

**OCCURRENCE AND DISTRIBUTION OF ANTIFOULING BIOCIDES
(DIURON) ALONG THE COASTAL AREAS OF ZANZIBAR: *THE CASE
STUDY OF UNGUJA ISLAND***

BY

ALI, FATMA KHAMIS

REG. NO. MSC/4/2016/04/TZ SUZA

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER
OF SCIENCE IN CHEMISTRY IN THE SCHOOL OF NATURAL AND
SOCIAL SCIENCES OF THE STATE UNIVERSITY OF ZANZIBAR**

DECEMBER 2022

DECLARATION

Candidate:

I, FATMA KHAMIS ALI, declare that the contents of this dissertation represent my own independently work and that the dissertation is not previously been submitted for academic examination towards any qualification.

.....

.....

Signature

Date

ALI, FATMA KHAMIS

REG. NO. MSC/4/2016/04/TZ SUZA

Supervisors:

We confirm that the work reported in this dissertation was carried out by the candidate under our supervision

.....

.....

Signature

Date

Dr. Hassan Rashid Ali

Department of Natural Science, The State University of Zanzibar, Tanzania

.....

.....

Signature

Date

Dr. Miza Ali Kombo

Department of Natural Science, The State University of Zanzibar, Tanzania

DEDICATION

This work is dedicated to my late grandfather Abdallah Omar, my beloved brother Suleiman Khamis Ali and my charming grandmother Bikombo Salum, who dreamt big in me about my studies. May the Almighty *Allah* rest their souls in paradise, Amiina.

Once again, the work is dedicated to my lovely and hardworking parents; Maua Abdalla Omar, and Khamis Ali Hamad. Besides, it is devoted to my wonderful and caring husband Omar Ali Juma, my dearest sons Akram Ali Hamad and Aasim Omar Ali plus my sisters and brothers who have supported for my education throughout. Thank you, and long live all.

ACKNOWLEDGEMENTS

I am immensely thankful to the Almighty *Allah*, the Omniscient and the Lord of all creatures, as He has kept me psychologically strong throughout my studies. I express my special thanks to the State University of Zanzibar through the support of its Building Stronger University (BSU II) project. Through it, I managed to use the analytical instruments and some of reagents to the completion of my research.

Besides, I would like to express my deep and sincere gratitude to my supervisors, Dr. Hassan Rashid Ali and Dr. Miza li Kombo for their constructive comments and supports. Their tireless devotion, mutual guidance, encouragements and suggestions meant a lot to me and have let me think creatively and critically on my research work.

Furthermore, I greatly appreciate all the Lecturers, Assistance Lecturers and laboratory technicians of the State University of Zanzibar for their encouragement, guidance, advice and moral support. Specifically, Prof. Hamad, Mr. Salim, Mr. Ali and Mr. Rahib Abdallah Abbas deserve special thanks for their advice, guidance and help during the entire period of my study. Similarly, special thanks are due to Prof. Sheikh for his guidance and research knowledge and Mr. Ali from Ifakara Health Institute Bagamoyo for his help in statistical data analysis.

Moreover, I extend my thanks to Mr. Msafiri Samweli Mwasyeba a Laboratory technologist at the Government Chemists Laboratory Authority for guiding and teaching me the use and procedure of HPLC. In addition, I thank the government and non- government officials and other authorities for their keen interest and great

support during my study. Distinguished respondents are acknowledged for their focused participation during my data collection sessions.

Equally, I would like to express my deep and sincere gratitude to my entire fellow masters' students at the State University of Zanzibar. Definitely, I will never forget the support and contribution of my best friends Zeana, Mwantum, Kombo, Lila, Khamis, Halima and Ali in this work.

To my humble and caring husband! What should I tell? Your long-lasting support and consistent inspiration in every step of this academic journey mattered most. You have realistically continued to stand on my side and support the course of this exercise. Equally, I extend deep gratitude to my beloved kids Akram and Aasim for their patience and understanding during my absence Thank you very much.

Above all, words are inadequate to express my thanks to my lovely and wonderful father Mr. Khamis Ali Hamad and mother Maua Abdallah. Thank you very much for your unlimited love, wholehearted prayers, distinct caring and inexplicable sacrifices for my education. In the same mood, I acknowledge the kindness and prayers of my brothers Ahmad, Abdul-Aziz and Nouman, as well as sisters; Sabra and Nahla and other relatives and friends. Lastly, but not for importance I salute all the people unstated here but have helped me all the way through this journey. Thank you all.

Contents

DECLARATION	ii
DEDICATION	ii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATION AND ACRONYMS	xi
ABSTRACT	xiii
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background of the Study	1
1.2 Problem Statement and Justification	4
1.3 Objectives of the Study.....	6
1.3.1 Main Objective	6
1.3.2 Specific Objectives	6
1.4 Research Questions.....	6
1.5 Significance of the Study.....	6
CHAPTER TWO	8
LITERATURE REVIEW	8
2.1 Pollution.....	8
2.2 Historical usage of antifouling chemicals	8
2.3 TBT COMPOUNDS – applications and history	9
2.4 International regulation on the TBT use.....	16
2.5 Adverse effects on living organisms	23
2.6 Importance and toxicity of new antifouling chemicals.....	26
2.7 Importance and toxicity of Diuron	27
2.8 Chemical structures and behavior of antifouling chemicals in the environment....	28
2.9 Diuron.....	29
CHAPTER THREE	32
METHODOLOGY	32
3.1 Background of Study Areas	32

3.1.1 Sampling Sites	32
3.1.2 Malindi	34
3.1.3 Bwawani	34
3.1.4 Mtoni	34
3.1.5 Bawe	35
3.1.6 Chapwani (Grave Island)	35
3.1.7 Chumbe	36
3.1.8 Mnemba	36
3.1.9 Kwale	37
3.1.10 Kizimkazi	37
3.1.11 Pange	38
3.2 Material and Methods	40
3.2.1 Sample Collections	40
3.2.2 Sample Extraction	40
3.2.3 Analysis of Diuron from Seawater	42
3.3 Quality Assurance of Diuron	43
3.4 Data Analysis	44
CHAPTER FOUR	46
RESULTS AND DISCUSSION	46
4.1 Levels of Diuron in Water	46
4.2 Level of diuron in sampling sites which exceeding the Maximum Permissible Concentration (MPC)	48
4.3 Comparison of Diuron level from different clusters	51
CHAPTER FIVE	56
CONCLUSIONS AND RECOMMENDATIONS	56
5.1 Conclusions	56
5.2 Recommendations	57
REFERENCES	59
APPENDICES	71

LIST OF TABLES

Table 1.1: Description of the major AF strategies past and present	2
Table 2.1: Summary of some Eco toxicological effects of TBT compounds indicated by taxonomic group.....	24
Table 3.1: Location, physical parameters and characteristics of sampling stations.....	39
Table 3.2: Recovery test data of Diuron spiked in remote samples.....	43
Table 3.3: Calibration curve data for Diuron (ppm) analysis using HPLC	44
Table 4.1: Levels of Diuron (ng/L) in coastal water of Unguja Island	47
Table 4.2: Mean Diuron concentration by study sites.....	48
Table 4.3: Spatial variation of concentration of diuron in ng/L using Tukey HSD.....	53
Table 4.4: Mean pairwise comparison for sites group	54
Table 4.5: Maximum concentrations of Diuron (ng/L) detected worldwide	55

LIST OF FIGURES

Figure 2.1: The TBT moiety. 3D image adapted after being generated as the “Tributyltin hydride model” by the open source software trial <i>Accelrys Discovery Studio Visualizer 2.0</i>	10
Figure 2.2: Pictures of marine biofouling on: (A) a ship hull, (B) a propeller of a vessel in dry dock and (C) the submerged portion of an anchor chain. Adapted from Ali, H.R. et al., (2014).	12
Figure 2.3: Diagram of the three types of TBT-based AF paints by the respective biocide dispersal mode over time; a) contact leaching; b) ablative; c) copolymer. Adapted from de Mora (1996).....	14
Figure 2.4: <i>Crassostreas gigas</i> . TBT effects on oysters: a) acute shells malformation – “ball shape” of a specimen collected in the Bay of Cadiz (Spain); b) shell from the Bay of Arcachon (France) with one type of growth during the TBT exposure period (left white arrow) and another after the TBT-based AF paints prohibition (right white arrow). Adapted from Alzieu (1996).	16
Figure 2.5: Pictures of some Greenpeace activists’ actions, carried out in September and October 1999, calling for a ban on the use of the TBT-based AF paints. Adapted from the organization international website www.greenpeace.org	20
Figure 2.6: Scheme of photoelectron transport system (photosynthesis light reaction). Broken red line showing site of action of diuron (DCMU); (modified from Vandermeulen <i>et al.</i> , 1972).	28
Figure 2.7: Schematic diagram showing routes and fate of antifouling chemicals (OTC, diuron and Irgarol) in coastal environment. Adapted from Ali, H. R., (2014).	29
Figure 2.8: Chemical structure of diuron	30
Figure 2.9: Chemical degradation of diuron	31
Figure 3.1: Map of Unguja coastal island shows sampling areas of this study.	33
Figure 3.2: Photo of Mtoni as one of the study area for diuron.	35
Figure 3.3: Analytical method for Diuron analysis in laboratory	41
Figure 3.4: Solid Phase Extraction (SPE) as way of sample preparation before analysis ..	42
Figure 3.5: Calibration curve of Diuron.....	44
Figure 4.1: Diuron concentration (ng/L) recorded from each study site.....	48

Figure 4.2: Spatial variation of average diuron concentration (ng/L) in coastal waters of Unguja Island 50

Figure 4.3: Comparison of average diuron concentration (ng/L) in coastal waters of Unguja Island with Maximum Permissible Concentration (MPC)..... 51

Figure 4.4: Mean concentrations of Diuron from four categories 52

LIST OF ABBREVIATION AND ACRONYMS

µg:	Microgram
°C:	Degree of Celsius
AF:	Antifouling
BDL	Below Detected Limit
BF ₃ :	Borontriflouride
Cu:	Copper
DBT:	Dibutyltin
DDTe:	Dichlorodiphenyltrichloroethane
EC:	European Commission
EDCs:	Endocrine disruptor chemicals
g:	gram
HPLC:	High Performance Liquid Chromatography
h:	hour
IPCS:	International Program on Chemical Safety
ICP:	International Chamber Program
IMO:	International Maritime Organization
ISO:	International Standards Organization
ISF:	International Shipping Federation
kg:	kilogram
l:	litre
m:	meter
MBT:	Monobutyltin
MPC:	Maximum Permissible Concentration
mg:	milligram
min:	minutes
ng:	nanogram
OT:	Organotin
OTs:	Organotins
ppm:	part per million
PVC:	Polyvinylchloride
SD:	Standard Deviation

Sec:	second
SPC:	Self-Polishing Copolymer
SPE:	Solid Phase Extraction
TBT:	Tributyltin
TCMTB:	Thiocyano methylthio benzothiazole
TPT:	Triphenyltin
Zn:	Zinc

ABSTRACT

Diuron (N-(3,4-dichlorophenyl)-N,N-dimethylurea) is one amongst the booster biocides presented to substitute tributyltin (TBT) as an antifouling agent. It has continued to be used ever since, though little is known about their levels in the maritime setting of Zanzibar. This thesis details the occurrence and distribution of Diuron around the coastline area of Unguja Island. Total of 33 samples from 11 sampling sites with different characteristics and activities such as tourisms, dockyards, fishing, coral reef areas, cargo and passengers' ports were collected. Samples were prepared and extracted automatically using Solid Phase Extraction (SPE) technique. Finally, Diuron was analyzed using High Performance Liquid Chromatography HPLC (an Agilent Technologies series 1260 infinity II, USA) equipped with DAD (G1315D). The concentrations of diuron in water ranged from Below Detection Limit (BDL) to 1321ng/L maximum were detected. The average concentration observed in harbor and ports (commercial and fisheries) (mean \pm SD) were 1006.67 \pm 90.6 ng/L (Malindi), 1321.67 \pm 52.3 ng/L (Bwawani), BDL (Mtoni). Average concentration results observed in small isles and sand sandbanks were 263.67 \pm 4.0 ng/L (Bawe), 1047.33 \pm 45.4 ng/L (Pange), BDL (Kwale). The average concentration observed in coastal area is 449.33 \pm 91.2 ng/L (Kizimkazi). Average concentration results from Coral reef islands were 1126.33 \pm 31.1 ng/L (Chapwani), 620.33 \pm 5.7 ng/L (Mnemba) while the results from Chumbe and Murongo were below detection limit (BDL). Majority of the sites showed higher levels of Diuron above the maximum permissible concentration (MPC) of 430 ng/L as restricted by the Dutch Authorities for marine organisms except Bawe (263.67ng/L). Average concentration between all sites and permissible concentration showed significant difference ($p < 0.05$). In general the results of this study suggest that the long term future studies on the antifouling biocides particularly on long term monitoring, chronic exposure, risk assessment, organisms' responses and pollution models would add special value towards better understanding the mechanisms and sustainable marine ecosystem health.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Biological fouling is one of the most critical topics in marine environmental studies. It refers to growth and accumulation of undesirable microorganisms, plants and animals on natural and artificial surface such as boats, fishing nets, cages, oilrig supports and buoyant immersed in seawater. Mostly it involves community of organisms that attach and grow on external surface of both natural and artificial objects either submerged or semi submerged in seawater (Lewis 1998).

One among the risk of marine environment is biofouling. This is danger because it brings countless socio- economic problems. Yet, it is normal in the maritime setting, despite being a key setback for the shipment business (Dafforn 2011; Lewis 2011; Johnston 2011). Biofouling accounts for approximately 90% of global and seaborne trade over the last three decades (ICS and ISF 2009). It has also been revealed that the growth of organisms on a vessel hull increases frictional drag, reducing ship speed or necessitating increased fuel consumption to maintain speed (Abbott *et al.* 2000). Slime films alone have been reported to cause powering penalties of 21%, with heavy calcareous bio-fouling increasing this penalty to 86 percent (Schultz 2007). The economic costs of hull fouling have been a driving force behind the development of antifouling technologies, which has been growing to a global industry worth nearly four billion US dollar annually (Wright 2009).

To overcome the biofouling problem to marine vessels such as boats and ships antifouling are used in order to prevent fouling organisms from settling and growing onto them. Antifouling paints usage in boats and ships, and their importance has been recognized for over 2000 years back (Yebra *et al.* 2004). The various methods used on coatings vessel hulls to inhibit the settlement of marine organisms are summarized in Table 1.1.

Table 1.1: Description of the major AF strategies past and present

Anti-fouling system	Mode of action
TBT Self-polishing copolymer (SPC) coatings	TBT biocide chemically bonded in copolymer resin – hydrolysis with seawater results in slow and consistent release of TBT biocide
Tin-free SPC coatings	Cu/Zn/Silyl copolymer resin with Cu particles and booster biocides dispersed through the paint matrix – hydrolysis with seawater results in slow and consistent release of biocide
Tin-free conventional coatings	Cu particles and booster biocides dispersed through soluble or insoluble paint binder – dissolution in seawater results in slow and decreasing release of biocide
Booster biocides	Most often herbicides/pesticides incorporated into Tin-free Conventional and SPC AF paints to increase efficacy against Cu-tolerant algae
Foul-release coatings	Low energy, minimally adhesive surfaces, mostly silicone elastomers and often incorporating silicone oils
Biomimetic	Incorporation of natural AF compounds produced by marine organisms (e.g., secondary metabolites) or surfaces based on natural micro topography

Among the compositions of antifouling agents, biocides are the important ingredients due to their role in preventing colonization of macro-organisms and microorganisms on hull surfaces. Biocides are usually made to slowly leach out into the seawater, and consequently affecting marine life that happen to attach themselves to the ship hulls and other solid surface intended for protection.

Apart from their importance in the shipping industry, studies have shown that biocide persist in the water, endangering sea life, harming the environment, and possibly entering the food chain. According to Lewis 1998, biocides are toxicant and can slowly leaches in water bodies, as a result they contaminate marine environment. As a result, environmentalists have become increasingly concerned about the effects of biocide on marine ecosystems, with many advocating for stricter regulation and monitoring (Alzieu *et al.* 1986; Claisse & Alzieu 1993; Thomas & Brooks 2010). As a result of these environmental concerns, some antifouling substances, particularly those with higher environmental risks, such as copper and tributyltin paints, have been banned and replaced by less toxic alternatives.

Diuron (N-(3,4-dichlorophenyl)-N,N-dimethyl-urea) is a biocide ingredient in antifouling agents that was developed to replace the more toxic biocide tributyltin. Diuron is the herbicide that inhibits the growth of a wide variety of broadleaf and grass species.

Though it was once thought to be environmentally friendly, recent research has revealed that it causes eye, respiratory, and skin irritation. Furthermore, diuron has been associated with harmful effect to developing fetus and cause reproductive system defects, as well as anemia and methemoglobinemia in animals. The LD50 of this

pesticide chemical in rats is 360–1250 mg/kg (Sathiyaseelan K *et al.* 2011). Endocrine disruption, immunotoxicity, teratogenicity, and acetyl cholinesterase inhibition cause diuron toxicity in non-target organisms such as fish, amphibians, and invertebrates (Sathiyaseelan K *et al.* 2011).

Diuron is one of the antifouling agents that cause damage to key marine ecosystem organisms such as coral reefs, which are under threat from a variety of anthropogenic stresses all over the world. Diuron occurrences in the marine environment require special attention in order to prevent further harm to marine life that is already stressed due to other environmental factors (Sano *et al.* 1984 &1987, Sheppard *et al.* 2002, Sathiyaseelan K *et al.* 2011).

Tanzania is one among the African countries member of IMO therefore ban of IMO on antifouling paints under the control of harmful antifouling systems for ship may considered. Since there is little information about contamination of antifouling chemical levels in Zanzibar, this study focused on determining the level of the antifouling biocide agent diuron around the coasts of Unguja Island. Diuron levels were measured in the study stations of Chumbe, Bawe, Murongo, Kizimkazi, Pange, Chapwani, Mnemba, Mtoni, Harbour, Bwawani, and Kwale.

1.2 Problem Statement and Justification

For more than a century, antifouling paints that are designed to release one or more biocides chemicals continuously through the paint surface have been the primary method of biofouling prevention on ships and other marine vessels. These chemicals have

contributed to the growth of the maritime transportation industry by addressing issues such as ship fuel consumption and speed. However, because these chemicals are toxic, allowing them to accumulate and persist in high concentrations in the marine environment can have a secondary environmental impact. Although diuron was previously considered an alternative to the more toxic biocide TBT they have also reported to pose some threat to aquatic ecosystem. Recent studies demonstrate the several effects of diuron on plants, corals and number of ecological effects as well as human being (Jones & Kerswell 2003). It has been classified as a hazardous substance by the European Commission (Malato *et al.* 2002; 2000a,b), that why some European countries, such as the United Kingdom, Sweden, Denmark, and France, have banned the use of Diuron in antifouling paints (Konstantinous & Albanis 2004; Giacomazzi & Cochet 2004).

In Tanzania specifically little attention has been given to these environmental hazardous chemical. Because most countries have authorized the use of Irgarol as a booster biocide in place of Diuron, little attention has been paid to assessing the environmental risk of diuron. However, because the maritime environment is a global trade, and most developing countries have poor biocide monitoring strategies, diuron is still used in this part. By considering the fact that, Zanzibar depends on shipping activities, tourism and agriculture as economic activities the chance of accumulation of antifouling agents such diuron is higher. Furthermore, based on the fact that the level of biocides especially diuron in marine water has not much given attention; the present study aimed to fill the knowledge gap by determine occurrence and distribution of diuron in seawater along the coasts of Unguja Island.

1.3 Objectives of the Study

1.3.1 Main Objective

The study aimed to assess the occurrence and distribution of diuron in the seawater around the coastal areas of Unguja Island.

