Doctoral Dissertation

MONITORING OF CHEMICALS AND TOXICITY DISCHARGED FROM RESIDENTIAL AREAS IN DEVELOPING COUNTRIES

アシュラフ　シュケリ　アハミド エルシェク
Ashraf Shokry Ahmed Elsheikh

Division of System Design and Engineering
Graduate School of Science and Engineering
Yamaguchi University
Japan
March 2018

博士論文
Doctoral Dissertation

開発途上国の居住地域から排出される化学物質および毒性のモニタリング

MONITORING OF CHEMICALS AND TOXICITY DISCHARGED FROM RESIDENTIAL AREAS IN DEVELOPING COUNTRIES

アシュラフ　シュケリ　アハミド エルシェク
Ashraf Shokry Ahmed Elsheikh

A dissertation submitted to the Division of System Design and Engineering of Yamaguchi University in partial fulfilment of the requirement for the degree of Doctor of Engineering

Advisor: Prof. Masahiko Sekine
(Division of Civil and Environmental Engineering)

Committee Members:
Prof. Masahiko Sekine
Prof. Masakazu Niinae
Prof. Tsuyoshi Imai
Assoc. Prof. Koichi Yamamoto
Assoc. Prof. Tasuma Suzuki

山口大学大学院理工学研究科
Division of System Design and Engineering
Graduate School of Science and Engineering
Yamaguchi University, Japan
March 2018

ABSTRACT

Assessment of water is not only for suitability for human consumption but also in relation to its agricultural, industrial, recreational, commercial uses and its ability to sustain aquatic life. Water quality monitoring is a fundamental tool in the management of freshwater resources. However, water pollution is one of the most serious problems especially in the developing countries, where, surface water is under excessive stress due to population growth and urban development. Few urban centers have wastewater treatment facilities mostly producing ill-treated effluents. The ease of the accessibility of surface water makes them the best choice for wastewater discharge. People of rural areas in developing countries still rely on untreated surface water as their basic source of domestic water supply. The quality of surface water is a major factor affecting human health and ecological systems, especially around residential areas, since rivers and their tributaries passing through cities often impacted by chemical pollution, originating from municipal and industrial wastewater effluents, airborne deposition as well as runoff from urban and agricultural areas. Therefore, the investigation of chemicals and their corresponding toxicity effect is very important.

Since, the complex mixtures of toxic substances occurring in surface waters are difficult to characterize by chemical analyses because each compound occurs at a very low concentration and requires a specific analytical method to be identified. Ecotoxicological tests on water extracts can be used as a screening tool to evaluate quickly and simply the overall quality of a water body with regard to micropollutant contamination.

Timor-Leste is a developing country with inadequate pollution control facilities, surface water and sanitation systems are very poor quality. Since, there are few researches about the toxicity from residential areas in developing countries, then the purpose of this study was to : 1) Introduce information about chemicals and their corresponding toxicity that discharged from residential areas into water streams in Dili city, Timor-Leste. 2) Investigate the applicability of passive sampling for larval medaka acute toxicity assay.

In chapter 3, the toxicity of organic chemicals that discharged from residential areas into water streams in Dili city was evaluated using concentrated water samples via Sep-Pak® Plus PS-2 cartridges combined with larval medaka acute toxicity assay.

The possible sources of organic pollutants were identified using GC/MS simultaneous analysis. The detection of coprostanol and many hydrocarbon components of fuel oils in Timor-Leste streams reflects the negative effects of anthropogenic activities on water streams as a result of discharging the house hold wastewater without any treatment. Toxicity levels of water streams in Timor-Leste were comparable or higher than those of the Japanese water streams that were investigated in 2013. Those results were interesting and referred to the need for regular monitoring of the toxicity conditions in Timor-Leste water streams, more frequently samples would be important but it is difficult using grab sampling because of unsafe monitoring sites, limited resources in Timor-Leste like shortage in laboratory, transportation facilities and unstable electricity therefore another sampling technique should be considered to avoid such these problems.

In Chapter 4, we investigated the applicability of passive sampling using chemcatcher Styrene Divinyl Benzene (SDB) disks for larval medaka acute toxicity assay, to evaluate the surface water quality. In order to select the most suitable passive sampler disk among SDB disks, a field and laboratory experiments were conducted and the results indicated that styrene divinyl benzene reverse phase sulfonated (SDB-RPS) disks were the most suitable to conduct a comparative toxicity study with active sampling via Sep-Pak® Plus PS2 cartridges and 10-L river water sample. SDB-RPS disks were deployed along 10 and 4 days as a long and short investigation periods, respectively. The Long deployment results showed that no toxicity was observed neither in the PS 3-day sample nor in any of the interval passive samples. Even though the amount of adsorbed chemicals in the PS 3-day and PS 2nd interval samples were higher than that in the PS 7- and 10-day samples, which both showed a little toxicity only at 100-fold concentrated samples. The amount of adsorbed chemicals in the PS 10-day sample was not equivalent to the sum of chemicals adsorbed in the individual PS interval samples. Whereas, the short deployment results showed that, the 4-day deployment period showed the highst bioassay toxicity even it had the lowest adsorbed chemicals amount. Whereas, more chemicals were detected by other deployment periods and showed same bioassay toxicity value, even their adsorbed chemicals amounts were different. The decomposition of adsorbed chemicals increased with longer deployment. Almost all chemicals (80%) might be decomposed during the four days deployment period. Whereas, about 25% of chemicals only might be decomposed

into other chemicals during the 1-day deployment period. According to these results, the application of SDB-RPS passive sampler disks with 1-day or shorter deployment might be considered to evaluate toxicity levels using medaka acute toxicity assay.
Key words: concentrated water sample, toxicity bioassay, Timor-Leste

要旨

先進国では一般家庭からも化学物質が排出され、生態毒性が検出されることが明らかになってきた。しかし生活様式が異なる開発途上国の住居地区からの化学物質と生態毒性の排出状況はほとんど知られていない。本研究では、近年急速に人口集中が進行している東ティモールの首都ディリを例にとり、化学物質と生態毒性の排出状況を調査して、先進国である日本のそれらとの比較を行った。また、時間変化が大きいこれらの水質項目を正しく測定するためには、連続ないし高頻度の採水が欠かせないが、電力の安定性や治安の問題から開発途上国では実現が難しい。この問題に対応できる方法として近年パッシブサンプリングが注目されている。本研究では、化学物質と生態毒性研究へのパッシブサンプリングの適用可能性について検討した。

本博士論文は５章で構成されており、その内容は以下の通りである。

第１章では、本研究の背景と目的、本論文の構成について述べている。

第２章では、化学物質と生態毒性の測定方法およびパッシブサンプリングに関する従来の研究についてまとめている。

第３章では、流域のほとんどが山林と住居地域からなる東ティモールの首都ディリの 4 水路 4 地点および宇部市の 2 水路 3 地点における化学物質と生態毒性について、既存手法であるグラブサンプリングによる比較を行い、それぞれの国の生活様式を反映して化学物質の組成は異なるもののいずれからも生態毒性が検出され、一人当たりの生態毒性排出量は両国を通じて単一の流域面積の関数でおおむね説明できることを示した。

第４章では、パッシブサンプラーとして Chemcatcher を使用し、ディリの 1 水路において 2~10 日間、0.5~4 日間の異なるタイムスパンで短期間のパッシブサンプリングが長期間のパッシブサンプリング結果を説明できるかを検討した。その結果、2~10 日間では短期間のサンプリング結果と長期間のサンプリング結果がほとんど一致しないこと、0.5~4 日間においても、サンプリング期間が長くなると化学物質の組成変化が大きくなることが明らかになった。これま

での単一の化学物質に注目したパッシブサンプリング研究では 1 週間から 1 か月程度の長期のサンプリング例が多いが、多くの化学物質を一度に取り扱う生態毒性研究では、0.5~1 日程度以下の短いサンプリング期間設定が必要であることを明らかにした。

第５章では、以上をまとめて結論とし、今後の展望について述べている。

キーワード：濃縮水サンプル , 毒性バイオアッセイ , 東ティモール

ACKNOWLEDGEMENTS

I owe my deepest gratitude to Allah who supported me and gives me the force to complete my study. It is honor for me to spend this space to express my sincere gratitude to:

1. My supervisor, Prof. Masahiko Sekine, for his guidance, helpful advices and warm encouragement to complete this study.
2. Prof. Tsuyoshi Imai, Assoc. Prof. Koichi Yamamoto, Assoc. Prof. Takaya Higuchi, Assistant Prof. Ariyo Kanno and Ms. Toshimi Yamamoto for their expert and meaningful advices as well as their attention in any aspects.
3. Jun Terasaki and Chiharu Hirabayashi for good cooperation, sharing ideas and knowledge .
4. All of my Japanese and International friends whose I cannot mention one by one, for friendship, help and our good times together.
5. My family; my beloved wife Shereen, my lovely sons: Abdulrahaman and Abdullah, my brothers Ehab and Ahmed, my sister Naglaa. My parents for their prayers and supplication to Allah to support me.

Ashraf Shokry Ahmed Elsheikh

March, 2018

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 01
INTRODUCTION

1.1 General

Assessment of water is not only for suitability for human consumption but also in relation to its agricultural, industrial, recreational, commercial uses and its ability to sustain aquatic life. Water quality monitoring is therefore a fundamental tool in the management of freshwater resources. However, water pollution has become one of the most serious problems in many countries, especially in the developing countries (Hunter et al. 2009; Tsuzuki, 2008).

Many people in developing countries of the world still rely on untreated surface water as their basic source of domestic water supply. This problem is exacerbated in rural areas. Surface water is under excessive stress due to population growth and increased industrialization. The ease of the accessibility of surface water makes them the best choice for wastewater discharge. Most quantities of wastewater generated in developing countries do not undergo any form of treatment. In few urban centers, various forms of wastewater treatment facilities exist but most of them are producing ill-treated effluents, which comprises of several contaminants all find their way to surface water resources causing loss of biodiversity in the aquatic ecosystem, and possibly health risk to humans. Surface water, therefore, should be protected from pollution.

Anthropogenic activities specially in developing countries result in the release of organic compounds into wastewaters that can have toxic, carcinogenic, mutagenic or/and endocrine disrupting properties. Of these organic pollutants, pesticides, pharmaceuticals and personal care products (PPCPs), that are a particular source of concern because of the growing recognition of the potential threats that they pose to the health of humans and ecosystems. Therefore It becomes an urgent need to give efficient evaluation on the water quality safety in water bodies so as to focus the environmental investigation and management efforts towards those sites showing low safety levels (Edokpayi et al., 2014).

East Timor is a developing country, 57% of its population doesn't have access to improved sanitation system as the sewerage system is not yet developed properly and 12% of urban people don't obtain drinking water from improved sources In

addition there is lack of solid waste management and their water sources are not well protected as well. As a result the surface water is polluted due to the various human activities. Consequently, a remarkable number of people, 19.7% children under 5 year have died in each year for diarrhea in Dili city. As more 45,973 cases of malaria was reported in 2008 to the public health facilities (Ministry of Finance, 2009-10). The WHO estimates that 88% of diarrheal diseases is attributed to unsafe water supply and over 2 million people die each year from water-related diseases.

The assessment of environmental pollution is a considerable and ongoing challenge since the variability, number and amount of potential hazardous chemicals of industrial use is tremendous (Lepom et al., 2009; Thomaidis et al*.,* 2013). Concentration of contaminants in aquatic environment and their effects need to be assessed taking into account the impacts and threats to the ecosystem (Hagger et al., 2008). Therefore monitoring approaches should have an integrative character combining chemical and ecological aspects with abiotic and biotic parameters (Schettino et al*.,* 2012). Regular monitoring programs rely on the availability of efficient and robust tools and technologies able to deliver appropriate and reliable data (Allan et al*.,* 2006, Brooks et al*.,* 2009 and Galloway et al*.,* 2004).

Prediction of the toxic effects of chemicals on organisms is the primary aim of ecotoxicology, one of the effective procedures of which is the bioassay. In this regard, the medaka fish (*Oryzias latipes*) serves as an excellent fish model for determining acute and chronic toxicities, including the endocrine disrupting activity of chemicals (Wei et al*.,* 2006). An efficient larval medaka assay has been developed by (Liu *el al.,* 2006), using organic pollutants that were concentrated 10 to 100 times from 4 L of river water with disposable commercial adsorption cartridges. The toxicity of these concentrated solutions was determined by exposing 48–72 h post-hatch aged larvae to the solution for 48 h. The median lethal concentration ratio (LCR_{50}) was used to evaluate the fish safety level of the river water. The key point of the method is the need to process only relatively small volumes of samples in the toxicity test using larvae, which are as small as 2–3 mg in weight and 2–3 mm in length, and therefore require only 20 ml of test solution in an acute toxicity test. Moreover, the larvae are usually among the most sensitive stage to toxicant exposure of the entire life cycle. (Yamashita et al*.,* 2012), proposed a semi quantitative toxicity test using medaka early fry and 100-

fold concentrated water sample to obtain results quickly, but as quantitatively as possible.

Most aquatic monitoring programs rely on collecting discrete grab, spot or bottle samples of water at a given time. Often, where pollutants are present at only trace levels, large volumes of water need to be collected. The subsequent laboratory analysis of the sample provides only a snapshot of the levels of pollutants at the time of sampling and does not provide information on the truly dissolved fraction of contaminants to which recipients are exposed. In the last two decades, alternatives have been sought to overcome such these problems. Among these, passive sampling methods have shown considerable promise as tools for measuring aqueous, dissolved concentrations of a wide range of priority chemicals (Vrana et al*.,* 2005).

1.2 Objectives

There were two objectives of this study;

- The first was to introduce information about chemicals and their corresponding toxicity that discharged from residential areas into water streams in Dili city, Timor-Leste.
- The second was to investigate the applicability of passive sampling for larval medaka acute toxicity assay.

1.3 The scope of dissertation

This dissertation comprises 5 chapters; chapter 1 explains the background and objectives of this study. Chapter 2 present literature review on Timor-Leste as the study area, water quality monitoring approaches, active and passive sampling, chemcatcher passive sampler disks, medaka fish as a biological indicator, international regulation for toxicity tests, medaka (*Oryzias latipes var*.) acute toxicity test and gas chromatography / mass spectrometer (GC/MS) simultaneous analysis data base. In chapter 3, the toxicity of organic chemicals that discharged from residential areas in Timor-Leste water streams was investigated using active sampling via Sep-Pak® Plus PS-2 cartridges combined with larval medaka acute toxicity assay. GC/MS simultaneous analysis showed the possible sources of organic pollutants. Detected plasticizers, coprostanol and fuel oils refers to the negative impacts on surface water quality as a result of discharging solid wastes including plastics in addition to house

hold waste water directly into water streams without any treatment in Timor-Leste. Toxicity levels of water streams in Timor-Leste were comparable or higher than those of the Japanese water streams that were investigated in 2013. Those results were interesting and referred to the need for regular monitoring of the toxicity conditions in Timor-Leste water streams, but it is difficult because of unsafe monitoring sites, limited resources in Timor-Leste like shortage in laboratory, transportation facilities and unstable electricity therefore another sampling technique should be considered to avoid such these problems. In chapter 4, we investigated the applicability of passive sampling (as one of the water sampling methods), using Empore^{TM} styrene-divinylbenzene reverse-phase sulfonated disks (hereafter SDB-RPS disks) to evaluate the toxicity level via bioassays using larvae of the medaka fish (*Oryzias latipes var.*). Then Chapter 5 is the conclusions and future work.

1.4 References

1. Allan I, Vrana B, Greenwood R, Mills G, Roig B, Gonzalez C (2006) A "toolbox" for biological and chemical monitoring requirements for the European Union's Water Framework Directive. *Talanta*., 69:302–322.
2. Brooks S, Lyons B, Goodsir F, Bignell J, Thain J (2009) Biomarker responses in mussels, an integrated approach to biological effects measurements. *J. Toxic. Environ. Health A*., 72:196–208.
3. Edokpayi JN, Odiyo JO, Olasoji SO (2014) Assessment of heavy metal contamination of Dzindi River, in Limpopo Province, South Africa. *International Journal of Natural Science Res.*, 2:185–194.
4. Galloway T, Brown R, Browne M, Dissanayake A, Lowe D, Jone M (2004) Ecosystem management bioindicators: the ECOMAN project a multi-biomarker approach to ecosystem management. *Mar. Environ. Res*., 58:233–237.
5. Hagger J, Jones M, Lowe D, Leonard D, Owen R, Galloway T (2008) Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: a case study. *Mar. Pollut. Bull*., 56:1111–1118.
6. Hunter, P.R, Zmirou-Navier, D., Hartemann, P. (2009). Estimating the impact on health of poor reliability of drinking water intervantions in developing countries. Science of the total environment. Vol. 407: 2621-2642.

7. Lepom P, Brown B, Hanke G, Loos R, Quevauviller P, Wollgast J (2009) Needs for reliable analytical methods for monitoring chemical pollutants in surface water under the European Water Framework Directive. *J. Chromatogr*., A. 1216: 302–315.
8. Liu R, Kameya T, Sugimura Y, Sawai A, Shigeoka T, Urano K. (2006) A larval medaka (*Oryzias latipes*) acute toxicity assay combined with solid-phase extraction to efficiently determine the toxicity of organic contaminants in river water. *Water Science & Technology*, 53:213-219.
9. Ministry of Finance, Democratic Republic of Timor-Leste, Dili, Timor-Leste.

   Demographic and Health Survey, Timor-Leste 2009-10.
10. Schettino T, Caricato R, Calisi A, Giordano M, Lionetto M (2012) Biomarker approach in marine monitoring and assessment. *Open Environ*. *Sci*., 6: 20–27.
11. Thomaidis N, Asimakopoulos A, Bletsou A (2013) Emerging contaminants: a tutorial mini-review. *Global NEST J*., 14:72–79.
12. Tsuzuki, Y. (2008). Relationship between water pollutant discharges per capita and indicators of economic level, water supply and sanitation in developing countries. Ecological economics. Vol. 68: 273-287.
13. Vrana B, Mills A, Allan J, Dominiak E, Svensson K, Knutsson J, Morrison G, Greenwood R (2005) Passive sampling techniques for monitoring pollutants in water. *Trends in Analytical Chemistry*., 24:No. 10.
14. Wei B, Kisuno A, Kameya T, Urano K (2006) A new method for evaluating biological safety of environmental water with algae, daphnia and fish toxicity ranks. *Science of the Total Environment*., 371:383-390.
15. Yamashita H, Haribowo R, Sekine M, Oda N, Kanno A, Shimono Y, Shitao W,Higuchi T, Imai T,Yamamoto K (2012) Toxicity test using medaka (*Oryzias latipes*) early fry and concentrated sample water as an index of aquatic habitat condition. *Environ. Sci. Pollut. Res*., 19:2581-2594.

CHAPTER 02
LITERATURE REVIEW

2.1 Introduction

Assessment of water is not only for suitability for human consumption but also in relation to its agricultural, industrial, recreational, commercial uses and its ability to sustain aquatic life. Water quality monitoring is therefore a fundamental tool in the management of freshwater resources. However, water pollution has become one of the most serious problems in many countries, especially in the developing countries (Hunter et al. 2009; Tsuzuki, 2008).

2.2 Timor-Leste Geography, Ggeology and Climate

The Democratic Republic of Timor-Leste is one of the world´s newest nations of the 21st century. Timor-Leste became an independent country in 2002 after a long history of colonization, first as a colony of Portugal and then under Indonesian occupation. The country declared independence from Portuguese rule on November 28 of 1975, but it was invaded by Indonesian military forces on December 7 of 1975. On August 30 of 1999, under an UN-sponsored referendum, a majority of Timorese people voted for independence from Indonesia. Following a period of violence and an UN administration period of three years, Timor-Leste was internationally recognized as an independent country on May 20 of 2002.

East Timor is located in the island of Timor, belonging to the driest and least developed parts of the Indonesian archipelago (Hiorth, 1985). The nation comprises approximately of the island: 18,899 km2 (including the Oecussi enclave in west Timor) (Hiorth, 1985). The forward thrust of the Australian tectonic plate toward the Asian plate has formed en extraordinary set of multi-island ridges of which Timor is the most prominent (Fox and Soares, 2003). With mountain ranges dividing the island lengthwise with summits exceeding 2000m (Hiorth, 1985) this rough and irregular mountainous interior is the heartland of the Timorese. Almost half of the country experiences slopes of approximately 40% which, combined with heavy rainfall, enhances soil erosion (UNDP, 2006).

The soft, scaly Bobonaro clay (named after a central region of the island) is the dominant soil type (Fox and Soares, 2003). This Bobonaro clay substratum is overlaid

with limestone and associated marl, a mix of clay and lime (Fox and Soares, 2003). These Timor clays do not support heavy vegetation, soaking up rain in the wet season and drying out in the dry season (Fox and Soares, 2003).

Differences in temperature are relatively small, so the rainfall regime describes the climate and determines the vegetation, the soil and consequently population settlements. The tropical climate exhibits cyclones unique to this part of the Indonesian arcgipelago and erratic rainfall seasons (Hiorth, 1985). A brief but intense monsoonal rain ranging from December through to April is followed by a prolonged dry season (Fox and Soares, 2003) which varies for different regions of the island. East Timor can be divided into three climatic zones classified by precipitation, temperature and altitude:

- The north, stretching from the north coast to the 600m mark, featuring annual average temperature over 24 degrees Celcius, weak precipitation (below 1500mm annually) and a pronounced dry period of five months.
- The southern zone, stretching from the south coast to the 600m mark, greater rainfall than the northern zone, average temperature higher than 24 degrees and a dry period of three months.
- The mountainous zone sandwiched between the northern and southern zones above the 600m level, comprising of temperatures under 24 degree, high precipitation (greater than 1500mm) and a dry period of four months (Government of the Democratic Republic of Timor-Leste, 2006).

The natural environment is important to many East Timorese as they are reliant on the use of fuel-wood as a major source of energy. Electricity is supplied to only 10% of the rural population (UNDP, 2006).

2.2.1 Public Health Condition in East Timor

- Child Health

Under-5 mortality for the most recent period (0-4 years before the survey or, roughly, during the calendar years 2005-2009) is 64 deaths per 1000 live births. This means that 1 in 16 children born in Timor-Leste dies before the fifth birthday. Sixteen percent of all children under the age of 5 had diarrhea in the 2 weeks before the survey and 1 percent had diarrhea with blood which are mainly related to water consumption. 53% Timorese children age 12-23 months are fully immunized and

23% received no vaccination at all. For under 5 years children are mainly affected by various infectious diseases (2%), 19% fever, 16% diarrhea and 38% anemic.

2.2.2 Housing Characteristics

There is a strong correlation between the socioeconomic condition of households and the vulnerability of their members, especially children, to common diseases. The amenities and assets available to households are important in determining the general socioeconomic status of the population. The availability of and accessibility to improved drinking water may, to a large extent, minimize the prevalence of waterborne diseases among household members, especially the young children. There are 45% populations is under 15 years of age and only 4% people are over 65 years old in East Timor.

The source of drinking water is important because potentially fatal diarrheal diseases, such as typhoid, cholera, and dysentery, are common in Timor-Leste. Overall, 63 percent of households obtain their drinking water from an improved source. Eighteen percent of households have access to piped water in their dwelling, yard or plot, while 27 percent access drinking water from a public tap. Nine percent of households get their drinking water from a tube well or borehole or a protected dug well, and 7 percent have access to protected spring water. There are 36% of household use non-improved sources of drinking water. In total 88 % urban people has access to improved source of drinking water and 56% rural people has access to improved source of drinking water. For rural area, 33% people use unprotected spring water for drinking, 27% use tap or stand pipe and 45% use the piped drinking water. There are 17% people do not treat water prior to drinking. The common treatment methods are 83% is boiling and 61% straining through clothes.

2.2.3 Sanitation facilities

Sanitation is very poor in East Timor. Open defecation is common in rural communities. On-site sanitation with pit latrines (with direct or off-set pits) is widely used with limited number of septic tanks. There does not exist a sewerage system even in Dili. Concentrated on-site toilets in Dili and other towns pose a high risk of contaminating ground water and surface drains. The situation sometimes become even worse when the low level ground is flooded with the surface run off. A temporary

arrangement has been made in Dili for cleaning and desludging of the septic tanks using vacuum trucks through contractors and treating them collectively in a small lagoon constructed near Dili. Existing drains for surface water runoff in Dili and other towns are blocked with siltation and are left open without a proper cover on them. It provides a good place for mosquito breeding. (WHO, 2001)

2.2.4 Diseases

- Diarrhea

Dehydration caused by severe diarrhea is a major cause of morbidity and mortality among young children in Timor-Leste, although the condition can be easily treated with oral rehydration therapy (ORT) and Zinc. Exposure to diarrhea-causing agent is frequently related to the use of contaminated water and to unhygienic practices in food preparation and disposal of excreta.

- Malaria

Malaria remains a leading public health problem in Timor-Leste. Most of the estimated one million population in the country is at high risk of malaria, with about 80 percent of the cases reported from 4 of the 13 districts-Dili,Viqueque,Covalima and Lautem (WHO SEAR, 2010). The existing climatic conditions in Timor-Leste are conducive to the spread of mosquitoes and the perennial transmission of malaria. The number of reported cases peak during the post –wet season of November to May (Cooper, et al., 2010). Still, a relatively large number of cases are recorded throughout the rest of the year.

2.3 Environmental Monitoring of Water Quality

Historically, environmental monitoring programs have tended to focus on organic chemicals, particularly those that are known to resist degradation, bioaccumulate in the fatty tissues of organisms, and have a known adverse toxicological effect. The Stockholm Convention on Persistent Organic Pollutants (http://chm.pops.int) identified several classes of chemicals of environmental concern. Recently, it has been recognized that risks to aquatic and terrestrial organisms, including humans, are not limited to chemicals fitting the classical POP definition. An examination of the complex mixtures of chemicals present in natural water reveals the presence of organic chemicals covering a wide range of water solubilities and

environmental half-lives. Many of these chemicals have been termed Emerging Contaminants (ECs) by the scientific community

Emerging contaminants (ECs) is a phrase commonly used to broadly classify chemicals which do not fall under standard monitoring and regulatory programs but may be candidates for future regulation once more is known about their toxicity and health effects (Glassmeyer, 2007). Chemicals such as polybrominated diphenyl ether (PBDE) flame retardants, musk fragrances, and pharmaceuticals have been present in the environment since their first use decades ago (Garrison et al., 1976; Hignite and Azarnoff, 1977; Yamagishi et al., 1981; Dewit, 2002), but only recently they have emerged into the spotlight due to advances in monitoring techniques and the increased understanding of their toxicological impact. Effluents, treated and non-treated, from wastewater treatment plants (WWTPs) and industrial complexes, leaking septic tanks, rural and urban surface runoff, and improper disposal of wastes are all common sources of ECs. ECs commonly include complex mixtures of new generation pesticides, antibiotics, prescription and nonprescription drugs (human and veterinary), personal care products, household and industrial compounds such as antimicrobials, fragrances, surfactants, and fire retardants (Alvarez et al., 2005).

