[illegible] OF CADMIUM IN [illegible] (CHEOCHROMIS NILOTICUS)

PREM KUMAR

FACULTY OF MARINE STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2007

In-vitro genotoxic effects of cadmium in tilapia fingerlings (Oreochromis niloticus) / Prem Kumar.

IN-VITRO GENOTOXIC EFFECT OF CADMIUM IN TILAPIA FINGERLINGS (*OREOCHROMIS NILOTICUS*)

By

Prem Kumar

Research Report submitted in partial fulfillment of
The requirements for the degree of
Bachelor of Science (Marine Science)

Department of Marine Science

Faculty of Maritime M and Marine Science

UNIVERSITI MALAYSIA TERENGGANU

2007

JABATAN SAINS MARIN
FAKULTI PENGAJIAN MARITIM DAN SAINS MARIN
UNIVERSITI MALAYSIA TERENGGANU

PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

In-Vitro Genotoxic Effect of Cadmium in Tilapia Fingerlings (*Oreochromis nioticus*) oleh **Prem Kumar**, no.matrik:**UK10616** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Marin sebagai memenuhi sebahagian daripada keperluan memperolehi **Ijazah Sarjana Muda Sains (Sains Samudera)** Fakulti Pengurusan Maritim dan Sains Marin, Universiti Malaysia Terengganu.

Disahkan oleh:

.....

Penyelia Utama

Nama: PROF MADYA DR. MOHD. EFFENDY ABD WAHID

Cop Rasmi: Pengarah, Institut Bioteknologi Marin, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu.

Tarikh: May 6, 2007

.....

Penyelia Kedua (jika ada)

Nama:

Cop Rasmi

Tarikh: .....

.....

Ketua Jabatan Sains Marin

Nama: DR. RAZAK ZAKARIYA

Cop Rasmi: Ketua Jabatan Sains Marin, Fakulti Pengajian Maritim dan Sains Marin, Universiti Malaysia Terengganu (UMT)

Tarikh: 2/3/08

ACKNOWLEDGEMENT

Supreme thanks to God for permitting me to successfully complete this thesis. My warmest gratitude also goes to Assoc. Prof. Dr. Effendy Abdul Wahid for his advice and precious suggestions fort the completion of this research. Also, my special thanks to Dr. Nor Antonina Abdullah for her kind support and encouragement that I receive from her during my research.

Deepest sincere gratitude goes to Mr. Vijayendran Govindasamy for his guidance, advices and full time monitoring and provided valuable information throughout the completion of this research. I would also like take this opportunity to thanks Miss Harmeeta Kaur for helping me from the beginning till the end of this research. Thanks to science officers in INOS for their advices.

Not forgetting my mother Mrs. Saraswathy for her boundless support and encouragement which kept me motivated. In a nutshell, thanks to everyone who were involved directly or indirectly in the achievement of this research.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVATIONS/SYMBOLS

ICP-MS	-	Inductively Coupled Plasma – Mass Spectrometry
Cd	-	Cadmium
Cd^{2+}	-	Cadmium in ionic form
ppb	-	parts per billion equivalent to ug L^{-1}
ppm	-	parts per million equivalent to mg L^{-1}
HNO_3	-	Nitric Acid
H_2SO^4	-	Sulfuric Acid
HCI	-	Hydrochloric acid
H_2O_2	-	Hydrogen Peroxide
Mg L^{-1}	-	milligram per liter
Ug L^{-1}	-	microgram per liter
L	-	Liter
PCR	-	Polymerase Chain Reaction
RAPD	-	Random Amplified Polymorphism DNA
mg	-	milligram
cm	-	centimeter

ABSTRACT

The ultimate aim of this study is to determine the genotoxic effect which takes place in aquatic organisms following exposure of heavy metal. *O. niloticus* fingerlings (2.5 cm ± 0.5) was exposed for a period of 21 days to various sub-lethal Cadmium concentrations (0.4683 ppm, 0.9366 ppm, 1.8552 ppm and 2.8098 ppm), designed from 96-h LC_{50} value (4.688 ppm) which was obtained from 96 hours acute toxicity test. The exposed fingerlings were harvested at each 7days for determination of Cadmium concentration in different body parts as well as determination of Cadmium induced genotoxic effect on fingerlings. Detection through ICP-MS indicated that significant mean difference for cadmium concentrations were found in gills, muscle and viscera only for exposure concentrations 1.8552 ppm and 2.8098 ppm when compared to control at all time intervals. However significant differences were found in whole body of every fingerling treated in all the exposure concentrations at all time intervals. Fluctuating pattern of Cadmium concentration which was found in all parts studied with increasing concentrations at various time intervals could be attributed to varying bioavailability as well other factors of temperature, size and physiological response towards heavy metal between individuals. The ICP-MS detection also indicated that Cadmium accumulated the most in muscle tissues, followed by viscera, gills and last, whole body for all time intervals. RAPD fingerprinting of *O.nilotiucs* fingerlings revealed appearance/disappearance of stable bands (400bp in OPA 9 and 900bp, 700bp in OPB 8) and changes in band intensity among samples treated with various concentrations at various time intervals, indicating damage had occurred at genomic levels. The Genomic

