UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF TRIBUTYLTIN-RESISTANT BACTERIA FROM CONTAMINATED SURFACE SEDIMENT AT KONG KONG LAUT, JOHOR, MALAYSIA

ABUSSAMAD ABUBAKAR

FPAS 2016 5

ISOLATION AND CHARACTERIZATION OF TRIBUTYLTIN-RESISTANT BACTERIA FROM CONTAMINATED SURFACE SEDIMENTS AT KONG KONG LAUT, JOHORE, MALAYSIA

By

ABUBAKAR ABDUSSAMAD

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

April 2016

DEDICATION

This thesis is dedicated to my late father Abubakar Bala Adamu for encouraging me to embark on this MSc. program whom could not live long to witness its end. May Allah (SWT) forgive his sins and grant him Jannatul Firdaus.

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

ISOLATION AND CHARACTERIZATION OF TRIBUTYLTIN-RESISTANT BACTERIA FROM CONTAMINATED SURFACE SEDIMENTS AT KONG KONG LAUT, JOHORE MALAYSIA

By

ABUBAKAR ABDUSSAMAD

April 2016

Chairman : Ferdius @ Ferdaus Binti Mohamat Yusuff, PhD
Faculty : Enviromental Studies

Tributyltin (TBT) is a species of organotin compound (OTC) under butyltin (BT) group, used as antifouling biocide in boat and ships paints to prevents the attachments of marine organism on their hull surface. Tributytin is very toxic to variety of targeted and none targeted organisms and has high persistence in sediments (can stays for up to thirty (30) years). Despite its global ban in 2008 by the International Maritime Organization (IMO), high concentrations of TBT in sediments from fresh and marine waters in many places across the world including northwest coast of Portugal, Strait of Johore Malaysia, South Africa, Australia among others was reported years after the total global ban. Malaysia is one of the countries with busiest maritime activities and well documented with high level of TBT in the marine environments, thus there is an urgent need to remediate TBT contamination. This study aimed at isolating the best indigenous bacterial specie(s) from TBT and heavy metals contaminated sediment as a prospect in bioremediation of TBT contamination based on the TBT and heavy metals tolerance ability. Sediment samples were collected from TBT contaminated surface sediments of Kong Kong Laut area. Bacteria were isolated in Minimal Salt Media (MSM) and screened in different concentrations of TBT treated media (1000, 1200 and 1500 μg/L). Selected TBT-resistant isolates were identified using morphological, biochemical (Gram staining) and polymerase chain reaction (16S rRNA) techniques. Growth tolerance of the best isolate was ascertained in MSM treated with different TBT (500, 1000, 1500, 2000 and 2500 μg/L) and heavy metals (0, 1, 5, 15, 25, 40 and 100 mg/L) concentrations. The metals were copper, zinc, lead and cadmium. Thirty-five (35) bacterial colonies were isolated on MSM from the sediment samples. Fourteen (14) isolates (KK1, KK2, KK3, KK4, KK5, KK6, KK7, KK8, KK12, KK14, KK15, KK16, KK17 and KK18) were found to resisted 1000 μg/L of TBT, a concentration level above the reported contaminated level (790 μg/L) at the sampling area. All the 14 isolates were Gram negative and three (3) isolates (KK1, KK3 and KK7) amongst were found to resisted TBT up to 1200 μg/L. The 3 isolates were identified as *Citrobacter* sp. FIRD 1, *Klebsiella* sp. FIRD 2 and *Acinetobacter* sp. FIRD 3 with accession no KR027917, KR005669 and KR013742, respectively. This study managed to isolate, screened and identified. The best TBT-resistant bacterium from Kong Kong Laut area, named *Klebsiella* sp. FIRD 2, a new species with high growth tolerance against TBT (1500 μg/L) and heavy metals (Cu 25, Pb 40, Zn 100 and Cd 40 mg/L). This study established

that *Klebsiella* sp. FIRD 2 isolated from Kong Kong Laut Johore has great potentials to be used as a bioremediation agent of TBT because of its high growth tolerance to TBT and heavy metals contaminated media.

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

PENGASINGAN DAN PENCIRIAN BAKTERIA TAHAN-TRIBULTILTIMAH DARI PERMUKAAN SEDIMEN YANG TERCEMAR DI KONG KONG LAUT, JOHORE MALAYSIA

Oleh

ABUBAKAR ABDUSSAMAD

April 2016

Pengerusi : Ferdius @ Ferdaus Binti Mohamat Yusuff, PhD
Fakulti : Pengajian Alam Sekitar

Tributiltimah (TBT) adalah spesies kompaun organotimah (OTC) di bawah kumpulan butiltimah (BT), yang digunakan sebagai biosida cegah-kebusukan dalam cat bot dan kapal-kapal untuk menghalang lekatan organisma marin di permukaan badan kapal. Tributiltimah adalah sangat toksik kepada pelbagai organisma sasaran dan bukan sasaran serta mempunyai ketahanan yang tinggi dalam sedimen (boleh kekal sehingga tiga puluh (30) tahun). Walaupun pengharaman diperingkat global oleh Pertubuhan Maritim Antarabangsa (IMO) pada tahun 2008, namun kepekatan TBT masih sangat tinggi dalam sedimen air tawar dan laut di banyak tempat di seluruh dunia termasuk pantai barat laut Portugal, Selat Johor Malaysia, Afrika Selatan dan Australia. Malaysia adalah salah satu negara mempunyai aktiviti maritim paling sibuk dan telah didokumentasikan mempunyai kepekatan TBT dalam persekitaran marin, dengan itu terdapat keperluan segera untuk mengatasi pencemaran TBT. Kajian ini bertujuan untuk mengasingkan spesies bakteria asal terbaik daripada sedimen yang dicemari TBT dan logam berat sebagai prospek dalam pemulihan persekitaran marin dicemari TBT berdasarkan keupayaan toleransi bakteria terhadap TBT dan logam berat. Sampel sedimen dikumpulkan dari sedimen permukaan kawasan dicemari TBT di Kong Kong Laut. Bakteria telah diasingkan dalam Media Begaram Minimum (MSM) dan didedahkan dengan media yang dirawat dengan TBT pada kepekatan yang berbeza (1000, 1200 dan 1500 µg/L). Asingan tahan TBT yang dipilih telah dikenal pasti berdasarkan teknik morfologi, biokimia (pewarnaan Gram) dan tindak balas rantaian polimer (16S rRNA). Toleransi pertumbuhan asingan yang terbaik telah ditentukan dengan MSM yang dirawat dengan TBT berkepekatan berbeza (500, 1000, 1500, 2000 dan 2500 µg/L) dan logam berat berkepekatan berbeza (0, 1, 5, 15, 25, 40 dan 100 mg/L). Logam yang dipilih adalah tembaga, zink, plumbum dan kadmium. Tiga puluh lima (35) koloni bakteria ttelah diasing daripada sampel sedimen pada MSM. Empat belas (14) asingan (KK1, KK2, KK3, KK4, KK5, KK6, KK7, KK8, KK12, KK14, KK15, KK16, KK17 dan KK18) didapati tahan 1000 µg/L TBT, tahap kepekatan di atas tahap yang dilaporkan mencemari (790 µg/L) kawasan persampelan. Kesemua 14 asingan adalah Gram negatif dan tiga (3) asingan (KK1, KK3 dan KK7) di kalangannya didapati tahan TBT sehingga 1200 µg/L. Tiga (3) pencilan telah dikenal pasti sebagai *Citrobacter* sp. FIRD 1, *Klebsiella* sp. FIRD 2 dan *Acinetobacter* sp. FIRD 3 dengan nombor kesertaan masing-masing KR027917, KR005669 dan KR013742. Kajian ini berjaya mengasing, menyaring dan mengenal pasti bakteria terbaik tahan TBT dari kawasan Kong Kong