Urban streams are impacted by EC contamination due to the concentration of people and potential point sources; however, surface and groundwater systems in rural areas can also be at risk due to less efficient waste treatment systems and non-point source contamination from agricultural practices (Barnes et al., 2008; Focazio et al., 2008). Releases of ECs into the environment, although at trace (parts per billion and parts per trillion) concentrations, have the potential to cause adverse biological effects across a range of species (Daughton and Ternes, 1999; Sumpter and Johnson, 2005). Pharmaceuticals designed for human or veterinary use have a specific biological mode of action; however, the impact on non-target species is rarely known. Since ECs are released into the environment as complex mixtures, and not single compounds, the possibility exists for synergistic or antagonistic interactions resulting in unexpected biological effects. The concentrations of ECs in water supplies are likely to be below any level of direct risk to humans; however, the presence of antibiotics in the environment may result in the development of antibiotic-resistant strains of bacteria which could become a serious threat to human health (Schwartz 2003; Kümmerer 2004; Josephson et al., 2006; Schwartz 2006).

The first step in understanding the potential biological impact of ECs in the environment is to identify and quantify the types of ECs that are present. To do so, innovative sampling methodologies need to be coupled with analytical techniques which can confirm the identity of targeted and unknown chemicals at trace concentrations in complex environmental samples

2.4 Monitoring Approaches of Chemical Pollution

The assessment of environmental pollution is a considerable and ongoing challenge since the variability, number and amount of potential hazardous chemicals of industrial use is tremendous (Lepom et al., 2009; Thomaidis et al., 2013). Concentration of contaminants in aquatic environment and their effects need to be assessed taking into account the impacts and threats to the ecosystem (Hagger et al., 2008). Therefore monitoring approaches should have an integrative character combining chemical and ecological aspects with abiotic and biotic parameters (Schettino et al., 2012). Regular monitoring programs rely on the availability of efficient and robust tools and technologies able to deliver appropriate and reliable data (Allan et al., 2006, Brooks et al., 2009 and Galloway et al., 2004).

2 .4.1 Traditional water sampling approach (Active sampling)

Most a quatic monitoring programs rely on active sampling via collecting discrete grab, spot or bottle samples of water at a given time. Often, where pollutants are present at only trace levels, large volumes of water need to be collected. The subsequent laboratory analysis of the sample provides only a snapshot of the levels of pollutants at the time of sampling. However, there are drawbacks to this approach in environments where contaminant concentrations vary over time, and episodic pollution events can be missed. One solution to this problem is to increase the frequency of sampling or to install automatic sampling systems that can take numerous water samples over a given time period. This is costly and in many cases impractical, since a secure site and significant pre-treatment of water are required. Such systems are rarely used in widespread monitoring campaigns. Spot sampling yields different apparent concentrations of pollutants depending on the pre-treatment applied (e.g., filtering) and does not provide information on the truly dissolved, bioavailable fraction of the contaminants. (Vrana et al., 2005)

2.4.2 Biomonitoring Approach

Certain aquatic living organisms are known to provide reliable information on the truly dissolved bioavailable fraction of organic contaminants in the water environment. Persistent organic pollutants such as PCBs, OCPs and PAHs are strongly lipophilic and although such contaminants may be present at very low concentrations in water, they slowly move across animal membranes (e.g. fish gills) and concentrate in the fat tissues of such aquatic organisms (Gorecki and Namiesnik, 2002; Kot et al., 2000). Information on the equilibrium concentration of the water-borne contaminants can be obtained from analysis of the lipid or tissue extracts of the organisms. Biomonitors have some limitations in their application. For example, organisms may not survive in certain environmental conditions and age, size, sex, and physical condition might affect the uptake rates of compounds. The organisms should also be abundant and less mobile in the environment so as to achieve reliable long-term monitoring. Moreover, extraction procedures of analytes from the tissues of animals prior to instrumental analysis are tedious and complex (Vrana et al., 2005).

2.4.3 Passive Sampling Approach

Passive sampling constitutes any sampling technique based on the free flow of analyte molecules from the sampled medium (e.g. water) to a collecting medium as a result of a difference in chemical potentials of the analyte between the two media (Rubio and Perez-Benedito, 2009; Paschke et al., 2005; Vrana et al., 2005; Gorecki and Namiesnik, 2002). The net flow of analyte molecules from one medium to the other continues until equilibrium is established in the system or until the sampling session is terminated. Thus, the quantity of the collected analyte by the sampler is dependent on both its concentration in sampled medium and the exposure time (Gorecki and Namiesnik, 2002). The ratio of analyte distribution between the two media involved or the experimental calibration of the device can then be used to determine the analyte's concentration. Therefore, use of integrative passive samplers can enable estimation of TWA concentrations of pollutants of interest and permits sequestration of residues from episodic events commonly not detected with grab sampling. In addition, this technique can allow the concentration of ultra-trace, yet, toxicologically relevant contaminant mixtures to be determined over extended periods of time (Yu et al., 2006).

A passive sampler is designed to mimic the parts of animals that cause bioconcentration. The device is left in the water for a few days to several weeks, during which it sequesters hydrophobic or hydrophilic water-borne contaminants depending on the sampler design. At the end of the period, the sampler is removed and then analysed for the contaminants. Parameters such as water temperature, fluctuation of analyte concentrations and turbulence can affect passive sampling. The last two or so decades has witnessed an exponential growth in the application of passive sampling (Vrana et al., 2005). Several designs of passive devices used for water monitoring are available either as experimental prototypes or as commercial products. Passive samplers, both experimental prototypes and commercial products, have been used in a variety of aqueous matrices (Fig. 2.1). However, the majority of the reported deployments of passive samplers have been in surface waters (Zabiegała et al., 2010)

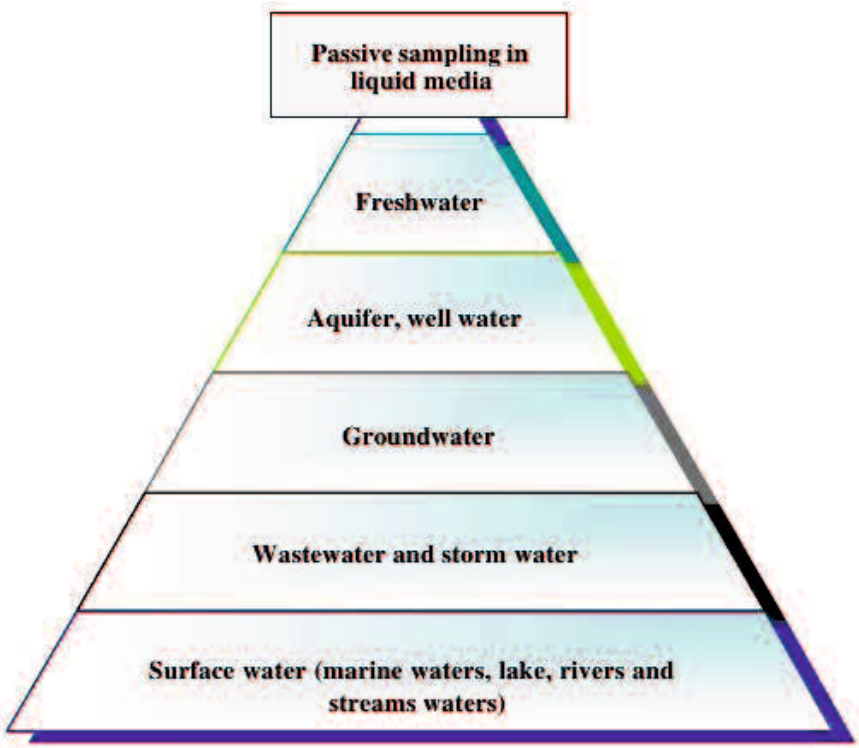


Fig. 2.1 Use of passive sampling in the aquatic environment

Source: (Zabiegała et al., 2010)

2.5 Working Principle of Passive Sampler Devices

Passive sampling can be defined in its broadest sense as any sampling technique based on free flow of analyte molecules from the sampled medium to a receiving phase

in a sampling device, as a result of a difference between the chemical potentials of the analyte in the two media. The net flow of analyte molecules from one medium to the other continues until equilibrium is established in the system, or until the sampling period is stopped (Vrana et al., 2002).

Analytes are trapped or retained in a suitable medium within the passive sampler, known as a reference or receiving phase. This can be a solvent, chemical reagent or a porous adsorbent. Pollutant adsorption or absorption from water into most passive sampling systems generally follows the pattern shown in (**Fig. 2.2**)

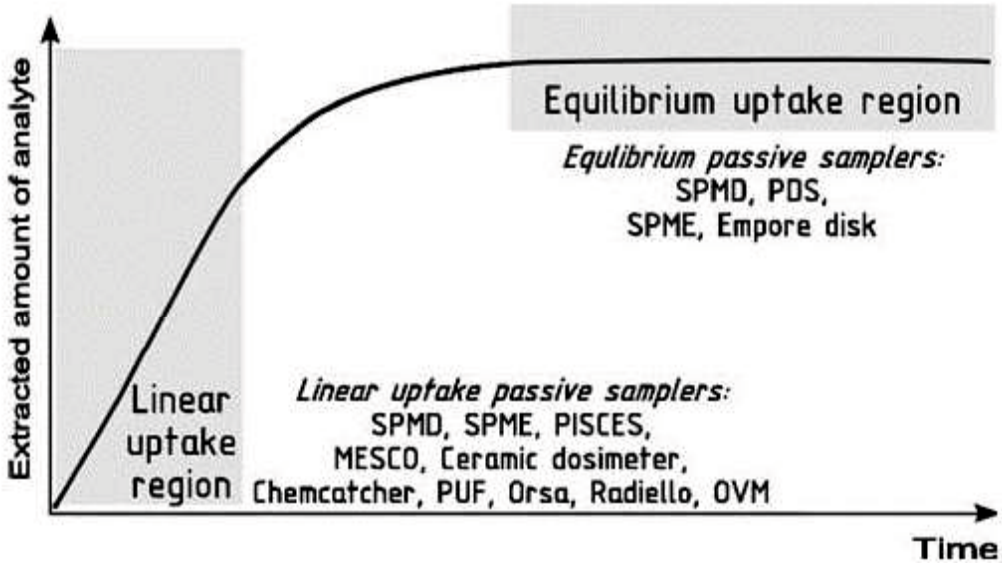


Fig. 2.2 Analyte mass uptake profile in passive sampling devices. Two different accumulation regimes of passive sampling devices can be distinguished)
Source: (Zabiegała et al., 2010)

The exchange kinetics between a passive sampler and water phase can be described by a first-order, one-compartment mathematical model:

$$C_s(t) = Cw\ (K_1/K_2)\ (1- e^{-k_2 t}), \qquad (1)$$

where Cs(t) is the concentration of the analyte in the sampler at exposure time t, C_W is the analyte concentration in the aqueous environment, and $k_1$and k_2 are the uptake and offload rate constants, respectively. Two main uptake regimes, either linear or equilibrium, can be distinguished in the operation of a sampler during field deployment.

2.5.1 Equilibrium-Passive Samplers

In equilibrium sampling, the exposure time is sufficiently long to permit the establishment of thermodynamic equilibrium between the water and reference phases. In this situation, equation (1) reduces to:

$$C_s(t) = C_w (K_1/K_2) = C_w K \quad (2)$$

Knowledge of the phase-water partition coefficient (K) allows estimation of dissolved analyte concentration (Mayer et al., 2003). The basic requirements of the equilibrium sampling approach are that stable concentrations are reached after a known response time, the sampler capacity is kept well below that of the sample to avoid depletion during extraction and the device response time needs to be shorter than any fluctuations in the environmental medium.

2.5.2 Kinetic-Passive Samplers

With kinetic sampling, it is assumed that the rate of mass transfer to the receiving phase is linearly proportional to the difference between the chemical activity of the contaminant in the water phase and that in the receiving phase. In the initial phase of sampler exposure, the rate of desorption of analyte from the receiving phase to water is negligible, the sampler works in the linear uptake regime, and equation (1) reduces to:

$$C_s(t) = C_w K_1 t \quad (3)$$

Equation (3) can be rearranged to an equivalent relationship:

$$M_s(t) = C_w R_s t, \quad (4)$$

where Ms(t) is the mass of analyte accumulated in the receiving phase after an exposure time (t) and Rs is the sampling rate, which may be interpreted as the volume of water cleared of analyte per unit of exposure time by the device. When Rs is known, Cw [the time-weighted average (TWA) concentration of a pollutant in the water phase] may be calculated from the sampling rate (Rs), exposure time (t) and the amount (Ms(t)) of the analyte trapped by the receiving phase. For most devices operating in the kinetic mode, Rs does not vary with Cw, but is often affected by water flow or turbulence, temperature and biofouling. The advantages of kinetic or integrative sampling are that they sequester contaminants from episodic events commonly not detected with spot sampling, and can be used where water concentrations are variable. They permit

measurement of ultra-trace, yet toxicologically relevant, contaminant concentrations over extended time periods. (Vrana et al., 2005)

2.6 Factors Affecting Performance of Passive Samplers

Water sampling rates (Rs) of specific analytes by passive sampler devices depend on a complex set of interacting environmental variables including temperature, water flow, sorption of the compounds to dissolved organic carbon, biofouling, photodegradation and the geometry of the mounting cages (Stuer-Lauridsen, 2005; Vrana et al., 2005; Booij et al., 1998).

- **Flow velocity**

(Gunold et al., 2008), studied the influence of flow velocity (0.135 m/s and 0.4 m/s) on the uptake of 12 polar and semi polar pesticides. Under the investigated conditions with high flow 0.4 m/sec, no influence of the flow velocity on the uptake kinetics was expected since the uptake should be governed only by the sampler's resistance to mass transfer and not primarily by diffusion through the aqueous boundary layer. By contrast, this would be expected for nonpolar compounds and was demonstrated by Vrana and Schuurmann (2002) for SPMDs for very slow flow (0.0006 m/s, 0.0028 m/s).

(Gunold et al., 2008), observed that, the sampling rates exhibited significant differences when compared at flow velocities of 0.135 m/sec and 0.4 m/sec. However, after removal of the elevated water concentration at 0.4 m/s, the Rs values would not be significantly different. Hence they suggested that the differences between the sampling rates could be attributed to variability in the analyte concentrations rather than to differences in uptake kinetics. The authors concluded that, the influence of flow velocity on the sampling rate seemed to play a minor role for hydrophilic compounds. Nevertheless, more studies are needed regarding the influence of flow velocity on the sampling rate, as this is a very important environmental variable in field deployment.

- **Biofouling**

The growth of bacterial mats, periphyton and even microfauna (biofouling) can have a major impact on analyte uptake rates (Mason et al., 2005). By randomly forming on the membrane surface, the biofilm layer increases the overall mass transfer resistance of the compounds by decreasing or even blocking pores in the diffusion limiting membrane. A study by (Richardson et al., 2002) revealed that biofouling of the membranes reduced amounts of absorbed contaminants by about 30 - 40% when compared to unfouled controls (Booij et al., 2007).

- **Temperature**

The sampling rates of compounds in an environmental media generally increase with an increase in temperature. (Michel et al., 2009) observed an increase in the mass transfer of triazole compounds in a supported liquid membrane with an increase in temperature. The effects of temperature on sampling rates have been also been observed in semipermeable membrane devices (SPMDs) (Yusa et al., 2005) and in membrane enclosed sorptive coating (MESCO) sampler (Vrana et al., 2001). Knowing the prevailing temperatures during field deployment of samplers is important in addition to evaluating the influence of temperature on each analyte of interest in the laboratory. However, use of in situ calibration methods by incorporating PRCs in the samplers before deployment still remain the best bet in mitigating temperature effects.

2.7 Chemcatchers® Passive Sampler

Among the passive sampler devices, the Chemcatcher® passive sampler which has the particularity of being adapted to organic, organo- metallic and inorganic contaminants depending on the receiving phase and membrane.

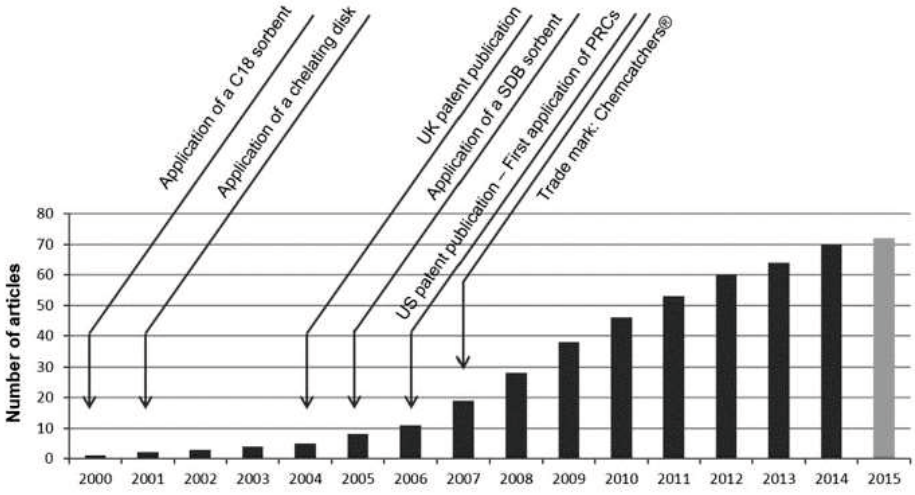


Fig. 2.3 Main steps and publication history for the development and uses of the Chemcatcher® (Source; Charriau et al ., 2015)

The first application of this sampler was reported by (Kingston et al., 2000) for organic contaminants (**Fig 2.3**). One year later, the first application for inorganic contaminants with a chelating disk was developed by (Bjorklund Persson et al., 2001). Two patents were published; the first in 2004 in the United Kingdom and the second in 2006 in the United States by (Kingston et al.,2004, 2006). The name Chemcatchers® appears only in 2007 when the trademark was deposited. Since, this tool has been adapted for a wide range of organic and inorganic pollutants due to the assorted materials available for the receiving phase and membrane.

2.7.1 Chemcatcher® Body Designs

The Chemcatcher® is composed of a disk and, optionally, a membrane sealed into a PolyTetraFluoroEthylene (PTFE) or polycarbonate support (PC). Three different housing geometries were successively developed (Fig. 2.4).

Fig. 2.4 Different housing designs of Chemcatcher® device.
(Source; Charriau et al ., 2015)

The first one is composed of two PTFE parts which are screwed to seal the disk and the membrane. A copper mesh can be added to protect the disk from mechanical damage and biofilm develop- ment. In this design, the disk is located inside a 20 mm deep cavity in the front of the sampler body (Kingston et al., 2000; Vrana et al., 2005). In the second design, two molded PC parts are clipped together around the membrane and the disk to seal the device. Compared to the previous housing, the depth of the cavity is reduced to 7 mm. This reduced depression allows higher sampling rates due to the thinner water boundary layer. However, in this configuration, the Chemcatcher® is more sensitive to variations of flow velocity and turbulence than the other designs This Chemcatcher® body is designed to be single-use. Disposability, which makes cleaning unnecessary, may be seen as an advantage (Lobpreis et al., 2008). However, for cost considerations, it must be balanced with the large number of samplers needed during field deployments and calibration experiments. The third design is composed of two PTFE parts which screw together to seal the membrane and the disk. The depression of this design is approximately 2 mm and thus results in increased sampling rates, as for the second design. During transport prior to deployment, a cap can be used to protect the disk and the membrane. All Chemcatcher® designs are also equipped at the back with a fastening lug which allows its suspension, facing downward, during field exposure. The sampling area is comprised between 14.5 and 17.5 cm².

2.7.2 Available Materials for the Receiving Phase and Membrane

Four types of Empore disks are used as receiving phases: C18, SDB-RPS, SDB-XC and chelating disks SDB-XD. C18, a silica sorbent bonded with octadecyl groups, is more appropriate for low polarity to nonpolar compounds. SDB-RPS (styrenedivinylbenzene-reverse phase sulfonated) is a poly (styrenedivinylbenzene) copolymer modified with sulfonic acid groups to make it hydrophilic and SDB-XC (styrenedivinylbenzene-exchange) is a poly(styrenedivinylbenzene) copolymer used as a reversed phase sorbent. These last two phases consist of 100% copolymeric particles that are spherical, porous and cross-linked and are suitable for polar and low polarity compounds. Chelating disks are made up of a polystyrene–divinylbenzene copolymer that has been modified with iminodiacetic acid groups and are suitable for metals. These described receiving phases can be overlaid with different types of membranes or applied in a “naked” version (Stephens et al, 2009). The most commonly used

membranes are cellulose acetate (CA, 0.45 mm pore size, 135‑152 mm thickness), low-density poly- ethylene (LDPE, 10 Å pore size, 40 mm thickness), polysulfone (PS, 0.2 mm pore size, 152 mm thickness) and polyethersulfone (PES, 0.1–0.2 mm pore size, 40–146 mm thickness) (Kingston et al, 2000).

2.7.3 Considerations with the use of a Covered or Uncovered Chemcatcher® Configuration

Chemcatchers® are often used with a membrane covering the receiving disk. The role of this membrane is threefold: (1) protection of the disk, (2) selectivity of the accumulated compounds depending on the material used and (3) control of analyte uptake. Additionally, the Chemcatcher® device can be used without a membrane covering the receiving disk, i.e. in a "naked" configuration.

Firstly, Empore disks employed without membrane accumulate a wider range of analytes with higher sampling rate values (Tran et al*.,* 2007). Indeed, for arrange of highly hydrophobic compounds the use of a membrane over the C18 disk lowered the accumulation 12–270 times for a low density poly ethylene (LDPE) membrane and 270–2500 times for a poly ether sulfone (PES) membrane (Cal et al*.,* 2008). In an artificial stream exposure, the insecticide thiacloprid was sampled on a SDB-XC disk at a rate of 0.035 L day^{-1} and 0.071 L day^{-1} respectively with or without a PES membrane (Schafer et al*.,* 2008a).

Secondly, the "naked" configuration leads to a decrease in the Integrative period of linear uptake, which would limitits use to only short deployment periods. In a laboratory flow-through exposure, Camilleri et al*.,* 2012, compared the accumulation of several endocrine disruptors on two C18 disks: uncovered or covered with a PES membrane. A 16 fold increase of the Rs was observed for Bisphenol A with uncovered C18 disks and a 126 fold increase for 4-tert-Octylphenol but the linear accumulation period was reduced to only 4 days. From these results, the authors concluded that analytes first adsorbed onto the membrane, then diffused through the membrane and finally accumulated on the disk. These results were inaccordance with other observations showing that the use of a membrane induces a lag-time between sampler deployment and the beginning of accumulation (Schafer et al*.,* 2008a ; Tran et al*.,* 2007).

The third constraint encountered when applying uncovered Empore disks is the higher extent of biofouling and the risk of deterioration during field deployment. Passive samplers are highly Prone to biofilm development at their surface. This biofilm, whose development essentially depends on the material in contact with water, is known to potentially affect sampling rates by reducing the permeability of the membranes (Schafer et al., 2008a ; Harman et al., 2009). As aconsequence of the smaller range of linear uptake and the higher risk of biofouling, it is recommended that uncovered chemcatchers are deployed in the field for shorter periods ; less than 1 week for (Kennedy et al., 2012 ; Page et al., 2010-2011; Fernandez et al., 2014), 4-13 days for (Shaw et al., 2010 ; Schäfer et al., 2008b). Taking advantage of the accelerated uptake kinetics of this "naked" sampler, this configuration has been suggested for the integration of short-term variations in environmental concentrations (Schäfer et al., 2008a).

2.7.4 Chemcatcher® Calibration

Chemcatcher® calibration is primarily needed in order to infer TWACs from the amounts accumulated in the sorbent. The sampler is operating in the kinetic regime of accumulation and, in that case, sampling rates (Rs) and exposure times during which accumulation remains in the linear phase are needed. Chemcatcher® calibration was generally performed by exposing samplers to known analyte concentrations for fixed periods under controlled conditions. **Table 2.1** gathers the methodologies applied by different authors for chemcatcher® calibration.

Table 2.1 Calibration methodology (design, duration, tested parameters, matrix) (green checks indicate applied and red crosses unused parameters).

