



C/N 5223

1100054374

Perpustakaan Sultanah Nur Zahirah (UMT)  
Universiti Malaysia Terengganu



LP 46 FMSM 2 2007



1100054374  
In-vitro genotoxic effects of cadmium in tilapia fingerlings  
(Oreochromis niloticus) / Prem Kumar.

PERPUSTAKAAN SULTANAH NUR ZAHIRAH  
UNIVERSITI MALAYSIA TERENGGANU (UMT)  
21030 KUALA TERENGGANU

1100054374		

Lihat sebelah

HAK MILIK  
PERPUSTAKAAN SULTANAH NUR ZAHIRAH UMT

**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**

**1100054374**



**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 memperoleh **Ijazah Sarjana Muda Sains (Sains Samudera)** Fakulti Pengurusan Maritim dan Sains Marin, Universiti Malaysia Terengganu.

Disahkan oleh:

Penyelia Utama

**PROF. MADYA DR. MOHD. EFFENDY ABD WAHID**  
Pegawai  
Institut Bioteknologi Marin  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu, Terengganu.

Nama:

Cop Rasmi:

Tarikh: May 6, 2007

.....  
Penyelia Kedua (jika ada)

Nama:

Cop Rasmi

Tarikh: .....

.....  
Ketua Jabatan Sains Marin

**DR. RAZAK ZAKARIYA**  
Ketua Jabatan Sains Marin  
Fakulti Pengajian Maritim dan Sains Marin  
Universiti Malaysia Terengganu  
(UMT)

Nama:

Cop Rasmi:

2/3/08  
Tarikh: .....

## 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 for 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

ACKNOWLEDGEMENT	ii
LIST OF CONTENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF APPENDICES	ix
LIST OF ABBREVIATIONS	x
ABSTRACT	xi
ABSTRAK	xiii
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	5
2.1 Nile Tilapia ( <i>Oreochromis niloticus</i> )	5
2.2 Heavy Metal	6
2.3 Cadmium	7
2.4 Gills, Muscle and Viscera of Fish	8
2.5 Median Lethal Concentration	9
2.6 DNA	10
2.7 Comet Assay	11
2.8 Randomly Amplified Polymerase Chain Reaction (RAPD-PCR)	12
2.8.1 Primers	13
CHAPTER 3 MATERIALS AND METHODS	14
3.1 Background of Study Area	14
3.2 Experimental Design for Toxicity Tests	14
3.2.1 Equipment	14
3.2.2 Water Source	15
3.2.3 Fish Source	15
3.2.4 Stock Solution of Cadmium	15
3.2.5 Control	16

3.3	Experimental Design for Acute Toxicity Testing	16
3.3.1	Range Finding Test	16
3.3.2	LC <sub>50</sub> Acute Toxicity Test	17
3.3.3	Statistical Analysis	
3.4	Experimental Design for Sub-Lethal Exposure Test	7
3.4.1	Continuous Flow Through System	18
3.5	Water Treatment	19
3.6	ICPMS Analysis	20
3.5.1	Sample Preparation	20
3.6.2	Open Acid Digestion	21
3.6.3	Detection of Heavy Metal Through	21
3.6.4	Calculation and Statistical Analysis	21
3.6.5	Recovery Test	
3.7	Single Cell Gel Electrophoresis	22
3.7.1	Protocol	22
3.7.2	Microscope Examination	23
3.7.3	Statistical Analysis	23
3.8	Genome Study	23
3.8.1	DNA Isolation	23
3.8.2	Purification and Quantification of DNA	24
3.8.3	Primer	25
3.8.4	DNA Amplification by RAPD-PCR	25
3.8.5	Database Establishment	26
3.8.6	Data Analysis	26
CHAPTER 4	RESULTS	28
4.1	Part A: Determination of 96-h LC <sub>50</sub> Cd <sup>2+</sup> and detection of Cadmium concentrations accumulated in exposed tilapia fingerlings via ICP-MS	28
4.1.1.	Toxicity Tests Data	
4.1.2.	Cadmium Analysis in Tilapia Fingerlings via ICP-MS	29
4.2	Part B: Effects in the genome of exposed tilapia fingerlings using Randomly Amplified Polymerase chain Reaction (RAPD-PCR)	36
4.2.1.	DNA Purification and Quantification	36
4.2.2.	DNA Extraction	37
4.2.3.	RAPD Fingerprinting pattern	38
4.2.4.	Genomic DNA Template Stability	44
4.2.5.	Dendogram	47

