UNIVERSITI PUTRA MALAYSIA

PURIFICATIONS AND CHARACTERIZATIONS OF CHOLINESTERASE FROM DIFFERENT ORGANS OF LATES CALCARIFER BLOCH

NURSABRINA BT MOHD HAYAT@AHMAD

FBSB 2015 3

PURIFICATIONS AND CHARACTERIZATIONS OF CHOLINESTERASE FROM DIFFERENT ORGANS OF *LATES CALCARIFER* BLOCH

By

NURSABRINA BT MOHD HAYAT@AHMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the Requirements for the Degree of Master of Science

September 2015

In Malaysia, many rivers have been greatly polluted by industrial effluents. Fish are ubiquitous organisms that have many features with a potential as a biomarker of heavy metals pollution. Recently, cholinesterase (ChE) from inhibition studies on fish has emerged to be one of great potential biomarkers for heavy metals monitoring. This study was aimed to assess the capability of ChE from selected *Lates calcarifer* organs namely brain, gill, liver, muscle, and kidney to detect metal ions because they tend to bioaccumulate and will give a great threat towards living organism. ChE was purified through ammonium sulphate precipitation and ion exchange chromatography. The optimum ChE activity for all organs determined to be at 25°C and in 0.1 M Tris-HCl buffer, pH 8.0. Each organ was able to hydrolyse different synthetic substrates. Brain, gill, and kidney showed a strong affinity towards acetylthiocholine iodide (ATC). Liver ChE hydrolysed butyrylthiocholine iodide (BTC) at a faster rate than other organs, while muscle ChE showed an optimum enzyme activity when propionylthiocholine iodide (PTC) was used as the substrate. Ten heavy metals namely argentum (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) were chosen for the inhibition study. When tested with a specific substrate for each organ, the results showed that the brain ChE was inhibited by Ag, As, Cd, Cr, and Hg. Different results were obtained for gill ChE, which was inhibited by Cu, Hg, and Pb only, while liver ChE was inhibited by almost all of the heavy metals used, but only Cd and Co did not show an inhibition of >50%. Muscle ChE was inhibited by Pb, while kidney ChE was very sensitive towards Pb. The results can be further used in biomarker studies for addressing heavy metals pollution in water bodies.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PURIFICATIONS AND CHARACTERIZATIONS OF CHOLINESTERASE FROM DIFFERENT ORGANS OF *LATES CALCARIFER* BLOCH

By

NURSABRINA BT MOHD HAYAT@AHMAD

September 2015

Chairperson : Siti Aqlima Ahmad, PhD

Faculty : Biotechnology and Biomolecular Sciences

In Malaysia, many rivers such as Sungai Juru and Sungai Merbok have been greatly polluted by industrial effluents from electronics, basic and fabricated metal products, chemical plants, and transport equipment industries. Fish are ubiquitous organisms that have many features with a potential as a biomarker of heavy metals pollution. Recently, cholinesterase (ChE) from inhibition studies on fish has emerged to be one of the great potential biomarkers for heavy metals monitoring. The aim of this study was to assess the capability of ChE from selected *Lates calcarifer* organs namely brain, gill, liver, muscle, and kidney to detect metal ions because they tend to bioaccumulate and will give a great threat towards living organism. The ChE was purified through ammonium sulphate precipitation and ion exchange chromatography. The optimum ChE activity for all organs was determined to be at 25°C and in 0.1 M Tris-HCl buffer, pH 8.0. Each organ was able to hydrolyse different synthetic substrates. Brain, gill, and kidney showed a strong affinity towards acetylthiocholine iodide (ATC). Liver ChE hydrolysed butyrylthiocholine iodide (BTC) at a faster rate than other organs, while muscle ChE showed an optimum enzyme activity when propionylthiocholine iodide (PTC) was used as the substrate. Ten heavy metals namely argentum (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) were chosen for the inhibition study. When tested with a specific substrate for each organ, the results showed that the brain ChE was inhibited by Ag, As, Cd, Cr, and Hg. Different results were obtained for gill ChE, which was inhibited by Cu, Hg, and Pb only, while liver ChE was inhibited by almost all of the heavy metals used, but only Cd and Co did not show an inhibition of >50%. Muscle ChE was inhibited by Pb, while kidney ChE was very sensitive

towards Pb. The results showed that different substrates gave different inhibition effects towards the heavy metals. The results from this study can be further used in biomarker studies for addressing heavy metals pollution in water bodies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENULENAN DAN PENCIRIAN ENZIM KOLINESTERES DARIPADA BERLAINAN ORGAN IKAN SIAKAP

Oleh

NURSABRINA BT MOHD HAYAT@AHMAD

September 2015

Pengerusi : Siti Aqlima Ahmad, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Di Malaysia, banyak sungai seperti Sungai Juru dan Sungai Merbok yang teruk dicemari oleh efluen perindustrian daripada industri elektronik, produk asas dan fabrikasi logam, loji kimia dan peralatan pengangkutan. Ikan merupakan organisma yang boleh didapati di mana-mana dan mempunyai banyak ciri yang boleh dijadikan sebagai biopenanda bagi pencemaran logam berat. Sejak akhir-akhir ini, kolinesterase (ChE) daripada kajian perencatan terhadap ikan telah diselidik dan berkembang sebagai salah satu biopenanda yang berpotensi bagi memantau logam berat kerana mereka berkemampuan untuk termendap dan menyebabkan ancaman yang besar terhadap organisma hidup. Tujuan kajian ini adalah untuk menilai kemampuan ChE daripada beberapa organ *Lates calcarifer* (Siakap), iaitu otak, insang, hati, otot, dan buah pinggang bagi mengesan ion logam. ChE ditulenkan dengan kaedah pemendakan amonium sulfat dan kromatografi pertukaran ion. Aktiviti ChE optimum bagi semua organ tersebut ditentukan pada suhu 25°C dan dengan menggunakan 0.1 M penimbal Tris-HCl pada pH 8.0. Setiap organ mampu menghidrolisis substrat sintetik yang berbeza. Otak, insang, dan buah pinggang menunjukkan keafinan yang kuat terhadap asetiltiokolin iodida (ATC). Selain itu, ChE hati menghidrolisis butiriltiokolin iodida (BTC) pada kadar yang lebih pantas, manakala ChE otot menunjukkan aktiviti enzim yang optimum apabila propioniltiokolin iodida (PTC) digunakan sebagai substrat. Sepuluh jenis logam berat, iaitu perak (Ag), arsenik (As), kadmium (Cd), kromium (Cr), kuprum (Cu), cobalt (Co), merkuri (Hg), nikel (Ni), plumbum (Pb), dan zink (Zn) dipilih dalam kajian perencatan. Apabila ujian substrat khusus bagi setiap organ dijalankan, keputusan awal menunjukkan bahawa ChE otak direncat oleh Ag, As, Cd, Cr, dan Hg. Organ-organ lain pula menunjukkan hasil yang berbeza. ChE insang direncat

oleh Cu, Hg, dan Pb. ChE hati pula direncat oleh hampir semua logam berat berkenaan, tetapi hanya Ag, As, Cr, Cu, Hg, Ni, Pb, dan Zn menunjukkan perencatan >50%. Aktiviti ChE otot direncat oleh Pb, sementara ChE buah pinggang amat sensitif terhadap Pb. Ringkasnya, substrat berbeza memberikan kesan perencatan yang berbeza terhadap logam berat. Oleh itu, keputusan daripada kajian ini boleh digunakan dengan lebih lanjut sebagai biopenanda bagi pencemaran air yang disebabkan oleh logam berat.