		Conditions						Design							R_s or k calculation		Other tested parameters
								Type of tank		Type of stirring			Spiking				
	Compounds	Matrix	Duration	Temperature	Equilibration time	Stirring	Container	Artificial stream	Carousel	Magnetic or mechanic stirrer	Frontal pumping	Spike	Flow through	Water analysis	Corrected by [water]	
Inorganics	Metals	Low saline tap water	14 days	-	48 hours	✓	24 L glass tank	✗	✓	✗	✗	-	-	✓	✓	Temperature Flow velocity
	Metals	River water	3 days	-	-		220 L PET	✗	✓ 30 rpm	✗	✗	✓	✗	No R_s calculation		-
	Metals	River water	28 days	18°C	-	✓ 70cm/s	-	-	-							-
	Metals	Natural water	10 days	Various	2-3 days	✓	1 L beaker	✗	✓ sampler rotate 400 rpm	✓ on sampler	✗	-	-	✓	✓	pH, temperature, concentration
	Metals	-	5 hours	Various	-	✓	1 L beaker	✗	✗	✓	✗	-	-	-	-	Metal concentration
Organotins	Organotin compounds	Tap water, artificial seawater	14 days	11°C	48 hours	✓	24 L glass tank	✗	✓	✗	✗	-	-	✓	✓	pH, salinity, biofouling
	Organotin compounds	Tap water	14 days	-	48 hours	✓	24 L glass tank	✗	✓	✗	✗	-	-	✓	✓	Temperature Flow velocity
Organics	PAH		7 days	18°C	48 hours	✓	20 L glass tank	✗	✓ 40 rpm	✗	✗	✗	✓	✓	✓	Sampler geometry
	PAH	Distilled water	14 days	11°C	48 hours	✓	20 L glass tank	✗	✗	✓	✗	✗	✓	✓	-	Gap filling fluid between membrane and disk
	Pesticides, PAH, PCB	River water	5 days	-	-	✓	200 L stainless-steel	✗	✓ 30 rpm	✗	✗	✓	✗	No R_s calculation		-
	Pesticides, PAH	-	14 days	Various	48 hours	✓	20 L glass tank	✗	✓	✗	✗	✗	✓	✓	✓	Temperature, flow velocity
	Pesticides PCB, PAH	-	14 days	15°C	48 hours	✓	20 L glass tank	✗	✗	✓ 30 rpm	✗	✗	✓	✓	✓	Membranes
	PCB, other	Tap water	56 days	22.6°C	4 days	✓	200 L tank	✗	✗	✓ 33 rpm	✗	Dosing sheets	✗	✓	✗	
	Pesticides Other	Distilled water	14 days	11°C	48 hours	✓	25 L stainless steel tank	✗	✗	✓ 20 rpm	✗	✗	✓	✓	✓	-
	Pesticides	Distilled water	7 days	13 °C	-	-	4.5 L reservoir	✗	-	-	-	✗	✓	✓	✗	pH, time, membranes, photolysis
	Pesticides	River water	6 days	10-16°C	-	✓	50 L channels	✗	✓	✗	✓ 0.15-0.2 m/s	✓	✗	✓	✓	
	Pesticides	Tap water	21 days	25°C	-	✓	6 L Perspex™ tank	✗	✗	✓	✗	✓	✗	✓	✓	
	Pesticides	Tap water	14 days	14.25 °C	-	✓	20 L vessel	✗	✓	✗	✗	✗	✓	✓	-	Flow velocity
	Pesticides, PAH, PCB	Distilled water	9 days	Various	48 hours	✓	19.5 L tank	✗	✗	✓	✗	✗	✓	✓	-	Membranes, temperature, flow velocity
	Pesticides	Tap water	10 days	Average 18.5°C	-	✓	1400 L stainless steel tank	✗	✓	✗	✗	✓	✗	✓	-	Flow velocity
	Pesticides	Tap or river water	21 days	27°C	-	✓	250 L Perspex™ tank	✗	✗	✓	✗	✗	✓	-	-	-
	Pesticides	Tap water	9 days	21.3°C	-	✓	✗	✓	✗	✗	✓	✓	✗	✓	✓	Biofouling, membranes
	Pesticides	-	30 days	21.4°C	-	✓	1500 L stainless steel	✗	✓ 12 rpm 0.14 m/s	✗	✗	✓	✗	✓	?	Membrane or no membrane
	Pesticides	-	28 days	22°C	-	✓	1500 L stainless steel	✗	✓ 12 rpm 0.14 m/s	✗	✗	✓	✗	✓	✓	Membrane or no membrane
	Pesticides	Tap water	11 days	20-25°C	-	✓	1400 L stainless steel	✗	✓ 0.14 m/s	✗	✗	✓	✗	✗	✗	-
	Pesticides	Tap water	21 days	23°C	-	✓	250 L storage 5 L chamber	✗	✗	✓	✗	✗	✓	✓	✓	Membranes, disks
	Pesticides, pharmaceuticals	Effluent water	3-24 days	16-17 °C	-	✓	✗	✓	✗	✗	✓	"*in situ*" concentration		✓	✓	Membranes, disks, flow velocity
	Pesticides, pharmaceuticals	River or tap water	8 days or 25 days	-	2-4 days	✓	✓ Circular tank	✓	✗	✓ 0.12-0.14 m/s	✓	✗	✓	✓	-	Matrix, calibration design
	Pesticides, pharmaceuticals	Tap water	17 days	27°C	-	✓	1400 L stainless steel tank	✗	✓ 11 rpm 0.23 m/s	✗	✗	✓	✗	✓	✓	-
	Pharmaceuticals	Effluent or river water	1-8 days	Data logger (between 8.2 and 19.7°C)	-	✓	✗	✓	✗	✗	✓	"*in situ*" concentration		✓	✓	Flow velocity, matrix
	Pharmaceuticals, pesticides	Tap water	28 days	20°C	-	✓	50 L glass aquaria	✗	✗	✗	✓ 10 cm/s	-	✓	✓	✓	-
	Pharmaceuticals	Distilled water	48 hours	21°C	-	✓	25 L stainless steel tank	✗	✗	✓ 45 rpm	✗	✗	✓	✓	✓	Membranes

Source; Charriau et al ., 2015

Some methodologies for Chemcatcher® calibration are explained below ;

- Flow through water is generally fortified in a mixing chamber and then transferred by means of a peristaltic pump to the exposure tank. This is the most commonly used system for Chemcatcher® calibration but practical design (container and stirring) varies among the authors (e.g. a 20-liter glass tank with carrousel device (Vrana et al., 2006), a 25-liter stainless steel tank with over- head stirrer (Cal et al., 2008) or a 50-liter glass aquarium with water nozzles in front of the samplers (Camilleri et al., 2012). The delivery of spiked solution is adjusted in order to

maintain a constant analyte concentration during all exposure. However, (Vrana et al., 2006; Vrana et al., 2005) noticed a decrease of dissolved analyte concentrations as a result of sampler uptake, vaporization, degradation or adsorption on tubing, tank walls and sampler body.

- Artificial streams or channels - Outdoor artificial streams (20 m length, total volume of 1000 L and closed circulation) were constructed by (Schäfer et al, 2008a) to evaluate the influence of biofouling on sampling rates. After a thiacloprid high-level spiking, a rapid decrease of the concentration was observed as a result of adsorption to sand and gravel in the system. In (Vermeirssen et al., 2008), the exposure system consists of several channels running with spiked water, sewage treatment effluents or naturally contaminated water from a stream or river. Compared to the previous laboratory designs, it has the advantage of approximating real field conditions. In the case of effluent or river water, calibration is limited to naturally present compounds.
- In situ calibration Chemcatcher® deployments and high frequency grab water samplings are jointly performed in streams. Sampling rates are calculated from the amounts accumulated in samplers and from time average water concentrations. This procedure has been successfully applied by (Moschet et al., 2015) for the calibration of 87 compounds (mainly pesticide and pharmaceutical residues) over the 322 that were analyzed. In situ sampling rates were considered as robust because several quality criteria were applied (number of detections in grab water samples and distribution of data points) and samplers were deployed in five streams under various conditions (temperature, flow velocity, and contamination level).

2.8 Bioassay Applications of Chemcatchers® Passive Sampler

(Escher et al., 2006) used the Maxi Imaging Pulse Amplitude Modulation (PAM) method to measure concentration effect curves of Chemcatcher® extracts on two algal species (Desmodesmussubspicatus and Phaeodactylumtricornutum). The same procedure was applied on SPE extracts of water samples in order to compare biological effects. Excellent agreement was obtained between chemical analysis of the extracts and the Maxi Imaging PAM bioassay.The herbicides diuron and simazine,

which were analytically detected, proved to contribute the most to the overall phytotoxicity of the extracts.

(Muller et al., 2007) used three different bioassays: Maxi Imaging PAM (phytotoxicity), Microtox^{TM} (bacterial toxicity) and umuC assay (genotoxicity) to evaluate the toxicity of effluent from sewage treatment plants (STP). All bioassays showed an effect of STP effluents. The results allowed determination of the toxicity of STP effluents at different treatment steps and thus endpoints of concern. This provides a powerful method for assessing effects of specific mixtures of organic pollutants resulting from various biota compartments.

(Tan et al., 2007) studied the estrogenic effect of grab samples and Passive sampler extracts of different matrices of a WWTP (influent, aerobic and anaerobic bioreactors , return activated sludge, clarifier, effluent, river water at the point of discharge and 1 km down stream).These authors used the E-Screen assay, based on increased growth of MCF-7 cells in the presence of estrogenic substances. The estrogenic activity obtained with passive sampler extracts was lower than that obtained with grab samples, mainly due to biofouling.

(Shaw et al., 2009) investigated four different bioassays to evaluate the sensitivity of coral reef biota to mixtures of organic pollutants sequestered in passive samplers deployed in the Great Barrier Reef (GBR, Australia) . The four bioassays were :coral larval settlement (on Acroporamillepora), sea urchin larval development (on Heliocidaristuberculata), bacterial luminescence (on Vibrio fischeri) and micro algal photo synthesis with Maxi Imaging PAM (on Phaeodactylumtricornutum). As for (Muller et al., 2007), the four tests showed that the passive sampler mixture impacted the tested populations. The combination of tests on indigenous populations and passive sampler extracts of GBR water allowed an understanding of the local impacts of pollution.

2.9 Toxicity test models for ecological risk assessment

Industrial chemicals, pharmaceuticals and pesticides, are controlled by authorized systems under the laws of individual nations, and several screening methods are performed to evaluate the toxicity of each chemical. In the case of ecological risk assessment It is necessary to conduct several tests with diverse fauna from bacteria to vertebrates. However, it is almost impossible to evaluate environmental influences for

all species on the earth, with the result that some representative species covering the diverse fauna are selected as models for testing. Generally, in aquatic ecological evaluations bacteria and algae are used to model bacteria and phytoplankton, crustaceans represent the invertebrate model, and fish represent vertebrate model. Fish toxicity test are conducted in many nations of the world. From the view of international regulations, the Organization for Economic Co-operation and Development (OECD) proposed test guidelines for chemical evaluation, and the majority guidelines using fish recommend the Japanese medaka as one model test species. Much attention has been paid to the medaka by many scientists and researchers for the following reasons:

- The lifecycles is shorter than with other species testing can be conducted within a year.
- Fish size is smaller than other species, so the volume of test water can be reduced, such that cost of treating waste can be lessened.
- It is easy to identify both the physiological sex type by external sex characters and genetic sex type by the detection of the male specific gene.
- In particular, because the medaka is a local species in east Asian countries like Japan, Korea and China, the scientist in these countries have a great deal of interest in the development of medaka toxicity.

2.10 Breeding of medaka fish (*Oryzias latipes var.*)

In our laboratory 2 tanks (made of polystyreen) with volumes about 25 - 40 L are used for breeding medaka. The tanks are placed on shelves and up to 30 – 50 adult fish are kept in those tanks, respectively. Tap water (dechlorinated by holding it for at least two days) is used for medaka breeding. Lights are installed to illuminate the tanks for 16 h /day. To avoid the effects of room illumination, black curtains were used to cover the shelves. Water is continuously aerated using small air pumps and the water quality parameters are maintained as follow ; temperature kept in the range 25 - 28 ^{o}C, pH of 6.8-7.5; conductivity, 200-450 μS/cm; ammonia, <0.2 mg/L nitrite, <0.1 mg/L; nitrate, <20 mg/L. The frequency of feeding is more important than how much feed is given, it is recommended that fish finish all the feed placed in the tank with in 10 minutes. Feed is supplied tow times /day at 10:00 and 17:00. These conditions are helpful to get eggs every day from adult fish. For maintenance, debris and left overfeed at the bottom of the tanks were removed by a plastic pipette with a cut tip.

2.11 Importance of Medaka as a Toxicity Test Model

Medaka is the tiny, fresh water, rice-field fish. In japan, scientists have used medaka as a model animal, especially since the work of Aida in 1921 (Kinoshita et al., 2009). Since his work, many Japanese scientists have strived to establish specific strains of medaka and to advance additional experimental methodologies using medaka fish as a model animal. These developments have resulted in the accumulation of the basic biological knowledge of medaka, which has contributed to the discovery of new biological facts in both human and other animal systems. They have helped to identify the functional mechanisms of many newly discovered phenomena in areas of both basic and applied research. Furthermore, recent advances in medaka genomics have provided new insight not only into vertebrate genome evolution but also into basic biology, ecological science, medical science and agricultural science, by comparative analyses with the substantial genomic information that now exists for the vertebrates such as humans, mice, puffer fish, stickleback and zebrafish. Another recent important activity is a large-scale mutagenesis screening of mutants with specific developmental defects.

2.12 International Regulation for Toxicity Tests

The purpose of toxicity test is to understand the effect of substances such as industrial chemical, pharmaceutical and personal care products. These substances are indispensable in most human activities and they are produced for domestic consumption and also international trade. Moreover, chemical migration occurs through the effect of climatic and/or geographic conditions. For example, contaminated air is carried by monsoons and wastewater is carried to others countries in international rivers. Therefore, the international regulation of toxicity test is necessary to regulate chemicals with a consensus between countries. Based on this, some of testing methods have been standardized by some international organization such as the International Organization for Standardization (ISO), the European Commission and the OECD. The most typical is the chemical toxicity test guidelines standardization by the OECD. From this, the fish chemical test guidelines are updated for some recommended species. In OECD test guidelines, the medaka is recommended as a model for the following test:

1. Fish acute toxicity test (TG203)
2. Fish prolonged toxicity test: 14 days (TG204)
3. Fish early-life stage toxicity test (TG210)

4. Fish short-term toxicity test on embryo and sac-larvae stages (TG212)
5. Fish juvenile growth test (TG215)

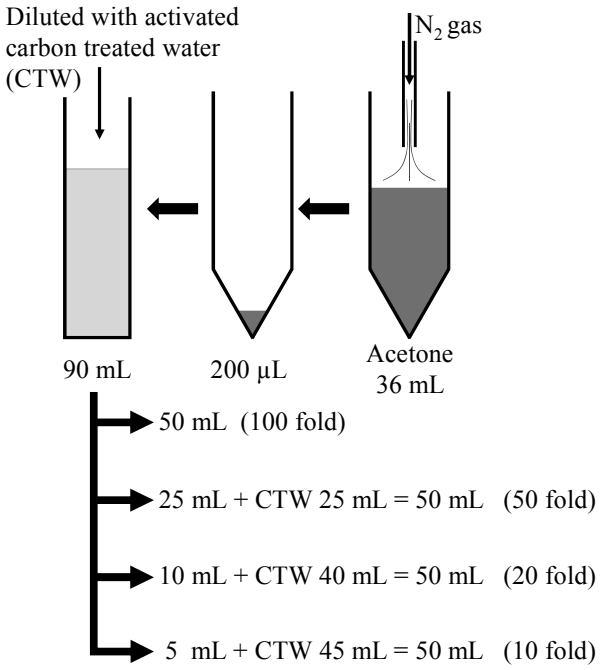
2.13 Medaka (*Oryzias latipes var.*) Acute Toxicity Test

(Liu et al., 2006), developed an efficient larval medaka assay. Organic toxicants were concentrated from 4-L of river water using disposable commercial adsorption cartridges. This concentrated solution was then diluted to prepare 10-, 20-, 50-, and 100-fold concentrated solutions and these solutions were used to examine toxicity. Toxicity was expressed as the median lethal concentration ratio (LCR_{50}). Depending on the mortality percentage of larval medaka exposed to different concentration ratios, the LCR_{50} was calculated using the TOXDAT Multi-Method Program (US EPA). The higher the LCR_{50}, the lower is the toxicity level of river water. (Liu et al., 2007), proposed a simplified procedure for the acute toxicity test for screening purposes, in which only a 100-fold concentrated sample was used in a 48-hour test, and toxicity was expressed as the inverse of the median lethal concentration (LC_{50}^{-1}).

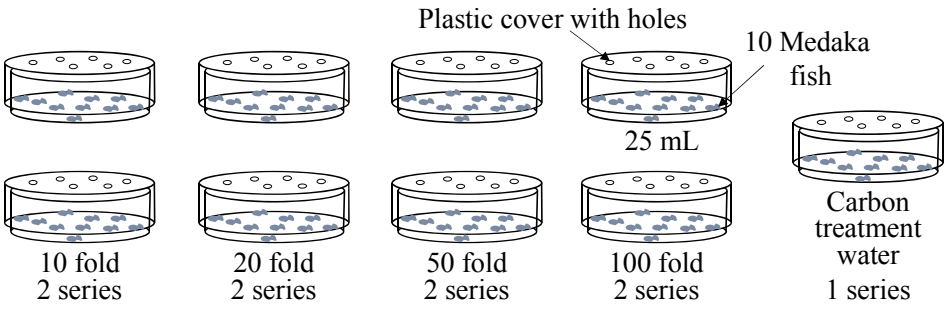
(Yamashita et al., 2012), modified this procedure in order to evaluate toxicity as quantitatively as possible. They counted the number of dead medaka at 0.5, 1, 2, 3, 6, 12, 24, and 48 h during a test, and expressed toxicity as the inverse of the median lethal time (LT_{50}^{-1}) using Probit analysis. The concentration of organic micropollutants from river water allows the prediction of potential effects on aquatic organisms as a result of bioaccumulation of chemical pollutants. (Yamashita et al., 2012), revealed the relationship between toxicity (LT_{50}^{-1}) of 100-fold concentrated river water sample and aquatic habitat conditions:

- Ratio of benthic animal sharply decreased at $LT_{50}^{-1} > 0.25\ h^{-1}$
- Tolerant fish become dominant at $LT_{50}^{-1} > 0.3\ h^{-1}$

In the present study, we employed LDR_{50} (lethal dilution ratio, which is the inverse of LCR_{50}) in addition to LT_{50}^{-1} as two toxicity indices. The higher the value of both indices are, the higher was the toxicity level of the sampled water. The reliable range of LT_{50}^{-1} is between 0.02 and 2.0 h–1, and that of LDR_{50} is between 0.01 and 0.10, depending on the concentration steps used.



(a)



(b)

Fig. 2.5 Toxicity test procedure. (a) Preparation of different folds. (b) Test dishes with different dose levels.

For excessively toxic samples ($LT_{50}^{-1} > 2.0\ h^{-1}$), the LDR_{50} was evaluated for each sample by conducting the toxicity test using four folds (10-, 20-, 50-, and 100-fold samples), and then LT_{50}^{-1} values were obtained for four folds to cover the over-range toxicity samples. Lethal dilution ratio LDR_{50} values were calculated using the same Probit analysis method as used for LT_{50}^{-1} calculation. The strong point of LDR_{50} is that it is a ratio scale value (no unit) and it can be handled as concentration. **(Fig. 2.5)**.

2.14 Gas Chromatography / Mass Spectrometer (GC/MS) Simultaneous Analysis Data Base

A gas chromatograph GC-2010 coupled with a mass spectrometer QP2010 (Shimadzu, Kyoto, Japan) was used for GC/MS analysis. The gas chromatograph was fitted with a fused silica capillary column J&W DB-5 ms (Agilent, Santa Clara, USA); 30 mm × 0.25 mm i.d., 0.25-μm film thickness). The initial oven temperature was 40°C, and this was then increased to 310°C at a rate of 8°C/min. The carrier gas was helium supplied at a constant flow of 40 cm/s. Injector, interface, and ion source were maintained at 250, 300, and 200°C, respectively. The splitting ratio was 20:1. Electron impact mass spectra were obtained at 70 eV, with scans at 0.20 scans/s from 33 m/z to 600 amu. In order to identify compounds in the collected samples, a GC/MS simultaneous analysis database was used, which can identify and quantify a total of 942 chemical compounds without the need for reference standards (Kadokami et al., 2005). To measure the amount of chemical adsorbed to SDB-RPS disks and Sep-Pak cartridges, the acetone eluate portion (4-mL) specified for GC/MS analysis was evaporated completely using nitrogen gas, and then 2 mL of hexane was added. Sodium sulfate was applied to remove moisture and was then removed. The hexane was subsequently evaporated to 1 mL and this volume was used for GC/MS analysis. The amounts of chemicals adsorbed to both SDB-RPS disks and Sep-Pak cartridges were calculated as the sum of the measured values of chemicals of each sample, and expressed as μg/3 disks and μg/4 Sep-Pak cartridges, respectively.

2.15 References

1. Allan I, Vrana B, Greenwood R, Mills G, Roig B, Gonzalez C. (2006), A"toolbox" for biological and chemical monitoring requirements for the European Union's Water Framework Directive. *Talanta.,* 69:302–322
2. Alvarez A, Stackelberg P, Petty J. (2005), Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream. *Chemosphere.,* 61:610-622.
3. Augulyte L, Bergqvist P. (2007), Estimation of Water Sampling Rates and Concentrations of PAHs in a Municipal Sewage Treatment Plant Using SPMDs with Performance Reference Compounds, *Environmental Science and Technology.,* 41 (14) : 5044 - 5049.

4. Barnes K, Kolpin D, Furlong E, Zaugg S, Meyer M, Barber L. (2008), A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States. *Ground water. Sci Total Environ.,* 402:192-200.
5. Blasco C, Picó Y (2009), Prospects for combining chemical and biological methods for integrated environmental assessment. *TrAC-Trend. Anal. Chem.*, 28 :745–757
6. Booij K, Sleiderink M, Smedes F. (1998), Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. *Environmental Toxicologal Chemistry.,* 17:1236 – 1245
7. Booij K, Smedes F, Van Weerlee M. (2002), Spiking of performance reference compounds in low density polyethene and silicone passive water samplers. *Chemosphere.*, 46 (8): 1157 - 1161.
8. Booij K., Vrana B, Huckins J (2007), Theory, modelling and calibration of passive samplers used in water monitoring. in: Greenwood, R., Mills, G.,Vrana, B. (Eds.), Passive sampling techniques in environmental monitoring. Comprehensive Analytical Chemistry Series, D. Barceló (series ed.).*Elsevier, Amsterdam.,* 48: 141 - 169
9. Brooks B, Lyons F, Goodsir J, Bignell J. (2009), Thain Biomarker responses in mussels, an integrated approach to biological effects measurements. *J. Toxic. Environ. Health A.*, 72:196–208
10. Cal A, Kuster M, Alda M, Eljarrat E, Barceló D. (2008), Evaluation of the aquatic passive sampler Chemcatcher for the monitoring of highly hydrophobic compounds in water, *Talanta.,* 76 : 327–332.
11. Charriau A, Lissalde S, Poulier G, Mazzella N, Buzier R, Guibaud G. (2015), Overview of the Chemcatcher for the passive sampling of various pollutants in aquatic environments Part A: Principles,calibration, Preparation and analysis of the sampler,*Talanta, http://dx.doi.org/10.1016/j.talanta*., 2015.06.076, inpress.
12. Cooper, R.D., M.D. Edstein, S.P. Frances and N.W.Beebe 2010. Malaria vectors of East Timor-leste. Malaria Journal 9:40.
13. Daughton C, Ternes T.(1999), Pharmaceuticals and personal care products in the environment: agents of subtle change. *Environ Health Perspect.,* 107 : 907-938.
14. Dewit A. (2002), An overview of brominated flame retardants in the environment. *Chemosphere.,* 46:583-624

15. Escher B, Quayle P, Muller R, Schreiber U, Mueller J. (2006), Passive sampling of herbicides combined with effect analysis in algae using a novel high through put phytotoxicity assay (Maxi-Imaging-PAM), *J.Environ.Monit*., 8 : 456–464.
16. Fernandez D, Vermeirssen E, Bandow N, Munoz K, Schäfer B. (2014), Calibration and field application of passivesampling for episodic exposure to polar organic pesticides in streams, *Environ.Pollut.,* 194 : 196–202.
17. FocazioJ, Kolpin W, Barnes K. (2008), A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States II) Untreated drinking water sources. *Sci Total Environ*., 402:201-216.
18. Fox, J.J. and Soares, D.B. (Eds.) (2003). Out of ashes: destruction and reconstruction of East Timor, ANUE Press, Canberra.
19. Gaag B, Volz J. (2008), Real-time On-line Monitoring of Contaminants in Water. Developing a Research Strategy from Utility Strategy from Utility Experiences and Needs. *Nieuwegein, The Netherlands*.
20. Garrison A, Pope J, Allen F. (1976), GC/MS analysis of organic compounds in 46 domestic wastewaters. In Identification and analysis of organic pollutants in water. *Ann Arbor, MI: Ann Arbor Science Publishers*, pp. 517-556.
21. Galloway T, Brown R, Browne M, Dissanayake A, Lowe D, Jones B. (2004), Ecosystem management bioindicators: the ECOMAN project - a multi-biomarker approach to ecosystem management. *Mar. Environ. Res*., 58 : 233–237.
22. Glassmeyer S.(2007), The cycle of emerging contaminants. *Water Res Impact*, 9 :5-7.
23. Gorecki T, Namiesnik J. (2002) Passive sampling, *Trends in Analytical Chemistry.*, 21, pp. 4.
24. Government of the Democratic Republic of Timor-Leste (2006).
25. Gunold R, Schafer R, Paschke A, Scharmann G, Liess M. (2008) Calibration of the chemcatchers passive sampler for monitoring selected polar and semi polar pesticides in surface water, *Environ.Pollut*.,155: 52–60.
26. Hagger J, Jones B, Lowe D, Leonard P, Owen R, Galloway S. (2008), Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: a case study. *Mar. Pollut. Bull*., 56 :1111–1118.

27. Harman C, Boyum O, Thomas K, Grung M. (2009), Small but different effect of fouling on the uptake rates of semipermeable membrane devices and polar organic chemical integrative samplers, *Environ.Toxicol.Chem*., 28 : 2324–2332.
28. Hignite C, Azarnoff L. (1977), Drugs and drug metabolites as environmental contaminants: Chlorophenoxyisobutyrate and salicylic acid in sewage water effluent. *Life Sci.,* 20 : 337–341.
29. Hiorth, F. (1985). Timor, past and present, James cook University of North Queensland, Townsville.
30. Huckins J, Petty J, Lebo J, Almeida F, Booij K., Alvarez D,Cranor W, Clark R, Mogensen B. (2002), Development of the permeability/performance reference compounds approach for in situ calibration of semipermeable membranee devices, *Environmental Science and Technology.*, 36: 85 - 91.
31. Huckins J, Petty J, Booij K. (2006), Monitors of organic chemicals inthe environment: Semipermeable Membrane Devices; *Springer: New York.*
32. Hunter, P.R, Zmirou-Navier, D., Hartemann, P. (2009). Estimating the impact on health of poor reliability of drinking water intervantions in developing countries. Science of the total environment. Vol. 407: 2621-2642.
33. Josephson J. (2006), The microbial resistome. *Environ Sci Technol.,* 40:6531-6534.
34. Kadokami K, Tanada K, Taneda K. (2005) Novel gas chromatography mass spectrometry database for automatic identification and quantification of micropollutants. *J Chromatogr*., 1089:219-226.
35. Kingston J, Greenwood R, Mills G, Morrison G, Persson L. (2000), Development of a novel passive sampling system for the time averaged measurement of arrange of organic pollutants in aquatic environments, *J. Environ. Monit.*, 2 : 487–495.
36. Kingston J, Greenwood R, Mills G, Morrison G, Persson L. (2004),Aquatic Passive Sampling Device and Methods for its Use, UK Patent No.:GB2353860.
37. Kingston J, Greenwood R, Mills G, Morrison G, Persson L. (2006), Aquatic Passive Sampling Device and Methods for its Use, US Patent No.:US7,059,206B1.
38. Kennedy K, Devlin M, Bentley C, Lee K, Paxman C, Carter S, Lewis S, Brodie J, Guy E, Vardy S, Martin K, Jones A, Packett R, Mueller J. (2012), The influence of a season of extreme wet weather events on exposure of the World Heritage Area Great Barrier Reef to pesticides, *Mar.Pollut.Bull.*, 64 : 1495–1507.

39. Kinoshita M, Murata K, Naruse K, Tanaka M. (2009), *A John Wiley & Sons, Ltd*., Publication.
40. Kot A, Zabiegala B, Namiesnik J. (2000), Passive sampling for long-term monitoring of organic pollutants in water, *Trends in Analytical Chemistry.*, 19(7), pp. 446 - 459
41. Kümmerer K. (2004) Resistance in the environment. *J Antimicrob Chemother.,* 54:311–320.
42. Lepom P, Brown B, Hanke G, Loos R, Quevauviller P, Wollgast J. (2009), Needs for reliable analytical methods for monitoring chemical pollutants in surface water under the European Water Framework Directive. *J. Chromatogr. A.*, 1216 : 302-315.
43. Liu R, Kameya T, Sawai A, Urano K. (2007b) Application of a larval medaka assay to evaluate the fish safety level in Sagami River. *Japan. Environ. Monit Assess*., 130:475-482.
44. Liu R, Kameya T, Sugimura Y, Sawai A, Shigeoka T, Urano K. (2006) A larval medaka (*Oryzias latipes*) acute toxicity assay combined with solid-phase extraction to efficiently determine the toxicity of organic contaminants in river water. *Water Science & Technology*, 53:213-219.
45. Lobpreis T, Vrana B, Dominiak E, Dercova K, Mills G, Greenwood R. (2008), Effect of housing geometry on the performance of Chemcatcher (TM) passive sampler for the monitoring of hydrophobic organic pollutants in water, *Environ. Pollut.,* 153:706–710.
46. Mason S, Hamon R, Nolan A, Zhang H, Davidson W. (2005), *Analytical Chemistry.*, 77: 6339.
47. Mayer P, Tolls J, Hermens J, Mackay D. (2003), *Environ. Sci. Technol*., 37: 184A.
48. Michel M, Chimuka L, Cukrowska E, Wieczorek P, Buszewski B. (2009), Influence of temperature on mass transfer in an incomplete trapping single hollow fibre supported liquid membrane extraction of triazole fungicides. *Analytica Chimica Acta.*, 632 : 86 - 92.
49. Muller R, Tang J, Thier R, Mueller J. (2007), Combining passive sampling and toxicity testing for evaluation of mixtures of polar organic chemicals in sewage treatment plant effluent. *J.Environ.Monit.*, 9 : 105-110.

