



UNIVERSITI PUTRA MALAYSIA

**FATE OF BOOSTER BIOCIDES IN AQUATIC ECOSYSTEM AND ITS
EFFECTS ON BLOOD COCKLE, *Tegillarca granosa* (LINNAEUS, 1758)
OF SOME SELECTED CULTIVATION AREAS IN PENINSULAR
MALAYSIA**

'AQILAH BINTI MUKHTAR

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By

'AQILAH BINTI MUKHTAR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

February 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

FATE OF BOOSTER BIOCIDES IN AQUATIC ECOSYSTEM AND ITS EFFECTS ON BLOOD COCKLE, *Tegillarca granosa* (LINNAEUS, 1758) OF SOME SELECTED CULTIVATION AREAS IN PENINSULAR MALAYSIA

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February 2021

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The booster biocides are mainly used in the shipping activities, agro-industries, and domestic use. The use of booster biocides is known to cause adverse effects on marine ecosystems, including inhibiting the process of photosynthesis in marine plants, and potential to accumulate in marine organisms. In this study, booster biocides of Irgarol 1051, diuron, 3,4-dichloroaniline (3,4-DCA), and chlorothalonil were measured in blood cockle, *Tegillarca granosa*, and sediment from some selected cockle cultivation areas in Peninsular Malaysia. Sampling activities were conducted in Bagan Pasir in Perak, Sungai Buloh and Kamparin Selangor, and Sungai Ayam in Johor for screening the booster biocides concentrations. The liquid-liquid extraction (LLE) follow by High-Performance Liquid Chromatography (HPLC) was performed to identify the concentration of booster biocides in the collected samples. The highest Irgarol 1051 mean was found in the blood cockle with a value of $98.92 \pm 13.65 \mu\text{g}/\text{kg}$ in Kapar, Selangor, while the means of diuron and its metabolites and 3,4-DCA showed the highest values of 40.31 ± 7.61 and $41.42 \pm 21.58 \mu\text{g}/\text{kg}$ in Kapar, Selangor and Sungai Ayam, Johor, respectively. Sungai Ayam, Johor also exhibited the highest amount of chlorothalonil of $29.76 \pm 8.80 \mu\text{g}/\text{kg}$. The highest mean concentration of Irgarol 1051 and diuron were detected in sediment from Kapar and Sungai Buloh, Selangor, respectively that are significantly different from other locations (Tukey's HSD, $p < 0.05$). Meanwhile, the highest mean concentrations of 3,4-DCA and chlorothalonil were 25.73 ± 6.40 and $23.87 \pm 13.87 \mu\text{g}/\text{kg}$, respectively which were not significantly different in locations (Tukey's HSD, $p > 0.05$). Among the booster biocides detected in blood cockle and sediment, only Irgarol 1051 was significantly correlated ($r=0.70$, $p<0.05$).

Referring to sediment quality guidelines, approximately 72% and more than 90% of sediment samples exceeded the environmental risk limits (ERLs) and maximum permissible concentration (MPC) for Irgarol 1051 and diuron, respectively. Thus, contaminants in marine ecosystem caused by booster biocides are highlighted as a serious problem in sediment.

Increased use of biocides has the potential for bioaccumulation in food webs. Distribution and bioaccumulation of biocides were studied in selected macrobenthos from subtidal food web in the Sungai Ayam, Johor. Stable isotope carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) ratios were used to describe the structure of food web. Nine species of organisms including phytoplankton, zooplankton, mollusk, and crustacean were collected to represent the benthic food web in the cockle cultivation areas. Higher concentration of diuron and chlorothalonil were detected in the hermit crab, *Clibanarius signatus* at a concentration of 109.29 and 61.03 $\mu\text{g}/\text{kg}$ wet weight, respectively, while 3,4-DCA concentration was highest in bivalve, *Tegillarca granosa*. The identified biomagnification factor (BMF) values indicate that biomagnification of these compounds have occurred in the studied subtidal food chain.

