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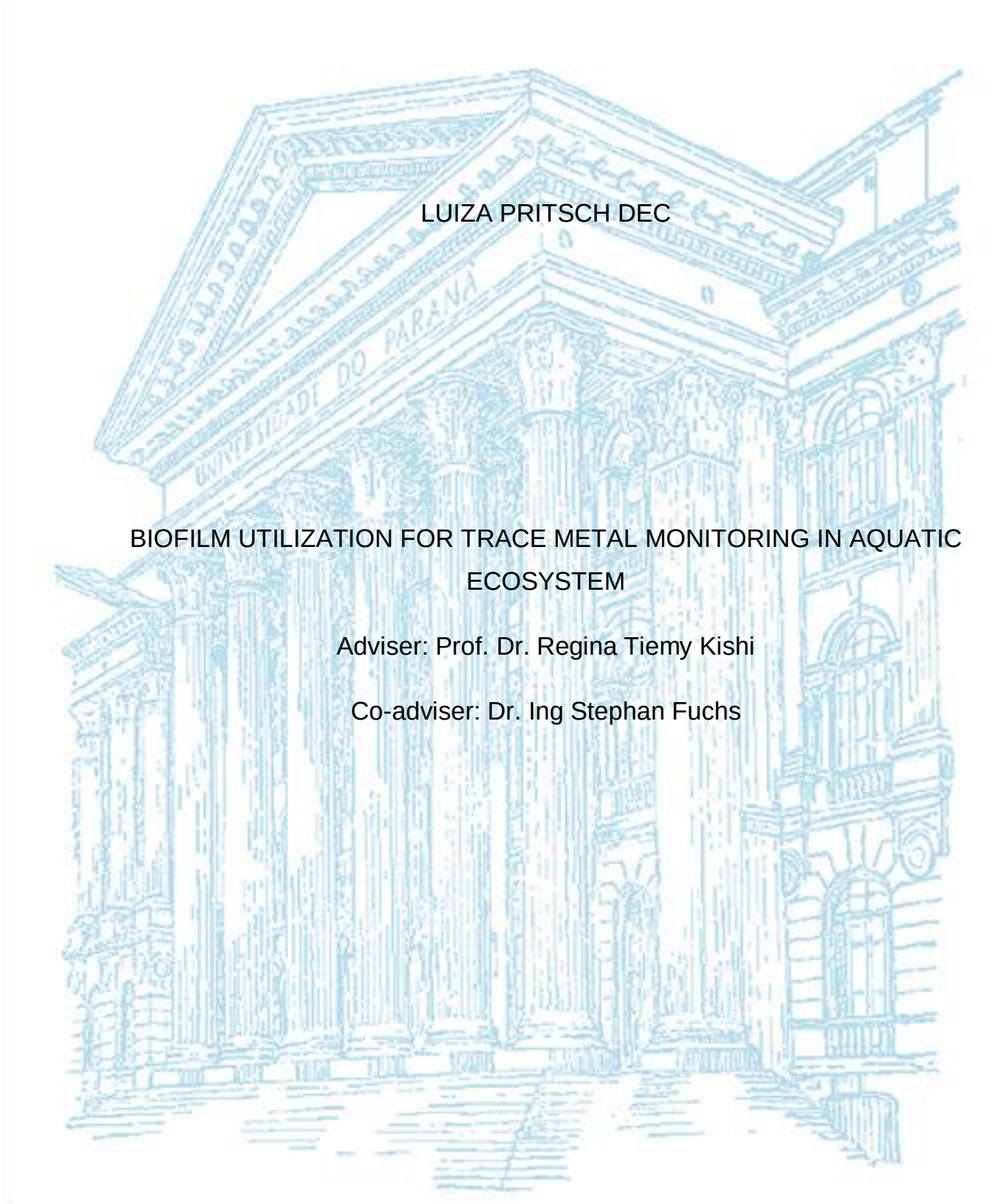

LUIZA PRITSCH DEC

BIOFILM UTILIZATION FOR TRACE METAL MONITORING IN AQUATIC ECOSYSTEM

Adviser: Prof. Dr. Regina Tiemy Kishi

Co-adviser: Dr. Ing Stephan Fuchs

CURITIBA

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Dissertation submitted as partial requirement for the Degree of Master at the Graduate Program in Water Resources and Environmental Engineering, Technology Sector, Federal University of Paraná.

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CURITIBA

2016

Dec, Luiza Pritsch
Biofilm utilization for trace metal monitoring in aquatic ecosystem / Luiza Pritsch Dec . – Curitiba, 2016.
85 f. : il.

Dissertation (master) – Federal University of Paraná, Technology Sector, Graduate Program in Water Resources and Environmental Engineering.
Advisors: Regina Tiemy Kishi, Stephan Funchs

1. Biofilm. 2. Metals – Environment . 3. Environmental monitoring I. Kishi, Tiemy. II. Funchs, Stephan. III. Title.

CDD 579.17

UFPR

PPGERHA

TERMO DE APROVAÇÃO

LUIZA PRITSCH DEC

"Biofilm Utilization for Heavy Metal Monitoring in Aquatic Ecosystem"

Dissertação aprovada como requisito parcial à obtenção do grau de Mestre, pelo Programa de Pós-Graduação em Engenharia de Recursos Hídricos e Ambiental do Setor de Tecnologia da Universidade Federal do Paraná, pela comissão formada pelos professores:

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Curitiba, 15 de março de 2016

ACKNOWLEDGMENTS

Primeiramente gostaria de agradecer à toda disposição, energia e saúde que me foi dada. Aprendi e evolui muito durante estes dois anos, tanto tecnicamente e profissionalmente como espiritualmente e psicologicamente.

Gostaria de agradecer à CAPES pelo apoio financeiro para que esta pesquisa pudesse ser realizada. Ao PPGERHA por todo o apoio intelectual e estrutural. Aos laboratórios LABEAM, LAMIR, LPH e Laboratório de Plantas e Nutrição pelo apoio técnico e estrutural. À Elisiane por ter sido tão querida e ter me ensinado todos os procedimentos no LAMIR.

Aos professores por todos os ensinamentos que me foram passados, em especial ao Professor Cristóvão e Sérgio que sempre me apoiaram nessa jornada. Ao Secretário Celmar, por estar sempre presente e pronto para ajudar, mesmo em momentos difíceis. Ao meu coorientador Fuchs e ao professor Hilgert por todo o apoio intelectual. A minha orientadora Regina que me guiou nesta etapa e mesmo sem recursos ou tempo fez com que tudo acontecesse.

Aos meus colegas e amigos, em especial à Adelita, Ellen, Ju, Lívia, Mayra, Pedro, Ricardo, Júlio e Robinson por todas as conversas acadêmicas e não acadêmicas. Ao Arthur, por toda a ajuda técnica e por sempre estar disposto a ajudar. Á Luciane, que se tornou uma grande amiga; ensinou-me muito no laboratório e também na vida, sempre disposta a aconselhar e ajudar. As minhas grandes amigas Ana e Carol, que estiveram ao meu lado em momentos bons e ruins, dizendo "coragem" e "vai dar tudo certo". As minhas melhores amigas Fa e Lis pela companhia e por entenderem os diversos momentos em que estive ausente.

A minha família: minha mãe, por sempre estar ao meu lado me apoiando. Ao meu pai, por fazer o mesmo à sua maneira de ser. Ao meu irmão, que apesar de todas as brigas e discussões sempre fez o melhor por mim. Aos meus bichinhos que trazem tanta graça à minha vida.

Finalmente, ao meu noivo Guilherme, por ser a pessoa mais compreensiva e querida da minha vida. Por estar ao meu lado quando eu estava estressada, feliz, triste, doente. Por ser o homem da minha vida e me apoiar em minhas decisões.

ABSTRACT

Trace metals are used in industries and agriculture and can be present in mining and sewer effluents. In such context, these elements can enter the environment and be very harmful to organisms, environment and people. A representative monitoring is essential for water resources management and, consequently, pollution prevention. Conventional water monitoring do not always show real environment condition. That happens because of effluent release conditions, pollution arrival system conditions, available technology to identify element concentrations and monitoring substance characteristics. Specifically for trace elements, they tend to adhere to other particles (suspended matter, soil, sediment, DOC) and deposit in riverbed. Other factor is that trace metal sources are usually from intermittent discharges and collections are not continuous. Besides that, usual techniques have high quantification limit and do not identify these elements in water. Thus, biofilm is an alternative monitoring technique for trace metals evaluation since it analyses contamination level in a time space. For this study, two biofilm samplers were constructed. Biofilm, water and sediment samples were collected for an eight month period in two monitoring sites, Barigüi and Miringuava watershed. Water quality parameters, granulometry, and trace metals content were estimated. Results showed that biofilm identified metals in almost every campaign, while water samples did not. On the other hand, sediment samples represented pollution but it was not possible to determine the contamination time by the used collection method. Biofilm also represented differences in soil use and occupation, representing consistent pollution potential for each basin.

Keywords: biofilm, trace metals, monitoring, Miringuava River, Barigüi River.

RESUMO

Os metais traço são utilizados na indústria e na agricultura e podem estar presentes em efluentes de mineração e esgoto. Assim, esses elementos atingem o ambiente e podem ser prejudiciais aos organismos, meio ambiente e às pessoas. Um monitoramento representativo é essencial para a gestão dos recursos hídricos e consequente prevenção à poluição. Monitoramentos convencionais da água algumas vezes podem não revelar a real condição do ambiente. Isto acontece devido às condições de lançamento e chegada dos poluentes ao sistema, à tecnologia disponível para quantificar a concentração e devido às características da própria substância monitorada. No caso dos metais traço, estes têm uma atração maior por outras partículas (sólidos suspensos, solo, sedimento, carbono orgânico dissolvido), as quais sedimentam, não permanecendo na coluna d'água. Outro fator a ser considerado é que as fontes de metais traço são geralmente intermitentes e as coletas são não contínuas. Além disto, os limites de detecção são altos e não detectam tais elementos na água. Desta forma, o biofilme é uma técnica alternativa de monitoramento, pois analisa o nível de contaminação em um intervalo de tempo. Para este estudo foram construídos dois amostradores. Amostras de biofilme, água e sedimento foram coletadas ao longo de oito meses para dois pontos de monitoramento, um na bacia do rio Barigüi e outro na bacia do rio Miringuava. Parâmetros de qualidade da água, granulometria e conteúdo de metais traço foram estimados. Os resultados mostram que o biofilme identificou os metais traço em praticamente todas as campanhas, enquanto que para as amostras de água isto não aconteceu. As amostras de sedimento representaram a poluição, porém não foi possível determinar o tempo da contaminação pelo método de coleta utilizado. O biofilme representou as diferenças no uso e ocupação do solo, representando poluição consistente com cada bacia hidrográfica.

Palavras-chave: biofilme, metais traço, monitoramento, rio Miringuava, rio Barigüi.

LIST OF FIGURES

LIST OF TABLES

LIST OF SIMBOLS

Al	Aluminium
ANA	National Water Agency (*Agência Nacional de Águas*)
Cd	Cadmium
COD	Chemical Oxygen Demand
CONAMA	National Council on the Environment (*Conselho Nacional de Meio Ambiente*)
Cr	Chromium
Cu	Copper
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EPS	Extracellular Polymeric Substances
Fe	Iron
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
H_2SO_4	Sulfuric Acid
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectometer
LABEAM	Borsari Neto Environmental Engineering Laboratory (*Laboratório de Engenharia Ambiental Borsari Neto*)
LAMIR	Mineral and Rocks Analysis Laboratory (*Laboratório de Análise de Minerais e Rochas*)
LOD	Limit of detection
LOQ	Limit of quantification

LPH	Hydrogeological Research Laboratory (*Laboratório de Pesquisas Hidrogeológicas*)
Mn	Manganese
Pb	Lead
TOC	Total organic carbon
UV	Ultraviolet Visible Spectrophotometer
Zn	Zinc

TABLE OF CONTENTS

1 INTRODUCTION

Water is an essential element to human survival; it is employed in public supply, trade, industries, electric power generation, agriculture, livestock, sanitation, and recreation. These activities together with accelerated population growth, high industrialization levels, and inappropriate or lack of wastewater treatment have been a source of unwelcome substances in the aquatic ecosystem (ESTEVES, 1998).

Within these substances, trace metals call attention because of their long-term effects (SAWYER; MCCARTY; PARKIN, 2003). Also, they are able to bioaccumulate along food chain and cause irreplaceable damage to aquatic biota and human health. Some of these damages are microorganisms procreation failure, abnormal fetus development, among others (ZHOU et al., 2008).

In order to enhance water resources quality and prevent its degradation, wastewater treatment, environmental awareness and other factors are required. Besides that, to carry out an appropriate and representative monitoring strategy for trace metal evaluation is crucial. Thereby, it is possible to evaluate water conditions, to develop scientific knowledge for pollutants removal techniques and to be able to control and preserve environment quality.

To select an appropriate monitoring technique it should be taken into account what is being analyzed, whether environment is already contaminated in a specific moment (with punctual samples) or being contaminated in a period of time. In other words, monitoring strategy should be planned thinking about its objective and how to achieve it. Likewise, understanding interest substances main characteristics like its concentration magnitude, pollution source (continuous or intermittent), and the processes it undergoes is important. Analytical measurement for trace metals is arduous because of their low concentrations in water (SAWYER; MCCARTY; PARKIN, 2003); further, they have intermittent pollution source in several places. In that way, a continuous environment analysis should be made. However, this sort of study demands plenty money and time (KISHI; FUCHS, 2008).

Thus, biofilm is one monitoring alternative for trace metal investigation in water due to its low cost and simple use. Fuchs et al. (1997) have been analyzing biofilm in Alb River on Germany and this research together with Kishi and Fuchs

(2008) state that biofilm analyzes contamination level in a time space, not only on the current situation. According to Ancion et al. (2010), biofilm retains trace metals quickly and release them slowly and is important in transferring trace metals between organisms in water column. Sediment and water analysis are also an option, but it is difficult to determine sediment representativeness, once it can be transported, like water that is in constant movement. In such manner, according to Zhou et al. (2008), biofilm provides the more integrated data on current or long-term time.

Other researches have been studying trace metals in Barigüi River since 2008 (GONÇALVES, 2008; KISHI; FUCHS, 2008; GOMES, 2010; CASTOLDI, 2014). Besides that, other studies were made in Bílina River in Czech Republic (KOHUŠOVÁ et al., 2011) and in several rivers in New Zealand (ANCION et al., 2013).

In such manner, water quality maintenance is essential to aquatic community survival. Besides, decent treatment and control of water and wastewater should be taken as crucial to decrease trace metals presence on environment (SAWYER; MCCARTY; PARKIN, 2003). Thus, biofilm monitoring is a practical and low cost option to identify trace metals in aquatic environment. In this way, to understand better biofilm behavior and its representativeness to show trace metal pollution during a specified period of time, this research covers monitoring of two different rivers of Curitiba Metropolitan Region.

1.1 OBJECTIVES

1.1.1 General Objectives

Considering that trace metals are present normally in concentrations under quantification limit in water for usual techniques, and presents discontinuous discharge, the objective of this work was to evaluate biofilm as a monitoring technique to quantify trace metals in aquatic environment.

1.1.2 Specific Objectives

- To collect biofilm that was formed in a determined time period and also collect sediment and water samples;
- To determine trace metals samples content;
- To evaluate biofilm formation through formed weight, flow, and trace metals content;
- To evaluate differences in land use and water quality considering trace metals content in biofilm.

2 LITERATURE REVIEW

2.1 TRACE METALS

Trace metals are usually found at low concentrations (ppm or less) (ESTEVES, 1998; SAWYER, MCCARTY, PARKIN, 2003; MANAHAN, 2005) and are mainly transported in association with fine particles (ANCION et al., 2013). In nature, they are usually found in concentrations that do not affect negatively the human health (SAWYER; MCCARTY; PARKIN, 2003; ANCION et al., 2013). Although, anthropogenic activities and urbanization have significantly increased metal concentrations in surface water (ANCION et al., 2013).

These elements are part of EPA (Environmental Protection Agency) priority pollutants. Among 126 toxic elements listed, 13 are trace metals, including Chromium (Cr), Copper (Cu), Zinc (Zn), Cadmium (Cd), and Lead (Pb) (USA. CODE OF FEDERAL REGULATIONS, 1982).

The following sections present how these elements get into environment and how they affect organisms, human beings, and ecosystem. Also, it is presents the main processes these metals suffer and some legal limits.

2.1.1 Sources

Substances sources are divided in point and diffuse sources. Point sources are single, readily identified entry point and tend to be from human activities. Diffusive sources are originated from large areas and harder to identify and monitor (MANAHAN, 2005). Besides that, these substances can be part of the natural environment or come from anthropogenic sources. Diffusive and point sources (natural or anthropogenic) for trace metals are illustrated in FIGURE 1.

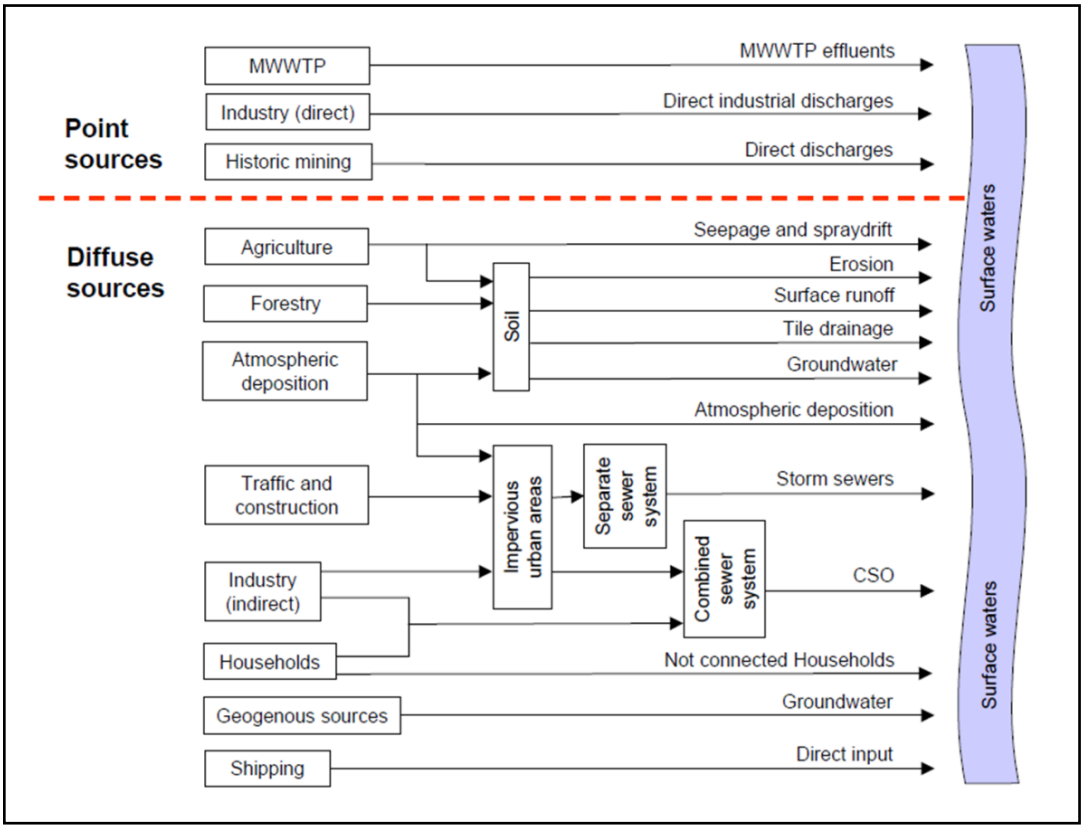


FIGURE 1 – DIFFUSE AND POINT SOURCES OF TRACE METALS
MWWTP - municipal wastewater treatment plants; CSO - combined sewer system
SOURCE: FUCHS et al. (2002)

Point sources cover municipal wastewater treatment plants (MWWTP), direct industrial discharges and direct discharges originated from mining activities (ESTEVES, 1998; FUCHS et al., 2002). Industrial wastes from manufacturing and metal-finishing operations are the main source of trace inorganic elements (SAWYER; MCCARTY; PARKIN, 2003).

Diffusive main natural source is rock weathering (ESTEVES, 1998). For example, iron is naturally found in soils and minerals generally as insoluble iron oxides and iron sulfide; manganese is found as manganese dioxide, insoluble in water (SAWYER, MCCARTY, PARKIN, 2003).

Diffusive anthropogenic pathway covers erosion, spray drift, tile drainage, and groundwater from agriculture (that uses agriculture pesticides containing Cd, Pb, Cu, and other trace metals). It also covers atmospheric deposition through the soil, which in some polluted regions can be the main source of trace metals into water. This can happen through natural burning, dust, and industrial emissions (ESTEVES, 1998; FUCHS ET AL 2002). Other diffusive sources are traffic and construction, not

connected household and indirectly industry waste, groundwater, and direct input from shipping.

Anthropogenic activities are the main sources of cadmium (ESTEVES, 1998). Some of them are (MANAHAN, 2005):

- industrial discharges;
- mining wastes release;
- metal plating for corrosion protection;
- batteries manufacture;
- paints and plastics uses.

Chromium is used to make (SAWYER; MCCARTY; PARKIN, 2003):

- alloys;
- refractories;
- catalyst;
- chromic oxide (plating industry);
- chromate salts (paints, laboratories).

Another important trace metal diffusive source is runoff from urban and rural areas. It is one of the main causes of superficial water quality deterioration. Some common concentrations found in urban runoff are zinc between 20 μgL^{-1} and 5000 μgL^{-1}, Cu and Pb between 5 μgL^{-1} and 20 μgL^{-1}, and Cd bellow 12 μgL^{-1} (GONÇALVES, 2008).

Historically, the first registered trace metal to become a problem was lead (SAWYER; MCCARTY; PARKIN, 2003), first used to build service pipes and still present in water supply systems on pipe connectors. Lead is also present on industry, mining and gasoline, being expelled as lead oxide to the atmosphere. After, Pb, Zn, and Cu became a problem; they started being used for galvanized services. Besides that, until today old copper pipes corrosion has released substance to environment (SAWYER; MCCARTY; PARKIN; 2003; MANAHAN, 2005).

2.1.2 Health and Environmental Effects

Some trace metals are essential to organisms. For instance, Cu, Iron (Fe), Manganese (Mn), and Zn are crucial for nearly all bacteria and fundamental for enzyme activity (SAWYER; MCCARTY; PARKIN, 2003). Likewise, Fe, Zn, and Cu

have an important role in metabolism (ESTEVES, 1998; MANAHAN, 2005). Additionally, Fe and Cu participate in respiratory chain (ESTEVES, 1998).

On the other hand, all these metals are toxic in higher concentration. For example, 1 ppb of any organomercury is enough to reduce phytoplankton growth by half. There is no growth at all above 50 ppb (ESTEVES, 1998). Several harmful effects have been shown due to aquatic metal exposure. Some of them are procreation failure associated to lead (MANAHAN, 2005), fetus abnormal development, and immunodeficiency (ZHOU et al., 2008).

Additionally, mercury, cadmium, and lead can cause kidney damage. Chromium can cause allergic dermatitis, skin disorders, and liver damage. Copper can cause kidney, lung, and gastrointestinal problems (SAWYER; MCCARTY; PARKIN, 2003; WHO, 2004; MANAHAN, 2005). Lead is highly toxic and carcinogen causing brain and central nervous system damage, high blood pressure, and red blood cells destruction (SAWYER; MCCARTY; PARKIN, 2003; MANAHAN, 2005).

As they are persistent in environment, when they are absorbed by organisms they become part of the food supply to higher trophic level organisms and therefore contaminate the whole food chain.

2.1.3 Physical, Chemical and Biological Processes in Surface Waters

The understanding of physical, chemical, and biological processes is crucial to evaluate trace metals in surface waters. For instance, it should be comprehended if elements will stay in dissolved or particulate form after entering a system, to which element they tend to connect with, and what chemical processes they will suffer. This is relevant because a considerable contaminants proportion can be transported in association with solid particles. This happens because metal ions bind easily with suspended particles like silt, clay, and organic matter (ANCION et al., 2013).

After reaching a water body, toxic substances are portioned into particulate and dissolved fractions in water and sediment compartments, depending on its charge and element type (CHAPRA, 1997; ESTEVES, 1998). Whether toxic contaminants are in dissolved or in particulate form depends on partition coefficient values and suspended solids concentration. This coefficient, called partition or distribution coefficient (Kd), expresses relation between the quantity of an element adsorbed by solid mass and quantity that remains in solution. When Kd>1, elements

prefer to adsorb with solid phase. When Kd<1 most, elements concentration are in solution (BRAZ, 2011). Some trace metals partition coefficients are presented in TABLE 1.

TABLE 1 - PARTITION COEFFICIENT FOR SOME TRACE METALS

Trace Metal	Soil / Water	Suspended matter / Water	Sediment / Water	DOC / Water
Cadmium	2.9	4.7	3.6	5.2
Chromium III	3.9	5.1	4.5	-
Chromium IV	1.1	-	-	-
Copper	2.7	4.7	4.2	5.5
Lead	4.2	5.6	5.1	5.0
Zinc	3.1	5.1	3.7	4.9

SOURCE: Adapted from ALLISON AND ALLISON (2005)

As it can be seen in TABLE 1, in all cases (soil - suspended matter – sediment – DOC / water) Kd>1, so these metals have more affinity with organic phases than with water. In that way, trace metals will be more associated to soil, suspended matter, sediment or DOC than to water. As an example: at Elbe River in Germany, trace metals concentration in sediment can be 1000 times greater than in water (ESTEVES, 1998).

