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“TRACKING HEAVY METAL POINT SOURCES IN GRAVITY  
SEWERS USING BIOFILM CARRIERS”

Master thesis (30 EAP)

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Tartu  
2021

## Kokkuvõte

# BIOKILEKANDJATE KASUTAMINE RASKMETALLI- REOSTUSE PUNKTALLIKA JÄLITAMISEKS KANALISATSIOONIS

Biokiledel on omadus akumulierida endasse mitmesuguseid saasteaineid, sealhulgas ka raskmetalle. Seetõttu on biokilesid võimalik kasutada bioindikaatorina raskmetallireostuse määramiseks. Käesoleva magistritöö eesmärgiks oli katsetada biokiledel põhinevat meetodikat raskmetallide punktreostusallika identifitseerimiseks kanalisatsioonis. Selleks viidi läbi katseid nii laboritingimustes kui ka isevoolse ühiskanalisatsioonis. Töö tulemused kinnitasid, et biokiled on võimelised endasse akumulierima reovees olevaid raskmetalle nii laboratoorses tingimustes kui ka kanalisatsioonis, võimaldades biokilede abil identifitseerida suuremaid reostusallikaid.

**Märksõnad:** raskmetallid, biokiled, tööstusreovesi, reovee seiramine

CERCS: T270 Keskkonnatehnoloogia, reostuskontroll.

## Abstract

# TRACKING HEAVY METAL POINT SOURCES IN GRAVITY SEWERS USING BIOFILM CARRIERS

It has been reported that biofilms have the ability to accumulate different pollutants, including heavy metals. Hence, biofilms can be used as a bioindicator to detect heavy metal pollution. The aim of this master's thesis was to test a biofilm carrier-based wastewater sampling method to detect illegal heavy metal discharges into the public sewer. For that, experiments were done in laboratory settings and in the public sewer. The results showed that biofilms are able to accumulate heavy metals from wastewater in both laboratory setting and sewer system, making it possible to track the major sources of upstream heavy metal pollution.

**Keywords:** heavy metals, biofilm sampling, industrial discharges, sewerage monitoring

CERCS: T270, Environmental technology, pollution control

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# 1. Introduction

The main by-product of the wastewater treatment process is sewage sludge, which is a potential fertilizer due to its high nitrogen and phosphorus content (Sörme & Lagerkvist, 2002). Further use of sewage sludge depends on the quality of the sludge, which itself relies upon the chemical composition of the influent wastewater and its treatment processes.

On a general level, one of the problematic groups of pollutants for the reuse of sludge are heavy metals, the key source of which in sewage sludge is industry (Fijalkowski et al., 2017). Therefore, careful management of industrial wastewaters is important for preventing the accumulation of heavy metals in sludge. None of the usual sludge treatment methods like composting, anaerobic digestion, or drying have significant effects on removing heavy metals from sludge. Hence, industrial release of heavy metals into the public sewers should be avoided. On-site heavy metal removal can often be done at a relatively reasonable cost by chemical precipitation, adsorption, or ion exchange. Unfortunately, not all industries follow this practice, making industrial wastewater discharge monitoring a necessity.

Currently, the preferred method for wastewater discharge monitoring is with grab samples that characterize wastewater only at the time of sample collection. However, industries do not usually discharge their wastewaters continuously and wastewater composition may change over time, which means that the determination of pollutants through individual grab sampling of water might give incorrect evaluations.

To locate a specific polluter every suspicious sewerage branch should be monitored by taking numerous composite samples. However, this approach is time-consuming and resource-intensive for local water companies. Due to legislation, the sewage service provider might have to cover the costs of wastewater analyses. This is the case in many countries, including Estonia. Therefore, there is a need for a continuous monitoring system that can locate illegal industrial discharges easily and cost-effectively. Biofilm carrier-based sampling might be a potential solution to the problem.

Biofilms are a complex aggregation of microorganisms that are found on almost every surface exposed to water. It has been reported that biofilms have the ability to accumulate heavy metals from the environment and this feature can be easily used for heavy metal detection in public sewers (Fuchs et al., 1997).

The objective of this thesis is to test a biofilm carrier-based wastewater sampling method to assess the suitability of methodology for non-compliant wastewater discharge. In other words, to clarify if the method is suitable in Estonian conditions, by verifying if elevated heavy metal levels are recorded in biofilms. For that, experiments were conducted in laboratory conditions and in the sewage system.

## 2. Literature review

### 2.1. Heavy metals

A widely accepted definition is that heavy metals are a group of elements with a density greater than  $5 \text{ g/cm}^3$ . From an environmental standpoint, they are defined as metals that have high toxicity at low concentrations with a long residence time in soil (Binkley & Simpson, 2003). Unlike organic contaminants, heavy metals are not biodegradable, thereby they can accumulate in the soil and enter the food chain (Fijalkowski et al., 2017). Many heavy metals are known to be toxic or carcinogenic and tend to accumulate in living organisms. (Fu & Wang, 2011). Toxic heavy metals of particular concern are enacted in European Directive 86/278/EEC which sets limits for seven metals for sewage sludge. Those metals are Cd, Cr, Cu, Pb, Zn, Ni, and Hg. Henceforth this master thesis will focus only on these metals (except Hg).

Metals in wastewater treatment plant (WWTP) influent can exist in a variety of states: free metal ions, chelated compounds, organometallic complexes, and elemental metals. These substances may be dissolved in water or associated with colloidal or particulate matter (Binkley & Simpson, 2003; Cantinho et al., 2016). Each metal acts differently in wastewater and the distribution of metals between particulate and soluble fraction varies between different wastewaters. Several factors such as influent chemical oxygen demand (COD), metal concentrations, pH, redox potential, and influent suspended solid concentration may influence metal speciation (Ziolko et al., 2011). For example, at acidic pH values heavy metals tend to exist as free metal ions, but around neutral pH values (6-9) precipitate as hydroxides or another insoluble state (Binkley & Simpson, 2003). In general, it has been found that Cu, Pb, Zn, and Hg in wastewater influent are mostly in particulate form while Ni is mostly in the soluble fraction. Zn is usually the most abundant heavy metal in municipal wastewaters, whilst Hg values are often the lowest (Carletti et al., 2008; Hargreaves et al., 2018)

### 2.2. Sewage sludge

Sewage sludge is a byproduct of the wastewater treatment process, which consists of water, inorganic and organic solid particles, and the microorganism developed during the activated sludge process. Approximately 1% of treated wastewater is turned into sludge (Lester et al., 1983). Sewage sludge consists of high concentrations of organic matter and biogenic compounds, like phosphorus and nitrogen which are crucial for plant growth (Tytła, 2019).

The final quality of sewage sludge can vary due to the influent characteristics and applied treatment technology (Gherghel et al., 2019)

Depending on the treatment stage, sludge generated in a typical WWTP can be divided into three categories: primary sludge, waste activated sludge, and chemical sludge (Andreoli et al., 2007). Primary sludge is produced during the mechanical wastewater treatment, where readily settleable and floatable solids from the wastewater are separated. The solids content of primary sludge is about 3-9% and consists of a high proportion of organic matter. Activated sludge is produced during biological treatment and consists mainly of microorganisms and inert materials. The total solids concentration is between 0.8 and 3.3% and the organic portion contains around 10-15% nitrogen and 1-3% phosphorus (Gherghel et al., 2019). Chemical sludge is produced by precipitation of suspended solids or specific substances and is most commonly a by-product of chemical phosphorus removal. Chemical sludges can contain a considerable amount of metals (Fe, Al), which is a result of using inorganic coagulants. (Sörme & Lagerkvist, 2002).

Quite often all previously mentioned sludges are combined for further treatment and disposal. The resulting mixed sludge is most commonly treated subsequently: thickening, stabilization (anaerobic/aerobic), mechanical dewatering, and finally composting or thermal treatment (drying and/or incineration). Thermal treatment is usually applied only in very large treatment plants due to economical and environmental reasons (Gherghel et al., 2019), but is gaining more popularity due to increasing concerns over organic micropollutants in the sludge.

Sewage sludge is often seen as a waste, but it also may be a source of energy when anaerobic or thermal treatment is applied. Due to the nutritional value of sewage sludge, it can be used as a land fertilizer, soil conditioner, or even as a source to extract phosphorus (Fijalkowski et al., 2017). Either way, the fact is that nutrient reuse of treated sewage sludge is getting more and more crucial due to the global depletion of phosphorus resources and the need for sustainable management strategies (Gherghel et al., 2019). Due to low population density in Estonia mainly small and medium-sized wastewater treatment plants dominate the water sector. For such treatment plants investment and operating costs for novel nutrient reuse or extraction technologies are too high and require further development. Therefore, land application is still the main sludge disposal and nutrient recovery method in Estonia.

Although land application provides several benefits, there are also some risks associated with it. For instance, if the influent contains heavy metals, then they are predominantly transferred to sewage sludge. Therefore sewage sludge can also contain toxic heavy metals such as cadmium, chromium, copper, mercury, nickel, lead, and zinc (Tytła, 2019). Considering that only 1% of the total flow is produced as sludge and this sludge contains more than 50% of the total influent heavy metal load, metals are concentrated to a significant extent (Lester et al., 1983). Since the metals can concentrate to a level where they can be environmentally hazardous, it sets significant restrictions on treated sludge use and disposal. In addition to that, the sludge can contain different organic pollutants, pathogens, and foreign matter, which can also limit further use of sludge (Fijalkowski et al., 2017). Unlike most organic contaminants, heavy metals are not degradable even with advanced oxidation technologies and therefore the presence of heavy metals is considered one of the main obstacles for the use of sewage sludge in the natural environment (Zhang et al., 2017)

All previously mentioned metals are present in sewage sludges in Estonia as indicated by the data collected from different Estonian WWTP's from 2011 to 2017 (Eesti Keskkonnauuringute keskus, 2018). Typical values of these metals are displayed in the table below.

Table 1. Minimal, maximal, average, and median heavy metal values (mg/kg of dry matter) from different sewage sludges in Estonia

<b>Heavy metal</b>	<b>Minimal values in sludge [mg/kg DM]</b>	<b>Maximal values in sludge [mg/kg DM]</b>	<b>Average values in sludge [mg/kg DM]</b>	<b>Median values in sludge [mg/kg DM]</b>	<b>Detection frequency in samples</b>
<b>Cr</b>	1.6	60	27.7	23.0	100%
<b>Ni</b>	5	29	15.5	15.0	100%
<b>Cu</b>	14	320	162.2	157.5	100%
<b>Zn</b>	120	830	534.5	555.0	100%
<b>Cd</b>	<1	16	3.1	1.3	60%
<b>Pb</b>	5	23	11.7	11.0	100%



### 2.3. Sources of heavy metals in wastewater and sewage sludge

In general, the main input sources of heavy metals to the WWTP influent can be divided into two: large point sources like industries or hospitals and small point sources such as businesses and households. With combined sewer systems surface (stormwater) runoffs from paved areas, roofs and gutters can contribute to additional heavy metal load to WWTP as a nonpoint source (Cantinho et al., 2016).

The load of heavy metals entering a municipal WWTP depends largely on the type of industries connected with the public sewer. High heavy metal content can be emitted by different industries whose specialties are for example surface treatment processes with metals, smelting, alloying, electronics (semiconductors, printed circuit boards) if no adequate treatment is used (Andreoli et al., 2007; Karvelas et al., 2003). Surprisingly car washing has also been identified as a major source of heavy metals, particularly for Zn, Cu, but also for Pb, Cr, and Cd (Sörme & Lagerkvist, 2002; Tekere et al., 2016)

The composition of the runoff is strongly influenced by the local pollution conditions, such as traffic intensity, air pollution, and proximity of industrial districts. The industrial area stormwater runoff can often have higher levels of contamination compared to other sources (Milik & Pasela, 2018). The most abundant metals in the runoff are Zn and Cu, which mainly originate from car tyres, brake linings, roofs, and other similar metal surfaces (Hargreaves et al., 2018).

In domestic wastewater from regular households, the main sources of heavy metals are everyday household items, chemicals and cosmetics. The main sources of Zn have been found to include personal care products like shampoos, toothpastes but also household agents such as laundry detergent. The majority of Cu in the wastewater comes from copper pipes used for drinking water distribution within buildings (Mattsson et al., 2012). Stainless steel products are confirmed to be an important source of Ni (Hargreaves et al., 2018). Even food contains some heavy metals, e.g., the essential dietary components Cu and Zn (Sörme & Lagerkvist, 2002).

In addition to the different sources of heavy metals to the influent, additional pollutant load can also be added during the wastewater treatment steps. For example, the coagulants used for phosphorus precipitation (mainly Fe- or Al-based) contain traces of other metals, such as Ni and Zn, significantly contributing to the total WWTP metal load (in Estonia respectively 11% of total Ni and 14% of total Zn loads) (Lember, 2020; Sörme & Lagerkvist, 2002).

Even though heavy metals are transmitted to the WWTP from countless sources, industrial wastewater has a remarkable impact on the total heavy metal concentration. Buzier et al. compared in their study nine WWTP's, in the Paris area where one received only industrial wastewater, five received domestic wastewater with a combination of industrial wastewater, and the other three received only domestic wastewater. It was found that metal concentrations were generally lower at WWTPs receiving wastewater only from households. For example, Cd was only detected at the inlet of WWTP's that received also industrial wastewater. A very high concentration of Cr was measured at WWTP which received mostly industrial wastewaters, whereas Cr was not detected in WWTP where the influent originated only from households (Buzier et al., 2011).