50. Moschet C, Vermeirssen E, Singer H, Stamm C, Hollender J. (2015), Evaluation of insitu calibration of Chemcatcher passive samplers for 322 micropollutants in agricultural and urban affected rivers. *Water Res.,*71 : 306–317
51. Quansah B,.Engel L. (2010), Rochon Early warning systems: a review early warning systems: *a review J. Terr. Obs*., 2 : 23 - 44
52. Page D, Dillon P, Mueller J, Bartkow M. (2010), Quantification of herbicide removal in a constructed wetland using passive samplers and composite water quality monitoring. *Chemosphere.,* 81 : 394–399.
53. Page D, Khan S, Miotlinski K. (2011), Asystematic approach to determine herbicide removals in constructed wetlands using time integrated passive samplers. *J.Water Reuse Desalin*., 1 : 11–17.
54. Paschke A, Vrana B, Popp P, Wennrich L, Lorenz W, Schuurman G. (2005), Novel passive samplers for monitoring organic pollutants in surface and ground water based on membrane enclosed silicone material. *Journal of Environmental Monitoring.,* 24 : 486 - 492.
55. Persson L, Morrison G, Friemann J, Kingston J, Mills G, Greenwood R. (2001), Diffusional behaviour of metals in apassive sampling system for monitoring aquatic pollution. *J.Environ. Monit.*, 3 : 639–645.
56. Richardson B, Zheng G, Tse E, Deluca S, Siu S, Lam P. (2002), A comparison of polycyclic aromatic hydrocarbon and petroleum hydrocarbon uptake by mussels (perna viridis) and semipermeable membrane devices (SPMDs) in Hong Kong coastal waters. *Environmental pollution.,* 122 : 223 - 227.
57. Rodriguez S,Marco P, Alda L, Barceló D. (2004), Biosensors for environmental applications: future development trends *Pure Appl. Chem*. 76 : 723–752
58. Rubio S, Perez D. (2009), Recent advances in environmental analysis. *Analytical Chemistry.*, 81: 4601 - 4622.
59. Salem H. (2003), Issues in chemical and biological terrorism. *Int. J. Toxicol*., 22: 465-471.
60. Schafer B, Paschke A, Vrana B, Mueller R, Liess M. (2008), Performance of the Chemcatchers passivesamplerwhenusedtomonitor10polarandsemi- polar pesticides in 16 Central European streams, and comparison with two other sampling methods. *Water Res*., 42 : 2707–2717.

61. Schafer R, Paschke A, Liess M. (2008), Aquatic passive sampling of a short term thiacloprid pulse with the Chemcatcher : impact of biofouling and use of a diffusion limiting membrane on the sampling rate. *J.Chromatogr.A*., 1203 : 1–6.
62. Schettino T, Caricato R, Calisi A, Giordano E, Lionetto G. (2012), Biomarker approach in marine monitoring and assessment. *Open Environ. Sci*., 6 : 20-27.
63. Schwartz T, Kohnen W, Jansen B, Obst U. (2003), Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol Ecol.,* 43:325-335.
64. Schwartz T, Volkmann H, Kirchen S. (2006), Real-time PCR detection of Pseudomonas aeruginosa in clinical and municipal wastewater and genotyping of the ciprofloxacin-resistant isolates. *FEMS Microbiol Ecol.,* 57:158-167.
65. Shaw M, Negri A, Fabricius K, Mueller J. (2009), Predicting water toxicity : pairing passive sampling with bioassays on the Great Barrier Reef. *Aquat Toxicol.,* 95 :108–116.
66. Shaw M, Furnas M, Fabricius K, Haynes D, Carter S, Eaglesham G, Mueller J. (2010), Monitoring pesticides in the Great Barrier Reef. *Mar.Pollut.Bull.*, 60 : 113-122.
67. Stephens B, Kapernick A, Eaglesham G, Mueller J. (2009), Event monitoring of herbicides with naked and membrane covered Empore disk integrative passive sampling devices. *Mar.Pollut.Bull*., 58 : 1116–1122.
68. Stuer-Lauridsen F. (2005), Review of passive accumulation devices for monitoring micropollutants in the aquatic environment. *Environmental pollution.*, 136: 503 – 524.
69. Sumpter J, Johnson C. (2005), Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment. *Environ Sci Technol.,* 39:4321–4332.
70. Tan B, Hawker D, Müller J, Leusch F, Tremblay L, Chapman H. (2007), Comprehensive study of endocrine disrupting compounds using grab and passive sampling at selected waste water treatment plants in South East Queensland, Australia. *Environ.Int.*, 33 : 654–669.
71. Thomaidis N, Asimakopoulos G, Bletsou A. (2013),Emerging contaminants: a tutorial mini-review. *Global NEST J.*, 14 : 72–79.

72. Tsuzuki, Y. (2008). Relationship between water pollutant discharges per capita and indicators of economic level, water supply and sanitation in developing countries. Ecological economics. Vol. 68: 273-287.
73. United Nations Development Program (2006). In Timor-Leste Human Development Report 2005 United Nations Development Program, Dili.
74. Vermeirssen E, Dietschweiler C, Escher B, Vander J, Hollender J. (2012), Transfer kinetics of polar organic compounds over polyethersulfone membranes in the passive samplers pocis and chemcatcher. *Environ.Sci.Technol.,* 46 : 6759–6766.
75. Vermeirssen E, Asmin J, Escher B, Kwon J, Steimen I, Hollender J. (2008), The role of hydrodynamics, matrix and sampling duration in passive sampling of polar compounds with Empore SDB-RPS disks. *J.Environ.Monit.*, 10 : 119–128.
76. Verweij F, Booij K, Satumalay K, Vander N, Vander R.(2004), Assesment of bioavailable PAH, PCB and OCP concentrations in water, using semipermiable membrane devices (SPMDs), sediments and caged carp. *Chemosphere.*, 54 : 1675 - 1689.
77. Vonderhaar S, Macke D, Sinha R, Krishnan R, Corporation T, Road C.(2003), Drinking Water Early Warning Detection and Monitoring Technology Evaluation and Demonstration in: *29th Annu. Environ. Energy Symp*., pp. 1–7.
78. Vrana B, Paschke A, Popp P, Schuurmann G. (2001a), Use of semipermeable membrane devices (SPMDs): determination of bioavailable, organic, waterborne contaminants in the industrial regions of Bitterfield, SaxonyAnhalt, Germany. *Environmental Science and Pollution Research.*, 8 (1) : 27 - 34.
79. Vrana B, Schuurmann G, 2002. Calibrating the uptake kinetics of semipermeable membrane devices in water: Impact of hydrodynamics. *Environmental Science and Technology*., 36 (2): 290-296.
80. Vrana B, Mills G, Allan I, Dominiak E, Svensson K, Morrison G, Greenwood R. (2005), Passive sampling techniques for monitoring pollutants in water, *Trends in Analytical Chemistry.*, 24, pp. 845 - 868.
81. Vrana B, Mills G, Greenwood R, Knutsson J, Svensson K, Morrison G. (2005), Performance optimisation of a passive sampler for monitoring hydrophobic organic pollutants in water. *J.Environ.Monit.*, 7: 612–620.

82. Vrana B, Mills G, Dominiak E, Greenwood R. (2006), Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water. *Environ. Pollut.,* 142 : 333–343.
83. World Health Organization Regional Office for South-East Asia, Environmental Sanitation in East Timor, Assignment Report: 16 October-20 December, 2001.
84. WHO Regional office for South-East Asia (WHO SEAR). 2010. Making Pregnancy safer: a health sector strategy for reducing maternal and perinatal morbidity. New Delhi, India.
85. Yamagishi T, Miyazaki T, Horii S, Kaneko S. (1981), Identification of musk xylene and musk ketone in freshwater fish collected from the Tama River. *Tokyo. Bull Environ Contam Toxicol*., 26 :656 - 662.
86. Yamashita H, Haribowo R, Sekine M, Oda N, Kanno A, Shimono Y, Shitao W,Higuchi T, Imai T,Yamamoto K. (2012) Toxicity test using medaka (*Oryzias latipes*) early fry and concentrated sample water as an index of aquatic habitat condition. *Environ. Sci. Pollut. Res*., 19:2581-2594.
87. Yu J, Bouwer E, Coelhan M. (2006), Occurrence and biodegradability studies of selected pharmaceuticals and personal care products, *Agric. Water Manage.*, 86, pp. 72.
88. Yusa V, Pastor A, Guardia M. (2005), Microwave assisted extraction of OCPs, PCBs and PAHs concentrated by semipermeable membrane devices (SPMDs), *Analytica Chimica Acta.*, 540 : 355 - 366.
89. Zabiegała B, Kot-wasik A, Urbanowicz M, Namieśnik J. (2010), Passive sampling as a tool for obtaining reliable analytical information in environmental quality monitoring. *Anal Bioanal Chem.,* 396:273–296.

CHAPTER 03
COMPARISON OF SURFACE WATER TOXICITY AND CHEMICALS FROM RESIDENTIAL AREAS IN TIMOR-LESTE AND JAPAN USING LARVAL MEDAKA (*ORIZIAS LATIPES VAR.*) ACUTE TOXICITY ASSAY

3.1 Introduction

Water pollution has become one of the most serious problems in many countries, especially in the developing countries, then water quality assessment is very important, not only for suitability for human consumption but also in relation to its agricultural, industrial, recreational, commercial uses and its ability to sustain aquatic life. Water quality monitoring is therefore a fundamental tool in the management of freshwater resources (Hunter et al., 2009; Tsuzuki, 2008).

Anthropogenic activities result in the release of organic compounds into wastewaters that can have toxic, carcinogenic, mutagenic or/and endocrine disrupting properties. Of these organic pollutants, plasticizers, that are a particular source of concern because of the extent of their use in commercial applications and the growing recognition of the potential threats that they pose to the health of humans and ecosystems (Barnabe et al., 2008). Unfortunately, because they are not chemically bound to the polymers, plasticizers can migrate from plastic products during normal use and following their disposal (Fromme et al., 2002). Thus, as many studies have shown, they have become widely distributed in the environment and are frequently found in the influents, effluents, sludge of wastewater treatment plants, and in surface waters that receive treated effluents (Bago et al*.,* 2005; Fromme et al*.,* 2002; Fauser et al*.,* 2003; Gavala et al*.,* 2003; Marttinen et al*.,* 2003b; Petrovic et al*.,* 2001).

Toxicity is a valuable indicator of water quality, and is used to assess the effects of organic chemicals. Prediction of the toxic effects of chemicals on organisms is the primary aim of ecotoxicology, one of the effective procedures of which is the bioassay. In this regard, the medaka fish (*Oryzias latipes*) serves as an excellent fish model for determining acute and chronic toxicities, including the endocrine disrupting activity of chemicals (Wei et al., 2006).

The traditional approach to environmental risk assessment couples monitoring of pollutant levels with the toxicity testing of individual chemicals. Application of such toxicological studies to realistic environmental risk assessment is, however, limited as it does not address the fact that these compounds do not exist in the environment in isolation, but are instead present in complex mixtures. Difficulties associated with identifying the risks posed by mixtures of pollutants might be addressed by pairing the enrichment of pollutants via active sampling using Sep-Pak® Plus PS-2 cartridges with the assessment of extracts via bioassays (Liu et al., 2006).

In this study, we investigated the toxicity level in water streams that run through Dili, the capital city of Timor-Leste. The organic toxicants were concentrated from the 10-L grab water samples using the disposable Sep-Pak® Plus PS-2 (Waters, USA) adsorption cartridges. Later, the extracts were used for toxicity evaluation via larval medaka fish acute toxicity assay, and then identifying the possible sources of organic pollutants using GC/MS simultaneous analysis. The toxicity test results of Timor-Leste water streams were compared with those of the Japa-nese water streams that were investigated in 2013, to show the difference in surface water toxicity and chemicals between the developed and developing countries.

3.2 Materials and Methods

3.2.1 Study Area

- ***Timor Leste***

Timor-Leste was selected as the sampling region because it is a developing country with inadequate pollution control facilities. Fifty-seven percent of its total population does not have ac-cess to an improved sanitation system. Moreover, there is a lack of solid waste management, and water sources are not well protected. Consequently, the surface water is often polluted (Ministry of Finance 2009-10).

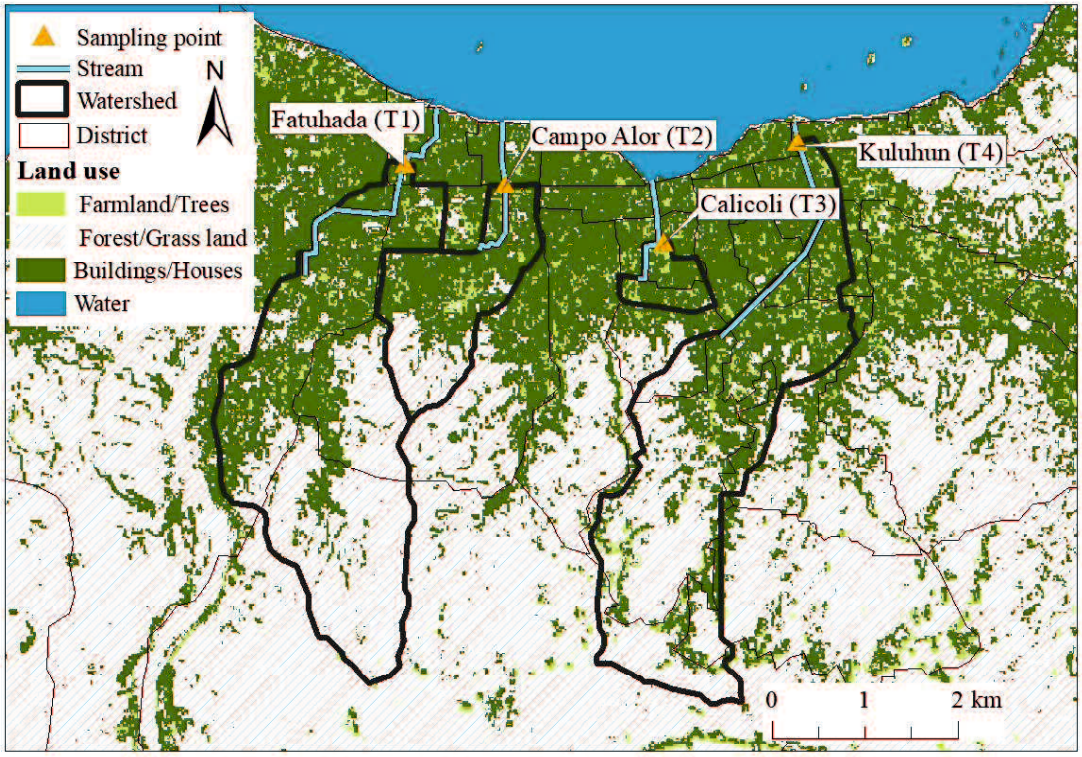


Fig. 3.1 Investigated water streams in Dili, the capital city of Timor-Leste

We conducted a preliminary survey in Dili City, the capital of Timor-Leste, and on the basis of this survey, subsequently selected four fresh water streams namely, Fatuhada, Campo Alor, Caicoli and Kuluhan (hereafter; T1, T2, T3 and T4, respectively). T1 has a relatively better water quality than other streams. T2 is close to T1, but its water quality is bad. T3 is running in the center of Dili city and it has the worst water quality. T4 has not so bad water quality and it is almost dry in some parts during dry season, different from T1, T2, T3 which keep water along the year (dry and wet seasons). Those streams are running through residential areas and mainly exposed to the discharged waste water and solid wastes from the surrounding houses.(**Fig. 3. 1**)

- ***Japan***

Japan is one of developed countries. It produces and consumes a huge number of chemicals in its industries and from human daily activities; therefore, it was expected that the toxicity level in the water streams of Japan would be higher than that in water streams of Timor-Leste as a developing country. The toxicity was investigated in three fresh water streams J1, J2, and J3, which are the tributaries of the Myojin River and

run through residential areas in the Ube City, Japan as shown in **Fig. 3.2**. These streams are mainly exposed to the dis-charged wastewater from the surrounding houses and commercial activities, almost the same conditions as Timor-Leste streams.

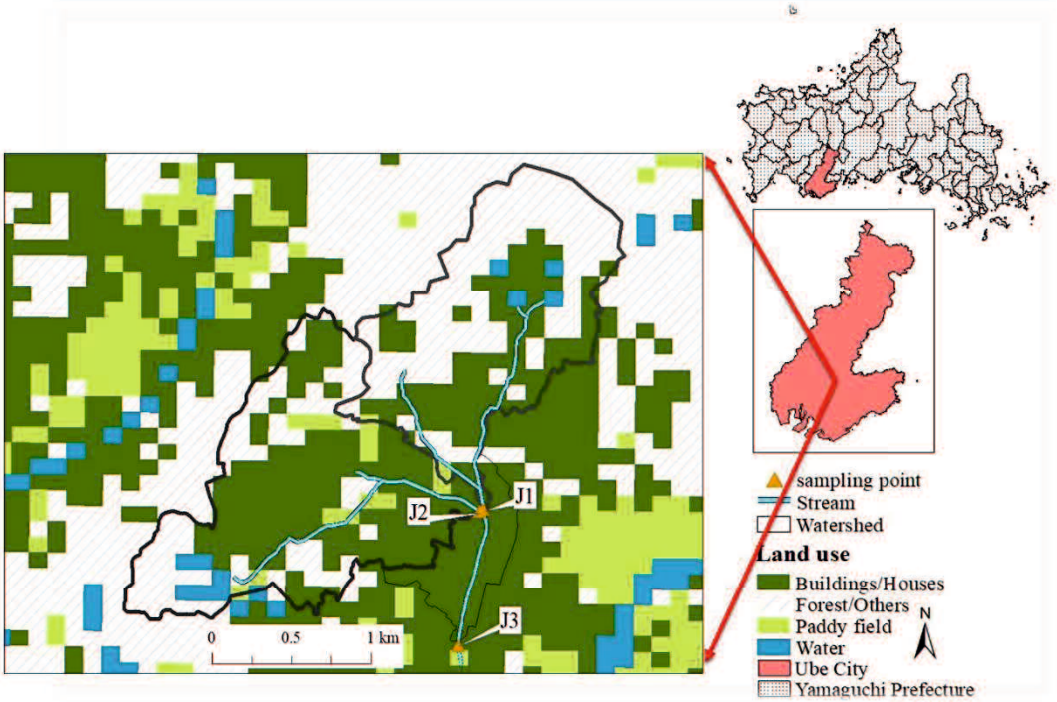


Fig. 3.2 Investigated tributaries of Myojin river (J1, J2, J3) in Ube city, Japan.

3.2.2 Sanitation facilities in both Timor-Leste and Japan

Table 3.1 Human waste disposal facilities/methods of each basin in both Dili and Ube cities.

Stream	Sewer treatment (people)	Gappei-syori johkasou (people)	Flush toilet/ septic tank (people)	Vault toilet/Pit latrine (people)	Shore/ open field (people)	No facility (people)
T1			9696	3451	1857	75
T2			3755	1493	582	107
T3			1136	565	72	57
T4			4486	1283	559	33
J1	1	1308	629	1436		
J2	154	3080	245	1084		
J3	1026	4582	986	2844		

Table 3.1 shows the human waste disposal facilities in the basins of investigated streams in both Dili and Ube cities. In Dili city, the conventional septic

tanks and pit latrines are used as onsite sanitation systems in the residential areas. Pit latrines (night soil tanks) in Timor-Leste, it is a hole in the ground to collect human waste, closed after it is filled then people moved to a new one. Septic tanks receive the living wastewater such as kitchen and bathroom wastewater. Most of septic tanks are in very poor condition due to insufficient maintenance. There is no sewerage system in Dili. In most urban centers, the domestic wastewater from households and other public uses is generally discharged to onsite septic tanks or open drains. In rural communities, open defecation is common (WHO, 2001). The data were obtained from the census report "Timor-Leste Dis-tribuisaun Populasaun tuir Area Administrativu Volume 2," published in 2015.

Whereas in Ube city, the domestic wastewaters are mainly treated through wastewater facilities, rural community sewerage systems, vault toilets / pit latrines (night soil tanks) and onsite wastewater treatment tanks (Johkasou in Japanese). In the past, Johkasou could treat flush toi-let wastewater before discharging it into water streams, whereas the night soil and Johkasou sludge are collected and transported to a treatment plant to be treated and recycled. The other living wastewater, such as kitchen and bathroom wastewater, was directly discharged without any treatment into water streams, leading to water pollution. This system is called Tandoku-syori johkasou (i.e., separate treatment). The new wastewater regulation, which stated that every kind of living wastewater must be treated before discharging into water streams, was set up in 2006. Subsequently, a flush toilet wastewater treatment tank was used. This new system is called Gappi-shori Johkasou (i.e., combined treatment). It can treat all kinds of living wastewater (Ministry of Environment, 2012).These data were collected from the local government of Ube city, Japan in 2013.

3.2.3 Sampling

Grab water samples (10-L) were collected from the investigated Japanese streams J1, J2 and J3, during summer season in June 2013. For Timor –Leste streams (T1, T2, T3 and T4), 10-L grab water samples were collected during the dry season in September 2015. Those samples were filtered with a 1-µm glass filter. Water quality parameters; temperature, DO, pH and EC, were measured using U-10 and U-52 multi parameter water quality meter (Horiba). BOD measurements were carried out using Japanese Industrial Standard (JIS) K0102 method for water samples that collected from

ube city streams. While manometric BOD measuring devices named OxiTop®IS6 were used for water samples that collected from Dili city streams.

3.2.4 Concentration and elution of adsorbed chemical compounds

Chemical compounds were concentrated from the collected water samples using the preconditioned Sep-Pak® Plus PS-2 cartridges (four cartridges were used for each 10-L grab water sample). These chemical compounds were subsequently eluted using acetone. **(Fig. 3.3)**.

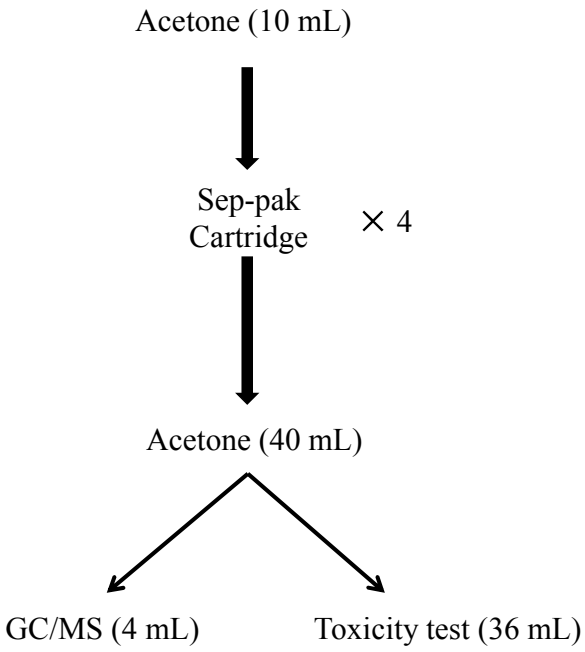


Fig. 3.3 Elution procedure of adsorbed chemicals from Sep-Pak® Plus PS-2 cartridges**.**

3.2.5 Medaka (Oryzias latipes var.) acute toxicity test

In the present study, we employed the same procedures as (Yamashita et al*.,* 2012) to determine the toxicity level in Timor-Leste water streams **(Fig. 2.5, chapter 2)**.

3.2.6 GC/MS simultaneous analysis database

In order to identify chemical compounds in the collected samples, we used the GC/MS simultaneous analysis database, which can identify and quantify a total of 942 chemical compounds without the need for reference standards (Kadokami et al*.,* 2005). (**Chapter 2, 2.14**)

3.3 Results and Discussion

3.3.1 Water quality measurements

Table 3.2 Water quality parameters of Timor-Leste and Japanese streams and characteristics of their basins

Sample	Date	Temperature (℃)	DO (mg/L)	pH (-)	EC (mS/cm)	BOD (mg/L)	Basin area (km^2)	Distance (sqrt(area)) (km)	Population (people)	Discharge rate (m^3/sec)	Toxicity load (m^3/sec)
T1	15th Sep. 2015	27.5	5.3	7.0	0.54	2.50	7.45	2.73	24756	0.026	0.0003
T2		27.7	3.9	7.5	0.82	24.50	2.24	1.50	14241	0.069	0.0048
T3		29.3	2.6	7.6	1.26	78.50	0.50	0.70	3854	0.008	0.0016
T4		30.2	6.8	7.8	0.57	24.50	6.55	2.56	20492	0.007	0.0002
J1	11th June 2013	19.5	6.6	7.5	0.36	10.40	1.75	1.32	3374	0.140	0.0098
J2		21.1	7.8	7.4	0.43	11.95	1.99	1.41	4915	0.030	0.0027
J3		21.0	6.9	7.5	0.40	10.32	4.08	2.02	9439	0.120	0.0012

Table 3.2 shows the water temperature in Timor-Leste streams was high as it is a hot country. In addition, the biochemical oxygen demand (BOD) concentration was high, especially in T3 (78.50 mg/L) followed by T2 and T4 with the same concentration (24.50 mg/L). This refers to high organic contamination, which might be related to the household waste water directly discharged without any treatment into those streams. On the other hand, the BOD concentration was relatively lower in the Japanese streams (J1, J2, and J3), where all waste water was treated via Tandoku-syori johkasou before discharging into water streams.

3.3.2 Acute toxicity test results

- ***Dili City Streams***

Table 3.3 shows the toxicity test results of the streams of Dili. The chemicals eluted from the concentrated water samples collected from T3, T2, and T4 showed strong toxicity ranging from 20- to 100-fold, whereas no toxicity was detected in the sample collected from T1. The LDR_{50} values were >0.10, 0.070, 0.030, and <0.010 for the streams T3, T2, T4, and T1, respectively.

