

Norwegian University of Life Sciences

Master's Thesis 2024 45 ECTS Faculty of Environmental Sciences and Natural Resource Management

Using a chipwood-biofilter for removal of dissolved metals and metalloids from tunnel wash water

Hanna Marie Kjernsby Environmental Sciences

Acknowledgements

I want to thank my head counsellor, Hans-Christian Teien, for all the help regarding planning, thinking and proofreading, my counsellor, Lene S. Heier, for starting this project for me, and for all the good advice and suggestions for improvements. I also want to thank my counsellor, Roger Roseth, for planning and setting up the biofilter with me and sharing his knowledge. I also want to thank the Norwegian Public Road Association (NPRA) for funding my master's project.

Of course, a special thanks is given to all beloved friends and family who went through this with me; none mentioned, none forgotten; and above all, Krister Hovde for all support and good advice from start to end.

And thank you to the MINA faculty for funding a new toaster for the soil building; it was highly needed.

Abstract

Tunnels accumulate road-related pollutants and are regularly washed to maintain traffic safety and upkeep of the tunnels. Tunnel wash water contains high concentrations of pollutants, including metals and metalloids, which are known to have the potential to cause negative effects on aquatic organisms. To protect organisms and water quality in receiving recipients, sedimentation ponds inside or outside of the tunnels are commonly used to clean tunnel wash water. Particles will settle in the sedimentation ponds, but not all metals and metalloids are bound to particles in the tunnel-wash water. Dissolved species of metals and metalloids are not removed through sedimentation and can pose a threat to aquatic life in the recipient.

In the present study, grey alder woodchips were used as a biosorbent, combined with reducing conditions to reduce levels of metals and metalloids in the tunnel-wash water. The biofilter was tested as an additional treatment after sedimentation. The tunnel wash water was collected after a half wash and was stored in tanks with volumes of one cubic metre for five days before the test started. The metals and metalloids investigated were Arsenic (As), Chromium (Cr), Copper (Cu), Nickel (Ni), Antimony (Sb) and Zinc (Zn), chosen based on their abundance in tunnel wash water and their potentially high dissolved fractions. *In situ*, size- and charge fractionation techniques were used to obtain information about the concentration and speciation of the metals and metalloids in the tunnel wash water before and after biofiltration.

Results show that after five to eight days of storage in the tanks, the tunnel wash water contained high concentrations of metals and metalloids. Cr, Cu and Zn were mainly present as particles, and Ni and Sb were mainly present as low molecular mass (LMM) species. After biofiltration, the concentrations were lower for As, Cr, Cu, Ni, Sb and Zn, with total removal efficiencies of 76, 82, 91, 60, 90 and 95 %, respectively. Removal of the particulate and LMM fractions were the main drivers for reduced metal and metalloid concentrations.

The changes in metal and metalloid concentrations from biofiltration were associated with increased LMM concentrations of calcium (Ca), magnesium (Mg), manganese (Mn), dissolved organic carbon (DOC) and reduced conditions (from +150 to -400 mV through the biofilter) in the effluent. Thus, the biofilter was able to remove metals and metalloids, most likely by either or both sorption and sulphide precipitation mechanisms. Longer hydraulic retention times were shown to have a higher capacity for metal and metalloid removal. Oxygenation of biofiltered tunnel wash water showed no impact on the metal concentrations or speciation in the water.

The work also identified that a large part of the metals and metalloids such as Cr, Cu and Ni were present as mainly negatively charged species in tunnel wash water, possibly as complexes with detergents. As anionic species have lower sorption to particles, this can be one of the reasons why some metals and metalloids are mobile and are not efficiently removed through sedimentation. More research should be done to investigate these findings.

The results of the present study show that biofiltration, in combination with reducing conditions, can decrease concentrations of metals and metalloids in tunnel-wash water after sedimentation.

Sammendrag

Tunneler akkumulerer veirelaterte forurensinger og blir vasket jevnlig for å opprettholde trafikksikkerhet og vedlikehold av tunnelene. Tunnelvaskevann inneholder høye konsentrasjoner av forurensninger som metaller og metalloider. Noen av disse er kjent for å ha potensiale til å forårsake negative effekter på akvatiske organismer. For å unngå slike negative effekter, blir tunnelvaskevann ofte dirigert til sedimentasjonsbasseng inne i eller utenfor tunnelen. Etter sedimentasjon blir tunnelvaskevannet sluppet ut i en naturlig resipient, eller til det kommunale avløpsnettet. Partikler i tunnelvaskevannet vil sedimentere i sedimentasjonsbassenget, men ikke alle metaller og metalloider er bundet til partikler. Løste tilstandsformer av metaller og metalloider fjernes ikke gjennom sedimentasjon, og kan utgjøre en trussel for akvatiske organismer i resipienten.

I denne studien ble flishogd trevirke fra gråor brukt som biosorbent, kombinert med reduserende forhold i forsøk på å rense tunnelvaskevann for metaller og metalloider. Renseløsningen ble prøvd ut som en tilleggsbehandling etter sedimentasjon. Tunnelvaskevann ble hentet etter en halvvask og oppbevart i 1 m³-tanker i fem dager før teststart. Metaller og metalloider som ble undersøkt var arsen (As), krom (Cr), kobber (Cu), nikkel (Ni), antimon (Sb) og sink (Zn). Disse ble valgt på grunn av tidligere rapporterte forekomster i tunnelvaskevann, og at de potentielt forekommer til stor del i løst form. For å undersøke konsentrasjoner og tilstandsformer til metaller og metalloider i tunnelvaskevann før og etter behandling, ble *in situ* størrelse- og ladningsfraksjoneringstekniker brukt.

Resultatene viste at etter fem til åtte dagers oppbevaring i tankene, inneholdt tunnelvaskevannet fortsatt høye konsentrasjoner av metaller og metalloider. Cr, Cu, og Zn var i hovedsak bundet til partikler, og Ni og Sb ble til en stor del påvist som lavmolekylære (LMM) spesier. Etter biofiltrering var konsentrasjonene lavere for As, Cr, Cu, Ni, Sb og Zn, og renseeffektiviteten var på henholdsvis 76, 82, 91, 60, 90 og 95 %. Fjerning av partikler og LMM fraksjon var den viktigste årsaken til reduserte konsentrasjoner av disse metallene.

Endringene i metallkonsentrasjoner under biofiltrering var assosiert med økte LMM konsentrasjoner av kalsium (Ca), magnesium (Mg), mangan (Mn) og løst organisk karbon (DOC), samt reduserende forhold (fra +150 til -400 mV gjennom biofilteret) i utløpet. Dermed var biofilteret i stand til å fjerne metaller og metalloider, trolig både på grunn av sorpsjon og utfelling av sulfider. Lengre hydraulisk oppholdstid viste seg å gi bedre kapasitet for fjerning av metaller og metalloider. Oksygenering av tunnelvaskevannet etter biofiltrering viste ingen effekter på metall og metalloid konsentrasjonene eller fordeling av tilstandsformene i vannet.