As shown in TABLE 1, cadmium is more likely to be present in DOC or in suspended matter than in water; in water it is present as +2 oxidation state. Cadmium experience geochemical processes together with Zinc (MANAHAN, 2005), in that way most part of cadmium is produced as a sub product of zinc fusion (BAIRD, 1995). Cadmium is adsorbed by suspended solids and carried out, having a great mobility; it can also become incorporated with sediment (MANAHAN, 2005). As for chromium, its soluble form is chromate (Cr (IV)); it's essential form is Cr (III) and it is hydrolyzed and precipitates (SAWYER; MCCARTY; PARKIN, 2003; MANAHAN, 2005). Lead is inorganic with charge +2 (MANAHAN, 2005) and as other presented metals, have more affinity with suspended matter than with water.

Between dissolved and particulate forms, sorption can operate. Sorption is one substantial process for contaminants transport, it happens when contaminants concentration goes from one phase to another (SAWYER; MCCARTY; PARKIN,

2003). For weakly sorbing contaminants and environment with low suspended solids concentration, the dissolved contaminant form will be predominant. On the other hand, for strong sorbers and in turbid environment, contaminant will be more associated with suspended solids (CHAPRA, 1997).

When contaminant is partitioned from one phase to another, process is called adsorption. This mechanism can raise trace metals concentration in organisms from 10^2 to 10^6 times higher than in the water (ESTEVES, 1998). Adsorption is a phenomenon that depends on particle surface area. There are physical, chemical, and exchange adsorption. Physical adsorption is reversible and happens as a result of weak attraction forces (van der Waals' force between molecules). In this case molecules are free to move over the surface. Also, these molecules can form superimposed layers. Chemical adsorption involves stronger forces and is not easily reversible. Under this circumstance, molecules form one molecule thick layer and are not free to move. This process can be reversed with high temperatures. Finally, exchange adsorption refers to electrical attraction in which ions exchange happens. Substance and surface are attracted when there is opposite charge and ions with higher charge are attracted strongly (SAWYER; MCCARTY; PARKIN, 2003).

Another meaningful process is metal speciation; it is a decisive step in environmental chemistry because metal properties rely upon the characteristics of metal species dissolved in water. A metal atom can bond to neutral or negatively charged ligands and become a complex (neutral, positively or negatively charged). When it is reversibly bounded to organic compounds or inorganic anions it becomes a metal complex. Metals can be solubilize or insolubilized by complexation, changing metal oxidation state. Metal ions can be removed from solution by the formation of insoluble complex compounds. Also, complexation affects metal bioavailability, toxicity, adsorption, distribution, and transport (MANAHAN, 2005).

In aquatic ecosystems, metal ions react chemically to become stable (MANAHAN, 2005) and sometimes more dangerous, like mercury that is transformed to methyl mercury (ESTEVES, 1998). Among these reactions are acid base, precipitation, and oxidation-reduction. Also, two or more metals can be bonded through OH^- and precipitate; some of these metals are Fe (III), Cu (II), and Pb (II) (MANAHAN, 2005).

Inside the system elements can suffer several processes. These mechanisms are shown in FIGURE 2, where a mass balance for trace metal in a

water-sediment system is presented. One important process is diffusion; it can transport substances between dissolved fraction in water and in sediment pore water (CHAPRA, 1997).

For dissolved and particulate fractions also acts settling and burial mechanisms. Settling occurs when elements are transported from water to sediment. These substances can return to water and be available, as a result of wind action through resuspension. Within sediment system substances do not move physically and are buried in it (CHAPRA, 1997).

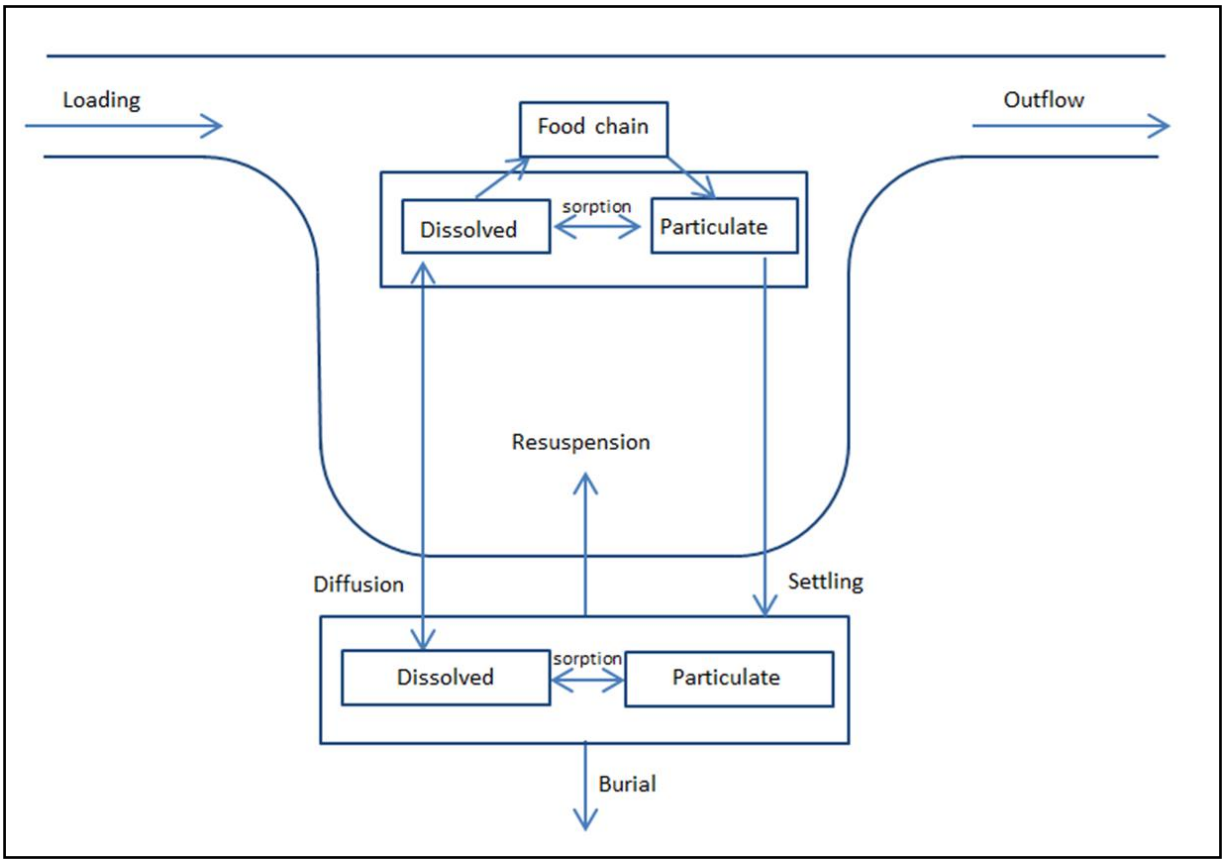


FIGURE 2 – TRACE METAL PROCESSES IN A WATER BODY
SOURCE: Adapted from CHAPRA (1997)

Sediment has a high capacity to accumulate compounds, being organics (insecticide and herbicide) or inorganics (trace elements) (ESTEVES, 1998). Clays, organic (humic) substances, and complexes between these two are the most frequent sediment type to bond with organic matter. Also, this bonding is not strong because of weak anion exchanges sites. That way, compounds are rather mobile and biodegradable (MANAHAN, 2005). Vertical distribution is also important to

demonstrate sediment contamination, once it reveals anthropic impacts evolution in a region (ESTEVES, 1998).

There is also interaction between environment and organisms, as presented in FIGURE 3. Organisms can be correlated with suspended matter, pore water, sediment, surface water, groundwater, soil, vegetation, and anthropogenic activities. Organisms can incorporate metals, but it depends on the portion of elements that are available for their use, it depends on metal bioavailability. Not necessarily all metal concentration is available for organisms. Bioavailability depends on many factors as pH, temperature, total organic content, suspended particle content, water velocity, among others (JOHN; LEVENTHAL, 1995).

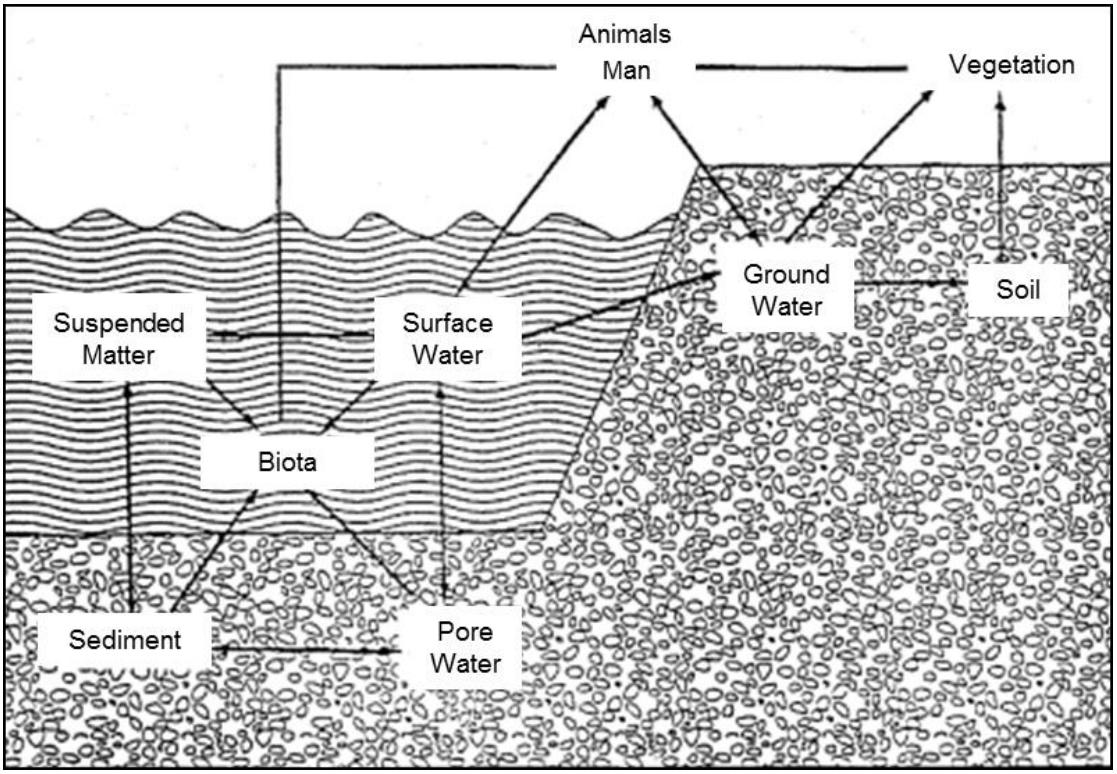


FIGURE 3- RELATION BETWEEN AQUATIC ENVIRONMENT AND ORGANISMS
SOURCE: Adapted from JOHN, LEVENTHAL (1995)

An organism can receive a contaminant from sediment when it is released to water or through a biological membrane as shown in FIGURE 4. This scheme represents trace metal transference between sediment and water, also between sediment and/or water with biota in a general way.

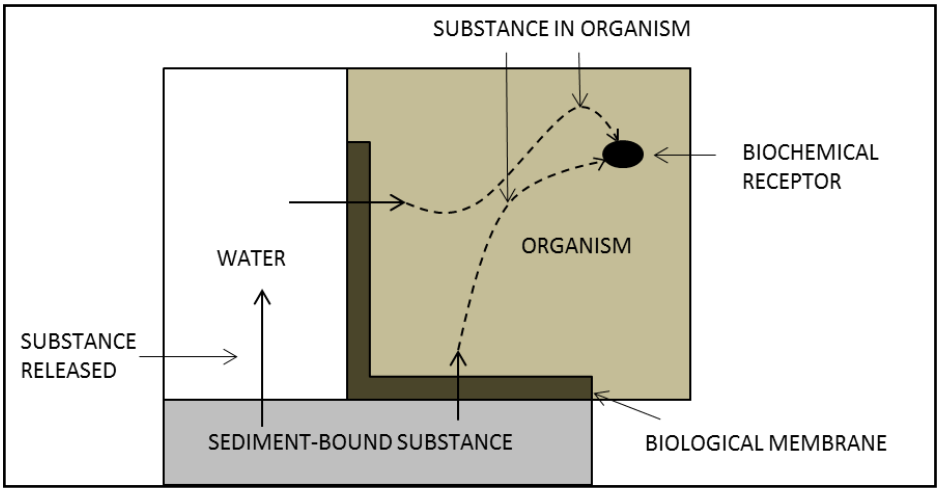


FIGURE 4 - CONTAMINANTS TRANSFER FROM SEDIMENT TO ORGANISMS
SOURCE: adapted from MANAHAN, 2005

Contaminants transfer from sediment to organisms may have toxic effect when substance reaches organism biochemical receptor and traverses gastrointestinal wall.

Trace metals can return to water through organism excretion or mainly through microbial organic waste decomposition. Also, organic waste part reaches sediment before it is completely broken down and can return to water column under specific conditions through resuspension (ESTEVES, 1998).

Another important fact to be analyzed is trace metal availability; it depends on dissolved organic matter concentration (ESTEVES, 1998). Interaction between organic compounds, suspended material, and sediment can be meaningful for organic pollutants transport in surface waters. For instance, an important source of herbicides in sediment is organics that carry contaminants (MANAHAN, 2005).

In addition to those processes, there is seasonal variation, during drought some elements can be mineralized; during floods elements return to solution in greater magnitude. Also, geological and ecological characteristics of drainage basin and human activity style affect total trace metals load (ESTEVES, 1998). Besides, some metals like mercury can volatilize.

Trace metals can present harmful effects to whole environment. In order to preserve organism's life all these presented processes must be understood. These substances suffer many modifications and are influenced by climatic changes, water quality, chemical reactions, physical changes, and biota. Thus, trace metals monitoring must be done having in mind all those influences for it to be

representative. Single water samples generally do not represent actual environment situation and other techniques should be used to show ecosystem's reality.

2.1.4 Legal Limits

In Brazil, limits for metal concentrations in drinking water are given by the Portaria 2914/2011 (BRASIL, MINISTÉRIO DA SAÚDE, 2011), in European Union by the Directive 80/778/CEE (EUROPEAN UNION, 1980), in United States by the EPA (UNITED STATES, ENVIRONMENTAL PROTECTION AGENCY, 2009), and by the World Health Organization (WHO, 2004). TABLE 2 presents some of these values.

TABLE 2– DRINKING WATER METAL LIMITS

Metal (mgL^{-1})	Brazil	WHO	US EPA	EU
Cd	0.005	0.003	0.005	0.005
Cr (total)	0.05	0.05	0.1	0.05
Cu	2.0	2.0	1.3	3.0*
Pb	0.01	0.01	0.015	0.05
Zn	5.0	3.0	5.0	5.0*
Al	0.2	0.1/0.2**	0.2***	0.2
Fe	0.3	-	0.3	0.2
Mn	0.1	0.4	0.05	0.05

*Limit over which astringent flavor is noticed. **Guidelines for small and large water treatment facilities respectively. ***Value measured in milligrams of substance per liter of water.
SOURCE: Adapted from BRASIL, MINISTÉRIO DA SAÚDE (2011); EUROPEAN UNION (1980); UNITED STATES, ENVIRONMENTAL PROTECTION AGENCY (2009); WHO (2004)

National Council on the Environment (CONAMA) Resolution number 357/2005 (BRASIL, MINISTÉRIO DO MEIO AMBIENTE, 2005) classifies fresh water bodies in five categories: special class, class one, two, three, and four; being class four, the worse water quality classification. Brazil limits are equal to other countries for several elements, like Cd, Zn, and Aluminium (Al). In some cases the limit is even lower than in other countries, for example for Cr, Cu, and Pb.

Limits for some parameters are shown in TABLE 3. Dissolved oxygen established values for class one, two, and three are 6 mgL^{-1}, 5 mgL^{-1}, and 4 mgL^{-1} respectively. The pH value settled for classes one, two, and three is from six to nine.

TABLE 3– WATER QUALITY PARAMETERS LIMITS FOR EACH CLASS

	Categories limit		
Metal (mgL^{-1})	**Class 1**	**Class 2**	**Class 3**
Cd	0.001	0.001	0.01
Cr	0.05	0.05	0.05
Cu*	0.009	0.009	0.013
Pb	0.01	0.01	0.033
Zn	0.18	0.18	5.0
Al*	0.1	0.1	0.2
Fe*	0.3	0.3	5.0
Mn	0.1	0.1	0.5

*In dissolved form.
SOURCE: Adapted from BRASIL, MINISTÉRIO DO MEIO AMBIENTE (2005)

According to CONAMA Resolution number 430/2011 (BRASIL, MINISTÉRIO DO MEIO AMBIENTE, 2011) concentration limits for effluent discharge are for Cd 0.2 mgL^{-1}, for Cr 1.1 mgL^{-1} (Cr^{+3} + Cr^{+6}), for Cu 1.0 mgL^{-1} (dissolved), for Pb 0.5 mgL^{-1}, and for Zn 5.0 mgL^{-1}.

Regarding soil quality, CONAMA Resolution number 420/2009 (BRASIL, MINISTÉRIO DO MEIO AMBIENTE, 2009) establishes limits for substances in the soil. Values for some metals are shown in TABLE 4. Values for Al, Fe, and Mn were not established by this Resolution.

TABLE 4– SOIL LIMITS FOR EACH SOIL USE

	Zone		
Metal ($mgkg^{-1}$)	**Rural**	**Residential**	**Industrial**
Cd	3	8	20
Cr	150	300	400
Cu	200	400	600
Pb	180	300	900
Zn	450	1000	2000

SOURCE: Adapted from BRASIL, MINISTÉRIO DO MEIO AMBIENTE (2011)

These are the acceptable concentration in soil for different land use. Rural zones present the lowest values for trace metals while industrial zones have the highest limits.

2.2 FIELD MONITORING

Field monitoring is a substantial step to evaluate water resources conditions (to comprehend environment processes and to validate hypothesis) and to be able to control and preserve environment quality. It must be planed having in mind its objective and in regard to the selection of the adequate sampling strategy. Usually, most water samples are taken punctually and instantaneously, resulting in a punctual sample at a specific time evaluation. In this context, intermittent discharges may not be recorded adequately. Consequently, it is important that quantification level corresponds with trace metal concentrations found in water. Composite sample systems can be used, although if there is no adequate place available to install it, the technique becomes unfeasible. In addition to it, this is a complex and expensive system comparing to other sampling strategies.

According to Zhou et al. (2008), water and sediment chemical analysis are the most direct approach to show trace metal pollution status on environment. If the objective is to evaluate long term environmental conditions, sediment monitoring can be a good method. On the other hand, if the interest is to monitor contamination in a specific period than water or sediment cannot be adequate (KISHI; FUCHS, 2008).

Sediment and water samples do not consider the impact on organisms and on ecosystem. According to Fuchs et al. (1997), trace metal quantification, based on sediment analysis has some disadvantages. They are: amount of sample needed, time-consuming procedure and needed equipment. Besides, it is difficult to identify from which period the sediment was sampled. Also, depending on the river, it is not possible to take sediment samples due to high depth or rocky bottom. Sometimes the environment does not present fine sediment on bottom, so metal analysis is not manageable. Thus, metal analyses associated with biofilms can be a more relevant indicator of potential risk to aquatic organisms than metal analyses in sediments (ANCION et al., 2013).

According to Kishi and Fuchs (2008), biomonitoring depends on organisms mobility. Thus, if the objective is to determine spatial water quality and source of pollution location, this fact should be taken in consideration. In addition, if the intent is to represent the contamination temporally, it should be taken in mind that bioaccumulation rate changes depending on the age of the organism. Although, when compared with conventional chemical analysis of aquatic environmental matrix, biomonitoring has some advantages as well as biofilm.First, it identifies pollutants monitoring at low levels which were below quantification limits of instrumental analytical techniques. Second, it avoids conventional analysis limits such as continuous sampling, expensive instruments needs. So, microbial community can be used as a viable ecological indicator because of its high sensitivity and prompt response (ANCION et al., 2010).

According to Fuchs, Haritopoulou and Wilhelmi (1996), some other biofilm technique advantages are that the method does not need complex equipment. Besides that, the results are of ecologically relevance and can be replicated for a meaningful sample quantity. Also, biofilm technique can be applied under different river conditions like flow dynamics and substrate types. Hence, biofilms constitute an integrative metal contamination indicator that occurs over a period of days or weeks (FUCHS; HARITOPOULOU; WILHELMI, 1996; KISHI; FUCHS, 2008; ANCION et al., 2010).

2.3 BIOFILM

Biofilm covers practically every surface in freshwater environments (ANCION ET AL,. 2013). According to Fuchs, Haritopoulou, and Wilhelmi (1996), the main biofilm characteristic is their ability to absorb and incorporate material. They are a microbial community with plenty “inhabitants” such as sessile bacteria, protozoa, fungi, and algae.

According to Flemming (2002), biofilms are the most successful form of life on earth tolerating almost any environmental condition, for instance, high biocide quantities. They grow in every environment with enough moist, nutrient flow and when surface attachment is accomplished (SINGH; PAUL; JAIN, 2006). Microbial

layers development on surfaces is sometimes an undesired phenomenon (biofouling) and can cause problems in ship hulls, drinking water reservoirs and distribution systems. Although, they can have useful effects like self-purification of running waters and toxic materials removal (MAGES et al., 2004).

Biofilms behave as gels with constant partner groups responsible for adhesion. Micro colonies number and sizes vary from one biofilm to another and also from one location to another within the same biofilm (LEWANDOWSKI; BEYENAL, 2003). According to Singh, Paul, and Jain (2006), biofilms are microenvironments and its processes must be understood in order to understand its dynamics. It should be noted how biofilms grow and detach, and how organisms absorb and release elements.

2.3.1 Physical, Chemical and Biological Processes

Biofilm formation is an important process to be understood, so metal input, fixation and output is considered. Since its formation, biofilm is subject of physical, chemical and biological processes. The first step for biofilm formation is initial attachment. Then, it involves layers of cells formation, attachment, bioconversion, and biofilm detachment. Formation begins with initial attachment within seconds by physic–chemical interactions or by extracellular matrix protein secretion (SINGH, PAUL, JAIN, 2006) as shown in FIGURE 5.

After initial attachment, cells form a monolayer where they gain some mobility and proliferate within minutes or hours. Then, other microbes attach and an active biofilm is formed. At this point, development and distortion are affected by environmental factors (hydrodynamic and mechanical stress). After some days a mature biofilm is formed where cells are motile and undergo chemotaxis[1] (spreading biomass and transferring genes) (SINGH; PAUL; JAIN, 2006).

After days or weeks, cells die and bioconversion and/or biodegradation leads solute transfer to or from bulk liquid[2] (SINGH; PAUL; JAIN, 2006). According to Kohušová et al. (2011), when a biofilm decomposes, it can release sorbed

[1] Movement of an organism in response to a chemical stimulus (SINGH, PAUL, JAIN 2006)
[2] Liquid between cells in biofilm (SINGH, PAUL, JAIN 2006)

substances. While releasing these substances, it can detach. Formation and detachment happens in a cycle (SINGH; PAUL; JAIN, 2006).

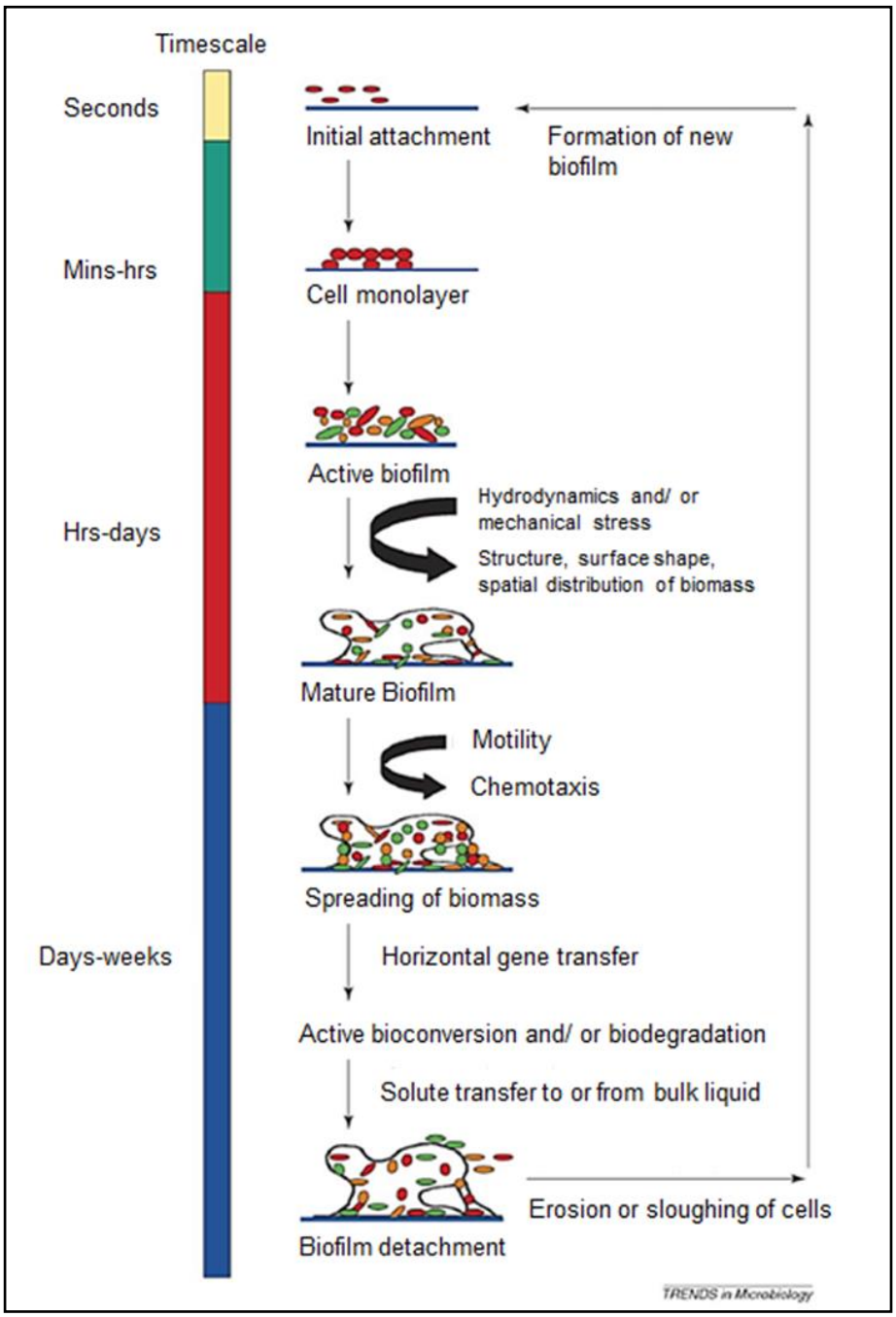


FIGURE 5 – BIOFILM FORMATION STEPS
SOURCE: Adapted from SINGH; PAUL; JAIN, (2006)

Organism is embedded in EPS - extracellular polymeric substances (a matrix of microbial origin), which can bind metal ions. These ions can also be bound to cytoplasm (MAGES et al., 2004) as can be observed in FIGURE 6 (bacterium structure). Biofilms have various properties that promote metal ions binding and it

can be very important to introduce them to higher trophic levels (ANCION et al., 2013).