Table 3.3 Toxicity test results for Dili streams during the dry season, September 2015

	Dili city streams							
	T1		T2		T3		T4	
folds	LT_{50}^{-1} (h^{-1})	LDR_{50}	LT_{50}^{-1} (h^{-1})	LDR_{50}	LT_{50}^{-1} (h^{-1})	LDR_{50}	LT_{50}^{-1} (h^{-1})	LDR_{50}
100 f	<0.020	<0.01	>2.0	0.07	>2.0	>0.10	>2.0	0.03
50 f	<0.020		>2.0		>2.0		>2.0	
20 f	<0.020		0.24		>2.0		<0.020	
10 f	<0.020		<0.02		0.14		<0.020	
Adsorbed amount (μg)	5.92		18.09		42.06		16.04	

- ***Ube City Streams***

Table 3.4 shows the toxicity test results of the Ube City streams. J2 showed the highest toxicity level followed by J1, whereas no toxicity was detected in J3. The LDR_{50} values were 0.09, 0.07, and <0.01 for the streams J2, J1, and J3, respectively.

Table 3.4 Toxicity test results for Ube city streams during summer season, June 2013

	Ube city streams					
	J1		J2		J3	
folds	LT_{50}^{-1} (h^{-1})	LDR_{50}	LT_{50}^{-1} (h^{-1})	LDR_{50}	LT_{50}^{-1} (h^{-1})	LDR_{50}
100 f	>2.0	0.07	>2.0	0.09	<0.020	<0.01
50 f	0.29		>2.0		<0.020	
20 f	0.06		0.38		<0.020	
10 f	<0.020		<0.020		<0.020	
Adsorbed amount (μg)	38.74		70.16		38.65	

Although we cannot prove the statistical significant difference in toxicity among the investigated river water samples because we have just one sample for each stream with four concentrations and two replicates for each concentration, the toxicity test results showed that the toxicity of collected water samples from Timor-Leste water streams were higher or comparable to those of Japanese water streams. (**Fig. 3.4**)

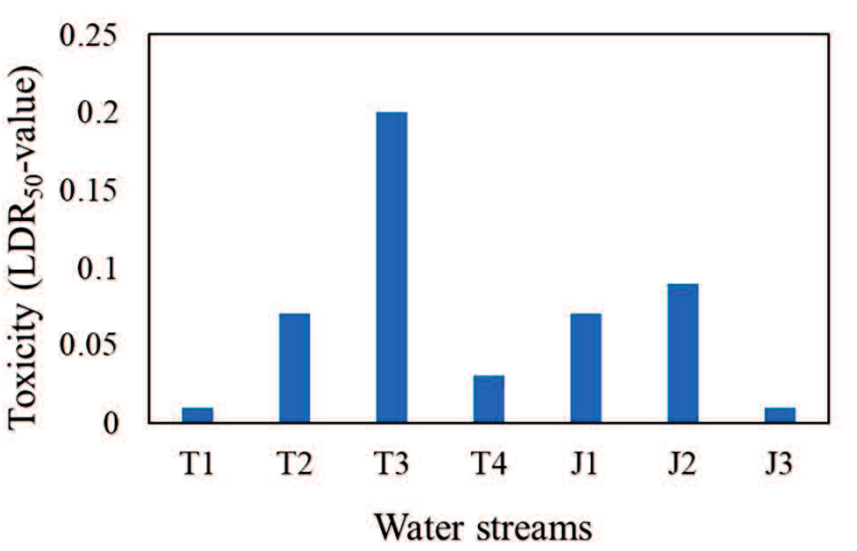


Fig. 3.4 Toxicity test resuls for both Dili and Ube streams.

3.3.3 Relationship between toxicity and population

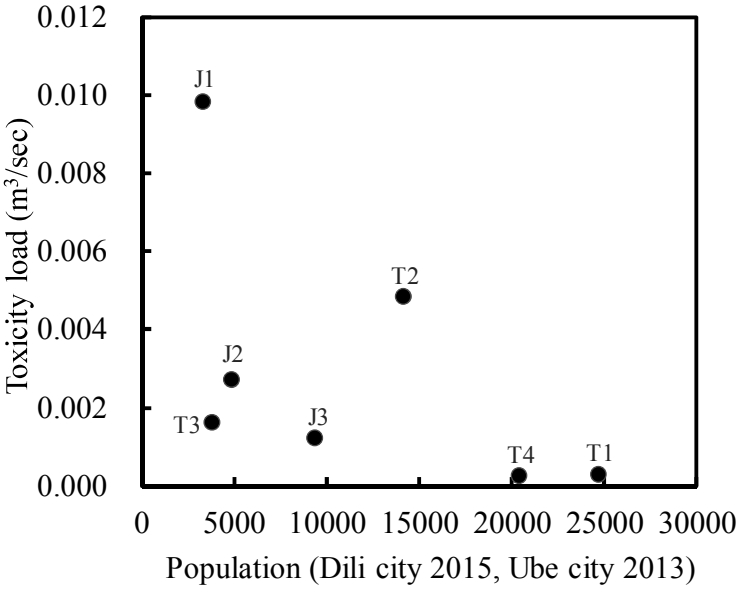


Fig.3.5 Relationship between toxicity load and population for both Ube and Dili streams.

If we assume that each person discharges the same amount of chemicals, the toxicity load and population should be proportional. Here, toxicity load is defined as the estimated toxicity (LDR_{50}) value multiplied by the discharge rate (m^3/ sec) since LDR_{50} can be handled as concentration. Nevertheless, it was noticed that the basins

with higher population showed lower toxicity load for both Dili and Ube streams, as shown in **Fig. 3.5** for both Dili and Ube streams. The distance from the pollution source might affect the decomposition of toxic chemicals.

3.3.4 Relationship between toxicity and distance from the source of pollutants

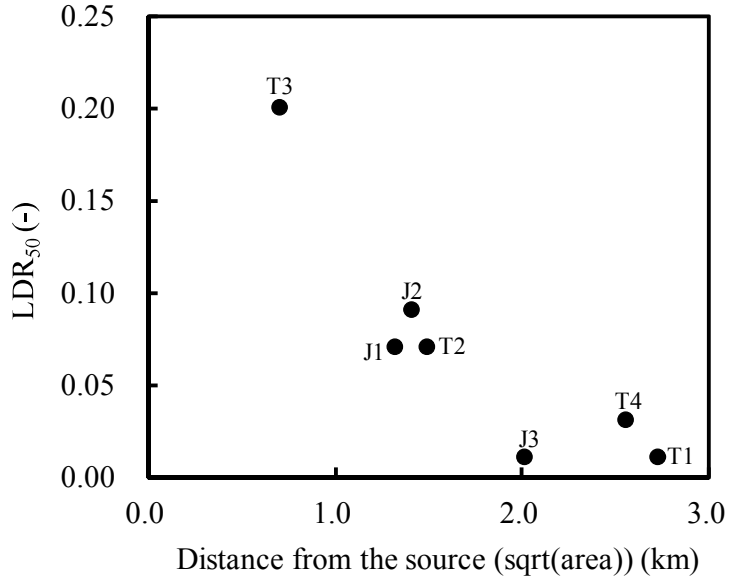


Fig.3.6 Relationship between toxicity and distance from pollution source.

Although it is difficult to determine pollutants flow-out distance in non-point source pollution analysis like in residential area, square-root of basin area have been used as a substitute of the distance (Sekine et al., 1991). **Figure 3.6** shows the relationship between the flow-out distance and LDR_{50}. In the figure, a longer distance shows a lower toxicity. The distance might affect the decomposition of toxic chemicals.

3.3.5 GC/MS Analysis Results of collected water samples from both Dili and Ube streams

According to the GC/MS analysis results, the detected chemicals were categorized as: OC; oxygen containing compounds (ethers, ketones, phenols, phthalates, fatty acid ester and others), PPCPs; pharmaceutical and personal care products; pesticides; and HC, hydrocarbons (fuel oils)

Table 3.5 GC/MS analysis combined with toxicity test results for Japanese and Timor Leste streams (only compounds with concentration ⩾ 0.01 µg/L are shown).

Chemical	Category	Medaka-LC_{50} (mg/L)		J1	J2	J3	T1	T2	T3	T4
				µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Phenylethyl alcohol	PPCPs: Pharmaceuticals & Personal care products			0.074						
Isosafrole				0.059						
Ethenzamide					5.49					
Crotamiton					3.24					
alpha-Terpineol		96hr-test	13.7	0.155			0.024	0.524	1.685	0.451
Caffeine		96hr-test	87.0	2.02	6.85		0.011	1.687	0.577	0.361
Aspirin				2.48				0.387	0.723	0.294
Diethyltoluamide		96hr-test	100	0.024		0.044	0.284	2.573	2.329	2.83
Cholesterol								0.348	1.920	1.150
N-(2-Phenylethyl)acetamide								0.373	1.434	0.069
L-Menthol		96hr-test	18.9				0.134	1.084	8.310	0.678
Ibuprofen								0.272	2.425	0.684
Dicyclopentadiene	OC: Oxygen containing compounds				0.120					
Anthraquinone					0.300					
Dimethyl phthalate				0.089		0.120				
1-Acetoxy-2-methoxyethane					0.060					
2-Methyl-2,4-pentandiol		96hr-test	100			0.030				
Diethyl phthalate			0.86	0.10			0.016	0.367	0.589	0.306
Diisobutyl phthalate			3	0.18	0.454	0.143	0.437	2.37	2.00	2.206
Di-n-butyl phthalate			2.8	0.72	1.31	0.620	0.178	0.813	1.04	0.519
Bis(2-ethylhexyl)phthalate			0.212	3.63		0.390	0.561	0.638	1.03	0.890
2-Phenoxyethanol				9.45	20.8	0.035		0.117	0.491	0.054
2-Ethyl-1-hexanol				0.035			0.084	0.279	3.84	0.07
4-Chloro-2-nitroaniline										0.247
Dicyclohexyl phthalate		96hr-test	2.00				0.328			0.532
Di(2-ethylhexyl)adipate		96hr-test	50.0				0.022	0.420	2.62	
Coprostanol								0.060	0.370	0.330
Methamidophos	Pesticides			17.2	30.2					
Terbufos					0.045					
Terbacil		48hr-test	40.0		1.00					
Dimethametryn		24hr-test	3.20			2.62				
Cypermethrin 1						1.61				
Bromobutide		48hr-test	10.0			9.84				
Tribenuron-methyl						2.42				
Norflurazon						2.09				
Bensulide				0.488		0.494		0.333	0.573	
Butachlor		96hr-test	0.280			0.142				
Pyriminobac-methyl Z						0.584				
Hymexazol				0.351		0.744				
Pyraclostrobin						1.59				
TCMTB						0.100				
Tricyclazole		48hr-test	9.50			2.13		0.333	0.573	
Triadimenol 2								0.201	0.133	
3-Hydroxycarbofuran										0.604
Pyrethrin 3								0.082		0.283
n-C_9H_{20}	HC: Hydrocarbons (Fuel oils)			0.281			0.012	0.012	0.004	
n-$C_{10}H_{22}$				0.045					0.007	
n-$C_{14}H_{30}$				0.035		0.016	0.011	0.057	0.102	0.007
n-$C_{16}H_{34}$				0.121		0.051	0.317	0.460	0.515	0.480
n-$C_{18}H_{38}$				0.175	0.176	0.016	0.774	0.696	0.803	0.195
n-$C_{20}H_{42}$						0.036	0.897	0.872	0.901	0.435
n-$C_{22}H_{46}$						0.054	0.762	0.739	0.769	0.499
n-$C_{24}H_{50}$				0.198			0.408	0.402	0.415	0.330
n-$C_{27}H_{56}$				0.067			0.116	0.087	0.095	0.079
n-$C_{28}H_{58}$				0.108			0.128			
n-$C_{26}H_{54}$							0.254	0.241	0.256	0.222
n-$C_{32}H_{66}$								0.024	0.028	
Bioassay toxicity values (LDR_{50})				0.07	0.09	<0.01	<0.01	0.07	>0.10	0.03
Normalized toxicity values				0.14	0.72	4.83	0.41	2.02	2.98	1.65

* : National Institute of Technology and Evaluation (NITE), acute toxicity 96h-LC_{50} with adult medaka, except Terbacil and Bromobutide were 48h a cute toxicity.

Blank sample that have been detected by Yamashita et al., 2012

** Plasticizers included among Endocrine disruptors.

As shown in **Table 3.5** most chemicals in the OC group were industrial raw materials, including solvents and plasticizers, except Coprostanol, which is an index of fecal pollution. Coprostanol was detected only in Timor-Leste. A high-boiling solvent, 2-Phenoxyethanol, was detected at a noticeably high concentration in Japan. Bis (2-ethylhexyl) phthalate, Diethyl phthalate, Di-isobutyl phthalate and Di-n-butyl phthalate are plasticizers and were detected in a relatively high concentration, comparing to other detected pollutants, specially in Timor-Leste water streams, that is may be related to the disposal of solid wastes including plastics, into water streams in Dili city. Those compounds have a toxic effect on medaka fish with LC_{50} values (0.212, 0.86, 3.04, 2.75 mg/L) respectively and also included among endocrine disrupting chemicals, then it may cause serious problems not only for aquatic organisms but also for the human health. Bis(2-ethylhexyl)phthalate, di-n-butyl phthalate, diisobutyl phthalate, and diethyl phthalate were detected in the streams of both Dili and Ube. The plasticizers di(2-ethylhexyl)adipate, 2-ethyl-1-hexanol, and dicyclohexyl phthalate and their raw materials were detected only in the streams of Dili.

Among PPCPs, caffeine, ethenzamide, aspirin (pain relief), and crotamiton (antipruritic) were detected in the streams of Ube. Caffeine, ibu-profen (pain relief), L-menthol (tooth wash, etc.), alpha-terpineol (aromatic oil), and diethyltol-uamide (insect repellent) were detected in the Dili streams. These chemicals seem to reflect the difference in the usage of PPCPs in both countries.

In the pesticides group, methamidophos was detected in the Ube streams J1 and J2, although it is prohibited in Japan. Besides, rather diverse chemicals, such as bromobutide, dimethametryn, tribenuron-methyl, norflurazon (herbicide), tri-cyclazole, pyraclostrobin (disinfectant), cyper-methrin 1 (insecticide), etc., were detected. In Timor-Leste, bensulide (herbicide), tricyclazole, triadimenol 2 (disinfectant), pyrethrin 3, 3-hydroxycarbofuran (insecticide), etc., were detected in the Dili streams. Compared to the Japanese streams, the kinds and concentrations of pesticides were smaller in Timor-Leste.

The number of hydrocarbon compounds detected in the Timor-Leste streams was greater than those detected in the Japanese streams. They are mainly the components of fuel oils and lubricants. This might be related with the fact that the people in Timor-Leste wash their automobiles in the streams.

3.3.6 Relationship between Normalized toxicity and bioassay toxicity

As a trial to explain about the toxic effect of detected chemicals in each stream, we calculated the normalized toxicity of chemicals which have known median lethal concentration (LC_{50}), by dividing the detected concentration of each chemical by its median lethal concentration and then multiplying with 1000 to magnify the obtained values. (**Table 3.6**) Stream T3 had high normalized toxicity value and it was in agreement with its corresponding LDR_{50} value. Whereas, no agreement was observed in case of stream J3, which had the highest normalized toxicity value, but its LDR_{50} was very low. As GC/MS analysis cannot detect all the compounds present in river water, the normalized toxicity was not enough to explain about the whole toxicity in each stream. In addition, many of detected chemicals still with unknown LC_{50}. Then bioassay toxicity would be helpful to detect the whole toxicity in the river water, a complex mixture with many unknown contaminants.

3.4 Conclusion

Chemicals eluted from Sep-Pak cartridges were used in toxicity tests and subjected to GC/MS analysis, showed the following results:

- The toxicity levels of water streams in Timor-Leste from residential areas were comparable or higher than in Japan.
- The basins of investigated streams with higher population showed lower toxicity, both in Timor-Leste and Japan.
- As the flow-out distance increases, the toxicity load decreases. The distance seems to affect the decomposition of toxic chemicals.
- According to the GC/MS analysis, the detected chemicals showed a difference in the usage of these chemicals in both countries.
- Plasticizers were detected in a relatively high concentration, comparing to other detected pollutants, specially in Timor-Leste water streams, that might be related to the disposal of solid wastes including plastics, into water streams in Dili city.
- No clear relationship was observed between the normalized toxicity values of the chemicals detected in each stream and their corresponding bioassay toxicity.
- According to our toxicity and GC/MS analysis results, we could not find a clear relationship between the detected chemicals and bioassay toxicity, but we suspect that some chemicals are decomposed into more toxic compounds, even if those

chemicals did not have any toxicity (further investigation will be needed). Then the measures that we can suggest will be common as follow:

- Environmental awareness programs for public towards the negative impacts of environmental pollution as a result of human activities and bad practices.
- Coverage of wastewater treatment facilities in both urban and rural area Using an improved sanitation facilities such as onsite treatment tanks in addition to establishing waste water treatment plants.
- Enforcement of water and environmental laws must be in place to protect the environment and the health of numerous people that still depend on surface water as their major source of water supply.

Although there is limitation in accuracy which comes from the sample number and/or analysis methods, current situation of toxicity and chemical pollution of residential area in Timor-Leste compared to Japan is illustrated. A simultaneous GC/MS analysis might help the identification of the possible sources of toxicity, but it is limited by the number of chemicals that can be identified. It is difficult to detect all the compounds present in river water, which is a complex mixture with many unknown contaminants; therefore, more bioassay tests are required.

3.5 References

1. Bago B, Martin Y, Mejia G, Broto-Puig F, Diaz-Ferrero J, Agut M, Comellas L. (2005), Di-(2-ethylhexyl) phthalate in sewage sludge and post-treated sludge: quantitative determination by HRGC-MS and mass spectral characterization. *Chemosphere,* 59:1191-1195.
2. Barnabe S, Beauchesne I, Cooper G and Nicell J. (2008) Plasticizers and their degradation products in the process streams of a large urban physicochemical sewage treatment plant. *Water Research*, 42:153-162.
3. Fauser P, Vikelsoe J, Sorensen B, Carlsen L. (2003) Phthlates, nonylphenols and LAS in an alternately operated wastewater treatment plant—fate modelling based on measured concentrations in wastewater and sludge. *Water Res*., 37:1288-1295.
4. Fromme H, Kuchler T, Otto T, Pilz K, Muller J, Wenzel A. (2002) Occurrence of phthalates and bisphenol A and F in the environment. *Water Res*., 36:1419-1438.

5. Gavala N, Alastriste-Mondragon F, Iranpour R, Ahring K. (2003) Biodegradation of phthalate esters during the mesophilic anaerobic digestion of sludge. *Chemosphere*, 52:673-682.
6. Hunter R, Zmirou-Navier D, Hartemann P. (2009) Estimating the impact on health of poor reliability of drinking water intervantions in developing countries. *Science of the total environment*, 407:2621-2642.
7. Kadokami K, Tanada K, Taneda K. (2005) Novel gas chromatography mass spectrometry database for automatic identification and quantification of micropollutants. *J Chromatogr*., 1089:219-226.
8. Marttinen K, Kettunen H, Rintala A. (2003b) Occurrence and removal of organic pollutants in sewages and landfill leachates. *Sci. Tot. Environ*., 301: 1-12.
9. Ministry of Finance, Democratic Republic of Timor-Leste, Dili, Timor-Leste. Demographic and Health Survey, Timor-Leste 2009-10.
10. NITE-化学物質管理分野 CHRIP(化学物質総合情報提供システム HP, www.safe.nite.go.jp/japan/db.html.
11. Petrovic M, Barcelo D. (2001) Analysis of ethoxylated nonionic surfactants and their metabolites by liquid chromatography/ atmospheric pressure ionization mass spectrometry. *J. Mass Spectrom*., 36:1173–1185.
12. Sekine, M., Ukita, M., and Nakanishi, H. (1991) systematic pollutegraph simulation for real scale river basin , *J. Wat. Sci. Tech.,* Vol. 23, Kyoto, pp. 141-150, 1991.
13. Timor-Leste Distribuisaun Populasaun tuir Area Asministrativu Volume2，2010
14. Tsuzuki Y(2008) Relationship between water pollutant discharges per capita and indicators of economic level, water supply and sanitation in developing countries. *Ecological economics*, 68:273-287.
15. Water Environment Management in Japan, August 2012 WEPA Dialogue in Sri Lanka, Tadashi Kitamura Director, Water Environment Division Environment Management Bureau, the Ministry of Environment, Japan.
16. Wei B, Kisuno A, Kameya T, Urano K. (2006) A new method for evaluating biological safety of environmental water with algae, daphnia and fish toxicity ranks. *Science of the Total Environment,* 371:383-390.
17. World Health Organization Regional Office for South-East Asia, Environmental Sanitation in East Timor, *Assignment Report*: 16 October-20 December, 2001.

18. Yamashita H, Haribowo R, Sekine M, Oda N, Kanno A, Shimono Y, Shitao W,Higuchi T, Imai T,Yamamoto K. (2012) Toxicity test using medaka (*Oryzias latipes*) early fry and concentrated sample water as an index of aquatic habitat condition. *Environ. Sci. Pollut. Res*., 19:2581-2594.

CHAPTER 04
A STUDY ON THE APPLICABILITY OF PASSIVE SAMPLING TECHNIQUE FOR ACUTE TOXICITY ASSAY USING LARVAL MEDAKA (*ORYZIAS LATIPES VAR.*)

4.1 Introduction

In recent years, the presence of trace chemicals, such as pesticides, pharmaceuticals, and personal care products, in the aquatic environment has emerged as one of the most urgent environmental concerns. Thus, there is a continuing need for new technologies and techniques to provide reliable data for assessing the potential threats associated with low levels of complex mixtures of environmental contaminants (Al-Odaini et al., 2010).

Most water monitoring programs are based on the collection of grab, spot, or bottle samples of water at a given time. Where pollutants are present in only trace levels, it is necessary to collect large volumes of water. Subsequent laboratory analysis of such samples provides only a snapshot of the levels of pollutants at the time of sampling. This approach accordingly has drawbacks for sampling environments where the concentration of pollutants varies over time, and thus episodic pollution events can be missed. One solution to this problem is to increase the sampling frequency or to install automatic sampling systems that can collect numerous water samples over a given time period. This, however, is costly and in many cases impractical, since a secure site and significant pre-treatment of water are required. Such systems are therefore rarely used in widespread monitoring programs. Spot sampling yields different apparent concentrations of pollutants depending on the pre-treatment applied (e.g., filtering) and does not provide information on the actual dissolved bioavailable fraction of the contaminants to which recipients are exposed. Passive sampling can overcome these difficulties by combining sampling, analyte isolation, and pre-concentration into a single step, and also enabling time-weighted average sampling of compounds during the deployment period (Vrana et al*.,* 2005).

To determine the concentrations of pollutants using passive samplers after field exposure requires the use of so-called substance-specific sampling rates (i.e., a volume

of water sampled per unit time), which allow users to compute time-weighted average concentrations from the compound mass in the receiving phase (Gunold et al., 2008). However, our interest in the present study was to assess the toxicity corresponding to the amounts of organic contaminants adsorbed by passive sampler disks as a simple indicator of chemical pollution, and not to identify the concentrations of individual contaminants. Accordingly, we did not need to identify the sampling rate for each individual organic compound.

Toxicity is a valuable indicator of water quality, and is used to assess the effects of organic chemicals. The traditional approach to environmental risk assessment couples monitoring of pollutant levels with the toxicity testing of individual chemicals. Application of such toxicological data to realistic environmental risk assessment is, however, limited, as it does not address the fact that these compounds do not exist in the environment in isolation, but are instead present in complex mixtures. Difficulties associated with identifying the risks posed by mixtures of chemicals might be addressed by pairing the enrichment of chemicals with the assessment of extracts via bioassays (Shaw et al., 2009).

In this research, we investigated the most suitable passive smpler disks among Empore^{TM} styrene-divinylbenzene (SDB disks) and its applicability to evaluate the toxicity level in water streams via bioassays using the larvae of medaka fish (*Oryzias latipes var.*).

4.2 Materials and Methods

4.2.1 Selection of The Most Suitable Passive Sampler Disks

a. Field and Laboratory Experiments

There are three chemcatcher passive sampling disks; SDB-RPS, SDB-XC and SDB-XD, which mainly made of styrene-divinyl benzene copolymer which relatively close to the sorbent material of Sep-pak® Plus PS-2 cartridges that used in previous study (yamashita et al*.,* 2012). We conducted a field and laboratory experiments to select the most suitable passive sampler disks among styrene-divinyl benzene (SDB) disks, which achieve the highest adsorption efficiency comparing to active sampling using Sep-pak® Plus PS-2 cartridges, and then decide the necessary number of disks and the length of deployment time required for sampling .

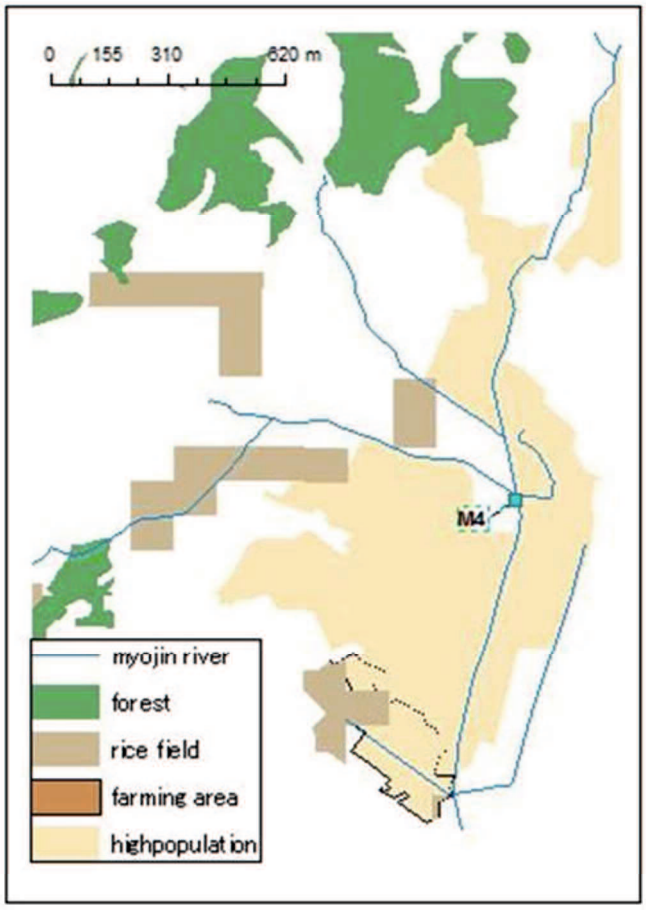


Fig. 4.1 Sampling point M4 from Myojin river

For the field experiment, **Fig 4.1** shows the sampling point M4 from Myojin river in Japan which is exposed mainly to discharged waste water from residential area. Three disks of each SDB-XC, SDB-XD and SDB-RPS disks were washed and conditioned as recommended by the manufacturer (3M company, USA); using10 mL acetone followed by 10 mL methanol twice respectively via suction filtration then the disks were fitted into the chemcatcher bodies till be deployed in the river between January 8 and 11, 2016, at which time the river had an average flow velocity of 0.17 m/s, average discharge of 0.11 m^3/s, average pH of 7.5, average conductivity of 0.3 mS/cm, average dissolved oxygen of 11.1 mg/L, and average water temperature of 11.6°C. During the deployment period, ten liters composite water sample was collected (10-L grab water sample each day) and then concentrated using four pre-conditioned Sep-pak® Plus PS-2 cartridges. Adsorbed chemicals were eluted from both passive sampler disks and sep-pak cartridges using 10 mL acetone twice/each passive sampler

disk and 10 mL acetone/each Sep-Pak cartridge. Eluted chemicals were prepared for both GC/MS analysis and toxicity test using larval medaka (*Oryzias latipes var.*).

