (Food & Drug Administration of the United States). 1990. US Food and Drug Administration Shellfish Sanitation Branch, Washington, DC.

FDA (Food and Drug Administration). 2001. *Fish and Fisheries Products Hazards and Controls Guidance, third ed. Center for Food Safety and Applied Nutrition*, United State Food and Drug Administration.

Food and Nutrition Board. 2001. *Dietary reference intakes for Vitamin A, Vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*. National Academy Press, Washington, DC.

Fung, C. N., Lam, J. C. W., Zheng, G. J., Connell, D. W., Monirith, I., Tanabe, S., Richardson, B. J. & Lam, P. K. S. 2004. Mussel-based monitoring of trace metal and organic contaminants along the east coast of China using *Perna viridis* and *Mytilus edulis*. *Environment Pollution*, **127**: 203– 216.

Gagnon, F., Tremblay, T., Rouette, J. & Cartier, J. F. 2004. Chemical risks associated with consumption of shellfish harvested on the north shore of the St. Lawrence River's lower estuary. *Environment Health Perspect,*, **112**: 883–888.

Goldberg, E. D. 1975. The mussel watch – a first step in global marine monitoring. *Marine Pollution Bulletin*, **6**: 111.

Goldberg, E. D., Bowen, V. T., Farrington, J. W., Harvey, G., Martin, J .H., Parker, P. L., Riseborough, R. W., Robertson, W., Schneider, E. & Gamble, E., 1978. The Mussel Watch. *Environmental Conservation*, **5:** 101–126.

Govind, P. & Madhuri S. 2014. Heavy Metals Causing Toxicity in Animals and Fishes *Research Journal of Animal, Veterinary and Fishery Sciences,* **2** (2): 17-23.

Hamed, A. E. S., Hamdy, A., Fahad, A. M., Magdy, B., Hesham, S., Khaled, A. R., Mona, K. & Heba, E. 2013. The potential use of the bivalve *Donax trunculus* as Bio-indicator for heavy metal pollution of Port Said western coast on the Mediterranean Sea. *Life Science Journal,* **10** (4).

Hays, A. W. 1989. *Principles and methods of toxicology.* 2 Ed. Raven Press, New York, pp 89-115.

Hernroth, B. E., Conden-Hansson, A. C., Rehnstam-Holm, A. S., Girones, R., & Allard, A. K. 2012. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: The First Scandinavian Report. *Applied and Environmental Microbiology*, **68** (9).

HKEPD (Hong Kong Environmental Protection Department). 1987. Marine water quality in Hong Kong in 1997. *Government Printer, Hong Kong.*

HKEPD (Hong Kong Environmental Protection Department). 1997. *Environment Hong Kong*, Environmental Protection Department (EPD), Hong Kong.

Hummel H. 1985. Food intake of *Macoma balthica* (Mollusca) in relation to seasonal changes in its potential food on a tidal flat in the Dutch Wadden Sea, Netherlands. *Journal of Sea Research,* **19**: 52-76.

ICES (International Council for the Exploration of the Seas).1988. *Report of the working group on environmental impacts of mariculture,* ICES CM 2012/SSGHIE: 16.

Ikem, A. & Egiebor, N. O. 2005. Assessment of trace elements in canned fishes (Mackerel, Tuna, Salmon, Sardines and Herrings) marketed in Georgia and Alabama (United State of America). *Journal of Food Composition Analysis*, **18**: 771–787.

Ismail, A. & Rosniza, R. 1997. Trace metals in sediments and molluscs from an estuary receiving pig farms effluent. *Environmental Technologies*, **18**: 509–515.

Ismail, A., Yap, C. K., Zakaria, M. P., Tanabe, S., Takada, H. & Ismail, A. R. 2000. Green-lipped mussel, *Perna viridis* (L.), as a biomonitoring agent for heavy metals in the West coast of Peninsular Malaysia. In: M. Shariff, F. M. Yusoff, N. Gopinath, H. M. Ibrahim, R. A. Nik Mustapha (Eds.), *Towards Sustainable Management of the Straits of Malacca*, pp. 553–559. Malacca Straits Research and Development Centre (MASDEC), Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Ismail, H. M. 2005. The role of omega-3 fatty acids in cardiac protection. *An overview of Frontier Bioscience*, **10**: 1079–1088.

