DNA CHARACTERIZATION OF NORMAL AND DAMAGED GILLS FOLLOWING HEAVY METAL EXPOSURE

FACULTY OF SCIENCE AND TECHNOLOGY
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
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By

Vijayendran Govindasamy

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FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
(KUSTEM)

PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk :

DNA Characterization of Normal and Damaged Gills Following Heavy Metal Exposure oleh **Vijayendran Govindasamy, No Matrik : UK7228** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Samudera sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains- Biologi Marin, Fakulti Sains and Teknologi , Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: PROF MADYA DR. KAMARUZZAMAN YUNUS Tarikh:

Cop Rasmi: *Timbalan Pengarah*
Institut Oseanografi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu

Penyelia Kedua

Nama: PROF. MADYA DR. MOHD EFFENDY ABD. WAHID Tarikh:

Cop Rasmi: *Ketua*
Pusat Pembangunan dan Kebajikan Pelajar
Bahagian Hal Ehwal Pelajar
Kolej Universiti Sains Dan Teknologi Malaysia
Mengabang Telipot, 21030 K. Terengganu.

Ketua Jabatan Sains Samudera

Nama: DR. AHMAD SHAMSUDDIN B. AHMAD Tarikh: 29/3/05

Cop Rasmi: Ketua
Jabatan Sains Samudera
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu

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ABSTRACT

The ultimate aim of this study is to predict how genomic of aquatic organisms respond to heavy metal exposure. Nile/ Red tilapia fingerlings siblings (*Oroechromis niloticus)* (2.5 cm ± 0.5) were exposed to lead (Pb^{2+}). The study was conducted into 3 parts whereby each part is interrelated. The first part was the lead acute toxicity test. Using probit method (computer program), the 96–h LC_{50} value for tilapia fingerlings was 2.7313 ppm. This value was then used to design a sub-lethal concentration (2.7313 ppm, 2.0484 ppm, 1.3656 ppm, 0.6828 ppm and 0 ppm), by which the fishes were exposed to a period of 30 days. At the end of each 10 days, 3 fishes were killed. The changes of the genomic of tilapia `s gills was detected by using the RAPD-PCR technique. Heavy metal analysis (open acid digestion technique and ICP-OES) was conducted to determine the accumulation of lead in the dry weight of the gills. No significant changes occurred in the genomic of the gills after 10 days of exposure to Pb^{2+} in all of the exposure concentration.17 μgmg^{-1} to 50 μgmg^{-1} of lead accumulation was found from the lowest to the highest concentration. After 20 days of exposure to Pb^{2+}, 2 stable bands (range 600-800 bp) from fishes exposed at 2.7313 ppm have disappeared; with 52μgmg^{-1} of Pb^{2+} have been accumulated in the dry tissues of gills. However, there is not much difference in terms of the number of bands and lead accumulation in other concentration. After 30 days, 2 bands (range 600-800 bp) disappeared in fish exposed at 2.7313 ppm, and 1 band (1200 bp) disappeared at 1.3656 ppm. Again in 2.7313 ppm, clusters of bands appeared at

the range of 600-900 bp in the genomic of the tilapia. A new band was merged at range the 400-500 bp in fish exposed at 2.0484 ppm. The accumulation of lead was 22.8 μgmg^{-1} at 1.3656 ppm, 53.6 μgmg^{-1} at 2.0484 ppm and 142.1 μgmg^{-1} at 2.7313 ppm. As a conclusion, the genomic of the tilapia gills started to show changes with the accumulation of Pb^{2+} as low as 52 μgmg^{-1} with continuous exposure to at least 2.0484 ppm.

ABSTRAK

Ciri-ciri DNA bagi insang normal and rosak selepas pendedahan dengan logam berat

Tujuan utama kajian ini adalah untuk meramal perubahan genomik organisma aquatik terhadap pendedahan kepada logam berat.Anak ikan tilapia Nile/ merah (*Oroechromis niloticus*) (2.5 cm ± 0.5) telah didedahkan kepada Plumbum (Pb^{2+}). Kajian ini telah dijalankan dalam tiga peringkat yang saling berkaitan di antaranya. Bahagian pertama ialah ujian penentuan ketoksikan akut Plumbum. Dengan menggunakan keadah probit, 96–h LC_{50} Pb^{2+} adalah 2.7313 ppm. Nilai ini kemudian digunakan mereka 5 jenis kepekatan logam Pb^{2+} (2.7313 ppm, 2.0484 ppm,1.3656 ppm, 0.6828 ppm dan 0 ppm) yang mana anak ikan didedahkan selama 30 hari. 3 ikan dibunuh pada setiap 10 hari. Perubahan pada genomic insang tilapia dikesan dengan menggunkan kaedah RAPD-PCR. Analisis logam berat (keadah asid terbuka dan ICP-OES) juga dijalankan untuk menentukan akumulasi Pb^{2+} pada tisu insang yang kering. Tidak ada sebarang perubahan yang dapat dilihat pada genomik insang tilapia selepas 10 hari didedahkan kepada semua kepekatan Pb^{2+} yang diperkenalkan.17 μgmg^{-1} hingga 50 μgmg^{-1} berat kering akumulasi Pb^{2+} telah dijumpai dalam ikan yang didedahkan dari kepekatan rendah kepada yang tertinggi. Selepas 20 hari didedahkan kepada Pb^{2+}, 2 jalur stabil DNA (jarak dari 600-800 bp) telah hilang pada ikan-ikan yang didedahkan pada kepekatan 2.7313 ppm dengan 52 μgmg^{-1} akumulasi Pb^{2+} pada tisu kering insang. Namun begitu, tiada sebarang perubahan jelas didapati dalam ikan –ikan yang didedahkan pada kepekatan yang lain. Selepas 30

hari, didedahkan kepada Pb^{2+}, 2 jalur DNA (jarak 600-800 bp) hilang pada ikan yang didedahkan pada kepekatan 2.7313 ppm dan 1 jalur DNA (1200 bp) hilang pada kepekatan 1.3656 ppm. Didapati juga dalam kepekatan 2.7313 ppm, terdapat kelompok DNA wujud pada jarak 600-900 bp. Terdapat satu jalur DNA baru (jarak 400-500 bp) wujud pada kepekatan 2.0484 ppm. Akumulasi Pb^{2+} adalah 22.8 μgmg^{-1} pada kepekatan Pb^{2+} 1.3656 ppm, 53.6 μgmg^{-1} pada kepekatan Pb^{2+} 2.0484 ppm dan 142.1 μgmg^{-1} pada kepekatan Pb^{2+} 2.7313.Kesimpulannya, perubahan genomik pada insang tilapia bermula apabila akumulasi adalah 52 μgmg^{-1} dan ikan tersebut didedahkan kepada kepekatan 2.0484 ppm untuk janga masa yang panjang.