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DNA CHARACTERIZATION OF NORMAL AND DAMAGE GILLS FOLLOWING
ZINC EXPOSURE

By
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This project report is submitted in partial fulfillments of the requirement for the degree of
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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk :

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2.1.1 Red Tilapia Fingerlings (*Orechromis niloticus*)

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LIST OF ABBREVIATIONS

bp	base per pair
cm	centimeter
dw	dry weight
gm	gram
HCl	Hydrochloric Acid
HNO ₃	Nitric Acid
H ₂ O ₂	Hydrogen Peroxide
H ₂ SO ₄	Sulfuric Acid
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
L	Liter
mg	miligram
mgL ⁻¹	milligram per liter
PCR	Polymerase Chain Reaction
RAPD-PCR	Randomly Amplified Polymerase Chain Reaction
ppm	parts per million / mgL ⁻¹
ppb	parts per billion / µgL ⁻¹
µgL ⁻¹	microgram per liter
µg	microgram
Zn	Zinc
Zn ²⁺	Zinc in ionic form

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ABSTRACT

The aim of this study is to predict how genomic of Nile/Red Tilapia (3.0cm - 4.0 cm) of normal and exposed respond to heavy metal (zinc) exposure. To complete this study, three parts of experiment were done whereby which each part are related to each other. The first part is to determine the 96-h LC₅₀ value for tilapia fingerlings using the Probit Method (computer programme) and the value was 5.91ppm. This value then was used to design a series of sub-lethal concentration which will be used in the next part (uptake and accumulation). The concentrations are 0 ppm 5.91 ppm, 4.43 ppm, 2.95 ppm and 1.48 ppm derived from 0%, 25%, 50% and 75% of the 96h LC₅₀ value. At this part, fishes were killed every 7 days and the duration of experiment was 21 days. Genomic changes of the death fish will be amplified using RAPD-PCR method and concentration in this tissue will be analysis by using ICP-MS. From the result, it shows that tilapia fingerlings can take up to 3317.713 μg^{-1} of Zn accumulation from 5.91 ppm of concentration and DNA study reveals no significant changes on the genomic at 7 days exposed to Zn. After 14 days exposed to the Zn, one band (900bp) was disappeared at the highest concentration (5.91ppm) with 481.853 μg^{-1} of Zn have been accumulated in the dry tissue of gills. For the fingerlings that were exposed to the other concentration, there were no significant changes. There was one band disappeared at the 5.91 ppm and one band at 4.43 ppm during 21 days exposed (900bp). The accumulation of Zn at 4.43 ppm during 21 days exposed are 1600.353 μg^{-1}

ABSTRAK

Ciri-ciri DNA Bagi Insang Normal dan Rosak selepas Pendedahan dengan Logam Berat Zink

Tujuan kajian adalah untuk meramal perubahan genetik organisma akuatik ikan Tilapia Merah (3.0cm-4.0cm) yang normal dan yang telah didedahkan kepada logam berat Zink. Bagi menyempurnakan kajian ini, tiga peringkat eksperimen telah dijalankan dimana ianya berkaitan diantara satu peringkat dengan yang lain. Bahagian pertama ialah ujian penentuan ketoksiikan akut Zn Nilai 96-h LC₅₀ yang telah diperolehi dengan menggunakan kaedah Probit-Method berkomputer ialah 5.91 ppm. Nilai ini kemudian digunakan untuk menghasilkan beberapa siri kepekatan yang akan digunakan dalam bahagian yang seterusnya iaitu ujian pengambilan dan pengumpulan zink dimana kepekatan yang digunakan ialah 0 ppm, 5.91 ppm, 4.43 ppm, 2.95 ppm dan 1.48 ppm setelah dikurangkan 0%, 25%, 50%, 75% daripada nilai 96-h LC₅₀. Pada bahagian ini ikan dibunuhi setiap 7 hari dan ujian memakan masa selama 21 hari. Ikan yang telah mati diambil untuk memerhatikan perubahan genomik insang ikan Tilapia dengan menggunakan kaedah RAPD- PCR dan juga untuk menganalisa logam berat Zn untuk menentukan akumulasi Zn pada tisu ikan yang kering dengan menggunakan ICP-MS Didapati tisu ikan yang kering boleh mengumpul logam Zn sehingga sebanyak 3317.713 µg⁻¹ berat kering yang didedahkan pada kepekatan 5.91 ppm. Berdasarkan ujian yang telah dilakukan, tidak ada

perubahan genomic pada insang yang didedahkan pada hari ketujuh pada semua kepekatan Zn. Selepas 14 hari didedahkan kepada Zn, terdapat satu jalur stabil DNA (900bp) telah hilang pada kepekatan 5.91 ppm iaitu kepekatan yang tertinggi dengan $481.853 \mu\text{g}^{-1}$ akumulasi Zn pada tisu kering insang.Pada ikan yang didedahkan pada kepekatan yang lain,tiada sebarang perubahan genomik yang jelas didapati.Terdapat satu jalur yang hilang pada kepekatan 5.91 ppm (900bp) dan satu jalur juga hilang pada kepekatan 4.43 ppm (900bp) pada hari ke 21.Akumulasi Zn adalah $1600.353 \mu\text{g}^{-1}$ pada kepekatan 4.43 ppm pada hari ke 21 ini.