Arbeidet identifiserte at en høy andel av metaller og metalloider som Cr, Cu og Ni var til stede som negativt ladede tilstandsformer i tunnelvaskevannet, trolig som komplekser med såpestoffer. Siden negative spesier har lav sorpsjon til partikler, kan dette være en grunn til at noen metaller og metalloider er mobile ikke blir tilstrekkelig fjernet i sedimentasjonsdammer. Mer forskning bør gjøres for å undersøke disse funnene.

Resultatene fra denne studien viser at biofiltrering, i kombinasjon med reduserende forhold, kan redusere konsentrasjonene av metaller og metalloider i forsedimentert tunnelvaskevann.

Table of Contents

1	I	ntro	du	iction	1
	1.1		A	Aim of study	5
2	1	Mate	ria	als and methods	6
	2.1		E>	xperimental setup	6
	2	2.1.1		Area description	6
	2	2.1.2		Tunnel wash water	8
	2	2.1.3		Construction of the biofilter	8
	2.2	2	C	hemical parameters	11
	2.3	3	W	Vater sampling and fractionation	12
	2	2.3.1		Sampling before and after biofiltration	12
	2	2.3.2		Oxygenation test after biofiltration	13
	2	2.3.3		Water fractionation	13
	2.4	ļ	Sa	ample handling and laboratory analysis	15
	2	2.4.1		Ion Chromatography analysis	15
	2	2.4.2		Organic carbon analysis	16
	2	2.4.3		ICP-MS analysis	17
	2.5	5	Da	ata handling	18
	2	2.5.1		Values below the reporting limit of ICP-MS analysis	18
	2	2.5.2	-	Additional adjustments	18
	2	2.5.3		Calculation of change in variables from before to after biofiltration	19
	2	2.5.4		Oxidation-reduction potential	20
	2.6	6	St	tatistical analysis and figures	20
	2	2.6.1		Principal Component Analysis	20
	2	2.6.2		Kruskal Wallis and Dunn's post-hoc test	21
	2.7	7	Μ	lodelling of sulphide precipitation	21
3	F	Resu	lts	s and discussion	22
	3.1		Pr	recision and accuracy of measurements	22
	3.2	2	Τι	unnel wash water	25
	3	3.2.1		Chemical composition of tunnel wash water	25
	3	3.2.2		Concentrations of metals and metalloids in tunnel wash water	29
	3	3.2.3		Size fractions of metals and metalloids in tunnel wash water	32
	3	3.2.4	-	Charge fractions of metals and metalloids in tunnel wash water	33
	3.3	3	In	npact of biofiltration on tunnel wash water	36
	3	3.3.1		Change of tunnel wash-water characteristics in the biofilter	36

	3.3.2	Impact of biofiltration on metal and metalloid concentrations in tunnel wash wate					
	3.3.3	Impact of reoxygenation on metals and metalloids in biofiltered tunnel wash water 43					
	3.3.4	Change of metals and metalloid concentrations across all hydraulic retention times45					
	3.3.5	Impact of hydraulic retention time on metal and metalloid concentrations46					
	3.3.6	Concentrations of metals and metalloids in tunnel wash water after biofiltration compared to environmental requirements48					
	3.3.7	Processes for removal of metals and metalloids in the biofilter49					
3	.4 1	racer test54					
3	.5 0	General discussion around the biofilter55					
	3.5.1	Future for biofiltration of tunnel wash water55					
	3.5.2	Opportunities for improvement of the biofilter					
	3.5.3	Durability and lifetime of the biofilter57					
4	Concl	usion59					
5	Refere	nce list60					
Арр	endice	s66					
А	ppendi	x A66					
A	ppendi	x B66					
A	ppendi	x C					
A	ppendi	x D68					
A	ppendi	x E69					
A	ppendi	x F70					
A	ppendi	x G72					
A	ppendi	x H74					
A	ppendi	x I77					
А	ppendi	x J81					
A	ppendi	x K82					
А	ppendi	x L82					

1 Introduction

Over the past decades, the traffic density in Norway has increased, putting higher pressure on existing road and tunnel networks (Statistisk sentralbyrå, 2024a; Statistisk sentralbyrå, 2024b). Roads have been identified as substantial sources of various pollutants in the air, soil and water (van Bohemen & Janssen Van De Laak, 2003). They are essential for transportation but consume vast land areas and contribute to heightened traffic noise and pollution levels (Statistisk sentralbyrå, 2021; Statistisk sentralbyrå, 2023). Consequently, tunnels are extensively built in Norway due to their low visual land footprint, higher traffic safety, and their role in shielding the urban environments from noise and pollution (Arenas, 2008).

However, studies have shown that tunnels accumulate road-related pollutants, originating from sources like vehicle exhaust, wear of vehicles, tyres and roadway, as well as road salts (Meland et al., 2010a; Meland, 2012). By preventing dusty conditions, poor visibility and unnecessary wear of tunnels and installations, tunnel washing plays a crucial role in maintaining traffic safety (Statens Vegvesen, 2014).

The frequency and extent of tunnel washing are dependent on the traffic load and are regulated through Handbook R610 by Statens Vegvesen (2014). The extent of cleaning in each tunnel wash event depends on whether it's a whole-, half, or technical wash. Typically, a tunnel wash involves a sweeper to clean dust and debris, followed by a washing car that uses significant amounts of water to cleanse the tunnel surfaces of dirt (Meland & Rødland, 2018; Statens Vegvesen, 2014). The details of the methodology will vary depending on the tunnel and the company performing the wash. The resulting tunnel wash water will contain the pollutants and dirt formerly deposited on the tunnel surfaces. Factors like tunnel length, time of the year, extent of the wash and the cleaning method used, like the amount of water and detergents, will impact the concentrations and composition of pollutants in the tunnel wash water (Meland & Rødland, 2018).

Several studies have revealed high concentrations of various pollutants in tunnel wash water, including particles, nutrients, organic chemicals, road salts, polycyclic aromatic hydrocarbons (PAHs) and a range of different metals and metalloids (Allan et al., 2016; Andersen & Vethe, 1994; Grung et al., 2021; Meland, 2010; Meland & Rødland, 2018). Each of these pollutants can harm aquatic organisms or their ecosystem. When mixed, these pollutants can cause multiple biological effects in aquatic organisms through a series of interactions with various target sites (Meland et al., 2010b). For example, tunnel wash water has been shown to cause accumulation of metals in gills and oxidative stress as well as molecular changes in liver tissue in brown trout (Meland et al., 2010b; Meland et al., 2011). Hence, without sufficient cleaning, tunnel-wash water poses a threat to aquatic organisms after discharge.