Laut, dinamakan *Klebsiella* sp. FIRD 2, iaitu satu spesies baru bertumbuh dengan toleransi yang tinggi terhadap TBT (1500 µg/L) dan logam berat (Cu 25, Pb 40, Zn 100 dan Cd 40 mg/L). Kajian ini menetapkan bahawa *Klebsiella* sp. FIRD 2 yang diasingkan daripada Kong Kong Laut, Johor mempunyai potensi yang besar untuk digunakan sebagai agen pemulihan persekitaran dicemari TBT kerana toleransi pertumbuhan yang tinggi kepada media dicemari TBT dan logam berat.

ACKNOWLEDGEMENTS

All thanks and glory to almighty Allah for sparing my life and for blessing me with all the opportunities in life up till this moment. My sincere and heartfelt thanks goes to my supervisors Dr. Ferdius @ Ferdaus Mohamat Yusuff, for her unending motherly support and guide throughout my work and beyond. To my co-supervisors; Associate Prof. Dr. Muskazli B. Mustafa, and Dr. Wan Lutfi Wan Johari for their objective criticism and guidance.

My appreciations are always with my sponsors, Bauchi State University, Gadau, My uncles Alh. (Dr) Ahmadu A. Muazu (Walin Bauchi) and Honorable Justice Ahmad Ramat Muhammed. I cannot finish without acknowledging the tremendous help and guide from all my lab members and my friends here in Malaysia for always being there for me. Their contributions were immeasurably contributed to the success of this work.
This acknowledgement would never be complete without mentioning one of the prayer warriors for the success of this journey, my caring mother Hajia Khadija Abubakar Adam (Umma). A special thanks goes to my step mum, siblings, friends, cousins, relatives and well-wishers for touching my life positively.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Ferdius @ Ferdaus Binti Mohamat Yusuff, PhD
Senior Lecturer
Faculty of Environmental Studies
Universiti Putra Malaysia
(Chairperson)

Muskazli B Mustafa, PhD
Associate Professor
Faculty of Sciences
Universiti Putra Malaysia
(Member)

Wan Lutfi Wan Johari, PhD
Senior Lecturer
Faculty of Environment Studies
Universiti Putra Malaysia
(Member)

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

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Ferdius @ Ferdaus Binti Mohamat Yusuff, PhD

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Muskazli B Mustafa

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Ferdius @ Ferdaus Binti Mohamat Yusuff, PhD

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Muskazli B Mustafa

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
%	Percentage
rpm	Revolution Per Minute
g	Gram
TBT	Tributyltin
DBT	Dibutyltin
MBT	Monobutyltin
µg	Microgram
L	Liter
OT	Organotin
BT	Butyltin
rRNA	Ribosomal Nucleic Acid
sp	Species
EFSA	European Food Safety Authority
ADI	Acceptable Daily Intake
TDI	Tolerable Daily Intake
CO_2	Carbon dioxide
ISO	The International Standard Organization
DNA	Deoxyribonucleic Acid
KK	Lab tagging for Isolates
IMO	International Maritime Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Tributyltin (TBT) is an organotin (OT) compound that has been largely used as part of antifouling biocide in boat and ships paints to restrain the attachment of marine organism on the hull surface of marine structures (Blanca Antizar-Ladislao, 2008). Organotin compounds are group of chemicals that have at least one tin–carbon bond, joined covalently (Rüdel, 2003). Tributyltin was classified as a very toxic compound, which poses danger to a wide range of organisms at the polluted environments due to the high concentrations (Fent, 1996). Leachates of tributyltin (TBT) from antifouling-based paint chemically bind themselves with suspended materials and deposited in benthic sediments via accumulation process. Accumulation of TBT in sediments is the cause of ongoing pollution sources because the residues from these compounds were slowly released back into aquatic phase (Chiron *et al.*, 2000). Tributyltin can be remobilised into aquatic phase by frequent disturbance of the contaminated sediments (Hussein *et al*., 2011). Concern arise as this disturbance activities could further recharge the sediments with TBT as re-suspension events may increase at sites that not yet widely affected by TBT (Svavarsson *et al.,* 2001) since the sediments act as 'reservoir' of the pollutants (Apitz *et al.,* 2005) (Zulkifli *et al.,* 2010). Therefore, site with highly contaminated with TBT requires management for remediation.

Serious organotin (OT) contamination, especially from TBT, was reported in the busy Straits of Malacca and the Strait of Johor (Sudaryanto *et al.,* 2004). Later study conducted by Harino *et al.* (2007) found that concentration of monobutyltin (MBT), dibutyltin (DBT) and TBT in sediments from coastal areas of Peninsular Malaysia within the ranged from 4 to 242 µg/kg dry weight, from 1 to 186 µg/kg dry weight and from 0.7 to 228 µg/kg dry weight, respectively (Harino *et al*., 2007). The author reported that highest concentration of MBT, DBT and TBT were found in samples collected from the Strait of Johor (MBT, DBT and TBT ranging from 83 to 542 µg/kg dry weight, from 30 to 232 µg/kg dry weight and from 41 to 492 µg/kg dry weight) respectively (Harino *et al*., 2007). The level of OT in marine mollusc (*Thais gradata*) from the Straits of Johor and southern part of the Straits of Malacca has been also reported at a high level (Mohamat-Yusuff *et al*., 2010). Its presence above the threshold limit of a particular species could cause some adverse effects such as imposex among female neogastropods (Ismail *et al*., 2004; Mohamat-Yusuff *et al*., 2010, 2011) and even up to carcinogenic effects with no significant evidence of immunotoxicity (Lee *et al*., 2005). Among all sites reported in the literature, Kong Kong Laut area in Johor could be labeled as the hot–spot of TBT contamination since the marine mollusk collected in this site experience with high level of imposex incidence and accumulated with high level of TBT. This was supported by recent study by Mohamat-Yusuff *et al.* (2013), TBT contaminations in sediments at Kong Kong Laut was as high as 790 µg/L even after the total global banning on the year 2008**.**

