WILD JAVA MEDAKA FISH (Oryzias javanicus Bleeker) AS A TOOL FOR TOXICOLOGICAL TEST OF HEAVY METALS IN ESTUARINE AREAS IN MALAYSIA

DARYOUSH KHODADOUST

FS 2012 65
WILD JAVA MEDAKA FISH (Oryzias javanicus Bleeker) AS A TOOL FOR TOXICOLOGICAL TEST OF HEAVY METALS IN ESTUARINE AREAS IN MALAYSIA

BY

DARYOUSH KHODADOUST

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia In Fulfilment of the Requirements for the Degree of Doctor of Philosophy

May 2012
DEDICATION

To the most patient and understanding person in my life that

I love more than ever Mahnaz
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

WILD JAVA MEDAKA FISH (*Oryzias javanicus* Bleeker) AS A TOOL FOR TOXICOLOGICAL TEST OF HEAVY METALS IN ESTUARINE AREAS IN MALAYSIA

By

DARYOUSH KHODADOUST

May 2012

Chairman: Professor Ahmad Ismail. PhD

Faculty: Science

Pollution of diverse environments is a result of population increase and industrialization in the Coastal areas and estuaries. Biomonitoring offers an appealing tool for the assessment of metal pollution in the aquatic ecosystem and for this reason there is a need for a suitable tool to assess coastal pollution. The overall hypothesis of this dissertation was to introduce *Oryzias javanicus* (Java medaka) fish as a new organism for ecotoxicological studies in the coastal and estuary areas. It was discovered that the Java medaka fish is available in all sizes and of all ages in all year around in the wild. Following the main aim of the research, biological and ecological condition and sex ratio of Java medaka were studied over a year in the Linggi estuary on the west coast of Peninsular Malaysia.
The results showed that the range of measured physico-chemical conditions for the fish to thrive were: salinity (4.93-19.7 ppt), pH (5.6-8.2), temperature (26.1-30.8 °C), and conductivity (80.3-220.6 mScm⁻¹), O₂ (4.3-8.9 mg/l) while the sex ratio was 1:1.6 (male/female). The second objective of this study was to determine the concentration of heavy metals (Cd, Cu, Zn, Fe, Ni and Pb) in different parts of Java medaka fish collected from four sites along the Linggi estuary. The concentration range of heavy metals in different tissues of Java medaka fish varied from 4.41-17.80 µg/g dry weight for Cu, 43.89-78.79 µg/g dry weight for Zn, 7.09-14.79 µg/g dry weight for Pb, 1.96-2.36 µg/g dry weight for Cd, 9.38-14.57 µg/g dry weight for Ni and 93.62-199.17 µg/g dry weight for Fe. The highest concentrations of Pb, Zn, Fe and Cu were found in the gill and the highest concentrations of Cd and Ni were found in the visceral organs; low concentrations of all metals were found in part of the caudal muscle. Sequential extraction technique (SET) was used to evaluate the four fractions (exchangeable, acid-reducible, oxidisable, and residual) in surface sediment of four sites of the Linggi estuary. Relationship of metals between each fraction of sediment and metal concentrations in different parts of fish (especially in the gills) were found between Cd, Fe, Zn and Pb. Therefore, *Oryzias javanicus* could be a useful biomonitoring agent for these metals in the environments like the Linggi estuary.

Acute toxicity of heavy metals (Cu, Zn and Cd) on Java medaka (*Orizyas javanicus*) fish, were studied in the next step based on O.E.C.D method, the LC50-96 h for Cu, Zn and Cd were determined 5.43 (5.32-5.54), 9.75 (9.65-9.85) and 6.02
(5.83-6.21) mg/l for juveniles and 8.64 (8.34-8.94), 14.32 (13.94-14.70) and 6.63 (6.31-6.95) for adults respectively.

In this study induction of metallothionein (MT) and levels of cadmium and zinc of the Java medaka fish were studied after long time (60 days) exposed of juvenile fishes to different concentrations of cadmium and zinc. Results showed statistically significant differences in Cd and Zn and MT’s content in different organs of fish groups exposed to those two metals were found between control group and other groups with different concentrations of metals (p<0.05). Correlation between Cd content and Mt’s in all body sections of Java medaka fish were statistically significant and the correlation was positive; increasing the Cd content in body sections, the Mt’s levels increased also (p<0.01). For Zn all body parts and Zn content significantly correlated (p<0.01) with r values ranging from 0.7343-0.969 whereas liver-MT and muscle-MT were significantly correlated (p<0.05) with value range 0.663. For metallothionein to be an effective biomarker, metal concentrations must be high enough to induce synthesis of the protein.