Metals and metalloids like As, Cr, Cu, Ni, Sb and Zn are well-reported contaminants in tunnel wash water (Meland et al., 2010a; Meland & Rødland, 2018; Rathnaweera et al., 2019; Rathnaweera et al., 2023; REHIRUP, 2022; Sternbeck et al., 2002). There is great concern about the negative effects metals and metalloids in tunnel-wash water can cause in aquatic organisms (REHIRUP, 2022). For example, Zn is a heavy metal known for its toxicity to aquatic

organisms. Excessive levels of Zn can reduce fish's physical performance and growth (Abdel-Tawwab et al., 2016). Ni can affect the respiratory system of aquatic animals and can cause cellular damage (Pyle & Couture, 2011). As, being a metalloid, is toxic at low concentrations, can bioaccumulate in aquatic organisms and, among others, lead to liver damage and oxidative stress (Canivet et al., 2001; Datta et al., 2009).

To prevent reduced water quality in the recipient, discharge of tunnel wash water often requires a separate permit from the County Governor under the Norwegian Pollution Act (Forurensningsloven, 1983). The County Governor can set conditions for cleaning measures, usually in practice as limit values for pollutants in the tunnel wash water discharge (Forurensningsloven, 1983, §16).

In addition, the water quality of the recipient is protected by European law through the Water Framework Directive (WFD), which is also implemented in Norwegian law as the Norwegian Water Regulation (NWR). The WFD and NWR provide environmental quality standards (EQS) for so-called prioritised metals and metals of concern, including As, Cr, Cu, Ni and Zn (Direktoratsgruppen vanndirektivet, 2018; Endr. i vannforskriften, 2012, Vedlegg VIIIA). According to the WFD, the concentrations of pollutants in the recipient should not exceed their corresponding limit value for chronic effects from long-term exposure (AA-EQS) (Direktoratsgruppen vanndirektivet, 2018). The AA-EQS for different metals are used as benchmark values when evaluating pollution levels in natural recipients. For polluted waters discharged to natural recipients, a limit of 10 × AA-EQS might be used to take into account dilution after discharge (Åstebøl et al., 2012). However, this approach needs to be assessed based on the specific water flow characteristics of the natural recipient.

Historically, tunnel wash water has often been discharged almost untreated to natural recipients, with gully pots and oil separators being the only treatment methods (Meland, 2012). Gully pots effectively capture larger particles, and oil separators help mitigate oil spread in case of traffic accidents. However, Meland and Rødland (2018) collected data from 34 tunnels in Norway, investigating the levels of contaminants in tunnel wash water collected directly from the outlet of the tunnels. They found that EQS for a range of metals, including Cr, Cu, Ni and Zn, was exceeded in over 50 % of the tunnels, possibly posing a risk to organisms when discharged to the recipient. Therefore, gully pots and oil separators are inadequate for cleaning tunnel wash water and additional treatment is required.

Hence, additional treatment in the form of sedimentation facilities has, since the year 2000, been introduced in several Norwegian tunnels (Meland, 2012). Sedimentation facilities were first built outside of the tunnels as open ponds. However, it's now recommended that treatment facilities be built as closed tanks inside the tunnels to prevent harm to animals established in and around the polluted ponds (Liane, 2018; Meland et al., 2019). Sedimentation facilities clean water through the sedimentation of particulate-bound pollutants and, to some extent, the degradation of organic compounds (Garshol et al., 2015). The treatment efficiency of sedimentation facilities is largely impacted by hydraulic retention time, which will depend on factors like the amount of water used in the tunnel wash, the amount of precipitation in the area (for open sedimentation ponds), and the volume of the pond. A big fraction of total metals in tunnel wash water are attached to particles and are therefore easily removed through sedimentation processes (Damsgård, 2011; Farm, 2002).

However, metals and metalloids can be present in a range of physicochemical forms, such as sorbed to particles and colloids, bound in complexes with inorganic ligands such as chloride (Cl⁻) and carbonate (CO_3^{2-}), or as complexes with naturally occurring organic molecules (Gadd, 2009). There are ongoing interactions between the physicochemical forms, impacted by factors like pH, oxidation-reduction potential (ORP), ionic strength and temperature (VanLoon & Duffy, 2017). Particles are often defined as the solid phase that will either settle in a water column due to gravity or defy gravity and stay in the water phase as suspended particles (Stumm & Morgan, 2012). The operational definition of particles has traditionally been the fraction retained by a 0.45 µm filter (Haygarth & Sharpley, 2000). Colloids are very small, dynamic particles with a high area-to-volume ratio, making them reactive and constantly changing in the aqueous environment through a range of processes (Stumm & Morgan, 2012). The range of colloids spans an overlap of small, suspended particles down to the size of viruses, and species smaller than colloids are on molecular size (Stumm & Morgan, 2012). Molecular species are distinguished based on mass, where LMM species are in the smaller range of molecules, sometimes defined as < 10 kDa (about 1.4 nm in size) (Sørlie Heier et al., 2010). The component size can determine the mobility and toxicity of the metal, and the LMM fraction is generally assumed to be more bioavailable and more easily bioaccumulated in organisms than bigger-size fractions (Paquin et al., 2002; Steinnes & Salbu, 1995).

The particulate bound fraction can vary significantly between pollutants, dependent on their chemical and physical properties (Meland et al., 2010a; Stumm & Morgan, 2012). Hence, some metals and metalloids can be present in a larger proportion as LMM species in the water phase and, to a lesser extent, be removed through sedimentation (Meland, 2010). For example, Meland et al. (2010a) reported that 24 % of the total load of trace metals in outlet water from a sedimentation pond was discharged as LMM species. Some trace metals and metalloids, such as As, Zn, Ni, Molybdenum (Mo), and Antimony (Sb), discharged mainly as LMM species (> 70 %) compared to their respective total fractions (Meland et al., 2010a).

The LMM fraction of metals and metalloids may also increase when detergents are used during tunnel wash events. Detergents are often added during tunnel wash to make metals and other pollutants more water soluble, allowing pollutants to be discharged with the tunnel wash water. Aasum (2014) reported that both the total concentration and the LMM-fraction of As, Cd, Cu, and Zn greatly increased in water with detergents compared to water without detergents after 21 days of sedimentation. Hence, adding detergents is a good way to enhance the effectiveness of the washing procedure, but might prevent sedimentation of some metals and metalloids.

Both in situ and laboratory studies have demonstrated that sedimentation alone is not enough to achieve sufficiently low levels of metals in tunnel wash water to meet limit values (Garshol et al., 2015; Hallberg et al., 2014; Meland et al., 2010a). Additionally, data from Oslo tunnels show that concentrations of Cu and Zn in tunnel wash water discharge, in some cases, exceed the threshold values set by the County Governor (personal communication, Lene S. Heier, 2023-12-08). Hence, additional treatment methods are needed to reduce the LMM fraction of metals and metalloids in tunnel-wash water.