Persistent of high concentrations of TBT in sediments from marine and fresh waters in many places across the world including the Strait of Johor, Malaysia even after the first global banning of the year 2003 (Harino *et al.*, 2008; Sakultantimetha *et al.*, 2011) and total global banging on the year 2008 (Mohamat-Yusuff *et al.*, 2013) has rose concern among scientist around the globe. The longer it stays, the higher the possibilities it transport up the food chain and end up in our diet. Unlike in non-tropical developed nations, there is still limited information on TBT degradation by microorganism in tropical countries particularly in Malaysia. Over the years, France, United Kingdom, and United States, many examples of TBT contamination and its degradation have been narrated (Cassi *et al.*, 2008; Kawai *et al.*, 1998). A potential TBT-degrading bacterium isolated from sediment/water from country of Ria de Aveiro (Portugal) successfully established by Cruz *et al.* (2007). The bacterium was identified as *Aeromonas veronii* Av27 which found tolerance to TBT up to 3 mM (Cruz *et al.*, 2007). The same author also reported that Gram-negative bacteria have more tolerance ability to TBT than Gram-positive bacteria (Cruz *et al.*, 2007). Similar works has been done by Adelaja and Keenan (2012). However, the work by Adelaja and Keenan (2012) isolated TBT-resistant bacteria and tested it for growth in the presence of methylmercury (MeHg). The isolated bacteria identified as *Pseudomonas flouresens*, *Enterobacter cloacae*, *Citrobacter braakii* and *Alcaligenes faecalis* were found to have great potentials to detoxify not only the TBT but also MeHg (Adelaja and Keenan, 2012). These bacteria could utilize carbon sources from the TBT compounds and recommended *Enterobacter cloacae* as the most preferable isolate for potential TBT and MeHg degradation (Adelaja and Keenan, 2012).

Another related study utilizing microorganism as biodegrading agent of TBT was the study of fungus, *Cunninghamella elegans* grown in Sabouraud medium added with 20 mg/L of TBT (Bernat and Długo, 2002). The study found that 70% of the chemical (TBT) was transformed into two less toxic forms (DBT and MBT) after 7 days of experiment. Concentration above than 20 mg/L was reported inhibited the growth of the fungi.

Even though microorganisms particularly bacteria and fungi, are natural degraders in their respective natural environments or elsewhere (Wuertz *et al.*, 1991), the ability to degrade pollutants in the environments could be slow due to many environmental dynamics. However, this process could be enhanced by the clever remediation technology (Abalos *et al.*, 2004). Therefore, invites for the need to isolate and study wild type microorganism, in order to explore their full potential in bioremediation process. Owing to limited information on TBT degradation in Malaysia and other tropical areas, this study is expected to make a significant contribution to studies on TBT remediation in tropical environments. Thus this work was done to identify the best indigenous bacterial candidate that has high resistance to extreme concentrations of Tributyltin (TBT) and to determine its unique characteristics as a prospect in biotechnology and bioremediation.

1.2 Problem Statement

TBT contamination is an issue of international concern because of its slow degradation. Many contaminated areas around the world are still required to be remediated years after the global ban. When deposited in sediment, TBT can stay up to 30 years and serves as

secondary source of contamination (Bray, 2006; Chiron *et al.,* 2000). In Malaysia, TBT concentrations in marine environment and the occurrence of imposex is higher and more serious than in other parts of Southeast Asia (Harino *et al.,* 2008). Above all, Kong Kong Laut at Johore has highest imposex incidence and highest TBT accumulation in the snails as compared to other sites in the southern part of Peninsular Malaysia (Mohamat-Yusuff *et al.,* 2010). In recent works, concentration of TBT in collected sediments from this sites has been reported up to 790 µg/L (Mohamat-Yusuff *et al.*,2013). TBT was found to have deleterious effects on both prokaryotic and eukaryotic organisms (B Antizar-Ladislao, 2008) while in humans it was found to inhibit the immune system and causes endocrine disruption (Dubey *et al.,* 2006). Tributyltin has been found to accumulate in the body of several marine organisms and these organisms are very important sources of food for human as well as other organisms and TBT was found to be carcinogenic to humans. Therefore, it is a need to remediate this compound in this area. Considering works on heavy metals by Zulkifli *et al*. (2010) and Wan and Ferdaus (2014) revealed that this particular site also experience heavy metal contamination. Thus, prior for the remediation works, identification and establishment of the bioremediation agent fit with the local condition especially tolerance with heavy metals must be done.

1.3 Scope of the study

Due to the fact that bioremediation using indigenous bacteria has been proposed as the most economically efficient and effective, this study focuses on the isolation of wild type TBT-resistant bacteria from Kong Kong Laut, the hotspot of TBT polluted area in Malaysia, identification of the morphology of bacteria and morphology of the colony, species identification of the best candidates using molecular technique and determination of the growth pattern in extreme concentrations of TBT along with tolerance ability with heavy metals.

1.4 General Objective

This study was aimed at isolating the best bacterial specie(s) as a prospect in bioremediation of TBT contamination based on their TBT and heavy metal tolerance ability.

1.5 Specific Objectives of the Study

This study embarks on the following specific objectives:

1. To isolate, screen and identify TBT-resistant bacteria from surface sediments of Kong Kong Laut, Johore.
2. To determine the growth performance of the best TBT-resistant bacterial species in different concentration of TBT treated media.
3. To ascertain the tolerance ability of the best TBT-resistant bacterial species in in different concentrations of heavy metals treated media.

REFERENCES

Abalos, a., Viñas, M., Sabaté, J., Manresa, a., & Solanas, a. (2004). Enhanced biodegradation of Casablanca crude oil by a microbial consortium in precence of rhamnolipid produced by *Pseudomonas aeruginosa. AT10 Biodegradation*, *15*, 249–260.

Acinas, S. G., Rodríguez-Valera, F., & Pedrós-Alió, C. (1997). Spatial and temporal variation in marine bacterioplankton diversity as shown by RFLP fingerprinting of PCR amplified 16S rDNA. *FEMS Microbiology Ecology*, *24*(1), 27–40.

Adelaja, O. A., & Keenan, H. E. (2012). Tolerance of TBT-resistant Bacteria Isolates to Methylmercury. *Reasearch Jounal of Environmental Science*, *6*(1), 1–13.

Albalat, A., Potrykus, J., Pempkowiak, J., & Porte, C. (2002). Assessment of organotin pollution along the Polish coast (Baltic Sea) by using mussels and fish as sentinel organisms. *Chemosphere*, *47*(2), 165–71.

Anderson, T. A., Guthrie, E. A., & Walton, B. T. (1993). Bioremediation. *Journal of Environmental Science Technology*, *27*, 2630–2636.