To achieve the main objective, this study addressed the following specific objectives:

1.3.2 Specific Objectives

- i. To determine level of diuron in different sampling areas along the Unguja Island
- ii. To measure the variation of diuron with reference to the Maximum Permissible Concentration (MPC)
- iii. To compare the data found in Zanzibar with other reported from those different countries.

1.4 Research Questions

- i. What is the level of diuron around the Unguja Island?
- ii. To what extent diuron concentration vary from the recommended Maximum Permissible Concentration?
- iii. To what extent does the level of diuron in Zanzibar differ with the level reported in other countries?

1.5 Significance of the Study

Toxic chemical substances are contributing to the maximum destruction of coral reef ecosystems. However, in Tanzania especially Zanzibar most of pollutants studies have been focus in on heavy metals in ports, marinas and islands, but there was no attention to

the availability, distribution and amount of antifouling chemicals like diuron in this region. Since there is significant gap of on research information regarding to the toxicity of new antifouling chemicals such as diuron in relation with the health of coral reef organisms worldwide. Therefore, there is a great significance to monitoring baseline level of diuron around the Zanzibar Island in order to realize and be alert to environmental causing damages to these vital marine natural resources. This thesis reports the levels and distribution of diuron in coastal waters of Zanzibar. This is evidence that coastal areas of Zanzibar face pollution threat of diuron. The findings can be used to fill the existing information gap of diuron in this region. Also the study exposed evidence of different concentration of diuron in some of coastal areas, which seems to exceed the maximum permissible concentration. Therefore the data may help Zanzibar Marine authorities to understand current level of diuron and pay attention towards controlling the usage of antifouling chemicals like diuron for sustainable conservation of vital marine resources.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pollution

Pollution and contamination are at the heart of our understanding of environmental quality degradation. The two concepts are distinguishable by the fact that pollution causes a decrease of resources for humans such as food sources from aquatic environment (Clark, 1992). In Ecotoxicology, and referring to chemicals, contamination is defined as an artificial increase above the background, whilst pollution implies damage to living resources or risks to human health (Chapman, 1995). Therefore, a pollutant must be a contaminant but not all contaminants are pollutants (Chapman, 2001). For a compound to be classified as a relatively pollutant to a given organism, the cause-effect relationship must be clearly determined (Clark, 1992). For example, Tributyltin (TBT) compounds are definitely contaminants of the marine and estuarine environments and, because of their adverse effects on natural resources; they can also be considered pollutants.

2.2 Historical usage of antifouling chemicals

It is becoming increasingly difficult to ignore the discussions about the importance of antifouling paints. These chemical antifouling paints have been recognized for more than 2000 years ago (Yebra *et al.*, 2004). It is reported that the ancient societies used wax, tar and asphaltum on ship bottoms and by the 3rd century B.C. Greeks and Romans used even lead sheathing (Yebra *et al.*, 2004) to prevent settlement of marine organisms on ship hulls. It is also reported that, lead sheathing was possibly the most tried scenario

for the protection of ship's hulls before the 18th century (WHOI, 1952). Besides, copper sheathing was mostly used in 1780s followed by various alternatives after introduction of iron ships at the period of time. Most of them included sheathings of zinc, lead, nickel, arsenic, galvanized iron and alloys of antimony, zinc and tin, followed by wooden sheathing, which was then coppered (WHOI, 1952).

Furthermore, a variety of antifouling chemicals was developed mid 1800s by the concept of dispersing a toxicant in a polymeric vehicle. During that time, copper oxide, arsenic, and mercury oxide were popular antifoulants and common solvents included turpentine oil, naphtha, and benzene while linseed oil, shellac varnish, tar, and various kinds of resin were used as binders (WHOI, 1952). Recently, the most antifouling compounds before introduction of Booster Biocides were a group of TBT, which was known as most dangerous compounds due to their effects on natural resources.

2.3 TBT COMPOUNDS – applications and history

The term “tributyltin”, as well as its acronym “TBT”, has been quite incorrectly accepted to represent a chemical compound. TBT is a chemical moiety, a part of an organotin (OT) compound that comprises 3 *n*-butyl chains covalently linked to a single atom of tin (Sn-C covalent bonds; see Figure 2.1) and not a compound on its own right.

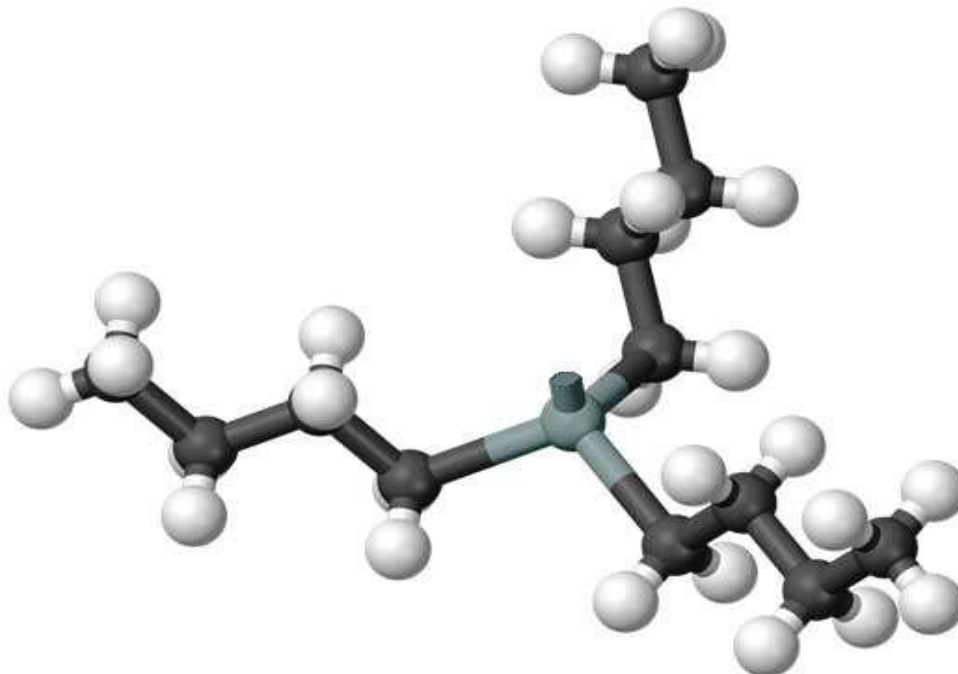


Figure 2.1: The TBT moiety. 3D image adapted after being generated as the “Tributyltin hydride model” by the open source software trial *Accelrys Discovery Studio Visualizer 2.0*.

The tri-substituted OT species have been described as the most toxic substances ever deliberately introduced into the marine environment by mankind (Goldberg, 1986; Evans *et al.*, 1995; Fent, 1996). Moreover, TBT compounds act as broad spectrum biocides and are endocrine disruptor chemicals (EDCs) with androgenic activity (Matthiessen and Gibbs, 1998; Oetken *et al.*, 2004; Oehlmann *et al.*, 2007).

Despite being used as polyvinyl chloride (PVC) stabilizers since the 1940s (Appel, 2004; Wilkes *et al.*, 2005), the industrial applications of OT compounds were followed by the recognition of biocidal properties of their tri-substituted derivatives, essentially TBT and triphenyltin (TPT) compounds, in the early 50’s (Bennett, 1996). It is reported that, TBT compounds were mostly applied in the preservation of wood, textiles and

paper as disinfectants, fungicides, insecticides, antibiotics and preventing degradation (Bennett, 1996). In addition to that, they have been used as active ingredients in antifouling (AF) paints, and constituted TBT compounds' main source to aquatic environment to date (de Mora, 1996; Yebra *et al.*, 2004). There are also scenarios whereby TPT compounds have been employed as TBT co-biocides in AF paints but their major application lied as fungicides in agriculture (Fent, 1996). Antifouling paints prevent bio incrustation – unwanted settling and growth of biological materials on surfaces immersed in water – in vessels hulls (Figure 2.2 A), buoys, platforms and other structures (Figure 2.2 B and C) namely related to aquaculture (Yebra *et al.*, 2004).

Bio incrustation, also known as bio fouling, has been described for more than 2000 marine species belonging to different taxonomic groups including: diatoms, green algae, sponges, hydroids, crustaceans, molluscs, among others (Alzieu, 1996). For instance, data from the International Maritime Organization (IMO) indicate that unprotected hulls may gather about 150 kg.m^{-2} of biological incrustation in less than six months at sea (IMO, 1999). For that reason, a super tanker with a submerged area of $40,000 \text{ m}^2$ loads approximately 6,000 tonnes of bio fouling in such a short period of time, which leads to a dramatic increase of water resistance to movement and a fuel consumption rise of 40 to 50% (IMO, 1999). Certainly, to a vessel owner, an AF system promotes fuel consumption reduction and an increase in the time between paint repairs in dry dock; a procedure with huge costs and that substantially reduces the vessel operational time (Susana, 2010).



Figure 2.2: Pictures of marine biofouling on: (A) a ship hull, (B) a propeller of a vessel in dry dock and (C) the submerged portion of an anchor chain. Adapted from Ali, H.R. et al., (2014).

It is reported that during early days of navigation, products such as quicklime, brimstone, rosin, pitch, tar and bitumen have been used to protect ships hulls (IMO, 1999; Yebra *et al.*, 2004). Currently, some of them continue to be used but over an extra wood sheath applied to the hull surface. This “sheathing method” allowed easier repairs, by the simple replacement of that last layer, in dry dock and at regular intervals. In addition to that, materials such as iron, zinc, lead and copper were subsequently tested. In practice, the later performed very well to protect the hull from bio fouling. Once in contact with water, it produces a kind of a “poisonous film” mainly of copper oxychloride (Kegley *et al.*, 2008). Furthermore, as this film is slightly soluble, it is gradually washed away, leaving no way in which marine life can attach itself to the

vessel submerged surface. Copper salts were the first agents to be integrated into biocidal AF systems and widely used until the 1950's. In order to increase their effectiveness, compounds of arsenic, mercury and dichlorodiphenyltrichloroethane (DDTe) were then introduced in paints formulations (IMO, 1999).

During the 1960's, the chemical industry produced effective and economic AF paints by using organometallic compounds, particularly the OT known as "TBT" (Crompton, 1997). In the 1970's, due to their durability and biocidal efficiency, most of the maritime shipping vessels had their hulls coated with TBT-based AF paints (Yebra *et al.*, 2004). The first TBT-based AF systems were known as "free association" paints in which a high amount of biocide was dispersed in a matrix that could be insoluble – "contact leaching" paints (Figure 2.3a) – or soluble – "ablative" paints (Figure 2.3b). These coatings lixiviation, by the contact with water, released the active agent by diffusion preventing the fixation of biological material on the submerged area (de Mora, 1996). The initial rate of the biocide release was fast although suffering an exponential decrease to a loss of effect only after 18 to 24 months (IMO, 1999).

By the end of the 1970's, new formulations were prepared using TBT paints copolymerized with methyl methacrylate (Yebra *et al.*, 2004; Yebra *et al.*, 2006). Thus, the leaching rate was controlled: the biocide was gradually released by the alkaline hydrolysis of the painting surface and the reaction only proceeded when the surface layer was depleted, resulting in a constant and relatively slow release of the biocide (Figure 2.3c). These systems reached a maximum life of 60 months (IMO, 1999).

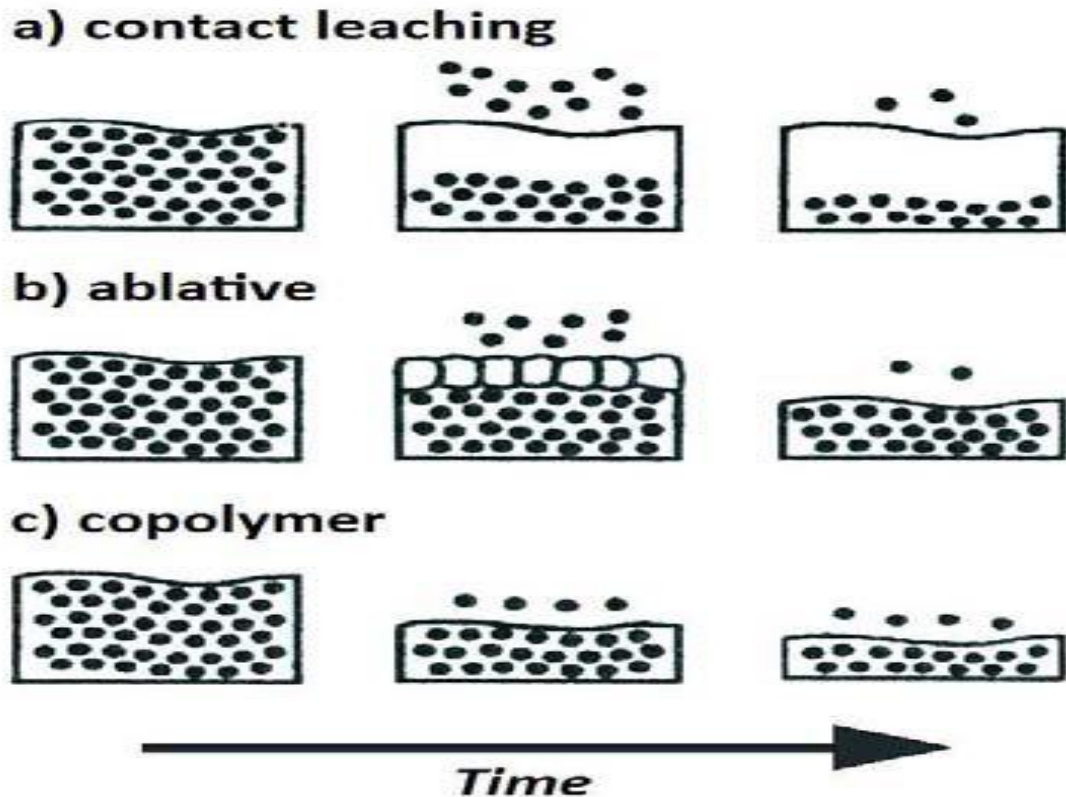


Figure 2.3: Diagram of the three types of TBT-based AF paints by the respective biocide dispersal mode over time; a) contact leaching; b) ablative; c) copolymer. Adapted from de Mora (1996)

In the marine environment, TBT degradation occurs by biotic and abiotic processes. In both cases, the compound sequential debutylation to its derivatives is involved: dibutyltin (DBT) and monobutyltin (MBT) to inorganic tin (Batley, 1996). The process has shown to be dependent mainly on temperature, organotins (OTs) concentration, salinity and light exposure.

These compounds degradation was proved to be accelerated at higher temperature, lower TBT concentrations, higher salinity and under light exposure [see complete reviews on the subject by Batley (1996), Fent (1996), Meador (2000) and Burton et al. (2006)].

Nevertheless, TBT compounds high toxicity has resulted in numerous and widespread adverse biological effects (Oehlmann et al., 2007). Despite some initial scepticism, the

problem of “TBT” as “large-scale pollutant” emerged as a result of the impact of public and political publications, specifically: (i) the description of shells malformations and reproductive failure in oysters by Alzieu et al. (1981) and (ii) reports of sexual abnormalities and decreased abundance of prosobranch gastropods in coastal ecosystems.

In the late 70’s, the oysters production at Arcachon Bay (France) was severely affected due to a process known as “shell thickening” or “chambering” – shells irregular calcification by hyper secretion of interlayer gel, overlap of a calcitic layer and disappearance of the gel, resulting in the formation of chambers that gave the shell a “ball shape” (Figure 2.4). Oysters’ shells chambering led to the animal’s body cavity reduction and to a break-down of the local production, with marked economic consequences. After several studies and field observations, the phenomenon was related to the TBT release into water at numerous marinas located in the bay. Besides chambering, TBT also revealed a negative effect on oysters larval growth and survival [see review by Alzieu et al. (2000)].

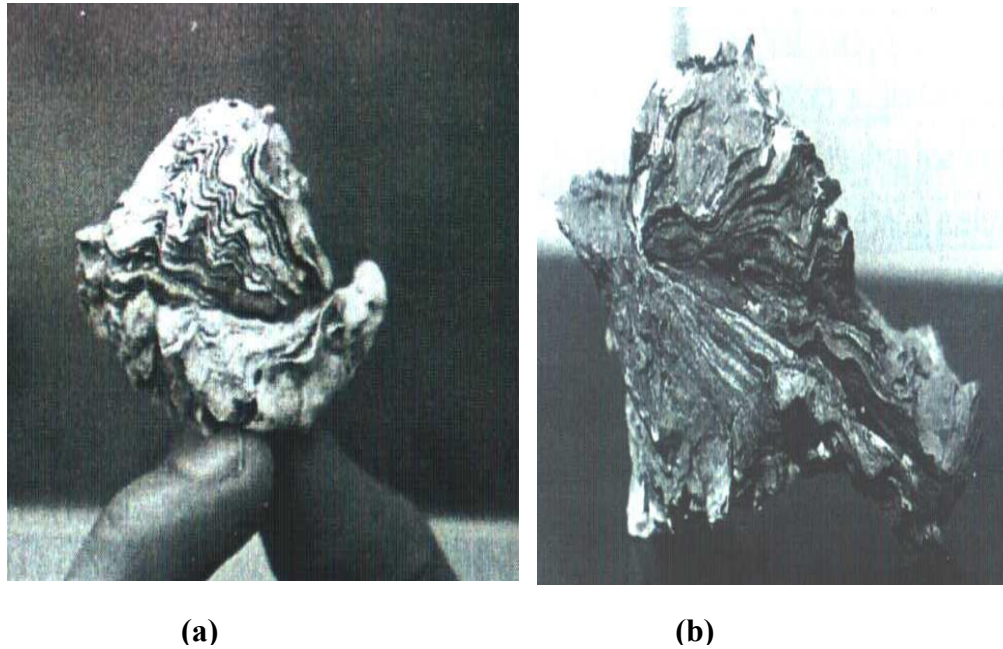


Figure 2.4: *Crassostrea gigas*. TBT effects on oysters: a) acute shells malformation – “ball shape” of a specimen collected in the Bay of Cadiz (Spain); b) shell from the Bay of Arcachon (France) with one type of growth during the TBT exposure period (left white arrow) and another after the TBT-based AF paints prohibition (right white arrow). Adapted from Alzieu (1996).

Oysters’ shells thickening was first described in France, then in UK, and few years later in Australia and New Zealand (de Mora, 1996), being the cause of the first legislative measure prohibiting the application of TBT-based AF paints in 1982.

2.4 International regulation on the TBT use

Subsequent to the above mentioned descriptions of oysters shell malformations, and to avoid the collapse of the oysters’ commercial industry from the Atlantic coast, the first legal restriction on the use of TBT compounds as biocides in AF paints was implemented by the French government in 1982. Such measure banned the use of AF systems with more than 3% of TBT on vessels <25 m in length, in areas of intense

oysters' production. This regulation was then extended to the entire coastline, except for aluminium structures, as they would suffer severe corrosion when protected only with copper preparations (Stewart, 1996).

Similar restrictions were subsequently introduced in most industrialized countries, specifically: United Kingdom in 1987; United States and New Zealand in 1988; Canada, Australia, Norway and Japan in 1989/90 (IMO, 2002). In these countries, it was also mandatory to register all TBT-based products as pesticides (Stewart, 1996). These measures led to a certain decline of TBT pollution in water, sediment and molluscs tissues, being accompanied by the recovery from abnormal shell growth in oysters and *imposex* intensity in gastropods at some locations (Gibbs and Bryan, 1994).

Concern on environmental pollution caused by TBT-based AF paints was first raised at IMO's Marine Environment Protection Committee (MEPC) in 1988, when the Paris Commission requested IMO to consider the need for measures under relevant legal instruments to restrict the use of TBT compounds on sea-going vessels (IMO, 2002). The MEPC concluded that, it was not viable to drastically abolish the use of AF systems with TBT on large commercial vessels for economic reasons (Champ, 1999).