Still, the current situation is not comparable with the previous century, where heavy metal contamination in sludge was strongly linked to only industrial wastewater discharge. Based on the results from the Rya WWTP in Gothenburg, the concentrations of seven regulated metals (except Cu) have decreased significantly, staying between one-tenth to one-third compared to the concentrations in the 1970s. (Mattsson et al., 2012). Most likely it is a coincidence of several factors, such as reduced global use of heavy metals in products (lead in gasoline), closures of heavy industries or their movement outside of urban areas, more stringent regulations on effluents, and more efficient pre-treatment technologies (Cantinho et al., 2016; Ziolkowski et al., 2011).

Remaining heavy metal levels in domestic wastewater reflect the general baseline of society, coming from food, construction materials, atmospheric downfall or other similar sources, and a substantial reduction of the metals in influent would require considerable changes in our material use or legislation (Mattsson et al., 2017). This approach for reducing diffuse sources of heavy metals is either impossible (i.e food) or extremely time-consuming and costly. Therefore, to reduce the general loads in the WWTP influents, the end of the pipe water treatment of industrial wastewaters is the best available solution.

#### 2.4. Heavy metals removal from wastewater

In conventional WWTPs heavy metals apart from nickel mostly end up in the sludge, and only a small portion is released to the receiving water body (Sörme & Lagerkvist, 2002). This sorption to sludge mainly takes place within two process steps: primary treatment and biological treatment (Brown & Lester, 1979). The purpose of primary treatment is to remove suspended solids from influent wastewater by settling, therefore insoluble and particulate

heavy metal fraction is also removed. Removal of the soluble heavy metal fraction is very limited during primary treatment (Hargreaves et al., 2018).

Both soluble and particulate heavy metal fraction process is believed to be reduced during biological treatment via a number of different mechanisms and pathways (Brown & Lester, 1979) :

- physical entrapment of insoluble metal by the biomass;
- binding of soluble metal to extracellular polymers and bacterial walls;
- accumulation of soluble metal by the cells.

When relatively large amounts of metals are present then precipitated metals may be physically entangled within the biomass. It is found that in absence of mixed liquor suspended solids (MLSS) only a small portion of insoluble metals is directly settled. Instead, the major removal mechanism for most metals is an MLSS interaction of precipitated metals (Stephenson & Lester, 1987).

Activated sludge microorganisms can remove heavy metals passively or metabolism-dependently. Bioaccumulation is a metabolism-dependent process, where soluble metals are accumulated intracellularly, which involves mechanisms such as metal binding on intracellular compounds and intracellular precipitation. On the other hand, biosorption is a passive process based on physico-chemical interaction between the functional groups on the cell or extracellular polymeric substances surface and metal. Although the specific efficiencies vary depending on the site and influent, the highest removal in biological treatment has been measured for mercury, lead and zinc, copper and chromium followed by cadmium. Because of its high mobility, nickel removal rate is the lowest (Ziolko et al., 2011).

Besides passive heavy metal removal during biological processes, there is a number of different technologies available to actively remove heavy metals from wastewater. Most of these methods are economically unfeasible for municipal wastewater with low heavy metal concentrations and are practically applicable only to concentrated industrial streams. While chemical precipitation is the most common of these technologies, ion exchange, adsorption, and membrane filtration can also be used.

Chemical precipitation is a process, where added chemicals react with heavy metal ions to form insoluble precipitates, which are subsequently separated from water by sedimentation or filtration. Common precipitants include hydroxides, sulphides, and carbonates. The

disadvantage of this method is that it produces a large amount of sludge that is difficult to treat (Blais et al., 2008; Metcalf & Eddy et al., 2003).

With ion exchange, specific materials (resins) are used to exchange cations on their surface with the metals in the wastewater. These materials commonly use either strongly acidic sulfonic acid groups (-SO<sub>3</sub>H) or weakly acid carboxylic acid groups (-COOH) on their surface. When wastewater containing heavy metals passes through the material, the hydrogen ions at the end of the sulfonic and carboxylic groups are exchanged with metal ions (Fu & Wang, 2011). The metal ions are held by the functional groups until washed out with a specific solution, resulting in a concentrated heavy metal solution and the regeneration of the resin material. The resin materials however can be easily contaminated with other substances (organics) and are also greatly dependent on the pH of the wastewater stream, severely limiting the potential uses of the technology (Metcalf & Eddy et al., 2003).

Adsorption is a process of accumulating substances, where they are transferred from the liquid phase onto a surface of a solid, and become bound by physical and chemical interactions (Hargreaves et al., 2018). A common adsorbent is activated carbon due to its high surface area and relative cheapness. The main drawback of adsorption is rapid saturation and clogging of the adsorption material, requiring frequent maintenance, replacement or regeneration which are costly and result in further expenditures, loss of the adsorption material and generation of secondary waste (Crini & Lichtfouse, 2019).

Membrane filtration technologies can remove heavy metal ions with high efficiency and small space requirements, mainly via reverse osmosis and nanofiltration. Although the membrane technology efficiency is excellent, its use is limited due to the membrane fouling, process complexity, low permeate flux, and high investment along with high operating costs (Fu & Wang, 2011). Out of the four technologies described in this section, membrane filtration, especially reverse osmosis, is considered multiple times more expensive than any other method.

Different researchers have also proposed several innovative methods to remove heavy metals from sewage sludge, such as electrokinetic technique, supercritical fluid extraction, treatment with chemical agents, and bioleaching process. At the moment, none of those named methods are economically feasible and are all in need for further development (Geng et al., 2020).

Therefore, heavy metal removal from wastewater is not a widely applicable practice in the WWTPs and preventive measures are still the most feasible solutions the reduce heavy metal

loads. Thankfully, most industries have introduced standard procedures for heavy metal removal which include better management and control of processes and materials. One of the main points specifically is removing and controlling the disposal of hazardous compounds before they reach the waste stream. Undoubtedly this approach requires time-consuming preliminary work to identify the sources but is essential to avoid heavy metal pollution. The streams that are already highly contaminated have to be separated or pre-treated properly before discharging them to public sewerage (Babel & del Mundo Dacera, 2006). Unfortunately, not all industries follow the practice as described previously, and still illegally discharge their polluted wastewater streams to sewerage, which is why industrial wastewater discharge should not be overlooked (Aydin et al., 2020).

## 2.5. Biofilms and their formation

IUPAC defines biofilm as an aggregate of microorganisms in which cells that are frequently embedded within a self-produced matrix of EPS (extracellular polymeric substances) adhere to each other and/or to a surface (Vert et al., 2012). It means that in the presence of water, microbial aggregates can accumulate at surfaces, but the definition can also include microbial aggregates like activated sludge flocs and granules, that are not attached to a surface (Flemming et al., 2021). Either way, all these aggregates are always embedded into a self-produced matrix, which makes up more than 90% of the biofilm dry mass. The matrix is a material that consists of different types of biopolymers, known as EPS. More specifically EPS comprises hydrated biopolymers such as polysaccharides, proteins, nucleic acids, and lipids, but it can also contain molecules and particles sorbed from the local environment (Flemming & Wingender, 2010).

The formation of EPS provides structural and functional benefits for the biofilm, for instance, it makes it possible for the microorganisms to stick to different supports as well as to form stable synergistic communities known as microconsortia. Most importantly, the matrix protects organisms against desiccation, biocides, some antibiotics and ultraviolet radiation (Flemming & Wingender, 2010; Mages et al., 2004). As a result, bacteria that dwell in the biofilm are more vigorous and resistant than free-living cells.

In natural conditions, biofilms are rarely single species and most biofilms are mixtures of microorganisms (Hullebusch et al., 2004). In sewers the majority of microorganisms dispersed on biofilms are bacteria, but also include archaea, fungi, and viruses (Li et al., 2019). Biofilms are influenced by the environmental and mechanical conditions they are

subjected to. Therefore, biofilms in the sewer system differ from other biofilm systems in two important ways: high organic loading rate and high shear stress at the biofilm surface due to high flow velocity. These two factors promote strong biofilm formation and enable them to grow rough and durable. As a result, multispecies biofilm with a very rough surface structure is formed. (Nielsen et al., 1992). Another important factor that influences biofilm characteristics in sewers is dissolved oxygen (DO). In high DO concentrations, aerobic and anoxic bacteria, such as sulphur-oxidizing and denitrifying bacteria are predominant on the surface of the biofilm and therefore the most active. As DO concentrations decrease gradually along poorly ventilated gravity sewer pipes, anaerobic conditions with dominant methanogenic archaea and sulphate-reducing bacteria (SRB) increase in the sewer systems (Li et al., 2019). The available DO concentrations for biofilms can also be seen from a visual point of view- if there is a lot of oxygen in the wastewater, the biofilm growth is strong and the colour of biofilm is light grey; under anoxic conditions, less biofilm is usually formed and it is coloured dark grey to black due to the presence of  $S^{2-}$  (Genuit, 2016).

The biofilm development has its own growing cycle on a biofilm carrier, which includes the following stages: attachment of free microorganisms on a surface, colonization, growth, aging, and finally detachment (Figure 1). After the detachment, the cycle starts all over again forming a new biofilm layer on the surface of the biofilm carrier (Butler & Boltz, 2014).

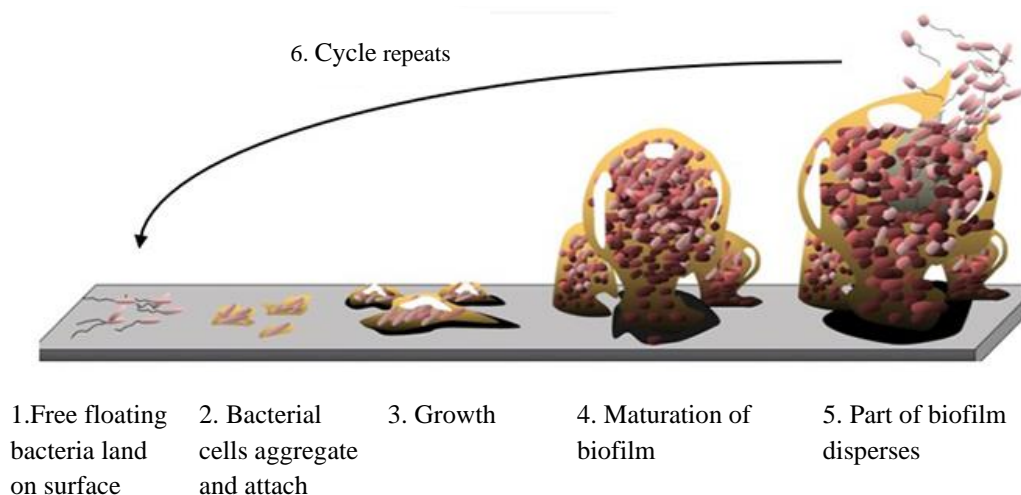


Figure 1. The five stages of biofilm development (Unosson, 2015)

## 2.6. Biomonitoring

Wastewater discharge is generally monitored with grab samples or composite samples. A grab sample is a single sample collected at a specific spot at a site over a short period, typically seconds or minutes. A composite sample is a combination of individual samples taken at selected intervals based on an increment of either flow or time. Composite samples can be obtained by combining portions of multiple grab samples or using automatic sampling devices. When a source is known to be relatively constant in composition over time, then the grab sample may represent a source longer period than the specific time at which it was collected (APHA, 2002). However industrial effluents are rarely constant in time and wastewater composition changes over time, which means that the determination of non-compliant wastewater discharge through individual grab samples may be challenging. Conventional pollution control, therefore, requires numerous samples to be examined before an emitter can be unequivocally identified. Especially when the public sewerage is extensively branched and, in that case, would need unreasonably much time and resources to locate the polluter (Kintrup & Wünsch, 2001). Hence, there is a need for a continuous monitoring system that can determine illegal industrial discharges.

Biomonitoring is one of the alternative methods to assess the presence of heavy metals in wastewater. Biofilm has a high sorption ability and can sorb a wide range of pollutants such as heavy metals and non-polar organic pollutants (Aydin et al., 2020; Flemming, 1995). Therefore, analysing the collected biofilm from sewers will indicate the amount of pollutants that were in contact with biofilm during the growing period. The main advantages of this method are easy deployment in the field with no need for the power supply or specific equipment, which significantly reduces the costs of analysis; and compared to single wastewater samples, temporary emissions are also recorded in the biofilm (Aydin et al., 2020). Since the growth of the biofilm is influenced by different physical (e.g. hydraulic conditions in the sewer, the temperature of the sewage), chemical (pH value, organic and inorganic composition of the wastewater), and biological (species of microorganisms and organism density in the biofilm) factors, no direct quantitative conclusions between the pollutant concentrations in the biofilm and wastewater cannot be obtained (Genuit, 2016). However, the biofilm will reach to equilibrium with contaminants in aqueous media and will give reliable indications for the determination of point sources (Aydin et al., 2020).

Biofilms exhibit a high sorption capacity and one of the mechanisms involved in the immobilization of metals within a biofilm is biosorption. The biofilm is enriched with various

functional groups (carboxyl, sulphate, phosphate and amino) which act as sorption sites where metal cations can be easily bound. Biosorption involves those physico-chemical mechanisms by which different metal particles and ions are removed from an aqueous solution. Biosorption's main metal-binding processes are (Figure 2) (Hullebusch et al., 2004):

- Complexation;
- Precipitation;
- Ion exchange;
- Adsorption.