Results of this study indicate that Java medaka fish is more useful and accurate to monitor particular hazardous chemicals and ecotoxicology studies in the estuary and coastal areas. Mapping the geographic area of Java medaka in Malaysia and Java region, genetic researches and study about forbidden chemicals effects such as TBT, recommended for future studies about this fish.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

LIAR JAWA MEDAKA IKAN (Oryzias javanicus Bleeker) SEBAGAI ALAT UNTUK UJIAN TOKSIKOLOGI LOGAM BERAT DI KAWASAN MUARA DI MALAYSIA

Oleh

DARYOUSH KHODADOUST

Mei 2012

Pengerusi: Prof Ahmad Ismail. PhD

Fakulti: Sains

Pencemaran persekitaran yang pelbagai adalah disebabkan oleh pertambahan penduduk dan perindustrian di kawasan muara dan Pantai. Biomonitor menawarkan satu alat yang menarik untuk penilaian pencemaran logam dalam ekosistem akuatik dan atas sebab ini terdapat keperluan untuk alat yang sesuai untuk menilai pencemaran pantai. Hipotesis keseluruhan disertasi ini adalah untuk memperkenalkan Oryzias javanicus (Java medaka) ikan sebagai organisma yang baru untuk kajian ecotoxicological di kawasan laut dan muara. Ia telah ditemui bahawa ikan Jawa medaka boleh didapati dalam semua saiz dan semua peringkat umur di sepanjang tahun di dalam hutan. Berikutkan tujuan utama penyelidikan, keadaan biologi dan ekologi dan nisbah jantina Jawa medaka dikaji lebih setahun

vi
dalam muara Linggi di pantai barat Semenanjung Malaysia. Keputusan menunjukkan bahawa pelbagai keadaan psiko-kimia yang diukur untuk ikan untuk berkembang maju adalah: kemasinan (4,93-19,7 ppt), pH (5,6-8,2), suhu (26,1-30,8 ° C), dan konduktiviti (80,3-220,6 mScm⁻¹), O₂ (4,3-8,9 mg / l) manakala nisbah jantina adalah 1:1.6 (lelaki / wanita). Objektif kedua kajian ini adalah untuk menentukan kepekatan logam berat (Cd, Cu, Zn, Fe, Ni dan Pb) dalam bahagian-bahagian yang berlainan ikan medaka Jawa yang dikutip dari empat tapak di sepanjang muara Linggi. Julat kepekatan logam berat dalam tisu yang berlainan ikan medaka Jawa berbeza dari 4,41-17,80 μg / g berat kering bagi logam Cu, 43,89-78,79 μg / g berat kering bagi Zn, 7,09-14,79 μg / g berat kering bagi Pb, 1,96 2,36 μg / g berat kering untuk Cd, 9,38-14,57 μg / g berat kering bagi Ni dan 93,62-199,17 μg / g berat kering bagi Fe. Kepekatan tertinggi Pb, Zn, Fe dan Cu ditemui di insang dan kepekatan tertinggi Cd dan Ni ditemui dalam organ dalaman; kepekatan rendah semua logam yang ditemui di sebahagian otot ekor. Teknik pengekstrakan berjujukan (SET) telah digunakan untuk menilai empat pecahan (boleh ditukar ganti, asid dikurangkan, oxidisable, dan sisa) dalam sedimen permukaan empat tapak muara Linggi. Hubungan logam antara pecahan setiap sedimen dan kepekatan logam di bahagian-bahagian yang berlainan ikan (terutamanya dalam insang) telah didapati antara Cd, Fe, Zn dan Pb. Oleh itu, *Oryzias javanicus* boleh menjadi ejen 'biomonitor' yang berguna untuk logam ini dalam persekitaran seperti muara Linggi.
Ketoksikan akut logam berat (Cu, Zn dan Cd) pada ikan Jawa medaka (*Orizyas javanicus*), telah dikaji dalam langkah seterusnya berdasarkan kaedah OECD, h LC50-96 bagi logam Cu, Zn dan Cd telah ditentukan 5,43 (5,32-5,54 ), 9,75 (9,65-9,85) dan 6,02 (5,83-6,21) mg / l untuk juvana dan 8,64 (8,34-8,94), 14,32 (13,94-14,70) dan 6,63 (6,31-6,95) untuk orang dewasamasing-masing.