For the laboratory experiment, Triclosan is a toxic chemical and had been detected in river water in previous studies (yamashita et al., 2012). So we prepared 5 L batches at concentrations of 0.2 µg/L (close to the concentration in river), 5 µg/L and 10 µg/L. Then SDB-RPS disk was deployed in each glass container of 5 L Triclosan solution. Deployment periods were 1, 2, 3 and 7 days. (**Fig. 4.2**)

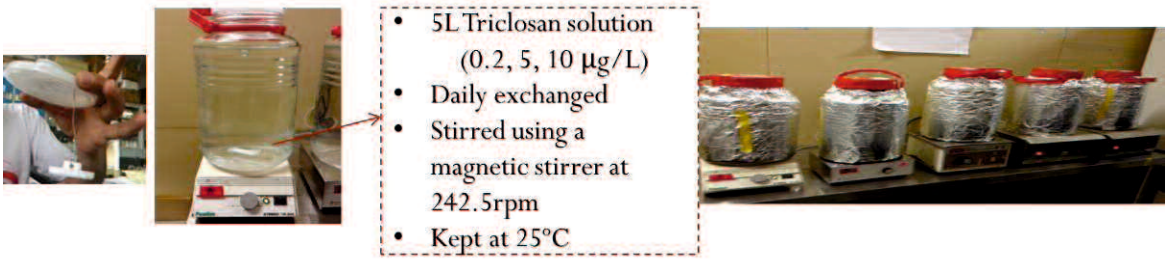


Fig. 4.2 Laboratory experiment

Triclosan solution was kept at constant temperature 25°C, stirred using a magnetic stirrer at 242.5 rpm and changed every 24 hours to maintain a constant concentration. As active sampling 5-L Triclosan 5 µg/L solution was stirred for 24 hours at constant temperature 25°C, then concentrated through two Sep-Pak cartridges. Adsorbed Triclosan had been eluted from both SDB-RPS disks and Sep-Pak cartridges and prepared for GC/MS analysis.

b. Medaka (Oryzias latipes var.) Acute Toxicity Test

In the present study, we employed the same procedures as (Yamashita et al., 2012) to determine the toxicity level **(Fig. 2.5, chapter 2)**.

c. Gas Chromatography / Mass Spectrometer (GC/MS) Simultaneous Analysis Data Base

In order to identify chemical compounds in the collected samples, we used the GC/MS simultaneous analysis database, which can identify and quantify a total of 942 chemical compounds without the need for reference standards (Kadokami et al., 2005). (**Chapter 2, 2.14**)

4.2.2 Investigation of Long Deployment of SDB-RPS Passive Sampler Disks

a. Study Area

Timor-Leste was selected as the sampling region since it is a developing country that has inadequate pollution control facilities. Fifty-seven percent of its total population does not have access to an improved sanitation system. Moreover, there is a lack of solid waste management, and water sources are not well protected. Consequently, surface water is often polluted (Ministry of Finance, 2009-10). A preliminary survey was conducted in Dili City, the capital of Timor-Leste, and on the basis of this survey, subsequently selected the Campo Alor River as our study site, as this is the city's most polluted river. The Campo Alor is a fresh water stream, with a basin area of 1.6 km2, width of 2 m, and water depth of 0.3–0.5 m, that runs through a residential area and is mainly exposed to the discharged waste water from the surrounding houses. Although some streams in Dili have no flow during the dry season, the Campo Alor River has a relatively high flow rate of between 0.07 and 0.13 m^3/s during the dry and rainy seasons, respectively. **(Fig. 4.3)**

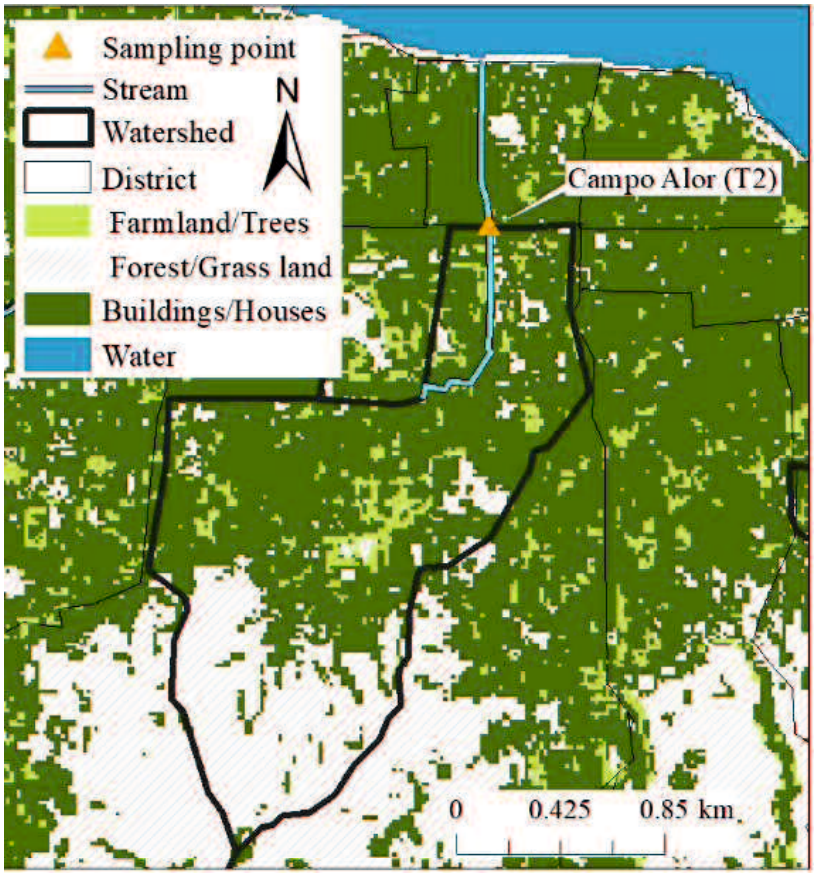


Fig. 4.3 Sampling site: Campo Alor river (March, 2016)

b. Sampling

For active sampling, we used Sep-Pak® Plus PS-2 cartridges (Waters, Milford, USA), which contain the same sorbent material (styrene-divinylbenzene copolymer) as used in in SDB-RPS passive sampler disks, to concentrate organic chemicals from river water samples. These are commonly used cartridges and we have used them in previous research (Yamashita et al., 2012). For passive sampling, we selected 47-mm SDB-RPS disks (3M, Saint Paul, USA), which contain a styrene-divinylbenzene copolymer that has been modified with sulfonic acid groups to make it hydrophilic and provide selectivity for polar organic chemicals. (Shaw et al., 2009), reported that, SDB-RPS disks capable of sequestering a broad range of organic pollutants, however, the range of compounds sequestered has not been comprehensively identified. In order to determine the necessary number of SDB-RPS disks and the length of deployment time required for sampling, preliminary tests were conducted both in the laboratory and in the field (Myojin River, Ube City, Japan). The results showed that in order to collect similar amounts of adsorbed chemicals and to show toxicity, it would be necessary to deploy three SDB-RPS disks in the river for at least 3 days.

Conditioned SDB-RPS disks were placed in a Chemcatcher™ passive sampler holder (3M) without a diffusion limiting membrane and this was then deployed in the Campo Alor River, 0.5 km upstream from its outlet into the sea. The survey was conducted during the rainy season between March 1st and 11th, 2016, at which time the river had an average flow velocity of 0.21 m/s, average discharge of 0.14 m^3/s, average pH of 7.6, average conductivity of 0.75 mS/cm, average dissolved oxygen of 4.5 mg/L, and average water temperature of 29.8 ^oC. All sampling activities were performed between 9.00 and 11.00 AM. The sampling schedule and data logger records for water level and temperature during the sampling period are shown in **Fig. 4.4**. For passive sampling, SDB-RPS disks were deployed for different time periods of 1, 2, 3, 7, and 10 days (hereafter, PS different period samples), and at 2-day intervals over the 10-day sampling period (hereafter, PS interval samples) to check the accumulation behavior of adsorbed chemicals in the 10-day PS sample and compared the amounts of chemicals adsorbed with those in the five 2-day interval samples. For active sampling, six 10-L grab samples were collected, an initial sample on day 1 and then five subsequent samples collected at the same time as the PS interval samples. These samples were concentrated using four conditioned Sep-Pak® Plus PS-2 cartridges (hereafter, GS

samples).

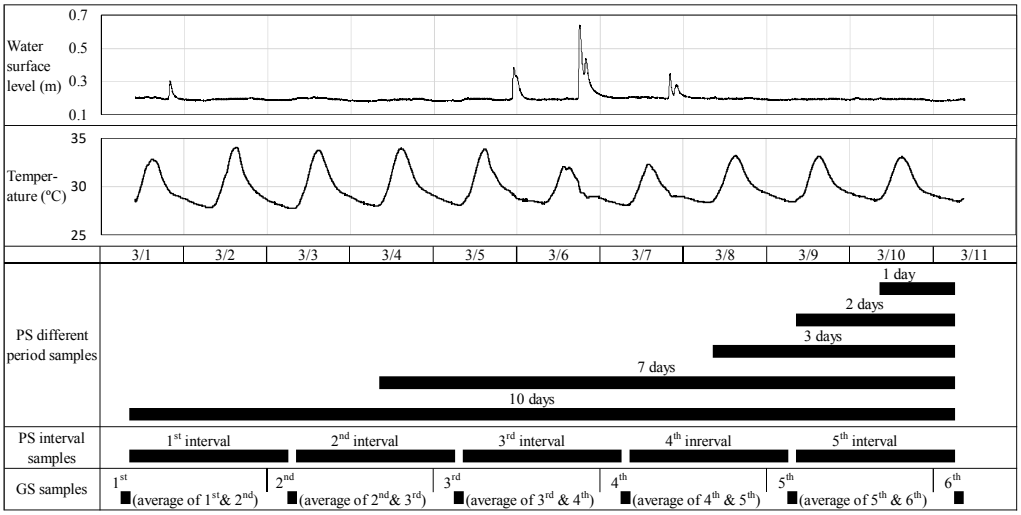


Fig. 4.4 Sampling schedule and data logger records during the sampling period.

c. Concentration and Elution of Adsorbed Chemical Compounds

Chemical compounds were concentrated from river water through both preconditioned Sep-Pak® Plus PS-2 cartridges and SDB-RPS disks. After concentration, Sep-Pak cartridges were dried by pumping air until all the water droplets inside were removed. Passive sampler SDB-RPS disks were dried in their Chemcatcher holders at room temperature (30°C), after which the holders were covered with their caps. The dried Sep-Pak cartridges and Chemcatcher samplers were wrapped in aluminum foil, refrigerated, and transferred to a cool bag until arrival in Japan. Adsorbed chemical compounds were subsequently eluted using acetone. Elution from the Sep-Pak® Plus PS-2 cartridges was based on the method of (Ishii et al., 2000), who reported that 9 mL of acetone could completely elute almost all organic microchemicals from each cartridge. Accordingly, 10 mL of acetone was flowed into each cartridge, and a total of 40 mL of acetone eluate was collected from the four Sep-Pak cartridges. Chemicals adsorbed onto SDB-RPS disks were eluted as recommended by the manufacturer (3M), using two 10-mL volumes of acetone for each passive sampler disk. The 60 mL of acetone eluate collected from three disks was then evaporated to a final volume of 40 mL.

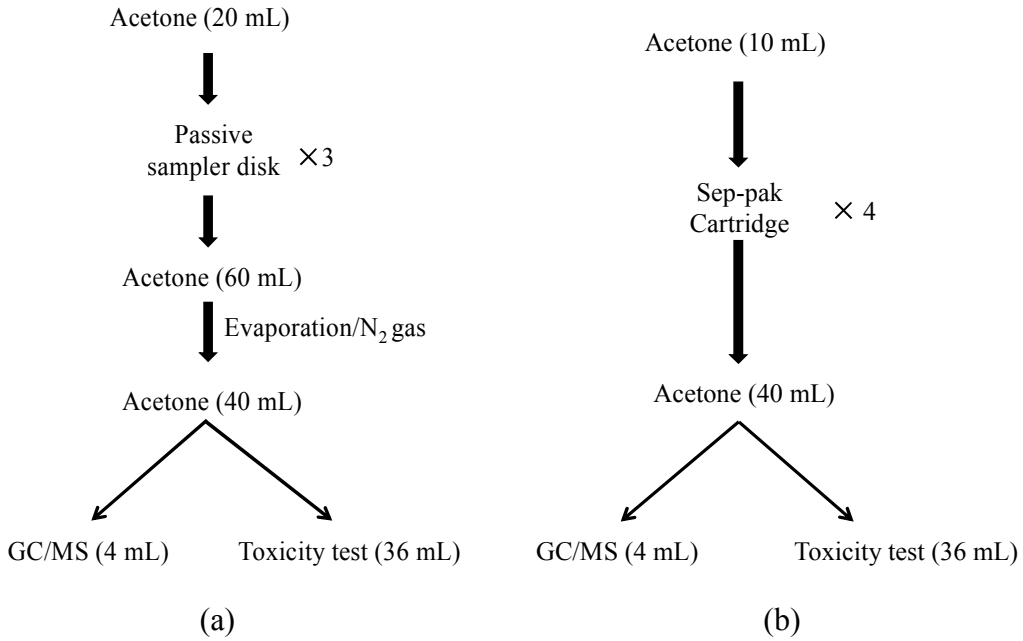


Fig. 4.5 Elution procedure of adsorbed chemicals, (a) from passive sampler (PS) samples, and (b) from grab samples (GS)**.**

Acetone eluates from both SDB-RPS disks (PS samples) and Sep-Pak® Plus PS-2 cartridges (GS samples) were divided into two samples: 4 mL for gas chromatography/mass spectrometry (GC/MS) analysis and 36 mL to prepare 10-, 20-, 50-, and 100-fold concentrated solutions for the toxicity test, as shown in **Fig. 4.5** (a, b).

d. Medaka (Oryzias latipes var.) Acute Toxicity Test

In the present study, we employed the same procedures as (Yamashita et al*.,* 2012) to determine the toxicity level **(Fig. 2.5, chapter 2)**.

e. GC/MS Simultaneous Analysis Database

In order to identify chemical compounds in the collected samples, we used the GC/MS simultaneous analysis database, which can identify and quantify a total of 942 chemical compounds without the need for reference standards (Kadokami et al*.,* 2005). (**Chapter 2, 2.14**)

4.2.3 Investigation of Short Deployment of SDB-RPS Passive Sampler Disks

a. Study Area

Caicoli river was selected as our study site, as it is one of the most polluted rivers in Dili. Caicoli is a freshwater stream, with a basin area of 0.70 km^2, width of 2 m, and water depth of 0.3 − 0.5 m, that runs through a residential area and is mainly exposed to the discharged wastewater from the surrounding houses. Caicoli River has a relatively low flow rate of between 0.008 and 0.021 m^3/s during the dry and rainy seasons, respectively.(**Fig. 4.6**)

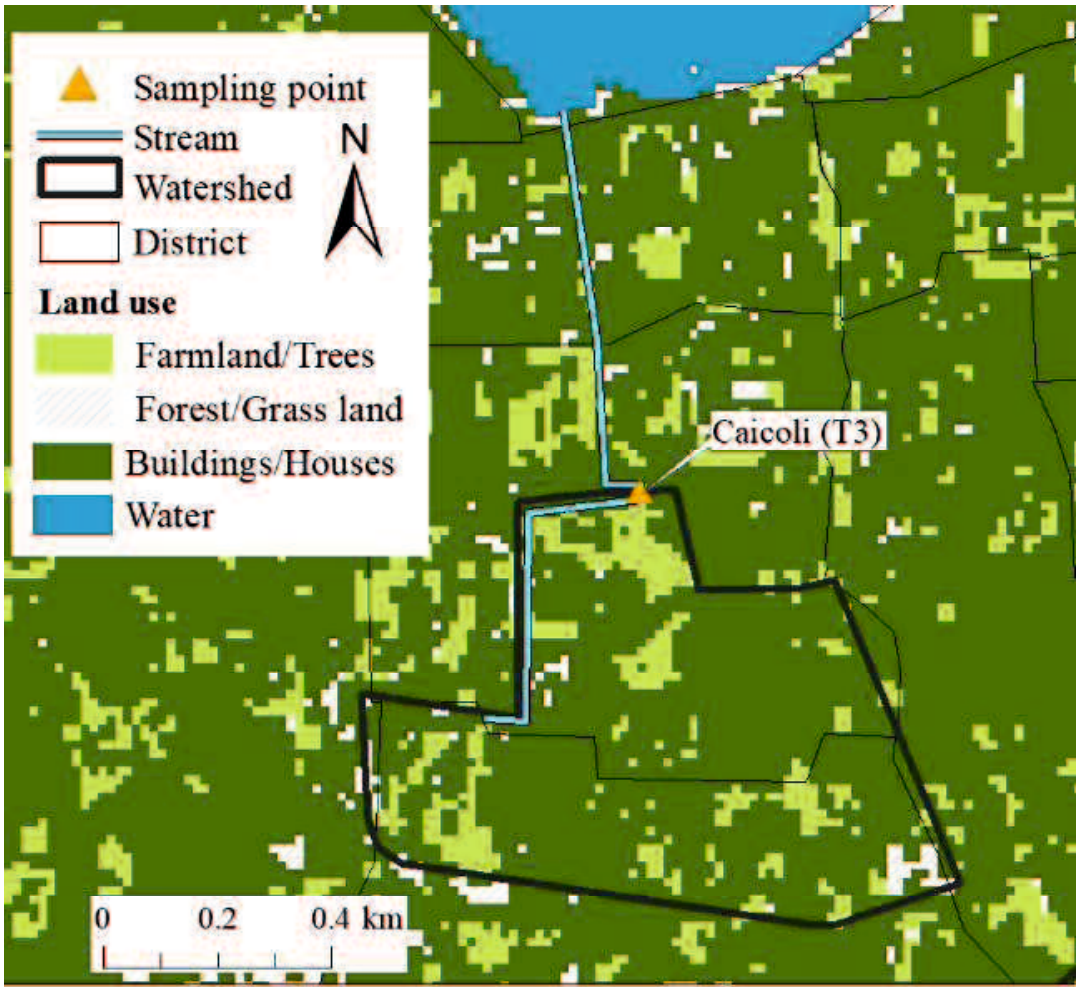


Fig. 4.6 Sampling site: Caicoli river (March, 2017)

b. Sampling

Conditioned SDB-RPS disks were placed in a Chemcatcher^{TM} passive sampler holder (3M) without a diffusion limiting membrane and this was then deployed in Caicoli river for ½-, 1-, 2-, and 4-days. Three disks were deployed for 2, and 4 days, whereas the number of deployed disks were increased as 6 and 12 disks for 1-, and ½-day samples, respectively. samples were duplicated as 1st half day, 2nd half day along 1-day deployment (hereafter, DH1, DH2). The same was done along 2-, and 4-day deployment periods (hereafter, D1-1, D1-2, D2-1, and D2-2), respectively.

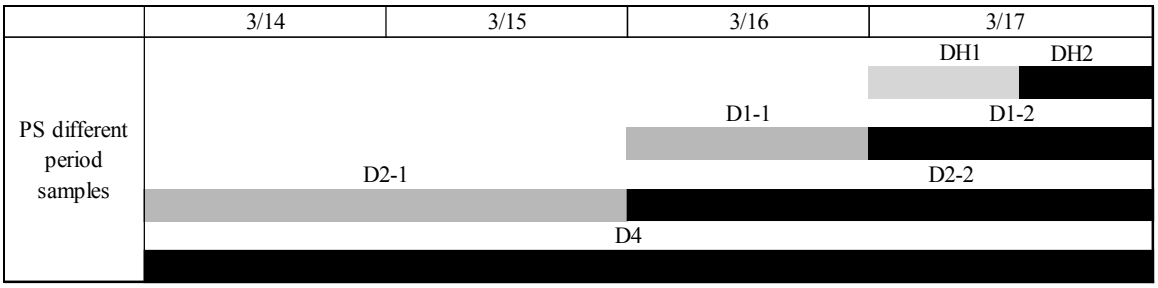


Fig. 4.7 Schedule of the deployment of passive sampler disks at Caicoli river, March 2017.

This study was conducted during the rainy seasons in March 2017, at that time the Caicoli river had an average flow velocity of 0.12 m/s, average discharge of 0.02 m^3/s, average pH of 7.45, average conductivity of 0.83 mS/cm, average dissolved oxygen of 2.01 mg/L, and average water temperature of 27.48°C. The Schedule of the deployment of passive sampler disks at Caicoli river, is shown in **Fig. 4.7.**

c. Concentration and Elution of Adsorbed Chemical Compounds

Fig. 4.5 (b).

d. *Medaka (Oryzias latipes var.) Acute Toxicity Test*

Chapter 2, Fig. 2.5

e. GC/MS Simultaneous Analysis Database

Chapter 2, (2.14)

4.3 Results and Discussion

4.3.1 *Selection of The Most Suitable Passive Sampler Disks*

a. Field Experiment

- ***GC/MS Analysis Results***

Table 4.1 : Chemicals eluted from SDB disks and Sep-pak cartridges.

Chemical	PS-2	SDB-RPS	SDB-XC	SDB-XD	Chemical	PS-2	SDB-RPS	SDB-XC	SDB-XD
1;1;0;n-C11H24	0.110	0.176		0.242	2;3;0;4-n-Octylphenol		0.018		
1;1;0;n-C12H26	0.042		0.005		2;3;0;4-sec-Butylphenol		0.028		
1;1;0;n-C13H28				0.071	2;3;0;Nonylphenol		4.498	0.549	0.585
1;1;0;n-C14H30				0.205	2;3;0;Phenol			0.096	
1;1;0;n-C16H34	0.099		0.011	0.111	2;3;1;3-&4-Chlorophenol	0.812	1.037	0.466	
1;1;0;n-C17H36	0.067		0.052	0.186	2;3;1;3,4-Dichlorophenol		0.139		
1;1;0;n-C18H38	0.093	0.107	0.096		2;4;0;Bis(2-ethylhexyl)phthalate	4.609	4.911	3.386	3.122
1;1;0;n-C19H40			0.089	0.065	2;4;0;Butyl benzyl phtalate		2.099		2.183
1;1;0;n-C20H42	0.183			0.103	2;4;0;Diethyl phthalate	0.244	0.201		
1;1;0;n-C21H44				0.037	2;4;0;Diisobutyl phthalate	0.148			
1;1;0;n-C22H46	0.134		0.077	0.068	2;4;0;Dimethyl phthalate	0.162			
1;1;0;n-C23H48	0.020			0.127	2;4;0;Di-n-butyl phthalate	1.334	0.950	0.524	0.317
1;1;0;n-C24H50	0.045				2;5;0;cis-11,14,17-Eicosatrienoic acid methyl ester	0.023			
1;1;0;n-C25H52		0.045		0.038	2;5;0;cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester			0.304	
1;1;0;n-C26H54	0.073	0.042			2;5;0;Methyl undecanoate			0.031	
1;1;0;n-C27H56		0.089			2;5;0;Tricosanoic acid methyl ester	0.033			
1;1;0;n-C28H58		0.138		0.014	2;9;0;1-Acetoxy-2-methoxyethane	0.063		0.014	
1;1;0;n-C29H60		0.033	0.154		2;9;0;1-Nonanol				0.300
1;1;0;n-C30H62			0.063		2;9;0;2-Butoxyethanol			0.028	
1;1;0;n-C31H64		0.170	0.021	0.244	2;9;0;2-Heptanol		0.020		0.020
1;1;1;Hexachloroethane		0.264			2;9;0;2-Hydroxy-4-meth. . .		0.033		
1;2;0;4-Cymene	0.032				2;9;0;3-Hexanol, 4-ethyl-		0.042		
1;2;1;1,4-Dichlorobenzene	0.019				2;9;0;Di(2-ethylhexyl)adipate	0.030			
1;2;1;3-Bromochlorobenzene				0.066	2;9;0;Ethanol, 2-phenoxy-	0.447	0.387		0.345
1;2;1;Benzyl chloride				0.013	2;9;1;1,3-Dichloro-2-propanol	0.034			
1;3;0;2,3-Benzofluorene		0.038			2;9;1;Tris(4-chlorophenyl)methanol		0.041		
1;3;0;2,6-Diisopropylnaphthalene	0.006				3;1;0;1-Naphthylamine		0.108		
1;3;0;2-Methylnaphthalene		0.101	0.032	0.018	3;1;0;2-Naphthylamine		0.091		
1;3;0;4,5-Methylene-phenanthrene	0.013				3;1;0;Acetamide, N-phenyl-	0.181			
1;3;0;Benzo(j&b)fluoranthene		0.016			3;1;0;Aniline		0.087		
1;3;0;Benzo(k)fluoranthene		0.017			3;1;0;N-Phenyl-1-naphthylamine		0.179		
1;3;0;Naphthalene	0.182	0.233	0.072	0.091	3;1;0;p-Phenylenediamine		0.504		
1;3;1;1,2,3,4,5,8-Hexachloronaphthalene	6.008				3;1;1;4-Bromo-2,6-dichloroaniline			0.159	
1;3;1;1,2,4,5,8-Pentachloronaphthalene				0.916	3;3;0;1,3-Dinitrobenzene			0.350	
1;3;1;1,2,5,8-&1,2,6,8-Tetrachloronaphthalene				0.360	3;3;0;2,6-Diamino-4-nitrotoluene				0.019
1;3;1;1,4-&1,6-Dichloronaphthalene			0.170		3;3;0;2-Nitronaphthalene			0.403	
1;4;1;PCB #114			0.010		3;4;0;N-Nitrosopyrrolidine				0.099
1;4;1;PCB #149				0.163	3;9;0;3-Methylpyridine	0.030	0.033		
1;4;1;PCB #52	0.028				3;9;0;Dibutylamine				0.009
1;4;1;PCB #77		0.015			3;9;0;e-Caprolactam			0.021	
2;1;0;Dibenzylether	0.016				3;9;0;N-Ethylmorpholine	0.019			
2;1;1;Bis(2-chloroethyl)ether		0.126			3;9;0;Nicotinonitrile				0.144
2;2;0;Acetophenone	0.189			0.176	4;1;0;2(3H)-Benzothiazolone		1.153		
2;2;0;Isophorone				0.216	4;1;0;Diphenyldisulfide		0.018		
2;3;0;2,6-Dimethylphenol	0.040				4;1;0;Phenothiazine				0.041
2;3;0;2-tert-Butylphenol		0.010			5;1;0;Diethyl-p-nitrophenyl phosphate	0.476			

Table (4.1) - (Continued)