ACKNOWLEDGEMENT

BISMILLAHIRRAHMANIRRAHIM
In the name of Allah who is Beneficent and Merciful

My warmest gratitude goes to my parents, Mohd Hayat@Ahmad Bin Mohd Yusuf and Raudzoh Bt Mawardi@Moin for their never-ending supports and advices that help me in the completion of my study. My biggest aprreciation also goes to my siblings for their encouragement that keeps me positive and strong throughout my study.

I would like to give my deepest gratitude to my supervisor, Dr. Siti Aqlima Ahmad, my co-supervisor, Prof. Dr. Mohd Arif Syed and Assoc. Prof. Dr. Mohd Yunus Abd Shukor for their constant supervisions and continuous suggestions that helped me in completing this project successfully. I would like to thank all the seniors and members of Enzymology and Bioremediation Lab (115 and 204) especially Khalizan, Kabiru, Ibrahim, Salihu and Abu Bakar for all the teachings and sharing of experiences despite of the knowledge that have been learned along with the technical assistance throughout the study. A very special gratitude to my dearest juniors, Wyatt, Weini, Izuan, Shakirah and Ain for their helps and concerns in the laboratory.

Last but not least, I would like to thank the authorities from Faculty of Biotechnology and Biomolecular Sciences who have provided a good environment and facilities for the completion of this study. This research project will be impossible without the helps from people that I have mentioned above. Thank you very much!

Nursabrina Mohd Hayat, 2015

I certify that a Thesis Examination Committee has met on 3 September 2015 to conduct the final examination of Nursabrina Bt Mohd Hayat@Ahmad on her Master of science thesis entitled "Purifications and Characterizations of Cholinesterase from Different Organs of *Lates calcarifer* Bloch" in Accordance with the Universities and University College Act1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science degree.

Members of the Examinations Committee were as follows:

Syahida Ahmad, PhD
Senior Lecturer,
Faculty of Biotechnology and Biomolecular Science,
Universiti Putra Malaysia
(Chairman)

Mohd Shukuri Mohamad Ali, PhD
Senior Lecturer,
Faculty of Biotechnology and Biomolecular Science,
Universiti Putra Malaysia
(Internal Examiner)

Jualang Azlan Gansau, PhD
Associate Professor,
School of Science and Technology,
Universiti Malaysia Sabah
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 January 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science.

The members of the Supervisory Committee were as follows:

Siti Aqlima Ahmad, PhD
Senior Lecturer,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia
(Chairperson)

Mohd Arif Syed, PhD
Professor,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia
(Member)

Mohd Yunus Abd Shukor, PhD
Associate Professor,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia
(Member)

Farrah Aini Dahalan, PhD
Senior Lecturer,
The School of Environmental Engineering,
Universiti Malaysia Perlis
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision,
- supervision responsibilities as slated in Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

(Siti Aqlima Ahmad, PhD)
Chairman

(Mohd Arif Syed, PhD)
Member

(Mohd Yunus Abd Shukor, PhD)
Member

(Farrah Aini Dahalan, PhD)
Member

TABLE OF CONTENTS

LIST OF EQUATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celcius
µg	Microgram
µL	Microlitre
Abs	Absorbance
Ag	Silver
As	Arsenic
ATC	Acetylcholine iodide
BSA	Bovine serum albumin
BTC	Butyrylthiocholine iodide
Cd	Cadmium
Cr	Chromium
Cu	Copper
DEAE	Diethylaminoethyl
DTNB	5, 5-dithio-bis-2-nitrobenzoate
et al.,	And others
g	Gram
HCl	Hydrochloric acid
Hg	Mercury
HPLC	High Performance Liquid Chromatography
kDa	Kilo Dalton
K_m	Michaelis Menten constant
L	Litre
M	Molar
mg	Miligram
mg L^{-1}	Miligram per litre
min	Minute
mins	Minutes
mL	Millilitre
mM	Milimolar
MW	Molecular weight
PAGE	Polyacrylamide gel electrophoresis
Pb	Lead
PMSF	Phenylmethylsufonyl fluoride

ppm	Part per million
PTC	Propionylthiocholine iodide
TEMED	Tetramethyl-ethylene diamine
U	Unit
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

In 2014, 56.5 kg of fish was consumed per person by Malaysians each year, making Malaysia among the world's top fish consumers (The Star, 2014). Asian sea bass is one of the most popular fish among Malaysians, followed by mackerel, squid, grouper and shrimp. Recently, the local fishery sector was seriously threatened by water pollution caused by heavy metals (The Star, 2014). For example, due to a 40-year accumulated pollution Juru River is dying mainly caused by industrial toxic wastes disposal. Juru River heavily polluted by heavy metal effluents from present industries such as electronics, basic and fabricated metal products, chemical plants, and transport equipment (Alkarkhi *et al*., 2008). They also stated that Juru River was highly polluted with Arsenic (As) and mercury (Hg) with 2.67 and 1.33 ppm respectively.

Fish is very sensitive to temperature changes, natural surroundings and water quality deterioration which made them into a favorite subject biomarker research (Skouras *et al*., 2003). Cellular responses considered as a suitable tool for the early and sensitive detection of chemicals exposure for the assessment of chemicals toxicity at cellular level (Monserrat *et al*., 2007). The use of biomarker for monitoring environmental quality in aquatic ecosystem had raised a great deal because of its economical method, early warning signal and give precise measurement (Paustenbach and Galbraith, 2006; Sarkar, 2006). Biomarkers represent changes that may arise due to the toxic effects of exposure to chemical pollutants from the molecular to the organism level. The response of biomarker occur prior to changes at the population and community levels, thus it has the ability to diagnose causes and act as early warning signals of ecosystem-level damage (Tsangaris *et al*. 2006).

The simplest estimation for toxicant existence was displayed by enzyme-based biomarker in which this method gave multiple advantages such as rapid determination, sensitive even exposed in low concentration of toxicant and low technical application needed (Sabullah *et al*., 2015). Cholinesterase (ChE) was used as a biomarker for metal and organochlorine compound in Kootenai River (Kruse and Scarnecchia, 2002). There are two basic types of ChEs that are the best known and characterized which are acetylcholinesterases (AChE) and butyrylcholinesterase (BChE) (Pezzementi *et al*., 2011). AChE hydrolyzes acetylcholine (Ach) at the neuromuscular junction of vertebrates while in higher vertebrates an evolutionarily related ChE, pseudocholinesterase (BChE and propionylcholinesterase (PChE)) also exist. The function of BChE is unknown but is suggested to play a role in growth and development and sometimes act as a scavenger of cholinergic toxins (Masson and Lockridge, 2010). Various sources of

ChE from aquatic organisms such as *Tilapia mossambica* (Al-Ghais, 2013), *Osteochillus hasselti* (Sabullah *et al*., 2013) and *Periophtalmodon schlosseri* (Sabullah *et al*., 2014) was reported to be a sensitive biomarker with toxicant especially heavy metals. Fish considered as one of a biomarker tool and a highly sensitive enzyme as sentinel species allows the lower contamination levels of pollution detection (Sabullah *et al*., 2015).