Antizar-Ladislao, B. (2008). Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environmental International*, *34*(2), 292–308.

Antizar-Ladislao, B. (2008). Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. a review. *Environment International*, *34*(2), 292–308.

Apitz, S. E., Davis, J. W., Finkelstein, K., Hohreiter, D. W., Hoke, R., Jensen, R. H., Stahl, R. G. (2005). Assessing and managing contaminated sediments: part I, developing an effective investigation and risk evaluation strategy. *Integrated Environmental Assessment and Management*, *1*(1), 2–8.

Appel, K. E. (2004). Organotin compounds: toxicokinetic aspects. *Drug Metabolism Reviews*, *36*(3-4), 763–786.

Arai, T., Harino, H., Ohji, M., & William, J. L. (2009). *Ecotoxicology of Antifouling Biocides*. (T. A. • H. Harino, M. O. • W. J. L. Editors, & Ecotoxicology, Eds.). Japan.

Awai, S. K., Rai, T. A., Hji, M. O., Amato, S. Y., Obayashi, N. K., & Iyazaki, N. M. (2008). Distribution of Organotin Compounds in Representative Coastal Areas from Japan : A Review, *32*(1), 88–95.

Bacun-Druzina, V., Butorac, A., Mrvcic, J., Dragicevic, T. L., & Stehlik-Tomas, V. (2011). Bacterial Stationary-Phase Evolution. *Food Technol. Biotechnol.*, *49*(1), 13–23.

Baker, G. C., Smith, J. J., & Cowan, D. A. (2003). Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods*, *55*, 541–55.

Barug, D. (1981). Microbial degradation of bis(tributyltin) oxide. *Chemosphere*, *10*(10), 1145–1154.

Bergey, D. H., John, G. H., Noel, R. K., & Peter, H. A. S. (1994). Bergey's Manual of Determinative Bacteriology (9th ed.). Lippincott Williams & Wilkins. ISBN 0-683-00603-7.

Bernat, P., & Długo, J. (2002). Degradation of tributyltin by the filamentous fungus Cunninghamella elegans , with involvement of cytochrome P-450, 1971–1974.

Bernat, P., & Długoński, J. (2006). Acceleration of tributyltin chloride (TBT) degradation in liquid cultures of the filamentous fungus Cunninghamella elegans. *Chemosphere*, *62*(1), 3–8..

Bernat, P., Siewiera, P., Soboń, A., & Długoński, J. (2014). Phospholipids and protein adaptation of Pseudomonas sp. to the xenoestrogen tributyltin chloride (TBT). *World Journal of Microbiology and Biotechnology*, *30*(9), 2343–2350.

Berto, D., Giani, M., Boscolo, R., Covelli, S., Giovanardi, O., Massironi, M., & Grassia, L. (2007). Organotins (TBT and DBT) in water, sediments, and gastropods of the southern Venice lagoon (Italy). *Marine Pollution Bulletin*, *55*(10-12), 425–35.

Bestawy, E. E., Helmy, S., Hussien, H., Fahmy, M., & Amer, R. (2012). Bioremediation of heavy metal-contaminated effluent using optimized activated sludge bacteria. *Applied Water Science*, *3*(1), 181–192.

Bettin, C., Oehlmann, J., & Stroben, E. (1996). TBT-induced imposex in marine neogastropods is mediated by an increasing androgen level. *Helgoländer Meeresuntersuchungen*, *50*(3), 299–317.

Bollag, J. M., & Lie, S. Y. (1990). Biological transformation of pesticides. In: pesticides in the soil environment. Chang, H.H (Ed), Soil Science Society of America, Madison (pp. 169–211).

Bravo, M., Lespes, G., De Gregori, I., Pinochet, H., & Gautier, M. P. (2005). Determination of organotin compounds by headspace solid-phase microextraction-gas chromatography-pulsed flame-photometric detection (HS-SPME-GC-PFPD). *Analytical and Bioanalytical Chemistry*, *383*(7-8), 1082–9.

Bray, S. (2006). *Tributyltin pollution on a global scale. An overview of relevant and recent research: impacts and issues. Contract*. UK.

Bryan, G. W., Gibbs, P. E., Burt, G. R., & Hummerstone, L. G. (2009). The effects of tributyltin (TBT) accumulation on adult dog-whelks, Nucella lapillus: long-term field and laboratory experiments. *Journal of the Marine Biological Association of the United Kingdom*, *67*(03), 525.

Buck-Koehntop, B. a., Porcelli, F., Lewin, J. L., Cramer, C. J., & Veglia, G. (2006). Biological chemistry of organotin compounds: Interactions and dealkylation by dithiols. *Journal of Organometallic Chemistry*, *691*(8), 1748–1755.

Cappucino, J. G., & Sherman, N. (2005). Microbiology: A Loboratory Manual 7th Edition. Pearson Education, Inc, San Francisco. (pp. 45–47).

Cassi, R., Tolosa, I., & de Mora, S. (2008). A survey of antifoulants in sediments from Ports and Marinas along the French Mediterranean coast. *Marine Pollution Bulletin*, *56*(11), 1943–8.

Cervantes, C., & Gutierrez-Corona, F. (1994). Copper resistance mechanisms in bacteria and fungi. *FEMS Microbiology Reviews*, *14*(2), 121–137.

Champ, M. a. (2000). A review of organotin regulatory strategies, pending actions, related costs and benefits. *Science of the Total Environment*, *258*(1-2), 21–71.

Chiron, S., Roy, S., Cottier, R., & Jeannot, R. (2000). Speciation of butyl- and phenyltin compounds in sediments using pressurized liquid extraction and liquid chromatography–inductively coupled plasma mass spectrometry. *Journal of Chromatography A*, *879*(2), 137–145.

Choudhury, R., & Srivastava, S. (2001). Zinc resistance mechanisms in bacteria. *Current Science*, *81*(7), 768–775.

Clarridge, J. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews*, *17*, 840–62.

Coelho, M. R., Bebianno, M. J., & Langston, W. J. (2002). Routes of TBT uptake in the clam Ruditapes decussatus. II. Food as vector of TBT uptake. *Marine Environmental Research*, *54*(2), 193–207.

Cooke, G. M. (2002). Effect of organotins on human aromatase activity in vitro. *Toxicology Letters*, *126*(2), 121–130.

Cooksey, D. a. (1993). Copper uptake and resistance in bacteria. *Molecular Microbiology*, *7*(1), 1–5.

Cruz, A., Anselmo, A. M., Suzuki, S., & Mendo, S. (2015). Tributyltin (TBT): A Review on Microbial Resistance and Degradation. *Critical Reviews in Environmental Science and Technology*, *45*(9), 970–1006.