The toxicity study of diuron in microalgae, *Isochrysis galbana* and blood cockle, *T. granosa* were conducted in short term-bioassay with the endpoints of growth performance and mortality, respectively. The 72-hours median effective concentration (EC_{50}) and 48-hours median lethal concentration (LC_{50}) of diuron were identified in the microalgae and blood cockle, respectively. The EC_{50} value for microalgae was 10.1 ppb, while LC_{50} for blood cockle was 1.5 ppm. The ability of the organisms to accumulate the biocides in tissues proved that *I. galbana* and *T. granosa* are potential biomonitoring organisms in marine ecosystem.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**TAKDIR BIOSID PENGGALAK DALAM SISTEM AKUATIK DAN
KESAN KEPADA KERANG DARAH, *Tegillarca granosa* (LINNAEUS, 1758)
DI SEBAHAGIAN KAWASAN PENTERNAKAN TERPIPIH DI
SEMENANJUNG MALAYSIA**

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Biosid penggalak digunakan terutama dalam industri perkapalan, kegiatan pertanian, dan penggunaan domestik. Penggunaan biosid penggalak diketahui menyebabkan kesan buruk terhadap ekosistem laut, termasuk menghalang proses fotosintesis pada tanaman laut, dan berpotensi untuk terkumpul dalam organisma laut. Dalam kajian ini, biosid penggalak Irgarol 1051, 3,4-DCA, dan klorotalonil diukur dalam kerang darah, *T. granosa* dan sedimen dari kawasan penternakan kerang terpilih di Semenanjung Malaysia. Aktiviti mengambil sampel dilakukan di setiap kawasan penternakan iaitu di Bagan Pasir di Perak, Sungai Buloh dan Kapar di Selangor dan Sungai Ayam di Johor untuk menyaring kepekatan biosid penggalak. Pengekstrakan cecair-cecair (LLE) diikuti oleh Kromatografi Cecair Prestasi Tinggi (HPLC) untuk mengenal pasti kepekatan biocides penggalak dalam sampel yang dikumpul. Purata Irgarol 1051 tertinggi ditemui dalam kerang darah dengan nilai $98.92 \pm 13.65 \mu\text{g}/\text{kg}$ di Kapar, Selangor, sementara diuron dan metabolitnya 3,4-DCA menunjukkan nilai tertinggi masing-masing 40.31 ± 7.61 dan $41.42 \pm 21.58 \mu\text{g}/\text{kg}$ di Kapar, Selangor dan Sungai Ayam, Johor. Sungai Ayam, Johor juga menunjukkan jumlah klorotalonil tertinggi iaitu $29.76 \pm 8.80 \mu\text{g}/\text{kg}$. Purata kepekatan tertinggi Irgarol 1051 dan diuron masing-masing dikesan dalam sedimen dari Kapar dan Sungai Buloh, Selangor, perbezaan yang bererti dari lokasi lain (Tukey's HSD, $p < 0.05$). Manakala, purata kepekatan 3,4-DCA dan klorotalonil adalah 25.73 ± 6.40 dan $23.87 \pm 13.87 \mu\text{g}/\text{kg}$, yang masing-masing perbezaan tidak bererti dari lokasi lain (Tukey's HSD, $p > 0.05$). Di antara biocides penggalak yang dikesan dalam kerang dan sedimen darah, hanya Irgarol 1051 yang berkorelasi secara signifikan ($r = 0.70$, $p < 0.05$).

Merujuk kepada garis panduan kualiti sedimen, sekitar 72% dan lebih daripada 90% sampel sedimen masing-masing melebihi had risiko persekitaran (ERL) dan kepekatan maksimum yang dibenarkan (MPC) untuk Irgarol 1051 dan diuron. Oleh itu, pencemaran dalam ekosistem laut yang disebabkan oleh biocides pengalag diklasifikasi sebagai masalah serius dalam sedimen.

Peningkatan penggunaan biosid berpotensi untuk biopengumpulan dalam jaringan makanan. Penyebaran dan biopengumpulan biosid dikaji dalam makrobentos terpilih dari jaringan makanan subpasang surut di Sungai Ayam Johor. Nisbah karbon ($\delta^{13}\text{C}$) dan nitrogen ($\delta^{15}\text{N}$) isotop stabil digunakan untuk menggambarkan struktur jaringan makanan. Sembilan spesies organisma termasuk fitoplankton, zooplankton, moluska, dan krustasea telah dikumpulkan mewakili jaringan makanan bentik di kawasan penternakan kerang. Kepekatan diuron dan klorotalonil dikesan tinggi dalam umang-umang, *Clibanarius signatus* pada kepekatan masing-masing 109.29 dan 61.03 $\mu\text{g}/\text{kg}$ berat basah, sementara kepekatan 3,4-DCA tertinggi di dwicangkerang, *Tegillarca granosa*. Nilai faktor biopembesaran (BMF) yang dikenal pasti menunjukkan bahawa biomagnifikasi sebatian ini telah berlaku dalam rantai makanan subtidal yang dikaji.