Complexation is a process of complex formation by electrostatic attraction or covalent bonds between metal ions and functional groups present on the biofilm surface (Ali Redha, 2020). Precipitation occurs due to chemical reactions between the functional groups of the cell wall of the biosorbent and the metal ion, which results in insoluble precipitates and remains attached to the microbial surface. For instance, sulphide precipitation occurs when microbiologically produced hydrogen sulphide by SRB reacts with the metal to form highly insoluble metal sulfide compounds (e.g. ZnS, CdS, CuS) (Hullebusch et al., 2004). Ion exchange mechanism involves the exchange of binary metal cations with the counter ions present on the surface of the biofilm. While physical adsorption of metals on biofilm surfaces takes place by physical interaction such as the van der Waals forces, between the biofilm and metal ions (Priyadarshane & Das, 2021). In addition to the above, simple diffusion is also a basic mechanism observed in bacterial cells (Figure 2). With all of those mechanisms, sequestered metals accumulate within the biofilm and are regarded as sorbed (Flemming, 1995). Metals that are attached to the cell surface can be intracellularly accumulated when metal ions are transported across the cell membrane (Priyadarshane & Das, 2021). The main sorption mechanisms are strongly dependent on several parameters, such as pH, temperature, binding site affinity, and density (D'Acunto et al., 2019)



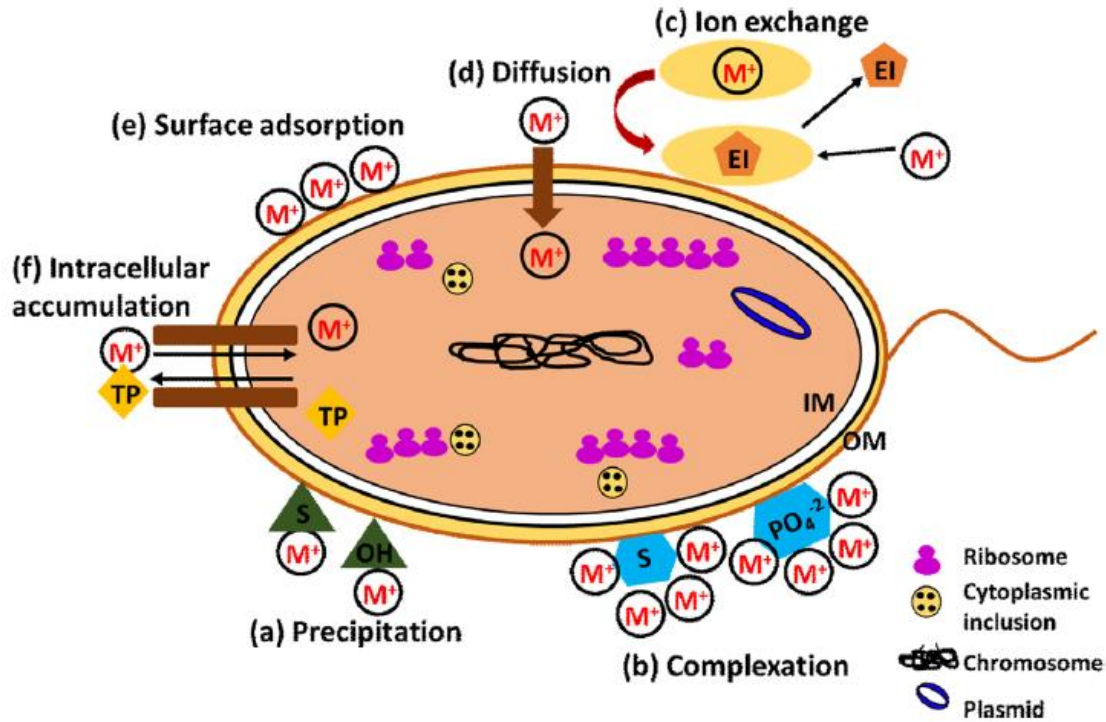


Figure 2. Mechanisms involved in heavy metal biosorption by biofilms (Priyadarshane & Das, 2021)

In biofilms, the metal ions can be sorbed to the bacterial extracellular polymer substances (EPS), to the cell walls, or by the cytoplasm (Mages et al., 2004). It has been reported that heavy metals are sorbed mostly into cellular fraction than into the EPS, although EPS has a high density of charged functional groups (carboxyl, hydroxyl, etc groups) which indicates that a small group of EPS could theoretically bind a large number of given metals (Flemming, 1995). Späth et al. examined Cd and Zn sorption to EPS and cell components finding that about 80% of Cd and more than 80% of Zn were sorbed to the cellular component compared to the EPS (Späth et al., 1998). Wuertz et al. similarly reported in their study that only 12,2% of Cd and 9,1% of Zn added were found in the EPS, while the vast majority of the hydrophobic compounds such as benzene, toluene, and xylene were sorbed in the EPS (Wuertz et al., 2001). Nevertheless, cellular products still play a crucial role in the biosorption of heavy metals. Several studies have shown that EPS-covered cells have a better affinity for adsorption of heavy metals than EPS-removed cells (Priyadarshane & Das, 2021). Sorption capacity can also depend on the bacteria, gram-positive bacteria have greater sorptive capacity than gram-negative bacteria thanks to a thicker layer of peptidoglycan, which provides additional acidic functional groups (Hullebusch et al., 2004).

## 2.7 Legislation in Estonia

The Sewage Sludge Directive (86/278/EEC), was taken into force in 1986 to encourage the application of sewage sludge and to regulate its use to prevent harmful effects in the natural environment. The directive regulates the allowable limits of Zn, Cu, Ni, Pb, Cd, Cr and Hg in sludge and sludge treated soil (Table 3)(European Commission, 1986).

Sewage Sludge Directive is one of the oldest obligatory directives in Europe and for now, it is considered as out-of-date. The European Commission recently opened a public consultation between 20 November 2020 and 5 March 2021 to re-evaluate the Sewage Sludge Directive (86/278). The roadmap underlines that the directive should provide the safe use of sludge in agriculture considering health risks due to the emerging contaminants, but also will aim to recover phosphorus as much as possible. At the moment, to fill the European legislative gap, more than half of EU countries have adopted stricter national regulations for sewage sludge use (Gianico et al., 2021). Estonia is not among those countries. The concentrations of heavy metals in sewage sludge are controlled by regulation No. 29 of the Minister for the Environment of 31 July 2019 according to the directive 86/27/EEC (Table 3).

If sewage sludge meets the set criteria in regulation No. 29, it can be used in agriculture, landscaping, or reclamation. However, according to the Waste Act, it is considered as waste, and therefore the use of sewage sludge must be registered with the Environmental Board. To make the sewage sludge compost more attractive to the end-user and safer for the environment Estonia took into force the end of waste (EoW) legislation for sewage sludge compost (Regulation No. 24 of the Minister for the Environment of 19 July 2017). When sewage sludge has undergone recovery operation and complies with the set criteria then sewage sludge will cease to be waste and can be used as a product. Requirements are set for stability, hygenisation, foreign matter and heavy metals (Table 2).

Table 2. Heavy metal limits in sewage sludge compost

<b>Parameter</b>	<b>86/278/EEC use in agriculture [mg/kg DM]</b>	<b>Regulation No. 29 for use in agriculture [mg/kg DM]</b>	<b>Regulation No. 24 for use in agriculture [mg/kg DM]</b>	<b>Regulation No. 24 use for landscaping and recultivation [mg/kg DM]</b>
Cd	20-40	20	0.15	2
Cu	1000-1750	1000	45	200
Hg	16-25	16	0.1	1
Ni	300-400	300	4	40
Pb	750-1200	750	7.5	130
Zn	2500-4000	2500	125	2500
Cr	-	1000	15	60

At the moment in Estonia, there aren't any certified sewage sludge products. Although some water companies have tried to certificate sewage sludge according to EoW regulation, the certification process's main obstacle has been too high heavy metal (Cd, Zn and Cu) concentration (Kriipsalu et al., 2020). The process also requires a considerable amount of paperwork and bureaucracy and as sludge use as waste per regulation No 29 is still allowed, the certification process is with low benefits and not often considered worthwhile by the WWTPs.

Sewage discharge to public sewers has been regulated since 1999 on the basis of the Public Water Supply and Sewerage Act. The limits of the heavy metals discharge into the sewers are set in Regulation No. 75 of the Minister for the Environment of 16 October 2003 (Table 3).

Public Water Supply and Sewerage Act also states that the water company has the right to regulate the customer contract which and how high metal concentrations are allowed to discharge into the public sewerage. Also, the same law provides for the establishment of precise procedures by local governments. By the local government regulation, water companies are obliged to receive wastewater from the client if their wastewater does not exceed the set values of various pollutants determined by the local government and it does not cause any disturbance to the WWTP cleaning process. In Table 3 are displayed the limit values of hazardous substances set by the Tallinn City Council. Usually, the limit values of

hazardous substances in local government regulations are the same as in Regulation No. 75 of the Minister for the Environment of 16 October 2003.

Table 3. The limit values in sewage discharge

<b>Metals</b>	<b>Regulation No. 75 [mg/l]</b>	<b>Tallinn City Council Regulation No. 37 [mg/l]</b>	
		<b>Limit value I</b>	<b>Limit value II</b>
Cd	0.2	0.2	0.8
Cu	2.0	2.0	8.0
Hg	0.05	0.05	0.2
Ni	1.0	1.0	4.0
Pb	0.5	0.5	2.0
Zn	2.0	2.0	8.0
Cr	0.5	0.5	2.0

### 3. Materials and methods

Both laboratory and field tests were performed within the scope of the thesis. The laboratory tests were carried out at the University of Tartu Institute of Chemistry Chair of Colloid and Environmental Chemistry laboratory and were divided into two phases; phase 1 commencing from 6th October and phase 2 from 17th of December 2020. The field tests were performed in April and May, 2021. The results of the analysis were plotted using MS Excel.

#### 3.1. Lab-scale tests

##### 3.1.1 First phase

The purpose of the first phase of laboratory tests was to find a suitable carrier type for biofilm growth. Two types of carriers were tested:

- String made of Bioflow 9 biofilm carriers
- Polyethylene strips



Photo 1. Bioflow 9 biofilm carriers

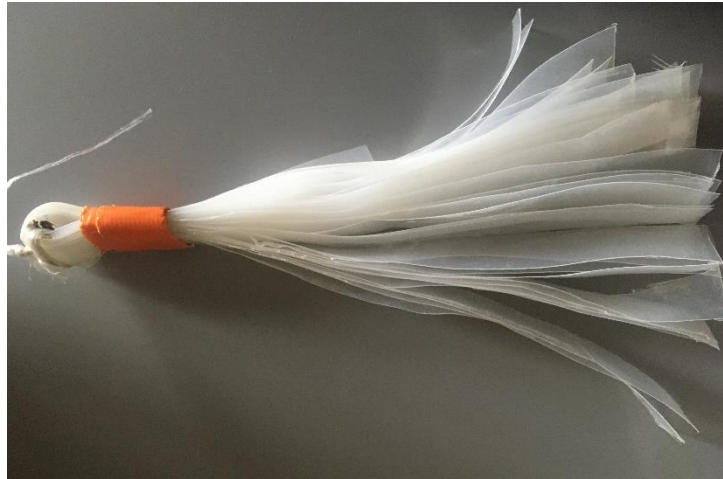


Photo 2. Polyethylene strips

Carriers (one of each type) were placed in a PVC sewage pipe that was connected with two 20L canisters (Figure 3). Canisters were filled with 40 L of municipal wastewater from Tartu WWTP grit chamber. To circulate the wastewater, a pump (JUWEL Ecoflow 1000) was placed in the left canister, which pumped water to the right canister. The pipe had a slight slope (0.5%) towards the left canister. As the right canister was filled, overflow passed through the pipe back to the left canister, creating a sewer-like flow. Wastewater in the reactors was replaced with newly collected wastewater after every 5-6 days. The first phase took place from 6<sup>th</sup> October till 5<sup>th</sup> of November, i.e. 30 days. The average parameters of wastewater during phase 1 are presented in Table 4.