Several studies have tested out new methods for cleaning tunnel wash water, such as the use of coagulants (Furunes-Olsen, 2023; Nersten, 2016; Rathnaweera et al., 2019), electrocoagulation (Normann, 2017) and precipitation with Ca and Sodium Hydroxide (NaOH) (Nersten, 2016),

often tested as additional treatments after initial sedimentation. However, such methods are often expensive and require extensive use of chemicals (Kovacova et al., 2020).

Rathnaweera et al. (2019) tested out anoxic/anaerobic biological treatment for the removal of Zn and Cu. The study found that anaerobic biological treatment was more effective than aerobic biological treatment for removing Zn and Cu. Rathnaweera et al. (2019) discusses that it might be sulphate ($SO_4^{2^-}$)-reducing bacteria indirectly driving decreases in metal concentrations. In strictly anaerobic conditions, where $SO_4^{2^-}$ -reducing bacteria are generally encountered, the main product from $SO_4^{2^-}$ reduction is bisulfide (HS⁻), with a small proportion of volatile hydrogen sulphide (H₂S) (Gadd, 2009). Sulphides are known to readily bond to metals in solution, forming metal sulphides such as ZnS and CuS (Gadd, 2009). Most metal sulphides have low solubilities and will precipitate, removing metals from the dissolved phase. Metal sulphide precipitation has been tested out as a solution for metal removal in different polluted waters, such as industrial wastewater and groundwater (Jong & Parry, 2003; Sahinkaya et al., 2012; Teclu et al., 2008).

Adsorption has also been tested as a method for metal removal from industrial wastewater. Using natural materials, also called biosorbents, can often be a low-cost solution by reusing local waste products as biosorbents. Various natural (biological) materials have been investigated, including agricultural by-products and waste materials from the forestry industry. Among the investigated biosorbents for water treatment are corn stalk, tree bark, and sawdust. These biosorbents can be used in either their natural form or be modified to enhance desired properties (Asadi et al., 2008; Božić et al., 2009).

Wooden sawdust was first identified as a biosorbent to remove Cu from wastewater 20 years ago (Yu et al., 2000). Today, many studies have investigated sawdust as a biosorbent for removing metals and metalloids. Sawdust consists of crude fibres, lignin, cellulose, hemicellulose, and hydroxyl groups (such as phenolic compounds and tannins), which can bind heavy metal ions through ion exchange and adsorption (Kovacova et al., 2020). Potassium (K), Sodium (Na), and Ca can participate in ion exchange processes. Sawdust has been identified as a cheap, efficient method for metal removal from different polluted waters (Bulut, 2007; Meez et al., 2021).

Most studies on wood used as biosorbent were done on polluted wastewater from industry, while studies on specific road- or tunnel-wash water runoff are scarcer. Only some organic media with modified properties from the commercial market have been tested for cleaning dissolved metals (< 0.45 µm in size) from tunnel wash water (Paruch & Roseth, 2008). The study found that metal and metalloid concentrations in tunnel wash water were efficiently decreased when using a combination of different organic media (Paruch & Roseth, 2008). Ashoori et al. (2019) combined the use of sawdust in a biofilter and the use of reducing conditions to remove metals from urban stormwater. They reported removal efficiencies above 80 % for dissolved Ni, Cu, Cd and Pb, showing that the combination of sulphide precipitation and biofiltration can reduce metal concentrations in urban stormwater.

A biofilter for treating tunnel wash water would have the potential to work as a cheap, efficient method of reducing pollutant concentrations.

1.1 Aim of study

This study aims to combine the use of woodchips as a biosorbent and the use of reducing conditions to clean tunnel-wash water from metals and metalloids as an additional treatment after sedimentation. The metals and metalloids investigated (As, Cr, Cu, Ni, Sb and Zn) were picked according to their relevance in tunnel wash water.

The composition of the biofilter material used has earlier been tested for nitrate (NO₃⁻) removal, both for runoff water from a blasting rock landfill and for discharge water from tunnel construction (Roseth et al., 2023; Roseth et al., 2024). The biofilter material has been shown to contribute to reducing conditions with possible increased concentrations of sulphide. Microbial activity in the biofilter is enhanced by nitrogen-rich water, using woodchips as a carbon source. Shell sand was added as a buffer since acidic conditions are common in anaerobic reactors as a consequence of production of fatty acids during digestion (Ali et al., 2019; Liu et al., 2008).

Tunnel wash water after biofiltration was expected to have reduced conditions from biological activity. A following discharge in a natural recipient would lead to reoxygenation of the reduced water, possibly shifting the size fraction distribution in tunnel wash water towards a higher colloidal and/or particulate content. This was expected due to soluble Fe and Mn species present in the tunnel wash water, which might form colloidal species and possibly precipitate when ORP conditions change (VanLoon & Duffy, 2017). Therefore, reoxygenation tests were performed to assess eventual changes in metal and metalloid speciation in the biofiltered tunnel wash water release to a stream.

Further, the areal use is a concern for a full-scale facility. Therefore, the study tested the effect of the biofilter at three different hydraulic retention times to see how the cleaning efficiency of metals and metalloids changed with hydraulic retention time.

A combination of filtration, ultrafiltration and ion exchange was used to obtain information about metal speciation regarding the size (molecular mass) and reactivity (charge) of raw tunnel wash water and tunnel wash water after biofiltration.

The hypotheses were:

- There will be lower concentrations in the LMM-fraction of As, Cr, Cu, Ni, Sb and Zn in tunnel wash water after biofiltration than before biofiltration.

- There will be differences in the ability of the biofilter to retain As, Cr, Cu, Ni, Sb and Zn from tunnel wash water dependent on the hydraulic retention time in the filter.

- Reoxygenation of biofiltered water will push the size fraction distribution to a higher particle content.

2 Materials and methods

2.1 Experimental setup

2.1.1 Area description

The Nordby tunnel is built between Frogn and Ås municipality in Norway. It is a 3.84 km long tunnel, divided into two separate tubes with four driving lanes and with walls and ceiling made of concrete (Jarslett, 2024; Meland, 2010). The Nordby tunnel is highly trafficked and has a traffic density, measured as Annual Average Daily Traffic (AADT) of over 38000 in 2022 (Statens Vegvesen, n.d.).

The Nordby tunnel is, in theory, washed 12 times a year, divided into two whole washes, four half washes and six technical washes (Statens Vegvesen, 2014). Wash water is led through several gully pots that remove coarse particles and are then pumped into the Vassum cleaning basin for further cleaning (Meland, 2010). The Vassum cleaning basin is open and consists of two ponds: a sedimentation basin, designed to remove coarse particles through settling, and the main pool, serving as a prolonged settling stage and possibly facilitating some breakdown of organic components before the water is discharged. The Vassum cleaning basin is shown in Figure 1. Sediments were removed from the sedimentation basin the day before tunnel wash water was collected for the present study.