In the light of the said descriptions and studies on biological effects of TBT on marine ecosystems, it was evidenced that these compounds were being harmful to aquatic organisms. Although, several countries had already restricted its use, it was obvious that international measures would need to be developed for total ban. In response, the European Commission by February 1988, proposed a legal restriction on marketing and use of OT compounds. Therefore, the Council Directive 89/677/ECC of 21 December amended, for the 8th time, Directive 76/769/EEC on the approximation of the laws,

regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations. Since then, “organostannic compounds may not be used as substances and constituents of preparations intended for use to prevent the fouling by microorganisms, plants or animals of: (a) the hulls of boats of an overall length, as defined by ISO (International Standards Organization) 8666, of less than 25 meters; (b) cages, floats, nets and any other appliances or equipment used for fish or shellfish farming; (c) any totally or partly submerged appliances or equipment.” Under this Directive, OTs were also forbidden as substances and constituents of preparations used in industrial waters treatment, irrespective of their use (Susana, 2010).

In April 1990, the “Third International Organotin Symposium”, held in Monaco, recognized the IMO competence to carry out the regulation regarding the use of AF systems. During the same year, in November, MEPC adopted Resolution 46 (30) on “Measures to Control Potential Adverse Impacts Associated with Use of Tributyltin Compounds in Antifouling Paints”. It was recommended that governments should adopt measures to eliminate use of TBT-based AF paints on non-aluminium hulled vessels <25 m in length and to forbid the use of AF systems with an average leaching rate $>4 \text{ mgTBT.cm}^{-2}.\text{day}$, as interim measures until IMO could consider a possible large-scale prohibition of TBT compounds on ships (IMO, 2002). The need for IMO to work on the AF issue was highlighted in the “United Nations Conference on Environment and Development”, held in Rio de Janeiro – Brazil, in 1992 (Eco-92 Conference). As a result, a document called Agenda 21 was developed to request each country’s commitment to reflect on social and environmental problems. For instance, it was

stipulated “States to take measures to reduce pollution caused by Organotin compounds used in Antifouling systems” (IMO, 1999, 2002).

Elsewhere, during MEPC 42nd session in November 1998, the success of Agenda 21 was confirmed because the United Nations Member States were encouraged to adopt alternatives to AF paints using OTs as biocides. Besides, the MEPC was assigned to develop a legal instrument to control its harmful effects by the 21st IMO Assembly of November 1999 (Figure 2.5). Fundamentally, through MEPC working group Resolution A.895 (21), IMO succeeded to establish global ban on the application and re-application of OT-based AF paints from 1st January 2003, and its total abolition from 1st January 2008 (IMO, 2001).

During diplomatic conference of the 5th October 2001, MEPC adopted the “International Convention on the Control of Harmful Antifouling Systems on Ships” (AFS Convention; IMO, 2001) rule to stop the use of OTs in AF paints and prevent introduction of new hazardous compounds in future formulations. In practice, the document became effective 12 months after being approved by 25 countries, whose fleets represent not less than 25% of world's merchant shipping tonnage (IMO, 2001). Following application and reapplication prohibition, the effective date was set for 1st January 2003 and the banning on the use and circulation, for 1st January 2008.

To increase the reliability of implementing the convention by the scheduled dates, the European Community (EC) in 2002 expected each member states to restrict OTs in AF paints regardless the document entry into force date. In practice, the EC intended to prohibit the application of OT compounds on ships flying flags of Member States from

1st January 2003, and the presence of such compounds on ships sailing to or from Member States ports from 1st January 2008 (Susana, 2010).



Figure 2.5: Pictures of some Greenpeace activists' actions, carried out in September and October 1999, calling for a ban on the use of the TBT-based AF paints. Adapted from the organization international website www.greenpeace.org.

Yet, the said regulation would be suspended, even though it could lead to competitive disadvantages for ship owners and shipyards of the European Union (EU), during the interim period (1st January 2003 to 1st January 2008) until the worldwide eradication.

Following the said measures, which would directly affect the functioning of OTs internal market; it was in thought necessary to approximate the laws of the Member States in this field. For that reason, Directive 2002/62/EC of 9th July adapted the already cited Directive 76/769/EEC for the 9th time and revised provisions regarding OTs used in AF

products to prohibit biocides in AF systems on ships by 1st January 2003. More specifically, the new Directive point (4) warned that a “regulation of the European Parliament and the Council” would “soon rule vessels bearing organostannic compounds”. In essence, the EU Member States were compelled to comply with the new Directive no later than 31st October 2002 and after the 1st July 2003 obligated by the Regulation No.782/2003j from 14th April, to apply the AFS Convention restrictions to their national fleets and to any vessel flying their flags.

In attempts to comply with AFS Convention and the new EU policy, Sweden, Netherlands, United Kingdom and Finland, had already announced the cancellation of the production and registration of all TBT-based AF products since March, 2002. Besides, the United States had begun legal procedures necessary to implement the AFS Convention in 2002 for immediate application once the rule was passed. It was recorded that until the 1st January 2003, only Denmark with 1.24% of the world’s merchant fleet tonnage had completely endorsed the AFS Convention and later followed by others. In the MEPC 49th session, in July 2003, five other countries approved the convention included Nigeria (0.07%), West Indies and Barbados (0.81%), Japan (2.53%) and Norway (3.93%) accounted only 8.58% of the world’s merchant fleet tonnage required for the convention worldwide application.

In addition to that, seven more countries had signed the document, pending the approval soon after national legislation amendment was finished including Australia (0.33%), Belgium (0.03%), Brazil (0.64%), Finland (0.28%), Morocco (0.51 %), Sweden (0.51%) and USA (1.91%). However, adding their merchant fleet tonnage to the first five would totalize 12.79% and still continued to be insufficient. Other countries had formally

announced the convention signing by the end of 2003 / early 2004 – Greece (4.99%), Spain (0.40%) and Italy (1.68%). United Kingdom (1.05%) and Panama (20.80%) had informally indicated their willingness to ratify the Convention in 2003 (IMO, 2009).

From the observed experiences, it was clear that the AFS Convention would not enter into force before the end of 2005. By the end of 2006, the document had been ratified by eighteen countries including Antilles and Barbados, Bulgaria, Croatia, Cyprus, Denmark, Greece, Japan, Latvia, Luxembourg, Mexico, Nigeria, Norway, Poland, Romania, Saint Kitts and Nevis, Spain, Sweden and Tuvalu totalizing 16.15% of the world's merchant shipping tonnage (IMO, 2009). It was until the 17th September 2007 when the AFS Convention entry into force was finally met following the 25th State endorsement of Panama representing a total of 38.00% of the world's merchant shipping tonnage. In view of that, the international ban on the use of TBT compounds as biocides in AF systems was scheduled for 17th September 2008 and by September, 2009; the document was approved by 40 States representing a total of 67.83% the world's merchant shipping tonnage (IMO, 2009).

Despite the need to verify measures on reducing TBT pollution, TBT compounds monitoring was also required because they were considered priority hazardous substances in EU legislation in the Water Framework Directive 2000/60/EC and the Marine Strategy Framework Directive 2008/56/EC which necessitated Member States to take measures for good marine environment status by the year 2020. Given that situation, the release of hazardous substances into the seas was set by Directive 2008/105/EC and for TBT compounds in surface waters is 0.0015 μ g TBT-Sn.l-1 (maximum allowed concentration).

The most recent European initiative regarding OTs is the Commission Decision 2009/425/EC of 28 May. This one restricted use of OTs compounds in consumer articles including: (i) Tri-substituted organostannic compounds, such as TBT and TPT, are not allowed after 1st July 2010, when the concentration is greater than the equivalent of 0.1% by weight of tin; (ii) DBT compounds are forbidden in mixtures and articles for supply to general public after 1 January 2012, when concentration is greater than the equivalent of 0.1% by weight of tin; (iii) Dioctyltin compounds shall not be used after 1 January 2012 in textile articles, gloves, footwear, female hygiene products, childcare articles and nappies, when the concentration (in the article, or part thereof) is greater than the equivalent of 0.1% by weight of tin.

However, by way of derogation, points (i) and (ii) shall not be applied until 1st January 2015 to some articles and mixtures for supply to the general public, namely: silicones, sealants, adhesives, soft PVC profiles, some items paints/coatings containing DBT compounds as catalysts, PVC coatings for outdoor applications containing DBT compounds as stabilizers, outdoor rainwater pipes, gutters and fittings, as well as covering material for roofing and facades.

2.5 Adverse effects on living organisms

Molluscs are amongst the most sensitive groups to TBT exposure, and the example of the oysters' shell malformations as mentioned in section 2.3, was just the beginning of the long process. The high toxicity of TBT resulted in numerous and widespread adverse biological effects to a wide range of organisms: from bacteria to mammals and from molecular to the community level as presented in Table 2.1. Now, it is clear that molluscs are the TBT most sensitive taxon due to compound high uptake rate (Fent,

1996) and weak ability to metabolize TBT and slow rate of elimination via excretory organs (Oehlmann *et al.*, 2007). Consequently, molluscs attain higher bioaccumulation than other taxa, so that the pollutant exhibits negative impacts at lower environmental concentrations; because of the extended time for tissue residues to reach toxic levels, as sublethal responses can also take a long time to develop (Meador, 2000).

Table 2.1: Summary of some Eco toxicological effects of TBT compounds indicated by taxonomic group.

Group	Effect	Important references
Bacteria	Toxic for bacteria (gram-positive more sensitive)	ICPS, 1990 Mendo <i>et al.</i> , 2003
Phytoplankton	Reduction of marine microalgae growth Alteration of photosynthetic pigment content Changes in the community structure	Petersen and Gustavson, 1998 Sidharthan <i>et al.</i> , 2002 EC, 2005
Plants	Impairment of macro algae spores motility Reduction of several marine angiosperms growth Stress induction, by bioaccumulation, in terrestrial plants used for human consumption	ICPS, 1990 Caratozzolo <i>et al.</i> , 2007 Azenha <i>et al.</i> , 2008 Lespes <i>et al.</i> , 2009
Crustaceans	Reproductive performance reduction Neonate survival decrease Juveniles growth rate decrease Community structure changes	ICPS, 1990 Waldock <i>et al.</i> , 1999 Dahllof <i>et al.</i> , 2001 Takeuchi <i>et al.</i> , 2001 EC, 2005 Aono and Takeuchi, 2008
	Abnormal shell growth Females virilisation (<i>Imposex</i> / Intersex) Sterility	Alzieu <i>et al.</i> , 1981 Bryan <i>et al.</i> , 1986 Bauer <i>et al.</i> , 1995

Molluscs	<p>Increased mortality</p> <p>DNA damage</p> <p>Teratogenic effects – embryos malformations;</p> <p>Community structure alterations</p>	<p>Page <i>et al.</i>, 1996</p> <p>Matthiessen & Gibbs, 1998</p> <p>Gabbianelli <i>et al.</i>, 2006</p> <p>Rank, 2009</p>
Fish	<p>Growth inhibition</p> <p>Females masculinization</p> <p>Sperm abnormalities induction and reduced fecundity</p> <p>Liver vacuolation</p> <p>Teratogenic effects – larvae malformations</p> <p>Hyperplasia of the hematopoietic tissue</p> <p>Disruption of intracellular energy production by inhibition of ATPase and ion-pump activities</p> <p>Neurotoxic through the modulation of the glutamate signalling pathway</p> <p>Thymus atrophy and thymocytes apoptosis</p> <p>Cytochrome P450 system inhibition</p>	<p>Fent, 1996</p> <p>McAllister and Kime, 2003</p> <p>Shimasaki <i>et al.</i>, 2003</p> <p>EC, 2005</p> <p>Zheng <i>et al.</i>, 2005</p> <p>Zhang <i>et al.</i>, 2008</p> <p>Zhang <i>et al.</i>, 2009</p> <p>Zuo <i>et al.</i>, 2009</p>
Mammals	<p>Behavioural changes in rats</p> <p>Spermatogenesis reduction in mice</p> <p>Foetal gonad morphology alterations by changes in gene expression profiles</p> <p>Teratogenic effects – embryos malformations</p> <p>Inhibition of the basal and calmodulin-dependent Ca²⁺ ATPase activity in rat brain synaptic membrane preparations</p> <p>Inhibition of mitochondrial oxidative phosphorylation or ATP synthesis</p> <p>Inhibition of natural killer cell cytotoxic function</p> <p>Suppression of osteoclastogenesis through the retinoic acid receptor (RAR) pathway</p>	<p>Fent, 1996</p> <p>Ema <i>et al.</i>, 1997</p> <p>Whalen <i>et al.</i>, 2002</p> <p>Grun <i>et al.</i>, 2006</p> <p>Aluoch <i>et al.</i>, 2006</p> <p>Ohtaki <i>et al.</i>, 2007</p> <p>Kishta <i>et al.</i>, 2007</p> <p>Yonezawa <i>et al.</i>, 2007</p> <p>Antizar-Ladislao, 2008</p> <p>Chen <i>et al.</i>, 2008</p>

	Adipose tissue differentiation rise – obesity induction; Disturbance of the Ca ⁺ homeostasis in human neutrophils Human lymphocytes inhibition	Yang <i>et al.</i> , 2009 Grun and Blumberg, 2009
--	--	---

2.6 Importance and toxicity of new antifouling chemicals

In practice, national and international legislations were introduced to restrict the use of TBT. Thus, in 1989, the European Community introduced a directive to prevent the use of TBT on boats under 25m. Following the directive, paint manufacturers and chemical companies were triggered to develop and sell a range of agents for new antifouling paints for the “small boat” market, which were also added to TBT-based paints to enhance efficacy for larger vessels. These compounds have since been termed “booster biocides” which have sometimes been assumed as environmentally sound, but their effects are poorly understood.

Studies show that accumulations of ‘booster’ biocides can reduce the germination and growth of non-*target algae* such as *Hormosira banksii* (e.g., diuron, zineb, DCOIT and zinc pyrithione; Myers *et al.*, 2006) and sea grasses such as *Zostera marina* (e.g., Irgarol 1051 and Diuron; Chesworth *et al.*, 2004), reduce the photosynthetic efficiency of symbiotic algae in corals (e.g., Irgarol 1051; Carbery *et al.*, 2006) and are toxic to sea urchin eggs and embryos (e.g., DCOIT, zinc and copper pyrithione; Kobayashi and Okamura, 2002). However, interactive effects of different biocides, their occurrence, degradation, bioaccumulation and transport remain mysterious, leading many researchers to recommend the precautionary principle when dealing with the regulation of these chemicals (Thomas, 2001).

Many of these compounds were known to be highly toxic. Negligible data, at that time, was, however, available concerning contamination, and (potential) effects/risks of these compounds in coastal and marine environments. Work undertaken through the Assessment of Antifouling Agents in Coastal Environments (ACE) project of the European Commission (MAS3-CT98-0178) (1999-2002) provided an overview of levels, behaviours and potential threats posed by the compounds. Several studies show that these new compound are abundant in environment with half-lives up to 200 days (Liu *et al.*, 1999) and could cause serious environmental damage (Evans *et al.*, 2000). Certainly, the new antifouling chemicals may impose more toxic effect in coral metabolisms than TBT.

2.7 Importance and toxicity of Diuron

Diuron is classified as photosystem II compounds (PSII). PSII is a pigment–protein membrane complex made up of the reaction centre D1/D2 heterodimer. The reaction centre of PSII carries out photochemical reactions, including primary charge separation and subsequent electron transfer from water plastoquinone (PQ). In the photosynthesis, excitation energy from light absorption in the light harvesting complex surrounding the reaction centre is transferred to a special chlorophyll species which is referred as P680*(Excited singlet state). P680* then form P680⁺ which then reduce to bound PQ called Q_A. Finally, the electron is passed to Q_A⁻ to Q_B on the D1 protein (Jones, 2005). PSII herbicides are plastoquinone analogs that inhibit photosynthesis by reversibly binding to the Q_B binding site of D1 protein (Jones, 2005).

Diuron may affect the host (animal) or/and symbionts (Jones, 2005). The studies showed diuron may reduce ¹⁴C incorporation in *Madracis mirabilis* (Owen *et al.*, 2002), the

reduction of $\Delta F/F_m'$ of *Stylophora pistillata*, *S. hystrix* and *Acropora Formosa*, (Jones and Kerswell, 2003), the loss of symbiotic algae in *M. digitata* and *S. hystrix* (Jones, 2004), and the detachment of soft tissue of juvenile of *Acropora tenuice* (Watanabe *et al.*, 2006).

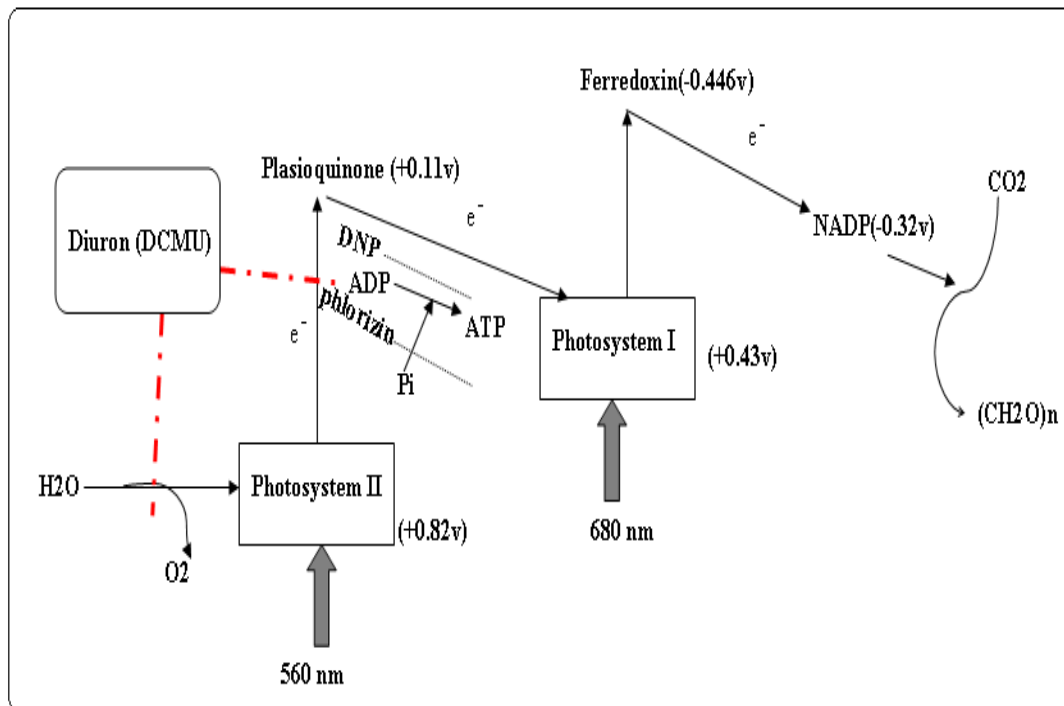


Figure 2.6: Scheme of photoelectron transport system (photosynthesis light reaction). Broken red line showing site of action of diuron (DCMU); (modified from Vandermeulen *et al.*, 1972).