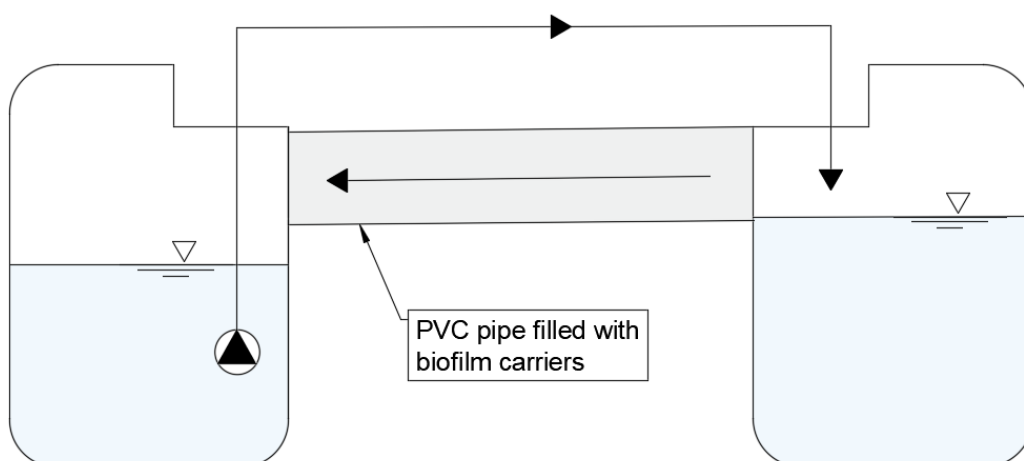


Figure 3. Setup for wastewater circulating

Table 4. Wastewater composition in 1st phase

Parameter	Unit	Value
COD	mg L <sup>-1</sup>	377.1 ± 54.6
NH <sub>4</sub> <sup>+</sup> -N	mg L <sup>-1</sup>	44.8 ± 12.9
NO <sub>2</sub> <sup>-</sup> -N	mg L <sup>-1</sup>	0.3 ± 0.5
NO <sub>3</sub> <sup>-</sup> -N	mg L <sup>-1</sup>	1.7 ± 0.2
PO <sub>4</sub> <sup>3-</sup> -P	mg L <sup>-1</sup>	6.5 ± 1.7
pH	-	7.60 ± 0.2

### 3.1.2 Second phase

The objective of the second phase was to test the passive sampling method - to see if biofilms are able to accumulate significantly more heavy metals if the wastewater circulated in the setup has elevated heavy metal concentrations. In order to provide necessary comparison, two setups, identical to the previous phase (Figure 3) were used, one with regular, and the other with spiked wastewater. Two sets of PE strips and one set of Bioflow 9 carriers were placed in both setups. Due to rapid COD degradation observed in the first phase, wastewater was now changed twice a week (Tuesdays and Thursdays) (Table 5). The second phase took place from 20<sup>th</sup> November till 17<sup>th</sup> of December, i.e., 30 days.

One of the reactors was spiked each time the wastewater was changed in the system (3-4 days) with 40 ml of heavy metal spiking solution. The solution was strongly acidic and contained significantly elevated concentrations of Cd, Zn, Cu, Cr, Ni and Pb. The heavy metal concentrations of spiked wastewater are detailed in Table 6. To avoid the rapid decrease of alkalinity, 40 ml of spiking solution was neutralised with an additional 1.8 ml of NaOH (10 mol) during each addition.

Table 5. Wastewater composition in the second phase

Parameter	Unit	Value
COD	mg L <sup>-1</sup>	344.23 ± 156.32
NH <sub>4</sub> <sup>+</sup> -N	mg L <sup>-1</sup>	53.31 ± 15.18
NO <sub>2</sub> <sup>-</sup> -N	mg L <sup>-1</sup>	0.25 ± 0.38
PO <sub>4</sub> <sup>3-</sup> -P	mg L <sup>-1</sup>	5.51 ± 1.39
pH		7.44 ± 0.15
Cd	µg L <sup>-1</sup>	0.07 ± 0.02
Pb	µg L <sup>-1</sup>	1.30 ± 1.40
Zn	µg L <sup>-1</sup>	34.00 ± 6.92
Ni	µg L <sup>-1</sup>	6.52 ± 5.02
Cr	µg L <sup>-1</sup>	6.15 ± 1.33
Cu	µg L <sup>-1</sup>	16.15 ± 4.73

Table 6. Spiked wastewater heavy metal concentrations

Metal	Concentration [µg/L]
Chromium (Cr)	11.93 ± 2.37
Nickel (Ni)	26.53 ± 1.51
Copper (Cu)	289.60 ± 6.08
Zinc (Zn)	3775.21 ± 72.43
Cadmium (Cd)	6.07 ± 0.68
Lead (Pb)	2.96 ± 2.09

### 3.2. Field tests

The purpose of the field tests was to investigate the applicability of the method in public gravity sewers, as numerous extra variables are present in in-situ experiments that cannot be foreseen in the laboratory tests. Five different sites were chosen for the field tests (Table 7). Four of them were located in Tallinn and one in Tartu. For every chosen site, multi-armed polyethylene strips (Photo 3) were installed in the connection point (manholes) of the sewer system. Since there was ample space in the field compared to the laboratory conditions longer



strips were used in the sewers (the use of PE strips is explained in section 4.2.1 “Biomass growth and composition”).

The organic matter content in metal industry effluents is usually low and can potentially contain hazardous substances inhibiting biofilm growth rate or causing biofilm detachment from the carrier. Therefore, as often as possible, biofilm carriers were also placed in manholes upstream and downstream from the connection point (Figure 4) - if the biofilm collected from the connection point doesn't provide any significant results, there is a possibility to compare the results of biofilms between upstream and downstream monitoring points. Grab wastewater samples were also taken from the same monitoring point to compare the results with biofilm samples (except site A). Biofilm carriers were placed in the sewer for 18 days, after which the biofilms were manually extracted from carriers with distilled water. Procured biofilms were thereupon dried, incinerated, and analysed.

Table 7. Selected monitoring points and their abbreviations

Site	Industry	Business area	Monitoring point	Notation
A.	Metalworking company	Manufactures products for the car, medical, and food industries	Upstream	A <sub>up</sub>
			Connection point	A <sub>cp</sub>
			Downstream	A <sub>down</sub>
B.	Electroplating	Electrolytic zinc galvanizing, electrolytic nickel and chrome plating, tin plating and cromating	Connection point	B <sub>cp</sub>
			Downstream	B <sub>down</sub>
C.	Car wash	Self-service car wash	Connection point	C <sub>cp</sub>
			Downstream	C <sub>down</sub>
D.	Car safety system components manufacturer	Stamping sheets, galvanization, isothermal hardening	Connection point	D <sub>cp</sub>
			Downstream	D <sub>down</sub>
E.	Electronics industry	Manufactures telecommunication and data communication equipment	Upstream	E <sub>up</sub>
			Connection point	E <sub>cp</sub>
			Downstream	E <sub>down</sub>



Photo 3. Biofilm carriers made of polyethylene strips were placed in the sewer

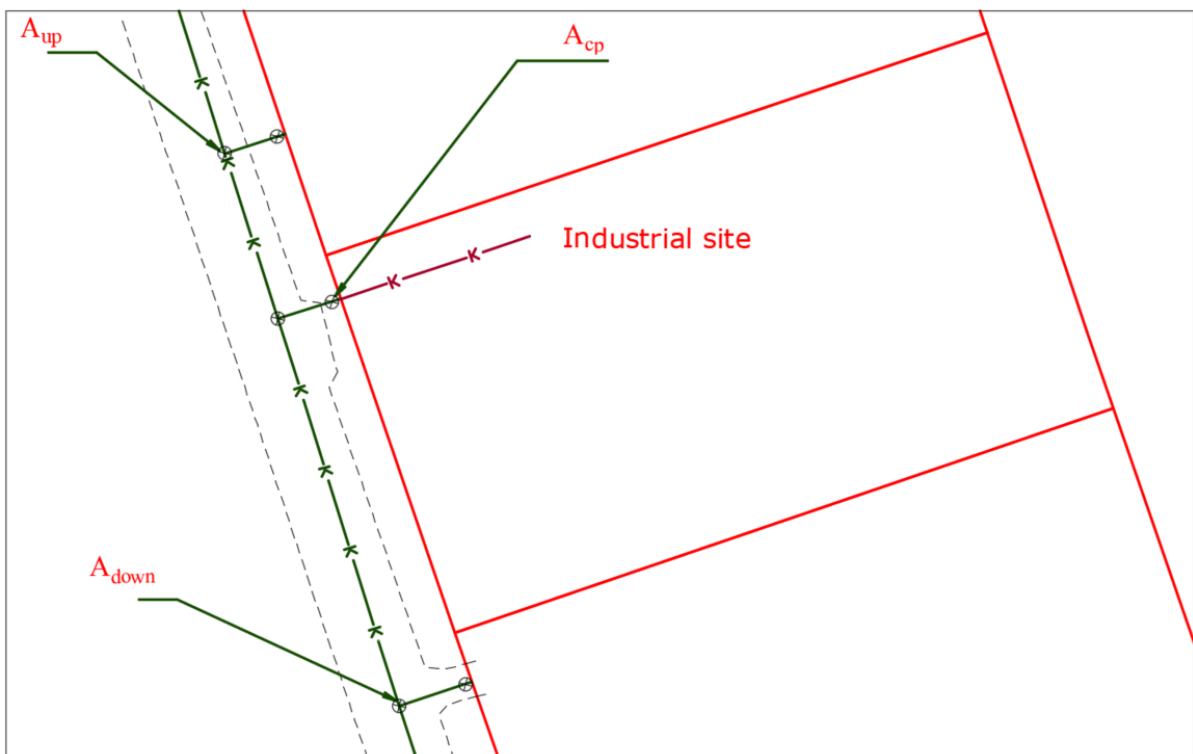


Figure 4. The monitoring points selection principle.  $A_{up}$  upstream monitoring point,  $A_{cp}$ - industrial property connection point,  $A_{down}$ - downstream monitoring point

### 3.3 Wastewater analysis

COD,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{N}$ ,  $\text{NO}_3^-\text{N}$ , pH, and  $\text{PO}_4^{3-}\text{-P}$  were determined from added wastewater.

The methods used to determine the parameters are given in Table 8.

Table 8. Used methods for wastewater analysis

Parameter	Methods	Apparatus
Chemical oxygen demand (CODCr)	ISO 6060:1989	Spectrophotometer Hach Lange DR 2800
$\text{NH}_4^+\text{-N}$	USEPA 8038 – Nessler method	Spectrophotometer Hach Lange DR 2800
$\text{NO}_2^-\text{N}$	SFS 3029	Spectrophotometer Hach Lange DR 2800
$\text{NO}_3^-\text{N}$	SFS 5752	Spectrophotometer Hach Lange DR 2800
pH	Potentiometric method	pH meter: Jenway 3520
$\text{PO}_4^{3-}\text{-P}$	EVS-EN ISO 6878:2004 Ammonium molybdate spectrometric method	Spectrophotometer Hach Lange DR 2800
Cr, Ni, Cu, Zn, Cd, Pb	EVS-EN ISO 17294-2:2016	Inductively coupled plasma mass spectrometry Agilent 8800 QQQ ICP-MS.

### 3.4 Determination of heavy metals from biomass

The heavy metal content of the dried residue was measured with inductively coupled plasma mass spectrometry (ICP-MS). Samples were treated accordingly:

1. Samples dried according to EVS-EN 12880 (2001 standard) or incinerated were ground with agate mortar. After that, samples were weighed to the Microwave digestion HVT50 vessels. The average sample size was 0.2 g.
2. 8ml of nitric acid was added to the samples and after initial reactions had subsided the vessels were capped
3. Samples were mineralised with Anton Par Multiwave Pro microwave reaction system in a 24HVT50 rotor. The acid digestion program was in accordance with the EPA 3051A method.
4. From digested samples, hundredfold dilutions were made with 2% of nitric acid. Heavy metals were determined with inductively coupled plasma mass spectrometry

on an Agilent 8800 QQQ ICP-MS using NIST 1643f for quality control and In as internal standard

To validate the correctness of the results, a certified reference standard – ERM-CC144 (sample nr 0053) was used, which was dissolved and analysed alongside unknown samples.

## 4. Results and discussion

### 4.1 Laboratory tests

#### 4.1.1 Effect of carrier material on biofilm growth and heavy metal accumulation

Figure 5 displays the amount of biomass (in dry weight) produced during the first phase of laboratory tests during 30-day growth period on both carrier types. Dried biomass from PE strips and Bioflow 9 biofilm carriers weighed respectively 2.71 g and 0.43 g, which means at least six times more biomass was extracted from PE strips than from the Bioflow 9 biofilm carriers. The biofilm on PE strips was rather evenly distributed over the surface area while on Bioflow carriers it was more scattered across the biofilm carrier surface area.

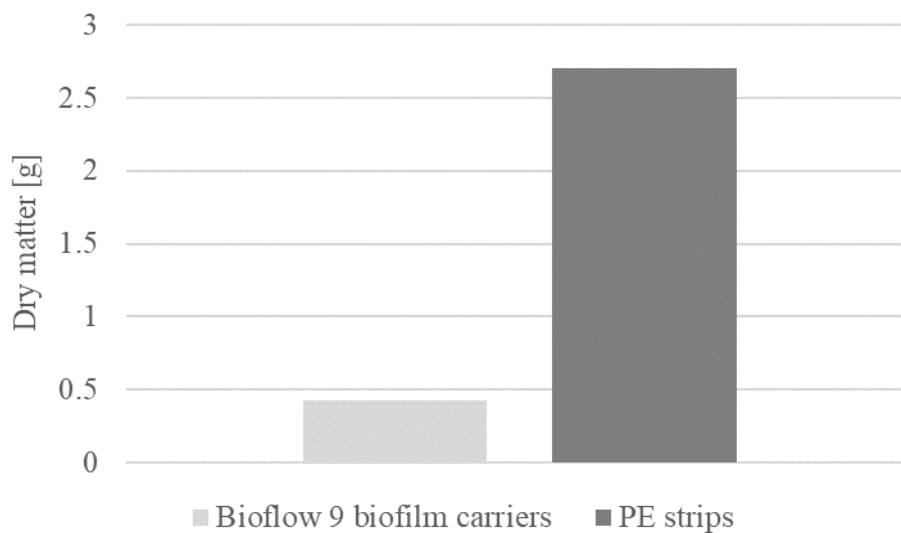


Figure 5. The amount of biomass produced during the first phase

One of the main problems identified during the first phase was the quick degradation of municipal wastewater in the system. While the wastewater in the system was changed every 5-6 days, the COD was already significantly degraded (from around 380 to 60-80 mg/L) within the first 3 to 4 days. This meant the organic carbon content in the circularly pumped wastewater was not high enough and may have inhibited the growth of biomass on the carriers. Based on these experiences, the exchange frequency of the wastewater was increased to 3-4 days (twice per week) to enable sufficient organic carbon concentration throughout the experiment.