Dalam induksi kajian ini metallothionein (MT) dan tahap kadmium dan zink ikan medaka Jawa dikaji selepas masa yang lama (60 hari) yang terdedah ikan juvenil yang berbeza kepekatan kadmium dan zink. Hasil kajian menunjukkan perbezaan statistik yang signifikan di Cd dan Zn dan kandungan MT dalam organ-organ yang berlainan kumpulan ikan yang terdedah kepada kedua-dua logam didapati di antara kumpulan kawalan dan kumpulan lain dengan kepekatan logam yang berlainan (p <0.05). Korelasi antara kandungan Cd dan Gunung di semua bahagian badan ikan medaka Jawa statistik yang signifikan dan korelasi positif; meningkatkan kandungan Cd dalam bahagian badan, tahap Gunung meningkat juga (p <0.01).

Bagi Zn semua bahagian badan dan kandungan Zn mempunyai hubungan yang signifikan (p <0.01) dengan nilai r antara dari 0.7343-0.969 manakala hati-MT dan otot-MT mempunyai hubungan yang signifikan (p <0.05) dengan pelbagai nilai 0,663. Untuk metallothionein untuk menjadi penanda bio yang berkesan, kepekatan logam mestilah cukup tinggi untuk merangsang sintesis protein.
Keputusan kajian ini menunjukkan bahawa Jawa medaka ikan adalah lebih berguna dan tepat untuk memantau bahan kimia berbahaya tertentu dan kajian Ekotoksikologi di kawasan muara dan pantai. Pemetaan kawasan geografi Jawa medaka di Malaysia dan rantau Jawa, kajian genetik dan kajian mengenai kesan bahan kimia yang dilarang seperti TBT, disyorkan untuk kajian masa hadapan kira-kira ikan ini.
ACKNOWLEDGEMENTS

I would like to thank my advisor, Prof. Ahmad Ismail and members of my supervisory committee Assoc. Prof. Dr. Nor Azwady Abd. Aziz and Dr. Syaizwan Zahmir Zulkifli for all their help. Without them this study would not be possible.

I would like to thank my wife Mahnaz Homauni and my family for all their support and understanding.

I would also like to thank all the members of Ecology Laboratory at the Department of Biology for their warm friendship.
APPROVAL

I certify that an Examination Committee has met on May 2012 to conduct the final examination of Daryoush Khodadoust on his Doctor of Philosophy thesis entitled “wild java medaka (Oryzias javanicus) as a tool for toxicological test for heavy metals in the estuarine areas in Malaysia” in accordance with University Pertanian Malaysia (Higher degree) Act 1980 and University Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy Degree. Members of the Examination Committee were as follows:

Assoc.Prof. Dr. Muskhazli Mustafa
Faculty of Science
University Putra Malaysia
(Chairman)

Assoc.Prof. Dr. Takaomi Arai
Faculty of Science
University Putra Malaysia
(Internal Examiner)

Dr. Hishamuddin Omar
Faculty of Science
University Putra Malaysia
(Internal Examiner)

Prof. Dr. Jiro Koyama
Faculty of Fishries
Kagoshima University Japan
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:
This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The Members of the Supervisory Committee were as follows:

**Ahmad Ismail, PhD**  
Professor  
Faculty of Science  
University Putra Malaysia  
(Chairman)

**Nor Azwady Abd Aziz, PhD**  
Associate Professor  
Faculty of Science  
University Putra Malaysia  
(Member)

**Syaizwan Zahmir Zulkifili, PhD**  
Lecturer  
Faculty of Science  
University Putra Malaysia  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institutions.

DARYOUSH KHODADOUST

Date: 15-Aug-2012
# TABLE OF CONTENT

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>iii</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>x</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xx</td>
</tr>
<tr>
<td>LIST OF ABBREVIATION</td>
<td>xxii</td>
</tr>
</tbody>
</table>