Jakimska, A., Konieczka, P., Skora, K. & Namiesnik, J. 2011. Bioaccumulation of metals in tissues of marine animals, Part 1: the role and impact of heavy metals on organisms. *Pollution Journal Environment Studies*, **20** (5): 1117-1125.

Jarup, L. 2003. Hazards of heavy metal contamination. *British Medical Bulletin*, **68**: 167-182.

JECFA (Joint FAO/WHO Expert Committee on Food Addictives). 2002. Limit Test for heavy metals in food additives specifications. *Joint FAO/WHO Expert Committee on Food Addictives (JECFA).*

JECFA (Joint FAO/WHO Expert Committee on Food Addictives). 2009. *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives.* At: http://apps.who.int/ipsc/database/evaluations/search.aspx. Accessed on November 27, 2015.

Jin H. B., Sung H. Y., & Sun Y. L., 2011. Heavy Metal Contents and Chemical Compositions of Atlantic (Scomber scombrus), Blue (Scomber australasicus), and Chub (Scomber japonicus) Mackerel Muscles. *Food Science Biotechnology,* **20** (3): 709-714.

Jocelyne, P. & Jean-Claude, A. 2009. Comparison of bioaccumulation of metals and induction of metallothioneins in two marine bivalves (*Mytilus edulis* and *Mya arenaria*). *Comparative Biochemistry and Physiology,* **150**: 186–195.

Kahle, J. & Zauke, G. P. 2003. Trace metals in Antartic copepods from the Weddell Sea (Antartic). *Chemospere,* **51**: 409.

Kamaruzzaman, B. Y., Shuhada, N. T., Akbar, B., Shahbudin, S., Jalal, K. C. A., Ong, M. C., Al-Barwani, S. M. & Goddard, J. S. 2011. Spatial concentrations of lead and copper in bottom sediments of Langkawi Coastal Area, Malaysia. *Journal in Environmental Science,* **5**: 179-186.

Kamaruzzaman, B. Y., Zahir, M. S. & John, B. A. 2011. Bioaccumulation of some metals by green mussel *Perna viridis* (Linnaeus 1758) from Pekan, Pahang, Malaysia. *International Journal of Biological Chemistry,* **5** (1): 54-60.

Kanakaraju, D., Connie, J. & Long, S. M. 2008. Heavy metal concentrations in the razor clam (*Solen* Spp) from Muara Tebas, Sarawak. *The Malaysian Journal of Analytical Sciences,* **12** (1): 53–58.

Kaufmann, D. B., Dinicola, W. & Macintosh, S. 1970. Acute potassium chromate poisoning. *American Journal Disease Children,* **119**: 374-376.

Keskin, Y., Baskaya, R., Ozyaral, O., Yurdun, T., Luleci, N. E. & O. Hayran. 2007. Cadmium, lead, mercury and copper in fish from the Marmara Sea, Turkey. *Bulletin of Environment Contamination and Toxicology,* **78** (3-4): 258-61.

Ketata I, Smaoui-Damak W, Guermazi F, Rebai T & Hamza-Chaffai A. 2007. In situ endocrine disrupting effects of cadmium on the reproduction of *Ruditapes descussatus.* *Comparative Biochemistry and Physiology,* **146** (5): 415-430.

Khaled, A. 2004. Heavy metal concentrations in certain tissues of five commercially important fishes from El-Mex Bay, Alexandria, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries,* **8** (51).

Kumar, S. R., Chavan, S. L. & Sapkale, P. H. 2007. Heavy metal concentrations in water, sediments and body tissues of red worm (*Tubifex* sp.) collected from natural habitats in Mumbai, India. *Environmental Monitoring and Assessment,* **129**: 471-481.

Langston, W. J., Bebianno, M. J. & Burt, G.R. 1998. *Metal Handling strategies in molluscs.* In: Langston, W.J., Bebianno, M.J. (Eds.), Metal metabolism in aquatic environments. Chapman Hall, London, pp 219–284.