The removal of xenobiotic compounds is needed to ensure a safer environment. The *in vitro* inhibition study of fish ChE activity by heavy metals gave multiple information aid standardization of environmental management and treatment to minimize and eliminate the toxicant (Sabullah *et al*., 2015) which *Lates calcarifer* was chosen as the sample because there is lack study of this species for biomonitoring pollution. Furthermore, *L. calcarifer* is important as a commercial and subsistence food fish in Malaysia.

1.1 Aim and Objectives

The main objective of this work is to purify cholinesterase enzyme from different parts of *Lates calcarifer* (Siakap) organ that will be later used as an alternative biosensor for bioremediation of aquatic environment. In this research, purification of the enzyme was performed to obtain a purified enzyme at the end of study. This study embarks the following objectives:

1. To purify cholinesterase from different parts of *Lates calcarifer* (Siakap) organs (brain, gill, kidney, liver and muscle).

2. To determine the optimum assay condition and substrate specificity of purified cholinesterase activity.

3. To characterise the cholinesterase activity by chemical approach using *in vitro* effects of metal ions on purified cholinesterase.

REFERENCES

Abdelhamid, R. F., Obara, Y. and Uchida, Y. (2007). Π-π interaction between aromatic ring and copper-coordinated His_{81} imidazole regulates the blue copper active-site structure. *Journal of Biological Inorganic Chemistry*, 12, 165–173.

Abramson, S. N., Radic, Z., Manker, D., Faulkner, D. J. and Taylor, P. (1989). Onchidal: A naturally occurring irreversible inhibitor of acetylcholinesterase with a novel mechanism of action. *Molecular Pharmacology*, 36, 349 –354.

Agrahari, S., Gopal, K. and Pandey, K. C. (2006). Biomarkers of monocrotophos in a freshwater fish *Channa punctatus* (Bloch). *Journal of Environmental Biol*ogy, 27, 453–457.

Al-Ghais, S. M. (2013). Acetylcholinesterase, glutathione and hepatosomatic index as potential biomarkers of sewage pollution and depuration in fish. *Marine Pollution Bulletin*, 74, 183–186.

Alkarkhi, F. M. A., Ismail, N. and Easa, A. M. (2008). Assessment of arsenic and heavy metal contents in cockles *(Anadara granosa)* using multivariate statistical techniques. *Journal of Hazardous Materials*, 150, 783–789.

Almroth, C., Sturve, B., Stephensen, J., Fredrik Holth, E. and Förlin, L. (2008). Protein carbonyls and antioxidative defenses in corkwing wrasse (*Symphodus melops*) from a heavy metal-polluted and PAH-polluted site. *Marine Environmental Re*search, 66, 271–277.

Al-Shami, S. A., Md Rawi, C. S., Ahmad, A. H., Abdul Hamid, S. and Mohd Nor, S. A. (2011). Influence of agricultural, industrial and anthropogenic stresses on the distribution and diversity of macro invertebrates in Juru River, Penang, Malaysia. *Ecotoxicology and Environmental Safety*, 74, 1195–1202.

Andres, C., M. el Mourabit, C., Stutz, J. M. and Waksman, A. (1990). Are soluble and membrane-bound rat brain acetylcholinesterase different?. *Neurochemical Research*, 15, 1065–1072.

Anglister, L., Haesaert, B. and McMahan, U. J. (1994). Globular and asymmetric acetylcholinesterase in the synaptic basal lamina of skeletal muscle. *The Journal of Cell Biology*, 125, 183–196.

Armentrout, P. B., Yang, B. and Rodgers, M. T. (2013). Metal cation dependence of interactions with amino acids: bond energies of Rb+ and Cs+ to Met, Phe, Tyr, and Trp. *Journal of Physical Chemistry B*, 117, 3771–3781.

Askar, K. A., Kudi, A. C. and Moody, A. J. (2011). Comparative analysis of cholinesterase activities in food animals using modified Ellman and Michel assays. *Canadian Journal of Veterinary Research*, 75, 261–270.

Assis, C. R. D., Castro, P. F. I., Amaral, P. G., Maciel Carvalho, E. V. M., Carvalho Jr, L. B. and Bezerra, R. S. (2010). Characterization of acetylcholinesterase from the brain of the Amazonian tambaqui (*Colossoma macropomum*) and in vitro effect of organophosphorus and carbamate pesticides. *Environmental Toxicology and Chemistry*, 29, 2243–2248.

Bandara, N. J. G. J. (2003). Water and wastewater related issues in Sri Lanka. *Water Science and Technology*, 47, 305–312.

Banni, M., Jebali, J., Daubeze, M., Clerandau, C., Guerbej, H., Narbonne, J. F. and Boussetta, H. (2005). Monitoring pollution in Tunisian coasts: application of a classification scale based on biochemical markers. *Biomarkers*, 10, 105–116.

Barata, C., Varo, I., Navarro, J. C., Arun, S. and Porte, C. (2005). Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds. *Comparative Biochemistry and Physiology C*, 140, 175–186.

Bargmann, C. (2005). Neuroscience: Genomics reaches the synapse. *Nature*, 436, 510–517.

Barron, M. G. and Woodburn, K. B. (1995). Ecotoxicology and chlorpyrifos. *Reviews of Environmental Contamination and Toxicology*, 144, 1–93.

Berg, J. M., Tymoczko, J. L. and Stryer, L. (2002). The purification of proteins is an essential first step in understanding their function. Biochemistry. 5th edition. New York: W H Freeman.

Bertrand, C., Chatonnet, A., Takke, C., Yan, Y. L., Postlethwait, J. and Toutant, J. P. (2001). Zebrafish acetylcholinesterase is encoded by a single gene localized on linkage group 7. *Journal of Biological Chemistry*, 276, 464–474.

Bhattacharyya, L. and Rohrer, J. S. (2012). Applications of Ion Chromatography for Pharmaceutical and Biological Products. New Jersey: John Wiley & Sons.

Bourne, Y., Kolb, H. C., Radić, Z., Sharpless, K. B., Taylor, P. and Marchot, P. (2005). Freeze-frame inhibitor captures acetylcholinesterasee in unique conformation. *The National Academy of Sciences*, 101, 1449–1454.

Bradford, M. M. (1976). A rapid and sensitive methods for the quantitation of microgram quantitaties of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.

Brestkin, A. P. and Rozengart's, E. V. (1965). Cholinesterase catalysis. *Nature*, 205, 388–389.

Brown, M., Davies, I. M., Moffat, C. F., Redshaw, J. and Craft, J. A. (2004). Characterisation of cholinesterases and their tissue and subcellular distribution in mussel (*Mytilus edulis*). *Marine Environmental Research*, 57, 155–169.

Bruch, T., Graalfs, H., Jacob, L. and Frech, C. (2009). Influence of surface modification on protein retention in ion-exchange chromatography evaluation using different retention models. *Journal of Chromatography A*, 1216, 919–926.

Burgess, R. R. (2009). Protein precipitation techniques. *Methods in Enzymology*, 463, 331–342.

Busca, G., Berardinelli, S., Resini, C. and Arrighi, L. (2008). Technologies for the removal of phenol from fluid streams: a short review of recent developments. *Journal of Hazardous Materials*, 160, 265–288.

Cai, A. L., Zipfel, G. J. and Sheline, C. T. (2006). Zinc neurotoxicity is dependent on intracellular NAD levels and the sirtuin pathway. *European Journal of Neuroscience*, 24, 2169–2176.