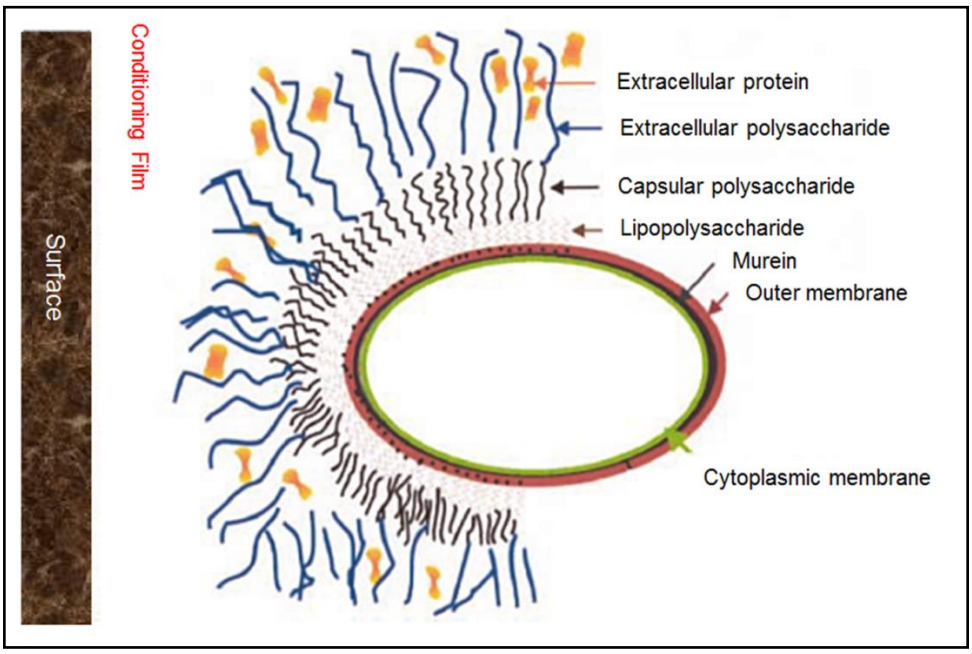


FIGURE 6 – BACTERIUM ENCOUNTERING A SURFACE IN WATER
SOURCE: Adapted from FLEMMING (2002)

Biofilm growth intensity is ruled mainly by nutrients availability and by shear forces (FLEMMING, 2002). It is also controlled by some environment properties, such as pH variations, oxygen availability, and nutrients and bacterial metabolites. These properties cause great difference in biofilm formation. Although, EPS provides resistance against shear forces, nutrient deprivation, pH changes, and antibiotics (SINGH; PAUL; JAIN, 2006). Even though, if velocity is higher than 3 ms^{-1} biofilm can be detached (CLOETE et al., 2003).

Biofilm is formed of single bacterial species or multiple populations' aggregation (many species of bacteria, fungi, algae, and protozoa). It is a microheterogeneous matrix due to microenvironments diversity that coexists in it. Also, it is formed of water (97% of biofilm matrix), microbial cells, secreted polymers, absorbed nutrients and metabolites, particulate material, and detritus from environment (SINGH; PAUL; JAIN, 2006).

Within the biofilm, substances are transported through diffusion processes. These processes depend on water-binding capacity and biofilm mobility. Solute transportation is conducted by diffusion in denser aggregates and by convective transport inside pores and water channels. Proteins and other cell surface properties

are important to organisms' attachment; and substrates availability influence biofilm structure which affects mass transport in biofilms. Also, these properties supply a competitive advantage to some organisms when there is a mixed community (SINGH; PAUL; JAIN, 2006).

Within biofilm also happens bioaccumulation that according to Zhou et al. (2008) occurs when an organism absorbs a toxic substance at a range greater than that at which substance is released. Thus, bioaccumulation results from a dynamic equilibrium between exposure from outside environment and uptake, excretion, storage, and degradation within organism.

2.3.2 Biofilm researches

Some biofilm development researches results are presented in TABLE 5. Fuchs, Haritopoulou and Wilhelmi (1996), and Fuchs et al. (1997) analyzed biofilm in three sites in Alb River comparing sediment and suspended solids data. For sediment analysis, fraction bellow 63 µm were used. Site one and two are presented in this work. Site one refers to an agriculture area and site two refers to a low urbanization area. Avarage flow for Alb River is 3.4 m^3s^{-1}. Metal content was higher at the highly degraded site than in the preserved site for the samples. Between three metals analyzed (Cd, Cu, and Pb), cadmium had the lowest concentration from all trace metals analyzed (bellow 1.9 $mgkg^{-1}$). Between samples and sites, copper varied between 25.0 $mgkg^{-1}$ and 73.3 $mgkg^{-1}$, and lead varied between 18.0 $mgkg^{-1}$ and 118.4 $mgkg^{-1}$. For suspended solids, Pb and Cu concentrations were higher at higher urbanisation level site.

Mages et al. (2004) investigated trace metal accumulation by natural biofilms living in catchment area of Tisza River in Hungary and biofilms cultivated in vitro. River was contaminated in 2000 by a gold mine wastewater accident and collection happened two years later. Biofilms were collected from biofilm supports in nature, like wood, stone, rubber, aluminium, and painted metal. Sample weight was under 1 mg, so sample homogeneity could not be guaranteed. Results chosen to be presented in TABLE 5 are for biofilm from stone on a site in Tisza River downstream dam broken. In vitro tests results showed that biofilms have an affinity for Cu and its bioaccumulation is rather fast. Hence, it may cause a very rapid uptake into food

chain if an environmental disaster occurs. Concentration differences up to two orders of magnitude were found between polluted and background sites.

Chalmers et al. (2007) evaluated trace metal (Cd, Cr, Cu, Pb, and Zn) in sediment and concluded that metal concentrations doubled when rural sites becomes suburban, and concentrations tripled when a suburban becomes urban.

Gonçalves (2008) analyzed trace metals content at Barigüi River in Almirante Tamandaré and collected water and sediment samples. This sampling site is the same one analyzed in this work. Metal concentration in water samples were bellow detection limit except for Cr in one campaign (0.02 mgL^{-1}); flow varied from 1.6 m^3s^{-1} to 3.8 m^3s^{-1}; and highest DO (dissolved oxygen) was 8.75 mgL^{-1}. According to Gonçalves (2008), low DO and pH can solubilize metals in water: as an example, for Cd and Zn when pH is lower than 4.0 and for Cr and Pb when pH is lower than 5. Total dissolved solids were high (300 mgL^{-1}), conductivity varied from 0.1 $mScm^{-1}$ to 0.32 $mScm^{-1}$, turbidity varied from 4 NTU to 7 NTU, and COD (chemical oxygen demand) was below 10 mgL^{-1}.

According to Gonçalves (2008), at Barigüi monitoring site silt was the most present sediment size. Correlations were high for pH and Cr (r=-0.92) only. Other correlation were Cr and DO (r=-0.66), and Cr and turbidity (r=0.47). Pearson coefficient was good for Cr and poor for Zn. The author concludes that urban area and metal concentration rises along basin. The author recommends to analyze also TOC (total organic carbon).

Ancion et al. (2010) exposed mature biofilm to different concentrations of zinc, copper, and lead for various period of time (1, 3, 5, 7, 14, and 21 days) in flow chamber microcosms. Biofilm samples were digested by hot HNO_3 in different concentrations, and then values were assessed using Flame Atomic Adsorption Spectrophotometry. Results showed that differences between exposed and unexposed populations to metal became significant within three days of exposure (rapid changes in bacterial community structure). In the same study, a recovery rate was analyzed, so after returning biofilm (already contaminated) to uncontaminated water, metals were released steadily but at a slower rate than they accumulated. Microbial community structure was affected by both high and low trace metal concentrations. Through exposition, bacteria diversity diminished and it was necessary only a few hours or days for metal to reach biofilm fixation bottom layer.

The relation between metal concentration in water and in biofilm obtained from Ancion et al. (2010) is presented in FIGURE 7. In this laboratory experiment, up to 1000 μgL^{-1} of Zn, 100 μgL^{-1} of Cu and 100 μgL^{-1} of Pb were added to water and trace metals concentrations were measured after 21 days of exposure. Zinc had the highest levels of concentration, both in water and biofilm. Between copper and lead, biofilm had more affinity with lead. Analyzing biofilm metal concentration the author observed that biofilm reaches equilibrium around 14 days after initial exposure. That was observed for three concentrations applied in different experiments in a steady state system.

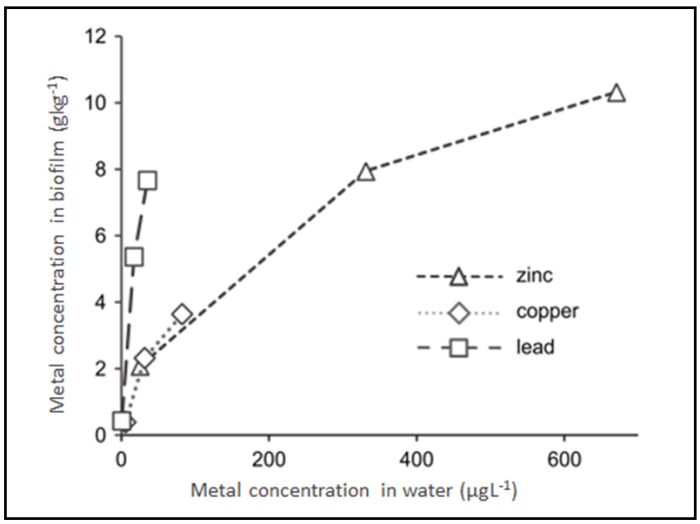


FIGURE 7 – CONCENTRATIONS OF TRACE METALS IN WATER AND IN BIOFILM
SOURCE: ANCION et al. (2010)

Biofilms can be used as well to evaluate faecal bacteria. Balzer et al. (2010) scraped biofilms from submerged stones and analyzed its bacteria content. Biofilm dry weight was 15.1%±9.1% indicating that its main content is water. Results showed that coliforms were more present at biofilm than in water and for *Escherichia coli* specifically its concentration was higher in sediment than in water.

Kohušová et al. (2011) investigated trace metals in biofilm, sediment, and surface water in Czech Republic in four sites of Bílina River (from less polluted to highly polluted areas) from 2004 to 2007. These results are presented in TABLE 5 for site one (a low urbanized area) and for site two (a highly industrialized area). Bottom sediments have similar properties as biofilms regarding cycling and pollutants fate in aquatic environment. Mixed biofilms samples were scraped off submerged surfaces

by a brush and spatula. Sediment sampling was about 10-15 cm depth from surface. Trace metals were determined by ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometer). Surface water reflected existing point and non-point pollution sources.

In Kohušová et al. (2011) research there was a relationship among pollutants concentration in biofilm and in sediments. Pollutant concentrations in these solid matrices differed from concentrations detected in water by three to six orders of magnitude. Comparing to dates from 1994 to 1996, pollutants concentration on surface water have decreased, but load to river ecosystem remains high as a consequence of long-term cumulative pollutants effect in solid matrices (biofilm and bottom sediments). Metal concentrations are still high due to anthropogenic load. Contamination in sediment and biofilm reflects existent pollution in the river. Some metals showed seasonal variation. Biofilm were correlated with sediment metal concentration.

Ancion et al. (2013) researched 23 sites across Auckland Region in New Zealand, from native forest to exclusively urban catchments. Biofilm was sampled from at least three rocks, totalizing an area of 100 cm^2, samples were from rocks collected within 30 m section of the stream. Presented values are from site one with basin area of 270 km^2 and covered with 100% forest; average water quality values are DO (dissolved oxygen) 10.1 mgL^{-1}, conductivity 16.8 $mScm^{-1}$, pH 7.7, and turbidity 2.5 NTU. Site two has 1647 km^2 basin area and is composed with 83% of forest and 17% of agriculture; average water quality values are DO 9.5 mgL^{-1}, conductivity 13.9 $mScm^{-1}$, pH 7.5, and turbidity 5.6 NTU. Site three has 334 km^2 basin area and is composed with 89.5% of urbanized area, 5.6 % of agriculture and 4.9% of forest; average water quality values are DO 7.3 mgL^{-1}, conductivity 51.4 $mScm^{-1}$, pH 7.5, and turbidity 14.0 NTU.

Ancion et al. (2013) results showed that concentrations of zinc, copper, and lead in both biofilm and sediment increased along urbanization gradient. Zinc and copper were higher in biofilm than in sediment. The author also analyzed weight loss during acid digestion. Results suggest that biofilms contain more organic matter and retain smaller inorganic suspended particles than sediment. Concentrations measured in biofilms can explain a greater proportion in variations observed in bacterial communities than concentrations measured in sediments.

TABLE 5 – SUMMARY OF TRACE METAL CONCENTRATIONS STUDIES

Sample	Place	Site	Cd	Cr	Cu	Pb	Zn	Source
Biofilm ($mgkg^{-1}$)	Alb River/ Germany	1	1.9	-	57.1	54.1	-	Fuchs et al. (1997)
		2	1.3	-	73.3	59.5	-	
	Tisza River/ Hungary	1	-	73.6	83.3	57.0	751.0	Mages et al. (2004)
	Barigüi River (At Tingui Park)/ Brazil	1	1.98	97.2	-	35.7	107.1	Castoldi (2014)
			<0.1	68.1	-	18.0	96.1	Gomes (2010)
	Bílina River/ Czech Republic	1	8.0	-	-	60.8	495.7	Kohušová et al. (2011)
		2	1.1	-	-	67.4	459.5	
	Auckland region/ New Zealand	1	-	-	60.0	3.0	120.0	Ancion et al. (2013)
		2	-	-	40.0	8.0	300.0	
		3	-	-	60.0	20.0	1100.0	
Sediment ($mgkg^{-1}$)	Alb River/ Germany	1	-	-	25.0	18.0	-	Fuchs et al. (1997)
		2	1.2	-	68.0	118.4	-	
	Barigüi River (At Almirante Tamandaré)/ Brazil	1	0.2	4.4	-	4.6	12.5	Gonçalves (2008)
	Bílina River/ Czech Republic	1	4.0	-	-	57.0	306.7	Kohušová et al. (2011)
		2	1.6	-	-	85.4	470.0	
	Auckland region/ New Zealand	1	-	-	80.0	5.0	100.0	Ancion et al. (2013)
		2	-	-	10.0	10.0	10.0	
		3	-	-	8.0	7.0	100.0	
Suspended solids (composed sample) ($mgkg^{-1}$)	Barigüi River (At Tingui Park)/ Brazil	1	4.4	166.0	-	51.7	284.3	Castoldi (2014)
Water (mgL^{-1})	Barigüi River (At Almirante Tamandaré)/ Brazil	1	LOD	0.02	-	LOD	LOD	Gonçalves (2008)
	Barigüi River (At Tingui Park)/ Brazil	1	<0.002	<0.05	-	<0.01	0.08	Gomes (2010)
	Bílina River/ Czech Republic	1	0.00016	-	-	0.001	0.036	Kohušová et al. (2011)
		2	0.00021	-	-	0.00792	0.035	

LOD-Bellow detection limit

Among these researches, most of them collected one single sample of each monitoring site and analyzed metal content. This presented research investigated

biofilm formation along a period of time to characterize environment actual situation. Sediment and water samples helped to analyze local pollution in a period of time.

3 MATERIALS AND METHODS

3.1 STUDY AREA

For the study two rivers were selected to investigate trace metals in superficial water: Barigüi and Miringuava Rivers. Sampling site's location, flow and precipitation monitoring sites are presented in FIGURE 8.

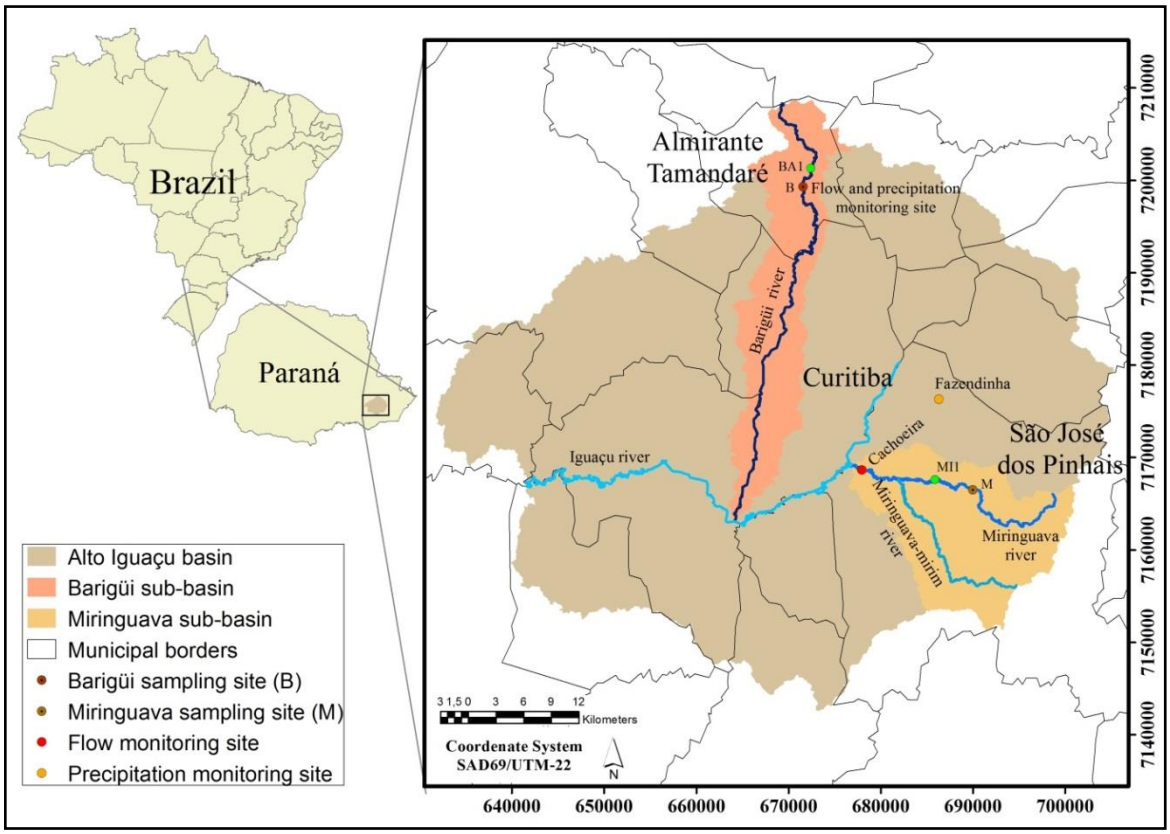


FIGURE 8 – SAMPLING SITE'S LOCATION MAP

Miringuava River is a tributary of Iguaçu River left bank. It is located in São José dos Pinhais municipality, in Curitiba Metropolitan Region (Paraná State). Miringuava basin has an area of 276 km² (Instituto das Águas do Paraná, 2007). Monitoring site (M) encompass 86.9 km² area, representing 31.4% of Miringuava basin, as shown in TABLE 6.

Barigüi River is a tributary of Iguaçu River right bank. It is located in Curitiba, Almirante Tamandaré, and Araucária municipalities. Barigüi basin has an area of 265

km² (Instituto das Águas do Paraná, 2007). Monitoring site (B) encompass 60.9 km², representing 23.0% of Barigüi basin, as it can be observed in TABLE 6.

TABLE 6 – SAMPLING SITE INFORMATION

Code	River	Coordinates	Municipality	Sampling site basin area (km²)
M	Miringuava	25° 36' 29.46" S 49° 6' 6.35" W	São José dos Pinhais	86.9
B	Barigüi	25° 18’ 46.2” S 49° 17’ 44.7” W	Almirante Tamandaré	60.9

SOURCE: PALMA-ACOSTA et al. (2015); GONÇALVES (2008)

Instituto das Águas do Paraná (2013) separates Miringuava basin in MI1 and MI2. Miringuava monitoring site is located within MI1, this river stretch is classified as category two according to legislation (CONAMA 357/2005 item 2.1.4). In the same way, Barigüi basin is divided in BA1, BA2, BA3 e BA4. Barigüi analysis site is located within BA1, this river stretch is also classified as category two.

MI1 embraces basin from headwater to a point 9.12 km downstream monitoring site along river. BA1 basin comprises an area from headwater to a point 2.74 km upstream monitoring site along river. MI1 and BA1 are shown in FIGURE 8. According to Instituto das Águas do Paraná (2013), Barigüi River within BA1 and Miringuava within MI1 are used for human consumption supply, amateur fishing, animal water supply, and mostly used for irrigation and agriculture.

TABLE 7 presents population distribution over sampling sites. On Miringuava (MI1), 30% of population is on urban area, 10% on rural area, 26% on superficial fountainhead supply, and 34% on environmental preservation area, amounting 3,850 people. On Barigüi (BA1), 95% of population is on urban mesh, 4.4% on rural area, and 0.6% on superficial fountainhead supply, amounting 27,215 people.

Miringuava monitoring site (M) drainage area is predominantly covered with forest (~68%), has 16% of pasture and field, 15% of agriculture (mainly vegetable cultivation), and around 1% of built-up areas, reforestation and exposed fields (PALMA-ACOSTA et al., 2015). São José dos Pinhais cultures use Cuprozeb and Manzate as pesticides, these compounds has Cu and Zn on its composotion (FURTADO, 2008). Miringuava River is surrounded by native forest and several

stretches have agriculture; region has mineral enterprises and the river is a water supplier for São José dos Pinhais (Instituto das Águas do Paraná, 2013).

TABLE 7 – POPULATION DISTRIBUTION OVER BASIN

Site	Urban mesh (% Pop)	Rural area (% Pop)	Protected areas	
			For water supply (% Pop)	Environmental preservation areas (%Pop)
MI1	30	10	26	34
BA1	95	4.4	0.6	-

SOURCE: INSTITUTO DAS ÁGUAS DO PARANÁ (2013)

On Barigüi monitoring site, basin is used for agriculture, livestock, and mineral extraction. Limestone is one of the extraction products; it is used as virgin lime for soil acidity correction on agriculture. Basin agriculture includes garlic, rice, and potatoes. These cultivation uses pesticides (Metiram, copper oxychloride, Mancozeb, and Propineb), which have Cu and Zn in their composition. There are no registered industries or landfill on basin. There is one cemetery (that can be a Pb and Zn provider), 0.11 km² of permanent cultivation, 3.48 km² of temporary cultivation, and 248.4 km² of soil with metal content. Monitoring site basin has plenty forest areas, low field, and dense urban area. Monitoring site is located at a low urban area (GONÇALVES, 2008).

According to Melo et al. (2008), soil composition of São José dos Pinhais (where Miringuava site is located) has 1.98 $mgkg^{-1}$ of Cd, 112.0 $mgkg^{-1}$ of Cr, 65.4 $mgkg^{-1}$ of Cu, 2.7 $mgkg^{-1}$ of Pb, 311.7 $mgkg^{-1}$ of Mn, and 5.8 $mgkg^{-1}$ of Zn. These metal concentrations are for silt, clay, and organic matter fractions. Soil presented more chromium concentration than other analyzed metals.

According to Gonçalves (2008), Barigüi basin is on first Paraná Plateau. Barigüi headwater is on Betara Serra (1210 m) and monitoring site is at 950 m. Monitoring site basin soil is composed of Podzolic (its composition have Fe, Al, Zn, Cd, Pb, Cr, and organic matter), Lactosoil (its composition have Cu, Pb, and Zn), and Cambisol soil. Monitoring site is at Cambisol, which has no metal on its composition.

In FIGURE 9A is shown Miringuava River bridge in which biofilm sampler (Item 0) was installed and in FIGURE 9B is shown analysis site surroundings with some animals.


A


B

FIGURE 9 – MIRINGUAVA SITE

Miringuava River receives an industrial load of 0.05 kgmonth^{-1} of Cr, 6.46 kgmonth^{-1} of Zn, 4,723 kgmonth^{-1} of suspended solids, and 19,511 kgmonth^{-1} of COD (Instituto das Águas do Paraná, 2007). It presents good water quality according to Instituto Ambiental do Paraná monitoring (IAP, 2009).

In FIGURE 10 is presented the bridge over which sampler (Item 0) was installed.

FIGURE 10 – BARIGÜI SITE

Barigüi River receives loads resulting of industrial discharges of 0.15 kgmonth^{-1} of Cd, 4.55 kgmonth^{-1} of Cr, 1.58 kgmonth^{-1} of Pb, 51.18 kgmonth^{-1} of Zn, 34,042 kgmonth^{-1} of suspended solids, and 120,903 kgmonth^{-1} of COD. Barigüi sub-basin is the one that receives the highest pollutant discharge of Alto Iguaçu basin (INSTITUTO DAS ÁGUAS DO PARANÁ, 2007).