This change seemed to play a significant effect in the second phase, especially when comparing the extracted dry matter weight from Bioflow 9 biofilm carriers between the two tests. While the dry weight of biomass on PE strips was similar to the first phase (2.24 g of

DM), the biomass on the Bioflow 9 carriers weighed approximately four times more than during the first phase (Figure 6). The biofilm carriers after the 30-day period were both quite dark in color (Photo 4), potentially indicating anaerobic conditions in the reactor.

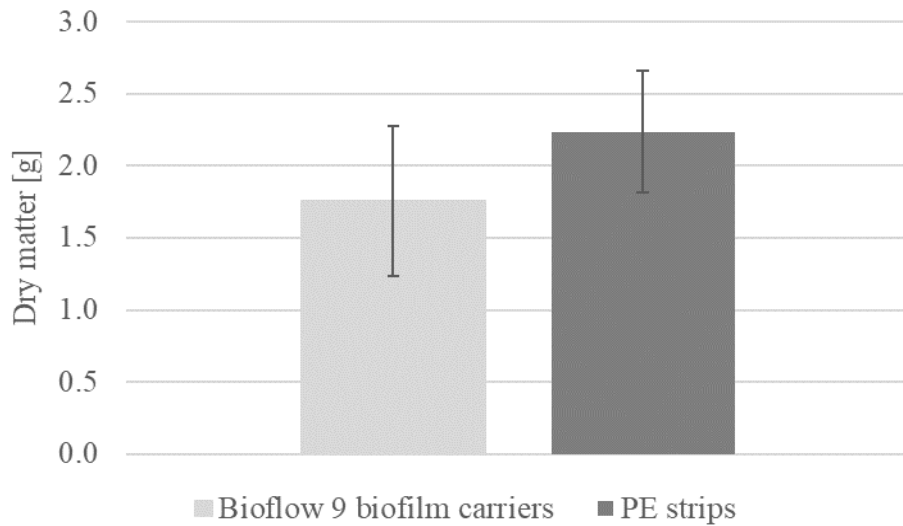


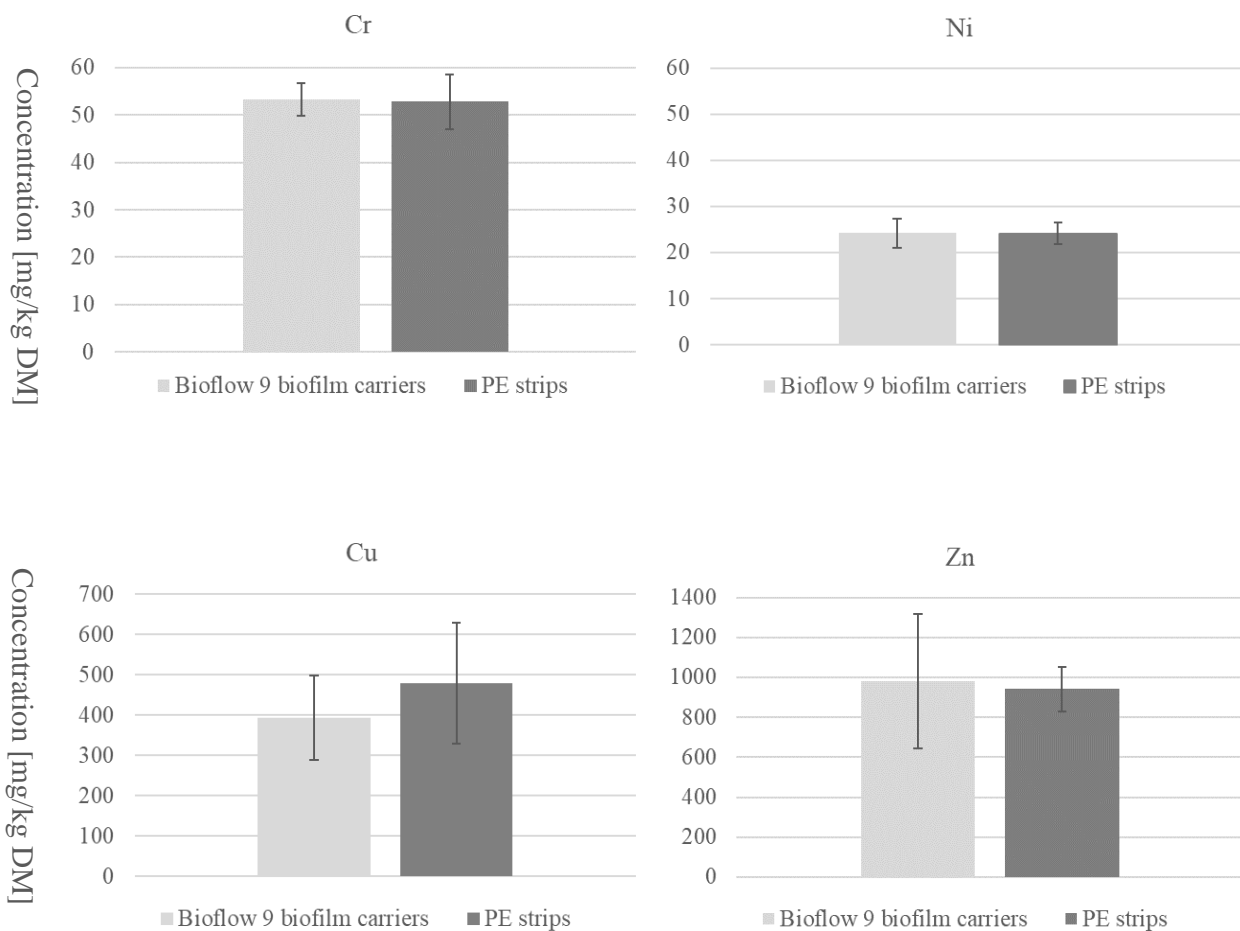
Figure 6. The amount of biomass produced during the second phase



Photo 4. Bioflow 9 biofilm carriers (left) and on the right PE strips (right) after the second phase

Analysis of heavy metals in biofilms shows that from six metals Zn appeared to be the most abundant in biofilm, while Cd was in the lowest concentration. The total concentration of heavy metals in the biofilms was Zn > Cu > Cr > Ni > Pb > Cd (from highest to lowest) and Zn > Cu > Ni > Cr > Pb > Cd in wastewater.

The differences in heavy metal accumulation during both phases between the two types of biofilm media are presented in Figure 7. As discussed before, the total amount of biomass extracted from the Bioflow 9 biofilm carriers was significantly lower during phase 1, but the heavy metal accumulation still showed similar results. While there are minor differences, the choice of biofilm media does not show a significant effect on heavy metal accumulation. In most cases, the difference was smaller than 5%. The exception was Cu and Pb, where Cu concentration was 21% higher on PE strips and Pb concentration was 14% higher on carriers. However, considering that standard deviations were higher than the difference, then the gap between the carriers is irrelevant.



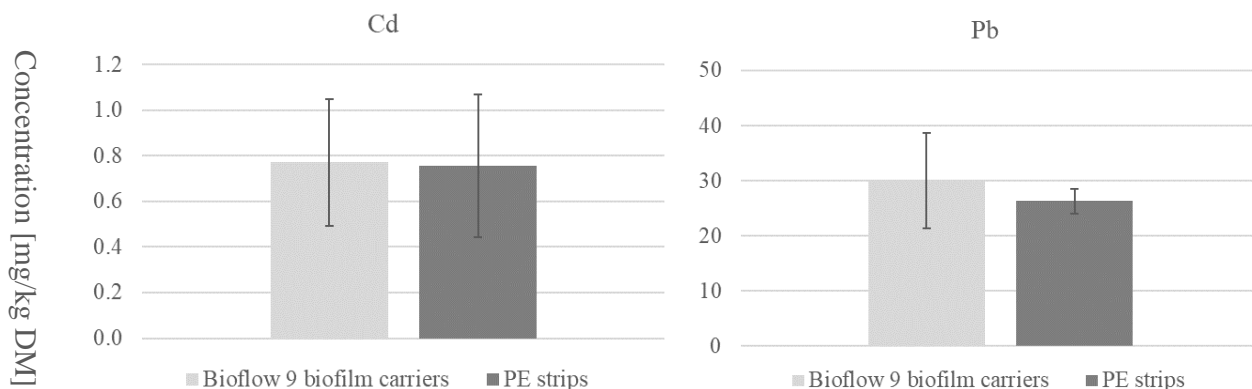


Figure 7. Cr, Ni, Cu, Zn, Cd and Pb concentrations in the biofilm collected from the Bioflow 9 biofilm carriers and PE strips

#### 4.1.2 Effect of spiked wastewater on heavy metal accumulation

In order to test the biofilm capability to accumulate more heavy metals from polluted streams, a second identical system was set up in the laboratory during the 2<sup>nd</sup> phase of testing. As mentioned previously, the wastewater in the second system was spiked with a concentrated heavy metal solution in order to mimic a more concentrated industrial wastewater stream. The spiking solution had high concentrations of Cd, Zn, Cu, Pb, Cr and Ni, the concentrations of the first three were aimed to be elevated around 20 times, the latter three around 10 times, based on average expected municipal wastewater heavy metal concentrations from previously measured Estonian WWTPs. The later measurements showed significantly lower real concentrations for Cd, Zn therefore the difference between regular and spiked wastewater was about 100 times. At the same time, Cr and Ni concentrations were 3-6 times higher than expected, lowering the difference between spiked and regular to 1.94 (Cr) and 4.07 (Ni).

Despite the differences in spiking ratios, all six metals exhibited several times higher values in spiked biofilms compared with regular biofilms, confirming biofilm's expected biosorption ability. The highest ratios were observed with Cd and Zn, (respectively 59 and 23 times higher concentrations in the spiked biofilm) (Figure 8), while as mentioned previously, these two were also spiked with the highest ratio.



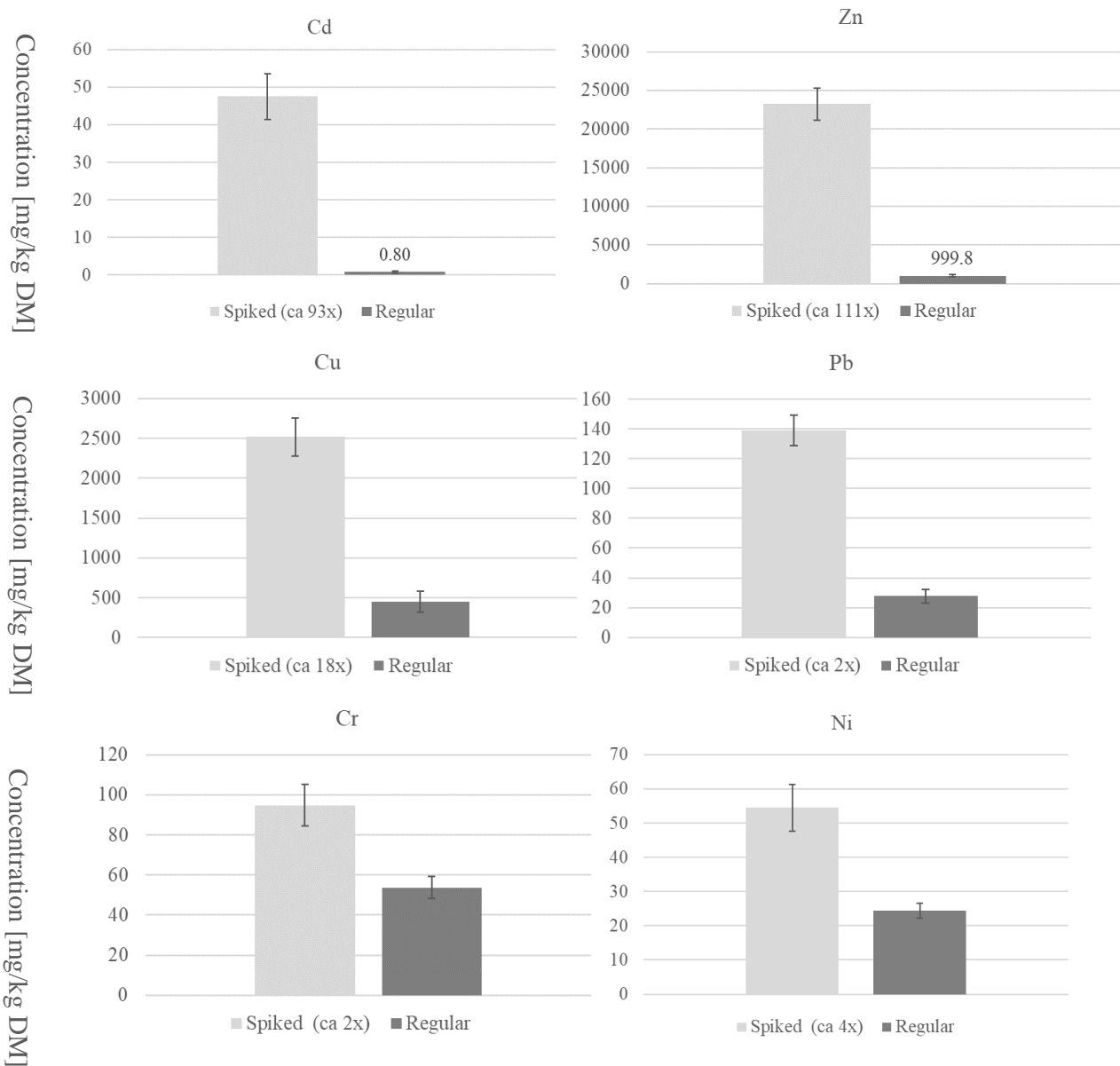


Figure 8. Cd, Zn, Cu, Pb, Cr and Ni concentrations differences between the biofilms collected from the setup circulated with spiked wastewater and with regular wastewater

The data summarized in Table 9 show the ratios between biofilms collected from spiked wastewater and regular wastewater, together with the difference in spike ratios. The tables also include an “accumulation coefficient” for each heavy metal, showing how strongly an increase in wastewater concentration is measured in the biofilm. The higher the coefficient the better the biofilm accumulation. According to the coefficients, heavy metal sorption efficiency in the first group (Cr, Ni and Pb) is in the following order: Pb> Cr> Ni>, which coincides with the literature review (Ziolko et al., 2011). Lead accumulation rate was more than two times higher than chromium (2.19 and 0.91 respectively), despite being spiked with a similar ratio. Nickel showed the lowest accumulation rate as expected with a coefficient of

0.55, while it might be somewhat influenced by higher spiking ratio compared to the other two heavy metals.