Campbell, A. K. (2014). Intracellular Calcium. John Wiley & Sons. United States of America. Pp 171.

Caussy, D., Gochfeld, M., Gurzau, E., Neagu, C. and Ruede, H. (2003). Lessons from case studies of metals: investigating exposure, bioavailability, and risk. *Ecotoxicology and Environmental Safety*, 56, 45–51.

Changeux, J. P. (1966). Response of acetylcholinesterase from *Torpedo marmorata* to saltsand curarizing drugs. *Molecular Pharmacology*, 2,369–392.

Chebbi, S. G. and David, M. (2009). Neurobehavioural responses of the freshwater teleost, *Cyprinus carpio* (*Linnaeus*) under quinalphos intoxication. *Biotechnology of Animal Husbandary*, 25, 241–249.

Chen, V. P., Xie, H. Q., Chan, W. K., Leung, K. W., Chan, G. K., Choi, R. C., Bon, S., Massoulié, J. and Tsim, K. W. (2010). The PRiMA-linked cholinesterase tetramers are assembled from homodimers: hybrid molecules composed of acetylcholinesterase and butyrylcholinesterase dimers are up-regulated during development of chicken brain. *Journal of Biological Chemistry*, 285, 27265–27278.

Coleman, J. E. (1992). Zinc proteins: enzymes, storage proteins transcription factors, and replication proteins. *Annual Review of Biochemistry*, 61, 897–946.

Colovic, M. B., Krstic, D. Z., Lazarevic -Pasti, T. D., Bondzic, A. M. and Vasic, V. M. (2013). Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. *Current Neuropharmacology*, 11, 315–335.

De Lima, D., Roque, G. M. and De Almeida, E. A. (2013). In vitro and in vivo inhibition of acetylcholinesterase and carboxylesterase by metals in zebrafish (*Danio rerio*). *Marine Environmental Research*, 91, 45–51.

Ding, Y. H., Wu, X. M. and Fang, J. B. (2011). Purification and characterization of acetylcholinesterase from brain tissues of *Oreochromis aurea* and its application in environmental pesticide monitoring. *Sciences in Cold and Arid Regions*, 3, 339–343.

Dvir, H., Silman, I., Harel., M., Rosenberry, T. L. and Sussman, J. L. (2010). Acetylcholinesterase: From 3D structure to function. *Chemico-Biological Interactions*, 187, 10–22.

Dziri, L., Boussaad, S., Tao, N. and Leblanc, R. M. (1998). Effect of pH on acetylcholinesterase Langmuir and Langmuir – Blodgett films studied by surface potential and atomic force microscopy. *Thin Solid Films*, 329, 56–59.

Ellman, G. L., Courtney, K. D., Andres, V. Jr. and Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7, 88–95.

Englard, S. and Seifter, S. (1990). Precipitation techniques. *Methods in Enzymology*, 182, 287–300.

Escartin, E. and Porte, C. (1997). The use of cholinesterase and carboxylesterase activities from *Mytilus galloprovincialis* in pollution monitoring. *Environmental Toxicology and Chemistry*, 16, 2090–2095.

Forget, J., Livet, S. and Leboulenger, F. (2002). Partial purification and characterization of acetylcholinesterase from the estuarine copepod *Eurytemora affinis* (Poppe). *Comparative Biochemistry and Physiology Part C*, 132, 85–92.

Frasco, M. F., Colletier, J. P., Weik, M., Carvalho, F., Guilhermino, L., Stojan, J. and Fournier, D. (2007). Mechanisms of cholinesterase inhibition by inorganic mercury. *FEBS Journal*, 274, 1849–1861.

Gagnaire, B., Geffard, O., Xuereb, B., Margoum, C. and Garric, J. (2007). Cholinesterase activities as potential biomarkers: Characterization in two freshwater snails, *Potamopyrgus antipodarum* (Mollusca, Hydrobiidae, Smith 1889) and *Valvata piscinalis* (Mollusca, Valvatidae, Müller 1774). *Chemosphere*, 7, 1–23.

Galloway, T. S., Brown, R. J., Browne, M. A., Dissanayake, A., Lowe, D., Jones, M. B. and Depledge, M. H. (2004a). A multibiomarker approach to environmental assessment. *Environmental Science and Technology*, 38, 1723–1731.

Galloway, T. S., Brown, R. J., Browne, M. A., Dissanayake, A., Lowe, D., Jones, M. B. and Depledge, M. H. (2004b). Ecosystem management bioindicators: the ECOMAN project-a multi-biomarker approach to ecosystem management. *Marine Environmental Research*, 58, 233–237.

Gao, J. R. and Zhu, K. Y. (2001). An acetylcholinesterase purified from the greenbug (*Schizaphis graminum*) with some unique enzymological and pharmacological characteristics. *Insect Biochemistry and Molecular Biology*, 31, 1095–1104.

Gbaye, O. A., Holloway, G. J. and Callaghan, A. (2012). Variation in the sensitivity of *Calloso bruchus* (Coleoptera: *Bruchidae*) acetylcholinesterase to the organophosphate insecticide malaoxon: effect of species, geographical strain and food type. *Pest Management Science*, 68, 1265–1271.

Ghazala, Mahboob, S., Sultana, S., Sultana, T., Ahmad, L. and Asi, M. R. (2014). Cholinesterases: Cholinergic Biomarkers for the Detection of Sublethal Effects of Organophosphorous and Carbamates in *Catla catla. International Journal of Agriculture and Biology*, 16, 406–410.

Girotti, S., Ferri, E. N., Fumo, M. G. and Maiolini, E. (2008). Monitoring of environmental pollutants by bioluminescent bacteria. *Analytical Chimica Acta*, 608, 2–29.

Giuliano, C., Parikh, V., Ward, J. R., Chiamulera, C. and Sarter, M. (2008). Increases in cholinergic neurotransmission measured by using choline-sensitive microelectrodes: enhanced detection by hydrolysis of acetylcholine on recording sites?. *Neurochemistry International*, 52, 1343–1350.

Gomes, I. D. L., Lemos, M. F. L., Soares, A. M. V. M., Barata, C. and Faria, M. (2014). The use of cholinesterase as potential biomarker: In vitro characterization in the polychaete *Capitella teleta*, *Marine Pollution Bulletin*, 85, 179–185.

Goyal, R. K. and Chaudhury, A. (2013). Structure activity relationship of synaptic and junctional neurotransmission. *Autonomic Neuroscience*, 176, 11–31.

Grodzki, A. C. and Berenstein, E. (2010). Antibody purification: Ion-exchange chromatography. Immuno cytochemical methods and protocols. *Methods in Molecular Biology*, 588, 27–32.

Gupta, R. C. (2006). Toxicology of organophosphate and carbamate compounds. Academic Press/Elsevier, Amsterdam.

Haddad, P. R. and Jackson, P. E. (1990). Ion chromatography – Principles and applications. *Journal of Chromatography Library Series*, 46, 409– 462.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C. and Fox, H. E. (2008). A global map of human impact on marine ecosystems. *Science*, 319, 948–952.

Hamzah, N. (2007). Assessment on water quality and biodiversity within Sungai Batu Pahat. Master of thesis. Universiti Teknologi Malaysia.