Lees, D. 2000. Viruses and bivalve shellfish. *International Journal of Food Microbiology,* **59** (12).

Lee, K., Nagler, J. J., Fournier, M., Lebeuf, M. & Cyr, D. G. 1999. Toxicological characterization of sediments from Baie des Anglais on the St. Lawrence Estuary. *Chemosphere,* **39**: 1019–1035.

Levinton J. 1972. Stability and trophic structure in deposit feeding and suspension-feeding communities. *American Naturalist,* **106**: 472-486.

Lias, L., Jamil, T. & Aliaa, S. N. 2013. A preliminary study on heavy metal concentration in the marine bivalves Marcia marmorata species and sediments collected from the coastal area of Kuala Perlis, North of Malaysia. *Journal of Applied Chemistry,* **4** (1): 48–54.

Liu, J., Goyer, R. A. & Waalker, M. P. 2008. *Toxic effects of metals.* The Basic Science of Poisons. McGraw-Hill, New York, pp 931-979.

Mahaffey, K. R. 2004. Fish and shellfish as dietary sources of methylmercury and the omega-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits. *Environment Respect,* **95**: 414–428.

Malaysian Regulations. 1985. *Malaysian Law on Food and Drugs. Kuala Lumpur.* Malaysia Law Publisher.

Meei-Fang, S., Wen-Der, C., Chia-Chia, S. & Ming-Chun, L., 2014. Heavy Metals in bivalve mollusks collected from Da-Peng bay lagoon in south-southwestern Taiwan. *Journal of Toxicology and Environmental Health,* **77**: 214-222.

MPHT (Ministry of Public Health, Thailand). 1986. *Residues in foods, Part 23, vol. 103 (pp.1123–1124). Special Issue, 16 February, 1986.* The Government Gazette, Bangkok, Thailand.

Mo, C. & Neilson, B. 1994. Standardization of oyster soft dry weight measurements. *Water Research,* **28:** 243–246.

Morrissey, J. F. & Sumich. J. L. 2012. *Introduction to the Biology of Marine Life.* 10th Ed. Jones & Bartlett Learning, USA.

Muhammad, S., Shah, M. T. & Khan, S. 2011. Health risk assessment of heavy metals and their source apportionment in drinking water of Kohistan region, northern Pakistan. *Microchemical Journal.* **98:** 334–343.

Murchie, L. W., Cruz-Romero, M., Kerry, J. P., Linton, M., Patterson, M. F. & Smiddy, M. 2005. High pressure processing of shellfish: a review of microbiological and other quality aspects. *Innovative Food Science & Emerging Technologies,* **6** (3).

Navarro, J. M. & Thompson, R. J. 1997. Biodeposition by the horse mussel *Modiolus modiolus* (Dillwyn) during the spring diatom bloom. *Journal of Experimental Marine Biology and Ecology,* **209**: 1-13.

Navarro, J. M., Elena, C., Marco, L. & Robert A. S. 2008. Feeding behavior of the infaunal bivalve *Tagelus dombeii* (Lamarck, 1818). Suspension *vs.* deposit feeding. *Biology Oceanography,* **43** (3).

NHMRC (National Health and Medical Research Council). 1987. *National food standard A 12: metal and contaminants in food. Canberra: Australia.* Australian Govemment Publishing Services.

Nirmala, K., Oshima, Y., Lee, R., Imada, N., Honjo, T. & Kobayashi, K. 1999. Transgenertaional toxicity of tributyltin and its combined effects with polychlorinated biphenyls on reproductive processes in Japanese Medaka (Oryzias latipes). *Environmental Toxicology and Chemistry*, **18**: 717- 721.

Numadia, A. A., Azrina, A., Amin, I., Mohd, Y., A. S., Mohd, I. E. H. 2013. Mineral contents of selected marine fish and shellfish from the west coast of Peninsular Malaysia. *International Food Research Journal*, **20** (1): 431-437.

Oehlmann, J. & Oehlmann, U. S. 2002. Molluscs as bioindicators. *Bioindicators and Biomonitors*, 577-635.