Miringuava precipitation data (daily accumulated data) was collected from a monitoring station 11 km away from river monitoring site, outside Miringuava basin.

Data are from Monitoring Station *Fazendinha* with Code 2549017 from National Water Agency (*Agência Nacional de Águas – ANA*).

Flow data was estimated for Miringuava monitoring site and also, daily data was obtained from National Water Agency. Available data were from Monitoring Station *Cachoeira* with Code 65015400 located 25.4 km along river downstream biofilm monitoring site (M). These data was calculated for Miringuava monitoring site (M) through area correlation. Estimated flow at M site was calculated multiplying its area with specific flow.

Considering that data obtained are from a station far from monitoring site (M), it was decided to measure flow at monitoring site (M). As access to site is difficult, it was not possible to use ADV (Acoustic Doppler Velocimetry). Also, river depth does not allow measuring cross-section with vessel. So, velocity estimation was made by timing floating using a cork on a ten meter stretch. The average of three measurements was considered as section medium velocity. As depth is lower than 2 m and width is lower than 5 m, velocity was measured at one point on the half of the width according to Santos et al. (2001) methodology. As velocity was measured on surface, a factor of 80% was used to correct velocity, due to bottom roughness (PALHARES et al., 2007). Then, flow was calculated with section measurement in the most accessible rectilinear stretch.

At Barigüi monitoring site there is a precipitation and flow station, so data were taken from that site. Precipitation and flow monitoring sites can be seen in FIGURE 8.

3.2 SAMPLING AND LABORATORY ANALYSIS

Water, sediment, and biofilm samples were collected for this study. Research steps were: building and installing the biofilm sampler, then letting it below water level for a period varying from 14 to 30 days in water course for biofilm formation. Campaigns were commanded by biofilm sampling, so after biofilm formation period, a biofilm, water, and sediment samples were collected. That way, product was biofilm and sediment samples from a period of time and water samples at the beginning and at the end of this period. Also, at the start and at the end of period: DO, pH,

temperature, turbidity, and conductivity were measured on field. The parameters measured for each matrix are presented in FIGURE 11.

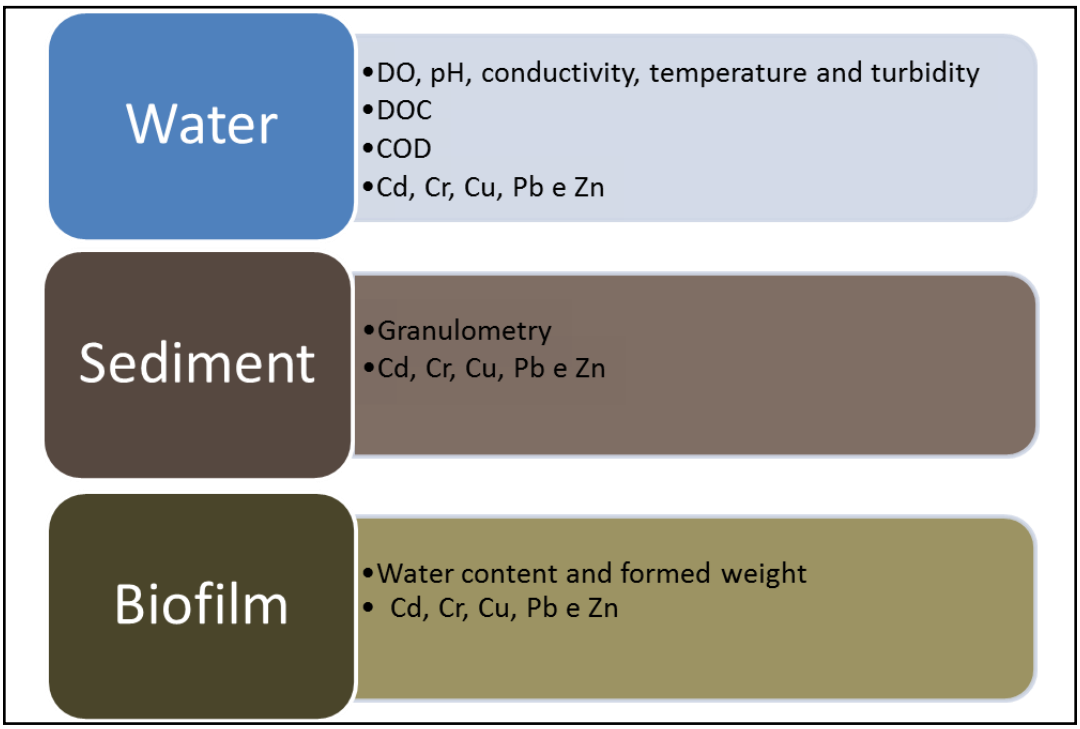


FIGURE 11 – ANALYZED PARAMETRS IN EACH MATRIX

In TABLE 8 is presented Miringuava campaign dates and biofilm formation period.

TABLE 8 – CAMPAIGN DATES AND BIOFILM FORMATION PERIOD OF MIRINGUAVA SITE

Campaign	Biofilm formation		
	Begin	End	Period (days)
1	01/19/2015	02/02/2015	14
2	02/02/2015	02/18/2015	16
3	02/18/2015	03/04/2015	14
4	03/04/2015	03/25/2015	21
5	03/25/2015	04/13/2015	19
6	04/13/2015	04/27/2015	14
7	04/27/2015	05/19/2015	22
8	05/19/2015	06/17/2015	29
9	06/17/2015	07/15/2015	28
10	07/15/2015	08/10/2015	26
11	08/10/2015	09/09/2015	30

For Miringuava site, monitoring began in January 2015 and ended in September 2015, including 11 campaigns in almost eight months. Biofilm formation time interval (and also time interval between campaigns) varied from 14 to 30 days.

At Barigüi site, ten campaigns were performed in nearly five months, beginning in April 2015 and ending in September 2015, as shown in TABLE 9. These campaigns were also ruled by biofilm sampling. For instance, for the seventh campaign biofilm sampler stayed 26 days at the site and after this period biofilm, water, and sediment samples were collected. Time interval of biofilm formation and campaigns varied from 14 to 26 days.

TABLE 9 – CAMPAIGN DATES AND BIOFILM FORMATION PERIOD OF BARIGÜI SITE

Campaign	**Biofilm formation**		
	Begin	**End**	**Period (days)**
1	04/10/2015	04/27/2015	17
2	04/27/2015	05/11/2015	17
3	05/11/2015	05/27/2015	16
4	05/27/2015	06/17/2015	21
5	06/17/2015	07/01/2015	14
6	07/01/2015	07/15/2015	14
7	07/15/2015	08/10/2015	26
8	08/10/2015	08/26/2015	16
9	08/26/2015	09/09/2015	14
10	09/09/2015	09/23/2015	14

Samples collected from both monitoring sites were preserved and conditioned in decontaminated bottles as presented in TABLE 10. Bottle and glassware cleaning and decontamination were according to method 3010B from APHA (1998). Decontamination for trace metal analysis glassware has one further step, before spending one day in HCl 5% they must spend one day in HNO_3 10%. All samples were carried from field to laboratory in a cool box with ice. In laboratory they were maintained in refrigerator until analysis.

TABLE 10 – BOTTLE TYPE, DECONTAMINATION, AND PRESERVATION FOR EACH ANALYZED PARAMETER

Sample	Parameter	Bottle type	Decontamination	Preservation
Biofilm	Trace metals (Cd, Cr, Cu, Pb, Zn)	Glass	HCl 5% for one day then HNO_3 10% for 24 h	Freeze
Sediment	Granulometry	Polyethylene sediment bag	HCl 5% for one day	Freeze
	Trace metals (Cd, Cr, Cu, Pb, Zn, Fe, Al, and Mn)	Polyethylene sediment bag	HCl 5% for one day then HNO_3 10% for 24 h	Freeze
Water	COD (Chemical oxygen demand)	Polyethylene	HCl 5% for 24 h	Made on collection day
	DOC (Dissolved Organic Carbon)	Glass	HCl 5% for 24 h	Filtration, 0.25 mL H_2SO_4 acidification for 50 mL sample and freeze (Expiration: six months)
	Trace metals (Cd, Cr, Cu, Pb, Zn, Fe, Al, and Mn)	Polyethylene	HCl 5% for 24 h then HNO_3 10% for 24 h	2.00 mL HNO_3L^{-1} sample and cooling at T <4°C (Expiration: six months)

SOURCE: APHA (1998)

Samples were analyzed at Borsari Neto Environmental Engineering Laboratory - LABEAM (*Laboratório de Engenharia Ambiental Borsari Neto*) located at Technology Sector at UFPR. For trace metal final analysis was used the Atomic Absorption Spectrometer (Spectra-100/Varian) from Hydrogeological Research Laboratory – LPH (*Laboratório de Pesquisas Hidrogeológicas*) located at Earth

Sciences Sector at UFPR. Also for trace metals was used the ICP/OES (Inductively Coupled Plasma – Optical Emission Spectrometer/ Varian/ 720-ES) from Plants Nutrition Laboratory (*Laboratório de Nutrição de Plantas*) located at Agrarian Sciences Sector at UFPR. Granulometry was made at Mineral and Rocks Analysis Laboratory - LAMIR (*Laboratório de Análise de Minerais e Rochas*) at UFPR. These information and applied method are presented in TABLE 11.

TABLE 11 – EQUIPAMENTS, METHODS, AND ANALYSIS LOCATION FOR EACH PARAMETER

Parameter	Method/Equipament	Analysis location
DOC (Dissolved Organic Carbon)	5310B from APHA (1998) /TOC-V CPH Shimadzu	LABEAM
COD (Chemical Oxygen Demand)	5220D from APHA (1998)/ /UV-1601PC Shimadzu	LABEAM
Granulometry	Weighting, sieving with water, drying in stove and weighting	LAMIR
Trace metals (Cd, Cr, Cu, Pb, Zn, Fe, Al, and Mn)	Water: 3030E from APHA (1998); Biofilm and sediment: 3050b from USEPA (1996)/ ICP/OES. Both acid extraction on hot plate	LABEAM, Plants Nutrition Laboratory and LPH
Conductivity, DO, pH, temperature, turbidity	U-50 Multiparameter Water Quality Checker - HORIBA	On-site

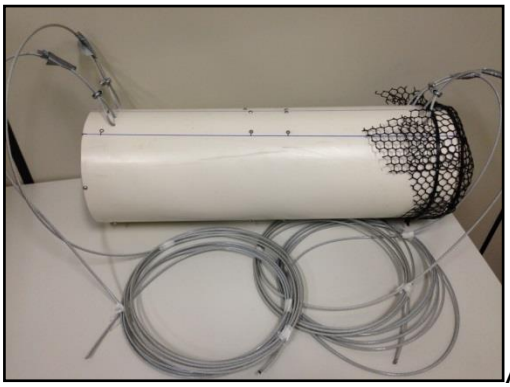
Among these equipment, Total Organic Carbon Analyzer measures total and inorganic carbon; through the difference of these two parameters it estimates organic carbon. When inorganic carbon concentration is higher than total carbon concentration, it is necessary to do a second step than the one presented in TABLE 11. It is necessary to eliminate inorganic carbon, so organic carbon can be estimated. This can be done by sparging[3] the sample with nitrogen. In this case, volatile organic carbon is lost according to User´s Manual (SHIMADZU CORPORATION, 2003).

[3] Sparging-bubbling a chemically inert gas to, in this case, eliminate the dissolved carbon dioxide (SHIMADZU CORPORATION, 2003).

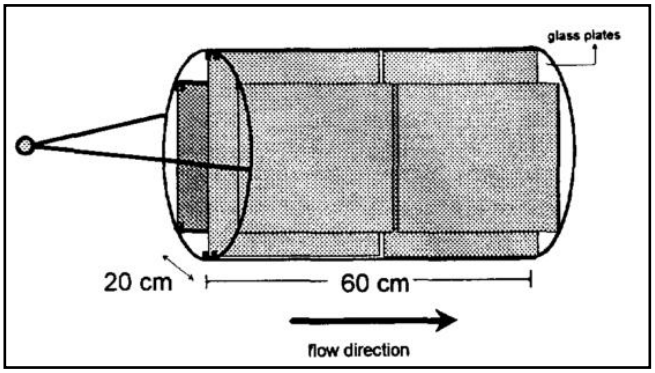
Attached to the presented parameters measured, a statistic investigation was made for all data by a Correlation analysis and by ANOVA with Tukey test. These results are presented together with each matrix section.

3.2.1 Biofilm Samples

Biofilm sampler was adapted from Fuchs, Haritopoulou, and Wilhelmi (1996) and Fuchs et al. (1997). FIGURE 12 illustrates the sampler made with a 60 cm length and 20 cm diameter pvc-pipe. Inside it, there are six glass sheets prepared with stearic acid and petroleum ether to facilitate biofilm formation. According to Flemming (2002), rough surfaces make microbial colonization more susceptible than smooth surfaces. Two glass sheets have 0.057 m² area (central ones) and four glass sheets have 0.044m² area (side ones).


A


B



C

FIGURE 12 – BIOFILM SAMPLER (A-SIDE VIEW, B-BACK VIEW, C-DIMENSIONS)
SOURCE: FUCHS, HARITOPOULOU, AND WILHELMI (1996) – FIGURE C

The sampler was installed with steel cable underneath a bridge structure. It has a mesh on upstream edge to avoid leaves and branches entrance. According to

Ancion et al. (2010) experiment, after 7 to 14 days stability between metals in water and in biofilm is reached, regardless of metal (when metal concentrations are in a steady state). Hence, campaigns were made initially with a 15 day interval (on average). After trace metal results, it was observed few changes in their concentration, so it was decided to extend the period that sampler stayed on field collecting samples.

After a specific time period, the biofilm sample was removed from sampler with a silicone spatula and then stored in a glass bottle. This bottle was weighed before and after collection, weight difference represented biofilm formed amount. To calculate water percentage, sample was weighed when it was wet and dry (after staying in a 40°C oven. According to USEPA (1994), this temperature avoids volatile metal loss).

Sample was maintained frozen until trace metal analysis moment, which was made with method 3050b from USEPA (1996) and with ICP/OES from Plants Nutrition Laboratory. Trace metal analysis (Cd, Cr, Cu, Pb, and Zn) were made when Plants Nutrition Laboratory equipment were available (around September 2015).

At the end, trace metal concentrations determined for biofilm were normalized by formation period (days), average daily flow (for biofilm formation period), formed biofilm dry weight and the combination of them. That way, trace metal concentration was divided by formation period and/or average daily flow of the period and/or formed biofilm dry weight of the period.

3.2.2 Water samples

Water samples were collected on center upper layer of river and stored in polyethylene bottle. Samples were collected every time biofilm was collected. For trace metal analysis (Cd, Cr, Cu, Pb, Zn, Fe, Al, and Mn), a sample fraction was conserved with 2 mL of $HNO_3 L^{-1}$ and refrigerated at T≤4°C. Particulate and dissolved parts were analyzed for Miringuava monitoring site for campaigns one to six and for Barigüi monitoring site campaigns one and two. For these fractions, filtration was made with cellulose acetate membranes of 0.45 µm. For other campaigns, total fraction was analyzed directly, without separating the fractions.

Trace metal analysis was made at LABEAM through acid digestion with HNO_3 on hot plate (method 3030E from APHA (1998)). Blank samples were also

analyzed. Atomic Absorption Spectrometer from LPH was used for first and second campaign water samples of Miringuava site. For other campaigns, ICP/OES from Plants Nutrition Laboratory was used. Trace metal analysis was made when LPH and Plants Nutrition Laboratory equipment were available (around September 2015). Analysis was made before expiration sample period (six months).

COD was measured for Miringuava site campaign seven and Barigüi site campaign three with an adapted method of 5220D from APHA (1998). The difference was in reagent proportion: 2 mL of sample, digestion solution, and sulfuric acid reagent. For other campaigns method 5220D (Closed refulux-colorimetric) was applied. It was not necessary to preserve water sample for COD analysis because the method was executed on the very campaign day. UV-1601PC (Uv visible spectrophotometer)/Shimadzu was used to analyzed COD concentration.

The following parameters were measured on-site: conductivity, DO, pH, temperature, and turbidity with U-50 Multiparameter Water Quality Checker/ HORIBA.

For DOC, samples were filtered with 0.45 µm cellulose acetate membrane to analyze dissolved fraction. Then, 0.25 mL H_2SO_4 was added for 50 mL sample and the sample was frozen. To estimate DOC concentration, it was used method 5310B (High –Temperature Combustion Method) from APHA (1998) and it was used TOC-V CPH (Total organic carbon analyzer) from LABEAM.

3.2.3 Sediment analysis

Sediment was taken with Petersen grab, next to the river bank that accumulated more sediment, and stored in polyethylene bag. It was taken 20 cm of superficial layer sediment. Samples were taken every time biofilm was collected. After collection, sediment was dried in oven at 40°C, this temperature avoid volatile metal loss (USEPA, 1994). Before granulometry, sediment was quartered, according to (USEPA, 2010) this method guarantees a better representativeness. Granulometry was made according to LAMIR methodology (presented in APPENDIX).

To assist the analysis, uniformity coefficient and effective diameter were calculated. Uniformity coefficient expresses how homogeneous is sediment. When values are lower than five, sediment is uniform, when it is higher than 15, it is uneven. Effective diameter represents soil thinness (ELIASSON, 2002).

For trace metals analysis (Cd, Cr, Cu, Pb, Zn Fe, Al, and Mn), fraction bellow 63 µm diameter was analyzed. Fuchs et al. (1997) used this fraction to analyze its sediment samples but according to Müller, Yahya, and Gentner (1993 apud Fuchs, Haritopoulou, and Wilhelmi, 1996) the fraction that expresses best trace metals in sediment is clay below 2 µm diameter. At LABEAM lowest sieve was of 63 µm, so that fraction was used for trace metal analysis and method 3050b from USEPA (1996) and ICP/OES from Plants Nutrition Laboratory was used. For granulometry at LAMIR, sieves of 5 mesh (4.0 mm), 9 mesh (2.0 mm), 16 mesh (1.19 mm), 32 mesh (0.5 mm), 60 mesh (250.0 µm), 115 mesh (125.0 µm), 250 mesh (63 µm), and 325 mesh (44.0 µm) were used.

4 RESULTS AND DISCUSSION

First, campaigns scheme was presented together with flow and rainfall data to a better description of hydrological conditions in this research monitoring period. Then, monitored data were presented in each matrix: water, sediment, and biofilm. For all matrices, Cd, Cr, Cu, Pb, and Zn were analyzed. For water: pH, turbidity, dissolved oxygen, water temperature and conductivity were also measured. For sediment: granulometry was also analyzed. For biofilm: water content and formed weight were estimated.

4.1 CAMPAIGN INFORMATION

For both sampling sites flow were measured. In Miringuava River, as mentioned on Item 3.1, there is no flow monitoring station close to monitoring site (M). So, flow data was adjusted for monitoring site (M) utilizing the closest flow station. Adjusted data for Miringuava monitoring site is shown in FIGURE 13.

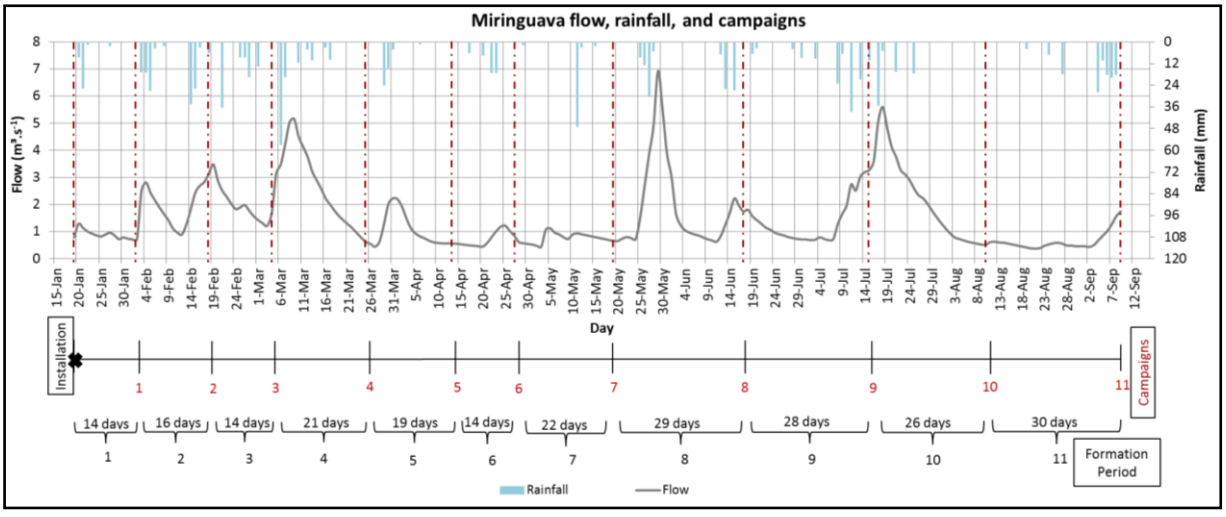


FIGURE 13 – MIRINGUAVA SITE FLOW, RAINFALL (OBTAINED DATA), AND CAMPAIGNS

It was also measured flow at monitoring site, these data is presented in TABLE 12. Difference between both estimates is relevant; reason could be distance between flow station and monitoring site. The difference becomes really relevant in

some campaign (as can be seen in TABLE 12), for instance in campaign 11 flow measured on field was 3.2 m^3s^{-1} while data obtained from ANA was 1.74 m^3s^{-1}. Also, data could be delayed, once flow behavior in the whole basin was assumed to be equal. Besides that, there are differences in soil type distribution, vegetation cover, rainfall distribution, humidity soil conditions, among others. Even though flow presented different values for monitoring site (M) and adjusted data, values utilized to analysis was the one presented in FIGURE 13 from ANA station, 25 km away from monitoring site (M).

TABLE 12 –MIRINGUAVA FLOW DATA (MEASURED ON FIELD AND FROM ANA)

Campaign	**On field measured flow (m^3s^{-1})**	**Flow from ANA station (m^3s^{-1})**
2	3.2	3.14
3	1.8	1.67
4	1.7	0.62
5	1.6	0.57
6	2.2	0.85
7	2.3	0.66
8	2.5	1.74
9	2.8	3.27
10	1.7	0.52
11	3.2	1.74

For Barigüi River, greatest observed flow was 6.2 m^3s^{-1} on July 17th and the smallest was approximately 0.6 m^3s^{-1} on September 22nd as can be seen in FIGURE 14. Average flow was 1.9 m^3s^{-1} and its standard deviation was nearly 0.7 m^3s^{-1}. The highest observed rainfall was almost 49 mm on November 08th, average rainfall was 3.6 mm and its standard deviation was 8.6 mm.

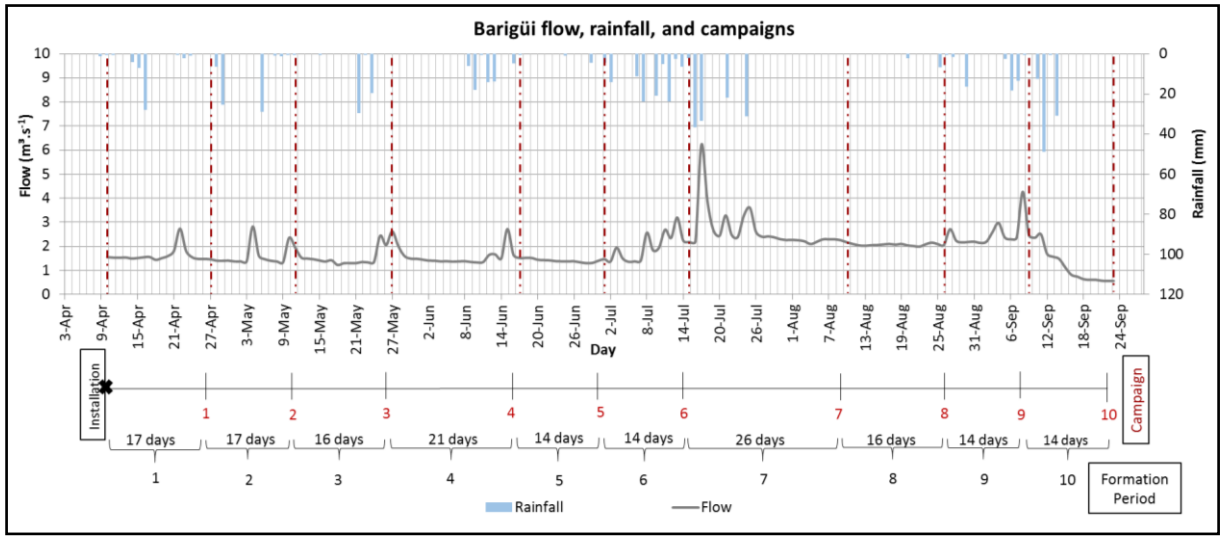


FIGURE 14 – BARIGÜI SITE FLOW, RAINFALL, AND CAMPAIGNS

4.2 WATER

Water samples were collected at the beginning and at the end of each biofilm formation period. With the campaign obtained values, average data (from analyzed parameters) was calculated between campaigns. Parameters values were compared with flow decrease or increase. For each biofilm formation, an average of water measured parameters was calculated, that way representing in a better way biofilm formation environment situation. Field data values for both monitoring site without average (as it was measured) are presented in APPENDIX.

4.2.1 Field Parameters

Data obtained from Multiparameter Water Quality Checker is shown in TABLE 13 for Miringuava. Within analyzed period, dissolved oxygen, pH, and conductivity varied not much. DO measured data was always above 7.0 mgL^{-1} and 73.4% representing good water oxygenation. Conductivity stayed always above 35 μScm^{-1}.

For Miringuava, turbidity and DO were high at the same campaign (between campaigns one and two). Conductivity and DO were lower at the same campaign (between campaigns two and three). Temperature varied from 13.6 °C to 19.7 °C.