Han, C. K., Park, Y. H., Jin, D. Q., Hwang, Y. K., Oh, K. B. and Han, J. S. (2007). SK-PC-B70M from *Pulsatilla koreana* improves scopolamine-induced impairments of memory consolidation and spatial working memory. *Brain Research*, 1184, 254–259.

Herrera, J. C., Naranjo, M. T. M., Campoy, F. J. and Vidal, C. J. (1994). G4 forms of acetylcholinesterase and butyrylcholinesterase in normal and dystrophic mouse muscle differ in their interaction with *Ricinus communis* agglutinin. *Biochimica et Biophysica Acta*, 1225, 283–288.

Holmstedt, B. (2000). Cholinesterase inhibitors: as introduction. In: Giacobini E (Ed.) Cholinesterase and cholinesterase inhibitors. Martin Duntz, London, 1–8.

Hong, Y., Chen, X., Guo, J., Xu, Z. , Xu, M. and Sun, G. (2007). Effects of electron donors and acceptors on anaerobic reduction of azo dyes by *Shewanella decolorationis* S12. *Applied Microbiology and Biotechnology*, 74, 230–238.

Hsiao, Y. M., Lai, J. Y., Liao, H. Y. and Feng, H. T. (2004). Purification and characterization of acetylcholinesterase from oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae). *Journal of Agricultural and Food Chemistry*, 52, 5340–5346.

Ibrahim, F., Guillaume, Y. C., Thomassin, M., and André, C. (2009). Magnesium effect on the acetylcholinesterase inhibition mechanism: a molecular chromatographic approach. *Talanta*, 79, 804–809.

Ingkaninam, K., De Best, C. M., Irth, H., Van Der Heijden, R., Hofte, A. J. P., Karabatak, B., Tjaden, U. R., Van Der Greef, J. and Verpoorte, R. (2000). High performance liquid chromatography with on-line couple UV-mass spectrophotometric-biochemical detection for identification of acetylcholinesterase inhibitors from natural products. *Journal of Chromatography*, 872, 61–73.

International Agency for Research on Cancer (IARC). (2015). Retrieved on 15 March 2015 at www.iarc.fr. France.

Ishihara, T., Kadoya, T. and Yamamoto, S. (2007). Application of a chromatography model with linear gradient elution experimental data to the rapid scale-up in ion-exchange process chromatography of proteins. *Journal of Chromatography A*, 1162, 34–40.

Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, 68, 167–82.

Jebali, J., Banni, M., Guerbej, H., Almeida, E. A., Bannaoui, A. and Boussetta, H. (2006). Effects of malathion and cadmium on acetylcholinesterase activity and metallothionein levels in the fish *Seriola dumerilli*. *Fish Physiology and Biochemistry*, 32, 93–98.

Jebali, J., Khedher, S. B., Sabbagh, M., Kamel, N., Banni, M. and Boussetta, H. (2013). Cholinesterase activity as biomarker of neurotoxicity: utility in the assessment of aquatic environment contamination. *Journal of Integrated Coastal Zone Management,* 13, 525–537.

Jokanović, M. and Prostran, M. (2009). Pyridinium oximes as cholinesterase reactivators: Structure-activity relationship and efficacy in the treatment of poisoning with organophosphorus compounds. *Current Medicinal Chemistry*, 16, 2177–2188.

Jonz, M. G. and Zaccone, G. (2009). Nervous control of the gills. *Acta Histochemica*, 111, 207–216.

Kato, G. , Tan, E. and Yung, J. (1972). Acetylcholinesterase. Kinetic studies on the mechanism of atropine inhibition. *Journal of Biological Chemistry*, 247, 3186–3189.

Kato, Y., Tanaka, T. and Miyata, T. (2004). Comparison of kinetic properties of a hydrophilic form of acetylcholinesterase purified from strains susceptible and resistant to carbamate and organophosphorus insecticides of green rice leafhopper (*Nephotettix cincticeps Uhler*). *Pesticide Biochemistry and Physiology*, 79, 64–73.

Katzung, B. G. (2001). Introduction to autonomic pharmacology. In: Basic and clinical pharmacology, 8th edition. USA: The McGraw Hill Companies, Inc, pp. 75–91.

Kavita, B., Limbachia, J. and Keharia, H. (2011). Hexavalent chromium sorption by biomass of chromium tolerant *Pythium* sp., *Journal of Basic Microbiology*, 51, 173–182.

Keane, S. and Ryan, M. F. (1999). Purification, characterisation, and inhibition by monoterpenes of acetylcholinesterase from the waxmoth, *Galleria mellonella (L.), Insect Biochemistry and Molecular Biology*, 29, 1097–1104.

Kelly, R. S. and Vineis, P. (2014). Biomarkers of susceptibility to chemical carcinogens: the example of non-hodgkin lymphomas. *British Medical Bulletin*, 1–12.

Kim, M. J., Choi, S. J., Lim, S. T., Kim, H. K., Kim, Y. J. and Yoon, H. G. (2008). Zeatin supplement improves scopolamine-induced memory impairment in mice. *Bioscience, Biotechnology and Biochemistry*, 72, 577–581.

Koenig, S and Solé, M. (2014). Muscular cholinesterase and lactate dehydrogenase activities in deep-seafish from the NW Mediterranean, *Marine Environmental Research*, 94, 16–23.

Kopecka, J., Rybakowas, A., Barsiene, J. and Pempkowiak, J. (2004). AChE levels in mussels and fish collected off Lithuania and Poland (Southern Baltic). *Oceanologia*, 46, 405–418.

Kopecka-Pilarczyk, J. (2009). In vitro effects of pesticides and metals on the activity of acetylcholinesterase (AChE) from different tissues of the blue mussel, *Mytilus trossulus L. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes*, 45, 46–52.

Kruse, G. O. and Scarnecchia, D. L. (2002). Assessment of bioaccumulated metal and organochlorine compounds in relation to physiological biomarkers in Kootenai River white sturgeon. *Journal of Applied Ichthyology*, 18, 430–438.

Kuca, K., Cabal, J. and Kassa, J. (2005). In vitro reactivation of sarin-inhibited brain acetylcholinesterase from different species by various oximes. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 20, 227–232.

Kwong, T. C. (2002). Organophosphate pesticides: biochemistry and clinical toxicology, *Therapeutic Drug Monitoring*, 24, 144–149.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.

Lam, P. K. S. and Gray, J. S. (2003). The use of biomarkers in environmental monitoring programmes. *Marine Polution Bulletin*, 46, 182–186.

Lang, G. J., Zhang, M. Y., Li, B. L., Yu, L. L., Lu, X. M. and Zhang, C. X. (2010). Molecular characterization and inhibition analysis of the acetylcholinesterase gene from the silkworm maggot, *Exorista sorbillans. BMB Reports*, 573–578.

Lawler, H. C. (1963). Purification and properties of an acetylcholinesterase polymer. *The Journal of Biological Chemistry*, 238, 132–137.

Leong, K. H., Benjamin, T. L. L. and Mustafa, M. A. (2007). Contamination levels of selected organochlorine and organophosphate pesticides in the Selangor river, Malaysia between 2002 to 2003. *Chemosphere*, 66, 1153–1159.

Levison, P. R. (2003). Large-scale ion exchange column chromatography of proteins Comparison of different formats. *Journal of Chromatography B*, 790, 17–33.