Cruz, A., Caetano, T., Suzuki, S., & Mendo, S. (2007). Aeromonas veronii, a tributyltin (TBT)-degrading bacterium isolated from an estuarine environment, Ria de Aveiro in Portugal. *Marine Environmental Research*, *64*(5), 639–50.

Cruz, A., Henriques, I., Sousa, A. C. a, Baptista, I., Almeida, A., Takahashi, S., … Mendo, S. (2014). A microcosm approach to evaluate the degradation of tributyltin (TBT) by Aeromonas molluscorum Av27 in estuarine sediments. *Environmental Research*, *132*, 430–437. d

Cruz, A., Moreira, R., & Mendo, S. (2013). An easy, rapid and inexpensive method to monitor tributyltin (TBT) toxicity in the laboratory. *Folia Microbiologica*, 0–4.

Dafforn, K. a, Lewis, J. a, & Johnston, E. L. (2011). Antifouling strategies: history and regulation, ecological impacts and mitigation. *Marine Pollution Bulletin*, *62*(3), 453–65.

Devereux, R., & Wilkinson, S. S. (2004). Amplification of ribosomal RNA sequences,”

A. D. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn, Eds., Kluwer Academic, Dodrecht, The Netherlands, 2nd edition. In *Molecular Microbial Ecology Manual* (pp. 1–17).

Dikshith, T. S. S. (2013). *Hazardous Chemicals: Safety Management and Global Regulations*. CRC Press. Retrieved from https://books.google.com/books?id=rpBE8zAQrPQC&pgis=1

Drancourt, M., Bollet, C., Carlioz, A., Martelin, R., Gayral, J. P., & Raoult, D. (2000). 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *Journal of Clinical Microbiology*, *38*(10), 3623–3630.

Du, J., Chadalavada, S., Chen, Z., & Naidu, R. (2014). Environmental remediation techniques of tributyltin contamination in soil and water: A review. *Chemical Engineering Journal*, *235*, 141–150.

Dubey, S. K., Tokashiki, T., & Suzuki, S. (2006). Microarray-mediated transcriptome analysis of the tributyltin (TBT)-resistant bacterium Pseudomonas aeruginosa 25W in the presence of TBT. *Journal of Microbiology*, *44*(2), 200–205.

Fent, K. (1996). Organotin compounds in municipal wastewater and sewage sludge: contamination, fate in treatment process and ecotoxicological consequences. *Science of The Total Environment*, *185*(1-3), 151–159.

Fent, K., & Looser, P. W. (1995). Bioaccumulation and bioavailability of tributyltin chloride: Influence of pH and humic acids. *Water Research*, *29*(7), 1631–1637.

Gadd, G. M. U. (2000). Microbial interactions with tributyltin compounds : detoxification , accumulation , and environmental fate.

Gianguzza, A. (1997). *Organotin — environmental fate and effects*. *Marine Chemistry* (Vol. 57). Springer Science & Business Media.

Gipperth, L. (2009). The legal design of the international and European Union ban on tributyltin antifouling paint: direct and indirect effects. *Journal of Environmental Management*, *90 Suppl 1*(September 2007), S86–95.

Gokulakrishnan, S., & Gummadi, S. N. (2006). Kinetics of cell growth and caffeine utilization by Pseudomonas sp. GSC 1182. *Process Biochemistry*, *41*(6), 1417–1421.

Guérin, T., Sirot, V., Volatier, J.-L., & Leblanc, J.-C. (2007). Organotin levels in seafood and its implications for health risk in high-seafood consumers. *The Science of the Total Environment*, *388*(1-3), 66–77.

Gueuné, H., Thouand, G., & Durand, M.-J. (2009). A new bioassay for the inspection and identification of TBT-containing antifouling paint. *Marine Pollution Bulletin*, *58*(11), 1734–8.

Guinn, D. A., Tumeo, M. A., & Braddock, J. F. (1996). Isolation and use of indigenous bacteria for bioremediation of soil from a former wood treatment site in southwestern Alaska. Retrieved from http://www.osti.gov/scitech/biblio/395276

Gummadi, S. N., Bhavya, B., & Ashok, N. (2012). Physiology, biochemistry and possible applications of microbial caffeine degradation. *Applied Microbiology and Biotechnology*, *93*(2), 545–54.

Harino, H., Arai, T., Ohji, M., Ismail, A. Bin, & Miyazaki, N. (2008). Organotin Contaminations in Malaysia. *Coastal Marine Science*, *32*(1), 96–101.

Harino, H., Fukushima, M., Kurokawa, Y., & Kawai, S. (1997). Degradation of the tributyltin compounds by the microorganisms in water and sediment collected from the harbour area of Osaka City, Japan. *Environmental Pollution*, *98*(2), 163–167.

Harino, H., Ohji, M., Wattayakorn, G., Adulyanukosol, K., Arai, T., & Miyazaki, N. (2007). Concentrations of organotin compounds in tissues and organs of dugongs from Thai coastal waters. *Archives of Environmental Contamination and Toxicology*, *53*(3), 495–502.

Hashimoto, S., Watanabe, M., Noda, Y., Hayashi, T., Kurita, Y., Takasu, Y., & Otsuki, A. (1998). Concentration and distribution of butyltin compounds in a heavy tanker route in the strait of Malacca and in Tokyo Bay. *Marine Environmental Research*, *45*(2), 169–177.

Hershkovitz, M. a, & Leipe, D. D. (1998). Phylogenetic analysis. In *Methods of biochemical analysis* (Vol. 39, pp. 189–230).

Hoch, M. (2001). Organotin compounds in the environment Ð an overview. *Applied Geochemistry*, *16*.

Hong, H. B., Nam, I. H., Kim, Y. M., Chang, Y. S., & Schmidt, S. (2007). Effect of heavy metals on the biodegradation of dibenzofuran in liquid medium. *Journal of Hazardous Materials*, *140*(1-2), 145–148. doi:10.1016/j.jhazmat.2006.06.049

Hong, H.-K., Takahashi, S., Min, B.-Y., & Tanabe, S. (2002). Butyltin residues in blue mussels (Mytilus edulis) and arkshells (Scapharca broughtonii) collected from Korean coastal waters. *Environmental Pollution*, *117*(3), 475–486.

Huang, J., Ye, J., Ma, J., Gao, J., Chen, S., & Wu, X. (2014). Triphenyltin biosorption, dephenylation pathway and cellular responses during triphenyltin biodegradation by Bacillus thuringiensis and tea saponin. *Chemical Engineering Journal*.

Hynninen, A. (2010). *Zinc , cadmium and lead resistance mechanisms in bacteria and their contribution to biosensing*.

Ibrahim, S., Shukor, M. Y., Syed, M. A., Arina, Nor Rahman, A., Abdul Khalil, K., & Ahmad, S. A. (2014). Bacterial Degradation of Caffeine : A Review. *Asian Journal of Plant Biology*, *2*(1), 24–33.