Comparing to CONAMA 357/2005, dissolved oxygen and pH values are in Class one (a good water quality classification).

TABLE 13 – AVERAGE FIELD PARAMETERS OF MIRINGUAVA SITE

Campaigns Average	T (°C)	OD (mgL^{-1})	% OD	pH	Turbidity (NTU)	Conductivity (μScm^{-1})
1-2	18.4	8.5	92.6	5.73	40	38
2-3	19.7	7.0	78.7	5.77	35	35
3-4	19.6	7.7	86.0	6.97	10	38
4-5	19.2	8.4	91.8	7.49	6	38
5-6	18.3	7.5	80.6	7.37	7	38
6-7	16.4	7.3	77.3	7.36	16	39
7-8	13.8	7.4	74.0	6.85	20	38
8-9	13.6	7.4	73.4	6.86	28	37
9-10	16.2	7.3	76.0	7.11	26	39
10-11	16.2	7.4	77.6	6.94	32	37
Min	13.6	7.0	73.4	5.73	6	35
Max	19.7	8.5	92.6	7.49	40	39
Average	17.7	7.6	80.8	6.85	22	38
Standard Deviation	2.2	0.5	7.0	0.62	12	1

Turbidity in Miringuava River varied between 6 NTU and 40 NTU. Variation was high because some measurements were made at flow peaks (high values) and at low flows (low values) as can be seen in FIGURE 15. High flows carry basin solids and increase suspended solids in water.

Field data for Barigüi is shown in TABLE 14. Temperature, pH, and DO were almost stable. Dissolved oxygen measured was always above 7.0 mgL^{-1} and 73.6 % representing good water oxygenation. Conductivity and pH lowest values happened at the same intervals (between campaigns five and six).

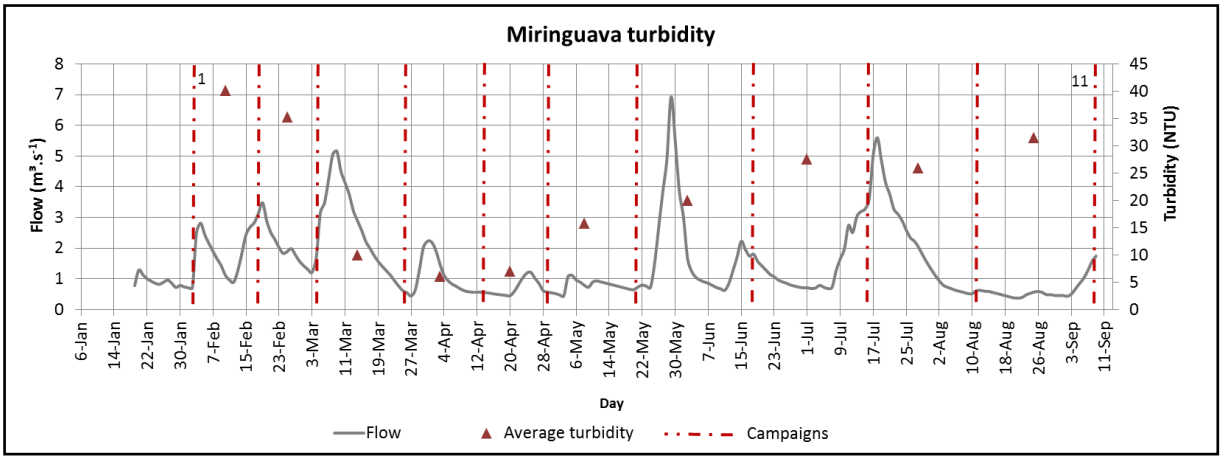


FIGURE 15 – MIRINGUAVA AVERAGE BETWEEN CAMPAIGNS TURBIDITY

For Barigüi, turbidity presented great variation on the observed period. Turbidity varied between 14 NTU and 197 NTU and high values happened with peak flows. Comparing to CONAMA 357/2005, dissolved oxygen and pH values are in Class one (a good water quality classification) for Barigüi River in this stretch.

TABLE 14 – AVERAGE OF FIELD PARAMETERS OF BARIGÜI SITE

Average Campaign	T (°C)	OD (mgL^{-1})	% OD	pH	Turbidity (NTU)	Conductivity (μScm^{-1})
1-2	17.5	7.6	81.5	7.76	20	320
2-3	17.4	7.4	79.4	7.86	198	280
3-4	16.5	7.0	73.6	8.20	189	290
4-5	15.1	7.6	78.2	7.70	15	310
5-6	16.5	8.8	92.7	7.68	32	230
6-7	17.5	8.6	92.4	7.75	33	240
7-8	17.2	8.1	86.4	7.71	14	330
8-9	16.4	8.0	84.3	7.86	46	300
9-10	18.5	7.4	81.2	8.04	117	310
Min	15.1	7.0	73.6	7.68	14	230
Max	18.5	8.8	92.7	8.20	198	320
Average	17.0	7.8	83.3	7.84	74	290
Standard Deviation	1.0	0.6	6.4	0.17	75	40

Although all these parameters are important for biofilm growth, they could not be evaluated more precisely due to few data. It would be necessary frequent water quality parameters data to observe its direct influence on biofilm's development.

4.2.2 COD (Chemical Oxygen Demand)

COD is a primary evaluation for organic content, in which trace metal can be attached to. COD values are presented in TABLE 15. Calibration curves values are presented in APPENDIX.

TABLE 15 – COD VALUES

Average Campaign	Miringuava COD (mgL^{-1})	Barigüi COD (mgL^{-1})
1-2	-	-
2-3	-	-
3-4	-	15.8
4-5	-	6.5
5-6	-	6.8
6-7	-	6.1
7-8	20.6	2.5
8-9	17.3	8.1
9-10	10.1	-
10-11	14.0	
Min	10.1	2.5
Max	20.6	15.8
Average	15.5	7.6
Standard Deviation	8.0	4.4

COD presented great variation for both sites and highest values happened in campaigns with high accumulated rain event and peak flows (as can be observed in FIGURE 16).

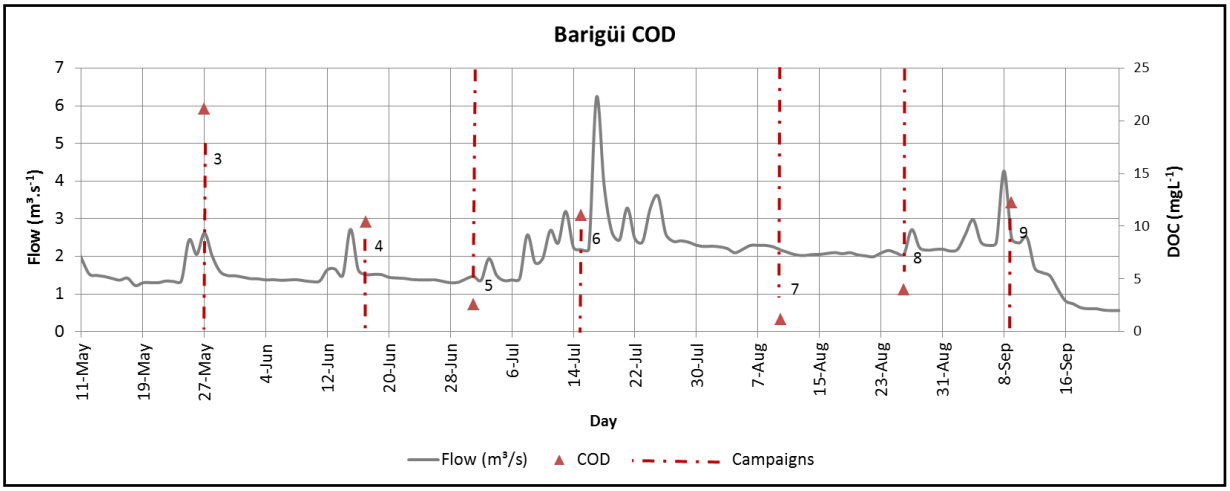


FIGURE 16 – COD VALUES FOR BARIGÜI RIVER (CAMPAIGNS 3 TO 9)

For analyzed period, both sites presented good water quality and low organic matter according to chemical oxygen demand values.

4.2.3 DOC (Dissolved Organic Carbon)

DOC is a direct measurement of organic matter, to which trace metals can be attached. DOC values are presented in FIGURE 17.

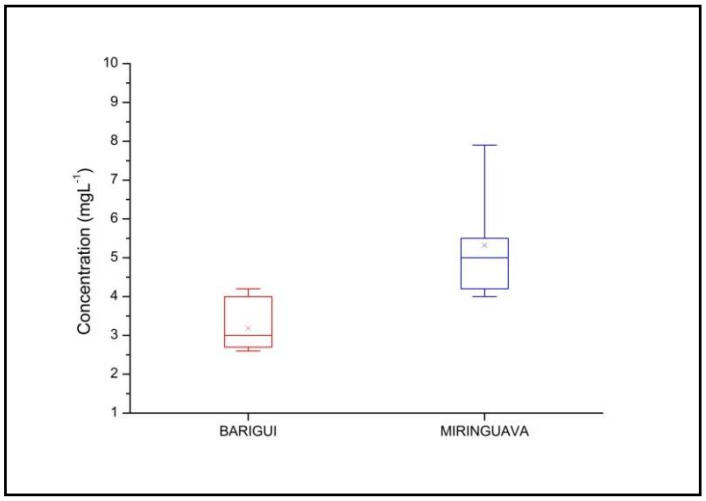


FIGURE 17 – DISSOLVED ORGANIC CARBON

For Barigüi River, inorganic carbon presented higher values than total carbon, that way the samples were sparged with nitrogen for 30 min and then read on the equipment (as presented in Item 3.2).

DOC highest values happened in campaigns with high accumulated rain event and peak flows (as can be observed in FIGURE 18 for Miringuava River).

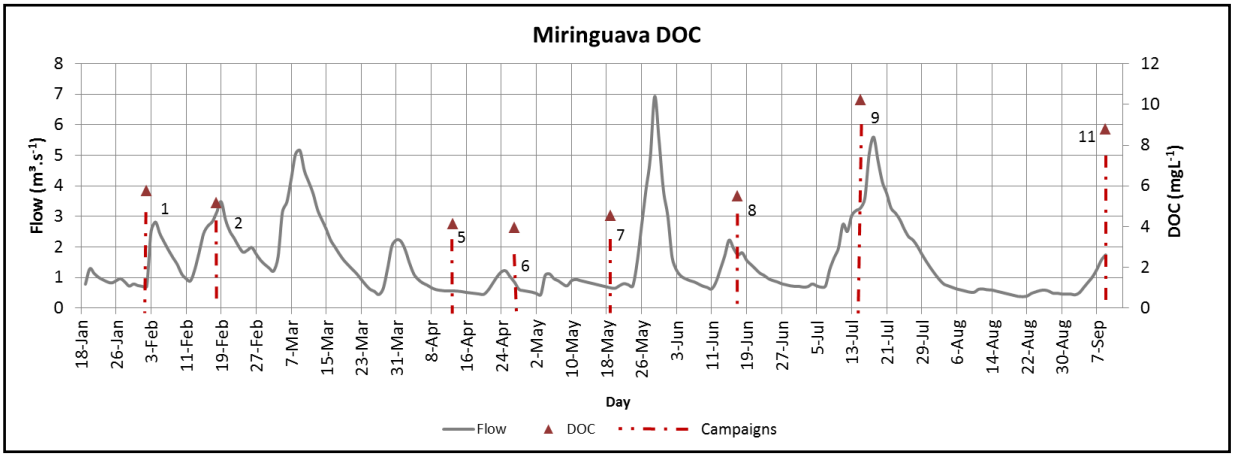


FIGURE 18 – DOC VALUES FOR MIRINGUAVA RIVER

Miringuava observed DOC endured a great variation, between 4.0 mgL^{-1} and 7.9 mgL^{-1}. For Barigüi, variation was low and values were lower than 4.2 mgL^{-1}. Miringuava concentrations were higher than Barigüi. Dissolved organic carbon indicates low organic matter quantity in both sites and good water quality.

4.2.4 Trace Metals in Water

Cd, Cr, Cu, Pb, and Zn were measured 11 times and their concentrations for Miringuava River are presented in TABLE 16.

TABLE 16 – MIRINGUAVA TRACE METAL VALUES IN WATER

Metal	Range (mgL^{-1})	Average (mgL^{-1})	Standard deviation (mgL^{-1})
Cd	LOQ - 0.016	0.005	0.005
Cr	LOQ - 0.36	0.087	0.146
Cu	LOD - 0.042	0.018	0.016
Pb	LOD - 0.045	0.030	0.022
Zn	0.02 - 0.41	0.12	0.13

LOD-Bellow detection limit, LOQ-Bellow quantification limit

Cd, Cu, Zn, and Cr presented their highest values between the same campaigns (seven and eight) in a high accumulated rain period (116 mm) and during

a peak flow, so these metals could have come through runoff. This information can be observed in FIGURE 19.

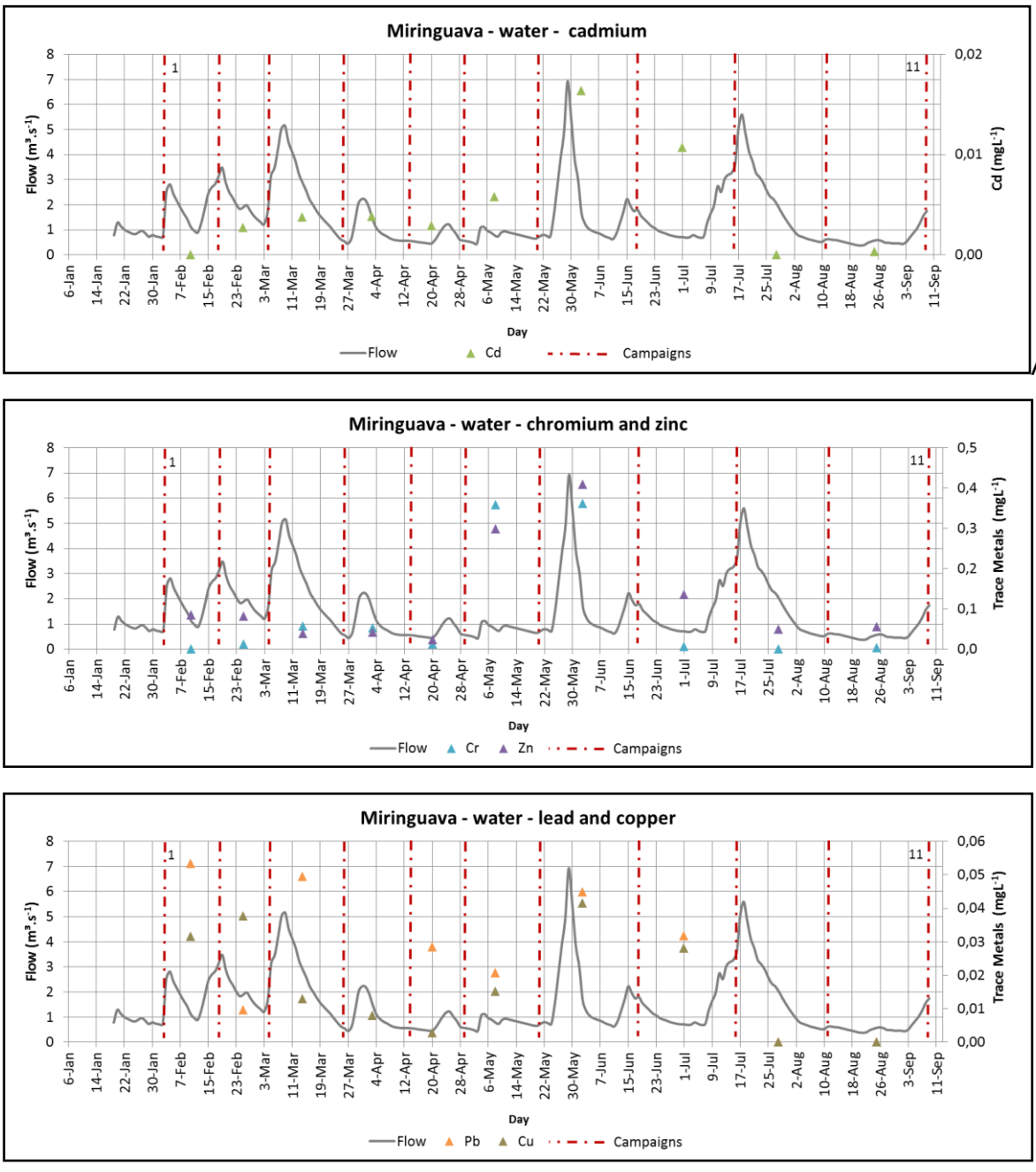


FIGURE 19 – TRACE METALS AVERAGE BETWEEN CAMPAIGNS FOR WATER IN MIRINGUAVA RIVER. Cd (A), Cr and Zn (B), and Pb and Cu (C)
*(C) - Pb was below quantification limit for campaigns 9-10 and 10-11, like Cu

For Miringuava River, Cd, Cr, Cu, and Pb presented lower values at the same campaign interval (nine and ten) in a high flow period (2.24 m^3s^{-1}), these elements could have dissolved (FIGURE 19). Accumulated rainfall varied from 43.5

mm to 130.0 mm and average daily flow from 0.74 m^3s^{-1} to 2.61 m^3s^{-1}. It is important to remember that these flow values was calculated through specific area off data from a point 11 km away from sampling site. That way, data could be lagged or even different due to spatial and temporal rainfall events.

In the course of the research, it was decided to also analyze Al, Fe and Mn. They were measured at the last campaigns and two values were obtained for Al and Fe, and three for Mn. These values are 0.50 mgL^{-1} for both Al measured campaigns, 0.96 mgL^{-1} and 1.15 mgL^{-1} for Fe. For Mn values were 0.04 mgL^{-1} for two campaigns and 0.10 mgL^{-1} for the other campaign.

In Miringuava basin it is common to use pesticides containing Cu and Zn. That way, some elements could have entered the system through runoff, considering Miringuava has plenty agriculture activities. Also, local soil has Cd, Cr, Cu, Pb, Mn, and Zn on its composition.

Comparing to CONAMA 357/2005, Zn, Cr, and Cu did not exceed water quality limits. Cadmium exceeded legislation 30% of the samples and lead 70% of the samples. Furthermore, in Miringuava River it was observed a significant and positive correlation in water between ion Zn and Cd ($R=0.7980$, $p=0.06$), Zn and Cr ($R=0.9258$, $p<0.001$) and Cd and Cr ($R=0.6652$, $p=0.036$). This indicates metals can be from the same source: soil or agrochemical substances. Miringuava River, on the analyzed site, is surrounded by agricultural activities that use pesticides with Cu and Zn in their cultures. Also, Cd, Cr, and Zn are present in the soil composition of the region. These data were presented in Item 3.1.

Correlating with literature values, Pb in Miringuava water presented the same value than Bílina River (KOHUŠOVÁ et al., 2011). Bílina River is located at an industrialized area, while Miringuava is located at a rural area. Zn concentration at Miringuava was lower than at Barigüi River (Tingui Park) (GOMES, 2010). Barigüi River, at this point, is at an urbanized area, supporting conclusions that the higher urbanization, the higher metal pollution (GONÇALVES, 2008; ANCION et al., 2013).

Trace metals concentration in Barigüi water is presented in TABLE 17. Cd, Cr, Cu, Pb, and Zn were measured ten times and Al, Fe, and Mn seven times. Cr, Cu, and Zn presented their highest values between the same campaign (seven and eight) when average flow was high (2.64 m^3s^{-1}), that way elements could have come from runoff. Pb, Cd, and Cu presented low values in the same period (between campaign nine and ten) when flow and accumulated rainfall were high (2.48 m^3s^{-1}

and 111.5 mm) so elements could have dissolved. Al, Fe, and Mn presented their lowest values between the same campaign (eight and nine). Average daily flow varied from 1.22 m^3s^{-1} and 2.64 m^3s^{-1} and accumulated rainfall varied between 9.9 mm and 124.7 mm.

TABLE 17 – BARIGÜI TRACE METAL VALUES IN WATER

Metal	Range (mgL^{-1})	Average (mgL^{-1})	Standard deviation (mgL^{-1})
Cd	LOD - 0.008	0.003	0.003
Cr	LOD - 0.066	0.026	0.026
Cu	LOD - 0.043	0.017	0.017
Pb	LOD - 0.024	0.008	0.010
Zn	0.01 - 0.09	0.047	0.027
Al	0.12 - 0.55	0.34	0.18
Fe	0.41 - 1.10	0.81	0.30
Mn	0.02 - 0.04	0.03	0.009

LOD-Bellow detection limit

Comparing to CONAMA 357/2005 (Values were presented in Section 2.1.3), Cu, Cr, and Zn did not exceed water quality limits. Lead exceeded legislation 40% of the time and cadmium 11% of the time.

Cr, Cu, Zn, Fe, and Mn highest values occurred at high flow periods, which could be due to materials carried through runoff. Cu presented high value when COD was low. Esteves (1998) states that trace metal availability depends on dissolved organic matter concentration. Cd higher value occurred with high turbidity, which can indicate high suspended solids content; cadmium could be adhered to these particles.

Moreover, in Barigüi River it was observed a significant and positive correlation in water between ion Zn and Cu (R=0.8310, p=0.006) and Cd and Pb (R=0.8772, p=0.002). This indicates metals can be from the same source, Zn and Cu could be from pesticides used in the cultures of the region, as presented in item 3.1 and Cd and Pb from sewage as found by Chowdhury et al., 2016.

Relating Barigüi and literature values, it is observed that for water samples: 30% of the samples were higher for Cr concentration than the ones found by Gonçalves (2008); both data are from the same monitoring site. Pb and Zn values

were higher than the one found by Gomes (2010) and Kohušová et al. (2011), both authors monitoring sites are at high urbanized areas. These results disagree with expectation of higher urbanized areas having higher metal concentrations.

Comparing water samples from both sampling sites by mean trace metals values, it can be observed that Cd, Cu, Cr, Pb, Zn, Al, and Fe were higher at Barigüi River. Cu, Pb, Zn, and Al are common in cemetery effluents and there is one Cemetery on Barigüi catchment drainage area. Iron is present on brake composition and there are plenty streets near monitoring site (GONÇALVES, 2008). These elements are also present in soil composition of the catchment. The only metal that presented higher concentration at Miringuava site was manganese, this metal were present in higher concentrations in sediment comparing to soil composition for all Miringuava campaigns. Also, Miringuava sampling site basin presented more rain than Barigüi.

Comparing to Miringuava, Barigüi exceeded legislation in less percent of the time but both sampling sites presented exceeding values for the same metals. Besides that, for water samples, Miringuava River presented more significant correlations.

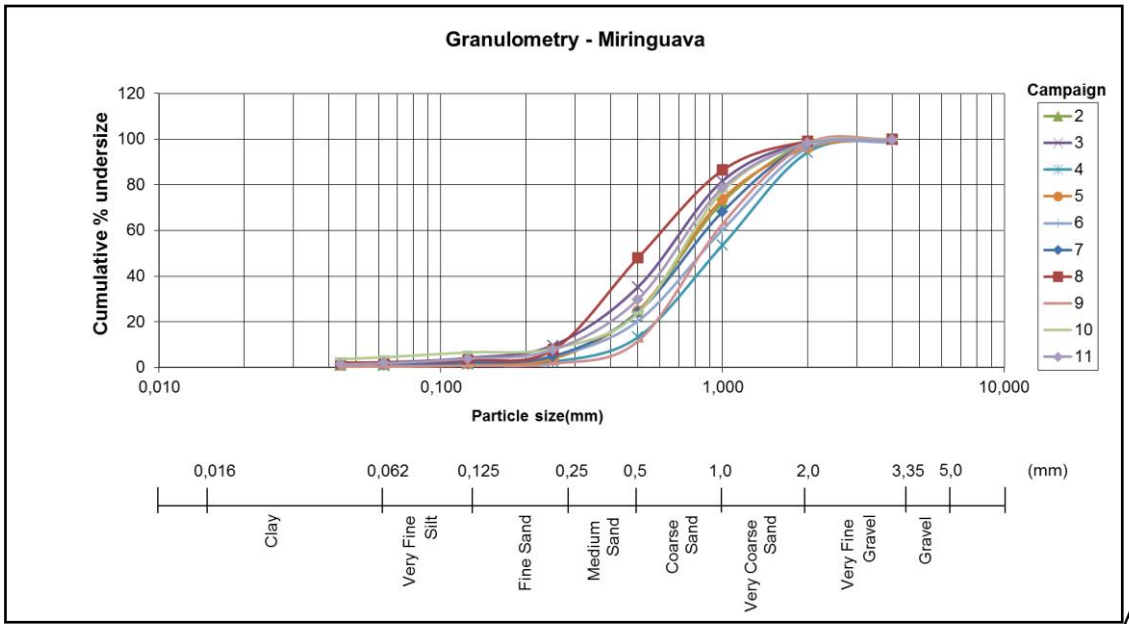
Trace metal concentrations specified for each monitoring site campaign are presented in APPENDIX.