Lionetto, M. G., Caricato, R., Calisi, A., Giordano, M. E. and Schettino, T. (2013). Acetylcholinesterase as a biomarker in environmental and occupational medicine: New insights and future perspectives. *BioMed Research International*, http://dx.doi.org/10.1155/2013/321213.

Lockridge, O. and La Du, B. N. (1986). Amino acid sequence of the active site of human serum cholinesterase from usual, atypical, and atypical-silent genotypes. *Biochemical Genetics*, 24, 485–498.

Lopes, P. R. M. and Bidoia, E. D. (2009). Evaluation of the biodegradation of different types of lubricant oils in liquid medium. *Brazilian Archives of Biology and Technology*, 52, 1285–1290.

Lopez-Roldan, R., Kazlauskaite, L., Ribo, J., Riva, M. C. and Gonzalez, S. (2012). Cortina: Evaluation of an automated luminescent bacteria assay for in situ aquatic toxicity determination. *Science of the Total Environment*, 440, 307–313.

Lund, S. A., Fulton, M. H. and Key, P. B. (2000). The sensitivity of grass shrimp, *Palaemonetes pugio*, embryos to organophosphate pesticide induced acetylcholinesterase inhibition. *Aquatic Toxicology*, 48, 127–134.

Malaviya, P. and Sharma, A. (2011). Impact of distillery effluent on germination behaviour of *Brassica napus*. *Journal of Environmental Biology*, 32, 91–94.

Marques, D., Almeida, M., Xavier, J. and Humanes, M. (2007). Biomarkers in marine sponges: acetylcholinesterase in the sponge *Cliona celata*. *Porifera Research: Biodiversity, Innovation and Sustainability*, 427–432.

Masson, P. and Lockridge, O. (2010). Butyrylcholinesterase for protection from organophosphorus poisons; catalytic complexities and hysteretic behaviour. *Archives of Biochemistry and Biophysics*, 494, 107–127.

Masson, P., Froment, M. T., Bartels, C. F. and Lockridge, O. (1996). Asp 70 in the peripheral anionic site of human butyrylcholinesterase. *European Journal of Biochemistry*, 235, 36–48.

Masson, P., Schopfer, L. M. and Bartels, C. F. (2002). Substrate activation in acetylcholinesterase induced by low pH or mutation in the π-cation subsite, *Biochemistry and Biophysics*, 1594, 313–324.

Massoulie, J., Pezzementi, L., Bon, S., Krejci, E. and Vallette, E. M. (1993). Molecular and cellular biology of cholinesterases. *Progress in Neurobiology*, 41, 31–91.

Massoulie´, J. (2002). The origin of the molecular diversity and functional anchoring of cholinesterases. *Neurosignals*, 11, 130–143.

Miller, J. N. and Miller, J. C. (2000). Statistics and chemometrics for analytical chemistry, Fourth Edition, United Kingdom: Pearson.

Minic, J., Chatonnet, A., Krejci, E. and Molgó, J. (2003). Butyrylcholinesterase and acetylcholinesterase activity and quantal transmitter release at normal and acetylcholinesterase knockout mouse neuromuscular junctions. *British Journal of Pharmacology*, 138, 177–178.

Monnet-Tschudi, F., Zurich, M. G., Boschat, C., Corbaz, A. and Honegger, P. (2006). Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Reviews in Environmental Health*, 21, 105–117.

Monserrat, J. M., Bianchini, A. and Bainy, A. C. D. (2007). Kinetic and toxicological characteristics of acetylcholinesterase from the gills of oysters (*Crassostrea rhizophorae*) and other aquatic species. *Marine Environmental Research*, 54, 781–785.

Moore, R. (1979). Natural sex inversion in the giant perch (*Lates calcarifer*). *Marine Freshwater Research,* 30, 803–813.

Moore, R. and Reynold, L. F. (1982). Migration patterns of barramundi, *Lates calcarifer* (Bloch), in Papua New Guinea. *Marine Freshwater Research*, 33, 671–682.

Nadji, S., Amrani, A., Mebarki, R. and El-Hadi Khebbeb, M. (2010). Acetylcholinesterase and catalase activities in several tissues of a bivalve mollusc (*Ruditapes decussatus*) fished from Mellah lagoon (North East of Algeria) after Malathion exposure. *Annals of Biological Research*, 1, 138–144.

Najimi, S., Bouhaimi, A., Daubeze, M., Zekhnini, A., Pellerin, J., Narbonne, J. F. and Moukrim, A. (1997). Use of acetylcholinesterase in *Perna perna* and *Mytilus galloprovincialis* as a biomarker of pollution in Agadir Marine Bay (South of Morocco). *Bulletin of Environmental Contamination and Toxicology*, 58, 901–908.

Nelson, J. S. (2006). Fishes of the World. Hoboken, NJ: Wiley.

Nguyen, V. T., Morange, M. and Bensaude, O. (1989). Protein denaturation during heat shock and related stress. *The Journal of Biological Chemistry*, 264, 10487–10492.

Obregon, A. D, Schetinger, M. R, Correa, M. M, Morsch, V. M, Da Silva, J. E., Martins, M. A, Bonacorso, H. G. and Zanatta, N. (2005). Effects per se of organic solvents in the cerebral acetylcholinesterase of rats. *Neurochemical Resources*, 30, 379–384.

Oliveira, M. M., Silva Filho, M. V., Cunha Bastos, V. L. F., Fernandes, F. C. and Cunha Bastos, J. (2007). Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes. *Marine Environmental Research*, 63, 303–312.

Oliveira, R. L., Seibt, K. J., Rico, E. P., Bogo, M. R. and Bonan, C. D. (2011). Inhibitory effect of lithium on nucleotide hydrolysis and acetylcholinesterase activity in zebrafish (*Danio rerio*) brain. *Neurotoxicology and Teratology*, 33, 651–657.

Oruc, E. O. and Usta, D. (2007). Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environmental Toxicology and Pharmacology*, 23, 48–55.

Pandey, G., Madhuri, S. and Shrivastav, A. B. (2012). Contamination of mercury in fish and its toxicity to both fish and humans: An overview. *International Research Journal of Pharmacy*, 3, 45–47.

Pathak, H. and Madamwar, D. (2010). Biosynthesis of indigo dye by newly isolated naphthalene-degrading strain *Pseudomonas* sp. HOB1 and its application in dyeing cotton fabric. *Applied Biochemistry and Biotechnology*, 160, 1616–1626.

Paustenbach, D. and Galbraith, D. (2006). Biomonitoring and biomarker: exposure assessment will never be the same. *Environmental Health Perspectives*, 114, 1143–1149.

Peakall, D. B. and Walker, C. H. (1994). The role of biomarkers in environmental assessment. *Vertebrates Ecotoxicology*, 3, 173–179.

Pezzementi, L., Nachon, F. and Arnaud, C. (2011). Evolution of acetylcholinesterase and butyrylcholinesterase in the vertebrates: An atypical butyrylcholinesterase from the Medaka *Oryzias latipes*. *Plos One Journal*, 6, 1–16.

Phyu, M. P. and Tangpong, J. (2014). Sensitivity of acetylcholinesterase to environmental pollutants. *Journal of Health Research*, 28, 277–283.

Postoarca, A. G., Ionescu, M., Piperea-Sianu, A., Sarbu, I. and Hinescu, L. G. (2015). Sodium Ion Effect on Separation Of Butyrylcholinesterase from Plasma by Ion Exchange Chromatography. *Current Health Sciences Journal*, 41, 165–171.