Kajian ketoksikan diuron pada mikroalga, *Isochrysis galbana* dan kerang darah, *T. granosa* dilakukan dalam bioassai jangka pendek dengan masing-masing melihat prestasi pertumbuhan dan kematian. Kepekatan berkesan median 72 jam (EC_{50}) dan kepekatan mematikan median 48 jam (LC_{50}) diuron masing-masing dikenal pasti dalam mikroalga dan kerang darah. Nilai EC_{50} untuk mikroalga adalah 10.1 ppb, sementara LC_{50} untuk kerang darah adalah 15 ppm. Keupayaan mengumpul biosid dalam tisu membuktikan bahawa *I. galbana* dan *T. granosa* berpotensi menjadi organisma biomonitor dalam ekosistem laut.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

$(\text{NH}_4)_6\text{MO}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$	Ammonium molybdate
$\mu\text{g}/\text{l}$	Microgram/litre
3,4-DCA	3,4 - dichloroaniline
ACN	Acetonitrile
ADI	Acceptable daily intake
AFS	Antifouling Systems
ANOVA	Analysis of variance
AOP	Adverse outcome pathway
ASE	Accelerated solvent extraction
BASF	Badische Anilin und Soda Fabrik
BoT	Balance of Trade
BW	Body weight
$\text{C}_{10}\text{H}_{15}\text{N}_2\text{O}_8$	[1,2-Bis[bis(carboxymethyl)amino]ethyl]radical
C18	Octadecylsilane
CAM	Crassulacean acid metabolism
CAS	Chemical Abstracts Service
C_{cockle}	Concentration of booster biocide in blood cockle
CF-IRMS-EA	Continuous flow isotopic ratio mass spectrometer with elemental analyzer
CO_2	Carbon dioxide
$\text{CoCl}_3\cdot 6\text{H}_2\text{O}$	Cobalt(III) Chloride Hexahydrate
Cu	Copper

CuSO ₄ .5H ₂ O	Copper sulfate pentahydrate
d	Day
DCM	Dichloromethane
DCOIT	4,5-Dichloro-2-octyl-4-isothiazolin-3-one
DCPMU	1-(3,4 dichlorophenyl)-3 methyl urea
DDS	Department Delivery System
DIC	Dissolved inorganic carbon
Disodium EDTA	Ethylenediaminetetraacetic acid disodium salt
DLLME	Dispersive liquid-liquid microextraction
DMSA	N'-dimethyl-N-phenyl-sulphamide
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOF	Department of Fisheries
DPR	Department of Pesticide Regulation
EC50	Effective concentration 50
EqP	Equilibrium partition
ERLs	Environmental risk limits
EUSES	European Uniform System for the Evaluation of Substances
FAO	Food and Agriculture Organization
g	Gram
GCMS	Gas chromatography-mass spectrometry
h	Hour
H ₃ BO ₃	Boric acid

HCl	Hydrochloric acid
hpf	Hours post fertilization
HPLC	High-performance liquid chromatography
HPLC-DAD	high-performance liquid chromatography with a diode-array detector
HPLC-ESI-MS/MS	High-performance liquid chromatography Electro spray Ionization with Mass Spectrometric
HPLC-MS/MS	High-performance liquid chromatography with Mass Spectrometric
HPLC-UV	High-Performance Liquid Chromatography-Ultraviolet
HRD	Human reference dose
IH _{cockle}	Blood cockle intake
IHCP	Institute of Health and Consumer Protection
IMO	International Maritime Organization
IPBC	3-iodo-2-propynyl N-butyl carbamate
IRMS	Isotope ratio mass spectrometry
IS	Internal standard
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KNO ₃	Potassium nitrate
LC ₅₀	Median lethal concentration
LC-ESI-MS/MS	Liquid Chromatography Electro spray Ionization with Mass Spectrometric
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-mass spectrometry with mass spectrometry
LLE	Liquid-liquid extraction