2.8 Chemical structures and behavior of antifouling chemicals in the environment

Antifouling chemicals are chemicals used to prevent the accumulation and growth of marine biofouling on submerged structures such as oil rig supports, buoyans, fishing nets and cages, and ships` hulls (Stupak *et al.*, 2003). Generally, antifouling chemicals including new antifouling (Irgarol and diuron) have complex cycle in the coastal environments (Figure 2.7)

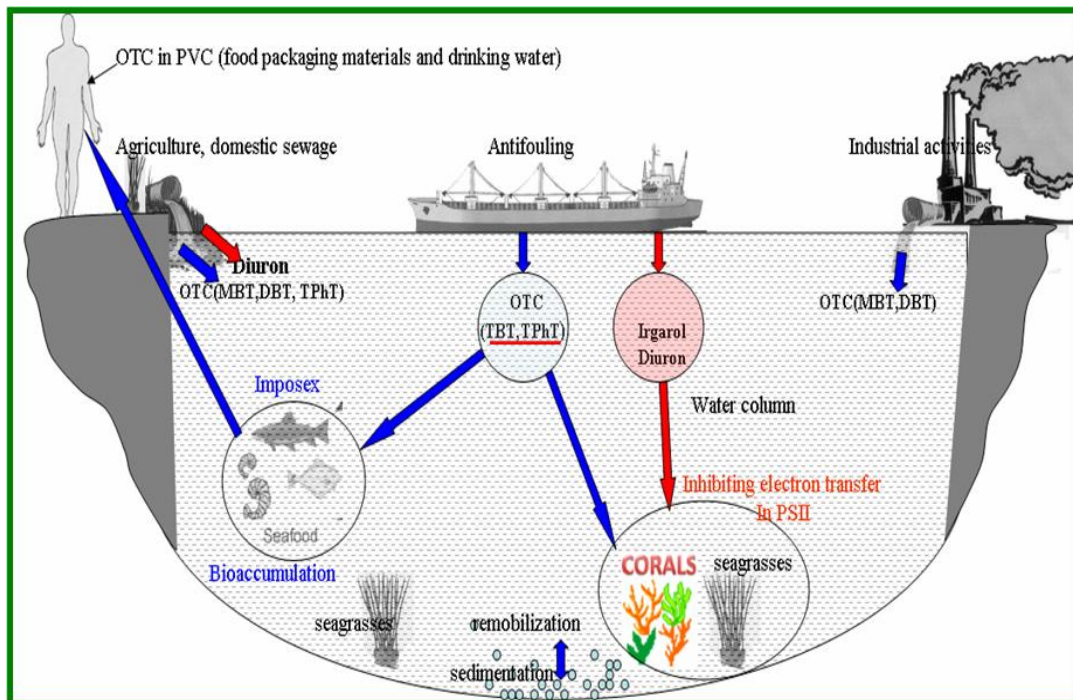


Figure 2.7: Schematic diagram showing routes and fate of antifouling chemicals (OTC, diuron and Irgarol) in coastal environment. Adapted from Ali, H. R., (2014).

2.9 Diuron

N-(3,4-dichlorophenyl)-*N,N*-dimethylurea (diuron) (Figure 2.8), is a herbicide belonging to the phenylamide family and the subclass of phenylurea. Diuron (CAS number: 330-54-1) is a colourless crystalline compound in its pure form, non-ionic with a moderate water solubility of 42 mg /L at 20 °C. It remains as a solid at ambient temperature with a melting point of 158–159 °C. Vapour pressure is 0.009 mPa at 25 °C and a calculated Henry's law constant of 0.000051 Pam³/mol suggesting that diuron is not volatile from water or soil (Giacomazzi and Cochet, 2004).

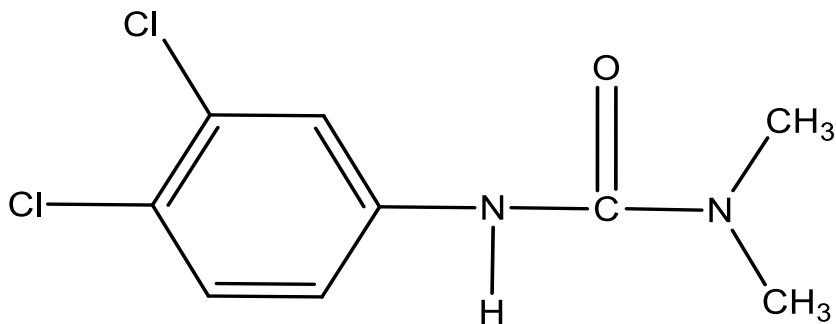


Figure 2.8: Chemical structure of diuron

Diuron is used to control weeds on hard surfaces, such as, roads, railway tracks, and paths, and to control weeds in crops, such as, pear and apple trees, forestry, ornamental trees and shrubs, pineapples, sugar cane, cotton, alfalfa and wheat. Furthermore, it is widely used as antifouling compound to inhibit photosynthesis in plants by binding site of photosystem II (PSII), which limits the electron transfer (Vandermeulen *et al.*, 1972)

Moreover, Diuron is very persistent in the environment, as it may remain from one month up to one year (Giacomazzi and Cochet, 2004). Diuron has been detected in marine environments from various regions such as western Japan (Okamura *et al.*, 2003; Sheikh *et al.*, 2009), UK (Boxall *et al.*, 2000), Spain (Ferrer and Barcelo 1997; Ferrer and Barcelo, 1999; Martinez *et al.*, 2000 and Martinez and Barcelo, 2001), The Netherlands (Lamoree *et al.*, 2002), Sweden (Dahl and Blanck, 1996). Diuron can undergo abiotic degradation such hydrolysis, photo degradation as well as biotic degradation (Figure 2.9) (Giacomazzi and Cochet, 2004).

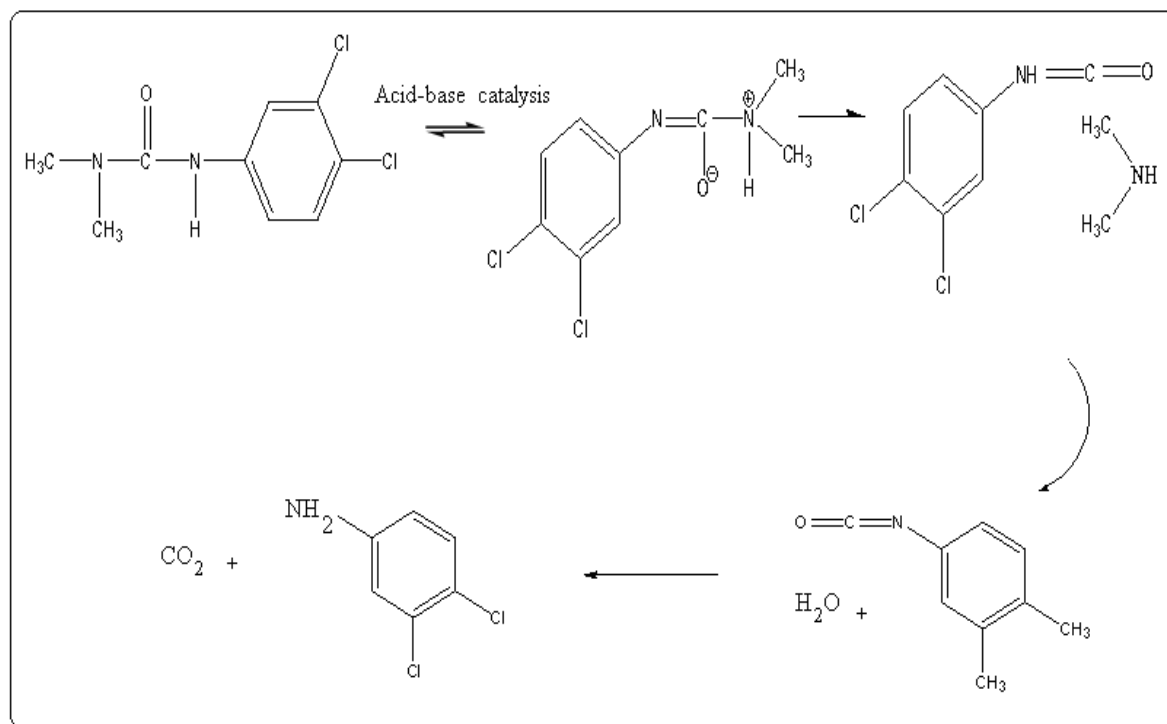


Figure 2.9: Chemical degradation of diuron

CHAPTER THREE

METHODOLOGY

3.1 Background of Study Areas

Zanzibar is a collection of small islands in the Indian Ocean 40 kilometers off the coast of Tanzania's main land. There are two main islands, Unguja and Pemba, and several smaller islands. Coral reefs, mangroves, sea grass beds, beaches, dunes, estuaries, rocky shores, and forests make up the coastal zone of the Zanzibar archipelago, which is rich in natural resources. The islands in the eastern, northern, and southern corridors surround fringing reefs (Muthiga *et al.* 2008). The most important economic players in Zanzibar are the marine transportation, tourism, and fishing industries.

3.1.1 Sampling Sites

The samples for this study were collected from 11 distinct locations along Unguja Island's coast (Figure 3.1). Several sampling sites with a range of different characteristics were chosen to guarantee that the sample collected was representative. The various socio-economic activities represented at the chosen sampling station included ports, recreation, fishing, agriculture, tourism, shipyards, cargo shipping, and ferries. A summary of the activities for each sampling station is shown in Table 3.1. Samples were taken at Malindi ports along the coastal area of Zanzibar, Bwawani, and Mtoni. Additionally, samples were taken from sand dunes and tiny islets in Bawe, Pange, and Kwale. Additionally, samples are taken from the coastal region of Kizimkazi and the coral reef islands of Chumbe, Murongo, Chapwani, and Mnemba.

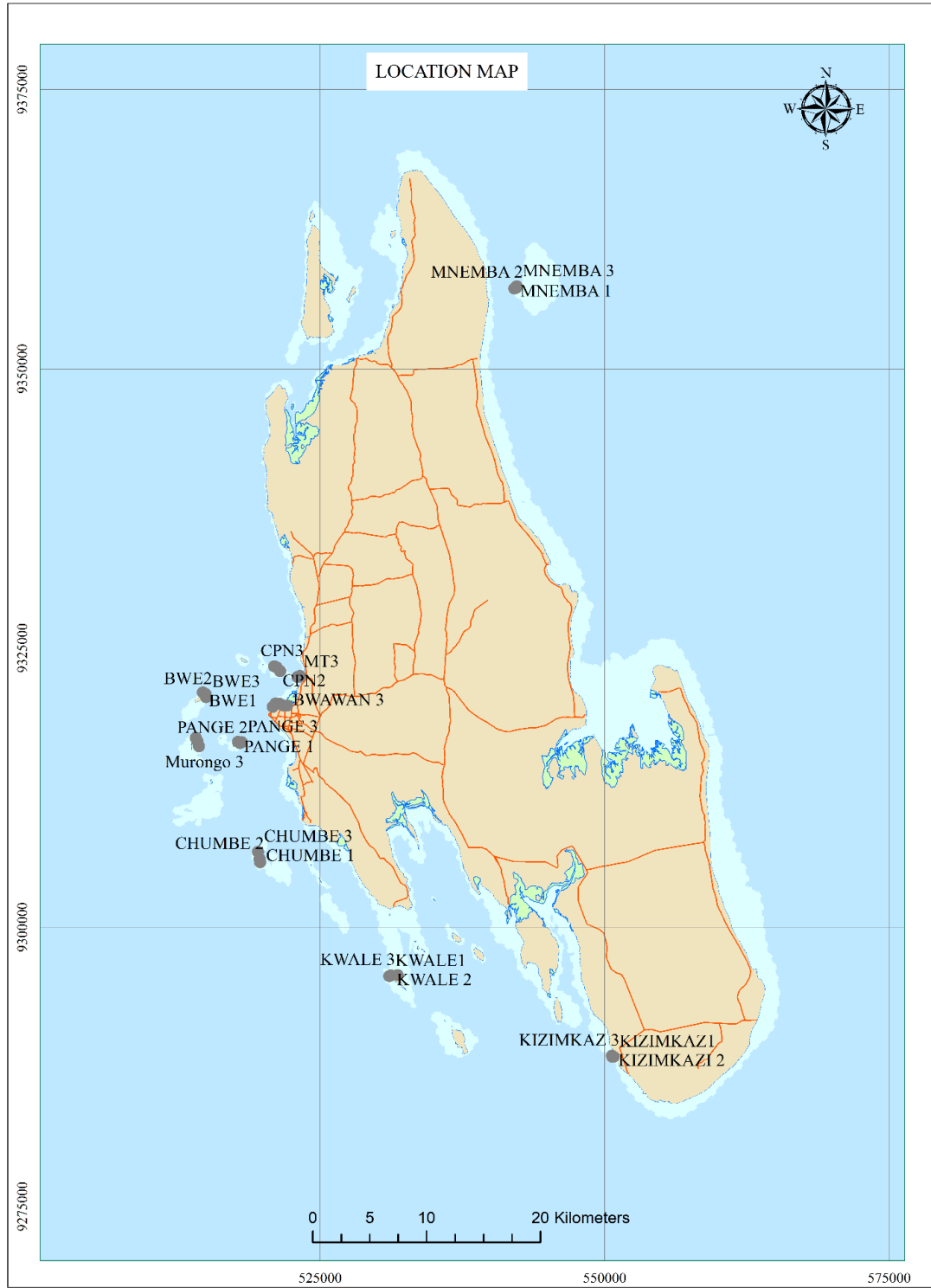


Figure 3.10: Map of Unguja coastal island shows sampling areas of this study.

3.1.2 Malindi

The site is located at 6° 09 south and 39° 11 east in Unguja Island, Zanzibar's Urban District. The site is located in an area surrounding Zanzibar City that has accumulated on a wide and shallow reef, flat and small islets off the coast of the Malindi port. Even in the strait, the sea off Malindi is shallow, with a depth of about 40 meters. It is a relatively calm sea area because the cays and shallow sea shelter it from the waves.

3.1.3 Bwawani

Another sampling station was Bwawani, which got its name from the swamps that once existed near Funguni. These swamps were created by a blockage of sea water that used to flow from the Indian Ocean to the current Darajani road's long creek. Bwawani is now known for the hotel built just beside the swamps, which was previously occupied by Funguni living houses before they were demolished to make way for the modern hotel. The site now has some wild vegetation and is home to some local birds as well as migratory birds from faraway places like Europe, depending on the weather each year.

3.1.4 Mtoni

Mtoni was another selected sampling location. The site is located within West 'A' District, which is part of the Urban West Region. It is bordered on the western side by the sea, with some residential houses on both sides, but the eastern one is completely full residential houses. Fishing, tourism (through hotels such as Hotel Verde) and tourist attractions such as of Mtoni Palace ruins, as well as petty business by locals, are the

main economic activities at Mtoni. The area is also known for the presence of deport and military camps mainly the Tanzania People's Defense Force – TPDF.



Figure 3.11: Photo of Mtoni as one of the study area for diuron.

3.1.5 Bawe

Bawe, a small island in Tanzania's Zanzibar Archipelago, was selected as another study area. It's about 10 kilometers off the coast of Stone Town, Zanzibar's capital, on the island of Unguja. Sultan Barghash bin Said of Zanzibar gave the island to the Eastern Telegraph Company at the end of the 18th century, which used it as an operation station for the underwater telegraph cable that connected Zanzibar to the Seychelles and Aden. Sultan Khalifa bin Said extended this agreement in 1889 in favor of Cable & Wireless, which built houses on the island to house their employees. Bawe is now primarily a tourist destination.

3.1.6 Chapwani (Grave Island)

Chapwani, formerly known as the "French Island," appears to contain a cemetery that was set aside for the burial of English people in 1870 by Seyyid Barghash (Sultan of

Zanzibar from 1870 to 1888). On the island, there are also a few gazelles that are common on the African continent. It is common to see numerous flying-foxes flying towards the town to feed in the evening.

3.1.7 Chumbe

Another sampling location was Chumbe Island, which is 12 kilometers southwest of Stone Town, Unguja, Zanzibar, and 6 kilometers from the nearest point on the Unguja coast (Chukwani). It is situated at the following coordinates: 6 16' S; 39 10' E. It is one of several MPAs in Tanzania, and only MPA in Zanzibar, among the four conservation areas. The Chumbe MPA closely borders the Menai Bay Conservation Area. A variety of marine species inhabit the Chumbe Reef Sanctuary (CRS). Out of the four the coral reef is the most diverse, hosting 90% of the hard coral species in East Africa least 16 coral families. It is known to be home to a total of 448 fish species that have been recorded within it. Swimming, snorkeling, underwater photography, education, and non-extractive research activities are among the permitted activities.

3.1.8 Mnemba

Mnemba was another study site. It is a single small island off the northeast coast of Unguja, Tanzania's largest island in the Zanzibar Archipelago, about 3 kilometers from beach at Matemwe. It has a roughly triangular shape with a diameter of 500 meters (1,640 feet) and a circumference of 1.5 kilometers (0.9 miles). An oval reef seven by four kilometers in size surrounds it. A marine conservation area has been established around these reefs. Mnemba Island is home to a diverse range of marine life and species. Diverse pelagic fish and marine mammals thrive in the warm waters of the Indian Ocean, while the coral reefs surrounding the island are home to a wide variety of reef

fish, anemones, and other marine specimens. In the ocean depths, humpback whales, dolphins of various species, sharks, and various types of rays can be found. Barracuda, mackerel, kingfish, sailfish, and marlin are among the predatory fish found in the area. The endangered green turtle, as well as a variety of bird species, including the rare crab plover, seek shelter on this island. The tiny suni antelopes and the recently introduced and highly endangered Ader's duikers can be found in the island's central forest.

Divers in the Mnemba area may come across the world's largest fish, the massive whale shark. Dolphin pods can be found in the warm tropical waters near Mnemba, and snorkelers and divers at Mnemba frequently come face to face with these telegenic deep dwellers. As a result, Mnemba is regarded as one of the world's most romantic ocean destinations along the African coast. With its white coral-sand beaches and spectacular coral reefs, this exquisite private island is known as one of the most romantic ocean destinations in the world.

3.1.9 Kwale

This is one of the Zanzibar Archipelago's many islands. It is only about 7 kilometers square. The island is surrounded by a large mangrove forest and the stunning turquoise waters of the Indian Ocean, which are teeming with natural wildlife. It is one of the best tourism aquatic attractions on Unguja Island's southern west tip, and it is famous for touristic activity known as "Safari blue".

3.1.10 Kizimkazi

This fishing town is located on Zanzibar's southern coast in Unguja. Three miles southeast of the Kizimkazi mosque, the village was once a walled city. According to the

National Census of 2012, the town is made up of two major villages: Kizimkazi Dimbani, which has a population of 1,760 people, and Kizimkazi Mkunguni, which has a population of 2,617 people. According to folklore, this town was built on the orders of a Sultan named 'Kiza,' and the builder was named 'Kizi,' thus the name 'Kizimkazi' was given to this town.

3.1.11 Pange

It is a sandbank in the Zanzibar Archipelago, off the coast of the Indian Ocean. During low tide, the area is sandy; during high tide, it is covered in seawater. Pange is located nearly at Bawe Island on the Western side of Unguja Island, directly across from Zanzibar's Stone Town, and is mostly visited as an aquatic tourist attraction during the trip from Prison Island.