4.3	Part C: Effects in the genome of exposed tilapia fingerlings	
	Using Single Cell Gel Electrophoresis/COMET Assay	50
4.3.1.	Microscopic Analysis	50
4.3.2.	Statistical Analysis	50
CHAPTER 5 DISCUSSION		52
CHAPTER 6 CONCLUSION		64
REFERENCES		66
APPENDICES		72
CURRICULUM VITAE		88



## LIST OF TABLES

<b>TABLES</b>		<b>PAGE</b>
Table 4.1	Physio chemical properties of test water	28
Table 4.2	Median lethal concentration value (LC <sub>50</sub> ) cadmium using Spearman Karber and Probit method	29
Table 4.3	Concentration design for continuous flow through recycled system	29
Table 4.4	Metal Recovery percentage for Cadmium	30
Table 4.5	Total accumulation of Cadmium in Tilapia Fries throughout 21 days of exposure to various Cadmium concentrations	30
Table 4.6	Concentration and quantity of extracted DNA, measured using Bio-photometer at 260 nm/280 nm absorbance ratio	35
Table.4.7.	Effect of various Cd <sup>2+</sup> concentrations at various time intervals on the mean tail length	50

## LIST OF FIGURES

FIGURES		PAGE
Figure 3.1	Overview of 21 days sub-lethal toxicity test	19
Figure 4.1	Calibration curve prepared using blank and standard	29
Figure 4.2	Cd <sup>2+</sup> concentration in gills of exposed tilapia fingerlings in Different Cd <sup>2+</sup> concentration for 7, 14 and 21 days	31
Figure 4.3	Cd <sup>2+</sup> concentrations in stomach of exposed tilapia fingerlings In different Cd concentration for 7, 14 and 21 days	32
Figure 4.4	Cd <sup>2+</sup> concentration in tissue of exposed tilapia fingerlings in different Cd <sup>2+</sup> concentration for 7, 14 and 21 days	33
Figure 4.5	Cd <sup>2+</sup> concentration in whole tilapia fingerlings in different Cd Concentration for 7, 14 and 21 days	34
Figure 4.6	Total Cd <sup>2+</sup> concentrations in parts of tilapia fingerlings at different time intervals	35
Figure 4.7	Replicate 1(A) and 2(B) of the electrophoresis pattern of <i>Oreochromis niloticus</i> genomic DNA	37
Fig.4.8.	RAPD profiles of genomic DNA from <i>Oreochromis niloticus</i> exposed to varying Cd <sup>2+</sup> concentrations at different time intervals tested with primer OPB 1	40
Fig.4.9.	RAPD profiles of genomic DNA from <i>Oreochromis niloticus</i> exposed to varying Cd <sup>2+</sup> concentrations at different time intervals tested with primer OPA 9	41
Fig.5.0.	RAPD profiles of genomic DNA from <i>Oreochromis niloticus</i> exposed to varying Cd <sup>2+</sup> concentrations at different time intervals tested with primer OPA 16	42
Fig.5.1.	RAPD profiles of genomic DNA from <i>Oreochromis niloticus</i> exposed to varying Cd <sup>2+</sup> concentrations at different time intervals tested with primer OPB 8	43