LOD	Limit of detection
MAE	Microwave-assisted extraction
MF-LPME	Micro funnel-supported liquid-phase microextractions
mg	Milligram
mg/l	Milligram/liter
mM	Millimolar
MMWQS	Malaysian Marine Water Quality Standard
MnCl ₂ .4H ₂ O	Manganese(II) chloride tetrahydrate
MOA	Ministry of Agriculture and Agro-based Industry
MPC	Maximum permissible concentration
N ₂	Nitrogen
N ₂ O	Nitrous oxide
Na ₂ SiO ₃	Sodium silicate
NAFP	National Agro-Food Policy
NaH ₂ PO ₄ .4H ₂ O	Sodium dihydrogen phosphate tetrahydrate
NaNO ₃	Sodium nitrate
NaOH	Sodium hydroxide
ND	Not detected
nM	Nanomolar
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
NOEC	No observed effect concentration
NOEL	No observable effect level

NO _x	Nitrogen oxide
NS	No sample
OTs	Organotins
PHD	Predicted human dose
PNEC	Predicted no effect concentration
POC	Particulate organic carbon
POPs	Persistent Organic Pollutants
Pow	Octanol-water partitions
ppb	Part per billion
ppt	Part per thousand
PSII	Photosynthetic system II
QuEChERS	Quick, easy, cheap, effective, rugged, and safe
RCR	Risk characterization ratios
rpm	Revolutions per minute
RSDs	Relative standard deviations
SBSE	Stir bar sorption extraction
SD	Standard deviation
SPC	Self-polishing copolymer
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SPSS	Statistical package for the social sciences
SQC	Sediment quality criteria
TBT	Tributyltin
TCC	Trichlorocarbanilide

TCMTB	2-(thiocyanomethylthio) benzothiazole
TGDRA	Technical guidance document on risk assessment
UAE	Ultrasound-assisted extraction
USD	United States Dollar
USEPA	United States Environmental Protection Agency
USGS	United States Geological Surveys
VA-DLLME	Vortex assisted liquid-liquid microextraction
VA-MSPD	Vortex-assisted matrix solid-phase dispersion
V-PDB	Vienna- Pee Dee Belemnite
ZIA	Aquaculture industrial zone
ZnCl ₂	Zinc chloride
ZPT	Zinc pyrithione

CHAPTER 1

INTRODUCTION

1.1 General introduction

In the last three decades, the economies in Malaysia have been growing rapidly. Environmental pollution is inevitable and closely linked to national development activities include human and industrial activities. The thriving trade industry has made the Straits of Malacca one of the world's busiest waterways. The shipping activities and agroindustries increase the pollution levels to the environment, especially to the marine ecosystem. In fact, the Malaysia marinas are contaminated with chemical compounds, for example, heavy metal (Sany et al., 2013; Noor et al., 2015; Mahat et al., 2018), Persistent Organic Pollutants (POPs) (Mohamad et al., 2013; Kaw and Kannan, 2016; Krishnan et al., 2016), and biocides (Sudaryanto et al., 2002, 2004; Harino et al., 2008, 2009; Ali et al., 2013, 2014; Hanapiah et al., 2017; Mukhtar et al., 2019).

Previously, the antifouling biocides known as organotin (OTs) have widely used in shipping industries. The marine organism such as algae, barnacles, and mussels attached to the submerged surface of ships and boats are a serious problem due to increased hydrodynamic traction that affects the maneuverability of ships and increases fuel consumption. (Konstantinou and Albanis, 2004). OTs compounds are used to prevent the colonization of the marine organism on ships and boat hulls. However, these compounds have adverse effects on the non-target organism due to uncontrolled usage. For example, in the field of ecotoxicological effects, OTs cause the imposex in gastropods (Mohamat-Yusuff et al., 2010, 2011). On this environmental problem, the International Maritime Organization (IMO) held a convention on "Control of Harmful Antifouling System on Ship" (IMO-AFS 2001) in October 2003 which banned the use of OT compounds started in January 2008 and encourage the manufacturers to produce tin-free antifouling paints.