Table 3.1: Location, physical parameters and characteristics of sampling stations

STATIONS	N	E	TEMP (° C)	pH	CHARACTERISTICS
KW1	6.37509	39.28192	30.25 ± 0.07	7.99 ± 0.00	Tourism
KW2	6.37450	39.28308	30.83 ± 1.27	7.84 ± 0.32	Tourism
KW3	6.37431	39.28846	29.17 ± 0.58	7.92 ± 0.84	Tourism
CHU1	6.28290	39.17922	28.83 ± 0.06	7.97 ± 0.06	Tourism
CHU2	6.28035	39.17878	28.8 ± 0.00	8.00 ± 0.00	Tourism
CHU3	6.27454	39.17746	28.90 ± 0.00	8.02 ± 0.01	Tourism
MTN1	6.13292	39.21033	28.2 ± 0.08	8.09 ± 0.08	Local cargo station
MTN2	6.13199	39.21078	28.30 ± 0.08	8.07 ± 0.01	Local cargo station
MTN3	6.13289	39.13289	28.20 ± 0.08	8.05 ± 0.08	Local cargo station
CPN1	6.12810	39.12810	28.55 ± 0.13	7.99 ± 0.01	Tourism
CPN2	6.12504	39.12504	28.66 ± 0.09	8.02 ± 0.01	Tourism
CPN3	6.12437	39.12437	28.95 ± 0.06	8.02 ± 0.00	Tourism
BWE1	6.14549	39.14549	28.45 ± 0.06	8.03 ± 0.01	Tourism, fishing, shipyard
BWE2	6.14669	39.14669	28.87 ± 0.12	8.01 ± 0.01	Tourism, fishing, shipyard
BWE3	6.14932	39.14932	29.33 ± 0.05	8.14 ± 0.01	Tourism, fishing, shipyard
MRG1	6.15904	39.1300	28.5 ± 0.00	8.03 ± 0.00	Tourism
MRG2	6.15973	39.13056	28.60 ± 0.00	8.03 ± 0.00	Tourism
MRG3	6.16168	39.13140	28.5 ± 0.00	8.02 ± 0.01	Tourism
PNG1	6.18541	39.16159	29.05 ± 0.06	8.06 ± 0.01	Tourism
PNG2	6.18643	39.16318	28.83 ± 0.05	8.04 ± 0.06	Tourism
PNG3	6.18566	39.16447	28.7 ± 0.00	8.04 ± 0.01	Tourism
HBR1	6.15701	39.18918	28.68 ± 0.05	8.02 ± 0.01	Cargo shipping, passenger speedboats, fishing, shipyard.
HBR2	6.15432	39.19152	28.88 ± 0.05	8.02 ± 0.00	Cargo shipping, passenger speedboats, fishing, shipyard
HBR3	6.15441	39.19415	28.48 ± 0.05	7.99 ± 0.00	Cargo shipping, passenger speedboats, fishing, shipyard
BWN1	6.15588	39.19752	28.95 ± 0.06	7.97 ± 0.01	Fishing.
BWN2	6.15617	39.19897	29.00 ± 0.00	7.99 ± 0.00	Fishing.
BWN3	6.15605	39.20052	29.45 ± 0.17	7.97 ± 0.01	Fishing.
KZM1	6.44037	39.46001	28.30 ± 0.00	7.98 ± 0.00	Fishing and tourism
KZM2	6.44031	39.45910	28.30 ± 0.01	8.00 ± 0.01	Fishing and tourism
KZM3	6.43944	39.45882	28.30 ± 0.00	8.0 ± 0.00	Fishing and tourism
MN1	5.81808	39.38077	28.53 ± 0.10	7.90 ± 0.00	Tourism
MN2	5.81734	39.38227	28.90 ± 0.00	7.90 ± 0.00	Tourism
MN3	5.81637	39.38271	29.00 ± 0.00	7.92 ± 0.00	Tourism

3.2 Material and Methods

3.2.1 Sample Collections

Total of 33 water samples were collected from sampling stations of Unguja Island. In the process Niskin water sampler was used to collect water 0.5m down the subsurface layer in all station to avoid taking the contaminated microlayer, after that 1L sample of water was poured in sampling bottles pre cleaned with acetone. The samples were then kept under 4°C using ice box and brought to laboratory for Diuron analysis within ten days from the sampling date.

Physical parameters such GPS coordinate, dissolve oxygen, salinity, pH, temperature, conductivity, turbidity, site depth and standard water qualities for each sampling site were recorded insitu using multi sonde (YSI ProDSS Multi-parameter water quality Meter).

3.2.2 Sample Extraction

Water samples were filtrated by vacuum filter with a glass fiber filter grade GF with normal pore size of 0.7µm and diameter of 25 mm. Diuron in water was analyzed following the solid phase extraction HPLC method of extracted of pesticides in drinking water as recommended by the Ministry of Health, Labour and Welfare, Japan (Okinawa Prefectural Enterprise Bureau, 2003) as later described by Ali, *et al.* 2014. Water samples were pre-concentrated in the solid phase extraction cartridges column (SUPELLEANTM, ENVITM-18 Sigma Aldrich, Germany).

Prior to the extraction of diuron the columns were conditioned with 10 mL of acetonitrile, followed by methanol and milli Q water respectively. 10 mL of 0.2M

EDTA was added to 1 L of water and then pH of water was adjusted to 3.5. Then 1 mL of 1 mg/L Diuron D-6 ($C_9H_4Cl_2D_6N_2O$) was spiked as surrogate standard in order to monitor the recovery of Diuron. Water samples were automatically concentrated in the solid phase extraction column by using solid phase extraction controller with flow rate of 20 mL/min. SupelcleanTM ENVITM-18 cartridges were then dried under nitrogen gas for 5 min. Diuron was eluted from the column using 5 mL of acetonitrile. Finally, acetonitrile was evaporated to 0.2 mL with a stream of pure nitrogen gas. The summary of samples extraction procedures is shown in Figure 3.3 and figure 3.4 respectively.

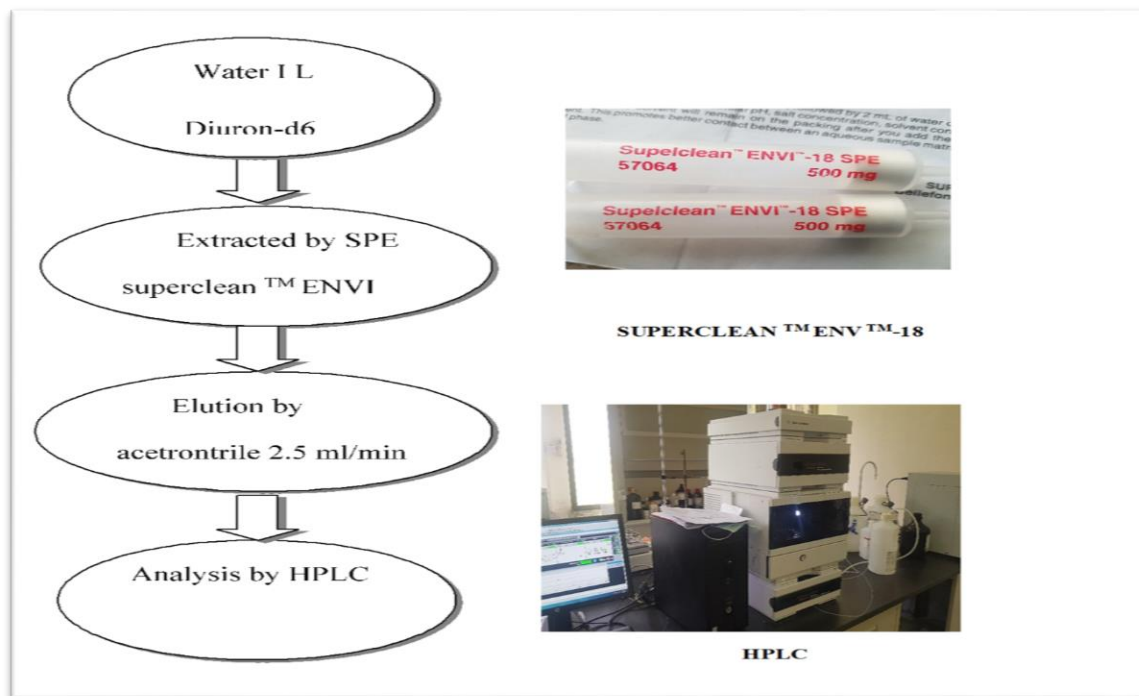


Figure 3.12: Analytical method for Diuron analysis in laboratory



Figure 3.13: Solid Phase Extraction (SPE) as way of sample preparation before analysis

3.2.3 Analysis of Diuron from Seawater

The Diuron was analyzed using an Agilent Technologies series 1260 infinity II High Performance Liquid Chromatography HPLC (Agilent USA) equipped with DAD (G1315D). The Supelcosil™ LC-18 HPLC column (25 cm x 4.6 mm 5 μm) (Agilent, USA) was used. Separation was obtained using gradient elution at flow rate of 1mL/min with solvent (80% A milli Q water) and solvent B (20% Acetonitrile). The determination wavelength was set at 254 nm and the column temperature was kept at 40°C, the retention time was 3.12 min and injection volume was 1.0 μL. The detection limit was

29.331 ng/L. The calibration curve and typically chromatogram showing from 0.05 standard sample is found in Figure 3.5 and calibration curve data are in table 3.3

3.3 Quality Assurance of Diuron

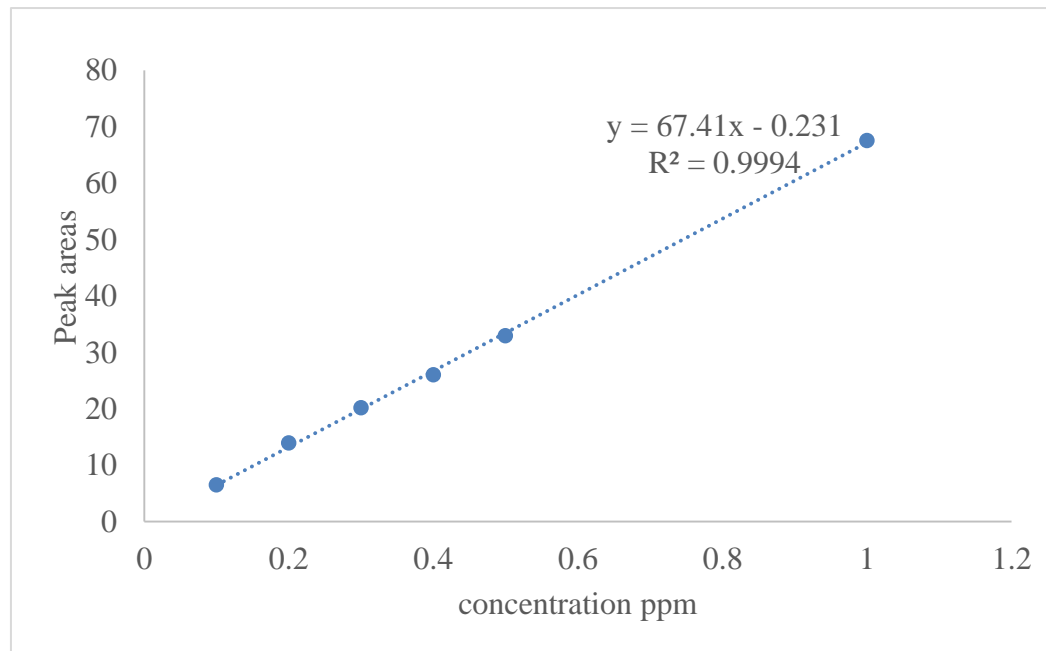
For the purpose of quality control of the analytical procedure, 1L of Milli Q water was treated the same as the real water samples for each batch of analysis as the water sample procedural blank. The recovery tests were also conducted for the purpose of monitoring the efficiency of the methodology. 1 L of water samples collected from remote areas was spiked with known concentration of Diuron D-6 was used to determine recovery. The sample was processed following same method as real water samples. The recoveries of Diuron D-6 are shown in Table 3.2.

Table 3.2: Recovery test data of Diuron spiked in remote samples

S.N	Spiked value (ppm)	Observed value (ppm)	%Recovery
1.	0	0.00	100.00
2.	0.1	0.0875	87.5
3.	0.2	0.1794	89.7
4.	0.4	0.3684	92.1
5.	0.6	0.5322	88.7
6.	0.8	0.788	98.5
7.	1.0	0.899	89.9
		Average	92.34
		SD	4.94

Table 3.3: Calibration curve data for Diuron (ppm) analysis using HPLC

Standard Name	Retention time	Peak Area
Standard 0.1	3.128	6.53677
Standard 0.2	3.128	13.928
Standard 0.3	3.128	20.18539
Standard 0.4	3.127	26.02167
Standard 0.5	3.126	32.92434
Standard 1.0	3.128	67.54146

**Figure 3.14: Calibration curve of Diuron**

3.4 Data Analysis

Average Diuron concentration was calculated from the triplicate measurements at each study site areas and summarized in Table 4.1. One-way repeated measures analysis of variance was used to test whether the mean Diuron concentration differs by the study

sites. Stata 15 standard editions (StataCorp, Texas, USA) was used for data analysis to compare spatial variation between all sample sites.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Levels of Diuron in Water

Diuron detected in seven (64.6%) sites among 11 sampled sites in Unguja Island with Bwawani 1 (BWN1) recorded a maximum value of 1366 ng/L (Table 4.1). The station is closer to the major dockyard in Zanzibar. It is also close to an overcrowded harbor in the archipelago. Furthermore, the station BWN1 is positioned in a bay area with little tides movement and water exchange, which may contribute to the elevation of Diuron. The lowest value of diuron among the detected sites was 260 ng/L at Bawe1 (BWE1). However, Diuron was below detection limit (BDL) in 4 (36.4%) sites, which are Chumbe, Kwale, Mtoni, and Murongo (Table 4.1). The detected values of diuron from each site are summarized in Figure 4. 1.

Table 4.1: Levels of Diuron (ng/L) in coastal water of Unguja Island

STATIONS	N	E	Concentration (ng/L)
KW1	6.37509	39.28192	ND
KW2	6.37450	39.28308	ND
KW3	6.37431	39.28846	ND
CHU1	6.28290	39.17922	ND
CHU2	6.28035	39.17878	ND
CHU3	6.27454	39.17746	ND
MTN1	6.13292	39.21033	ND
MTN2	6.13199	39.21078	ND
MTN3	6.13289	39.13289	ND
CPN1	6.12810	39.12810	1097
CPN2	6.12504	39.12504	1123
CPN3	6.12437	39.12437	1159
BWE1	6.14549	39.14549	260
BWE2	6.14669	39.14669	268
BWE3	6.14932	39.14932	263
MRG1	6.15904	39.1300	ND
MRG2	6.15973	39.13056	ND
MRG3	6.16168	39.13140	ND
PNG1	6.18541	39.16159	1096
PNG2	6.18643	39.16318	1006
PNG3	6.18566	39.16447	1040
HBR1	6.15701	39.18918	1026
HBR2	6.15432	39.19152	1086
HBR3	6.15441	39.19415	908
BWN1	6.15588	39.19752	1366
BWN2	6.15617	39.19897	1264
BWN3	6.15605	39.20052	1335
KZM1	6.44037	39.46001	554
KZM2	6.44031	39.45910	387
KZM3	6.43944	39.45882	407
MN1	5.81808	39.38077	625
MN2	5.81734	39.38227	622
MN3	5.81637	39.38271	614

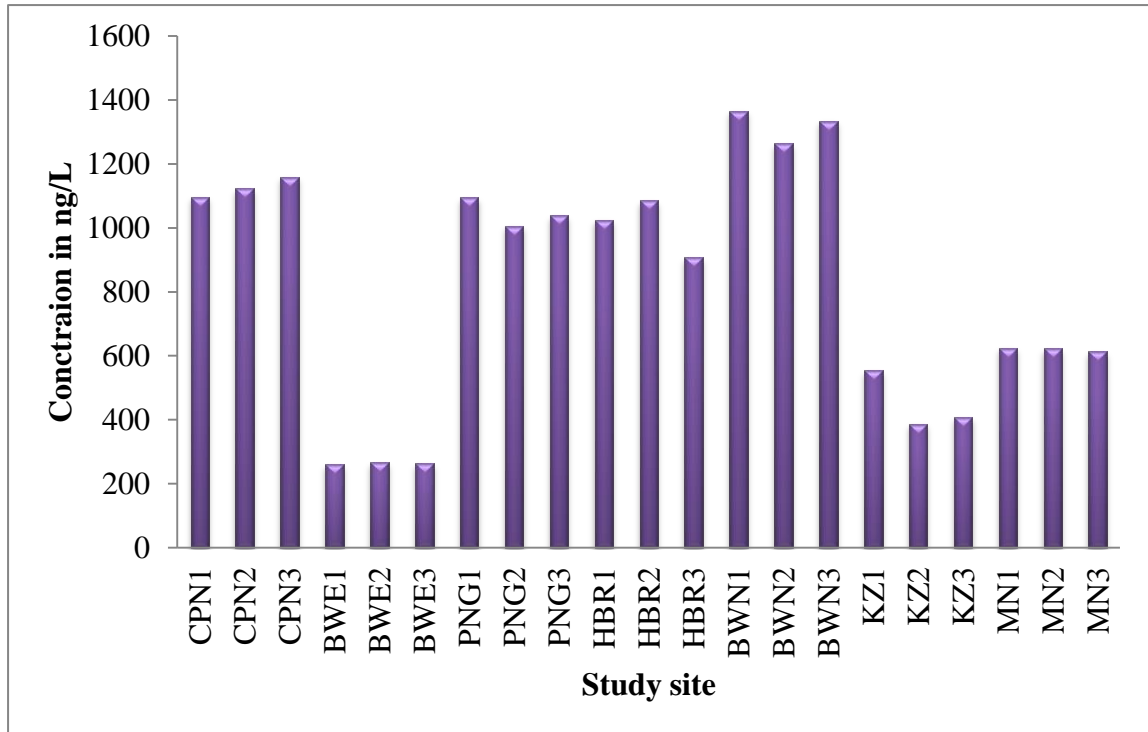


Figure 4.15 Diuron concentration (ng/L) recorded from each study site

Results from one-way repeated measures analysis of variance (replicate within the site), indicated no significance difference in Diuron concentration among measured concentrations at each site as shown in Figure 4.1.

4.2 Level of diuron in sampling sites which exceeding the Maximum Permissible Concentration (MPC)

The maximum permissible concentration of 430 ng/l for Diuron has been derived by the appropriate Dutch authorities (Lamoree et al., 2002). Diuron is no longer approved for use in the UK as an active ingredient in antifouling paints, on any size of vessel. In this study the highest Diuron concentration in average is 1321.67 ± 52.30 ng/L recorded at Bwawani while the lowest is 263.33 ± 4.00 recorded at Bawe (Table 4.2).

Table 4.2: Mean Diuron concentration by study sites

SITES	Mean \pm S.D (ng/L)	Maximum	Minimum
Bawe	263.67 \pm 4.00	268	260
Bwawani	1321.67 \pm 52.30	1366	1264
Chapwani	1126.33 \pm 31.10	1159	1097
Harbour	1006.67 \pm 90.60	1086	908
Kizimkazi	449.33 \pm 91.20	554	387
Mnemba	620.33 \pm 5.70	625	614
Pange	1047.33 \pm 45.40	1096	1006

Spatial variations in diuron mean concentrations were noted at Harbour, Bwawani and Kizimkazi and little variation in Pange (Figure 4.2). However, all sites except Bawe have Diuron concentration exceeding maximum permissible concentration (MPC) of 430 ng/L, as proposed by Dutch National Institute of Public Health and Environment (Giacomazzi and Cochet, 2004) for boats working in the water depth below 25m (Figure 4.3). The average Diuron concentrations detected around Unguja Island were considerably different ($p = 0.05$; at 5% significance level) from the allowed Diuron value of 430ng/L (Figure 4.3). Apart from the fact that, few study sites were not record the diuron concentrations, the results of this study indicating that diuron in Zanzibar is at alarming stage since the majority of the sites records the value above the maximum permissible concentration (MPC) of 340ng/L. The ubiquitous presence of diuron is unsurprising since it appears one of the most commonly used antifouling paint booster biocides whereas also having wide spread non-antifouling uses. Predominantly, the use of diuron has been associated with weed control in non-agricultural applications. Several studies have investigated the impact of non-antifouling use of diuron release into the aquatic environment (Albanis et al., 1994; House et al., 1997; De Almeida Azevedo et al., 2000; Gennaro et al., 1995; Field et al., 1997). It is suggested that the source of diuron in estuaries and coastal areas is supplemented by non-antifouling paint inputs

since the estuaries are subject to relatively low volumes of shipping and yachting when compared to marinas and ports water. There is the possibility that some freshwater and seawater from ports and marinas near estuaries might receive considerable agricultural drainage, containing diuron (Konstantinou & Albanis; 2004).