'Olafsson, E. B. 1986. Density dependence in suspension feeding and deposit-feeding populations of the bivalve *Macoma balthica* field experiment. *Journal Animal Ecology*, **55**: 517-526.

Patterson, J. 2002. Introduction-comparative dietary risk: balance the risks and benefits of fish consumption. *Comments Toxicology*, **8**: 337–344.

Paez-Osuna, F., Osuna-K'opaz, J. I., Izaguirre-Fierro, G. & Zazuetta-Padilla, H. M. 1993. Heavy metals in clams from a subtropical coastal lagoon associated with an agricultural drainged basin. *Bulletin Environment Contamination Toxicology*, **50**: 915-921.

Phillips, D. J. H. 1976. The common mussels *Mytilus endulis* as an indicator of Zinc, Cadmium, lead, Copper. *Marine Biology*, **38**: 59-69.

Phillips, D. J. H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments – a review. *Environmental Pollution*, **13**: 281–317.

Prowe, F., Kirf, M. & Zauke, G. P. 2006. Heavy metals in crustaceans from the Iberian deep sea plain. *Science Marine,* **70**: 271.

Rainbow, P. S. & Phillips, D. J. H. 1993. Cosmopolitan biomonitors of trace metals. *Marine Pollution Bulletin,* **26**: 593–601.

Reese, J. B., Lisa A. U., Michael L. C., Steven A. W., Peter V. M. & Robert B. Jackson. 2008. *Biology.* 9th Ed. Pearson, USA.

Richards, G. P. 1988. Microbial purification of shellfish: a review of depuration and relaying. *Journal of Food Protection,* **51** (3).

Ritterhoff, J. & Zauke, G. P. 2003. Trace metals in field samples of zooplankton from the Fram Strait and the Greenland Sea. *Science Total Environment,* **199**: 255.

Ruelas-Inzunza, J. R. & Paez-Osuna, F. 2000. Comparative bioavailability of trace metals using three filter-feeder organisms in a subtropical coastal environment (Southeast Gulf of California). *Environmental Pollution,* **107:** 437-444.

Robertson, L. J. 2007. The potential for marine bivalve shellfish to act as transmission vehicles for outbreaks of protozoan infections in humans: a review. *International Journal of Food Microbiology,* **120** (3).

Romalde, J. L., Estes, M. K., Szucs, G., Atmar, R. L., Woodley, C. M., & Metcalf, T. G. 1994. Insitu detection of hepatitis A virus in cell cultures and shellfish tissues. *Applied and Environmental Microbiology,* **60** (6).

Saiz-Salinas, J. I., Ruiz, J. M. & Frances-Zubillaga, G. 1996. Heavy metal levels in intertidal sediments and biota from the Bidasoa Estuary. *Marine Pollution Bulletin,* **32**: 69-71.

Shazili, N. A. M, Mohd-Lokman, H., Rashid, M. K. A., Yaakob, R., Shamsuddin, A. A. & Razali, M. S., 1995. Trace metal contents in the fish and bivalves from East Coast Malaysia. Proceedings of the International Seminar on Marine Fisheries Environment, 1995 March 9–10, Eastern Marine Fisheries Development Center and Japan International Cooperation Agency, Rayong, Thailand.

Shazili, N. A. M., Kamaruzaman, Y., Ahmad, S. A., Norantonina, A. & Kamil, M. A. R. 2006. Heavy metal pollution status in the Malaysian aquatic environment. *Aquatic Ecosystem, Health and Management*, **9**(2):137–145.

Singh, B. R. & Steinnes, E. 1994. Soil and water contamination by heavy metals. Stewart (eds.) Soil processes and water quality. Lewis, Boca Raton, Florida, pp 233-272.

Sirot, V., Guerin, T., Volatier, J. L. & Leblanc, J. C., 2009. Dietary exposure and biomarkers of arsenic in consumers of fish and shellfish from France. *Science Total Environment*, **407**: 1875–1885.

Sivaperunal, P., Sankar, T. V. & Viswanathan, N. P. G. 2007. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards. *Food Chemistry*, **102**: 612-620.

Sorimachi, F. 1987. Activation of alkaline phosphatase with Mg+2 and Zn+2 in rat hepatoma cells. *Journal Biological Chemistry*, **262**: 1535-1541.