Table 9. Cr, Ni, Pb, Cu, Zn and Cd concentration differences and relations between the biofilms collected from the setup circulated with spiked wastewater and regular wastewater

Heavy metal	Biofilm from spiked wastewater [mg/kg DM]	Biofilm from regular wastewater [mg/kg DM]	Spiked and regular wastewater biofilm ratio	Spiked and regular wastewater ratio	“Accumulation coefficient”
Cr	94.78 ± 10.39	53.73 ± 5.52	1.76	1.94	0.91
Ni	54.46 ± 6.83	24.48 ± 2.17	2.23	4.07	0.55
Pb	138.99 ± 10.42	27.79 ± 4.62	5.00	2.29	2.19
Cu	2519.58 ± 239.17	448.25 ± 133.04	5.62	17.93	0.31
Zn	23232.99 ± 2084.96	999.84 ± 168.57	23.24	111.04	0.21
Cd	47.47 ± 6.13	0.8 ± 0.29	59.36	93.06	0.64

Coefficients in the second group were in the following order: Cd > Cu > Zn, which do not coincide with what was prescribed in the literature (Ziolko et al., 2011). Considering that the concentration of Zn in spiked biofilm was tens of times higher than the other metals, one explanation might be that the spiked biofilm surface functional groups were already saturated with Zn. The competition of metal ions for the same functional binding sites within biofilms may also explain the difference. It is found that generally the concomitant presence of several metals affects the biosorption efficiency of single metals, whilst no influence is observed on the total metal binding capacity (Papirio et al., 2017). This should not pose a problem in real-life applications of the method however, if one heavy metal concentration is high enough to create competition between metals for functional binding sites, the industrial site can already be identified as a point-source pollution.

Nevertheless, metal removal by microorganisms is a complex process that depends on the chemistry of the metal ions, biofilm composition, cell physiology, and physicochemical factors such as pH, temperature, time, ionic strength, and metal concentration which can lead to different results. Therefore, no fundamental conclusions can be drawn as binding capacity

varies between specific test sites and conditions. This once again means that the method can be used for generally identifying potential pollution sources, not quantifying the pollution load.

## 4.2 Field tests

### 4.2.1 Biomass growth and composition

Before field tests commenced, the results of the laboratory tests were evaluated to choose the best materials to be used in the field tests. As it was clear that the carrier material did not significantly affect the uptake of heavy metals, the main parameter taken into account was the formation of biomass and the convenience of the overall method. The initial laboratory tests with periodically low COD showed lower biomass formation on the Bioflow 9 biofilm carriers than on PE strips. As metal and electronics industry wastewater may contain only a small amount of organics, better biomass retention was an important aspect to take into account. Laboratory tests also showed that it is much more convenient to remove biofilms from strips than from Bioflow carriers, especially in outdoor conditions with no access to ultrapure water. Therefore, for the field tests, only PE strips were chosen.

Due to time constraints the testing period was shortened, from 30 days of laboratory tests to 18 days in the field. Based on previous literature, two weeks were deemed sufficient for biofilm growth in field-scale tests. This also proved correct from the results - almost all PE strips placed in the sewer had grown full of biofilms, weighing up to 27 grams of DM.

Table 10. The amount of biomass produced on sewer biofilms.

<b>Monitoring point</b>	<b>Dry matter weight [g]</b>	<b>Organic fraction [%]</b>	<b>Inorganic fraction [%]</b>
A <sub>up</sub>	10.54	86.12	13.88
A <sub>cp</sub>	2.69	57.87	42.13
A <sub>down</sub>	27.04	80.37	19.63
B <sub>cp</sub>	3.86	63.82	36.18
B <sub>down</sub>	7.07	58.96	41.04
C <sub>cp</sub>	21.49	19.91	80.09
C <sub>down</sub>	19.54	50.51	49.49
D <sub>cp</sub>	9.96	48.59	51.41
D <sub>down</sub>	25.73	19.50	80.50
E <sub>up</sub>	10.14	60.46	39.54
E <sub>cp</sub>	6.87	62.49	37.51
E <sub>down</sub>	2.34	63.30	36.70

There were two exceptions where biomass yield was significantly lower; monitoring point  $A_{cp}$  and monitoring point downstream from site E (Table 10). That was likely caused by practical test setup reasons, not because of the toxicity of wastewater. More precisely PE strips placed in monitoring point  $A_{cp}$  were tangled with tissues, wipes, and paper towels; which most likely physically hindered the biofilm growth (Photo 5). As we all know, such items should not be disposed of into the sewer because they can cause blockages in the pipes and sewage pumps. This issue could not be accounted for in the field test setup. Similarly, the PE strips downstream from site E exhibited low biofilm growth because the sewer line had been partially clogged and instead of floating in the water the PE strips laid partly on garbage (Photo 6).



Photo 5. Waste tangled on the PE strips from site A



Photo 6. Fully grown biofilm from point  $E_{up}$  vs biofilm from  $E_{down}$

Lower than average biofilm growth rate was also observed in site B. In this case, the growth may have been slower due to the higher content of heavy metals in wastewater. From all of the grab samples, site B wastewater had the highest heavy metal content (Table 13).

Table 10 also shows the organic and inorganic content in the gathered biofilm, with significant variations. The lowest organic fraction percentages were observed in point Ccp and point Ddown, where total biomass dry masses were some of the highest. No specific explanation could be identified for that phenomenon. It is an interesting result which needs further research.

#### 4.2.2 Heavy metal accumulation

All of the gathered biomass samples were analysed for heavy metals and as expected, with a few exceptions, the highest heavy metal values were found at industrial connection points.

During the work, it became clear that comparing upstream and downstream concentrations do not always justify itself. When using site A as an example (Table 11), it is evident that metal concentrations are only slightly higher at downstream monitoring points compared with upstream monitoring points. Therefore, considering that the effect of dilution can be so great, comparing upstream and downstream biofilms may not always give reliable information.

Table 11. Heavy metal concentrations of biofilms from site A monitoring points

<b>Metal</b>	<b>Upstream [mg/kg DM]</b>	<b>Connection point [mg/kg DM]</b>	<b>Downstream [mg/kg DM]</b>
Cr	10.77	2236.65	66.06
Ni	3.28	808.38	25.71
Cu	19.69	363.41	35.65
Zn	77.57	1025.17	119.88
Cd	0.08	0.13	0.06
Pb	2.91	10.03	3.91

In order to evaluate the accumulated heavy metal values from specific monitoring points, background values showing municipal baselines had to be used. As no large-scale determination of background values in the sewer system was carried out within this research project, accumulated heavy metal concentrations from laboratory tests are used as one background value set (Table 12). Given that wastewater used in laboratory tests was obtained from Tartu WWTP influent it gives a good description of overall heavy metal levels in the city of Tartu and can be used as an initial empirical values.

For comparison, similar background values from literature are also presented in Table 12. It can be seen that the laboratory biofilms compared to other background values are mostly similar (Ni, Cd, Pb) or slightly higher (Zn). Only larger differences with background values were seen for Cr and Cu, which were nearly two times higher, while Cu values were even higher than the Konya industrial points.

It has been proven that the exceedance of the triple background value can be taken as a limit for further investigations (Aydin et al., 2020; Genuit, 2021). To make the methodology more precise, one additional option is to divide background values into two categories: domestic and industrial (Aydin et al., 2020). As there is not a sufficient amount of data collected to determine reliable industrial background values within this thesis, the Konya industrial background values from literature will be used in addition to values gathered from laboratory tests. Ideally, each city should be evaluated separately since the climatic conditions, wastewater characteristics, hydraulic regime, etc can strongly influence the biofilm formation and capacity. In the current situation biofilms collected from Tallinn's monitoring points are compared with biofilms from Tartu WWTP influent on assumption that wastewater from cities within one country does not differ significantly. Figures 9-14 illustrate the heavy metal concentrations in biofilm samples from different monitoring points in comparison with the Konya and Tartu WWTP reference values.

Table 12. Background values

Concentration in biofilm [mg/kg of DM]					
Element	Kintrup & Wünsch <sup>[1]</sup> 400 samples	Konya domestic points <sup>[2]</sup> (one point 14 samples)	Bielefeld <sup>[3]</sup> 38 points 1500 samples	Konya <sup>[2]</sup> industrial points (9 points)	Tartu WWTP influent
Cr	27	19	24	434	53.2
Ni	23	36	17	129	24.5
Cu	180	29	250	360	448.3
Zn	880	103	800	1872	999.8
Cd	1.2	0.2	0.6	0.7	0.8
Pb	42	9	34	128	27.8

[1] (Kintrup & Wünsch, 2001), [2] (Aydin et al., 2020) [3] (Genuit, 2016)

The highest values of chromium were found from site A property connection point (metalworks), with a concentration of 2236.65 mg/kg DM. Tartu WWTP and Konya industrial reference values were respectively 53.73 and 434 mg/kg DM hence Cr values found from A<sub>cp</sub> exceed Tartu WWTP reference values over 40 and Konya industrial reference values over five times indicating excessive discharge of Cr from the industry. High levels of Cr (1319.74 mg/kg DM) were also found at site B connection point (electroplating), where Cr concentration exceeded even the industrial reference value more than three times (Figure 9).

A similar trend was observed with nickel, where on connection points of sites A and B threefold exceedance of the industrial reference values took place (Figure 10). Very high values in biofilm were also measured from B<sub>down</sub>, especially compared A<sub>down</sub>. As a similar trend can be observed with other metals at site B as well, it probably means that the discharges to the sewer from other sources directly after point B are low and the industrial discharge is not yet diluted enough. In addition, higher concentrations of Ni were also found from site D (car components manufacturer) connection point and downstream point, respectively 288.57 and 376.90 mg/kg of DM. While some quite high values from site D were detected for other heavy metals as well, Ni was the only one exceeding both reference values.

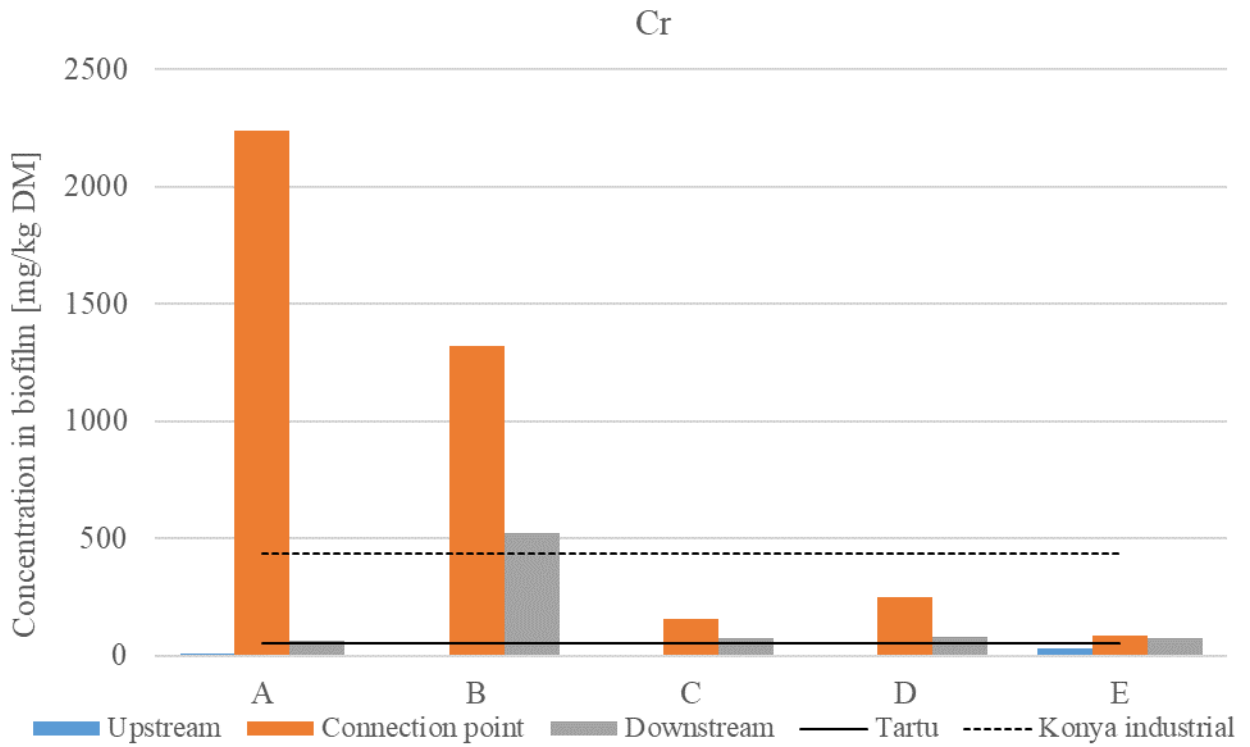


Figure 9. Cr concentrations in biofilm samples taken from different monitoring points in comparison with Konya industrial and Tartu WWTP influent reference