The Vassum cleaning basin receives water from three different nearby tunnels: the Nordby tunnel, the Vassum tunnel and the Smiehagen tunnel marked in Figure 2 (Meland, 2010). Since the basin is open and built outside of the tunnel, it will also receive runoff water from the nearby area during precipitation events. Water from the Vassum cleaning basin is discharged to Årungselva (Meland et al., 2010a), which ends up in Bunnefjorden. Årungselva is a spawning locality for sea trout (Meland, 2010; Meland et al., 2010a).



Figure 1: View over the Vassum cleaning basin to the left, with the opening of the Nordby tunnel in the background.



Figure 2: Map over the area. 1) The Vassum cleaning basin. 2) The location for installation of the biofilter. 3) The Nordby tunnel. 4) The Vassum tunnel. 5) The Smiehagen tunnel.

2.1.2 Tunnel wash water

Tunnel-wash water for use in the present study was collected following a half wash of one of the tubes of the Nordby tunnel. According to information from the Norwegian Public Roads Administration, 88m3 of water and 70 L of detergent (PG-IV1-X2–1000; Purify AS, Stavanger, Norway) were used in the tunnel wash.

Tunnel wash water was pumped from the sedimentation basin of the Vassum cleaning basin for storage in three IBC containers using a 230 V rainwater tank pump from Gardena (Q_{max} = 4700 L/h) connected to a hose (Figure 3). One of these containers (1000 L) was brand new, one (1000 L) was previously used for storage of glycerine, and one (800 L) was previously used for storage of glycerine, and one (800 L) was previously used for storage of tunnel water. The older containers were washed with Zalo and rinsed thoroughly with water before use. The tunnel wash water was left in the IBC containers for four days to sediment coarse particles before the first samples were collected.



Figure 3: To the left: The Vassum cleaning basin, with the pump used to fill up the tanks with tunnel wash water. To the right: One of the tanks is used to store tunnel-wash water.

2.1.3 Construction of the biofilter

To test its ability to retain metals and metalloids from tunnel wash water, a biofilter was constructed, consisting of a horizontal pipe containing biofilter material and gravel. The pipe was 3 meters long, 30 cm in diameter and made from polypropylene (PP). The pipe had a gasket and a 45-degree PP bend at each end, sealed with TEC7 black sealant (item no. T535106). A PP end cap, sealed with Illbruck sealing foam FM610, was fitted at the far end of the inlet, with drilled holes to ensure air exchange and prevent biofilter overpressure (Figure 4a). The pipe was

positioned at a slight incline, with the inlet end slightly higher than the outlet end, to create a gentle gravitational force that would encourage water to flow towards the lower end.

Biofilter material was taken from a container that was earlier used by Roseth et al. (2024) for filtering discharge water from tunnel construction over two months. Older chipwood was used to prevent the solubilisation of metals bound in the wood during start-up, as reported in sawdust biofilters by Lepine et al. (2021). The biofilter material consisted of a mix of grey alder woodchips (four to 60mm in length) and shell sand in volume ratios of approximately 80:20 (Figure 4c). Shell sand was added to maintain porosity and prevent a decrease in pH. The pipe was filled with 205 L of biofilter material. Throughout the filling process, the biofilter material was mechanically compacted multiple times to ensure complete coverage of the pipe's diameter. At the far end of each pipe, a layer of 12 L of washed gravel was added to keep the biofilter material in place (Figure 4b).

To establish near-reducing conditions in the biofilter before the experiment started, the biofilter was filled with water from the main pool of the Vassum cleaning basin 24 days before the experimental start-up.

To expose the biofilter to tunnel-wash water during the experiment, an inlet hole was drilled in the end cap, and a hose was fitted. The hose was connected to a small pump in one of the IBC containers. A digital timer was installed to control the pump, switching it on and off at defined times each day during the experiment.



Figure 4: a) Pictures the inlet side of the biofilter in the middle, with one tank filled with tunnel wash water on each side. The upside-down bucket on the left tank contains a box with the timer controlling the pump. b) Pictures the outlet side of the biofilter, including a thin hose for sampling and a digital multiparameter sensor. c) Pictures of the mix of wood chips and shell sand used as biosorbent in the biofilter.

Pumping rates and hydraulic retention time

Three tests using different hydraulic retention times were performed over four weeks from September to October. The tests were conducted to study whether the filter removed metals and metalloids from the tunnel wash water and to examine the impact of the hydraulic retention time on the removal efficiency.

The longest hydraulic retention time, 45 hours, was chosen based on its similarity with the hydraulic retention time tested in Roseth et al. (2024) and was regarded as the main test.

The pumping rate required to achieve the nominal hydraulic retention time of 45 hours was calculated using the following function. The volume of water-saturated biofilter material was 141.3 L, obtained from calculations displayed in Appendix A. The total waterfilled porosity of the biofilter material was estimated to be around 64 % after measurements done by Bruun et al. (2016) and reported by Hoffmann et al. (2019) on similar filter material.

Hydraulic retention time (HRT) was calculated using the following formula, also used by Audet et al. (2021).

$$HRT = \frac{Vs * \theta}{Q}$$

where Vs is the water-saturated volume of the filter material (in m^3), θ is the fraction of water-filled porosity of the filter material, and Q is the inflow of water in the biofilter (L/day).

Hence, a pumping rate of 48 L/day was required to achieve a hydraulic retention time of 45 hours. In the following tests, the initial pumping rate of 48 L/day was doubled to 96 L/day and then quadrupled to 192 L/day, corresponding to hydraulic retention times of 45, 23, and 11 hours, respectively.

A pumping test was conducted to determine how much water was pumped per minute. (Table 1). To resemble how a pump from a sedimentation pond would likely work, tunnel wash water was pumped in intervals. For example, for the test using a hydraulic retention time of 45 hours, the pump would be turned on for one minute once every four hours.

Nominal hydraulic retention time	Pumping rate	The time between each pumping	Length of each pumping event	Liters of water pumped per minute	Experiment duration
45 hours	48 L/day	4 hours	1 min	8 L/min	10 days
23 hours	96 L/day	4 hours	2 min	8 L/min	4 days
11 hours	192 L/day	2 hours	2 min	8 L/min	4 days

Table 1: Displays pumping specifications for the different hydraulic retention times.

To examine how the calculated hydraulic retention time compared to the actual hydraulic retention time in the biofilter, a tracer test was conducted on the doubled pumping rate of 96 L/day. Approximately 500 g of table salt (NaCl) from Seltin was mixed with about 500 L of water, increasing the electrical conductivity (EC) from 600 μ S/cm to 1700 μ S/cm. Before the start of the tracer test, the pump was pumping tunnel-wash water at a pumping rate of 96 L/day. The pump was simply relocated to the salted water when starting the tracer test. An automatic recorder was placed in the biofilter outlet to record the EC every 10 minutes until the EC curve had flattened out and stabilised.