Inoue, K., & Miyazaki, N. (2011). Pollution of hazardous chemicals in the coastal marine environment and their ecological effect (Project-4). *Atmosphere and Ocean Research Institute*, 87–101.

Intorne, A. C., de Oliveira, M. V. V, de M Pereira, L., & de Souza Filho, G. a. (2012). Essential role of the czc determinant for cadmium, cobalt and zinc resistance in Gluconacetobacter diazotrophicus PAl 5. *International Microbiology*, *15*(2), 69–

78.

Iwata, H., Tanabe, S., Mizuno, T., & Tatsukawa, R. (1995). High accumulation of toxic butyltins in marine mammals from japanese coastal waters. *Environmental Science & Technology*, *29*(12), 2959–2962.

Jeong, B. G., Hong, S. W., Choi, Y. S., Kumaran, R. S., Kim, M., Kim, S., & Kim, H. J. (2011). Isolation of tributyltin chloride resistance bacteria and rapid electrochemical determination of bacterial organotin degradation activity. *Bulletin of the Korean Chemical Society*, *32*(1), 356–358.

Jude, F., Capdepuy, M., Quentin, C., Lascourrèges, J. F., & Caumette, P. (1996, June). Evaluation of tributyltin resistance in marine sediment bacteria. *Canadian Journal of Microbiology*.

Kanazawa, S., & Mori, K. (1996). Isolation of Cadmium-Resistant Bacteria and Their Resistance Mechanisms. *Soil Science and Plant Nutrition*, *42*(4), 731–736.

Kannan, K., Grove, R. A., Senthilkumar, K., Henny, C. J., & Giesy, J. P. (1999). Butyltin Compounds in River Otters (Lutra canadensis) from the Northwestern United States. *Archives of Environmental Contamination and Toxicology*, *36*(4), 462–468.

Kawai, S., Kurokawa, Y., Harino, H., & Fukushima, M. (1998). Degradation of tributyltin by a bacterial strain isolated from polluted river water. *Public Health*, *102*(2-3), 259–263.

Khanolkar, D. S., Dubey, S. K., & Naik, M. M. (2015). Biotransformation of tributyltin chloride to less toxic dibutyltin dichloride and monobutyltin trichloride by Klebsiella pneumoniae strain SD9. *International Biodeterioration & Biodegradation*, *104*, 212–218.

Khanolkar, D. S., Naik, M. M., & Dubey, S. K. (2014). Biotransformation of Tributyltin chloride by Pseudomonas stutzeri strain DN2. *Brazilian Journal of Microbiology : [Publication of the Brazilian Society for Microbiology]*, *1245*(4), 1239–1245.

Kotrikla, A. (2009). Environmental management aspects for TBT antifouling wastes from the shipyards. *Journal of Environmental Management*, *90 Suppl 1*, S77–85.

Landmeyer, J. E., Tanner, T. L., & Watt, B. E. (2004). Biotransformation of tributyltin to tin in freshwater river-bed sediments contaminated by an organotin release. *Environmental Science and Technology*, *38*(15), 4106–4112.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. a, McWilliam, H., … Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics (Oxford, England)*, *23*(21), 2947–8.

Laughlin, J., & B., R. (1996). *OrganotinBioaccumulation of TBT by Aquatic Organisms*. (M. A. Champ & P. F. Seligman, Eds.). Dordrecht: Springer Netherlands.

Lima de Silva, A. A., de Carvalho, M. A. R., de Souza, S. A. L., Dias, P. M. T., da Silva Filho, R. G., de Meirelles Saramago, C. S., … Hofer, E. (2012). Heavy metal tolerance (Cr, Ag AND Hg) in bacteria isolated from sewage. *Brazilian Journal of*

Microbiology : [Publication of the Brazilian Society for Microbiology], *43*(4), 1620–31.

Lo, S., King, I., Alléra, A., & Klingmüller, D. (2007). Effects of various pesticides on human 5alpha-reductase activity in prostate and LNCaP cells. *Toxicology in Vitro : An International Journal Published in Association with BIBRA*, *21*(3), 502–8.

Lovley, D. R. (1993). Dissimilatory metal reduction. *Annual Review of Microbiology*, *47*, 263– 290.

Lovley, D. R. (2003). Cleaning up with genomics: applying molecular biology to bioremediation. Nature reviews. *Microbiology*, *1*(1), 35–44.

Lu, J. J., Perng, C. L., Lee, S. Y., & Wan, C. C. (2000). Use of PCR with universal primers and restriction Endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. *Journal of Clinical Microbiology*, *8*, 2076–2080.

Lutfi, W., Johari, W., Ghazali, N., Yunus, M., & Shukor, A. (2014). Biodecolorization of Azo Dyes by Microorganisms Isolated From Serdang and Merambong Soils. *Bioremediation Science & Technology Research*, *2*(1), 5–8.

Madigan, M. T., Martinko, J., & Parker, J. (2004). Brock Biology of Microorganisms (10th ed.). Lippincott Williams & Wilkins. (pp. ISBN 0–13–066271–2).

Maguire, R. J., Wong, P. T. S., & Rhamey, J. S. (1984). Accumulation and Metabolism of Tri- n -butyltin Cation by a Green Alga, Ankistrodesmus falcatus. *Canadian Journal of Fisheries and Aquatic Sciences*, *41*(3), 537–540.

Maidak, B. L., Larsen, N., McCaughey, M. J., Overbeek, R., Olsen, G. J., Fogel, K., … Woese, C. R. (1994). The Ribosomal Database project. *Nucleic Acids Research*, *22*(17), 3485–3487.

Martins, J., Jurado, A., Moreno, A., & Madeira, V. (2005). Comparative study of tributyltin toxicity on two bacteria of the genus Bacillus. *Toxicology in Vitro*, *19*(7), 943–9.

Meador, J. P. (2000). Predicting the fate and effects of tributyltin in marine systems. *Reviews of Environmental Contamination and Toxicology*, *166*, 1–48.

Mohamat-Yusuff, F., Ahmad, I., Harino, H., Emi, Y., Selvahganesh, P., & Zulkifli, S. (2013). Concentration of organotin compounds and the correlationwith physicochemical parameter in core sediment from two diffent scale piers at the Strait of Johore. In *NRCT-JSPS Joint International Seminar on Coaltal Marine Science in Southeast Asia* (p. 87). Chiang Mai, Thailand.

Mohamat-Yusuff, F., Zulkifli, S. Z., Ismail, A., Harino, H., Yusoff, M. K., & Arai, T. (2010). Imposex in Thais gradata as a Biomarker for TBT Contamination on the Southern Coast of Peninsular Malaysia. *Water, Air, & Soil Pollution*, *211*(1-4), 443–457.