4.3 SEDIMENT

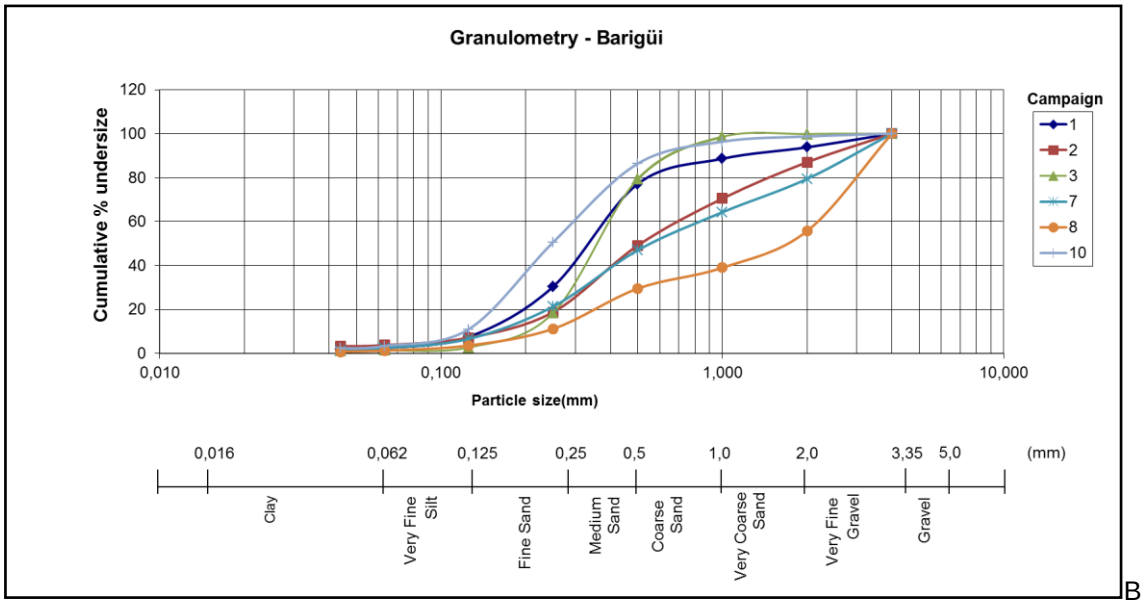
Sediment samples were collected at the end of each biofilm formation period. For these samples the following parameters were analyzed: granulometry, effective diameter, uniformity coefficient, Cd, Cr, Cu, Pb, Zn, Al, Fe, and Mn. In contrast to water, sediment was analyzed by period, not by campaigns average.

4.3.1 Granulometry

Sediment granulometry results are presented in FIGURE 20.



A



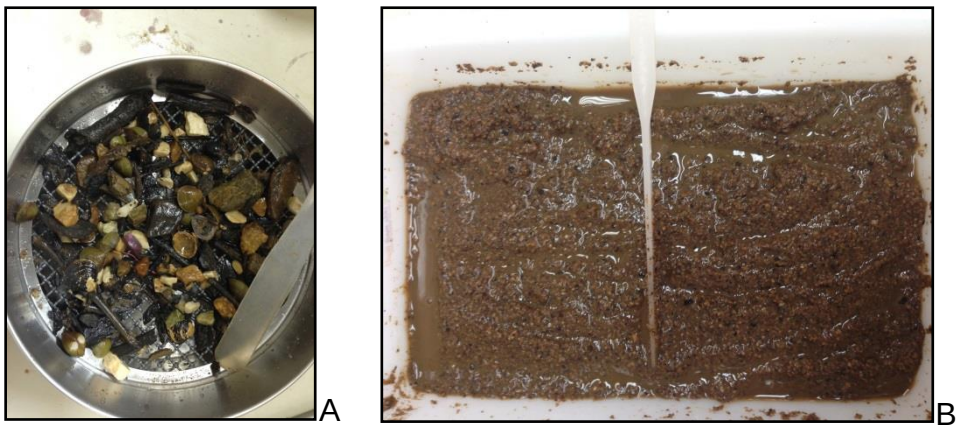
B

FIGURE 20 – SEDIMENT GRANULOMETRY (% WEIGHT): (A) MIRINGUAVA SITE; (B) BARIGÜI SITE

For Miringuava campaigns more than 95% of sediment was over 63 µm diameter in all campaigns and for Barigüi more than 96%. In Miringuava, grains lower than 63 µm varied between 0.29% (campaign nine) and 4.49% (campaign ten); average was 1.8% and standard deviation was 1.0%. In Barigüi, the higher quantity of small grains (<63 µm) was 3.75% in campaign two and the lower quantity was 1.28% in campaign eight; average was 6.1% and standard deviation 9.0%.

Although collection with Petersen grab did not allow temporal determination of sediment; it was noticed that percentage of sediment particle lower than 63 µm (with more surface area to adhere contaminants) were higher at a high flow period and precipitation period for both sampling sites. These events could have brought more suspended particles to analyzed section.

It was observed that at Barigüi there was a great quantity of grains > 2 cm as shown in FIGURE 21A, that way it was difficult to collect fine sediment, in some campaigns it was not possible to collect sediment. At Miringuava sampling site sediment presented to be more uniform (FIGURE 21B).



A B

FIGURE 21 – SEDIMENT SAMPLES FROM BARIGÜI (A) AND MIRINGUAVA (B).

To assist granulometry results, uniformity coefficient and D_{10} (effective diameter) were calculated for both sites and are shown in TABLE 18.

At Miringuava, uniformity coefficient varied from 2.10 to 3.03; average was 2.8 and standard deviation was 0.3; indicating low variance in sediment particle size among samples and uniform samples (unif. coef.< 5). Effective diameter varied between 0.25 and 0.46 and variation was low (standard deviation was 0.07 mm). On average, effective diameter was 0.33 mm expressing that 10 % of particles are below 0.33 mm.

As for Barigüi, uniformity coefficient values were between 2.29 and 11.83; average was 6.3 and standard deviation was 3.8. That means sediment particles size varied among campaigns and in half of the campaigns soil could be considered not uniform. Effective diameter values were between 0.12 and 0.31, average was 0.19 mm and standard deviation was 0.06 mm. In that way, particles with diameter of 0.19 mm or below represented 10 % of sediment sample.

TABLE 18 – UNIFORM COEFFICIENT AND EFFECTIVE DIAMETER FOR SEDIMENT SAMPLES

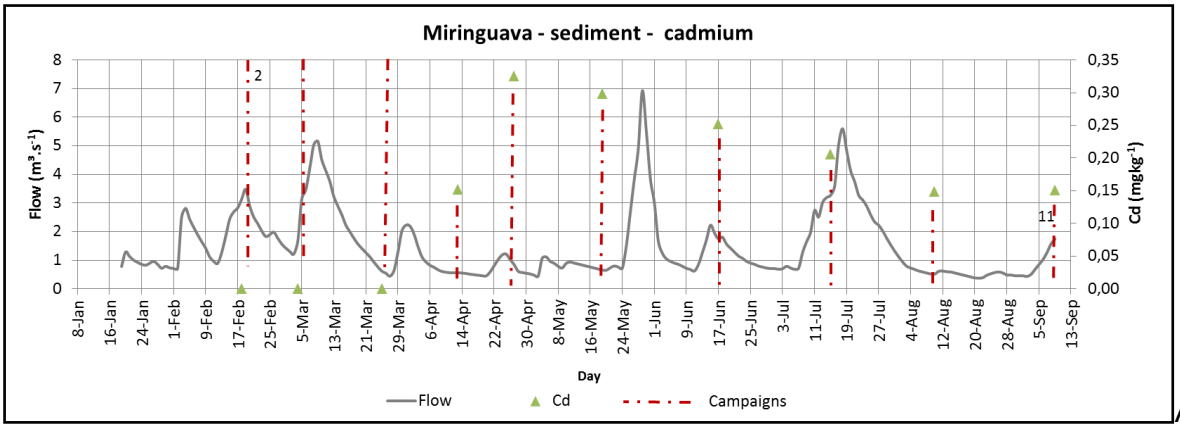
Campaign	Miringuava		Barigüi	
	Uniformity Coef.	D_{10} (effective Diameter-mm)	Uniformity Coef.	D_{10} (effective Diameter-mm)
1	-	-	2.99	0.14
2	2.67	0.33	8.00	0.19
3	3.03	0.25	2.29	0.18
4	2.72	0.43	-	-
5	2.65	0.32	-	-
6	3.00	0.33	-	-
7	2.86	0.32	9.73	0.18
8	2.48	0.26	11.83	0.31
9	2.10	0.46	-	-
10	3.02	0.28	2.76	0.12
11	2.93	0.28		

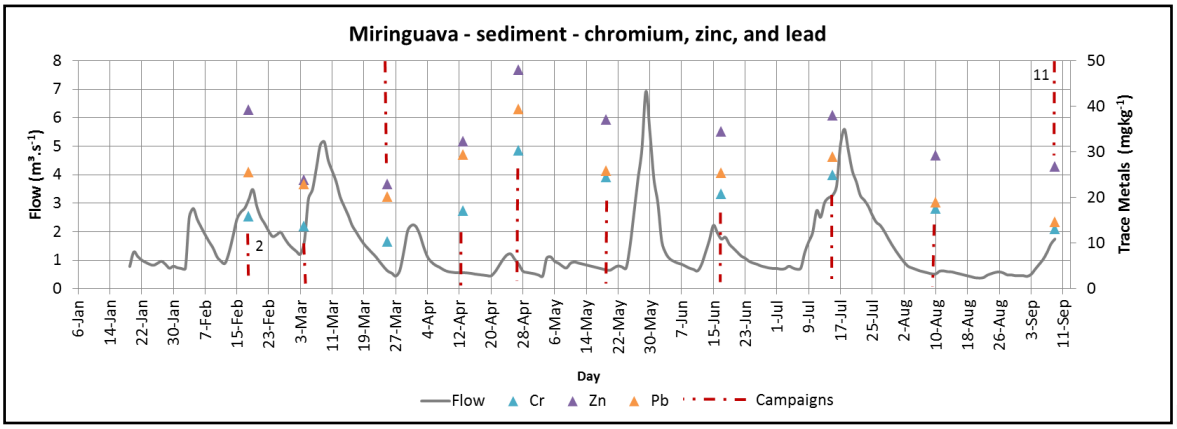
Comparing both monitoring sites Miringuava sediment was coarser and more uniform than Barigüi, even though majority of both sediment sites was sand.

4.3.2 Trace Metals in Sediment

Sediment samples were collected with Petersen grab, making it difficult to collect samples representing deposition on the same biofilm formation period. For trace metal analysis only the fine part of the sample was analyzed. Even though, it was noted that at Miringuava, highest concentrations of Cd, Cr, Cu, Pb, and Zn happened at the same campaign (six). In this period, flow was low (0.72 m^3s^{-1}) and metals could have concentrated. These analyses can be checked with FIGURE 22.

Al, Fe, and Mn highest value happened within a period with high precipitation (Campaign nine - 121 mm), in such manner, they could have entered system through runoff. Accumulated rainfall varied from 43.5 mm to 130.0 mm and average daily flow from 0.74 m^3s^{-1} to 2.61 m^3s^{-1}. Mn, Pb, and Zn values were higher than soil composition in all campaigns. Cd, Cr, and Cu concentrations show that sediment is not contaminated with these trace metals.





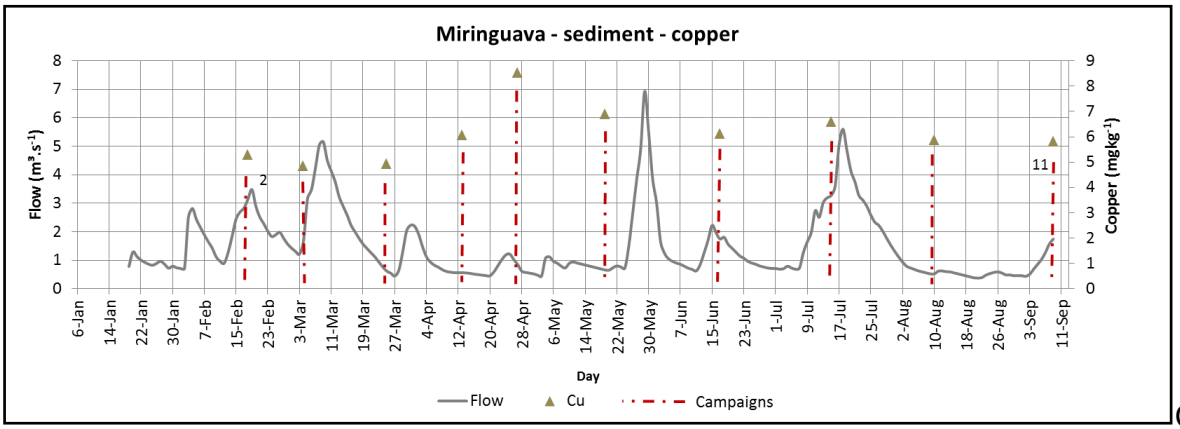


FIGURE 22 – TRACE METALS FOR SEDIMENT IN MIRINGUAVA RIVER. Cd (A), Cr, Zn, and Pb (B), and Cu (C)

Sediment metals concentration range, average, and standard deviation for Miringuava are shown in TABLE 19.

In Miringuava River it was observed significant and positive correlation in sediment between all trace metal analyzed indicating proportional accumulation between those metals in sediment.

Comparing sediment values found with literature data, it is observed that for Miringuava River: Cd values were lower than the one found by Gonçalves (2008) and

Castoldi (2014) for Barigüi River, in a low urbanized area and urbanized area respectively. On the other hand, Cr was lower than Castoldi (2014) values Barigüi River but higher than Gonçalves (2008). Cu values were similar from Ancion et al. (2013) for a similar land use area. For Pb, Miringuava River values were higher than Fuchs et al. (2006) for agriculture zone and Gonçalves (2008) for low urbanization area. Zn concentration was lower than values observed by Kohušová et al. (2011) in low urbanized areas but higher than the ones found by Gonçalves (2008).

TABLE 19 – MIRINGUAVA TRACE METALS VALUES IN SEDIMENT

Metal ($mgkg^{-1}$)	Range	Average	Standard deviation
Cd	LOD - 0.33	0.15	0.12
Cr	10.4 - 30.4	18.9	6.2
Cu	4.9 - 8.5	6.1	1.1
Pb	14.7 - 39.4	25.1	6.8
Zn	23.0 - 48.1	33.2	7.8
Al	11134 - 11865	1148	367
Fe	7113 - 9302	7950	1181
Mn	374 - 1180	752	406

LOD-Bellow detection limit

With respect to Barigüi sediment samples, it is important to remark that it was difficult to collect Barigüi River sediment. It was necessary more than one attempt to get enough sediment for analysis. It was also difficult to collect from the same site; there were cases when grab caught only solid waste.

For Barigüi: Cr, Cu, Pb, and Zn were higher at campaign two, in a high flow period (1.61 m^3s^{-1}). Fe presented its higher concentration in campaign seven, when flow was high (2.64 m^3s^{-1}). Average daily flow varied from 1.22 m^3s^{-1} and 2.64 m^3s^{-1} and accumulated rainfall varied between 9.9 mm and 124.7 mm. These five metals could have come through runoff (relation with flow can be observed in FIGURE 23). Among them, Cu, Pb, and Zn may compose cemetery effluent. Iron is present in car break composition.

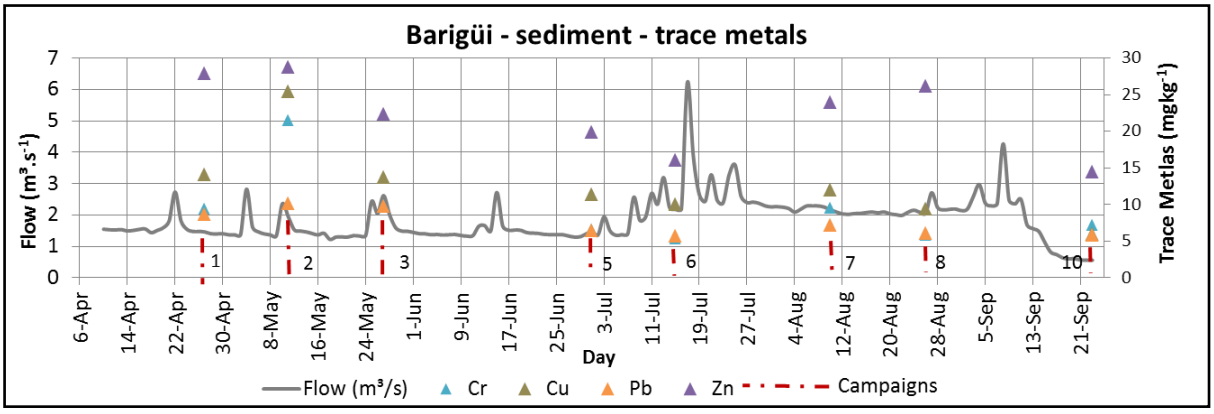


FIGURE 23 – TRACE METALS FOR SEDIMENT IN BARIGÜI RIVER

Another metal, Mn, presented high concentration while COD concentration was low. Concentration range, average and standard deviation of metals in sediment for Barigüi are shown in TABLE 20.

TABLE 20 – BARIGÜI TRACE METALS VALUES IN SEDIMENT

Metal(mgkg^{-1})	Range	Average	Standard deviation
Cd	0.06 - 0.21	0.16	0.05
Cr	5.4 - 21.5	9.4	5.2
Cu	5.9 - 25.4	12.8	5.7
Pb	5.8 - 10.2	7.5	1.8
Zn	16.1 - 28.8	22.5	5.3
Al	2775 - 4855	4382	1115
Fe	6099 - 7890	7362	835
Mn	79 - 210	161	61

In Barigüi River, it was observed significant and positive correlation in sediment between almost every trace metal analyzed indicating proportional growth between those metals in sediment.

Correlating literature values and Barigüi sediment metal concentration, it is observed that Cd presented 20% lower concentration than Gonçalves (2008) same sampling site. For the same analyzed site, Cr, Pb, and Zn presented 114%, 63%, and 80% higher concentrations than Gonçalves (2008) respectively. Cd, Cr, and Pb were lower than Castoldi (2014) suspended solids concentration for the same river at

a more urbanized site. Cu and Pb were lower than Fuchs et al. (1996) in low urbanized site.

Relating analyzed monitoring sites, Miringuava presented higher values of Cr, Pb, Zn, Fe, and Mn. In Miringuava, Zn is common in applied pesticides composition. Mn was higher than soil composition at Miringuava site for all samples; also Miringuava average flow was higher than Barigüi. Cd, Cu, and Al were higher for Barigüi site (being more urbanized than Miringuava). Cadmium and aluminium are common in urban pollution. Copper is present in pesticides commonly used in Barigüi culture areas (GONÇALVES, 2008). Also, for water and sediment samples, Miringuava River presented more significant correlations.

Neither in Miringuava, nor in Barigüi sediment samples presented trace metal concentration above legislation CONAMA 420/2009 (for soil, see Item 2.1.4), not for rural, residential or industrial zone. Furthermore, analyzing both sampling sites with ANOVA it was observed significant difference among average concentration of some trace metals in sediment. There was great difference for Cr ($p=0.0061$), Cu ($p=0.0050$), and Zn ($p=0.0041$). These differences certify that both environments presented different concentration of some trace metals due to differences in use and soil occupation.

Values specified for each monitoring site campaign are presented in APPENDIX. Correlation results with other matrices are presented in Item 4.4.2.

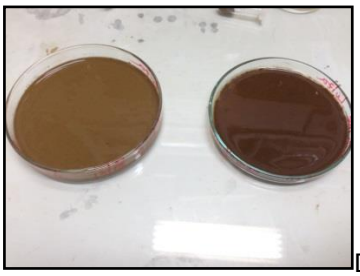
4.4 BIOFILM

For biofilm samples the following parameters were analyzed: water content, dry and wet formed weight, Cd, Cr, Cu, Pb, and Zn. It can be seen in FIGURE 24A a biofilm glass sheet after biofilm formation. In FIGURE 24B it can be observed biofilm collection. In FIGURE 24C is showed biofilm texture. FIGURE 24D presents differences between biofilm formed in each sampling site. Petri dish on the right (with darker biofilm) is from Miringuava and petri dish on the left (with lighter biofilm) is from Barigüi. Difference in biofilm color could be due to different organic matter concentration. Finally, dry biofilm is presented in FIGURE 24E.


A


B


C


D

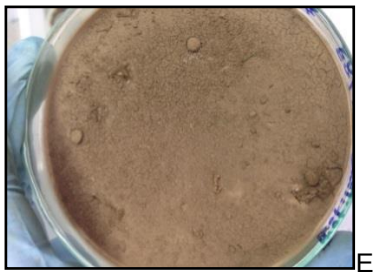

E

FIGURE 24 – BIOFILM: GLASS SHEET (A), COLLECTION (B), TEXTURE (C), FROM BARIÜI (LEFT) AND FROM MIRINGUAVA (RIGHT) (D), AND DRY (E).

Biofilm sampler was adapted from Fuchs, Haritopoulou and Wilhelmi (1996) to be installed under a bridge. Before, the sampler used to be installed on the riverbed, making it difficult to collect samples when water level was high. Although, when the water level was high, it was difficult to align sampler with water flow in Barigüi River due to peculiarities of the chosen stretch (see APPENDIX for some picture examples). Also, many times there were trash, leaves and branches stuck over sampler. For some campaigns, biofilm formed in one side but not in the other of the glass sheet, suspicion is that it happens because in some part of the time sampler was not aligned with flow.

4.4.1 Water Content and Formed Weight

Miringuava water content was higher than 80 % for 10 of 11 campaigns, average was 84.6% and standard deviation 5.3 %. As for Barigüi, for all campaigns biofilm presented more than 80 % of water content, average was 86 % and standard deviation was 4.7 %, as presented in FIGURE 25. Miringuava and Barigüi average water content was high, corroborating with water percentage in natural biofilms presented by Balzer et al. (2010). Although, water content was lower than Singh,

Paul, Jain (2006) values for pure biofilm. This could represent that formed biofilm is not pure, having other substances like suspended solids or organic matter.

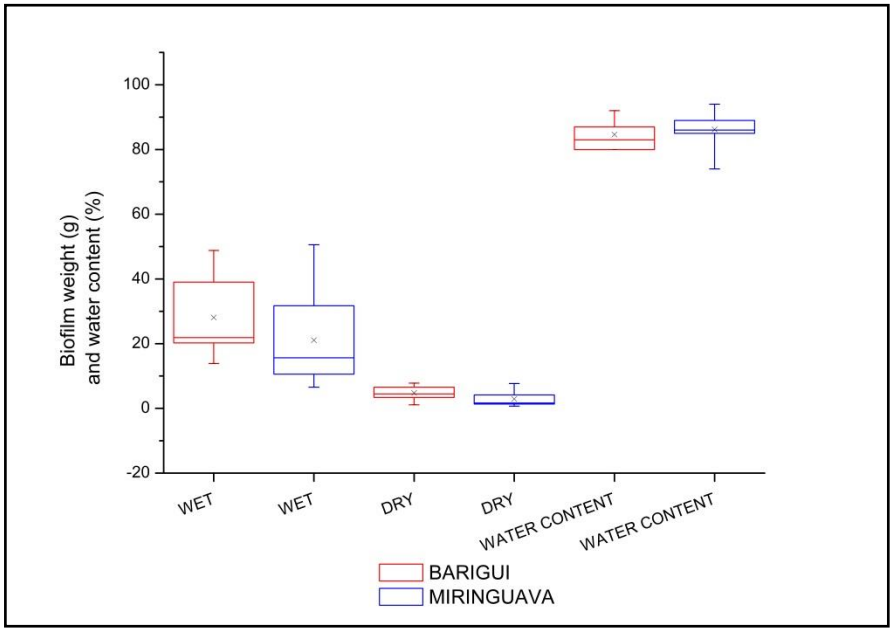


FIGURE 25 – BIOFILM WET AND DRY WEIGHT AND WATER CONTENT

For Miringava site, high dry and wet biofilm happened at high accumulated precipitation period. They varied from 7 g to 51 g for wet weight and from 0.7 g to 7.7 g for dry weight (as can be seen in FIGURE 25). Weight differences in biofilm formation for Miringuava River had no linear relation with flow variance. For Barigüi, during periods without significant changes in flow, higher weight biofilm was formed (campaigns one and two). Low biofilm weight (campaigns three and nine) were observed when the samples were collected during a peak flow. In Barigüi River, wet weight varied from 14 g to 49 g and dry weight from 1.1 g to 7.8 g (FIGURE 25).

Regarding biofilm values, wet and dry weight were directly proportional to each other. Comparing sampling sites, wet and dry weight and water content were higher for Barigüi, which also presented higher average flow.

4.4.2 Trace Metals in Biofilm

Trace metals in biofilm for both sampling sites can be observed in FIGURE 26.