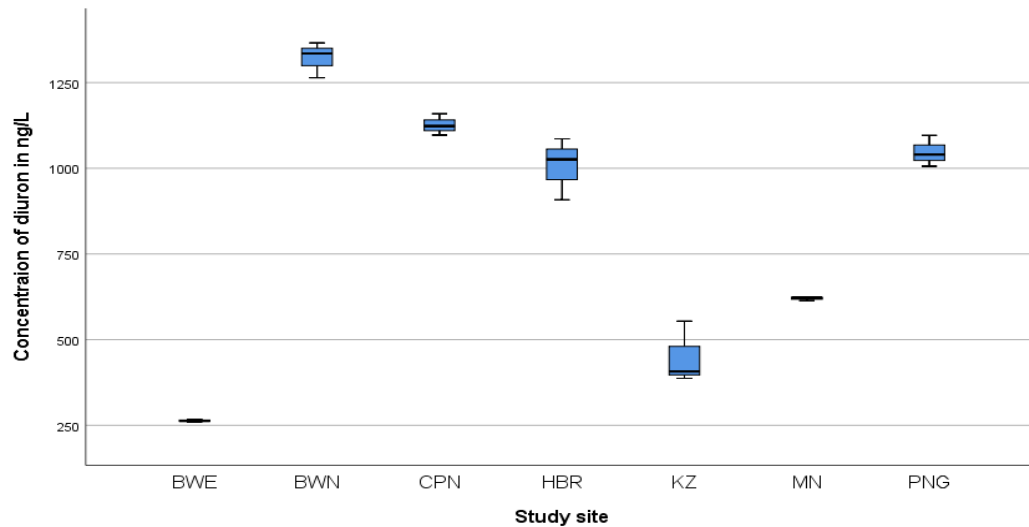


Figure 4.2: Spatial variation of average diuron concentration (ng/L) in coastal waters of Unguja Island

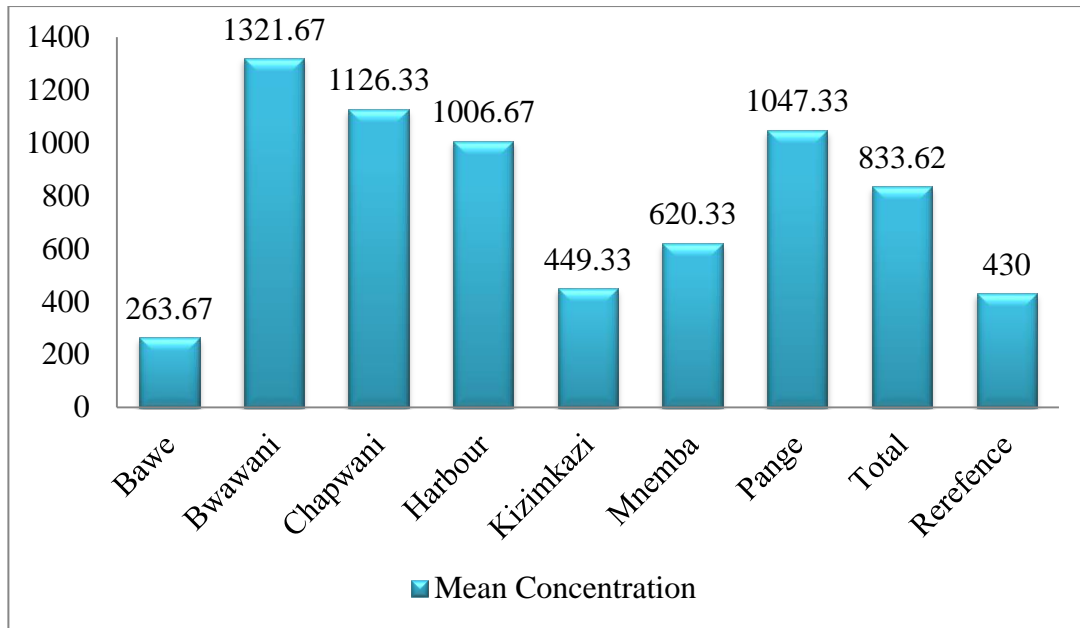


Figure 4.3: Comparison of average diuron concentration (ng/L) in coastal waters of Unguja Island with Maximum Permissible Concentration (MPC)

4.3 Comparison of Diuron level from different clusters

Diuron residues showed significant differences between average concentrations of the study sites (Table 4.3) with least of 0.000 for $p < 0.05$ (ANOVA). It evident that, distributions reflect anthropogenic activities as a possible source of diuron in the sampling areas (Ali *et al.* 2021; Sheikh *et al.* 2016; Lam *et al.* 2005; Hall *et al.* 1999).

Moreover, statistical analysis of the results between the clusters (Reef, Island, Harbor and Coast) showed a significant difference ($p < 0.05$) of the concentration of Diuron between the clusters (Table 4.4). The findings showed that the distribution of Diuron was based on the characteristics of the relative site. The highest average concentration of 1164.17 ± 184.78 ng/L was found at the area of dockyards and harbor because mainly economic activities conducted like cargo shipping, passenger speed boat, fishing, tourism activities such as snorkeling and local cargo increase the level of diuron

contamination (Fig 4.5). Liu et al., 1999 reported that fishing boats require heavy applications of antifouling paint to prevent the growth of fouling organisms, due to the faster wear off of the antifouling chemicals through their heavy use. The lowest average concentration (449.33 ± 91.19 ng/L) was detected at the coastal area. This is probably due to the fact that the economic activities in this area are mainly agriculture, which have low possibility of producing high amount of contamination of diuron (Fig 4.3). The average concentration in coral reefs areas was 925.20 ± 276.29 ng/L (Fig 4.4). The coral reefs positioned at the intermediate contamination of Diuron.

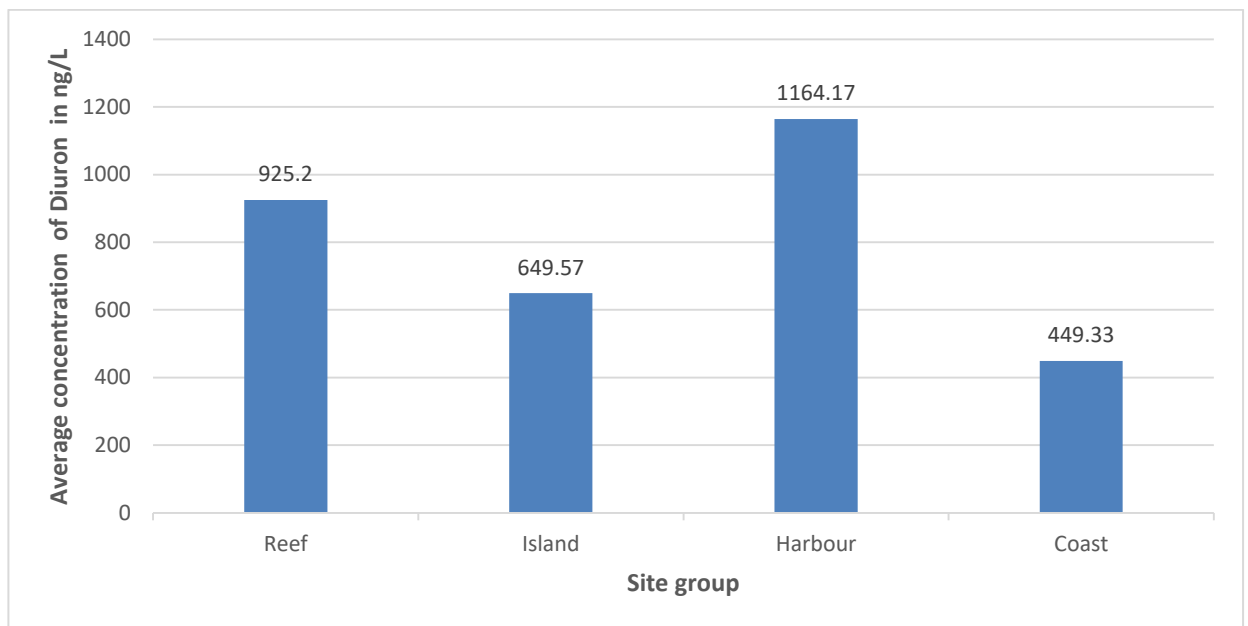


Figure 4.4: Mean concentrations of Diuron from four categories

Table 4.3: Spatial variation on concentration of diuron in ng/L using Tukey HSD

(I) Study site	(J) Study site	Mean Difference			95% Confidence Interval	
		(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
CPN	BWE	862.667*	46.121	.000	705.18	1020.15
	PNG	79.000	46.121	.619	-78.49	236.49
	HBR	119.667	46.121	.199	-37.82	277.15
	BWN	-195.333*	46.121	.011	-352.82	-37.85
	KZ	677.000*	46.121	.000	519.51	834.49
	MN	506.000*	46.121	.000	348.51	663.49
BWE	CPN	-862.667*	46.121	.000	-1020.15	-705.18
	PNG	-783.667*	46.121	.000	-941.15	-626.18
	HBR	-743.000*	46.121	.000	-900.49	-585.51
	BWN	-1058.000*	46.121	.000	-1215.49	-900.51
	KZ	-185.667*	46.121	.017	-343.15	-28.18
	MN	-356.667*	46.121	.000	-514.15	-199.18
PNG	CPN	-79.000	46.121	.619	-236.49	78.49
	BWE	783.667*	46.121	.000	626.18	941.15
	HBR	40.667	46.121	.970	-116.82	198.15
	BWN	-274.333*	46.121	.001	-431.82	-116.85
	KZ	598.000*	46.121	.000	440.51	755.49
	MN	427.000*	46.121	.000	269.51	584.49
HBR	CPN	-119.667	46.121	.199	-277.15	37.82
	BWE	743.000*	46.121	.000	585.51	900.49
	PNG	-40.667	46.121	.970	-198.15	116.82
	BWN	-315.000*	46.121	.000	-472.49	-157.51
	KZ	557.333*	46.121	.000	399.85	714.82
	MN	386.333*	46.121	.000	228.85	543.82
BWN	CPN	195.333*	46.121	.011	37.85	352.82
	BWE	1058.000*	46.121	.000	900.51	1215.49
	PNG	274.333*	46.121	.001	116.85	431.82
	HBR	315.000*	46.121	.000	157.51	472.49
	KZ	872.333*	46.121	.000	714.85	1029.82
	MN	701.333*	46.121	.000	543.85	858.82
KZ	CPN	-677.000*	46.121	.000	-834.49	-519.51
	BWE	185.667*	46.121	.017	28.18	343.15
	PNG	-598.000*	46.121	.000	-755.49	-440.51
	HBR	-557.333*	46.121	.000	-714.82	-399.85
	BWN	-872.333*	46.121	.000	-1029.82	-714.85
	MN	-171.000*	46.121	.030	-328.49	-13.51
MN	CPN	-506.000*	46.121	.000	-663.49	-348.51
	BWE	356.667*	46.121	.000	199.18	514.15
	PNG	-427.000*	46.121	.000	-584.49	-269.51
	HBR	-386.333*	46.121	.000	-543.82	-228.85
	BWN	-701.333*	46.121	.000	-858.82	-543.85
	KZ	171.000*	46.121	.030	13.51	328.49

Note: *The mean difference is significant at the 0.05 level.

Table 4.4: Mean pairwise comparison for sites group

(I) Site group	(J) Site group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Reef	Island	275.629	169.202	.390	-205.34	756.59
	Harbor	-238.967	174.978	.536	-736.35	258.42
	Coast	475.867	211.032	.148	-124.00	1075.74
Island	Reef	-275.629	169.202	.390	-756.59	205.34
	Harbor	-514.595*	160.766	.024	-971.58	-57.61
	Coast	200.238	199.406	.749	-366.59	767.06
Harbor	Reef	238.967	174.978	.536	-258.42	736.35
	Island	514.595*	160.766	.024	57.61	971.58
	Coast	714.833*	204.331	.013	134.01	1295.66
Coast	Reef	-475.867	211.032	.148	-1075.74	124.00
	Island	-200.238	199.406	.749	-767.06	366.59
	Harbor	-714.833*	204.331	.013	-1295.66	-134.01

Note: *The mean difference is significant at the 0.05 level

In addition to that the statistical information between each category varied, where their mean variation showed significant and others not. For example; the average concentration of Diuron between Reef category and Small Island showed statistically there is no significant variation ($p > 0.05$). On the other hand the average concentration between Reef and Harbour, Reef and Coast, and between Coast and Island show there was no significant. However the average concentration between Island and Harbour and between Harbour and Coast statistically showed significant variation (Table 4-4). Variation was probably due to difference of the activities and boat density (Sheikh *et al.*, 2016, Ali *et al.*, 2021). The main sources of the biocides could come from increasing in activities in harbor/dockyard such as passengers, cargo and fishing as well as tourism boating activities during snorkeling and dolphin watching. It is important to continue monitoring of the biocides.

The maximum concentrations measured from different regions are much higher compared to the maximum concentration found around coastal areas of Unguja Island. For example concentration up to 3,050 ng/L was detected in Seto Inland Sea, Japan (Okamura *et al.*, 2003); 42,000 ng/L in Lagoon water Italy (Gennaro *et al.*, 1995); 6742ng/L estuaries UK, (Thomas *et al.*, 2001) ; 2,000 ng/L in Mediterranean coast, Spain, (Martinez *et al.*, 2000). In contrast to the said examples, however, the findings of the current study demonstrate that the level of Diuron around coastal Unguja Island is relatively low.

Table 4.5: Maximum concentrations of Diuron (ng/L) detected worldwide

Location and Country	Maximum concentration (ng/L)	References
Seto Inland Sea, Japan	3,050	Okamura <i>et al.</i> , (2003)
Lagoon water, Italy	42,000	Gennaro <i>et al.</i> , (1995)
Estuaries, UK	6,742	Thomas <i>et al.</i> , (2001)
Mediterranean Coast, Spain	2,000	Martinez <i>et al.</i> , (2000)
Zanzibar	1,366	This study

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Diuron is no longer approved for use as an active ingredient in antifouling paints, on any size of vessel in many countries. Other European countries such as Denmark and Sweden have also restricted the use of paints containing Diuron to boats >25 m in length. A comparative general environmental assessment of biocides used in antifouling paints was recently reported by [Voulvoulis et al. \(2002\)](#), provided support for the use of the precautionary principle with respect to policies on antifouling products.

This thesis presents basic information of diuron *N*-(3, 4-dichlorophenyl)-*N*, *N*-dimethylurea (Diuron) contamination as a fingerprint of anthropogenic impacts on coral reef ecosystems around coastal area of Unguja Island. The study specifically, focused on spatial variation and quantification of Diuron concentration among the study sites. Additionally, results of this study were compared with the proposed Maximum Permissible Concentration (MPC) of 430 ng/L, as recommended by Dutch National Institute of Public Health and the Environment. Finally, the findings were compared to other reported concentrations from different countries as well as the acute exposure experimental data to corals and other marine organisms.

5.2 Conclusions

The results suggest that Unguja Island might be contaminated with Diuron from various sources such as agriculture, urban uses and shipping activities while coral reef Islands, might be contaminated from high boating activities. The results reveal that Diuron

contamination at Zanzibar is at levels of causing concern since most recorded values were above the maximum permissible concentration of 430 ng/L set by Dutch Authorities. It can be concluded that, although the data obtained are far below the lowest observable effects concentration (LOEC) to corals as shown by laboratories of ecotoxicological studies in the world, but still is undefined how the chronic exposure of ambient concentration of Diuron will affect corals in Zanzibar Island.

5.3 Recommendations

Based on the presence data of diuron residue found in Zanzibar which exceed the Maximum Permissible Concentration of 430 ng/L as proposed by Dutch National Institute of Public health and Environment the following are recommended:

Firstly, it is important for Zanzibar Maritime Authorities and other stakeholders in Zanzibar to pay particular attention and explore the existence of Diuron in other coastal region of Zanzibar. This will give clear understanding of the environmental assessment around coastal areas of the Islands.

Secondly, determination of diuron in other sources such as sediments and organisms should be given priority in future studies.

Thirdly, short and long term exposure on the antifouling biocide Diuron particularly on monitoring, chronic exposure, risk assessment, organism's responses and pollution models would add special value towards better understanding of the mechanisms and sustainable marine ecosystem health.

Fourthly, the findings of this study should be considered useful for the formulation of technical strategies to control new antifouling contamination, risk assessment and develop new alternative antifouling chemicals in coastal waters.

Finally, the data should be shared to many parties especially government agencies, private sectors, researchers and other stake holders for future plan and conserving the ecological marine biodiversity.

REFERENCES

- Abbott A, Abel PD, Arnold DW, Milne A. (2000). Cost-benefit analysis of the use of TBT: the case for a treatment approach. *Sci Total Environ.* 258:5–19
- Ali, H.R., Ariffin, M.M., Omar, T.F.T., Ghazali, A., Sheikh, M.A., Shazili, N.A.M., Bakari, S.S., & Bachok, Z (2021). Antifouling paint biocides (Irgarol 1051 and diuron) in the selected ports of Peninsular Malaysia: occurrence, seasonal variation, and ecological risk assessment. *Environmental Science and Pollution Research*; <https://doi.org/10.1007/s11356-021-14424-1>.
- Ali, H.R., Ariffin, M.M., Sheikh, M.A., Shazili, N.A.M., Bakari, S.S., & Bachok, Z (2014). Contamination of Diuron in coastal waters around Malaysian Peninsular. *Marine Pollution Bulletin* 85, 287–291.
- Almeida, E., Diamantino, T., De Sousa, O., (2007). Marine Paints: The Particular Case of Antifouling Paints, *Prog. Org. Coat.*, 59, 2-20.
- Aluoch, A.O., Odman-Ghazi, S.O., Whalen, M.M., 2006. Alteration of an essential NK cell signaling pathway by low doses of tributyltin in human natural killer cells. *Toxicology* 224, 229-237.
- Alzieu, C., Heral, M., Thibaud, Y., Dardignac, M.J., Feuillet, M., 1981. Influence of antifouling paints a base of organotin calcification of the shell of the oyster *Crassostrea gigas*. *Review of Works of the Institute of Maritime Fisheries* 45, 101-116.
- Alzieu, C.L., Sanjuan, J., Deltreil, J.P., Borel, M., (1986). Tin contamination in Arcachon Bay: effects on oyster shell anomalies. *Mar. Pollut. Bull.* 17, 494–498.
- Alzieu, C., 1996. Biological effects of tributyltin on marine organisms. In: de Mora, S.J. (Ed.). *Tributyltin*:
- Alzieu, C., 2000. Environmental impact of TBT: The French experience. *Science of the Total Environment* 258, 99–102.
- Anderson, C. Atlar, M. Callow, M. Candries, A. Milne, R. L. Townsin, 2003, “The development of foul-release coatings for seagoing vessels”, *Journal of Marine Design and Operations*, No. B4.
- Antizar-Ladislao, B., 2008. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment: a review. *Environmental International* 34, 292–308.

