UNIVERSITI PUTRA MALAYSIA

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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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

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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 ºC), and conductivity (80.3-220.6 $mScm^{-1}$), O_2 (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

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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 º 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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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:

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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.

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DARYOUSH KHODADOUST

Date: 15-Aug-2012

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