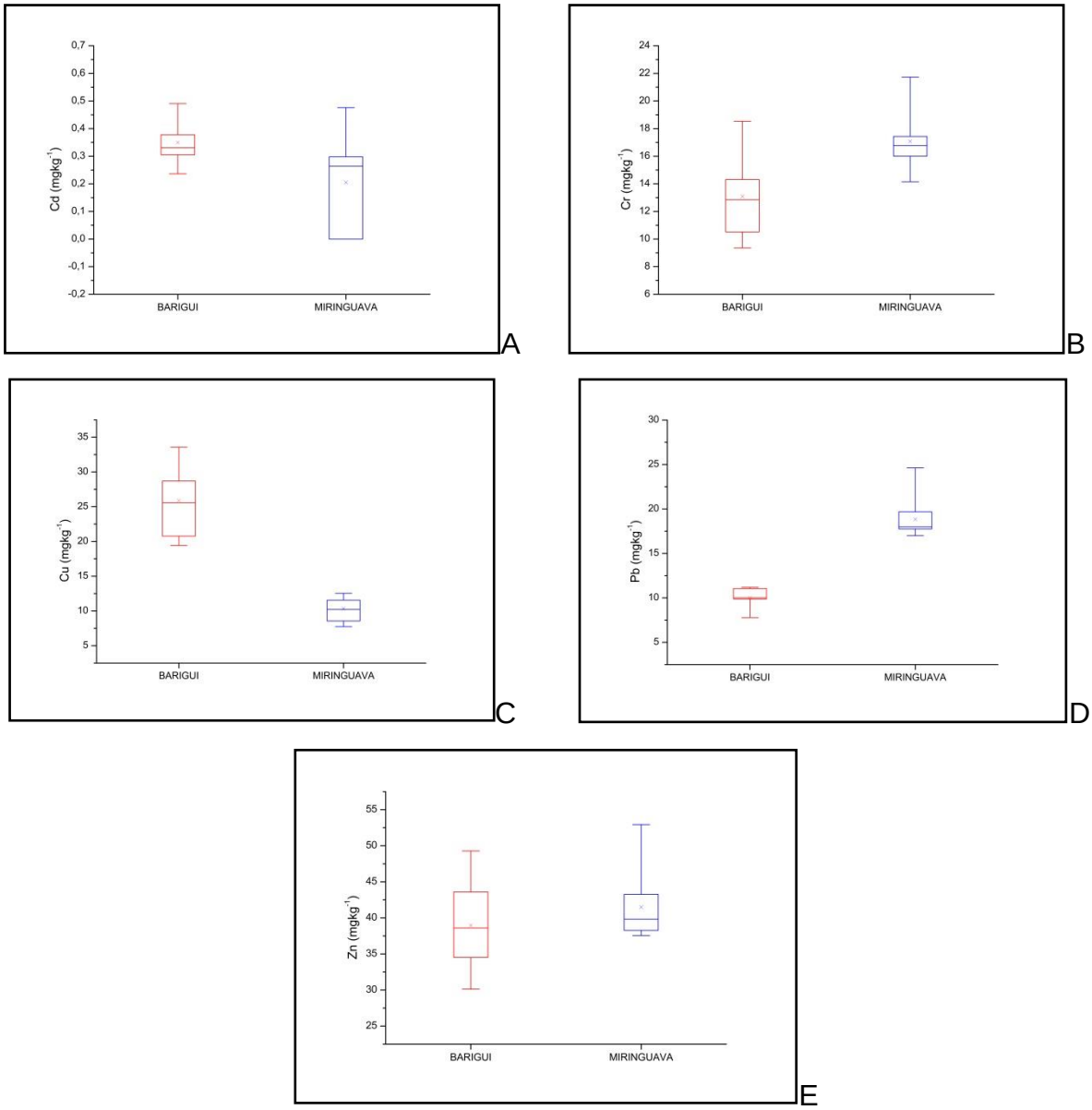


FIGURE 26 – TRACE METALS CONCETRATION IN BIOFILM FOR MIRINGUAVA (M) AND BARIGÜI (B). CADMIUM (A), CHROMIUM (B), COPPER (C), LEAD (D), AND ZINC (E)

Regarding Miringuava, Cu high value occurred at high average flow and accumulated rain period (campaign three - 2.07 m^3s^{-1} and 91.9 mm), that way these element could have entered the system through runoff. Zn, Cr, and Pb high value happened at a low average flow and accumulated rain period (campaign five - 1.03 m^3s^{-1} and 43.5 mm). Cd presented its higher value in campaign six, when average daily flow and accumulated rainfall was low (0.72 m^3s^{-1} and 47.8 mm). So, Zn, Cr, Pb, and Cd could have concentrated. Accumulated rainfall varied from 43.5 mm to 130.0 mm and average daily flow from 0.74 m^3s^{-1} to 2.61 m^3s^{-1}.Values show that

biofilm is not contaminated with Cd, Cr, Cu and Zn comparing it to natural soil composition. Trace metals fluctuation with flow can be observed in FIGURE 27.

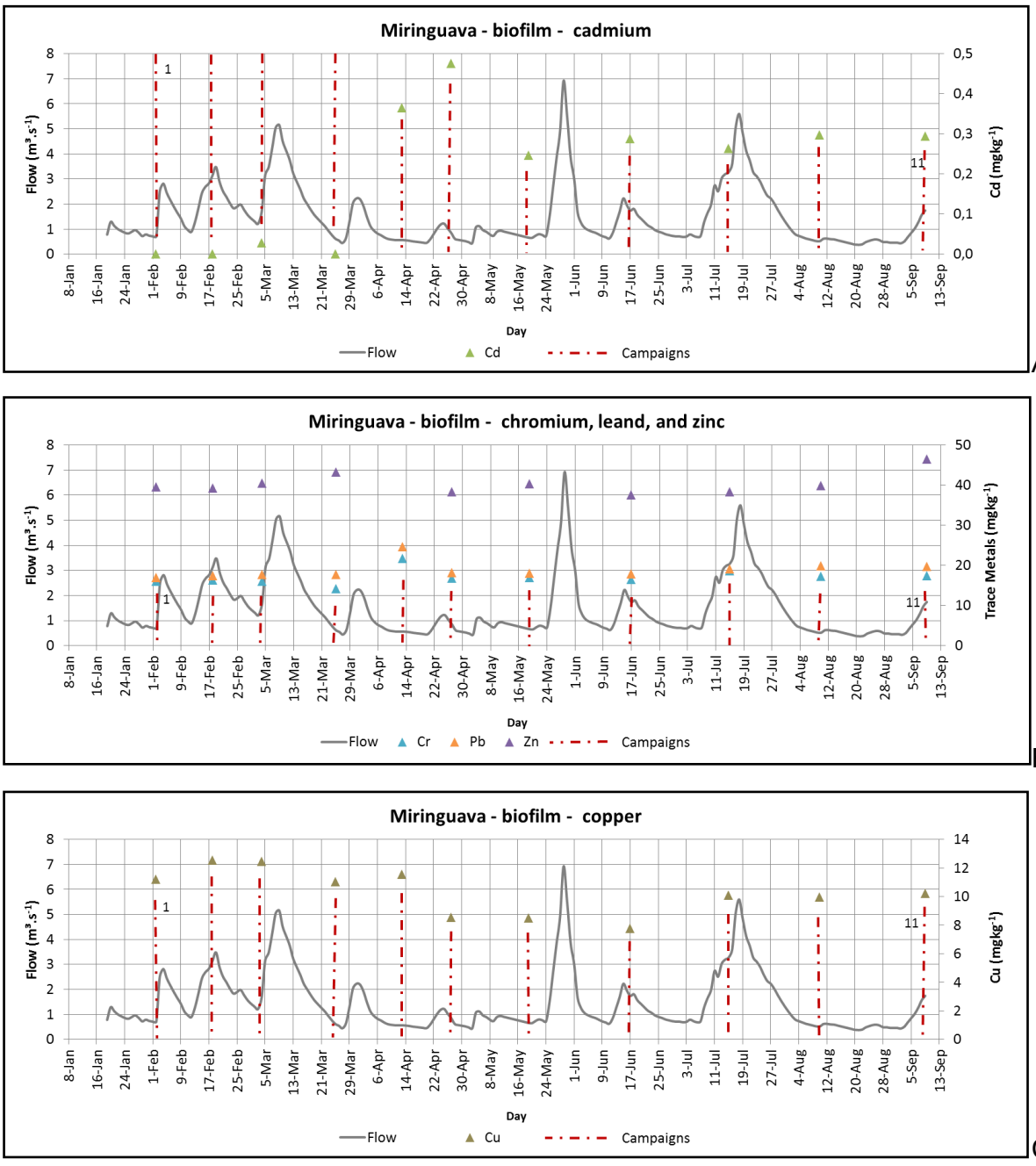
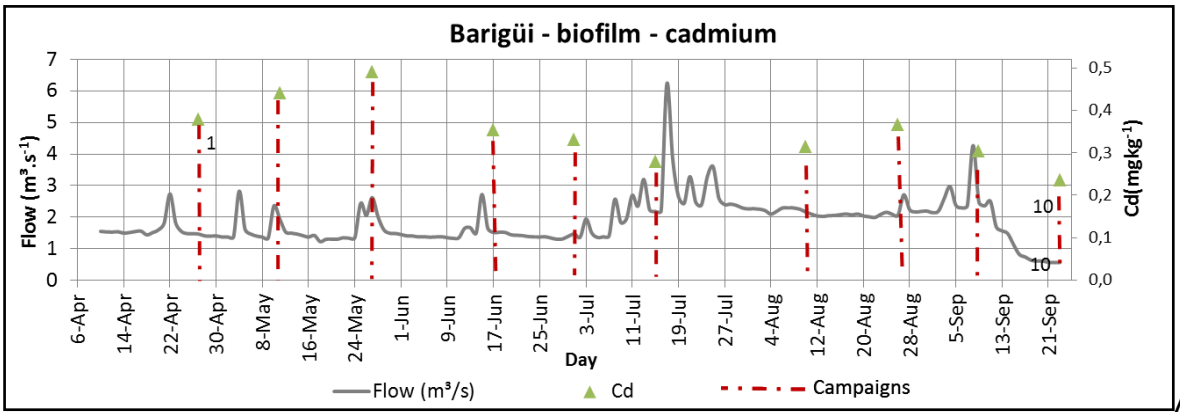


FIGURE 27 – TRACE METALS FOR BIOFILM IN MIRINGUAVA RIVER. Cd (A), Cr, Pb, and Zn (B), and Cu (C)

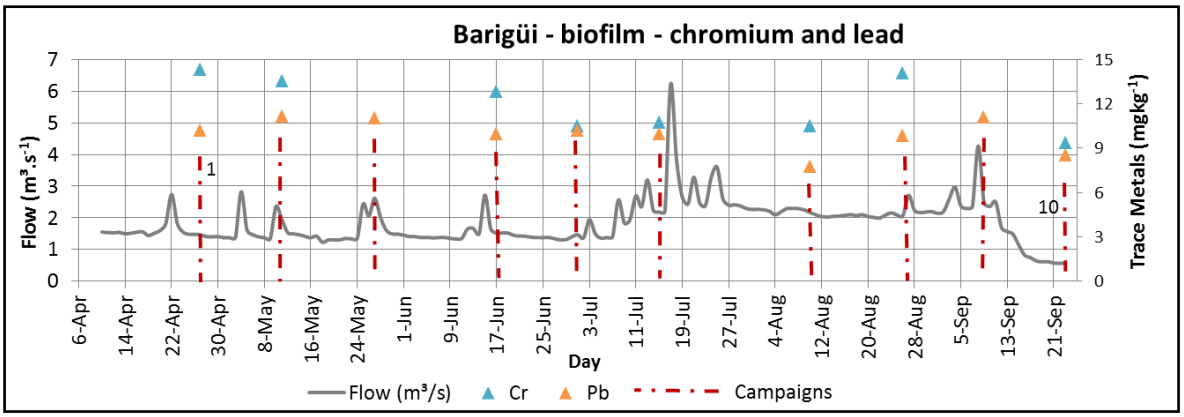
Comparing biofilm values with literature, for Miringuava, Cd, Cr, and Cu was lower than Fuchs et al. (1996), Mages et al. (2004), Gomes (2010), Kohušová et al. (2011), Ancion et al. (2013), and Castoldi (2014) even for similar land use areas. Only lead presented higher values than literature, it was higher than Gomes (2010),

Kohušová et al. (2011), and Ancion et al. (2013), being the first and third author monitoring site located at low urbanized sites and the second author site located at more urbanized site. Besides, when comparing biofilm Pb average value with soil composition, lead is higher at biofilm.

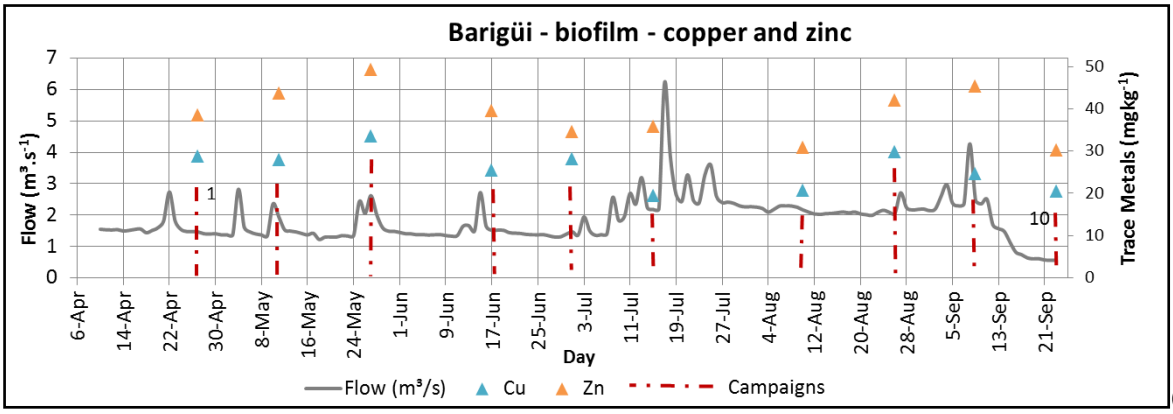
For Barigüi, Cd, Cu, Cr, Pb, and Zn highest values happened at low flow period (campaign three - 1.58 m^3s^{-1} and 32.3 mm), these elements could have concentrated as can be observed in FIGURE 28. Average daily flow varied from 1.22 m^3s^{-1} and 2.64 m^3s^{-1} and accumulated rainfall varied between 9.9 mm and 124.7 mm. For Barigüi, all trace metal concentrations in biofilm were lower than literature data (FUCHS et al., 1996; MAGES et al. 2004; GONÇALVES, 2008; GOMES, 2010; KOHUŠOVÁ et al., 2011; ANCION et al., 2013; CASTOLDI, 2014). Values specified for each monitoring site campaign are presented in APPENDIX.



A



B



C

FIGURE 28 – TRACE METALS FOR BIOFILM IN BARIGÜI RIVER. Cd (A), Cr and Pb (B), and Cu and Zn (C)

In Miringuava River, it was observed significant and positive correlation in biofilm between Cr and Pb, Cr and Zn, and Pb and Zn. There was significant and negative correlation between Cd and Cu in biofilm. Further investigation could analyze if there is competition between these metals or if they are from different sources. Correlation values for these metals can be observed in TABLE 21.

TABLE 21 – MIRINGUAVA BIOFILM CONCENTRATION CORRELATION

	Cd	Cr	Cu	Pb
Cr	0.5949 p=0.054			
Cu	-0.6627 p=0.026	0.0159 p=0.963		
Pb	0.5263 p=0.096	0.8895 p<0.05	0.1207 p=0.724	
Zn	0.1724 p=0.612	0.6024 p=0.050	0.3481 p=0.294	0.8275 p=0.002

* Red values - p<=0.05 (significant correlations)

In Barigüi River it was observed significant and positive correlation in biofilm between almost every trace metal analyzed except for Pb and Cd as it can be seen in TABLE 22. These results could indicate the same adsorption system in biofilm for original concentrations. For biofilm samples, Barigüi River presented more significant correlations than Miringuava River, probably because of higher concentrations of these metals dissolved in Barigüi water.

For both sampling sites none of the trace metal values in biofilm were above limits established by CONAMA 420/2009 for soil use. When comparing sites, biofilm and sediment presented the same aspects for trace metals concentration except for

Cd. Cd and Cu was higher for Barigüi than for Miringuava, as can be seen in FIGURE 26, these elements are common in urban pollution. Cr, Pb, and Zn concentration was higher for Miringuava biofilm samples.

TABLE 22 – BARIGÜI BIOFILM CONCENTRATION CORRELATION

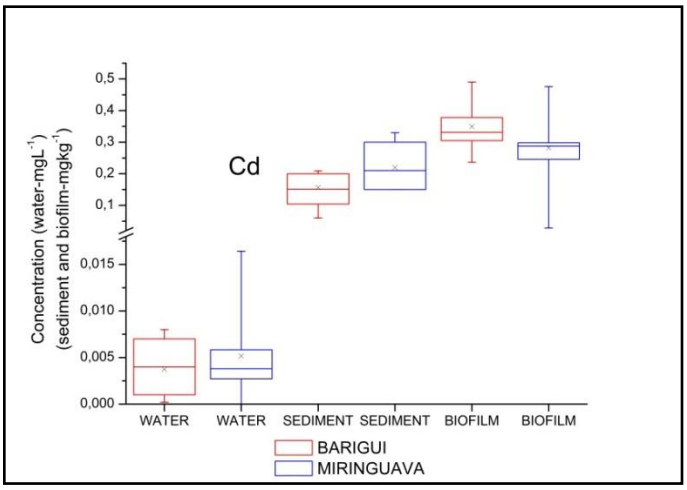
	Cd	Cr	Cu	Pb
Cr	0.7355 p=0.015			
Cu	0.8625 p=0.001	0.7443 p=0.014		
Pb	0.6139 p=0.059	0.7251 p=0.018	0.6454 p=0.044	
Zn	0.7612 p=0.011	0.9453 p<0.05	0.7413 p=0.014	0.8604 p=0.001

* Red values - p<=0.05 (significant correlations)

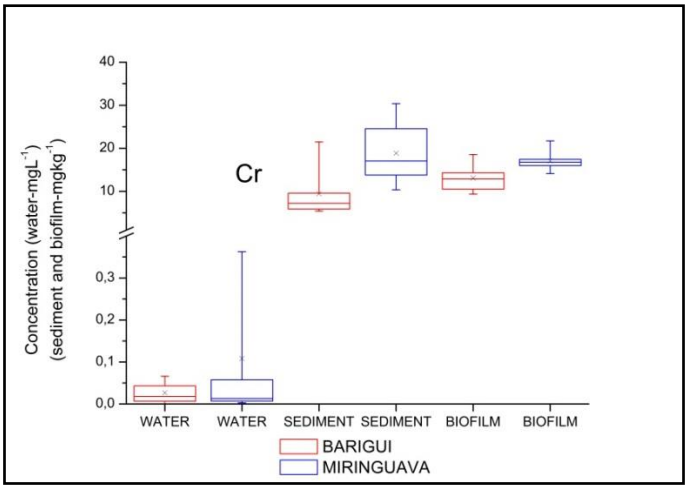
Analyzing data with ANOVA it was observed significant difference (95%) in accumulated biofilm between Barigüi and Miringuava River for Cr (p=0.0020), Cu (p=0.0002), and Pb (p=0.0002). These differences ratify that both environments presented different concentration of some trace metals due to differences in use and soil occupation.

Comparing Barigüi River matrices, it was observed positive correlations among the three matrices. Therefore, there is interaction among water, sediment, and biofilm. For instance, there was positive correlation between Cd in water and biofilm (0.9072/p=0.005), Cd in water and sediment (0.8486/p=0.016), Cd in sediment and biofilm (0.8574/p=0.006), and Pb in water and sediment (0.7663/p=0.045). Positive values could indicate no competition among some metals in these matrices. Also, Barigüi River presented more significant correlations among matrices.

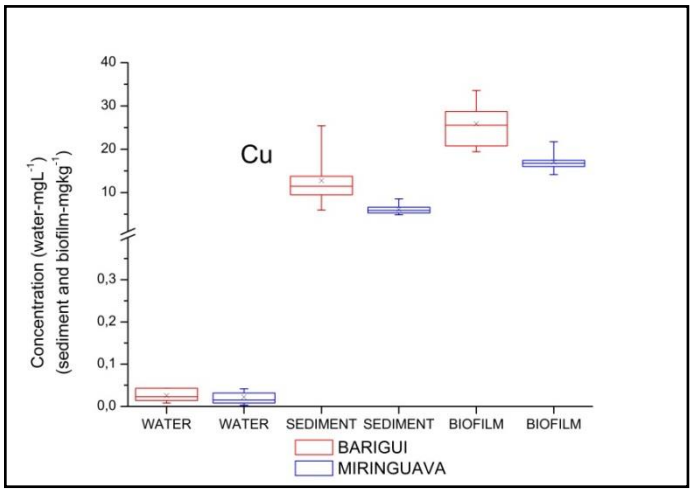
For Cd, Cu, and Zn, there were higher concentrations (on average) in biofilm for both sampling sites than on sediment, as can be seen in FIGURE 29 A, C and E. For Cr and Pb, there were higher concentrations in Barigüi River for biofilm and on Miringuava River for sediment, as can be seen in FIGURE 29B and D.



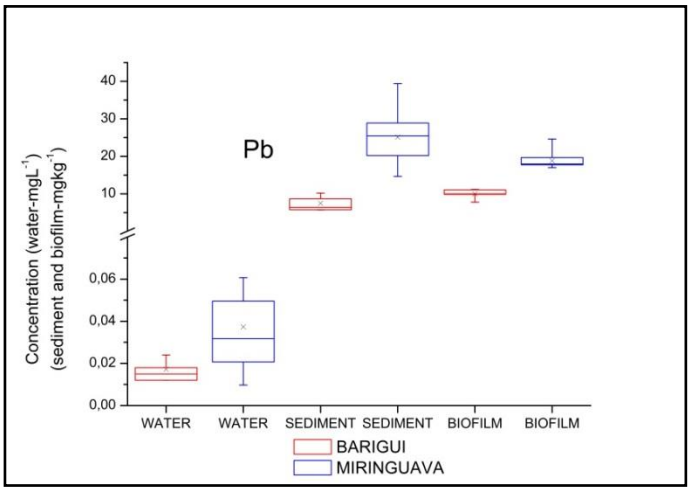
A



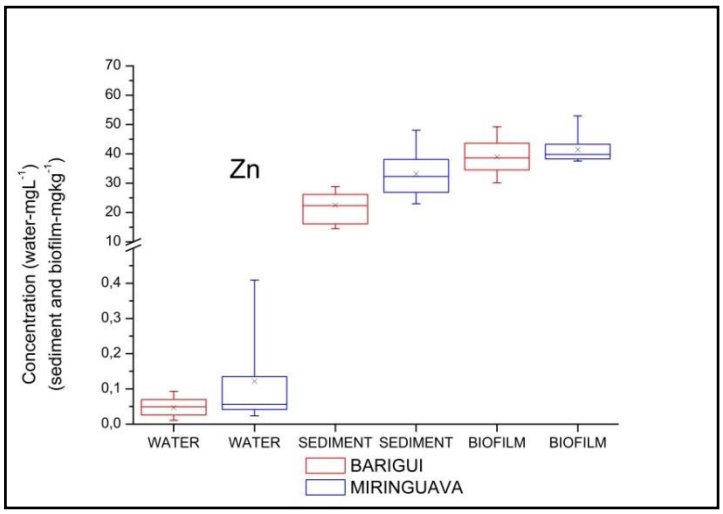
B



C



D



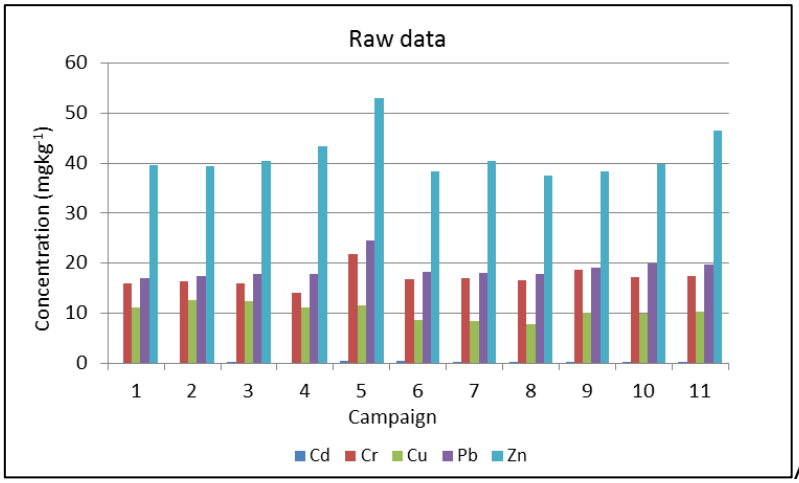
E

FIGURE 29 – TRACE METALS CONCETRATION IN THREE MATRICES FOR MIRINGUAVA FOR CADMIUM (A), CHROMIUM (B), COPPER (C), LEAD (D), AND ZINC (E)

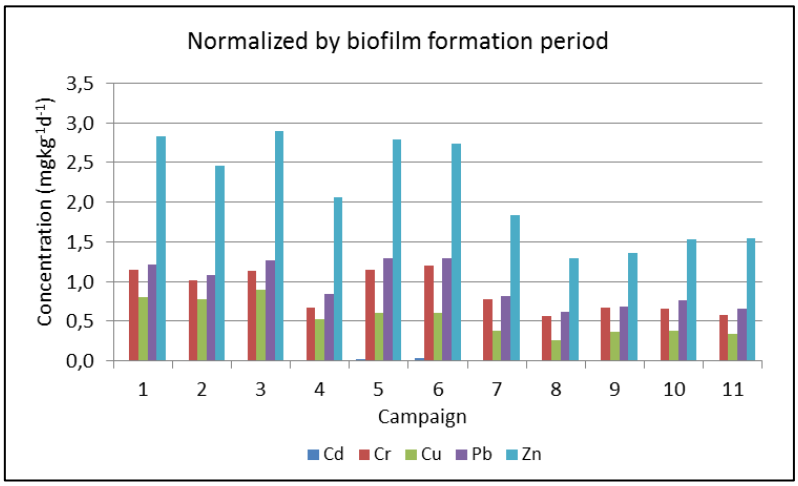
As can be checked on FIGURE 29, between monitoring sites it was observed that Miringuava River presented higher average concentration of Cr, Pb, and Zn for three analyzed matrices (water, sediment and biofilm). For water and sediment it was observed higher average concentration of Cd. Cu was the only trace metal that presented higher average concentration for all matrices in Barigüi River. Barigüi also presented higher Cd average concentration of biofilm.

4.4.3 Normalized data of trace metals in biofilm

Trace metal concentration in biofilm were normalized by formation period (days), average daily flow (for biofilm formation period), formed biofilm dry weight and the combination of them. Best and most significant results are show in FIGURE 30 for Miringuava River and in FIGURE 31 for Barigüi River.