- Aono, A., Takeuchi, I., 2008. Effects of tributyltin at concentrations below ambient levels in seawater on *Caprella danilevskii* (Crustacea: Amphipoda: Caprellidae). *Marine Pollution Bulletin* 57, 515-523.
- Appel, K.E., 2004. Organotin Compounds: Toxicokinetic Aspects. *Drug Metabolism Reviews* 36, 763-786.
- Apolinario, M & Coutinho, R. 2009. "Understanding the biofouling of offshore and deep-sea structures". In *Advances in marine antifouling coatings and technologies*, Edited by: Hellio, C and Yebra, D. 132-147. Cambridge, UK: Woodhead Publishing.
- Azenha, M.A., Evangelista, R., Martel, F., Vasconcelos, M.T., 2008. Estimate of the digestibility, assimilability and intestinal permeability of butyltins occurring in wine. *Food and Chemical Toxicology* 46, 767-773.
- Batley, G., 1996. The distribution and fate of tributyltin in the marine environment. In: de Mora, S.J.(Ed.). *Tributyltin: Case Study of an Environmental Contaminant*. Cambridge University Press, Cambridge, pp. 139-166.
- Bauer, B., Fioroni, P., Ide, I., Liebe, S., Oehlmann, J., Stroben, E., Watermann, B.T., 1995. TBT effects on the female genital system of *Littorina littorea*: a possible indicator of tributyltin pollution. *Hydrobiologia* 309, 15-27.
- Bennett, R.F., 1996. Industrial manufacture and applications of tributyltin compounds. In: de Mora, S.J.(Ed.). *Tributyltin: Case Study of an Environmental Contaminant*. Cambridge University Press, Cambridge, pp. 21-61.
- Boxall, A.B.A., Comber, S.D., Conrad, A.U., Howcroft, J., Zaman, N., (2000). Inputs, monitoring and fate modelling of antifouling biocides in UK estuaries. *Mar. Pollut. Bull.* 40, 898-905.
- Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., Burt, G.R., 1986. The Decline of the Gastropod *Nucella lapillus* Around Southwest England - Evidence for the Effect of Tributyltin from Antifouling Paints. *Journal of the Marine Biological Association of the United Kingdom* 66, 611-640.
- Burton, E.D., Phillips, I.R., Hawker, D.W., 2006. Tributyltin partitioning in sediments: Effect of aging. *Chemosphere* 63, 73-81.
- Callow, M E and Edyvean, R G V. (1990). "Algal fouling and corrosion". In *Introduction to Applied Phycology*, Edited by: Akatsuka, I. 367-387. The Netherlands: SPB Academic Publishing.

- Caratozzolo, R., Bellini, E., Melati, M.R., Pellerito, C., Fiore, T., D'Agati, P., Scopelliti, M., Pellerito, L., 2007. Interference of tributyltin (IV) chloride on the vascular plant cells. *Applied Organometallic Chemistry* 21, 66-72.
- Carbery, K., Owen, R., Frickers, T., Otero, E., Readman, J., 2006. Contamination of Caribbean coastal waters by the antifouling herbicide Irgarol 1051. *Marine Pollution Bulletin* 52, 635.
- Chambers, L.D., Stokes, K.R., Walsh, F.C., Wood, R.J.K., (2006). Modern approaches to Marine antifouling coatings. *Surface and Coatings Technology* 201, 3642-3652.
- Champ, M.A., 1999. The Need for the Formation of an Independent, International Marine Coatings Board. *Marine Pollution Bulletin* 38, 239-246.
- Chapman, PM., (1995). Ecotoxicology and pollution – Key issues. *Marine pollution Bulletin* 31, 167-177
- Chapman, PM., (2001). How Toxic. *Marine pollution Bulletin* 42, 1279 – 1280.
- Chen, Y.F., Zuo, Z.H., Chen, S.Z., Yan, F.H., Chen, Y.X., Yang, Z.M., Wang, C.G., 2008. Reduction of spermatogenesis in mice after tributyltin administration. *Toxicology* 251, 21-27.
- Chesworth, J.C., Donkin, M.E., Brown, M.T., 2004. The interactive effects of the Antifouling herbicides Irgarol 1051 and diuron on the seagrass *Zostera marina* (L). *Aquat. Toxicol.* 66, 293–305
- Claisse, D. and C. Alzieu, (1993): Copper contamination as a result of antifouling paint regulations? *Mar. Poll. Bull.* 26, (7), 395–397.
- Clark, R.B., (1992). *What is Pollution?* Clarendon Press, Oxford, p 172.
- Crompton, T.R., 1997. Toxicity Data. In: Crompton, T.R. (Ed.). *Toxicants in the aqueous ecosystem*. John Wiley & Sons Ltd., West Sussex, England, pp. 73-80.
- Dafforn, K., Lewis, J., Johnston, E., (2011). Antifouling strategies: history and Regulation, ecological impacts and mitigation. *Mar. Pollut. Bull.* 62, 453–465.
- Dahllof, I., Agrenius, S., Blanck, H., Hall, P., Magnusson, K., Molander, S., 2001. The effect of TBT on the structure of a marine sediment community - a boxcosm study. *Marine Pollution Bulletin* 42, 689-695.
- De' Mora, S.J., 1996. The Tributyltin debate: ocean transportation versus seafood harvesting. In: de Mora, S.J. (Ed.). *Tributyltin: case study of an environmental contaminant*. *Cambridge University Press*, Cambridge, pp. 1-15.

- EC., 2005. Environmental Quality Standards - Substance Data Sheet No. 30 - Tributyltin compounds. Common Implementation strategy for the Water Framework Directive. Expert Advisory Forum on Priority Substances / Expert Group on Quality Standards, European Community, Brussels.
- Ema, M., Harazono, A., Miyawaki, E., Ogawa, Y., 1997. Effect of the day of administration on the developmental toxicity of tributyltin chloride in rats. *Archives of Environmental Contamination and Toxicology* 33, 90-96.
- Evans, S.M., Leksono, T., McKinnell, P.D., 1995. Tributyltin pollution: A diminishing problem following legislation limiting the use of TBT-based anti-fouling paints. *Marine Pollution Bulletin* 30, 14-21.
- Evans, S. M., Birchenough A. C., Brancato, M. S., 2000. The TBT Ban: Out of the Frying Pan into the Fire? *Marine Pollution Bulletin* 40, 204-211.
- Dahl, B., Blanck, H., 1996. Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosms. *Marine Pollution Bulletin* 32, 342-350.
- Fent, K., 1996. Ecotoxicology of organotin compounds. *Critical Reviews in Toxicology* 26, 3-117.
- Ferrer, I., Ballesteros, B., Marco, M-P., Barceló, D., 1997. Pilot Survey for Determination of the Antifouling Agent Irgarol 1051 in Enclosed Seawater Samples by a Direct Enzyme Linked Immuno sorbent Assay and Solid-Phase-Extraction Followed by Liquid Chromatography-Diode Array Detection: *Environmental Science and Technology* 31, 3530-3535.
- Ferrer, I. and Barceló, D., 1999. Simultaneous Determination of Antifouling Herbicides in Marina Water Samples by on-line Solid-Phase Extraction followed by Liquid Chromatography-Mass Spectrometry, *Journal of Chromatography A*, v. 854, 197-206.
- Ferreira, C.E.L.; Floeter, S.R.; Gasparini, J.L.; Joyeux, J.C. 2004. Trophic structure patterns of Brazilian reef fishes: a latitudinal comparison. *J. Biogeogr.*, 31:1093-1106.
- Field, J.A., Reed, R.L., Sawyer, T.E., Griffith, S.M., Wigington, P.J., (2003). Diuron occurrence and distribution in soil and surface and ground water associated with grass seed production. *J. Environ. Qual.* 32, 171-179.
- Gabbianelli, R., Moretti, M., Carpena, E., Falcioni, G., 2006. Effect of different organotins on DNA of mollusk (*Scapharca inaequivalvis*) erythrocytes assessed by the comet assay. *Science of the Total Environment* 367, 163-169.

- Gennaro, M.C., Abrigo, C., Giacosa, D., Rigotti, L., Liberatori, A., (1995). Separation of phenyl urea pesticides by ion-interaction reversed phase high performance liquid chromatography: diuron determination in lagoon water. *J. Chromatogr. A* 718, 81–88
- Giacomazzi, S., Cochet, N., (2004). Environmental impact of diuron transformation: a review. *Chemosphere* 56, 1021–1032.
- Gibbs, P.E., Bryan, G.W., 1994. Biomonitoring of Tributyltin (TBT) Pollution using the *Imposex* Response of Neogastropod Molluscs. In: Kramer, K.J.M. (Ed.). *Biomonitoring of Coastal Waters and Estuaries. CRC Press Inc., Boca Raton*, pp. 205-226.
- Goldberg, J.I., 1986. TBT: an environmental dilemma. *Environment* 28, 17-44.
- Grun, F., Blumberg, B., 2009. Endocrine disrupters as obesogens. *Molecular and Cellular Endocrinology* 304, 19-29.
- Grun, F., Watanabe, H., Zamanian, Z., Maeda, L., Arima, K., Cubacha, R., Gardiner, D.M., Kanno, J., Iguchi, T., Blumberg, B., 2006. Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. *Molecular Endocrinology* 20, 2141-2155.
- Hall Jr., L.W., Giddings, J.M., Solomon, K.R., Balcomb, R., (1999). An ecological risk assessment for use of Irgarol 1051 as an algaecide for antifoulants paints. *Critical Reviews in Toxicology* 29, 367-437.
- ICPS, 1990. International Programme on Chemical Safety. Tributyltin compounds. Environmental Health Criteria 116. *World Health Organization*, Geneva.
- ICS & ISF, (2009). Overview of the international shipping industry. International Chamber of Shipping and International Shipping Federation, Web publication:
- IMO, 1999. Focus on IMO. Anti-fouling systems: moving towards the non-toxic solution. *International Maritime Organization*, London. Available via IMO website. <http://www.imo.org/home.asp>
- IMO, 2001. International Convention on the Control of Harmful Anti-Fouling Systems on Ships. AFS/CONF 26. *International Maritime Organization*, London, pp. 1-28.
- IMO, 2002. Focus on IMO. Anti-fouling systems. *International Maritime Organization*, London. Available via IMO website. <http://www.imo.org/home.asp>
- IMO, 2009. Summary of Conventions. *International Maritime Organization*, London.

- Jones, R., 2005. The ecotoxicological effects of Photosystem II herbicides on corals. *Marine Pollution Bulletin* 51 (5-7), 495-506.
- Jones, R.J., (2004). Testing the photo inhibition model of coral bleaching using chemical inhibitors. *Mar. Ecol. Progress Ser.* 284, 133–145.
- Jones, R.J., Kerswell, A.P., 2003. Phytotoxicity of photosystem II (PSII) herbicides to coral. *Mar. Ecol. Progress Ser.* 261, 149–159.
- Kegley, S.E., Hill, B.R., Orme, S., Choi, A.H., 2008. Copper oxychloride. PAN Pesticide Database [on-line] available from: http://www.pesticideinfo.org/Detail_Chemical.jsp.Rec_Id=PC35070. *Pesticide Action Network North America*, San Francisco.
- Kishta, O., Adeeko, A., Li, D., Luu, T., Brawer, J.R., Morales, C., Hermo, L., Robaire, B., Hales, B.F., Barthelemy, J., Cyr, D.G., Trasler, J.M., 2007. In utero exposure to tributyltin chloride differentially alters male and female fetal gonad morphology and gene expression profiles in the Sprague-Dawley rat. *Reproductive Toxicology* 23, 1-11.
- Kitada, Y., Kawahata, H., Suzuki, A., Oomori, T., 2008. Distribution of pesticides and bisphenol A in sediments collected from rivers adjacent to coral reefs. *Chemosphere* 71 (11), 2082 – 2090.
- Kobayashi, N., Okamura, H., 2002. Effects of new antifouling compounds on the development of sea urchin. *Marine Pollution Bulletin* 44, 748–751.
- Konstantinou, I.K., Albanis, T.A., (2004). Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environ. Int.* 30, 235–248.
- Lam, K.H., Cai, Z., Wai, H.Y., Tsang, W.H., Wah Lam, M.H., Hing Cheung, R.Y., Yu, H.X., Sing Lam, P.K., 2005. Identification of a new Irgarol-1051 related s-triazine species in coastal waters. *Environ. Pollut.* 136, 221–230.
- Lamoree, M.H., Swart, S.P., van der Horst, A., van Hattum, B., (2002). Determination of diuron and the antifouling paint biocide Irgarol 1051 in Dutch marinas and coastal waters. *J. Chromatogr. A* 970, 183–190.
- Lespes, G., Marcic, C., Heroult, J., Le Hecho, I., Denaix, L., 2009. Tributyltin and triphenyltin uptake by lettuce. *Journal of Environmental Management* 90, S60-S68.
- Lewis, J.A., (1998). Marine biofouling and its prevention on underwater surfaces. *Mater Forum* 22, 41–61.

- Liu, D., Pacepavicious, G.J., Maguire, R.J., Lau, Y.L., Okamura, H., Aoyama, I., 1999. Survey for the occurrence of the new antifouling compound Irgarol 1051 in the aquatic environment. *Water Res.* 33, 2833–43.
- Malato, S., Blanco, J., Fernandez-Alba, R., Aguera, A., Rodrigues, A., 2002. Photocatalytic treatment of water-soluble pesticides by photo Fenton and TiO₂ using solar energy. *Catalysis Today* 76, 209–220.
- Malato, S., Blanco, J., Fernández, A.R., Agüera, A., 2000c. Solar photocatalytic mineralization of commercial pesticides: acrinathrin. *Chemosphere* 40(4), 403–409.
- Malato, S., Blanco, J., Richter, C., Fernández, P., Maldonado, M.I., 2000b. Solar photocatalytic mineralization of commercial pesticides: Oxamyl. *Solar Energy Materials and Solar Cells* 64, 1–14.
- Malato, S., Blanco, J., Richter, C., Maldonado, M.I., 2000a. Optimization of pre-industrial solar photocatalytic mineralization of commercial pesticides. Application to pesticide container recycling. *Applied Catalysis B: Environmental* 25, 31–38.
- Mackie, C., Lloyd, G., 2002. Antifoulants and marine biocides. In: Knight, D.J., Cooke, M. (Eds.), *the Biocides Business*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, FRG.
- Martinez, K., Ferrer, I., Barcelo, D., (2000). Part-per-trillion level determination of antifouling pesticides and their byproducts in seawater samples by off-line solid-phase extraction followed by high-performance liquid chromatography atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr. A* 879, 27–37.
- Martinez, K., Barcelo, D., 2001. Determination of antifouling pesticides and their degradation products in marine sediments by means of ultrasonic extraction and HPLC-APCI-MS. *Fresenius' Journal of Analytical Chemistry* 370, 940–945.
- Matthiessen, P., Gibbs, P.E., 1998. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. *Environmental Toxicology and Chemistry* 17, 37–43.
- McAllister, B.G., Kime, D.E., 2003. Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinization and irreversible sperm damage in zebrafish (*Danio rerio*). *Aquatic Toxicology* 65, 309–316.
- Meador, J.P., 2000. Predicting the fate and effects of tributyltin in marine systems. *Reviews of Environmental Contamination and Toxicology* 166, 1–48.

- Mendo, S.A., Nogueira, P.R., Ferreira, S.C.N., Silva, R.G., 2003. Tributyltin and triphenyltin toxicity on benthic estuarine bacteria. *The Fresenius Environmental Bulletin* 12, 1361-1367.
- Moncada, A., (2004). Environmental fate of diuron. Environmental Monitoring Branch, Department of Pesticide Regulation, Sacramento, California.
- Muthiga N, Costa A, Motta H, Muhando C, Mwaipopo R, Schleyer M. (2008). Status of Coral Reefs in East Africa: Kenya, Tanzanian, Mozambique, and South Africa. Status of Coral Reefs of the World. https://digitalcollections.sit.edu/isp_collection
- Myers, J.H., Gunthorpe, L., Allinson, G., Duda, S., 2006. Effects of antifouling biocides to the germination and growth of the marine macroalga, *Hormosira banksii* (Turner) Desicaine. *Marine Pollution Bulletin* 52, 1048–1055.
- Negri, A.P., Hales, L.T., Battershill, C., Wolff, C., Webster, N.S., 2004. TBT contamination identified in Antarctic marine sediments. *Marine Pollution Bulletin* 48, 1142–1144.
- Ohtaki, K., Aihara, M., Takahashi, H., Fujita, H., Takahashi, K., Funabashi, T., Hirasawa, T., Ikezawa, Z., 2007. Effects of tributyltin on the emotional behavior of C57BL/6 mice and the development of atopic dermatitis-like lesions in DS-Nh mice. *Journal of Dermatological Science* 47, 209-216.
- Okamura H., (2002). Photodegradation of the antifouling compounds Irgarol 1051 and diuron released from a commercial antifouling paint. *Chemosphere* 2002; 48:43–50.
- Oehlmann, J., Di Benedetto, P., Tillmann, M., Duft, M., Oetken, M., Schulte-Oehlmann, U., 2007. Endocrine disruption in prosobranch molluscs: evidence and ecological relevance. *Ecotoxicology* 16, 29-43.
- Oetken, M., Bachmann, J., Schulte-Oehlmann, U., Oehlmann, J., 2004. Evidence for endocrine disruption in invertebrates. *International Review of Cytology - A Survey of Cell Biology. Elsevier Academic Press Inc, San Diego*, pp. 1-44.
- Okamura, H., Aoyama, I., Ono, Y., Nishida, T., (2003). Antifouling herbicides in the coastal waters of western Japan. *Mar. Pollut. Bull.* 47, 59–67.
- Oliveira. D., & Granha. L., (2020). Ship hull in-water cleaning and its effects on fouling-control coatings, *Biofouling*, 36:3, 332-350, DOI: 10.1080/08927014.2020.1762079.

- Owen, R., Knap, A., Toaspern, M., Carberry, K., 2002. Inhibition of coral photosynthesis by the antifouling herbicide Irgarol 1051. *Marine Pollution Bulletin* 44, 623–32.
- Page, D.S., Dassanayake, T.M., Gilfillan, E.S., 1996. Relationship between tissue concentrations of tributyltin and shell morphology in field populations of *Mytilus edulis*. *Bulletin of Environmental Contamination and Toxicology* 56, 500-504.
- Petersen, S., Gustavson, K., 1998. Toxic effects of tri-butyl-tin (TBT) on autotrophic pico, nano, and micro plankton assessed by a size fractionated pollution-induced community tolerance (SF-PICT) concept. *Aquatic Toxicology* 40, 253-264.
- Råberg, S., Nyström, M., Erös, M., Plantman, P., (2003). Impact of the herbicides 2, 4-D and diuron on the metabolism of coral porites cylindrical. *Marine Environmental Research* 56, 503-514.
- Railkin, A. I., 2004. *Marine biofouling: colonization processes and defenses*. CRC Press LLC, 303.
- Rank, J., 2009. Intersex in *Littorina littorea* and DNA damage in *Mytilus edulis* as indicators of harbor pollution. *Ecotoxicology and Environmental Safety* 72, 1271-1277.
- Sano M, Shimizu M, Nose Y. (1984). Changes in structure of coral-reef fish communities by destruction of hermatypic corals—observational and experimental views. *Pac Sci.* 28:51–79.
- Sano M, Shimizu M, Nose Y. 1987. Long-term effects of destruction of hermatypic corals by *Acanthaster planci* infestation of reef fish communities at Iriomote Island, Japan. *Mar Ecol Prog Ser.* 37: 191–199.
- Sathiyaseelan K & Stella D., (2011). Isolation, identification and antagonistic activity of marine actinomycetes isolated from the Muthupet mangrove environment. *International Journal of Pharmaceutical and Biological Achieves.* 2 (5); 1464-146
- Schultz, M.P., (2007). Effects of coating roughness and biofouling on ship resistance and powering. *Biofouling* 23,331-331.
- Sheikh, M.A., Fujimura, H., Miyagi, T., Uechi, Y., Yokota, T., Yasumura, S., Oomori, T., (2009). Detection and ecological threats of PSII herbicide diuron on coral reefs around the Ryukyu Archipelago, Japan. *Mar. Pollut. Bull.* 58, 1922–1952.