The commercial tin-free compounds widely used nowadays are Sea-nine 211, Irgarol 1051, zineb, ziram, diuron, chlorothalonil, dichlofluanid, tolyflua nid, copper omadine, zinc omadine, and pyridine-triphenylborane. The compounds increase their concentration significantly into the marine environment, mostly in marinas and harbors with heavy shipping activity. Leached biocides are readily transported into the marine environment which then settle in sediment creating

“hotspots” for contaminants. This is likely to pose a longer-term threat to various aquatic organisms (Kamarudin et al., 2020). Therefore, there is increasing concern about booster biocides due to their increasing use in shipping paints. Although the booster biocides do not threaten on endocrine system similar to TBTs, they are nonetheless toxic to microalgae and have the potential to destruct the primary producer on the base of the food web (Bao et al., 2011). Booster biocides such as Irgarol 1051 and diuron are pesticides widely used in agricultural activities. These compounds are among pesticides that most detected in aquatic environment (Konstantinou and Albanis, 2004). Coral, phytoplankton, and periphyton are highly susceptible to the herbicidal properties of booster biocides.

Past monitoring has indicated the widespread occurrence of booster biocides in the marine environment of Malaysia. Ali et al. (2013) have reported of Irgarol 1051 in the seawater from Peninsular Malaysia at spatial average range concentration from 40.8 to 663 ng/l. Diuron concentrations also detected in the seawater (Ali et al., 2014; Hanapiah et al., 2017). Studies in the west coast of Peninsular Malaysia and Strait of Johor detected booster biocide concentrations in sediment (Harino et al., 2009; Mukhtar et al., 2019). In the future, it is expected that the concentration of booster biocides will be higher in the marine environment as it is widely used in the shipping and agricultural activities.

The environmental change occurs on the west coast of Peninsular Malaysia may have an ecological knock-on effect such as the reduction of benthic organism population including the blood cockle, *Tegillarca granosa*, that is one of the main aquaculture products in Malaysia. Blood cockle species contribute about 35.8% of total brackish water production values in 2018 (RM 84,030.47) after marine fish and prawn (DOF, 2018). The highest production of blood cockle in Malaysia has recorded in 2010 with a total of 78,024 per year (DOF, 2011). However, the production of cockle show continuously declined in the following year. This situation may occur due to pollutants release into the marine ecosystem and inadequate management of the wild cockle population which result in high mortality of adult cockles and spats (Khalil et al., 2017). Therefore, it is important to determine the interaction between the marine organism, together with physical and chemical processes that trigger the environmental changes. Cockle is a filter feeder that has a great capacity to accumulate the contaminants in their tissue and are suitable as a biomonitor to monitor the distribution of contaminants in the coastal water. The investigation on cockle is also important to ensure the cockles are safe for a human consumption.

Usually, cockle cultivation areas are conducted in estuaries and coastal zones. They are abundant in complex intertidal and subtidal zones of plant and benthic organisms such as polychaete worm, snails, clams, sea urchins, and crabs. Benthic organism act as nutrient triggers from detritus and serve as a source of

protein for fish and cockle (Imevbore Bakare, 1970 and Walag et al., 2016). Detritus in the form of leaf litter in the subtidal areas largely come from the mangrove forest in the intertidal area (Krumhansl and Scheibling, 2012). By consuming debris, benthic organism responsible for carrying the nutrient circulation in the marine ecosystem. Therefore, it should rely on important estuarine areas to support the living systems of the offshore population by supplying food resources, and shelter in addition to acting as nursery habitats.

The discharge of chemicals and nutrients from the anthropogenic activities into aquatic ecosystem, may effect the ecological process such as nutrient dynamic and energy transfer, thus possible alter the system of food web structure (Warry et al., 2016). The food web is defined as the energy flow from the base to the highest trophic level that occurred among the organism within the ecosystem (Krumins et al., 2013). A few studies have recognized the trophic interaction in Malaysia's coastal areas (Zulkifli et al., 2014; Mukhtar et al., 2016; Joni et al., 2017). To understand the interactions of the benthic organisms, the study on the ecosystem circulation of the food web is conducted. The previous study of trophic interaction has used either gut content analysis or the observational study (Davis et al., 2012; Baker et al., 2014; Dromard et al., 2015; McClain-Counts et al., 2017). However, these conventional methods have the limitations in identifying interactions because they are used alone as the content cannot be separated into the prey species due to the unidentifiable digested materials (Baker et al., 2014). Thus, the method provides an unclear interpretation of the organism interaction where the research study requires a detailed explanation of organism interaction.