A



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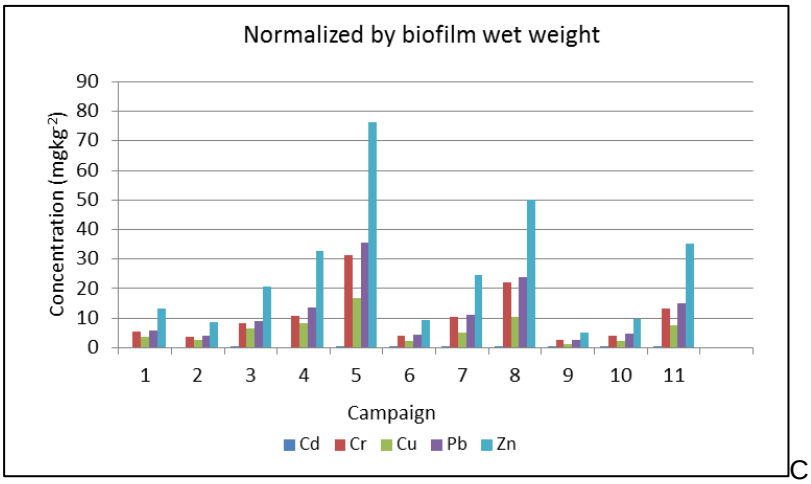

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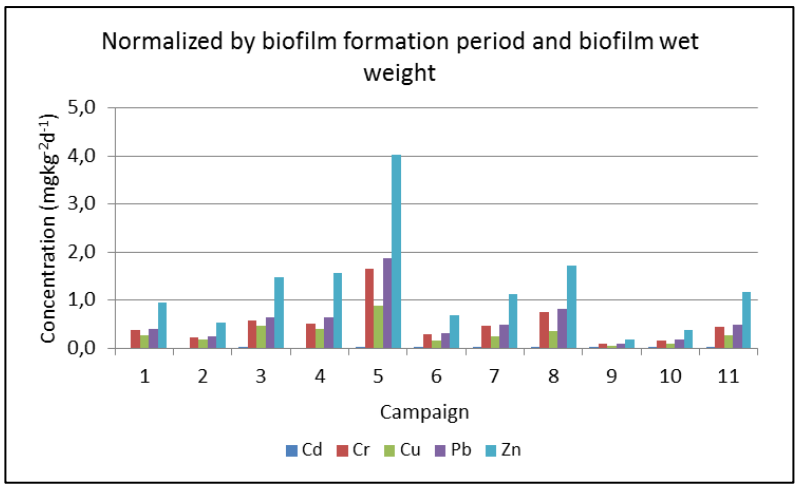
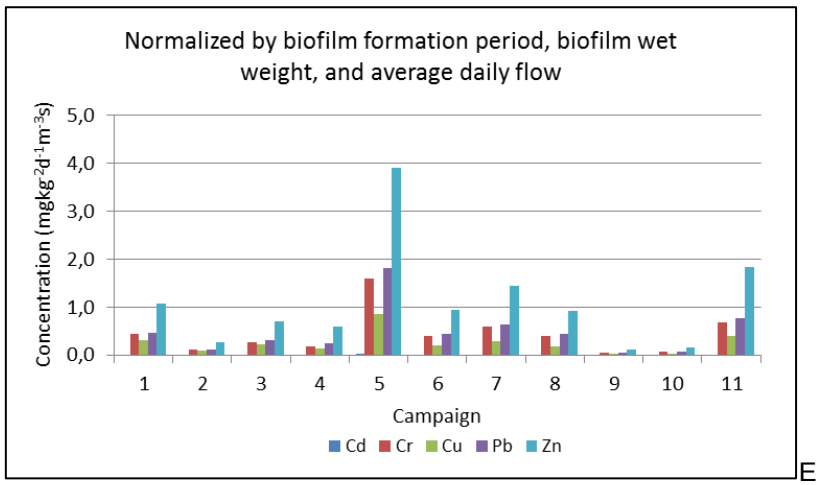


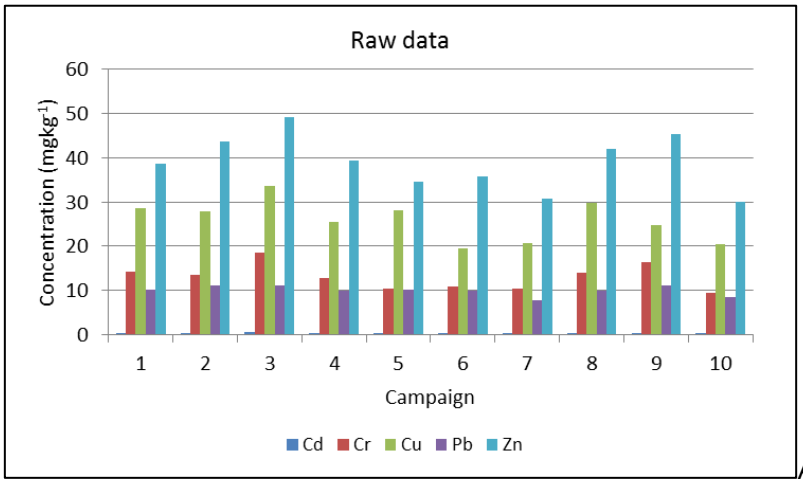
D



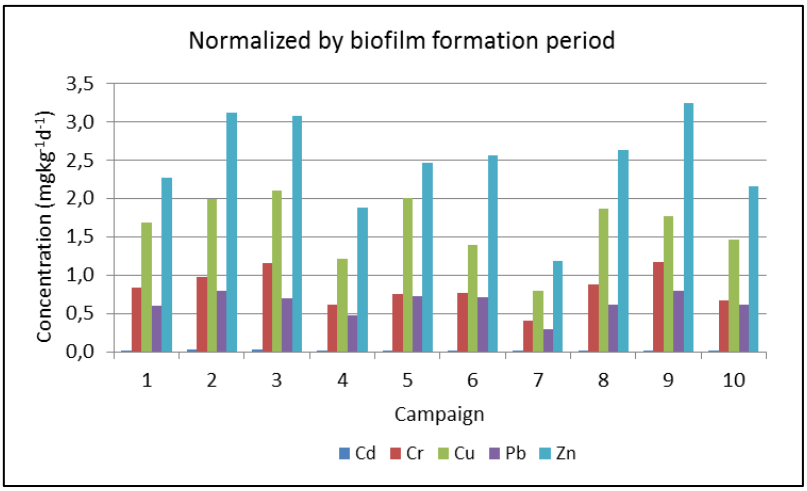
E

FIGURE 30 – TRACE METAL CONCENTRATIONS IN BIOFILM THROUGH CAMPAIGNS (NORMALIZED DATA) - MIRINGUAVA RIVER. RAW DATA (A), NORMALIZED BY BIOFILM FORMATION PERIOD (B), DRY BIOFILM WEIGHT (C), BIOFILM FORMATION PERIOD AND DRY BIOFILM WEIGHT (D), AND BIOFILM FORMATION PERIOD, DRY BIOFILM WEIGHT AND AVERAGE DAILY FLOW (E)

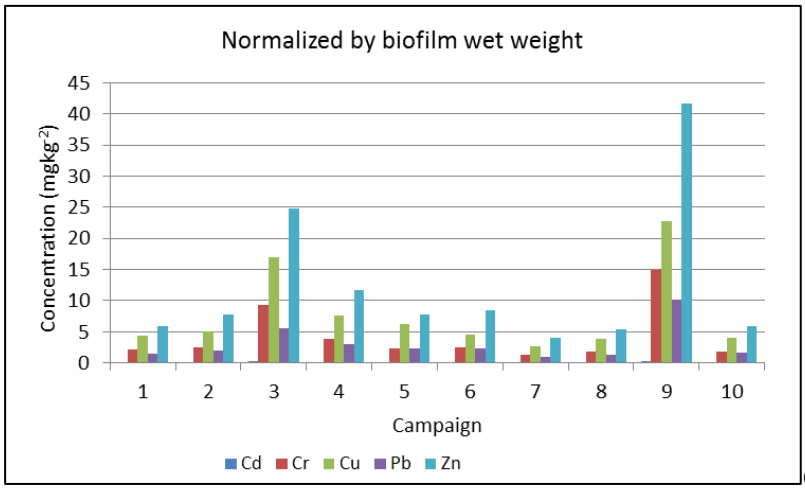
For Miringuava sampling site, by looking at no normalized data (FIGURE 30A), it can be observed that there were no big changes in trace metal concentration. On the other hand, if data is normalized by days of formation (FIGURE 30B), after campaign eight trace metal concentration decreased. From campaigns one to seven, formation period was on average 17 days. From campaigns 8 to 11, formation period was on average 28 days. For Barigüi River the same happen concerning data no normalized (FIGURE 31A) and normalized by formation period (FIGURE 31B). It can be seen in campaigns four and seven lower trace metal concentration, at this campaigns formation period was 21 and 26 days respectively. It can be concluded that a higher period is worse for metal concentration, a hypothesis is that material is lost or that metal concentration in biofilm is trying to achieve equilibrium with environment.



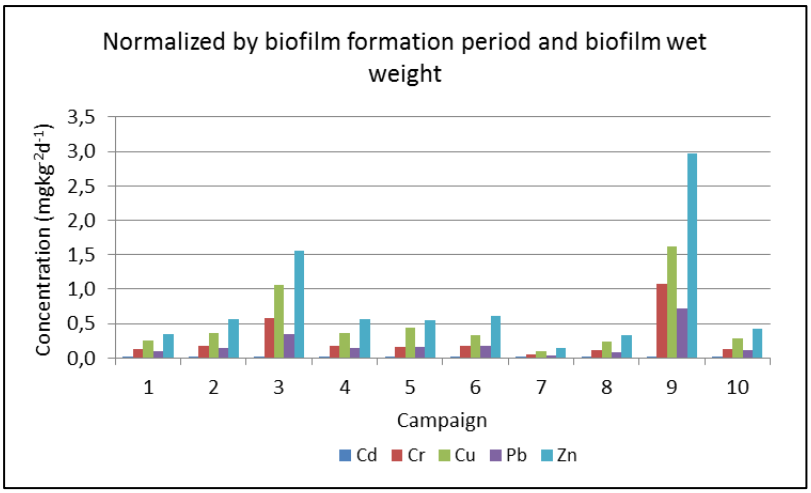
A



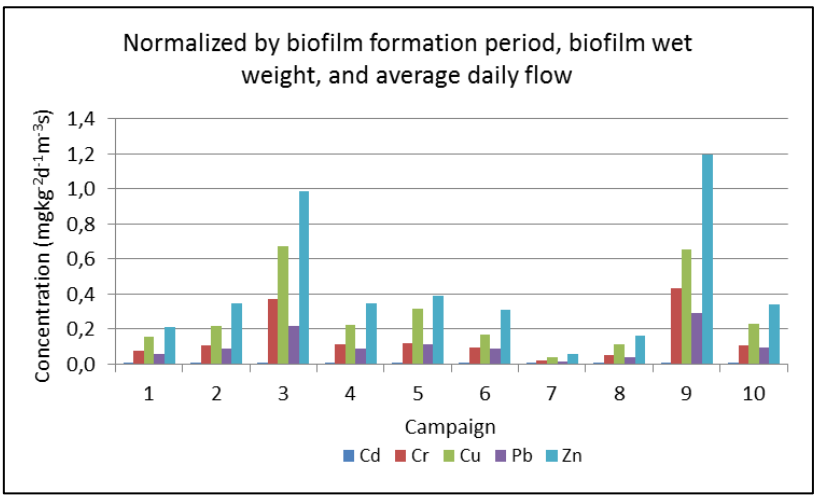
B



C



D



E

FIGURE 31 – TRACE METAL CONCENTRATIONS IN BIOFILM THROUGH CAMPAIGNS (NORMALIZED DATA) - BARIGÜI RIVER. RAW DATA (A), NORMALIZED BY BIOFILM FORMATION PERIOD (B), DRY BIOFILM WEIGHT (C), BIOFILM FORMATION PERIOD AND DRY BIOFILM WEIGHT (D), AND BIOFILM FORMATION PERIOD, DRY BIOFILM WEIGHT AND AVERAGE DAILY FLOW (E)

Comparing Miringuava normalized data by biofilm formed weight (FIGURE 30C), formation period and weight (FIGURE 30D), and by formation period, day and

weight (FIGURE 30E), it can be seen that the highest trace metal concentration occurred in campaign five and second highest concentration happened in campaign eight and three (except for FIGURE 30E). Campaign five presented the lowest dry formed weight of biofilm (0.69 g), a low peak flow (see FIGURE 13), low average flow (1.03 m^3s^{-1}) and low accumulated rainfall (43.5 mm). Campaign eight presented also low dry weight formed on biofilm (0.75 g) but, on contrary, a great peak flow (see FIGURE 14), high average flow (1.86 m^3s^{-1}) and high accumulated rainfall (116.0 mm). Low and high values comparing to the range for the sampling site (wet weight from 0.7 g to 7.7 g, average flow from 0.64 m^3s^{-1} to 2.24 m^3s^{-1}, and accumulated rainfall from 37.8 mm to 130.0 mm).

As for Barigüi, analyzing normalized data by weight (FIGURE 31C), formation period and weight (FIGURE 31D), and by formation period, day and weight (FIGURE 31E), it can be seen that the higher trace metal concentration occurred in campaign nine and the second highest concentration occurred in campaign three. Campaign nine presented the lowest dry biofilm formed weight (1.09 g), high average flow (2.48 m^3s^{-1}), and high accumulated rainfall (111.5 mm). Campaign three also presented low dry biofilm formed weight (1.98 g) but low average flow (1.58 m^3s^{-1}), and low accumulated rainfall (32.3 mm). Low and high values comparing to the range for the sampling site (wet weight from 1.1 g to 7.8 g, average flow from 1.22 m^3s^{-1} to 2.48 m^3s^{-1}, and accumulated rainfall from 9.9 mm to 124.7 mm).

For both sampling sites, it can be concluded that high trace metal values were observed when flow was low (so values could be concentrated) and also when flow was high, so this pollutants could have come from runoff.

5 FINAL NOTES

Miringuava and Barigüi Rivers at the monitored period (from January to September 2016) for the analyzed point (Miringuava - 25° 36' 29.46" S/ 49° 6' 6.35" W and Barigüi - 25° 18’ 46.2” S/ 49° 17’ 44.7” W) presented good oxygenation. They also presented low organic matter and good water quality according to COD and DOC concentrations. Few water quality data made it not possible to analyze pH, DO, and other parameters influence over biofilm formation and metal adsorption. Although, trace metals concentration in water indicated that these elements could have come from runoff and some of them could be from the same source, like agrochemical substances for both sampling sites, soil composition for Miringuava River, and sewer for Barigüi River. Also, trace metals content in water were higher for Barigüi, except for manganese.

Regarding sediment, for both sampling points little fine sediment was collected, sometimes hampering trace metals content determination. Besides, it was a challenge to collect sediment samples representing a specific time period with the used technique and available equipment. Even though, results showed that manganese, lead, and zinc presented higher concentration than soil composition for Miringuava River. Positive representative correlation among almost all metals in Barigüi and Miringuava River indicated proportional accumulation among trace metals. Besides, between sampled sites, Miringuava River presented higher concentration for all analyzed metals and difference in soil use and occupation were evident with trace metal analysis.

For both sampling sites, biofilm presented great water content corroborating with other authors values. In weight terms, Barigüi River formed more biofilm than Miringuava River; and for both sites, formed weight was higher when flow had few variation over the time period. Also, high trace metal concentration happened in low flow period. Further, Miringuava River results showed that there is no pollution regarding cadmium, chrome, zinc, and copper. In Barigüi River, correlation indicated that trace metals may have endured the same adsorption system. Additionally, differences in trace metal concentration between both sampling sites indicated different use and soil occupation.

Among matrices, there were significant correlations among metals in sediment and in biofilm. Biofilm presented higher concentration of cadmium, copper, and zinc than sediment. Further, normalized data showed that higher formation period (more than 15 days) is prejudicial for metal concentration, maybe because material is lost or because biofilm is trying to achieve equilibrium with metal concentration of the environment.

Concluding, biofilm represented environment situation for different land use sites. Also, the sampler is low cost when compared to frequent water analysis. Its representativeness is good, mainly when compared to water and sediment samples (trace elements were detected in almost every campaign in biofilm samples). By evaluating biofilm as a monitoring technique to quantify trace metals in aquatic environment it can be concluded that it is a good technique.

6 FURTHER STUDIES

Over the research some difficulties were found; to avoid these adversities some changes in methodology should be made. First, biofilm sampler should be improved; it should be adapted to join water flux and to prevent clogging of the mesh. To collect biofilm samples, a large bottle should be chosen to facilitate the collection. Also, sampler exposure should be changed (starting with two or three days and varying it). For trace metal analysis, organic matter content should be estimated and it should be chosen a place with higher urbanization

Besides, it should be chosen a better technique to collect sediment, one that represents only recent contribution. Regarding water samples, due to few water quality data it was not possible to conclude relation between some parameters and metal content. For further studies it is suggested to measure water quality parameters more frequently, during whole biofilm formation. Composed water sample could give a better idea to understand sample viability and be used to evaluate suspended solids concentration in water.

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8 APPENDIX

GRANULOMETRY METHODOLOGY

1 - RESULTADO ESPERADO

Distribuição granulométrica (8 mesh 500 micras).

2 - RECURSOS/MATERIAIS NECESSÁRIOS

Jogo de peneiras;

Desagregador;

Balança de 2 casas;

Quarteador Jhones;

Becker 1500 mL;

Granulômetro Cilas 1064

3 - METODOLGIA

Quartear e secar a amostra em estufa a 70oC;

Pesar 200g da amostra já seca;

Anotar o peso das peneiras 8, 14, 42 e 80 mesh na planilha de granulometria, na coluna peneira ;

Desagregar o material com 500 mL de água e passar pela peneira o sobrenadante, repetindo o processo até que o sobrenadante fique límpido, podendo assim colocar todo o material do Becker na peneira;

Peneirar a úmido, forçando a passagem pelas peneiras com água corrente retirando os excessos de amostra das paredes com a mão, sem forçar;

Após repetir o processo em todas as peneiras, colocar para secar em estufa até 70 ºC por cerca de 15 horas;

Retirar da estufa, esperar esfriar, pesar as peneiras novamente registrando o peso na coluna peneira + amostra ;

Subtrair da coluna peneira + amostra a coluna peneira e anotar os valores na coluna retido ;

Com outra alíquota quarteada, passar a amostra por uma peneira 80 mesh forçando a passagem com água corrente, reservando o passante para ser analisado no granulômetro. Para analisar no granulômetro, seguir a metodologia PP20;

Com esses dados preencher a planilha de Excel Integrada e então se obtém o gráfico de distribuição granulométrica.

4 - CUIDADOS ESPECIAIS

Controlar a corrente de água para o material não extravasar;

Não impor muita força nas peneiras para que não rompam as malhas. Ter sempre o cuidado de estar homogeneizando o passante para não decantar o material.

FIELD DATA FROM MIRINGUAVA SITE

Campaign	pH	Turbidity (NTU)	DO (mgL^{-1})	Water Temperature (°C)	Conductivity (μScm^{-1})
1	6.25	24.0	9.50	17.7	44.0
2	5.50	56.4	7.59	19.0	31.5
3	6.69	14.3	6.43	20.3	38.0
4	8.11	5.8	8.96	18.8	38.0
5	7.24	6.4	7.84	19.6	38.0
6	7.56	7.7	7.18	16.9	38.0
7	7.22	23.9	7.48	16.0	39.0
8	6.65	16.2	7.34	11.6	37.0
9	7.27	38.9	7.42	15.7	37.0
10	7.00	13.1	7.09	16.7	40.0
11	6.89	50.0	7.71	15.7	33.0

FIELD DATA FROM BARIGÜI SITE

Campaign	pH	Turbidity (NTU)	DO (mgL^{-1})	Water temperature (°C)	Conductivity (μScm^{-1})
1	7.98	6.5	7.45	18.5	341
2	7.62	33.0	7.65	16.6	303
3	8.45	362.0	7.11	18.2	260
4	8.04	15.7	6.81	14.8	315
5	7.51	13.8	8.40	15.4	308
6	7.96	50.3	9.15	17.5	144
7	7.61	16.0	7.99	17.6	329
8	7.85	12.0	8.11	16.9	334
9	7.87	80.3	7.86	15.9	261
10	8.31	153.0	6.95	21.2	366

DQO VALUES AND CALIBRATION CURVE DETERMINATION COEFFICIENT

Campaign	Miringuava site		Campaign	Barigüi site	
	DQO (mgL^{-1})	R^2		DQO (mgL^{-1})	R^2
7	23.8	0.98	3	21.1	0.98
8	17.5	0.99	4	10.4	0.99
9	17.1	0.99	5	2. 6	0.98
10	3.1	0.98	6	11.0	0.99
11	24.8	0.99	7	1.1	0.98
			8	3.9	0.98
			9	12.2	0.99

WATER TRACE METAL VALUES FOR MIRINGUAVA (values in mgL^{-1})

Campaign	Cd	Cr	Cu	Pb	Zn	Al	Fe	Mn
1	LOQ	LOQ	LOQ	0.11	0.11	-	-	0.033
2	LOQ	LOQ	0.063	LOQ	0.15	-	-	0.17
3	0.0054	0.026	0.012	0.019	0.090	-	-	-
4	0.0022	0.089	0.014	0.080	0.069	-	-	-
5	0.0055	0.018	0.0022	0.042	0.014	-	-	-
6	0.00028	0.0066	0.0035	0.015	0.033	-	-	-
7	0.011	0.71	0.027	0.026	0.56	-	-	-
8	0.022	0.014	0.056	0.064	0.25	-	-	-
9	0.000038	LOD	LOD	LOD	0.016	0.78	1.05	0.04
10	LOD	LOD	LOD	LOD	0.083	0.23	0.86	0.031
11	0.00060	0.0058	LOD	LOD	0.030	0.78	1.44	0.055

LOD-Bellow detection limit, LOQ-Bellow quantification limit

WATER TRACE METAL VALUES FOR BARIGÜI (values in mgL^{-1})

Campaign	1	2	3	4	5	6	7	8	9	10
Cd	0.000063	0.013	0.0038	0.0069	0.00039	LOD	0.0015	LOD	LOD	LOD
Cr	0.053	0.033	0.0032	0.058	0.024	0.11	0.024	0,013	LOD	LOD
Cu	0.011	0.034	0.011	0.016	LOD	LOD	0.086	LOD	LOD	LOD
Pb	0.030	0.018	0.071	0.029	LOD	LOD	LOD	LOD	LOD	LOD
Zn	0.020	0.048	0.050	0.091	0.0074	0.045	0.14	0.0029	0.018	0.024
Al	-	-	-	-	0.12	0.57	0.16	0.12	0.95	0.14
Fe	-	-	-	-	0.41	1.54	0.42	0.47	1.64	0.30
Mn	-	-	-	-	0.018	0.053	0.023	0.024	0.054	0.020

LOD-Bellow detection limit

SEDIMENT TRACE METAL VALUES FOR MIRINGUAVA (values in $mgkg^{-1}$)

Collection average	1	2	3	4	5	6	7	8	9	10	11
Cd	-	LOD	LOD	LOD	0.15	0.33	0.30	0.25	0.21	0.15	0.15
Cr	-	15.9	13.8	10.4	17.0	30.4	24.6	20.8	24.9	17.6	13.2
Cu	-	5.3	4.9	4.9	6.1	8.5	6.9	6.1	6.6	5.9	5.8
Pb	-	25.5	23.0	20.2	29.3	39.4	25.9	25.4	28.9	18.9	14.7
Zn	-	39.2	23.8	23.0	32.3	48.1	37.1	34.4	38.1	29.2	26.9
Al	-	-	-	-	-	-	-	-	11865	11134	11446
Fe	-	-	-	-	-	-	-	-	9302	7438	7113
Mn	-	-	-	-	-	-	-	-	1180	700	374

LOD-Bellow detection limit

SEDIMENT TRACE METAL VALUES FOR BARIGÜI (values in $mgkg^{-1}$)

Collection	1	2	3	4	5	6	7	8	9	10
Cd	0.21	0.20	0.20	-	0.18	0.10	0.15	0.15	-	0.06
Cr	9.4	21.5	9.9	-	6.6	5.4	9.6	5.9	-	7.2
Cu	14.1	25.4	13.8	-	11.4	10.1	12.0	9.5	-	5.9
Pb	8.7	10.2	9.8	-	6.4	5.7	7.2	6.1	-	5.8
Zn	27.9	28.8	2.,3	-	19.9	16.1	24.0	26.2	-	14.5
Al	-	-	-	-	-	5321	4855	4581	-	2775
Fe	-	-	-	-	-	7505	7890	7812	-	6100
Mn	-	-	-	-	-	147	205	210	-	80

PROBLEMS FACED BY THE BIOFILM SAMPLER


A


B


C

A – Sampler obstructed with leaves and branches. B – Sampler stucked in branches and other materials. C – Sampler misaligned with flux due to high flow.

BIOFILM TRACE METAL VALUES FOR MIRINGUAVA (values in $mgkg^{-1}$)

Collection average	1	2	3	4	5	6	7	8	9	10	11
Cd	LOD	LOD	0,03	LOD	0.36	0.48	0.25	0.29	0.26	0.30	0.29
Cr	16.01	16.36	15.97	14.15	21.73	16.76	16.98	16.49	18.67	17.29	17.43
Cu	11.20	12.54	12.47	11.05	11.56	8.55	8.50	7.74	10.10	9.94	10.21
Pb	17.00	17.41	17.78	17.76	24.61	18.16	17.98	17.84	19.11	19.93	19.68
Zn	39.62	39.30	40.49	43.27	52.94	38.27	40.39	37.56	38.26	39.83	46.50

LOD-Bellow detection limit

BIOFILM TRACE METAL VALUES FOR BARIGÜI (values in $mgkg^{-1}$)

Collection	1	2	3	4	5	6	7	8	9	10
Cd	0.38	0.44	0.49	0.35	0.33	0.28	0.31	0.37	0.30	0.24
Cr	14.31	13.59	18.53	12.87	10.50	10.77	10.52	14.12	16.37	9.36
Cu	28.70	27.88	33.57	25.55	28.07	19.44	20.76	29.76	24.72	20.47
Pb	10.22	11.19	11.06	10.01	10.19	9.97	7.79	9.88	11.09	8.54
Zn	38.60	43.61	49.27	39.48	34.52	35.90	30.76	42.08	45.38	30.14