- Sheikh, M.A., Juma, F.S., Staehr, P., Dahl, K., Rashid, J.R., Mohammed, M.S., Ussi, A.M., & Ali (2016). Occurrence and distribution of antifouling biocide Irgarol-1051 in coral reef ecosystems, Zanzibar. *Marine Pollution Bulletin* 109, 586–590
- Shimasaki, Y., Kitano, T., Oshima, Y., Inoue, S., Imada, N., Honjo, T., 2003. Tributyltin causes masculinization in fish. *Environmental Toxicology and Chemistry* 22, 141-144.
- Sheppard C.R.C, Spalding M, Bradshaw C, Wilson S. 2002. Erosion vs. recovery of coral reefs after El Niño: Indian Ocean. *Ambio*. 104:79–86.
- Sidharthan, M., Young, K.S., Woul, L.H., Soon, P.K., Shin, H.W., 2002. TBT toxicity on the marine microalga *Nannochloropsis oculata*. *Marine Pollution Bulletin* 45, 177-180.
- Stewart, C., 1996. The efficacy of legislation. In: De Mora, S.J. (Ed.). Tributyltin: case study of an environmental contaminant. *Cambridge University Press, Cambridge*, pp. 264-297.
- Stupak, M. E., Mónica, T.G., Miriam, C.P., 2003. Non-toxic alternative compounds for marine antifouling paints. *International Biodeterioration & Biodegradation*, 52, 49-52.
- Susana, G., (2010). Determinant factors for the use of imposex in TBT pollution monitoring. PhD thesis, Universidad de Aveiro Departamento de Biologia.
- Takeuchi, A., Takahashi, S., Tanabe, S., Miyazaki, N., 2001. Caprella watch: a new approach for monitoring butyltin residues in the ocean. *Marine Environmental Research* 52, 97-113.
- Thomas, K.V., (2001). The environmental fate and behavior of antifouling paint booster biocides: a review. *Biofouling* 17, 73–86.
- Thomas, K.V., McHugh, M., Hilton, M., Waldock, M., (2002). Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. *Sci. Total Environ.* 293, 117–127.
- Thomas, K.V., McHugh, M., Hilton, M., Waldock, M., (2002). Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. *Sci. Total Environ.* 293, 117–127.
- Townsin, R.L., Anderson, C.D., 2009. Fouling control coatings using low surface Energy, foul release technology. In: Hellio, C., Yebra, D. (Eds.), *Advances in*

Marine Antifouling Coatings and Technologies. Woodhead Publishing Limited

- Vandermeulen, J.H., Davis, N.D., Muscatine, L., 1972. The effect of Inhibitors of Photosynthesis on Zooxanthellae in Corals and other Marine Invertebrates. *Mar. Bio.* 16, 185-191.
- Voulvoulis N, Scrimshaw MD, Lester JN (2002). Comparative environmental assessment of biocides used in antifouling paints. *Chemosphere*; 4, 789-795.
- Waldock, R., Rees, H.L., Matthiessen, P., Pendle, M.A., 1999. Surveys of the benthic in fauna of the Crouch Estuary (UK) in relation to TBT contamination. *Journal of the Marine Biological Association of the United Kingdom* 79, 225-232.
- Watanabe, T., Yuyama, I., Yasumura, S., (2006). Toxicological effects of biocides on symbiotic and aposymbiotic juveniles of the hermatypic coral *Acropora tenuis*. *J. Exp. Mar. Biol. Ecol.* 339, 177–188.
- West, K.; van Woosik, R., (2001). Spatial and temporal variance of river discharge on Okinawa (Japan): Inferring the temporal impact on adjacent coral reefs. *Mar. Pollut. Bull.* 42 (10), 864-872.
- Whalen, M.M., Green, S.A., Loganathan, B.G., 2002. Brief butyltin exposure induces irreversible inhibition of the cytotoxic function on human natural killer cells, in vitro. *Environmental Research* 88, 19-29.
- WHOI. 1952. Marine Fouling and Its Prevention. Woods Hole Oceanographic Institute, United States.
- Wilkes, C.E., Summers, J.W., Daniels, C.A., Berard, M.T., 2005. PVC Handbook. In: Wilkes, C.E., Summers, J.W., Daniels, C.A., Berard, M.T. (Eds.). *Hanser Verlag, Munich*, p. 723.
- Wright, T. Marine Coatings Market. *Coatings World*; May 2009. http://www.coatingsworld.com/issues/2009-05/view_features/marine-coatings-market-2009-05-07-09-18-13/ (accessed November 25, 2021).
- Yang, J., Oshima, Y., Sei, I., Miyazaki, N., 2009. Metabolism of tributyltin and triphenyltin by Dall's porpoise hepatic microsomes. *Chemosphere* 76, 1013-1015.
- Yebara, D.M., Kiil, S. and Dam-Johansen, K. (2004) Antifouling Technology-Past, Present and Future Steps towards Efficient and Environmentally Friendly Antifouling Coatings. *Progress in Organic Coatings*, 50, 75-104.

- Yebera, D.M., Kiil, S., Weinell, C.E., Dam-Johansen, K., 2006. Effects of marine microbial biofilms on the biocide release rate from antifouling paints - A model-based analysis. *Progress in Organic Coatings* 57, 56-66.
- Yonezawa, T., Hasegawa, S., Ahn, J.Y., Cha, B.Y., Teruya, T., Hagiwara, H., Nagai, K., Woo, J.T., 2007. Tributyltin and triphenyltin inhibit osteoclast differentiation through a retinoic acid receptor dependent signalling pathway. *Biochemical and Biophysical Research Communications* 355, 10-15.
- Zhang, A.Q., Leung, K.M.Y., Kwok, K.W.H., Bao, V.W.W., Lam, M.H.W., 2008. Toxicities of antifouling biocide Irgarol 1051 and its major degraded product to marine primary producers. *Marine Pollution Bulletin* 57, 575-586.
- Zhang, J.L., Zuo, Z.H., Chen, R., Chen, Y.X., Wang, C.G., 2008. Tributyltin exposure causes brain damage in *Sebastes marmoratus*. *Chemosphere* 73, 337-343.
- Zhang, J.L., Zuo, Z.H., He, C.Y., Wu, D., Chen, Y.X., Wang, C.G., 2009. Inhibition of thyroidal status relate to depression of testicular development in *Sebastes marmoratus* exposed to tributyltin. *Aquatic Toxicology* 94, 62-67.
- Zheng, R.H., Wang, C.G., Zhao, Y., Zu, Z.H., Chen, Y.X., 2005. Effect of tributyltin, benzo(a)pyrene and their mixture exposure on the sex hormone levels in gonads of cuvier (*Sebastes marmoratus*). *Environmental Toxicology and Pharmacology* 20, 361-367.
- Zuo, Z.H., Cai, J.L., Wang, X.L., Li, B.W., Wang, C.G., Chen, Y.X., 2009. Acute administration of tributyltin and trimethyltin modulate glutamate and N-methyl-D-aspartate receptor signalling pathway in *Sebastes marmoratus*. *Aquatic Toxicology* 92, 44-49

APPENDICES

Appendix A. Solid phase extraction in progress in the laboratory.



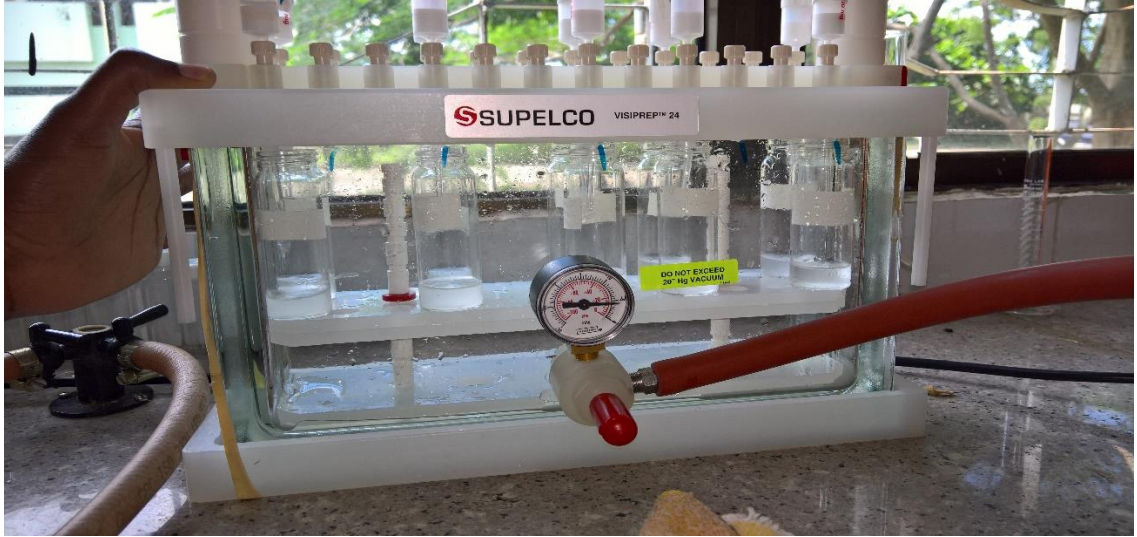
Appendix B. Solid phase Extraction process in the laboratory



Appendix C. Drying process of supel clean™ ENVI™ – 18 under nitrogen gas



Appendix D. Elution process on the laboratory



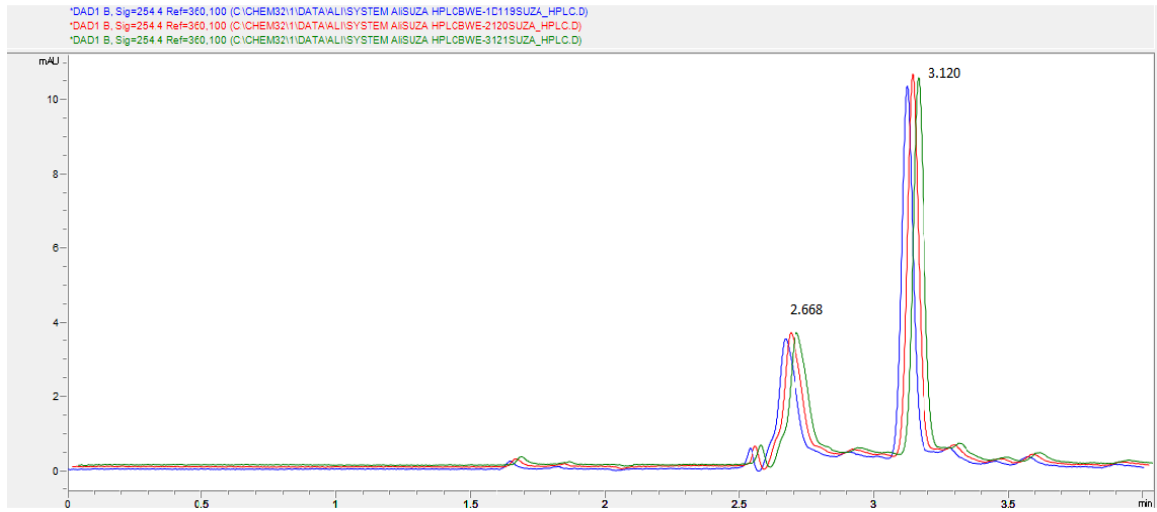
Appendix E. Collecting sample journey in various sampling sites around Unguja Island.



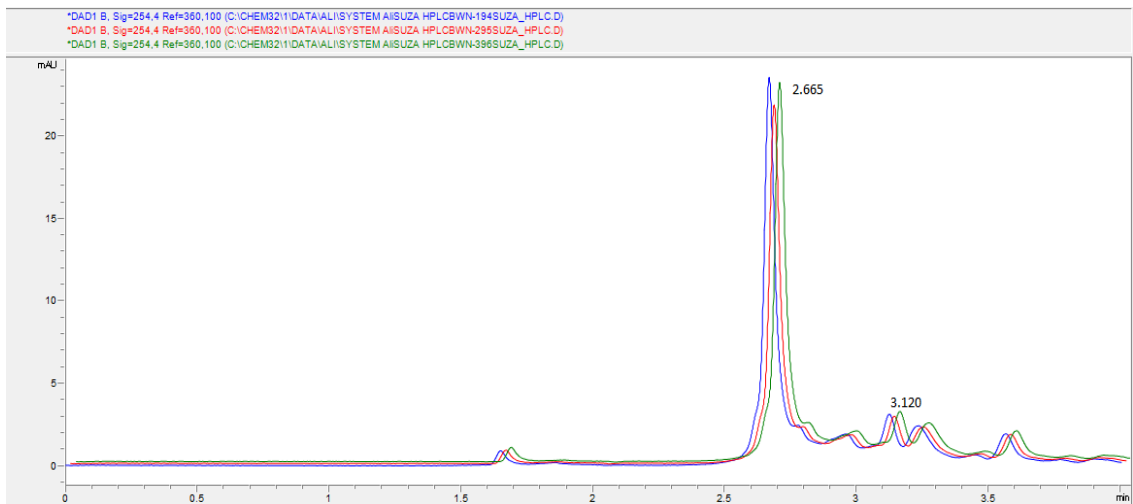
Appendix F. Collecting sample process using water collector.



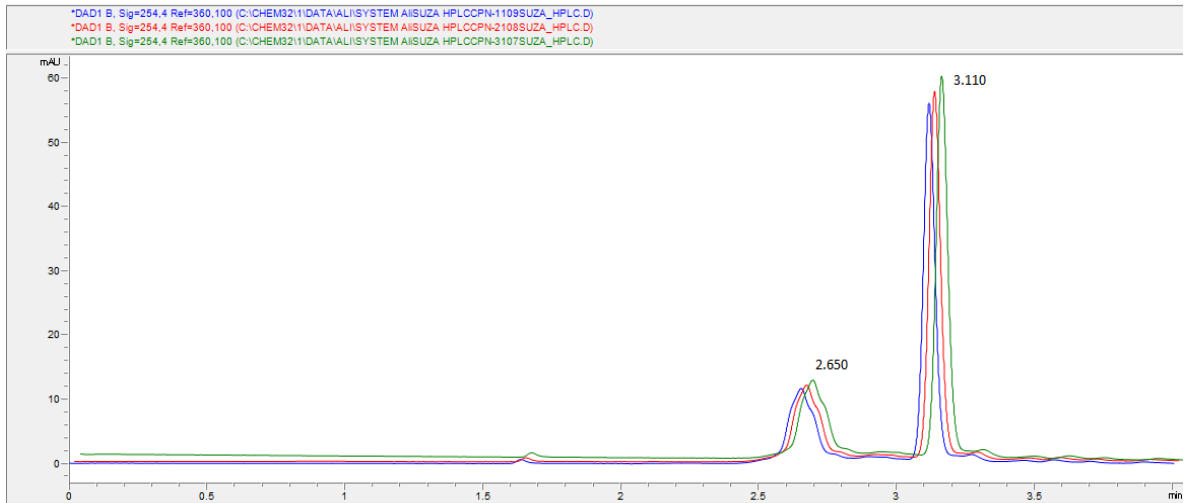
Appendix G. Overall Chromatograms



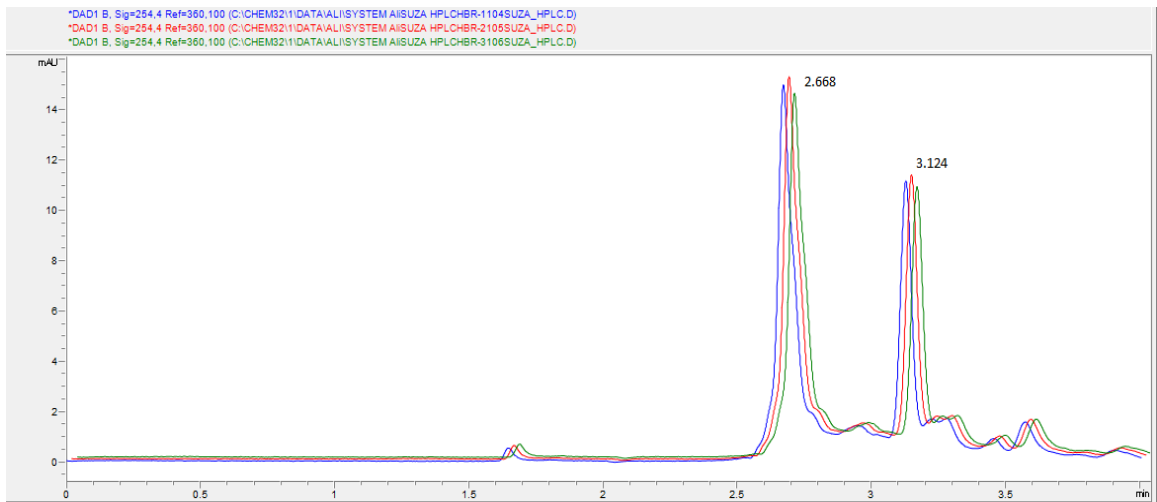
1. Chromatogram result of Bawe



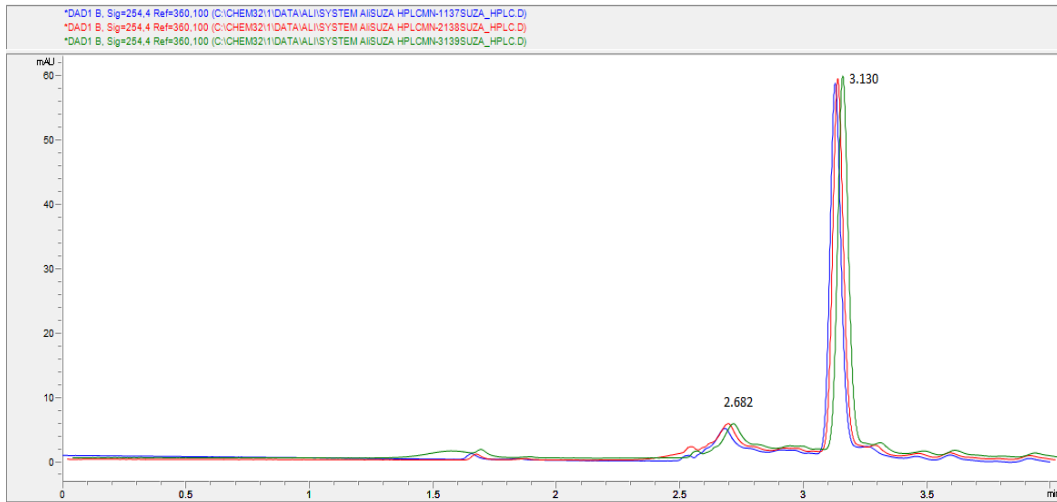
2. Chromatogram result of Bwawani.



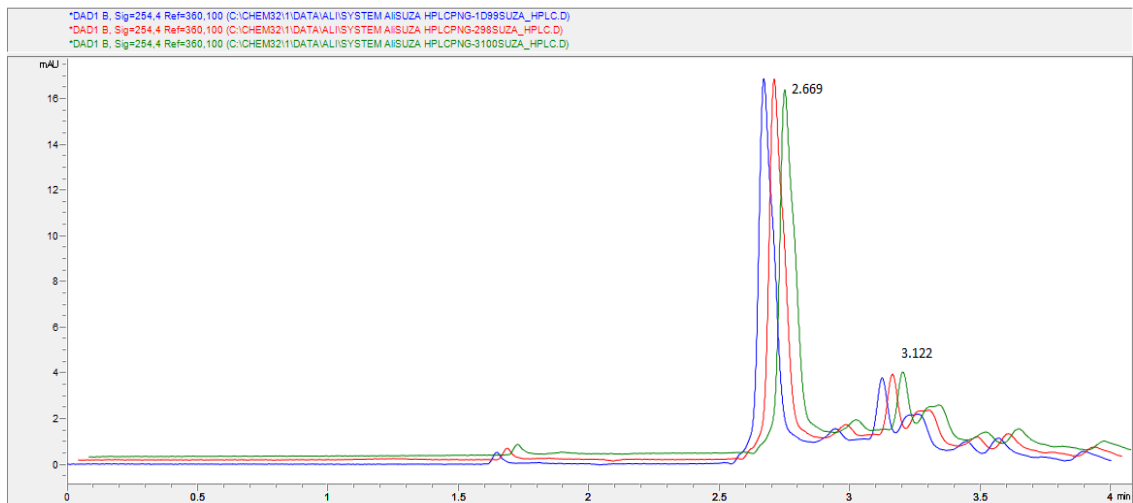
3. Chromatogram result of Chapwani.



4. Chromatogram result of harbor



5. Chromatogram result of Mnemba



6. Chromatogram result of Pange