2.2 Chemical parameters

EC, pH, temperature, and dissolved oxygen levels were recorded manually using a WTW[™] MultiLine[®] Multi 3620 IDS Multi-Parameter Portable Meter with suitable sensors every time water samples were collected. The following sensors were used:

pH was recorded with a WTW[™] SenTix[™] 41 pH Electrode. The pH sensor was calibrated at the start of every day using Hamilton DuraCal pH buffer 4.01 (REF = 238917) and Hamilton DuraCal pH buffer 7.00 (REF = 238918).

Dissolved oxygen was recorded with a WTW[™] FDO[®] 925 Optical IDS Dissolved Oxygen Sensor. The dissolved oxygen sensor was calibrated for temperature by putting the sensor casket in water at approximately the same temperature as the sample water before calibration.

EC was recorded with a WTW[™] Tetra Con325 conductivity sensor. The accuracy of the EC sensor was occasionally checked using the WTW control standard for conductivity cells 0.01 mol/L KCl. This sensor was also used for automatic recordings in the tracer test.

A digital multiparameter sensor SEBA Hydrometrie MPS-D8, equipped with pH-, EC-, oxidationreduction potential (ORP) and dissolved oxygen sensors, was installed in a bucket receiving outlet water from the biofilter. The multiparameter probe was programmed to automatically record all parameters every 30 minutes during the experiment. After the initial testing of the hydraulic retention times, the digital multiparameter sensor was placed in a full tank with tunnel wash water for six days to record ORP for later use as inlet ORP.

2.3 Water sampling and fractionation

2.3.1 Sampling before and after biofiltration

To obtain information about metal concentrations in tunnel wash water before and after biofiltration, water samples were collected from the tank (referred to as raw tunnel wash water) and directly from the outflow of the biofilter (referred to as tunnel wash water after biofiltration) for a minimum of three days for each hydraulic retention time. Samples of tunnel wash water after biofiltration were collected directly after pumping at 12 am, and samples of raw tunnel wash water were collected at one pm.

Total concentration samples were collected in 50- and 15-ml polypropylene (PP) tubes directly from the inlet or outlet of the biofilter using a single-use syringe to obtain the total concentrations of elements in the water. The syringe was rinsed three times with sample water before collection. Additional samples for further fractionation were collected in separate one-litre water flasks; all were rinsed three times with sample water before sampling and three times with deionised water post-sampling.

Both samples of raw runnel wash water for total concentrations and samples of raw tunnel wash water for further fractionation were obtained by submerging a flask or a single-use syringe into the tank through a hole in the top. Thus, raw tunnel wash water was gathered from the water surface to a depth of approximately one decimetre.

In tunnel wash water after biofiltration, reducing conditions were expected. Air mixing into the water might change the fraction distribution of metals and metalloids. To prevent this, a small hose connected to the bottom of the biofilter outflow was used for sampling. On every pumping occasion, the water level was elevated in the biofilter, causing water to drain through the hose. Samples were quickly collected by transferring the free end of the hose into a one-litre plastic flask. Three one-litre plastic flasks were filled and sealed with lids on each sampling occasion before further treatment.

2.3.2 Oxygenation test after biofiltration

An additional 3 × 1.2 L of water was collected from the 45-hour hydraulic retention time test to assess the impact of oxygenation on the metal and metalloid speciation in tunnel wash water after biofiltration. The water was collected directly into an open plastic bucket from the outlet of the biofilter right after pumping and sampling of biofiltered tunnel wash water at 12 am for three days. The water was oxygenated for 48 hours using an aeration pump. A tarpaulin was installed as a roof about one decimetre above the bucket to protect it from precipitation.

Water samples were collected directly from the bucket using techniques similar to those used in water tanks with tunnel-wash water and biofiltered tunnel-wash water.

2.3.3 Water fractionation

In situ, fractionation techniques were utilised to obtain information about the element distribution in the tunnel wash water. The transformation of elements in the biofilter was identified by differences in trace element distribution between raw tunnel wash water and tunnel wash water after biofiltration.

Changes concerning size- and charge distribution were investigated using filtration and *in situ* cation- and anion chromatography. This master thesis distinguishes between particulate (> 0.45 μ m), colloidal (0.45 μ m > 10kDa), and LMM-species (< 10kDa), obtained through filtration. The LMM fraction underwent further fractionation into anions and cations.

To exclude particles, a single-use syringe was equipped with a 0.45 μ m Supor Membrane filter with a diameter of 32mm. Before sampling, approximately five millilitres of water were filtrated and discarded to prevent sample contamination from the filter.

50- and 15-mL samples of 0.45 µm filtrate and 15 mL samples of ultrafiltrate and ion-exchanged samples, respectively, were stored in PP tubes, dark at four °C until further preparation and analysis. Gloves were used during all sampling.

Ultrafiltration

To separate between colloidal and LMM-species and to obtain information about charge distribution (anions, cations, and neutrals), ultrafiltration was performed using a Pall's Microza[™] hollow fibre cartridge with a nominal molecular mass cut-off level of 10 kDa, combined with cation- and anion- chromatography (Figure 5).

One-litre water flasks filled with raw or biofiltered tunnel wash water and an open plastic bucket containing oxygenated tunnel wash water were used for ultrafiltration separately. After sampling, ultrafiltration of raw or oxygenated tunnel wash water was performed within one hour. When performing ultrafiltration on biofiltered tunnel wash water, extra care was taken not to introduce air into the hose system since reducing conditions were expected in the biofilter and speciation can change with changing redox conditions. Ultrafiltration of tunnel wash water after biofiltration was performed approximately five to 10 minutes after sampling.

During ultrafiltration, sample water was pumped from the water flask or plastic bucket through a hose and into the flow-through filter cartridge. Firstly, one litre of sample water was pumped to flow through for rinsing. After that, the hose was transferred to a second flask with sample water. After most of the air had left the hose system, the outlet end of the hose was put into the flask to recirculate the water. Pressure was then applied to the hose, reaching 15 pSi, forcing water through the filter at approximately 15 mL/min. The first 50 mL of ultrafiltrate was discarded. The following ultrafiltrate was subject to further charge fractionation (chapter 0), and lastly, ultrafiltrate was collected in separate 15 mL PP tubes and stored dark at four °C until analysis.

The 10 kDa filter system was cleaned with deionised water after every filtering occasion. It was cleaned thoroughly about every fourth filtration or when filtration started to slow down, indicating clogging. When cleaning thoroughly, the filter was first rinsed with one litre of deionised water. 1.2 L of 0.01 M NaOH was then pumped through the system; the first 200 mL was discarded after filtration, and the following one litre was filtrated on circulation for 15 minutes. One litre of deionised water was then filtrated and discarded. The same process was repeated with first 1.2 L of water mixed with 11.5 g of EDTA (9.58 g/L) and then 1.2 L of 0.01 M HNO₃. After 15 minutes of HNO₃ circulation, about five litres of deionised water was pumped through the filter and discarded to ensure that any residues were removed before further use.