Fig.5.2.	Comparison among genomic template stability using OPB 1 in Population of <i>O.niloticus</i> exposed to various Cd <sup>2+</sup> concentrations at various time intervals	44
Fig.5.3.	Comparison among genomic template stability using OPA 9 in population of <i>O.niloticus</i> exposed to various Cd <sup>2+</sup> concentrations at various time intervals	44
Fig.5.4.	Comparison among genomic template stability using OPA 16 in population of <i>O.niloticus</i> exposed to various Cd <sup>2+</sup> concentrations at various time intervals	45
Fig.5.5.	Comparison among genomic template stability using OPB 8 in population of <i>O.niloticus</i> exposed to various Cd <sup>2+</sup> concentrations at various time intervals	45
Fig.5.6.	UPGMA (unweighted pair-group method using arithmetic averages) dendogram for tilapias exposed to various concentration of Cd <sup>2+</sup> at various time intervals based on nie and li cluster analysis using OPB1	47
Fig.5.7.	UPGMA (unweighted pair-group method using arithmetic averages) dendogram for tilapias exposed to various concentration of Cd <sup>2+</sup> at various time intervals based on nie and li cluster analysis using OPA 9	47
Fig.5.8.	UPGMA (unweighted pair-group method using arithmetic averages) dendogram for tilapias exposed to various concentration of Cd <sup>2+</sup> at various time intervals based on nie and li cluster analysis using OPA 16	48
Fig.5.9..	UPGMA (unweighted pair-group method using arithmetic averages) dendogram for tilapias exposed to various concentration of Cd <sup>2+</sup> at various time intervals based on nie and li cluster analysis using OPB 8	48
Fig.6.0	Photomicrographs of EtBr-stained cells processed for alkaline comet assay	50
Fig.6.1.	Effect of various Cd <sup>2+</sup> concentrations at various time intervals on the mean tail length (microns)	51

## LIST OF APPENDICES

APPENDICES		PAGE
APPENDICES I	Replicate 1 96 h LC50 value obtained from spearman kaber	72
APPENDICES II	Replicate 2 96 h LC50 value obtained from spearman kaber	73
APPENDICES III	Replicate 3 96 h LC50 value obtained from spearman kaber	74
APPENDICES IV	Total body length, length, Dry weight and wet weight of fish	75
APPENDICES V	Binary Matrix for OPA 9	76
APPENDICES VI	Binary Matrix for OPA 16	77
APPENDICES VII	Binary Matrix for OPB 1	78
APPENDICES VIII	Binary Matrix for OPB 8	79
APPENDICES IX	Two way ANOVA test for gills	80
APPENDICES X	Two way ANOVA test for Viscera	82
APPENDICES XI	Two way ANOVA test for Muscle Tissue	84
APPENDICES XII	Two way ANOVA test for Whole Body	86

## LIST OF ABBREVIATIONS/SYMBOLS

ICP-MS	-	Inductively Coupled Plasma – Mass Spectrometry
Cd	-	Cadmium
Cd <sup>2+</sup>	-	Cadmium in ionic form
ppb	-	parts per billion equivalent to ug L <sup>-1</sup>
ppm	-	parts per million equivalent to mg L <sup>-1</sup>
HNO <sub>3</sub>	-	Nitric Acid
H <sub>2</sub> SO <sub>4</sub>	-	Sulfuric Acid
HCl	-	Hydrochloric acid
H <sub>2</sub> O <sub>2</sub>	-	Hydrogen Peroxide
Mg L <sup>-1</sup>	-	milligram per liter
Ug L <sup>-1</sup>	-	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<sub>50</sub> value (4.688 ppm) which was obtained from 96 hours acute toxicity test. The exposed fingerlings were harvested at each 7 days 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. niloticus* 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  $\mu\text{m}$ ), with increasing time intervals. The present study concludes that Cadmium accumulates in *O.niloticus* at some significant level in various parts and induces genotoxic 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  $\pm$  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<sub>50</sub> 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 berdasarkan min panjang ekor comet) berlaku di dalam semua sample yang 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.