Chemical	PS-2	SDB-RPS	SDB-XC	SDB-XD	Chemical	PS-2	SDB-RPS	SDB-XC	SDB-XD
5;1;0;Tributyl phosphate	0.144				7;2;;Benoxacor				0.041
5;1;0;Trimethyl phosphate		0.013	0.010		7;2;;Bensulide	4.267			4.299
5;1;1;Tris(2-chloroethyl) phosphate	0.484				7;2;;Butafenacil		0.231		
6;1;;Caffeine	0.803	0.789		0.390	7;2;;Butamifos			0.019	
6;1;;Crotamiton	1.746	1.161	0.867	0.477	7;2;;Butylate	0.084			
6;1;;Diethyltoluamide	0.475	0.492	0.151	0.109	7;2;;Captan		0.177		
6;1;;Fenoprofen				3.230	7;2;;Carbetamide	0.315			
7;1;;3-Hydroxycarbofuran 2	0.116				7;2;;Carfentrazone-ethyl		0.077		
7;1;;Allethrin 1			0.509		7;2;;Chlorpropham		0.064		
7;1;;Bioresmethrin	0.027				7;2;;Dimethenamid			0.056	
7;1;;Carbaryl		0.099			7;2;;Fenoxaprop-ethyl				0.179
7;1;;Chlorfenapyr	0.066	0.062			7;2;;MCPA-thioethyl (Phenothiol)		0.521		
7;1;;Chlorpyrifos-methyl			0.028		7;2;;MCPB-ethyl	0.022			
7;1;;Cypermethrin 2	2.026				7;2;;Mefenacet				0.142
7;1;;Cypermethrin 3	0.231	0.403			7;2;;Metribuzin			0.160	
7;1;;Cypermethrin 4		0.909			7;2;;Metribuzin DADK	0.291			
7;1;;Cyromazine			0.144		7;2;;Metribuzin DK			0.609	
7;1;;DCIP		0.080			7;2;;Oxabetrinil		0.114		
7;1;;DDVP		0.433			7;2;;Oxyfluorfen				1.494
7;1;;Deltamethrin				0.763	7;2;;Pretilachlor			0.142	
7;1;;Demeton-S-methylsulphon			0.136		7;2;;Pyraflufen ethyl		0.239		
7;1;;Dicrotophos	0.108			0.041	7;2;;Pyrazoxyfen				
7;1;;Dimethoate	0.017				7;2;;Pyriminobac-methyl Z		0.113		
7;1;;Diofenolan 1			0.064		7;2;;Terbacil		0.188		
7;1;;Disulfoton			0.026		7;3;;Bitertanol	0.136	0.131	0.046	
7;1;;Ethiofencarb		0.134			7;3;;Captafol	0.048			0.059
7;1;;Ethoprophos			0.124		7;3;;Cyproconazole				0.216
7;1;;Etofenprox	0.017				7;3;;Dichlofluanid metabolite		0.238		
7;1;;Flucythrinate 1		0.477			7;3;;Fenpropimorph			0.014	
7;1;;Flucythrinate 2			0.368		7;3;;Flusilazole		0.188		
7;1;;Isocarbophos	0.065				7;3;;Flutolanil				0.051
7;1;;Methamidophos			0.315		7;3;;Hexaconazole				0.198
7;1;;Methidathion	0.059				7;3;;Hymexazol		0.488		
7;1;;Methoprene	0.163				7;3;;Oxpoconazole-formyl		0.263		
7;1;;Nereistoxin oxalate deg.		0.074			7;3;;Procymidone		0.089		
7;1;;o,p'-DDT			0.005		7;3;;Propiconazole 2	0.127		0.227	
7;1;;Permethrin 1				0.084	7;3;;Pyraclostrobin		0.170		
7;1;;Phosmet			0.090		7;3;;Tetraconazole		0.032		
7;1;;Piperonyl butoxide				0.074	7;3;;Triadimefon	0.028			
7;1;;Pyraclofos				0.456	7;3;;Zoxamide			0.044	
7;1;;Pyridaben			0.056		7;9;;Dicofol	0.076			
7;1;;Pyridaphenthion	0.254				7;9;;Dicofol-deg	0.296			0.141
7;1;;Thiocyclam	0.399	0.736			7;9;;Fenamiphos	0.060			
7;1;;Thiometon	0.024				7;9;;Prohydrojasmon	0.236			
7;1;;Xylylcarb			0.047		7;9;;Spirodiclofen			0.122	
7;2;;Acetochlor			0.139						

The results of GC/MS analysis showed that the Styrene-Divinyl Benzene Reverse Phase Sulfonated (SDB-RPS) passive sampler disks caught the highest number of detected chemicals mostly at higher concentration than other disks comparing to Sep-Pak cartridges, as shown in **Table (4.1)**. and summarized in the following **Table (4.2)**.

Table 4.2. Comparison between GC/MS Results for both SDB disks and Sep-pack Plus PS-2 cartridges

	Sep-Pak	SDB-RPS	SDB-XC	SDB-XD
Number of detected compounds	68	70	53	54
Adsorbed amount (µg)	29.25	27.38	11.70	23.15

- ***ToxicityTest Results***

The results of toxicity test according to probit statistical analysis, indicated that chemicals eluted from Sep-Pak cartridges (Composite sample) had toxicity effect only at 100 fold (concentrated sample) with inverse of median lethal time value (LT_{50}^{-1} = 0.3850) . While the chemicals that eluted from SDB-RPS disks only among other disks, showed also toxicity effect at 100 fold with inverse of median lethal time value (LT_{50}^{-1} = 0.1225), as shown below (**Fig. 4.8** and **Fig. 4.9**).

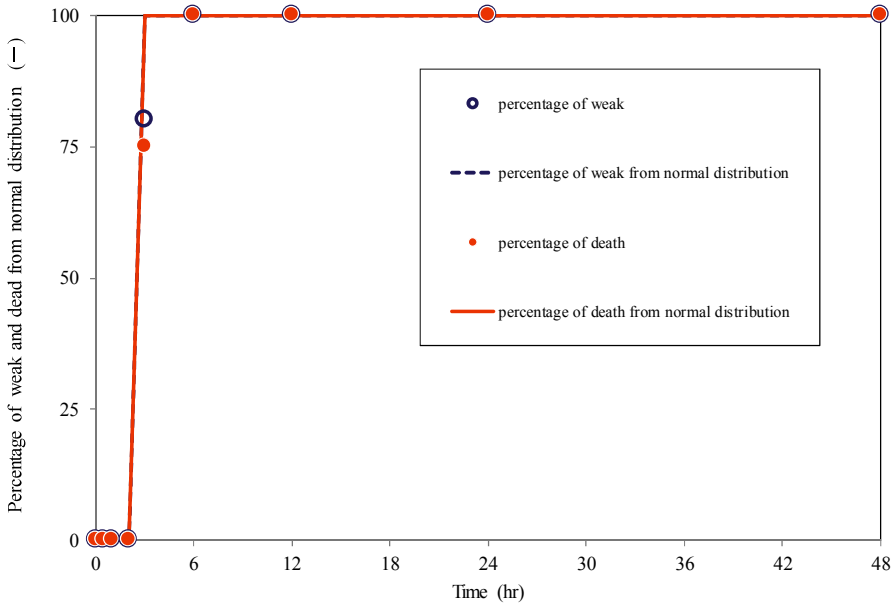


Fig. 4.8 Toxicity test result of composite sample (Sep-Pak cartridges).

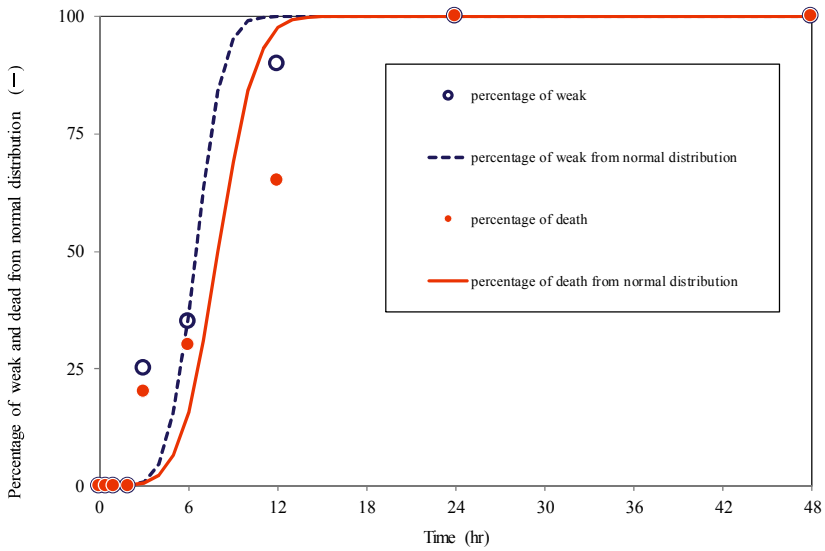


Fig. 4.9 Toxicity test result of PS (SDB-RPS disks).

b. Laboratory experiment

Results of laboratory experiment showed that the adsorbed amount of Triclosan was increasing during the deployment period from 1 day to 2, 3 till 7 days. In addition, there was a positive relationship between the concentration of Triclosan and its adsorbed amount for each deployment event, as shown in **Fig. 4.10**.

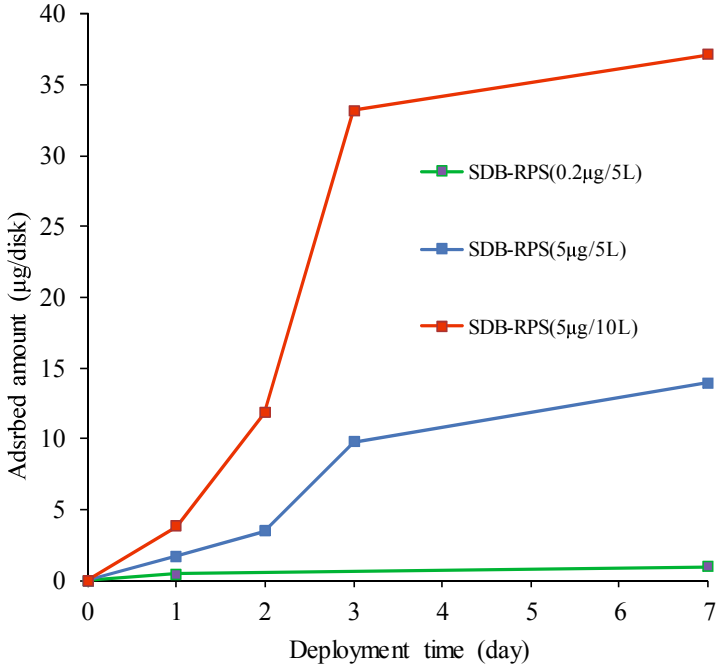


Fig. 4.10 Effect of concentration on the adsorbed amount.

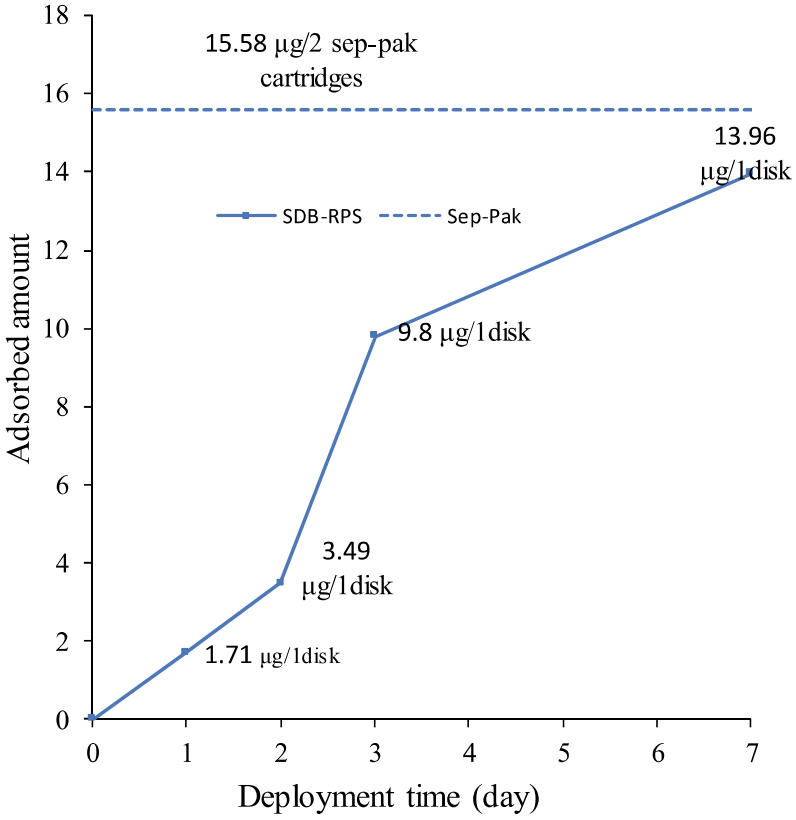


Fig. 4.11 Adsorbed Triclosan amount to SDB-RPS disk during the deployment period compared to adsorbed amount with Sep-Pak cartridges.

The adsorbed Triclosan amount by two Sep-Pak cartridges from 5 L Triclosan solution (5 µg/L) was 15.58 µg, while, its adsorbed amount by one SDB-RPS disk from 5 L Triclosan solution (5 µg/L) was 1.71, 3.49, 9.80 and 13.96 µg after the deployment for 1, 2, 3 and 7 days, respectively (**Fig. 4.11**). In case of active sampling in the field survey, 10 L water sample have to be collected to pass through 4 Sep-pak® Plus PS-2 cartridges (doubled volume in case of laboratory test), so it is expected that the adsorbed Triclosan amount will be 31.16 µg (2 x 15.58) by active sampling via 4 Sep-pak® Plus PS-2 cartridges. Then, the deployment of three passive sampler SDB-RPS disks for at least 3 days, would be able to adsorb 29.40 µg (3 x 9.80), almost close to the adsorbed Triclosan amount by 4 Sep-pak® Plus PS-2 cartridges via active sampling.

4.3.2 *Investigation of Long Deployment of SDB-RPS Passive Sampler Disks*

a. Acute Toxicity Test and GC/MS Analysis Results

Tables 4.3, **4.4**, and **4.5** show the toxicity test results for chemicals eluted from; the PS different period, PS interval, and GS samples, respectively. In spite of our pre-test designed to determine the appropriate number of disks to use, the amounts of adsorbed chemicals in the PS samples were lower than those in the GS samples. This might be related to the environmental conditions of the sampling location, such as biofouling, flow rate, or high temperature. **Table 4.3** shows that the chemicals eluted from the PS 10-day sample had highest toxicity at 100-fold, followed by the 7-day sample. The toxicities of PS 1-, 2-, and 3-day samples were lower than the limit of detection, even though the amount of adsorbed chemicals in the PS 3-day sample (32.5 μg/3 disks) was higher than that (32.2 μg/3 disks) in the PS 7-day sample. **Table 4.4** shows that no toxicity was observed in any of the PS interval samples, even though the amount of adsorbed chemicals in the PS 2nd interval sample (52.50 μg/3 disks) was higher than that in the PS 7- and 10-day samples, which both showed toxicity (**Table 4.3)**.

The results presented in **Table 4.5** show that GS samples had strong toxicity at different folds, ranging from 20- to 100-fold. The LDR_{50} values were the same for the 1st and 3rd, 2nd and 4th, and 5th and 6th GS samples. However, the combination between the two indices LDR_{50} and LT_{50}^{-1} showed that the highest toxicity level was detected in the 1st grab sample, which contained the highest amount of adsorbed chemicals, whereas the 6th grab sample had the lowest toxicity level.

In spite of our pre-tests designed to determine the appropriate number of disks to use, the amounts of adsorbed chemicals in the PS 3-, 7-, and 10-day samples were lower than those in the GS samples. This might be related to the difference in environmental conditions of the sampling locations, such as biofouling, flow rate, adsorbed chemicals, or high temperature. Furthermore, toxicity test results for PS 7- and 10-day samples were considerably lower than those for the GS samples. These findings are consistent with those of (Tan et al*.,* 2007), who studied the estrogenic effect of grab samples and passive sampler extracts of different matrices of wastewater treatment plants. The authors found that the estrogenic activity obtained using passive sampler extracts was lower than that obtained using grab samples, mainly due to biofouling.

Table 4.3. Toxicity test results for PS different period samples

Deployment time	Fold (Concentration ratio)	Adsorbed amount (μg/3 disks)	$(LT_{50}^{-1})^{\#}$	$(LDR_{50})^{\#\#}$
1 day	100	14.20	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	
2 days	100	16.70	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	
3 days	100	32.50	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	
7 days	100	32.15	0.20	0.014
	50		<0.02	
	20		<0.02	
	10		<0.02	
10 days	100	40.80	0.26	0.020
	50		0.20	
	20		<0.02	
	10		<0.02	

Table 4.4 Toxicity test results for PS interval samples

Deployment event	Folds (Concentration ratio)	Adsorbed amount (μg/3 disks)	$(LT_{50}^{-1})^{\#}$	$(LDR_{50})^{\#\#}$
1st interval	100	19.20	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	
2nd interval	100	52.50	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	
3rd interval	100	26.80	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	
4th interval	100	27.80	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	
5th interval	100	23.20	<0.02	<0.01
	50		<0.02	
	20		<0.02	
	10		<0.02	

Table 4.5 Toxicity test results for grab samples (GS)

	1st		2nd		3rd		4th		5th		6th	
Fold (Concentration ratio)	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}
100	>2		>2		>2		>2		>2		>2	
50	>2	0.07	>2	0.05	>2	0.07	0.91	0.05	0.71	0.03	0.42	0.03
20	0.11		0.02		0.09		0.02		<0.02		<0.02	
10	<0.02		<0.02		<0.02		<0.02		<0.02		<0.02	
Adsorbed amount (μg/4 sep-pak cartridges)	137.47		112.74		101.18		111.67		73.19		97.40	

LT_{50}^{-1}: the inverse of median lethal time

LDR_{50}: the lethal dilution ratio

Table 4.5 and **Fig. 4.12** show the relationship between the amounts of adsorbed chemicals and toxicity results for GS samples. The correlations between the chemical amounts and the corresponding LDR_{50} and LT_{50}^{-1} values, were not significant (r = 0.70, p-value = 0.12 and r = 0.57, p-value = 0.23, respectively).

Table 4.6 and **Fig. 4.13** show the relationship between the adsorbed chemicals in the PS interval samples and the GS averaged toxicity. As shown in the sampling schedule (**Fig. 4.4**), GS samples were collected during the deployment time of the PS interval samples, and then the average toxicity values for GS samples were calculated to represent the toxicity conditions during the deployment times of PS interval samples. The correlations between the chemical amounts in the PS interval samples and the corresponding GS averaged toxicity (LDR_{50} and LT_{50}^{-1}) values, were not significant (r = 0.30, p-value = 0.55 and r = 0.08, p-value = 0.86, respectively).

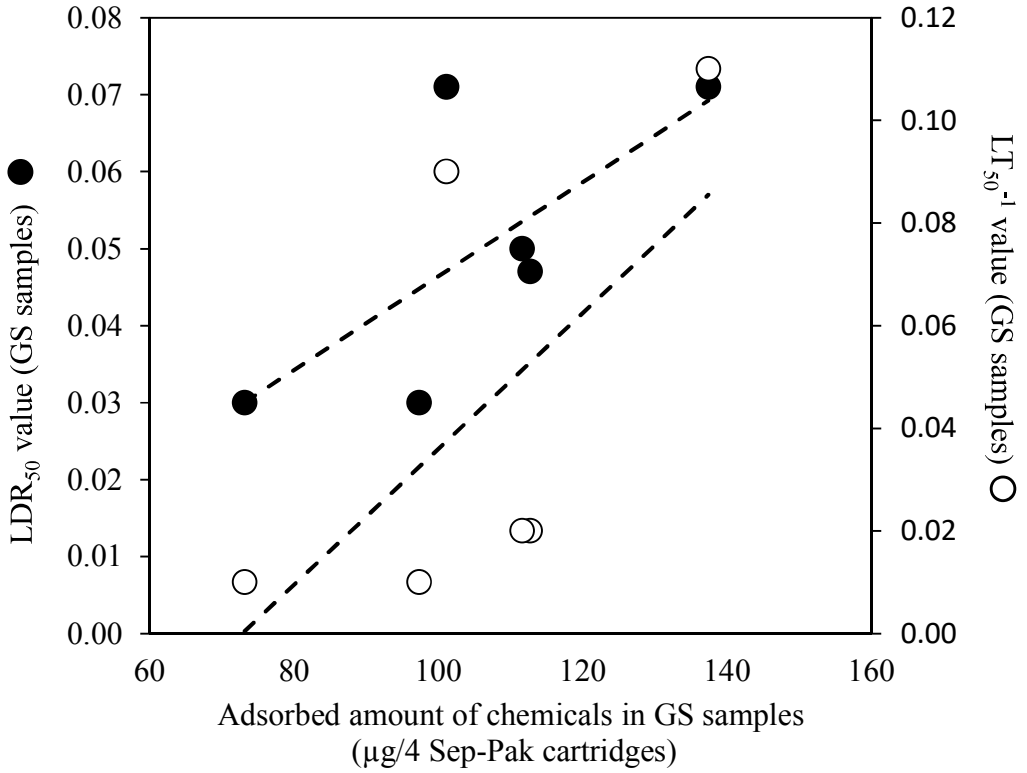


Fig. 4.12 Relationship between average adsorbed amount (μg/4 cartridges) of chemicals in GS samples and their corresponding average toxicity level.

Table (4.6) Adsorbed amount of chemicals in PS interval and 10-day samples & average toxicity test results for GS samples.

PS interval samples	1st	2nd	3rd	4th	5th	PS 10-day sample
adsorbed amount (μg/3 disks)	19.20	52.50	26.80	27.80	23.20	40.80
	Average toxicity of GS samples					
	1st & 2nd	2nd & 3rd	3rd & 4th	4th& 5th	5th & 6th	1st to 6th
LT_{50}^{-1} (20 fold) *	0.065	0.050	0.050	0.020	0.010	0.040
LDR_{50}	0.06	0.06	0.06	0.04	0.03	0.05

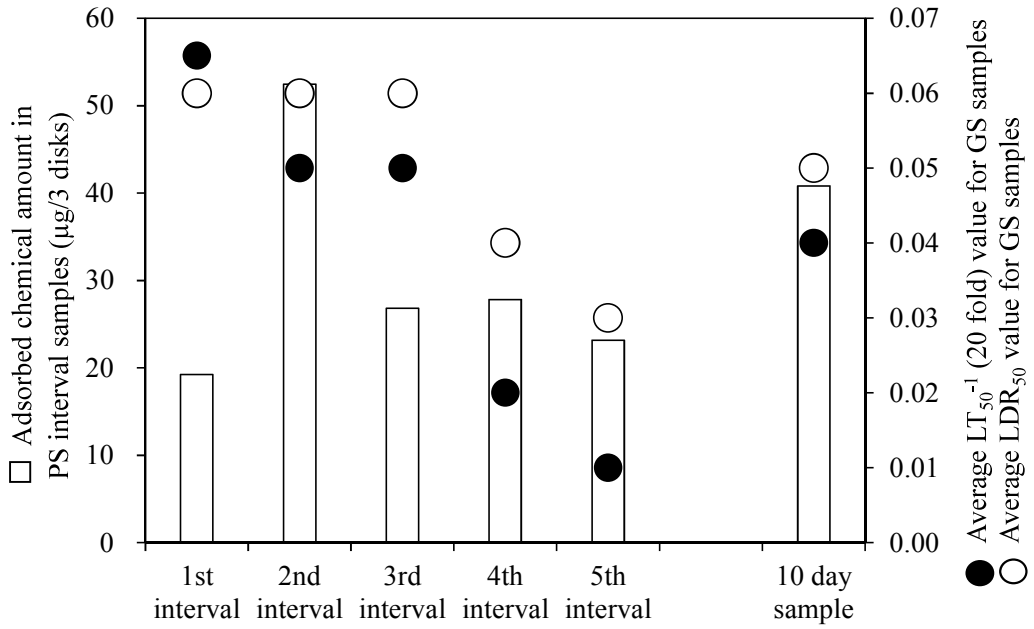


Fig. 4.13 Relationship between adsorbed amount (μg/3 disks) of chemicals in PS interval samples and average value of toxicity results for GS samples.

It was noticed that the amount of adsorbed chemicals in the PS 10-day sample did not represent an accumulation of chemicals adsorbed in the individual PS interval samples. We performed a comparison between the sum of the adsorbed amounts of each chemical in PS interval samples and their adsorbed amount in the PS 10-day sample for all 125 detected chemicals. As **Fig.4.14** shows, there was a correlation between them ($r = 0.91$, p-value $< 2.2\ e^{-16}$).

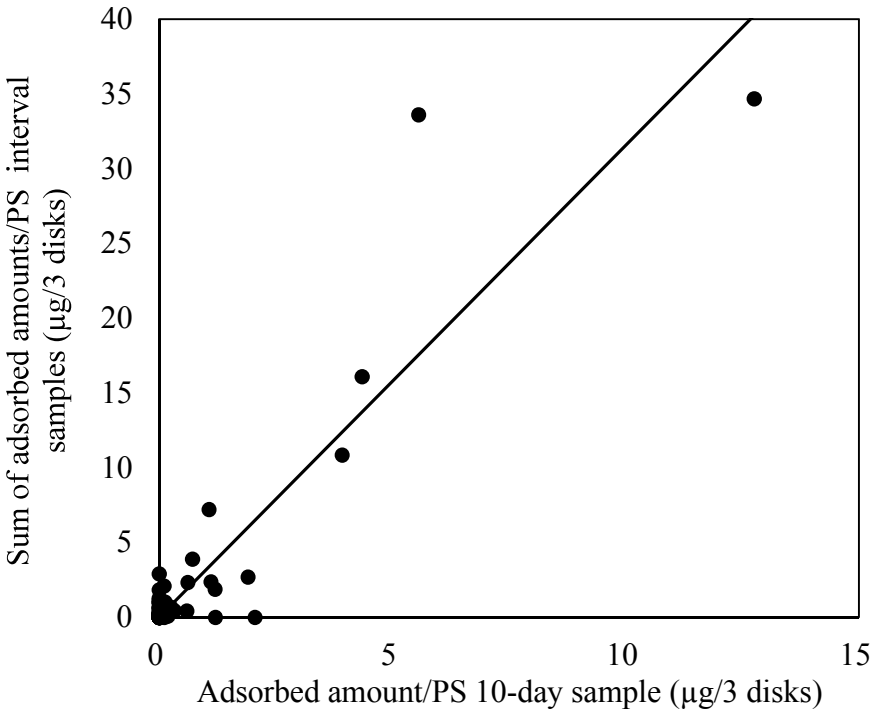


Fig. 4.14 Relationship between the amount of adsorbed chemicals in PS 10-days and the sum of adsorbed amount of chemicals in passive sampler (PS) interval samples.