From total and fractionated samples, the following size fractions were identified:

<u>Total</u> = Total (concentration measured in the unfiltered water)

<u>Particulate</u> = Total - $<0.45 \mu m$ (concentration in unfiltered water, subtracted concentration in 0.45 μm filtrate)

<u>Colloidal</u> = <0.45 μ m - <10 kDa (concentration in 0.45 μ m filtrate, subtracted concentration in ultrafiltrate)

LMM = <10 kDa (concentration in ultrafiltrate)

Charge fractionation

To obtain information about the concentration of positively charged species, 65 mL of ultrafiltrate was pumped directly into a cation-exchange column after ultrafiltration. The cation-exchange column contained 8 ml of BIO-RAD CHELEX-100 Resin 50 to 100mesh, Na form, and was used with a 15 mL/min flow rate (1.9 mL min⁻¹ bed volume⁻¹). The first 50 mL of water from the column was discarded for conditioning purposes, and the following 15 mL were collected and stored in a dark and cool environment until analysis. Since the Na form of CHELEX-100 was used, Na was excluded from concentration measurements in cation-exchanged samples.

The same procedure was repeated to obtain information about negatively charged species but with an anion-exchange column containing 8 ml of BIO-RAD AG 1-X8 Resin 50 to 100 mesh, Cl⁻ form, and was used with a 15 mL/min flow rate (1.9 mL min⁻¹ bed volume⁻¹). Cl⁻ was excluded from concentration measurements in anion exchanged samples.

All resins were exchanged for new ones about every fifth time a new water sample was fractionated to ensure high ion exchange capacity.

From fractionated samples, the following charge fractions were identified:

<u>LMM cation</u> = <10 kDa - <10 kDa cation exchanged (concentration measured in ultrafiltrate subtracted concentration measured in cation exchanged water)

<u>LMM anion</u> = <10 kDa - <10 kDa anion exchanged (concentration measured in ultrafiltrate subtracted concentration measured in anion exchanged water)

<u>LMM neutral</u> = <10 kDa - <10 kDa cation - <10 kDa anion (concentration measured in ultrafiltrate subtracted concentration measured in cation exchanged water and concentration measured in anion exchanged water)



Figure 5: Displays the equipment used for in situ ultrafiltration and following ion exchange chromatography.

2.4 Sample handling and laboratory analysis

2.4.1 Ion Chromatography analysis

Ion chromatography was used to determine concentrations of the major anions $SO_4^{2^2}$, Cl^- and fluoride (F⁻) in 0.45 µm- and ultrafiltrate samples. All vials for later analysis with ion chromatography were rinsed three times with sampling water before sampling. Samples for ion chromatography analysis were stored dark at 4°C for 2.5 months before analysis.

The analysis was performed by personnel in the NMBU inorganic lab. The ion chromatography system used was an ICS-6000 equipped with a Dionex ADRS 600 2mm Suppressor and a Dionex ICS-6000 CD Conductivity Detector. The suppressor removes the charge for all species that are not desired for the analysis. The columns used in the analysis were a Dionex[™] IonPac[™] AG28 Fast (2 × 150 mm) and a Dionex[™] IonPac[™] AS28 Fast (2 × 150 mm). The software of the instrument used was a PC with installed Thermo ScientificTM ChromeleonTM 7 Chromeleon Data System, Software Version 7.3.1.

Three calibration standards from Sigma Aldrich were used. The concentrations for F^- , Cl^- , and SO_4^{2-} used in the calibration standards are displayed in Appendix B. All chemicals used were of quality *pro analysis* or equivalent.

To avoid harming the ion chromatography equipment, all samples were diluted 100-fold before analysis. The results were then corrected back to the original concentrations. Certified reference samples (ION-96.4), (SANGAMON-03) and (QC3060) were included to test the accuracy of the analysis.

Blank correction

Samples that resulted in "n.a." signified that no peaks were detected, meaning they were indistinguishable from the baseline. N.a. results were only found in method blank samples.

Data from ion chromatography analysis was corrected for method blanks by subtracting the average concentration of the three method blanks from each sample.

2.4.2 Organic carbon analysis

Total Organic Carbon (TOC) analysis was performed by Eurofins. These samples were delivered for analysis on the same day as the sampling.

DOC analysis vials were rinsed three times with sampling water before sampling and stored dark at four °C for 2.5 months before analysis. While this is considered a long time for storage of samples before analysis, our primary interest was to determine the relative difference in DOC concentrations between samples from before to after biofiltration of the tunnel wash water. We reasoned that any potential degradation would occur equally in all samples.

Personnel in the inorganic lab on NMBU analysed DOC on samples filtered at 0.45 µm and ultrafiltered samples. The analysis used a TOC Organic carbon analyser, Model TOC Vcph/CPN, from Shimadzu Corporation. High-purity air from the cylinder was used as carrier gas at a pressure of approximately 300 to 600 kPa. The method used was NPOC 1-10ppm.met.

The uncertainty of the TOC instrument:

- < 1 mg/L: ± 25 %
- < 10mg/L: ± 10 %
- > 10mg/L: ± 5 %

Two standards were analysed to obtain information about the accuracy of the analysis. The standards consisted of three and six mg/L potassium hydrogen phthalate in deionised water. Both standards were within the uncertainty of the TOC instrument and are displayed in Appendix B.

Blank correction

Three method blank samples were run through all the same treatments and analyses as their sample siblings. Hence, if the analyte was measured in blank samples, it was assumed that other samples were also contaminated. All samples were, therefore, blank corrected by subtracting the mean concentration of the three blanks of the analyte from all the other samples.

2.4.3 ICP-MS analysis

Total and fractionated samples were analysed using Inductively coupled plasma mass spectrometry (ICP-MS) to determine concentrations of elements.

Preparation and digestion of unfiltered samples

Unfiltered samples were stored dark at four °C for two months before further treatment. The samples for ICP-MS analysis had previously been shaken, and 10 mL of sample had been extracted for TOC analysis. However, these TOC samples came out incorrect and are not included further in this study.

The samples were added two volumes per cent of ultrapure (UP) HNO₃ and left for 19 hours before 10 mL of each sample was transferred to 17 mL Teflon UltraClave tubes and weighed. The samples were evaporated for two days at 90°C until dryness and then digested. Digestion was performed to dissolve any particles, allowing determination of the total element concentrations, including elements bound inside the particles.

To gain information about the accuracy of the analysis, CRMs of water (1.5 mL) (NIST 1643f) and soils (10 mg) (NCS ZC 73007), (NCS DC 73325), (NCS DC 73324a) were transferred to Teflon tubes, added 8.5 to 10 mL of deionised water, and evaporated to dryness before digestion.