## CHAPTER

1 **INTRODUCTION**

1.1 General  
1.2 Research objectives

2 **LITERATURE REVIEW**

2.1 Marine organisms and biological agents as indicator of heavy metals pollution  
2.2 Medaka Fish, a New Test Organism for Freshwater, Brackish Water, and Sea water  
   2.2.1 Species of Oryzias  
   2.2.2 Java medaka (*Oryzias javanicus*)  
   2.2.3 Classification of medaka  
2.3 Heavy metals and Toxicity  
2.4 Coastal areas and Heavy metal pollution  
   2.4.1 Heavy metals in marine environment and organisms  
2.5 Bioaccumulation of Heavy Metals in Marine Organisms  
   2.5.1 Cadmium (Cd)  
   2.5.2 Zink (Zn)  
   2.5.3 Lead (Pb)  
   2.5.4 Nickel (Ni)  
   2.5.5 Copper (Cu)  
   2.5.6 Iron (Fe)  
2.6 Metallothionein in ecotoxicology study  
   2.6.1 Metallothionein Structure  
   2.6.2 Factors affecting metallothionein concentration in marine animals  
   2.6.3 Metal binding  
   2.6.4 Control of oxidative stress  
2.7 Biomarkers  
2.8 Estuaries Area
3 ASSESSMENT OF THE AVAILABILITY OF JAVA MEDAKA (Oryzias javanicus) IN COASTAL AREAS

3.1 Introduction 37
3.2 Java medaka 39
  3.2.1 Linggi River-Estuary 40
3.3 Materials and Method 44
  3.3.1 Sampling method 44
  3.3.2 Distinguishing females from males 47
3.4 Results and Discussion 49
  3.4.1 Linggi Estuary water physicochemical parameters and medaka fish tolerant 49
  3.4.2 Sex ratio of Java medaka fish in Linggi estuary 52
  3.4.3 Availability of all sizes (ages) of Java medaka fish around year in Linggi estuary 56
3.5 Conclusion 62

4 ASSESSMENT OF HEAVY METALS LEVELS IN THE MEDAKA FISH (Oryzias javanicus) AND SURFACE EDIMENTS COLLECTED FROM LINGGI ESTUARY IN THE WEST COAST OF PENINSULAR MALAYSIA

4.1 Introduction 64
4.2 Materials and Method 68
  4.2.1 Study area and method of sediment and Java medaka (O. javanicus) sampling 68
  4.2.2 Medaka fish tissues digestion 69
  4.2.3 Sediments preparation and digestion 69
  4.2.4 Speciation of Cd, Cu, Zn, Pb, Fe and Ni of sediment samples 70
  4.2.5 Ecological parameters 71
  4.2.6 Quality control 72
  4.2.7 Blank procedure 72
  4.2.8 Recovery test 73
  4.2.9 Statistical analysis 74
4.3 Results and Discussion 75
  4.3.1 Heavy metal concentrations of intertidal sediments of Linggi estuary 75
  4.3.2 Total Concentration of Cd, Cu, Fe, Ni, Pb and Zn 99
  4.3.3 Heavy metal concentrations in the different tissues of Java medaka fish from four different sampling locations in the three times of year 104
  4.3.4 Relationship between different speciation of heavy metal and their accumulation in different organs of O. javanicus. 112
  4.3.5 Heavy metal concentration in Linggi estuary 121
water

4.4 Conclusion 129

5 ACUTE TOXICITY TEST OF HEAVY METALS (ZN, CD AND CU) ON JAVA MEDAKA (Oryzias javanicus) FISH AS AN ESTUARY POLLUTION BIOINDICATOR

5.1 Introduction 132
5.2 Materials and Method 135
  5.2.1 Solution preparation 135
  5.2.2 Toxicity Tests 135
  5.2.3 Record and analysis of results 138
5.3 Results and Discussion 141
5.4 Conclusion 150

6 BIOACCUMULATION OF Cd AND Zn IN JAVA MEDAKA FISH (Oryzias javanicus) AND IDENTIFYING OF METALLOTHIONEIN-LIKE PROTEIN

6.1 Introduction 152
6.2 Materials and Method 155
  6.2.1 Animal treatment 155
  6.2.2 Cadmium and zinc analysis 157
  6.2.3 Metallothionein determination 157
  6.2.4 Statistical analysis 158
6.3 Result and Discussion 158
  6.3.1 Comparison of Cd, Zn and MT levels in Java medaka fish tissues exposed to different concentration of Cd and Zn 158
  6.3.2 Correlation between cadmium and zinc content with MTs levels 171
6.4 Conclusion 179

7 GENERAL DISCUSSION

7.1 Fish as Bioindicator and Biomonitoring tool 181
7.2 Heavy metals and estuary pollution monitoring 184
7.3 Recommendation for future researches 188

REFERENCES 189
BIODATA OF STUDENT 215
LIST OF PUBLICATIONS 216