Nowadays, the biomarkers of stable isotope have been used to complement the conventional dietary analysis for identifying the organic matter transport throughout the food web and the trophic relationship. This powerful analysis technique is time-integrated, non-lethal, and significantly faster than gut content analysis (Fry, 2006). The carbon ($\delta^{13}\text{C}$) and and nitrogen ($\delta^{15}\text{N}$) ratios are commonly used to construct the trophic interaction of the organism in the ecosystem. Studies on trophic relationship among organism provide insight about the energy transfer in the food web and generate the importunateness to conserve the ecosystem in order to maintain the function each of the organism in the trophic levels.

Microalgae and blood cockle are interconnected when they depend on each other for survival. Microalgae serve as primary producer for the primary consumer such as blood cockle. The reduction of primary producer has the potential to affect the food available to organism at different trophic levels especially the herbivores organism. Pollutant discharge and water quality of the coastal areas are factors that affect species production. However, limited studies have been performed on the toxic effects of booster biocides on these species.

Hence, this study also conducted to provide the baseline information on the effect of booster biocides of diuron on the microalgae, *Isochrysis galbana* as a primary producer and on blood cockle, *Tegillarca granosa* as primary consumer by determining the median effect (EC_{50}) and lethal effect concentration (LC_{50}), respectively, using Probit analysis.

1.2 Problem statement and justification

Since the restriction of OTs compound used as an ingredient in ship paints after 1 January 2008, many booster biocides has been manufactured. The previous study on booster biocides has mainly focused on the water column and sediment but no data recorded on aquatic life in Malaysia marinas. In the future, the concentration of booster biocides are expected to be higher in the marine environment as they are widely used in the painting system and herbicides in agroindustries activities. Moreover, booster biocides have broad-spectrum effects on environmental components and some compounds have the potential to persist for long periods of time into the aquatic environment. The accumulation of pollutants in marine ecosystem is possible to transmit into the aquatic organisms and transferred into the human body throughout the food intake.

The selected cockle cultivation areas in Peninsular Malaysia were chosen as the study sites to investigate the effect of booster biocide in the blood cockle and sediment. The decline in cockle production in Malaysia has now triggers speculation the factors about that contribute to this problem. "Does booster biocide affect cockle production in Malaysia?", "Does booster biocide disrupt the food web ecosystem in the cultivation area consequently affecting the cockle production?" and also "Does consuming a cockle that have been exposed to booster biocides affect the human health risks?" Therefore, to understand and answer the questions, the studies had been performed by conducting booster biocides extraction, human risk assessment, and stable isotope analysis as an effective approach to assess coastal ecosystems along with median effect concentration diuron on primary producer of microalgae, *Isochrysis galbana*, and median lethal concentration on primary consumer of blood cockle, *T. granosa*.

1.3 Research objectives

The study on the effects of booster biocide on cockle, *Tegillarca granosa*, and sediment collected from selected cockle cultivation areas of Peninsular Malaysia can be achieved by having the following study objectives:

- 1) To elucidate the concentration of booster biocides in the *T. granosa* and sediment collected from selected cockle cultivation areas of Peninsular Malaysia
- 2) To assess the health risk of booster biocides in *T. granosa* on human and predators
- 3) To construct the food web of cockle cultivation areas using dual-isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis
- 4) To investigate the distribution of booster biocides pattern in the food web of cockle cultivation areas
- 5) To determine the 72 hours median effective concentration (EC_{50}) of primary producer, *Isochrysis galbana* and 48 hours median lethal concentration (LC_{50}) of primary consumer, *T. granosa* in the optimum laboratory condition.



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LIST OF PUBLICATIONS

Published Article

- Zulkifli, S. Z., Mohamat-Yusuff, F., **Mukhtar, A.**, Ismail, A., & Miyazaki, N. (2014). Determination of food web in intertidal mudflat of tropical mangrove ecosystem using stable isotope markers: A preliminary study. *Life Science Journal*, 11(3), 427-431.
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