Munson, M. A., Banerjee, A., Watson, T. F., & Wade, W. G. (2004). Molecular analysis of the microflora associated with dental caries. *Journal of Clinical Microbiology*,

42, 3023–3029.

Naik, M. M., & Dubey, S. K. (2013). Lead resistant bacteria: Lead resistance mechanisms, their applications in lead bioremediation and biomonitoring. *Ecotoxicology and Environmental Safety*, *98*, 1–7.

Najirad, S., Alikhani, H. A., Hashemi, M. M., Naghavi, M. R., & Ghavidel, A. (2012). Hydrocarbon bioremediation efficiency by two indigenous bacterial strains in contaminated soils. *World Applied Sciences Journal*, *17*(6), 792–796.

Nath, S., Deb, B., & Sharma, I. (2012). Isolation and Characterization of Cadmium and Lead Resistant Bacteria. *Global Advanced Research Journal of Microbiology*, *1*(11), 2315–5116.

Nies, D. H. (1999). Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology*, *51*(6), 730–750.

Okoro, H. K., Fatoki, O. S., Adekola, F. a., Ximba, B. J., & Snyman, R. G. (2011). Sources, environmental levels and toxicity of organotin in marine environment - A review.

Owolabi, J. B., & Hekeu, M. M. (2015). Isolation and characterization of zinc resistant bacteria from a coil coating industrial wastewater treatment plant, *5*(5), 1030–1042.

Pain, A., & Cooney, J. J. (1998). Characterization of Organotin-Resistant Bacteria from Boston Harbor Sediments. *Archives of Environmental Contamination and Toxicology*, *35*(3), 412–416.

Park, K., Kim, R., Park, J. J., Shin, H. C., Lee, J. S., Cho, H. S., … Kwak, I.-S. (2012). Ecotoxicological evaluation of tributyltin toxicity to the equilateral venus clam, Gomphina veneriformis (Bivalvia: Veneridae). *Fish & Shellfish Immunology*, *32*(3), 426–33.

Rajendran, P., Muthukrishnan, J., & Gunasekaran, P. (2003). Microbes in heavy metal remediation. *Indian Journal of Experimental Biology*, *41*(9), 935–944.

Randhawa, G. J. (2013). *Isolation and Purification of Genomic DNA*.

Rolfe, M. D., Rice, C. J., Lucchini, S., Pin, C., Thompson, A., Cameron, A. D. S., … Hinton, J. C. D. (2012). Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *Journal of Bacteriology*, *194*(3), 686–701.

Roy, U., Dubey, S. K., & Bhosle, S. (2004). Tributyltin chloride-utilizing bacteria from marine ecosystem of west coast of India. *Current Science*, *86*(5), 702–705.

Roy, U., & Nair, D. (2007). Biodiversity of organotin resistant Pseudomonas from west coast of India. *Ecotoxicology*, *16*(2), 253–261.

Rüdel, H. (2003). Case study: bioavailability of tin and tin compounds. *Ecotoxicology and Environmental Safety*, *56*(1), 180–189.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for

reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425.

Sakultantimetha, A., Keenan, H. E., Beattie, T. K., Bangkedphol, S., & Cavoura, O. (2011). Bioremediation of tributyltin contaminated sediment: degradation enhancement and improvement of bioavailability to promote treatment processes. *Chemosphere*, *83*(5), 680–686.

Sambrook, J., F., F. E., & Maniatis, T. (1989). *Bacterial cell maintenance. Molecular cloning: a laboratory manual, vol. 1. New York: Cold Spring Harbor laboratory Press.*

Sangeeta, S. (2007). *Assessing impacts of TBT on multiple coastal uses*. *National Institute of Oceanography, India*.

Seligman, P. F., Valkirs, A. O., Stang, P. M., & Lee, R. F. (1988). Evidence for rapid degradation of tributyltin in a marina. *Marine Pollution Bulletin*, *19*(10), 531–534.

Sevgi, E., Coral, G., Gizir, A., & Sagun, M. (2009). Investigation of heavy metal resistance in some bacterial strains isolated from industrial soils. *Turk Journal of Biology*, *34*, 423–431.

Smith, B. (1971). Sexuality in the American mud-snail Nassarius obsoletus Say. *Proceedings of the Malacological Society of London*, *39*(5), 377–378.

Soumitesh, C., Danica, H., & David, A. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods*, *69*(2), 330–339.

Stasinakis, A. S., Thomaidis, N. S., Nikolaou, A., & Kantifes, A. (2005). Aerobic biodegradation of organotin compounds in activated sludge batch reactors. *Environmental Pollution (Barking, Essex : 1987)*, *134*(3), 431–8.

Stewart, C., & de Mora, S. J. (1990). A review of the degradation of tri(n -butyl)tin in the marine environment. *Environmental Technology*, *11*(6), 565–570.

Sturgeon, R. E., Wahlen, R., Brandsch, T., Fairman, B., Wolf-Briche, C., Alonso, J. I. G., … Willie, S. (2003). Determination of tributyltin in marine sediment: Comité Consultatif pour la Quantité de Matière (CCQM) pilot study P-18 international intercomparison. *Analytical and Bioanalytical Chemistry*, *376*(6), 780–7.

Suardana, I. W. (2014). Analysis of Nucleotide Sequences of the 16S rRNA Gene of Novel Escherichia coli Strains Isolated from Feces of Human and Bali Cattle. *Journal of Nucleic Acids*, *2014*, 475754.

Sudaryanto, A., Takahashi, S., Iwata, H., Tanabe, S., & Ismail, A. (2004). Contamination of butyltin compounds in Malaysian marine environments. *Environmental Pollution*, *130*(3), 347–358.

Sudaryanto, A., Takahashi, S., Monirith, I., Ismail, A., Muchtar, M., Zheng, J., … Tanabe, S. (2002). Asia-Pacific mussel watch: Monitoring of butyltin contamination in coastal waters of Asian developing countries. *Environmental Toxicology and Chemistry*, *21*(10), 2119–2130.

Svavarsson, J., Granmo, Å., Ekelund, R., & Szpunar, J. (2001). Occurrence and Effects of Organotins on Adult Common Whelk (Buccinum undatum) (Mollusca, Gastropoda) in Harbours and in a Simulated Dredging Situation. *Marine Pollution Bulletin*, *42*(5), 370–376. 8

Takahashi, S., Mukai, H., Tanabe, S., Sakayama, K., Miyazaki, T., & Masuno, H. (1999). Butyltin residues in livers of humans and wild terrestrial mammals and in plastic products. *Environmental Pollution*, *106*(2), 213–218.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(30), 11030–5.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–9.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *EXS. Author Manuscript;*, *101*, 133–64.

Terlizzi, A., Delos, A. L., Garaventa, F., Faimali, M., & Geraci, S. (2004). Limited effectiveness of marine protected areas: imposex in Hexaplex trunculus (Gastropoda, Muricidae) populations from Italian marine reserves. *Marine Pollution Bulletin*, *48*(1-2), 188–92.