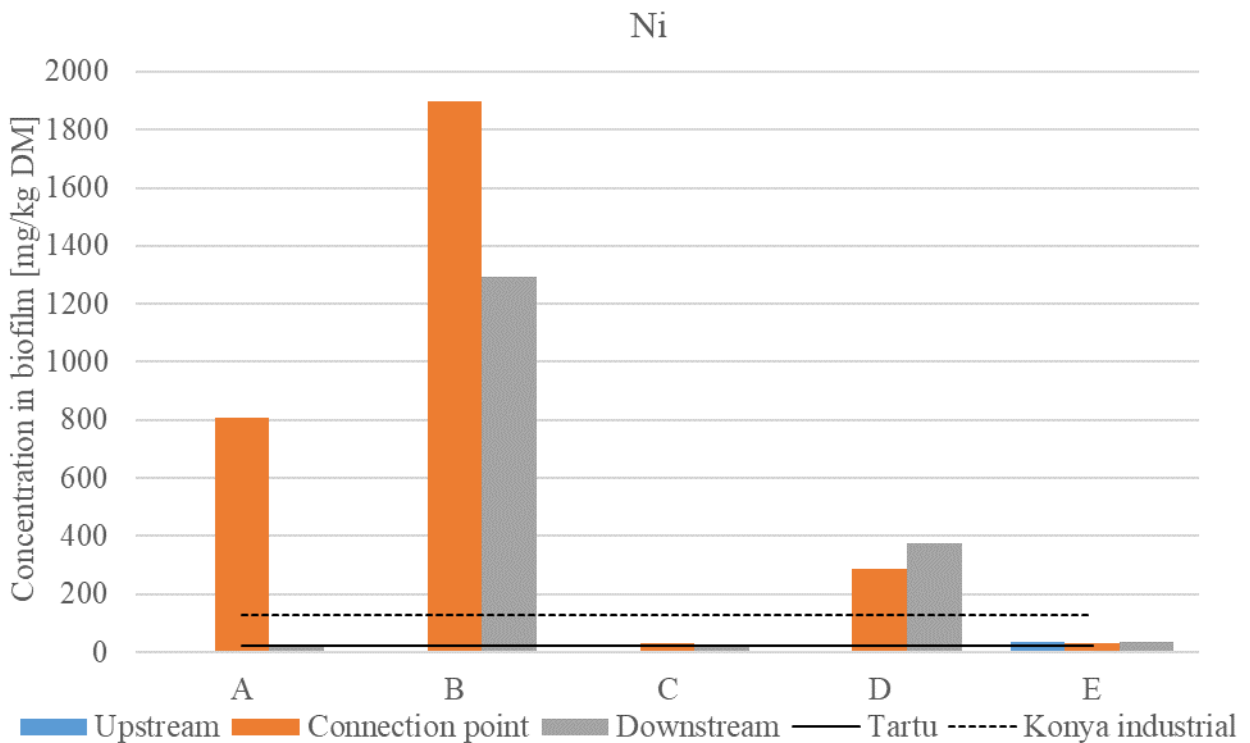


Figure 10. Ni concentrations in biofilm samples taken from different monitoring points in comparison with Konya industrial and Tartu WWTP influent reference values



Compared to other monitored heavy metals, Zn showed the highest deviation from reference values throughout all test points (Figure 11). The large deviation was found at the site B connection point, where Zn concentration was 38190.79 mg/kg of DM, exceeding the Tartu WWTP and Konya industrial reference values by 38 and 20 times respectively. All other monitoring points were near the reference values.

In contrast to Zn, the lowest deviation from reference values was measured for Cu (Figure 12). Surprisingly, the highest Cu (578.88 mg/kg DM) value was found from the site C connection point at a car wash. Although at that point Zn concentration was still higher, demonstrating value near the reference line (1097.60 mg/kg DM). As mentioned in the literature, Zn and Cu tend to be most abundant in the carwash effluent, derived mainly from tyres and brake pads respectively (Tekere et al., 2016).

None of the monitoring points exceeded the Cu reference values more than two times, which might also be influenced by the relatively high Cu background value from laboratory tests (higher than Konya industrial background value). The reference value might be significantly lower in Tallinn (lowest municipal reference value in literature shown as 29 mg/kg DM), but it's impossible to determine without significant further testing.

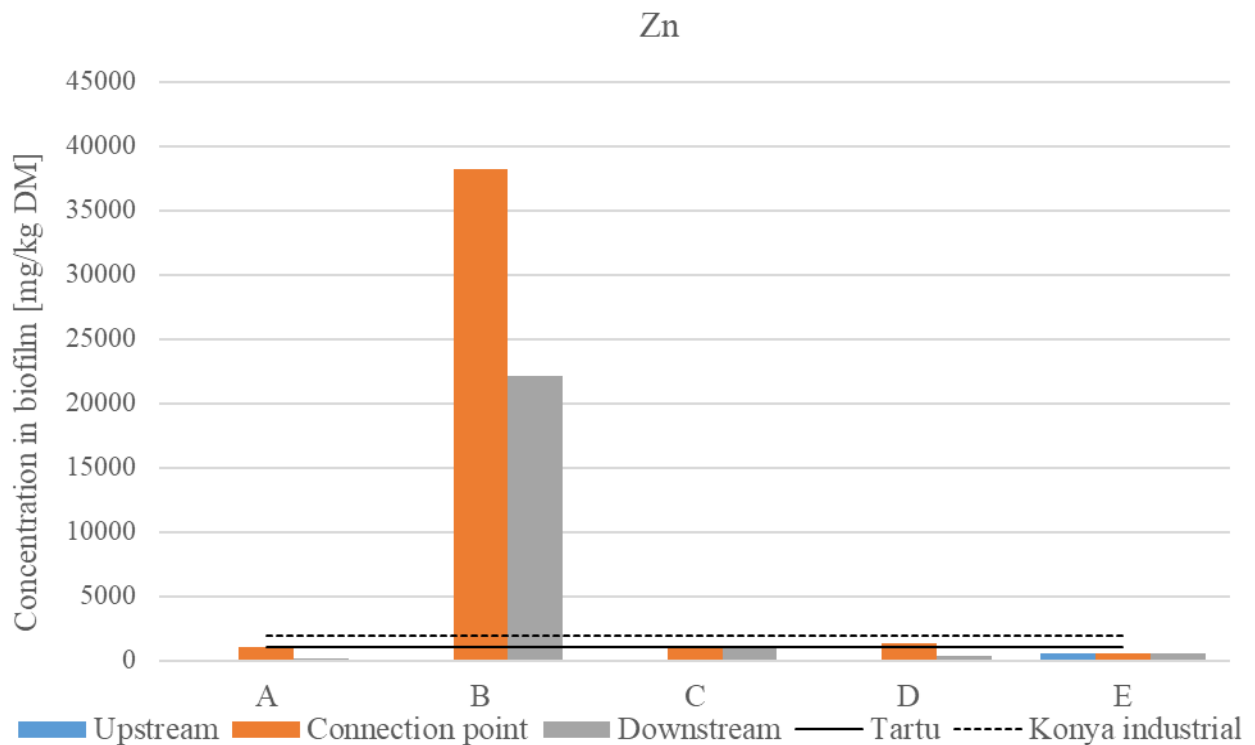


Figure 11. Zn concentrations in biofilm samples taken from different monitoring points in comparison with Konya industrial and Tartu WWTP influent reference

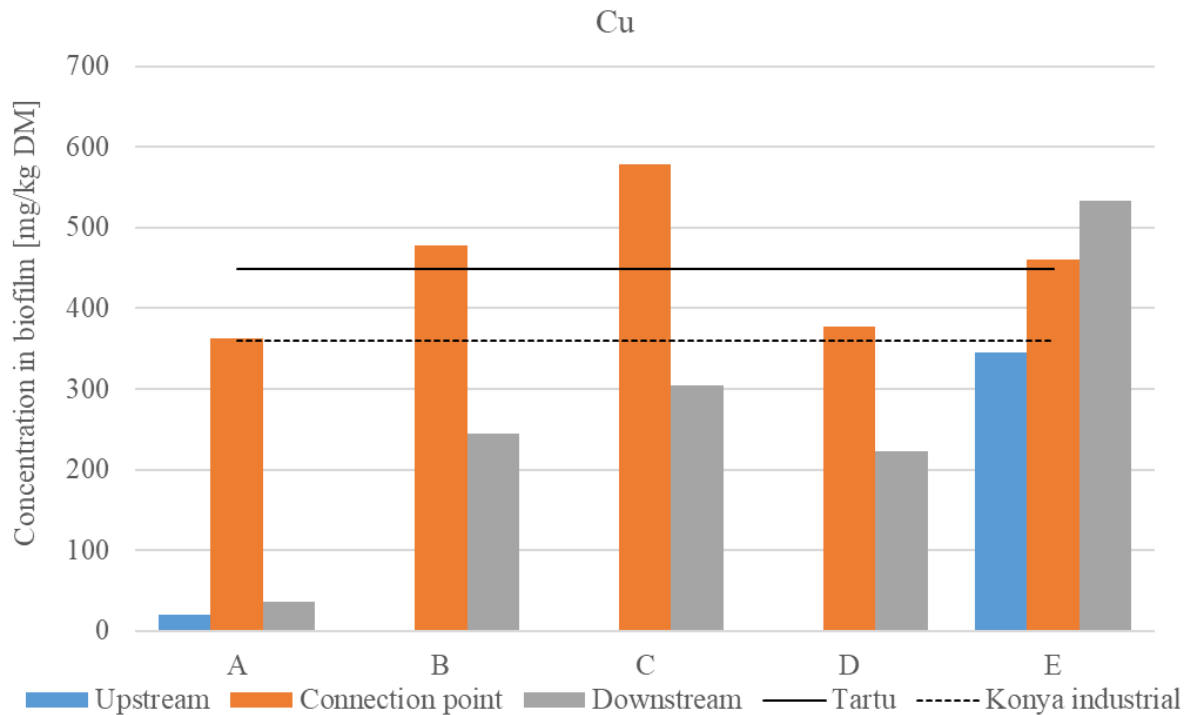


Figure 12. Cu concentrations in biofilm samples taken from different monitoring points in comparison with Konya industrial and Tartu WWTP influent reference

The Cd results are very similar to Zn results, as again only site B exceeded Cd reference values (both reference values were similar and were exceeded around 3 times). All other sites showed relatively low Cd concentrations (Figure 13), which considering the high toxicity of Cd is a very positive result. Compared to the few measurements from upstream sites, the difference with connection points and downstream sites is quite small compared to other targeted heavy metals.

The higher industrial reference value for lead was not exceeded by any measured points. Unexpectedly the highest concentration of lead (123.08 mg/kg) from biofilm was measured at a downstream point ( $D_{down}$ ), exceeding the Tartu reference value more than three times, while other points were near the reference line (Figure 14). The value was significantly lower (23.29 mg/kg DM) at the connection point itself. Similar situation at this test point was identified with nickel. Taking into account that other industrial facilities besides site D were situated on the same street, it may indicate that there may be another more significant polluter connected to the same sewer line.