Sow, A. Y., Ahmad, I. & Syaizwan, Z. Z. 2012. Heavy Metals Uptake by Asian Swamp Eel, *Monopterus albus* from Paddy Fields of Kelantan, Peninsular Malaysia: Preliminary Study. *Tropical Life Sciences Research*, **23** (2): 27–38.

Straalen, V. N. M., Donker, M. H., Vijner, M. G. & Gestel, C. A. M. V. 2005. Bioavailability of contaminants estimated from uptake rates into soil invertebrates. *Environmental Pollution*, **136** (3): 409–417.

Taweel, A., Shuhaimi-Othman, M. & Ahmad, A. K. 2013. Assessment of heavy metals in tilapia fish (*Oreochromis niloticus*) from the Langat River and Engineering Lake in Bangi,Malaysia, and evaluation of the health risk from tilapia consumption. *Ecotoxicology and Environmental Safety*, **93**(45–51).

Turkmen, M., Turkmen, A., Tepe, Y., Ates, A. & Gokkus, K. 2008. Determination of metal contaminations in sea foods from Marmara, Aegean and Mediterranean Seas: twelve fish species. *Food Chemical*, **108**: 794–800.

UNEP (United Nation Environment Programme). 1996. Final Reports on research projects dealing with eutrophication and heavy metal accumulation. *Journals of United Nation Environment Programme*, **104**: 238.

US-EPA (Environment Protection Agency), 2000. Guidance for Assessing Chemical Contamination Data for Use in Fish Advisories, vol. II. Risk Assessment and Fish Consumption Limits EPA/823-B94-004. *United States Environmental Protection Agency, Washington, DC.*

Usero, J., Morillo, J. & Gracia, I. 2005. Heavy metal concentration in mollusks from the Atlantic coast of southern Spain. *Chemosphere*, **59**: 1175–1181.

Usydus, Z., Richert, J. S., Polak-Juszak, L., Kanderska, J., Adamczyk, M., Ciecwierz, M. & Ruczynska, W. 2008. Food of marine origin: between benefits and potential risks. Part I. Canned fish on the polish market. *Food Chemical*, **111:** 556–563.

Vigh, P., Z. Mastala, & K. V.-Balogh. 1996. Comparison of heavy metal concentration of grass carp (*Ctenopharyngodon idella* Cuv. et Val.) in a shallow eutrophic lake and fish pond (possible effects of food contamination). *Chemosphere*, **32**: 691-701.

Waznah, S. A., Kamaruzzaman, B. Y. & Shahbudin, S. 2011. Spatial and temporal variation of zinc (Zn) and cadmium (Cd) in the bottom sediments of Pahang river-estuary, Pahang, Malaysia, in *Proceedings of the 10th Universiti Malaysia Terengganu International Annual Symposium (UMTAS '11)*, Kuala Terengganu, Malaysia, July 2011.

Yap, C. K., Ismail, A. & Tan, S.G. 2004. Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel Perna viridis (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. *Food Chemistry*, **84:** 569-575.

Yap, C. K., Kamarul, A. R. & Edward, F. B. 2009. Heavy metal concentrations (Cd, Cu, Ni, Pb, Fe and Zn) in different soft tissues and shells of *Pholas orientalis* collected from Sekinchan and Pantai Remis, Selangor. *Malaysian Applied Biology Journal*, **38**(1): 21–27.

Yebra, M. C., Cancela, S. & Cespon, R. M. 2008. Automatic determination of nickel in foods by using atomic absorption spectrometry. *Food Chemistry,* **108** (2): 774-778.

Yonge CM. 1949. On the structure and adaptations of the Tellinacea, deposit-feeding Eulamellibranchia. *Philosophical Transactions of the Royal Society*, **234**: 29-76.

Yu, G.B., Liu, Y., Yu, S., Wu, S.C., Leung, A.O.W., Luo, X.S., Xu, B., Li, H.B. & Wong, M.H. 2011. Inconsistency and comprehensiveness of risk assessments for heavy metals in urban surface sediments. *Chemosphere*, **85**: 1080–1087.