Rajesh, R. V., Balasubramanian, A. S. and Boopathy, R. (2009). Evidence for presence of Zn^{+2} binding site in acetylcholinesterase, *Biochimie*, 91, 526–532.

Rakhi, S. F., Mohsinul Reza, A. H. M., Hossen, M. S. and Hossain, Z. (2013). Alterations in histopathological features and brain acetylcholinesterase activity in stinging catfish *Heteropneustes fossilis* exposed to polluted river water. *International Aquatic Research*, 5, 1–18.

Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V. and Jackson, R. B. (2011). Campbell Biology, Ninth Edition, Boston: Benjamin Cummings/ Pearson Education.

Reed, C. J., Lewis, H., Trejo, E., Winston, V. and Evilia, C. (2013). Protein adaptations in archaeal extremophiles. *Archae*. http://dx.doi.org/10.1155/2013/373275.

Rico, E. P., Rosemberg, D. B., Senger, M. R., Arizi Mde, B., Bernardi, G. F. and Dias, R. D. (2006). Methanol alters ecto-nucleotidases and acetylcholinesterase in zebrafish brain. *Neurotoxicology and Teratology*, 28, 489–496.

Rodríguez-Fuentes, G., Armstrong, J. and Schlenk, D. (2008). Characterization of muscle cholinesterases from two demersal flatfish collected near a municipal wastewater outfall in Southern California. *Environmental Toxicology and Environmental Safety*, 69, 466–471.

Rosenberry, T. L., Johnson, J. L., Cusack, B., Thomas, J. L., Emani, S. and Venkatasubban, K. S. (2005). Interactions between the peripheral site and the acylation site in acetylcholinesterase, *Chemico-Biology Interaction*, 157–158.

Rothenberg, M. A. and Nachmansohn, D. (1947). Studies on cholinesterase. *Journal of Biological Chemistry*, 168, 223–231.

Rothernberg, M. A. and Nachmansonh, D. (1947). Purification of the enzyme from electric tissue by fractional ammonium sulfate precipitation. *The Journal of Biological Chemistry*, 168, 223–231.

Rotundo, R. L. (1984). Purification and properties of the hydrophobic, membrane bound form of acetylcholinesterase from chicken brain: evidence for two distinct polypeptide chains. *The Journal of Biological Chemistry*, 259, 13186–13194.

Sabullah, M. K., Ahmad, S. A., Ishak, I., Sulaiman, M. R., Shukor, M. Y., Syed, M. A. and Shamaan, N. A. (2013). An inhibitive assay for insecticides using the acetylcholinesterase from *Osteochillus hasselti*. *Bulletin of Environmental Science and Management*, 1.

Sabullah, M. K., Ahmad, S. A., Shukor, M. Y., Gansau, A. J., Syed, M. A., Sulaiman, M. R. and Shamaan, N. A. (2015). Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. *International Food Research Journal*, 22, 435–454.

Sabullah, M. K., Sulaiman, M. R., Shukor, M. Y., Syed, M. A., Shamaan, N. A., Khalid, A. and Ahmad, S. A. (2014). The assessment of cholinesterase from the liver of *Puntius javanicus* as detection of metal ions. *The Scientific World Journal*, 1–9.

Salles, J. B., Cunha, V. L., Bastos, M. V., Silva Filho, O. L., Machado, C. M., Salles, S., Giovanni de Simone, J. and Bastos, C. (2006). A novel butyrylcholinesterase from serum of *Leporinus macrocephalus*, a neotropical fish. *Biochimie*, 88, 59–68.

Sani, R. K., Rastogi, G., Moberly, J. G., Dohnalkova, A., Ginn, T. R., Spycher, N., Shende, R. V. and Peyton, B. M. (2010). The toxicity of lead to *Desulfovibrio desulfuricans* G20 in the presence of goethite and quartz. *Journal of Basic Microbiology*, 50, 160−170.

Santarpia, L., Grandone, I., Contaldo, F. and Pasanisi, F. (2013). Butyrylcholinesterase as a prognostic marker: a review of the literature. *Journal of Cachexia, Sarcopenia, and Muscle*, 4, 31–39.

Sarkar A., Ray, D., Shrivastava, A. N. and Sarker, S. (2006). Molecular Biomarkers: Their significance and application in marine pollution monitoring. *Ecotoxicology*, 15, 333–340.

Sarkarati, B., Çokuğraş, A. N. and Tezcan, E. F. (1999). Inhibition kinetics of human serum butyrylcholinesterase by Cd^{2+}, Zn^{2+} and Al^{3+}: comparison of the effects of metal ions on cholinesterases. *Comparative Biochemistry and Physiology, Part C*, 122, 181–190.

Sathesh Prabu, C. and Thatheyus, A. J. (2007). Biodegradation of acrylamide employing free and immobilized cells of *Pseudomonas aeruginosa*. *International Biodeterioration and Biodegradation*, 60, 69–73.

Scopes, R. (1993). Protein Purification: Principles and practice. Third Edition, New York: Springer, pp. 71–101.

Scopes, R. K. (1987). Protein purification, principles and practice. Second Edition, New York: Springer-Verlag.

Seidman, S. and Soreq, H. (2001). Acetylcholinesterase – new roles for an old factor. *Nature Reviews Neurosciences*, 2, 294–302.

Senger, M. R., Rico, E. P., de Bem Arizi, M., Frazzon, A. P., Dias, R. D. and Bogo, M. R. (2006). Exposure to Hg^{2+} and Pb^{2+} changes NTPDase and ecto-50-nucleotidase activities in central nervous system of zebrafish (*Danio rerio*). *Toxicology*, 226, 229–237.

Shah, M. D., Papoy, A. R., Ward, M. and Cooper, R. L. (2010). Roles of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, plasma membrane Ca^{2+}-ATPase and Na^{+}/Ca^{2+} exchanger in regulation of heart rate in larval *Drosophila*. *The Open Physiology Journal*, 3, 16–36.

Sharbidre, A. A., Metkari, V. and Patode, P. (2011). Effects of Diazinon on Acetylcholinesterase Activity and Lipid Peroxidation of *Poecilia reticulata*. *Research Journal of Environmental Toxicology*, 1–10.

Sharma, R. (2012). Enzyme Inhibition: Mechanisms and scope, enzyme inhibition and bioapplications, Prof. Rakesh Sharma (Ed.), InTech, Croatia, pp. 4–36.

Sheline, C. T., Ying, H. S., Ling, C. S., Canzoniero, L. M. and Choi, D. W. (2002). Depolarization-induced (65) zinc influx into cultured cortical neurons. *Neurobiology*, 10, 41–53.

Shugart, L. R. (2005). Biomarker Environmental. In: Enciclopedia of Toxicology, Wexler P. (Editor in Chief), Elselvier, New York, pp. 287–290.

Shukor, Y., Baharom, N. A., Rahman, F. A., Abdullah, M. P., Shamaan, N. A. and Syed, M. A. (2006). Development of a heavy metals enzymatic-based assay using papain. *Analytica Chimica Acta A*, 566, 283–289.

Silver, A. (1974). The biology of cholinesterase. Amsterdam: North-Holand Publishing Company.

Skouras, A., Broeg, K., Dizer, H., Von Westernhagen, H., Hansen, P. D. and Steinhagen, D. (2003). The use of innate immune responses as biomarkers in a programme of integrated biological effects monitoring on flounder *(Platichthys flesus)* from the southern North Sea. *Helgoland Marine Research*, 57, 190–198.