The above results showed that there was a positive relationship between the amount of adsorbed chemicals and toxicity for the GS samples. In contrast, although PS 7- and 10-day samples showed toxicity, the PS samples with shorter deployment times did not show toxicity even though they contained similar or higher amounts of adsorbed chemicals. Furthermore, the amount of adsorbed chemicals in the PS 10-day sample was not equivalent to the sum of chemicals adsorbed in the individual PS interval samples. These observations suggest that longer deployment of SDB-RPS disks might be associated with desorption or decomposition of some adsorbed chemicals over the course of the deployment period, the latter of which could yield compounds of higher toxicity.

b. Pattern of Occurrence and Proportions of Different Chemicals

Table 4.7 shows the chemicals in the log k_{ow} range −0.07 to 16.5 with a maximum adsorbed amount ≥ 0.20 µg/3 disks, which were detected in PS interval samples and the PS 10-day sample. The detected chemicals were categorized into five groups as shown in the table. Many compounds were absent from the PS 1st interval sample,

which can be attributed to the fact that this sample was spilt during the preparation, and accordingly only 25% of the sample was used for GC/MS analysis. Thus, it is assumed that only compounds with a high concentration were detected. **Figure 4.15** shows the pattern of occurrence and proportions of each chemical in the PS interval and PS 10-day samples with amounts > 0.50 μg/3 disks. The highest bars represent the highest adsorbed amount of the chemical, and the absence of a bar indicates that the chemical was not detected. There were wide variations in the occurrence and proportions of each chemical. In the PS 10-day sample, the amounts of diethyl phthalate, di-n-butyl phthalate, and nicotine represent between 60% and 140% of the accumulated amounts of these chemical in the PS interval samples. In contrast, the amounts of 2-ethyl-1-hexanol, elaidic acid methyl ester, aspirin, methyl palmitoleate, methyl palmitate, pyrethrin 2, 3-, and 4-methylphenol, and methyl myristate in the PS 10-day sample were considerably lower (or even absent) compared with the accumulated amounts of these chemicals in the PS interval samples. Pyrethrin 4 and pyrazoxyfen were only detected in the PS 10-day sample, whereas the amounts of other chemicals in the PS 10-day sample were between 10% and 50% of those accumulated in the PS interval samples. These differences in behavior do not, however, show a clear relationship with log Kow values. The pattern of occurrence and proportions of different chemicals support our assumption that chemicals adsorbed onto SDB-RPS disks might undergo desorption and/or decomposition, and that some of these could be converted to more toxic compounds.

Table 4.7 Organic chemicals eluted from PS interval and PS 10-day samples (only chemicals with maximum adsorbed amount ≥0.2 μg/3 disks are shown)

Name	"Log K_{ow}"	Cat-gory ***	PS interval samples							PS 10-days	Max. adsorbed amount
			1st (**)	2nd	3rd	4th	5th	Sum	Average		
Caffeine	-0.07		5.77	8.86	5.99	7.10	6.95	34.67	6.93	12.75	12.75
Diethyltoluamide	2.02		0.92	7.75	2.77	3.41	1.24	16.09	3.22	4.35	7.75
Aspirin	1.19	PPC	ND	ND	1.04	ND	ND	1.04	1.04	ND	1.04
L-Menthol	3.30	Ps	ND	0.06	0.07	0.87	0.04	1.04	0.26	0.12	0.87
Cholesterol	8.70		3.73	14.33	4.42	4.16	6.96	33.60	6.72	5.56	14.33
Bis(2-ethylhexyl)phthalate	7.50		ND	4.37	2.20	2.47	1.82	10.86	2.71	3.92	4.37
Coprostanol	8.82		2.34	2.31	0.52	0.49	1.55	7.20	1.44	1.07	2.34
Cholestanol	8.82		UL	1.92	0.48	0.47	1.03	3.90	0.97	0.71	1.92
Di-n-butyl phthalate	4.50		0.12	0.85	0.69	0.65	0.38	2.69	0.54	1.90	1.90
beta-Sitosterol	9.65		ND	1.77	ND	ND	0.56	2.34	1.17	0.61	1.77
Elaidic acid methyl ester	7.45		1.08	1.31	0.27	0.26	ND	2.91	0.73	ND	1.31
Diethyl phthalate	2.47		0.16	0.57	0.32	0.50	0.36	1.89	0.38	1.19	1.19
Diisobutyl phthalate	4.11		0.30	0.57	0.57	0.69	0.25	2.37	0.47	1.11	1.11
Methyl palmitoleate	7.08		ND	0.96	0.16	0.17	ND	1.28	0.43	ND	0.96
Methyl palmitate	7.38	OC	ND	0.90	0.47	0.48	ND	1.85	0.62	ND	0.90
3-&4-Methylphenol	4.74		ND	0.04	0.69	0.19	0.06	0.98	0.25	0.03	0.69
Methyl myristate	6.41		0.12	0.58	0.15	0.15	ND	0.99	0.25	ND	0.58
alpha-Terpineol	2.98		ND	0.03	0.03	0.49	0.08	0.63	0.16	0.21	0.49
3,5-Dimethylphenol	2.35		ND	0.12	0.05	0.47	0.13	0.78	0.19	0.11	0.47
Linolelaidic acid methyl ester	7.05		ND	0.38	0.16	0.13	ND	0.68	0.23	ND	0.38
Nonylphenol	5.76		ND	0.34	0.21	0.08	0.05	0.69	0.17	0.24	0.34
Stigmasterol	9.43		ND	0.34	ND	0.31	0.34	0.98	0.33	ND	0.34
Oleic acid methyl ester	7.45		0.26	0.32	0.07	0.06	ND	0.70	0.18	ND	0.32
Triclosan	4.76		ND	0.16	0.10	0.11	0.09	0.46	0.12	0.32	0.32

Table 4.7 Continued

Name	"Log K_{ow}"	Cate-gory ***	PS interval samples							PS 10-days	Max. adsorbed amount
			1st (**)	2nd	3rd	4th	5th	Sum	Aver-age		
2-Ethyl-1-hexanol	2.73		1.60	0.14	0.10	0.20	0.06	2.09	0.42	0.10	1.60
Butyl benzyl phthalate	4.73	OC	0.48	0.05	0.06	ND	0.01	0.60	0.15	ND	0.48
Pyrethrin 4	5.90		ND	ND	ND	ND	ND	ND	ND	2.05	2.05
Pyrazoxyfen	5.37		ND	ND	ND	ND	ND	ND	ND	1.20	1.20
Pyrethrin 2	4.30	P	ND	0.44	ND	0.74	ND	1.18	0.59	ND	0.74
Triadimenol 2	2.90		ND	ND	0.26	ND	ND	0.26	0.26	ND	0.26
Nicotine	1.17		ND	0.04	ND	0.19	0.20	0.43	0.14	0.59	0.59
Acetamide, N-(2 phenylethyl)-	1.19	NC	ND	ND	0.29	ND	0.10	0.39	0.20	ND	0.29
N-Nitrosopiperidine	2.63		0.22	0.08	0.05	ND	ND	0.35	0.12	ND	0.22
n-C32H66	16.06		ND	0.06	0.50	0.03	ND	0.59	0.20	0.07	0.50
n-C26H54	13.11		0.06	0.03	0.39	0.11	0.02	0.60	0.12	0.15	0.39
n-C29H60	14.58		ND	0.04	0.37	0.04	ND	0.44	0.15	0.05	0.37
n-C30H62	11.94		ND	0.02	0.33	0.01	ND	0.35	0.12	0.02	0.33
n-C28H58	14.09	HC	ND	0.03	0.33	0.04	ND	0.40	0.13	0.08	0.33
n-C27H56	13.60		0.27	0.05	0.31	0.11	0.03	0.76	0.15	0.09	0.31
Squalane	14.63		ND	0.26	0.19	0.14	ND	0.59	0.20	ND	0.26
n-C24H50	12.13		0.19	0.23	0.06	0.06	0.02	0.54	0.11	0.03	0.23
2(3H)-Benzothiazolone	2.35		ND	0.22	0.20	0.21	0.07	0.69	0.17	0.21	0.22
n-C33H68	16.50		ND	ND	0.22	ND	ND	0.22	0.22	ND	0.22

(**) 75% of the 1st passive sampler interval sample was spilt during preparation.
Category*** : PPCPs: pharmaceutical and personal care products; OC: oxygen-containing compounds (ethers, ketones, phenols, phthalates, fatty acid ester and others);P: pesticides; NC: nitrogen-containing compounds; HC; Hydrocarbons
"Log Kow" : Octanol-water partition coefficient, ND : Not detected

Compounds	Max. value of highest bar, µg/3 disks	Adsorbed chemical amounts 1st	2nd	3rd	4th	5th	PS 10-day	Accumulative ratio(#), %
Cholesterol	14.3							16.5
Caffeine	12.8							36.8
Diethyltoluamide	7.75							27.0
Bis(2-ethylhexyl)phthalate	4.37							36.1
Coprostanol	2.34							14.9
Pyrethrin 4	2.05							…..
Cholestanol	1.92							18.2
Di-n-butyl phthalate	1.90							70.6
beta-Sitosterol	1.77							26.1
2-Ethyl-1-hexanol	1.60							4.8
Elaidic acid methyl ester	1.31							0.00
Pyrazoxyfen	1.20							…..
Diethyl phthalate	1.19							63.0
Diisobutyl phthalate	1.11							46.8
Aspirin	1.04							0.00
Methyl palmitoleate	0.96							0.00
Methyl palmitate	0.90							0.00
L-Menthol	0.87							11.5
Pyrethrin 2	0.74							0.00
3-&4-Methylphenol	0.69							3.1
Nicotine	0.59							137
Methyl myristate	0.58							0.00

Fig. 4.15 Pattern of occurrence and proportions of each chemical in PS interval and PS 10-day samples with amounts >0.5 µg/3 disks (the highest bar represents the highest adsorbed amount; no bar indicates that compounds were not detected).

"Log K_{ow}": Octanol-water partition coefficient

4.3.3 *Investigation of Short Deployment of SDB-RPS Passive Sampler Disks*

a. Acute Toxicity Test Results

Table 4.8 Toxicity test results for passive sampler (PS) different period samples along 4 days.

	DH-1		DH-2		D1-1		D1-2		D2-1		D2-2		D4	
folds	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}	LT_{50}^{-1}	LDR_{50}
100 f	>2	0.040	>2	0.040	>2	0.040	>2	0.040	>2	0.040	>2	0.040	>2	0.066
50 f	0.83		0.71		0.53		0.67		0.04		0.11		>2	
20 f	<0.02		<0.02		<0.02		<0.02		<0.02		<0.02		0.03	
10 f	<0.02		<0.02		<0.02		<0.02		<0.02		<0.02		<0.02	
Adsorbed amount	75.6 µg/12 disks		101.5 µg/12 disks		96.4 µg/6 disks		83.2 µg/6 disks		82.6 µg/3 disks		31.6 µg/3 disks		27.8 µg/3 disks	

The results presented in **Table 4.8** shows that PS samples of different periods along 4 days deployment had strong toxicity comparing to that of PS samples of different periods along 10 days deployment, which have been investigated in the previous study conducted in 2016 (Elsheikh et al., 2017). The D4 sample showed the highest toxicity (LDR_{50} = 0.066) even it had the lowest adsorbed chemicals amount comparing to other samples along the four days deployment trial (27.8 µg/3 disks) . Whereas, LDR_{50} values were the same (LDR_{50} = 0.040) for the DH1, DH2, D1-1, D1-2, D2-1 and D2-2 samples, even their adsorbed chemicals amounts were different. However, the combination between the two indices LDR_{50} and LT_{50}^{-1} showed that the highest toxicity level was detected in the DH1 (75.6 µg/12 disks), followed by DH2 (101.5 µg/12 disks), whereas the D2-1 (82.6 µg/3 disks) showed the lowest toxicity level.

b. GC/MS Analysis Results

Table 4.9 Organic chemicals eluted from passive sampler (PS) samples along 4 days deployment period (with a maximum adsorbed amount ≥ 0.03 μg/ PS disks).

Name	Category*	D4 (μg/3 disks)	D2-1 (μg/3 disks)	D2-2 (μg/3 disks)	D1-1 (μg/6 disks)	D1-2 (μg/6 disks)	DH1 (μg/12 disks)	DH2 (μg/12 disks)
2-Butoxyethanol					0.124			
2-Methyl-2,4-pentandiol					1.137	0.703	1.618	0.845
Phenol			0.236	0.108	0.174	0.038	0.285	0.061
2-Ethyl-1-hexanol			0.100	0.056	0.087	0.067	0.052	0.092
Benzyl alcohol			0.177	0.199	0.532	0.050	1.229	0.141
2-Methylphenol					0.038		0.025	
Acetophenone				0.025	0.057	0.056	0.073	0.063
3-&4-Methylphenol			20.801	2.519	4.237	0.569	6.814	
Phenylethyl alcohol			0.685	0.465	1.060	0.119	1.805	0.203
2,4-Dimethylphenol		0.060						
3,5-Dimethylphenol							2.313	
1-Nonanol					0.073			0.077
alpha-Terpineol			0.275	0.279	0.484		0.607	
Ethanol, 2-phenoxy-			0.859	0.675	1.531	0.583	2.624	0.926
2-sec-Butylphenol							0.012	
Phenol, 2,6-dimethoxy-		0.278			0.260			0.218
Diethyl phthalate			0.932	0.520	1.120	0.734	1.359	1.134
Methyl myristate	OC	0.184	0.066		0.247	0.098	0.159	0.184
Diisobutyl phthalate			1.452	0.766	1.585	1.745	1.874	1.971
Methyl palmitoleate			0.565	0.022	0.820			
Methyl palmitate			0.451	0.241	1.154	0.965	1.087	1.739
Di-n-butyl phthalate			2.876	1.386	3.000	3.339	3.281	3.874
Methyl heptadecanoate					0.051	0.037	0.032	0.085
Linolelaidic acid methyl ester			0.061		0.455	0.300	0.303	0.661
Oleic acid methyl ester			2.599	0.014	0.391	4.025	0.342	
Elaidic acid methyl ester			10.587	2.155	15.292	16.375		30.810
Triclosan		0.550	0.355	0.189	0.387	0.480	0.458	
Stearic acid methyl ester			0.292	0.228	0.316	0.498	0.595	0.392
Di(2-ethylhexyl)adipate							0.073	0.113
Dicyclohexyl phthalate		3.744	2.503		4.359	2.395		
Bis(2-ethylhexyl)phthalate			4.322	2.841	7.365	4.140	6.975	7.775
Coprostanol			3.101	2.185	5.779	7.620	0.898	4.951
Cholesterol			7.108	3.849	11.776	11.137	9.237	12.896
Cholestanol		1.056	0.807		1.223	1.076		0.927
beta-Sitosterol					1.998	2.631	3.029	2.710

Table 4.9 Continue

Name	Category*	D4 (μg/3 disks)	D2-1 (μg/3 disks)	D2-2 (μg/3 disks)	D1-1 (μg/6 disks)	D1-2 (μg/6 disks)	DH1 (μg/12 disks)	DH2 (μg/12 disks)
L-Menthol	PPCPs		1.002	0.458	1.052	0.062	0.158	
Thymol		0.138						
Nicotine							1.317	
Diethyltoluamide			4.004	2.592	5.891	5.130	6.070	6.553
Caffeine			15.052	9.229	17.768	15.741	17.554	20.158
Cycloate	P		0.054					
Dimethametryn		21.660						
Piperonyl butoxide			0.178	0.088	0.232	0.229	0.211	0.275
Fenoxycarb							0.596	
Tebufenpyrad					0.020			
Pyridate						0.116		
Phthalimide	NC		0.042	0.021		0.063		0.099
2-(Methylthio)-benzothiazol	SC		0.122	0.085	0.170		0.211	
2(3H)-Benzothiazolone					0.888	0.781	0.837	0.979
n-C9H20	HC				0.123			0.138
n-C14H30					0.024		0.065	
n-C15H32			0.036	0.008	0.035	0.016	0.081	
n-C17H36			0.298		0.448	0.229	0.503	
Phenanthrene		0.100	0.068	0.034	0.067	0.050	0.072	0.050
n-C18H38					0.100	0.147	0.182	
n-C19H40				0.036			0.093	
2-Methylphenanthrene					0.012			
1-Methylphenanthrene		0.008						
n-C20H42						0.077	0.107	
n-C21H44			0.124	0.023				
n-C22H46					0.196	0.189		0.057
n-C23H48					0.056	0.093		
n-C24H50			0.119	0.117	0.196	0.081	0.163	0.263
n-C25H52			0.135		0.307	0.155		
n-C26H54					0.335	0.104		
n-C27H56			0.124		0.761			
n-C28H58					0.297	0.112		0.060
n-C30H62				0.160	0.326		0.181	

Category*: OC: oxygen-containing compounds (ethers, ketones, phenols, phthalates, fatty acid ester and others); PPCPs: pharmaceutical and personal care products; P: pesticides; NC: nitrogen-containing compounds; SC: sulfur-containing compounds; HC; Hydrocarbons

Table 4.9 shows the chemicals with a maximum adsorbed amount ≥ 0.03 µg/ PS disks, which were detected in PS samples along 4 days deployment period. The detected chemicals were categorized into six groups as shown above.

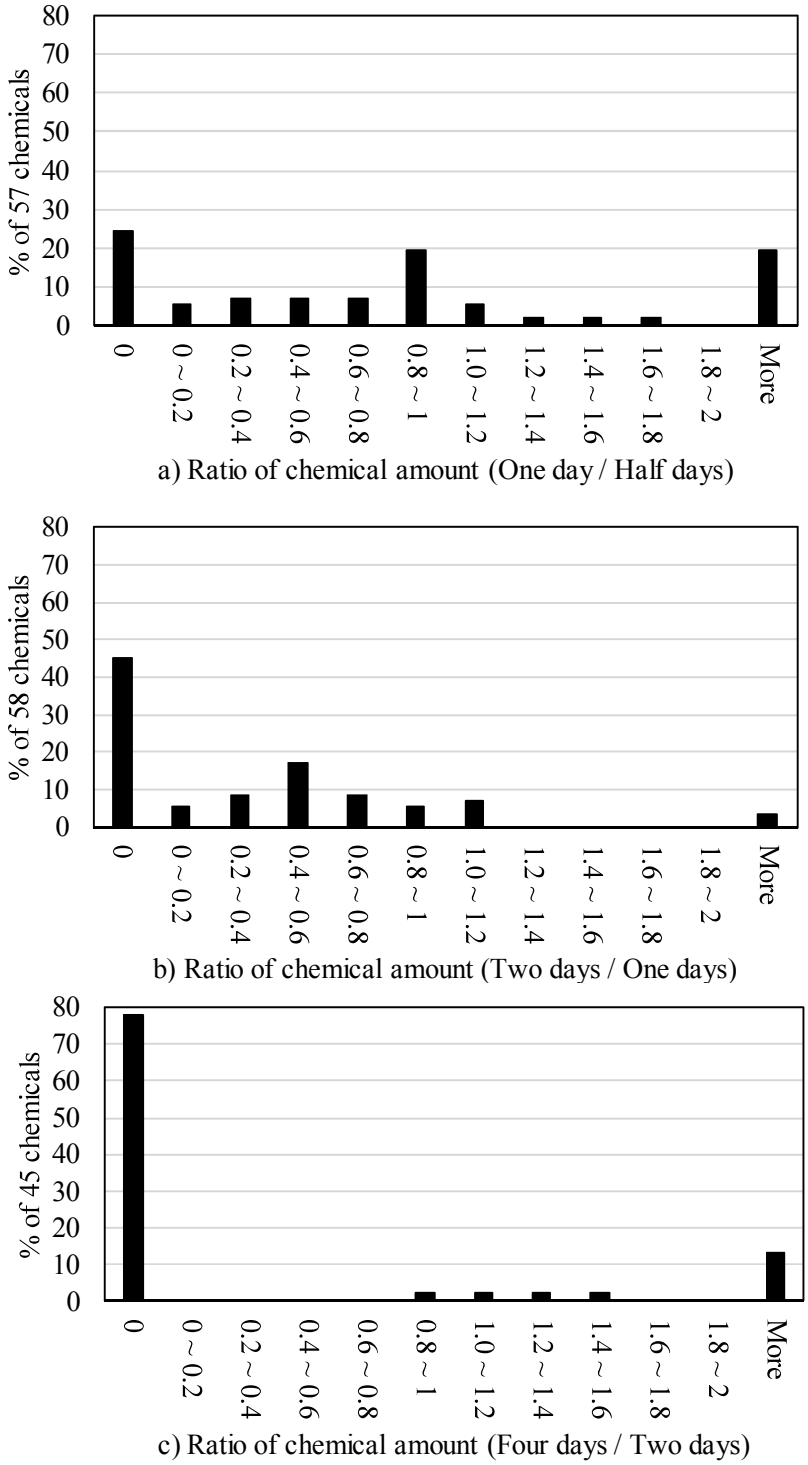


Fig. 4.16 (a, b, c) Percentage of detected number of chemicals & the ratio of chemicals amount remain in longer deployed disks

Figure 4.16 shows the percentage of detected number of chemicals vs. the ratio of chemicals amount which remain in longer deployment disks. For example, a chemical which has value 0 in ratio of chemical amount might be completely decomposed during the longer deployment period and disappeared. A chemical which has value 1 in ratio of chemical amount might be stable not decomposed. A chemical which has higher value than 1 might include a decomposition products of other chemicals. In (**Fig. a)**, 20% of chemicals has 0.8 to 1 for the ratio of chemical amount, whereas in (**Fig. b),** 17% of chemicals has 0.4 to 0.6 for the ratio of chemical amount. In (**Fig. a, b)**, around 45% of chemicals has 0.2 to 1.0 for the ratio of chemical amount. Whereas, in (**Fig. c)** only 2% of chemicals has 0.2 to 1.0 for the ratio of chemical amount. Instead, around 80% of chemicals disappeared from the four days deployment period (**Fig. c)**, whereas, around 50% and 25% disappeared from 2- and 1-day deployment periods as shown in Figures 4 b) and a) respectively.

These results showed that even for one day deployment, about 25% of number of detected chemicals might be decomposed into other chemicals, and almost 80% of number of detected chemicals might be decomposed during the four days deployment period. Even though, the bioassay toxicity of D4 was the highest (0.066) and its amount of adsorbed chemicals was 27.7 µg /3 disks. Whereas, more chemicals were detected by other deployment periods and showed almost same bioassay toxicity value (**Table 4.8**).

These observations revealed that longer deployment of SDB-RPS disks might be associated with desorption or decomposition of some adsorbed chemicals over the course of the deployment period. In addition, the deployment for one day might be applicable to show toxicity.

4.4 Conclusion

4.4.1 Selection of The Most Suitable Passive Sampler Disks

According to the field and laboratory experiments results it is cocluded that;

- Styrene-Divinyl Benzene Reverse Phase Sulfonated (SDB-RPS) disks showed higher adsorption efficiency comparing to other SDB disks.
- Using three passive sampler SDB-RPS disks to be deployed for at least three days in the water stream might be enough to achieve almost the same or close to adsorbed amount of chemicals as active sampling (10 liters water sample) via Sep-

pak® Plus PS-2 cartridges.

4.4.2 Investigation of Long Deployment of SDB-RPS Passive Sampler Disks

Chemicals eluted from GS, PS different period, and PS interval samples were used in toxicity tests and subjected to GC/MS analysis, with the following results:

- Chemicals eluted from GS samples showed strong toxicity at different folds ranging from 20- to 100-fold, , compared to the toxicity of those eluted from PS 7- and 10-day samples.
- Chemicals eluted from PS samples collected over a shorter period of time did not show any toxicity, especially for PS 3-day and PS 2nd samples even when their chemical amounts were higher than those of PS 7-day and 10-day samples, respectively.
- The amount of adsorbed chemicals in the PS 10-day sample did not represent an accumulation of the amounts of adsorbed chemicals in the PS interval samples, this support our assumption that chemicalas adsorbed onto SDB-RPS disks might be desorbed or decomposed.
- Accordingly, the application of SDB-RPS disks with 10-days cannot be considered to evaluate toxicity levels using medaka acute toxicity assay.

4.4.3 Investigation of Short Deployment of SDB-RPS Passive Sampler Disks

Chemicals eluted from PS different period samples were used in toxicity tests and subjected to GC/MS analysis, with the following results:

- The 4-day deployment period showed higher bioassay toxicity even the number of detected chemicals was the lowest. Whereas, more chemicals were detected by other deployment periods and showed almost same bioassay toxicity value.
- Almost all chemicals (80%) might be decomposed during the four days deployment period.
- Whereas, about 25% of chemicals only might be decomposed into other chemicals during the 1-day deployment period.

On the basis of our results, the overall conclusion is :

- Styrene-Divinyl Benzene Reverse Phase Sulfonated (SDB-RPS) disks showed higher adsorption efficiency comparing to other SDB disks.
- The chemicals adsorbed onto SDB-RPS disks might be subject to desorption or decomposition during the deployment periods along 10-, and 4-days.
- According to the GC/MS analysis and bioassay toxicity results, the application of SDB-RPS passive sampler disks for 1-day or shorter deployment might be considered to evaluate toxicity levels using medaka acute toxicity assay.

4.5 References

1. Al-Odaini NA, Zakaria MP, Yaziz MI, Surif S. (2010): Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A*, 1217, 6791–806.
2. Gunold R, Schafer B, Paschke A, Schüürmann G, Liess M. (2008): Calibration of the chemcatcher passive sampler for monitoring selected polar and semi-polar pesticides in surface water. *Environ. Pollut.*, 155, 52–60.
3. Ishii S, Urano K, Kameya T. (2000): General conditions for concentrating trace organic compounds in water with porous polystyrene cartridges. *J. Jpn. Soc. Water Environ.,* 23, 301–307.
4. Ministry of Finance, Democratic Republic of Timor-Leste, Dili, Timor-Leste. Demographic and Health Survey, Timor-Leste 2009–10.
5. Shaw M, Negri G, Fabricius K, Mueller JF. (2009): Predicting water toxicity: Pairing passive sampling with bioassays on the Great Barrier Reef. *Aquat. Toxicol.*, 95, 108–116.
6. Tan B, Hawker D, Müller J, Leusch F, Tremblay L, Chapman H. (2007): Comprehensive study of endocrine disrupting compounds using grab and passive sampling at selected waste water treatment plants in South East Queensland, Australia. *Environ. Int.,* 33, 654–669.
7. Vrana B, Mills A, Allan J, Dominiak E, Svensson K, Knutsson J, Morrison G, Greenwood R. (2005): Passive sampling techniques for monitoring chemicals in water. *Trends Anal. Chem*., 24(10), 845–868.

CHAPTER 05
CONCLUSION

5.1 Conclusions

1. Detected chemicals showed a difference in the usage between Timor-Leste and Japan. However, the toxicity levels in Timor-Leste were comparable or higher than in Japan.
2. Basins with higher population showed lower toxicity. The distance from the pollution source might affect the decomposition of toxic chemicals.
3. The chemicals adsorbed onto SDB-RPS disks might be subject to desorption or decomposition during the longer deployment periods.
4. According to GC/MS analysis and bioassay toxicity results, the application of SDB-RPS passive sampler disks with 1-day or shorter deployment might be considered to evaluate toxicity levels using medaka acute toxicity assay.

5.2 Future Work

Results of the deployment of SDB-RPS disks for 10- and 4-days showed that the adsorbed chemicals might be decomposed or desorbed even during 1 day deployment. Therefore, there is a need to investigate more shorter deployment periods that might not exposed neither to desorption nor to docomposition.