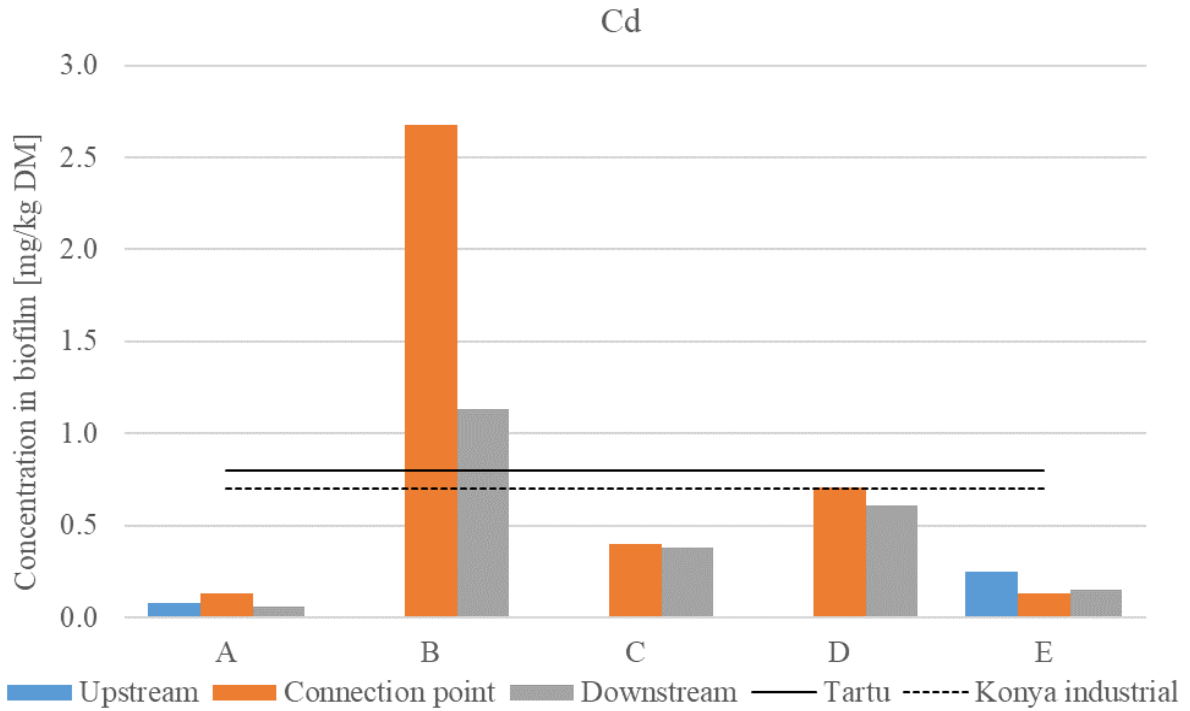


Figure 13. Cd concentrations in biofilm samples taken from different monitoring points in comparison with Konya industrial and Tartu WWTP influent reference

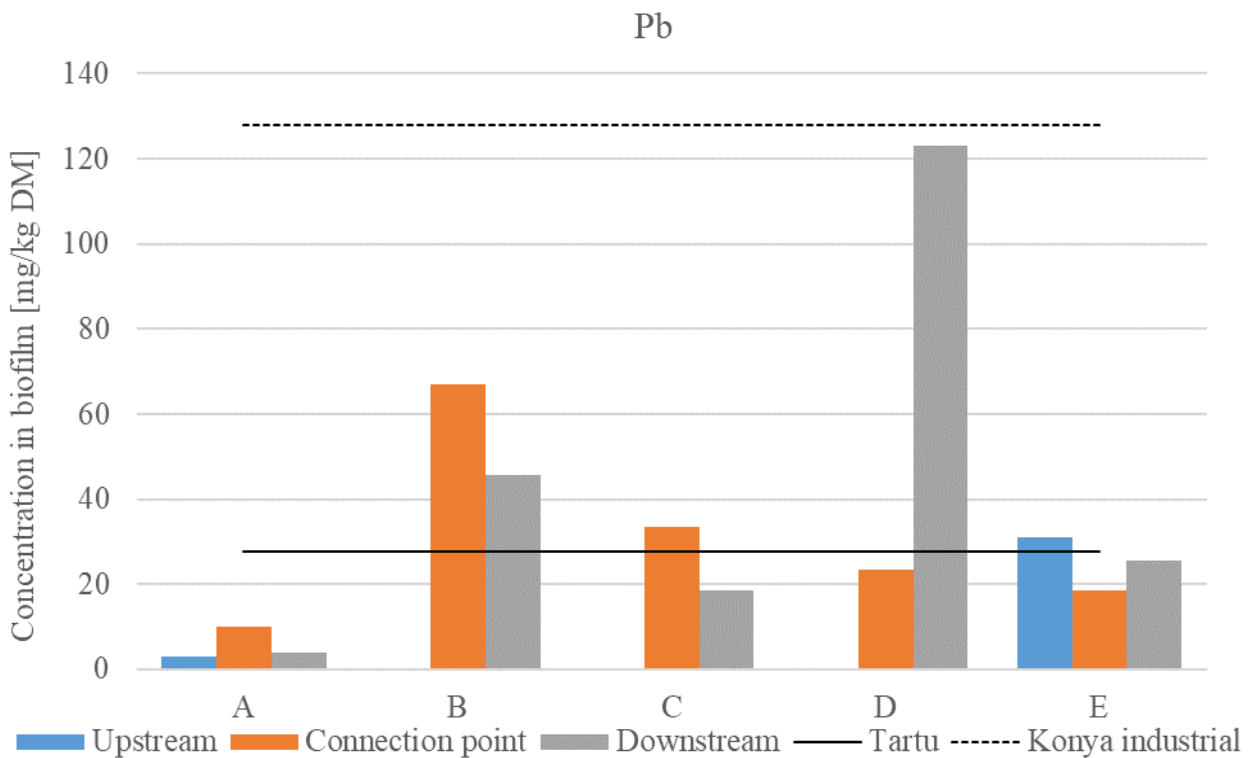


Figure 14. Pb concentrations in biofilm samples taken from different monitoring points in comparison with Konya industrial and Tartu WWTP influent reference

### 4.2.3 Method evaluation for detecting pollution sources

As one of the main objectives of current research was to evaluate the applicability of the biofilm sampling-based method for determining pollution point sources in the sewers, grab samples were also taken and evaluated alongside the biofilm samples (besides point A). The results are presented in Table 13. Based on the difference between the biofilm and water samples from the monitoring point and mean heavy metal concentrations obtained from laboratory tests, the results are coloured accordingly (green < 150%, yellow 150-300% and red >300%). As discussed previously, triple exceedance of background values should be considered a significant pollution source (Aydin et al., 2020; Genuit, 2021), which is used as a basis of this colouring. The same rule is applied to the wastewater grab samples, which are often used to identify a potential pollution source, to evaluate, if the biofilm-based method and grab samples show similar results.

Over threefold values of nickel from laboratory results can be seen for both water and biofilm in points B<sub>cp</sub>, B<sub>down</sub>, D<sub>cp</sub>. Similar results can be seen with Zn in points B<sub>cp</sub> and B<sub>down</sub>. This means both methods were successful in identifying the potential pollution source and support the reliability of the method. However, the values measured from wastewater grab samples exceeded the background more than threefold, they did not actually exceed the limit values allowed under current legislation for industrial inflows. However, based on the biofilm samples, the total load of the heavy metals from these industries might be quite significant.

On the other hand, the biofilm samples were able to identify potential pollution sources of chromium in B<sub>cp</sub>, B<sub>down</sub>, D<sub>cp</sub>, while the wastewater grab samples were practically the same as the wastewater used in the laboratory. This demonstrates perfectly the benefit of the proposed methodology since biofilm-based long-term sampling also catches discharges that only occur at specific times or conditions. Overall, similar pollution points were identified in B<sub>cp</sub> for Cd and D<sub>down</sub> for Pb.

At this point, it is also important to mention that Tartu WWTP and Konya industrial background values were obtained holding biofilms in wastewater for at least four weeks, while biofilms from sites A, B, C, D, and E were kept in the sewer for 18 days. Thus, the ratio between biofilm heavy metal concentrations from field samples and background values could actually be underestimated. Considering that sites A and B both exhibited approximately fortyfold exceedance (Cr and Zn respectively) from reference values further monitoring of these industries is recommended. It is also worth pointing out that while the

spiked wastewater contained approximately 3.8 mg/L of zinc (national limit value 2 mg/L (Table 3)) and 23233 mg/kg of DM was measured in biofilm after 30 days, the Zn concentration in the biofilm from point B<sub>cp</sub> after 18 days was 38190.79 mg/kg DM of Zn.

Based on both the results obtained from the laboratory and sewerage system, the biofilm carrier-based heavy metal point source tracking method should be suitable to assess discharges with high heavy metal content. As the method cannot be used to quantify the heavy metal loads, further analysis and research should be undertaken to determine if industries actually discharge non-compliant wastewater at certain periods. This could be achieved by determining more precise background values (larger sample size and different time periods) and employing statistical analysis and quality assurance methods on the data sets.

Table 13. Grab samples and biofilm samples from monitoring points. Colors indicate the difference between field and laboratory samples.

Point	Sample	Cr	Ni	Cu	Zn	Cd	Pb
<b>A<sub>up</sub></b>	Biofilm (mg/kg DM)	10.77	3.28	19.69	77.57	0.08	2.91
<b>A<sub>cp</sub></b>	Biofilm mg/kg DM	2236.5	808.38	363.41	1025.17	0.13	10.03
<b>A<sub>down</sub></b>	Biofilm mg/kg DM	66.06	25.71	35.65	119.88	0.06	3.91
<b>B<sub>cp</sub></b>	Water µg L <sup>-1</sup>	8.18	45.30	65.93	1387.43	0.08	0.10
	Biofilm mg/kg DM	1319.74	1897.41	477.5	38190.79	2.68	67.02
<b>B<sub>down</sub></b>	Water µg L <sup>-1</sup>	6.86	59.36	45.12	857.74	0.04	0.35
	Biofilm mg/kg DM	521.78	1292.57	244.38	22112.39	1.13	45.83
<b>C<sub>cp</sub></b>	Water µg L <sup>-1</sup>	11.65	7.93	131.68	561.74	0.11	3.54
	Biofilm mg/kg DM	159.56	33.10	578.88	1097.60	0.40	33.45
<b>C<sub>down</sub></b>	Water µg L <sup>-1</sup>	7.55	2.69	50.06	173.37	0.04	1.08
	Biofilm mg/kg DM	74.58	24.65	304.31	991.99	0.38	18.58
<b>D<sub>cp</sub></b>	Water µg L <sup>-1</sup>	6.69	25.27	15.75	15.96	0.03	0.27
	Biofilm mg/kg DM	250.58	288.57	376.96	1315.52	0.71	23.39
<b>D<sub>down</sub></b>	Water µg L <sup>-1</sup>	9.28	7.71	10.93	35.97	0.05	0.58
	Biofilm mg/kg DM	79.11	28.82	223.93	301.64	0.61	123.08
<b>E<sub>up</sub></b>	Water µg L <sup>-1</sup>	4.64	2.22	38.63	20.24	0.04	0.35
	Biofilm mg/kg DM	33.2	39.96	345.73	542.97	0.25	31.02
<b>E<sub>cp</sub></b>	Water µg L <sup>-1</sup>	6.19	3.35	55.18	36.28	0.04	0.49
	Biofilm mg/kg DM	87.09	30.06	459.77	528.94	0.13	18.54
<b>E<sub>down</sub></b>	Water µg L <sup>-1</sup>	5.24	4.52	35.57	24.00	0.04	0.85
	Biofilm mg/kg DM	77.11	33.67	532.65	529.78	0.15	25.72

## 5. Summary

Treated sewage sludge could be an excellent fertilizer due to its high nitrogen and phosphorus content. However, if heavy metals are present in the WWTP's influent then they are predominantly transferred to sewage sludge. If the quantity of heavy metals exceeds the set values in sewage sludge compost its further use in agriculture is forbidden. Unfortunately, there are no cost-efficient solutions to remove heavy metals from the sludge. Therefore, the most efficient strategy is to prevent illegal discharges of heavy metals into the sewerage. As that kind of approach would require more effort from water companies due to the need for more frequent sampling, there is a need for a continuous monitoring system that can locate illegal industrial discharges easily and affordably. This thesis aimed to test an alternative monitoring method using biofilm carriers in both the laboratory setting and field tests in the sewerage system.

The purpose of the laboratory tests was to determine the suitable carrier type and evaluate the metal uptake capacity of biofilms. Laboratory tests showed that there is no significant difference between different carrier types in terms of heavy metal sorption capacity. Therefore, PE strips were chosen for field tests based on higher biomass yield on low COD concentrations and the ease of use. Laboratory tests also successfully demonstrated the high heavy metal sorption ability of biofilms, showing a difference in uptake up to 60 times with Cd between regular and spiked wastewater streams. Considering the heavy metal concentrations between spiked and regular wastewater, Pb indicated the highest sensitivity and proportionally highest sorption.

During field tests in Tartu and Tallinn, the highest heavy metal values were generally found in property connection points. In some cases, due to high dilution, the pollution effect was not apparent when comparing upstream and downstream points. The results measured from biofilms kept in wastewater of different industrial sites were compared with the reference values obtained both from literature and laboratory testing. Two out of the five sites investigated exhibited approximately fortyfold exceedance of Cr and Zn from referenced background values, which might indicate illegal discharges.

In conclusion, the study confirmed that the pollution source tracking system based on the sorption properties of the biofilm is suitable for use in Estonian sewer systems. In order to determine if non-compliant wastewater is discharged from point sources, further research has to be done to see if measured biofilm concentrations might be quantifiable to assess total

heavy metal loads. Even as it is now, the method can be used to improve the effectiveness of monitoring industrial polluters and to help maintain or even decrease the heavy metal levels in sewage sludge.



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### **Legislation:**

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Regulation No. 24 of the Minister for the Environment. Entry into force: 19.07.2017, RT I 28.07.2017, 4

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– 0012

## Tänuavaldused

Töö autor soovib tänada juhendajat Markus Raudkivi oskusliku juhendamise ja igakülgse nõu ja jõu eest. Tänavaldused lähevad ka Päärn Paistele, kelle abiga viidi läbi ICP-MS analüüsid. Samuti soovib autor tänada Peeter Prassi ja Priit Kappakit Tallinna Veest ning Kaido Põhakot Tartu Veevärgist suure abi eest välitööde läbiviimisel.

## **Lihthtsents lõputöö reprodutseerimiseks ja üldsusele kättesaadavaks tegemiseks**

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**01.06.2021**