Thoonen, S. H. ., Deelman, B.-J., & Koten, G. van. (2004). Synthetic aspects of tetraorganotins and organotin(IV) halides. *Journal of Organometallic Chemistry*, *689*(13), 2145–2157.

Tong, S. L., Pang, F. Y., Phang, S. M., & Lai, H. C. (1996). Tributyltin distribution in the coastal environment of Peninsular Malaysia. *Environmental Pollution*, *91*(2), 209–216.

Trevors, J. T., Stratton, G. W., & Gadd, G. M. (1986). Cadmium transport, resistance, and toxicity in bacteria, algae, and fungi. *Canadian Journal of Microbiology*, *32*(6), 447–64.

Turenne, C. Y., Tschetter, L., Wolfe, J., & Kabani, A. (2002). Necessity of quality-controlled 16S rRNA gene sequence database: Identifying non-tubercluous Mycobacterium species. *Journal of Cinical Microbiology*, *6*, 2326.

Ukushima, K. F., Ubey, S. K. D., & Uzuki, S. S. (2009). Quantitative Analysis of Expression of Tributyltin (TBT) -Regulated Genes in TBT-Resistant Pseudomonas aeruginosa 25W. *Environmental Research in Asia*, 163–166.

Van de Peer, Y., Chapelle, S., & De-Wachter, R. A. (1996). Quantitative map of nucleotide substitution rates in bacterial rRNA. *Nucleic Acids Research*, *24*, 3381–3391.

Vegesna, R. S. B. (2007). *A biotectechnological approach for decaffeination*. *University of Mysore*.

Vidali, M. (2001). Bioremediation. An overview. *Journal of Pure Applied Chemistry*,

73(7), 1163–1172.

Vreysen, S., Maes, A., & Wullaert, H. (2008). Removal of organotin compounds, Cu and Zn from shipyard wastewaters by adsorption--flocculation: a technical and economical analysis. *Marine Pollution Bulletin*, *56*(1), 106–15.

Wan, E. C. K., & Mohamat-Yusuff1, F. (2014). Contamination of Trace Elements (Cu, Pb, Cr) in Kong Kong Laut, Johor, Malaysia. In *4th International and National Seminer on Fisheries and Marine Science, Indonesia*. Indonesia.

Wuertz, S., Miller, C. E., Pfister, R. M., & Cooney, J. J. (1991a). Tributyltin-resistant bacteria from estuarine and freshwater sediments. *Appl. Envir. Microbiol.*, *57*(10), 2783–2789.

Wuertz, S., Miller, C. E., Pfister, R. M., & Cooney, J. J. (1991b). Tributyltin-resistant bacteria from estuarine and freshwater sediments. *Applied and Environmental Microbiology*, *57*(10), 2783–9.

Wyszkowska, J., Kucharski, J., & Borowik, A. (2008). Response of Bacteria To Soil Contamination With Heavy Metals. *Chair of Microbiology*, *13*(3), 443–453.

Yáñez, J., Riffo, P., Santander, P., Mansilla, H. D., Mondaca, M. A., Campos, V., & Amarasiriwardena, D. (2015). Biodegradation of Tributyltin (TBT) by Extremophile Bacteria from Atacama Desert and Speciation of Tin By-products. *Bulletin of Environmental Contamination and Toxicology*, *95*(1), 126–30.

Zeng, L., Huang, J., Zhang, Y., Qiu, G., Tong, J., Chen, D., … Luo, X. (2008). An effective method of DNA extraction for bioleaching bacteria from acid mine drainage. *Applied Microbiology and Biotechnology*, *79*(5), 881–888.

Zulkifli, S. Z., Ismail, A., Mohamat-Yusuff, F., Arai, T., & Miyazaki, N. (2010). Johor Strait as a hotspot for trace elements contamination in Peninsular Malaysia. *Bulletin of Environmental Contamination and Toxicology*, *84*(MAY), 568–573.

Zulkifli, S. Z., Mohamat-Yusuff, F., Arai, T., Ismail, A., & Miyazaki, N. (2010). An assessment of selected trace elements in intertidal surface sediments collected from the Peninsular Malaysia. *Environmental Monitoring and Assessment*, *169*(1-4), 457–472.

LIST OF PUBLICATIONS

Conference proceedings:

Abubakar, A., Mustafa, M., Johari, W. L. W., & Ferdaus Mohamat-Yusuff, S. Z. Z. and A. I. (2014). Tributyltin-resistant bacteria from contaminated surface sediment. In C. Yong, S. Yien, W. Norhamidah, & W. Ibrahim (Eds.), *MALAYSIA INTERNATIONAL BIOLOGICAL SYMPOSIUM 2014 "Sustainable " Sustainable Bioresources for Bioeconomy "* (p. 142). Kuala Lumpur: ©Department of Biology Faculty of Science Universiti Putra Malaysia 43400 UPM Serdang Selangor Darul Ehsan Malaysia Tel: +603 8946 6613 http://www.science.upm.edu.my/biology.

Abubakar, A., Mustafa, M. B., Lutfi, W., Johari, W., Zulkifli, S. Z., Ismail, A., Yusuff, M. (2015). Isolation and identification of TBT-resistant bacteria from contaminated surface sediment. In *International Conference on Environmental Forensics "from sources to solutions"* (p. 110). Putrajaya.

Publications:

Abubakar, A., Ferdaus B, M. Y., Mustafa, M. B., Johari, L. W. W., Zulkifli, S. Z., & Ismail, A. (2015b). *Klebsiella* sp . FIRD 2 , a TBT-resistant bacterium isolated from contaminated surface sediment along Strait of Johor Malaysia ". *Marine Pollution Bulletin*. **Published**

Abubakar, A., Ferdaus B, M. Y., Mustafa, M. B., Johari, L. W. W., & Zulkifli, S. Z., (2016a). Tributyltin (TBT) tolerance of an indigenous and non-indigenous bacterial species. *Water, Air & Soil Pollution*. **Under review**

Abubakar, A., Ferdaus B, M. Y., Mustafa, M. B., Johari, L. W. W., & Zulkifli, S. Z., (2016b). Tolerance of *Klebsiella sp*. FIRD 2, a TBT-resistant bacterium to heavy metals (Cu, Pb, Zn and Cd). *Heliyon Journal*. **Under review**

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

ISOLATION AND CHARACTERIZATION OF TRIBUTYLTIN-RESISTANT BACTERIA FROM CONTAMINATED SURFACE SEDIMENTS AT KONG KONG LAUT, JOHORE, MALAYSIA

NAME OF STUDENT : ABUBAKAR ABDUSSAMAD

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from_____ until _____ (date) (date)

Approved by:

_____	_____
(Signature of Student)	(Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.:	Name:
Date :	Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]