



UNIVERSITI PUTRA MALAYSIA

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

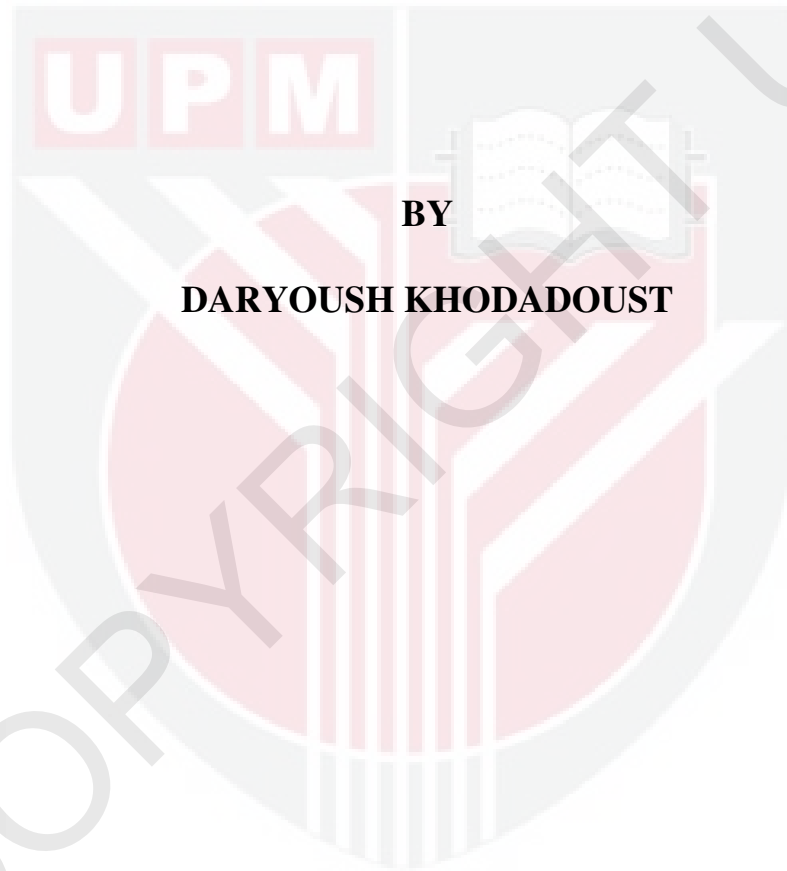
DARYOUSH KHODADOUST

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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

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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 (*Oryzias 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

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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. Berikutan tujuan utama penyelidikan, keadaan biologi dan ekologi dan nisbah jantina Jawa medaka dikaji lebih setahun

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 mScm¹) , 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.



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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:

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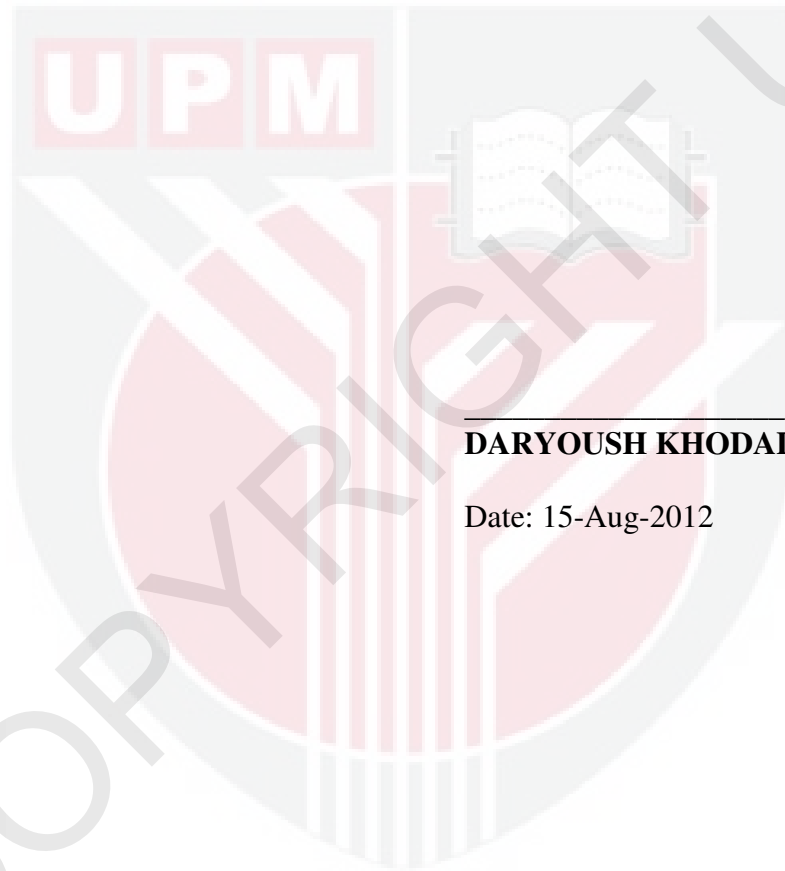
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DECLARATION

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

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xvii
LIST OF FIGURES	xx
LIST OF ABBREVIATION	xxii
CHAPTER	
1 INTRODUCTION	1
1.1 General	1
1.2 Research objectives	4
2 LITERATURE REVIEW	6
2.1 Marine organisms and biological agents as indicator of heavy metals pollution	6
2.2 Medaka Fish, a New Test Organism for Freshwater, Brackish Water, and Sea water	7
2.2.1 Species of <i>Oryzias</i>	8
2.2.2 Java medaka (<i>Oryzias javanicus</i>)	10
2.2.3 Classification of medaka	11
2.3 Heavy metals and Toxicity	12
2.4 Coastal areas and Heavy metal pollution	13
2.4.1 Heavy metals in marine environment and organisms	15
2.5 Bioaccumulation of Heavy Metals in Marine Organisms	16
2.5.1 Cadmium (Cd)	18
2.5.2 Zink (Zn)	19
2.5.3 Lead (Pb)	19
2.5.4 Nickel (Ni)	21
2.5.5 Copper (Cu)	21
2.5.6 Iron (Fe)	22
2.6 Metallothionein in ecotoxicology study	23
2.6.1 Metallothionein Structure	23
2.6.2 Factors affecting metallothionein concentration in marine animals	24
2.6.3 Metal binding	27
2.6.4 Control of oxidative stress	28
2.7 Biomarkers	30
2.8 Estuaries Area	33

2.9	Coastal areas in Peninsular Malaysia	35
3	ASSESSMENT OF THE AVAILABILITY OF JAVA MEDAKA (<i>Oryzias javanicus</i>) IN COASTAL AREAS	37
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 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 (<i>Oryzias javanicus</i>) AND SURFACE SEDIMENTS COLLECTED FROM LINGGI ESTUARY IN THE WEST COAST OF PENINSULAR MALAYSIA	64
4.1	Introduction	64
4.2	Materials and Method	68
4.2.1	Study area and method of sediment and Java medaka (<i>O. javanicus</i>) 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 <i>O. javanicus</i> .	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 (<i>Oryzias javanicus</i>) FISH AS AN ESTUARY POLLUTION BIOINDICATOR	132
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 (<i>Oryzias javanicus</i>) AND IDENTIFYING OF METALLOTHIONEIN-LIKE PROTEIN	152
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	181
7.1	Fish as Bioindicator and Biomonitoring tool	181
7.2	Heavy metals and estuary pollution monitoring	184
7.3	Recommendation for future reseachs	188
	REFERENCES	189
	BIODATA OF STUDENT	215
	LIST OF PUBLICATIONS	216