DNA template stability analysis also showed that significant decrease in genomic stability had occurred in all samples tested with OPB 1, OPA9, OPA 16 and OPB 8. Dendogram analysis meanwhile showed that genetic diversity occurred to some extent between all samples tested with primers mentioned. Comet assay had also revealed that significant Strand Breakage (assessed through analysis of mean comet tail length) occurred in all samples treated with various Cadmium concentrations. However, DNA repairing was also found occurring following exposure to the lowest sub-lethal concentration (0.4688 pm), with increasing time intervals. The present study concludes that Cadmium accumulates in *O.niloticus* at some significant level in various parts and induces genotocic effect as well in aquatic organisms.

KESAN GENOTOKSIK CADMIUM TERHADAP ANAK IKAN TILAPIA (*Oreochromis niloticus*)

ABSTRAK

Tujuan utama kajian ini adalah untuk menentukan kesan genotoksik terhadap organisma akuatik akibat pendedahan kepada logam berat. Anak ikan *O.niloticus* berukuran (2.5 cm ± 0.5) didedahkan selama 21 hari kepada beberapa kepekatan sub-lethal yang berbeza (0.4683 ppm, 0.9366 ppm, 1.8552 ppm and 2.8098 ppm) yang direka berdasarkan dari nilai 96-h LC_{50} yang diperolehi dari 96 hours ujian toksik akut. Anak-anak ikan yang didedahkan kepada Cadmium akan diambil seminggu sekali untuk mengaji kandungann Cadmium di dalam pelbagai bahagian ikan serta mengaji kesan genotoksik yang diakibatkan oleh logam berat Cadmium terhadap ikan tilapia. Melalui ICP-MS, adalah didapati bahawa terdapat perbezaan yang signifikan dalam kandungan cadmim di dalam insang, otot dan bahagian dalam perut hanya ditemui pada kepekatan dedahan 1.8552 ppm and 2.8098 ppm bila dibandingkan dengan kawalan. Walaubagaimanapun, kepekatan cadmium dalam badan menunjukkan perbezaan ang signifikan untuk semua kepekatan dedahan pada setiap masa. Bioavailability dikatakan sebagai punca utama kepada trend kandungan cadmium yang naik/turun di dalam semua bahagaian yang dikaji, selain factor lain seperti suhu, saiz dan respon fisiologi yang berbeza antara individual. Melalui ICP-MS juga diketahui bahawa cadmium berkumpul paling banyak di dalam tisu, diikuti bahagian dalaman perut, insang dan akhirny badan. Profil RAPD O.niloticus yang didedahkan kepada cadmium mendedahkan bahawa

kemunculan/kehilangan jalur stabil (400bp untuk OPA 9 dan 900bp, 700bp untuk OPB 8) dan perbezaan dalam kecerahan jalur berlaku. Ini menunjukkan bahawa terdapat kesan genotoksi berlaku pada peringkat genomic. Stabiliti templat DNA juga menunjukkan penurunan yang signifikan untuk semua sample yang diuji dengan primer OPB 1, OPA 9, OPA 16 dan OPB 8. Analisa dendogram juga menunjukkan bahawa diversity genetic berlaku antara pada takat tertentu dalam populasi yang didedahkan kepada cadmium. Comet assay turut mendedahkan berlakunya pemecahan jalur DNA yang signifikan (dinilai berdasatkan min pnjang ekor comet) berlaku di dalam semua sample yand diuji dengan cadmium. Walaubagaimanapun, pembaikpulihan DNA ditemui berlaku pada kepekatan pendedahan terkecil (0.4688 ppm), dengan pertambahan masa. Kajian ini menyimpulkan bahawa cadmium berkumpul di dalam O.niloticus pada taket yang signifikan di dalam pelbagai bahagian dan menyebabkan kerosakan genotoxic terhadap organisma akuatik.