Smart, T. G. and Paoletti, P. (2012). Synaptic neurotransmitter-gated receptors. *Cold Spring Harbour Perspective in Biology*, 4, 1–26.

Solé, M., Vega, S. and Varo, I. (2012). Characterization of type "B" esterases and hepatic CYP_{450} isoenzimes in Senegalese sole for their further application in monitoring studies. *Ecotoxicology and Environmental Safety*, 78, 72–79.

Solé, M., Lobera, G., Aljinovica, B., Ríos, J., García de la Parrab, L. M., Maynoua, F. and Cartesa, J. E. (2008). Cholinesterases activities and lipid peroxidation levels in muscle from shelf and slope dwelling fish from the NW Mediterranean: Its potential use in pollution monitoring. *Science of The Total Environment*, 402, 306–317.

Somdare, P. O., Nwani, C. D., Nwadinigwe, A. O., Nwani, J. C., Odo, G. E., Ugbor, O. N., Ukonze, J. A. and Ezeibe, A. B. C. A. (2015). Fenthion induced toxicity and histopathological changes in gill tissue of freshwater African catfish, *Clarias gariepinus* (Burchell, 1822). *African Journal of Biotechnology*, 14, 2103–2113.

Son, J. Y., Shin, S., Choi, K .H. and Park, I. K. (2002). Purification of soluble acetylcholinesterase from Japanese quail brain by affinity chromatography. *The International Journal of Biochemistry and Cell Biology*, 34, 204–210.

Srivastava, N., Nigam, A. K., Kumari, U., Mittal, S. and Mittal, A. K. (2013). Inhibition and recovery of acetylcholinesterase activity in the gills of the carp, *Cirrhinus mrigala* exposed to 'Nuvan®'. *International Journal of Zoological Research*, 3, 1–10.

Stanton, P. (2004). HPLC of Peptides and Proteins. Methods in Molecular Biology. New Jersey: Humana Press; 2004.

Storm, J. E., Rozman, K. K. and Doull, J. (2000). Occupational exposure limits for 30 organophosphate pesticides based on inhibition of red blood cell acetylcholinesterase. *Toxicology*, 150, 1–29.

Sturm, A., Wogram, J., Segner, H. and Liess, M. (2000). Different sensitivity to organophosphates of acetylcholinesterase and butyrylcholinesterase from three-spined stickleback (*Gasterosteus aculeatus*): application in biomonitoring. *Environmental and Toxicology Chemosphere*, 19, 1607–1615.

Talesa, V., Contenti, S., Mangiabene, C., Pascolini, R. Rosr, G. and Principato, G. B. (1990). Propionylcholinesterase from murex brandaris: comparison with other invertebrate cholinesterases. *Comparative Biochemistry and Physiology C*, 96, 3943.

Tas, E. C., Filipuci, I., Cakir, D. T., Beyaztas, S., Sunlu, U., Togulga, M., Ozaydin, O. and Arslan, O. (2011). Heavy metal concentrations in tissues of edible fish (*Mullus barbatus* L., 1758) from the Candarli Bay (Turkey). *Fresenius Environmental Bulletin*, 20, 2834–2839.

Tham, L. G., Perumal, N., Syed, M. A., Shamaan, N. A. and Shukor, M. Y. (2009). Assessment of *Clarias batrachus* as a source of acetylcholinesterase (AChE) for the detection of insecticides. *Journal of Environmental Biology*, 30, 135–138.

The Star News Paper (2014). Malaysians eat more fish than Japanese, reveals study. Retrieved on 15 March 2015 at www.thestar.com.my.

Tortora, G. and Grabowski, S. R. (2003). Principles of anatomy and physiology, control system of the human body. Volume 3. John Wiley & Sons. United States.

Tougu, V. (2001). Acetylcholinesterase: Mechanism of catalysis and inhibition. *Current Meicinal Chemistry – Central Nervous System Agents*, 1, 155–170.

Toutant, J. P. (1989). Insect acetylcholinesterase: catalytic properties, tissue distribution and molecular forms. *Progress in Neurobiology*, 32, 423–446.

Tripathi, A. and Srivastava, U. C. (2007). Histoenzymological distribution of acetylcholinesterase in the cerebral hemispheres of indian wall lizard, *Hemidactylus flaviviridis*. *Annals of Neurosciences*, 14, 387.

Tripathi, A. and Srivastava, U. C. (2008). Acetylcholinesterase: A versatile enzyme of nervous system. *Annals of Neurosciences*, 15, 200.

Tsangaris, C., Papathanasiou, E. and Cotou, E. (2006). Assessment of the impact of heavy metal pollution from a ferro-nickel smelting plant using biomarkers. *Ecotoxicology and Environmental Safety*, 66, 232–243.

Unwin, N. (2013). Nicotinic acetylcholine receptor and the structural basis of neuromuscular transmission: insights from *Torpedo* postsynaptic membranes. *Quarterly Reviews of Biophysics*, 46, 283–322.

Valavanidis, A., Vlahogianni, T., Dassenakis, M. and Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology Environmental Safety*, 64, 178–189.

Vallee, B. L. and Falchuk, K. H. (1981). Zinc and gene expression. *Philosophical Transactions of the Royal Society of London: Biological Sciences*, 294, 185–197.

Van der Oost, R., Beyer, J. and Vermeulan, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*, 13, 57–149.

Vanderheyden, P. M., Demaegdt, H., Swales, J., Lenaerts, P. J., De Backer, J. P., Vogel, L. K. and Vauquelin, G. (2009). Synergistic inhibition of the enzymatic activity of aminopeptidase N by divalent metal ion chelators. *Fundamental and Clinical Pharmacology*, 20, 613–619.

Vidali, M. (2001). Bioremediation. An Overview. *Pure and Applied Chemistry*, 73, 1163–1172.

Wang, J., Liu, X. D. and Lu, J. (2012). The 18th biennial conference of international society for ecological modelling. Urban river pollution control and remediation. *Procedia Environmental Sciences*, 13, 1856–1862.

Wang, R., Yan, H. and Tang, X. (2006). Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. *Acta Pharmacologica Sinica*, 27, 1–26.

Weinbroum, A. A. (2005). Pathophysiological and clinical aspects of combat anticholinesterase poisoning. *British Medical Bulletin*, 72, 119–133.

Wogram, J., Sturm, A., Segner, H. and Liess, M. (2001). Effects of parathion on acetylcholinesterase, butyrylcholinesterase, and carboxylesterase in three-spined stickleback (*Gasterosteus aculeatus*) following short-term exposure. *Environmental Toxicology and Chemistry*, 20, 1528–1531.

Wong, C., Capel, P., Nowell, L. H. (2004). National-scale, field-based evaluation of Biota Sediment Accumulation Factor Model. *Environmental Science and Technology*, 35, 1709–1715.

Yang, Y. X., Niu, L. Z. and Li, S. N. (2011). Purification and studies on characteristics of cholinesterases from *Daphnia magna*. *Journal of Zhejiang University Science B*, 14, 325–335.

Yap, C. K. and Pang, B. H. (2011). Assessment of Cu, Pb and Zn contamination in sediment of north western Peninsular Malaysia by using sediment quality values and different geochemical indice. *Environmental Monitoring and Assessment,* 183, 23–39.