1.5 mL of UP HNO₃ was added to all dried samples, CRMs, and blanks (n=3) before digestion in an UltraClave IV from Milestone, programmed to keep a temperature of 260 °C for 30 minutes. The digested samples were transferred to 15 mL tubes and diluted to 15.0 mL using type 1 water (ultrapure water).

Preparation of filtered samples

Filtered samples were stored dark at four °C for one month before further treatment. All filtered samples for ICP-MS analysis were acidified with two volumes per cent of UP HNO₃, shaken by turning them upside down ten times and stored three weeks before analysis.

Analysis

ICP-MS analysis was performed by personnel at the NMBU inorganic lab. Acidified samples were analysed using ICP-MS (Agilent 8900#100) in He-KED in oxygen- and NH₃ reaction mode. An online internal standard (IS) was used to correct physical interferences. The IS contained 20 μ g/L of Rhodium (Rh), Indium (In) and Bismuth (Bi) in 30 % sub-boiled ethanol and two per cent HNO₃. The IS was mixed into the sample with a dilution factor of 17. All acids used were of UP quality. The acids were sub-boiled in a Duopur from Milestone. The instrument was calibrated with certified standards in addition to a calibration blank.

A CRM, (NIST 1643f), was used to check the method's accuracy.

Correction for method blanks

Three method blank samples were run through all the same treatments and analyses as their sample siblings. Hence, if the analyte was recorded in blank samples, it was assumed that other samples were also contaminated. If the method blank samples were higher than the Limit of Quantification (LoQ), the mean of method blanks was subtracted from all the other samples.

2.5 Data handling

All calculations in data handling were performed using Microsoft Excel, version 2404.

2.5.1 Values below the reporting limit of ICP-MS analysis

The limit of detection (LOD) is the lower limit concentration under which the instrument cannot detect the analyte and is calculated as three times the Standard Deviation (SD) of the sample blanks. However, the uncertainty of the measurement is higher if it is close to the detection limit (Croghan, 2003), and the limit of quantification (LOQ) is therefore often calculated in addition. LOQ is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. It is calculated as 10 times the SD of the sample blanks. Data where at least one value is lower than LOQ is called left-censored data.

In the results from the ICP-MS analysis, certain data points fell below the LOD or LOQ. Values below LOD or LOQ required substitution with a numerical value to allow for identification of the concentrations of different size- and charge fractions. This is also important for statistical analyses, which require numeric data.

There are several methods for treating values below LOQ, and a simple and widely used method is the use of substitution (Helsel, 1990). Common substitutions are to replace values below LOQ with zero, with the LOQ-value, LOQ/2 or (LOQ+LOD)/2 (Zeghnoun et al., 2007). Similarly, values below LOD are commonly substituted with zero, with the LOD-value or LOD/2 (Zeghnoun et al., 2007).

Different substitution methods were used for different samples to minimise the uncertainty.

Values below LOD:

All blanks and samples treated with IEC (anion or cation-exchanged), with values below LOD, were substituted with zero. The argument is that no analyte concentrations were expected in method blanks unless otherwise proved by concentrations above LOD. Samples from IEC were substituted with zero since it was expected that if almost all ions in a sample were retained in the ion exchange resin column, all of them would.

All unfiltered, ultrafiltered, and samples filtrated on 0.45 μ m with values below LOD were substituted with LOD/2 to limit the risk of biasing the data too high or too low.

Values above LOD but below LOQ:

Values of all samples above LOD but below LOQ were substituted with (LOD+LOQ)/2 as described by Zeghnoun et al. (2007) to limit the risk of biasing the data, either high or low.

2.5.2 Additional adjustments

In the results of Cu and Cr from analysis of unfiltered samples using ICP-MS, the LOD was high due to a high variation between the three control samples. Two controls had similar concentrations, and the third control had concentrations five to 10 times higher than the other two, indicating contamination. The contamination was assumed to be only in this third control sample and was therefore deleted from calculations of LOD and LOQ for Cu and Cr. This adds additional statistical uncertainty since it can't be determined if samples other than the third blank were contaminated. Similarly, one of the blank samples put through anion exchange chromatography was clearly contaminated with Zn, showing blank concentrations three times higher than the other blank samples. It had the highest Zn concentration of all samples from anion exchange chromatography. Therefore, the contaminated blank sample was excluded from the calculation of LOD and LOQ for Zn for anion exchanged samples, resulting in the LOD and LOQ of this metal being based on two blank samples.

One of the 0.45 μ m filtered samples of Zn in the test of a 23-hour hydraulic retention time was clearly polluted compared to the other samples, resulting in the 0.45 μ m fraction being higher than the total fraction. This was adjusted by using the total fraction as a reference and adjusting the Zn concentration in the sample filtered on 0.45 μ m to the same Zn concentration recorded in the total fraction. This adjustment affects the colloidal and particulate fraction of Zn.

2.5.3 Calculation of change in variables from before to after biofiltration

To investigate the impact of biofiltration on elements and chemical parameters in tunnel wash water, changes in these variables were calculated based on measurements taken in tunnel wash water before and after biofiltration. In advance, recordings of raw tunnel wash water were paired with recordings of biofiltered tunnel wash water. The recordings were paired to ensure that the hydraulic retention time in the biofilter during sampling corresponded to the time between the recording of raw tunnel wash water and the biofiltered tunnel wash water recording. The pairing was done to introduce less uncertainty in the data than in the case of using the average or median of the recordings in raw tunnel wash water and biofiltered tunnel wash water.

One recording of raw tunnel wash water for each hydraulic retention time was missing. Therefore, one theoretical raw tunnel wash water measurement was calculated for each hydraulic retention time, based on the average of measurements recorded in raw tunnel wash water the day before and after, thereby assuming linearity.

The datum used for pairing is as follows. The inlet date is to the left, and the outlet date is to the right.

45 h hydraulic retention time: 27/9 – 29/9 29/9 – 1/10 (average of 29/9 and 3/10) – 2/10

23 h hydraulic retention time: 3/10 – 4/10 (average of 3/10 and 5/10) – 5/10 5/10 – 6/10

11 h hydraulic retention time: 6/10 – 7/10 (average of 6 and 9) – 8/10 9/10 – 9/10

The change in pH was calculated in hydronium ions [H⁺] using the following equation for each recording: $[H^+] = 10^{(-pH)}$

2.5.4 Oxidation-reduction potential

Due to equipment failure, no manual recordings of ORP were taken. Hence, ORP values used for tunnel wash water after biofiltration are an average based on recordings from the automatic meter placed at the outlet end of the biofilter. Since sampling may have distorted the measurements of the automatic meter on the sampling occasions, the average is based on ORP from the pumping occasion closest to the time before and after the sampled pumping. To ensure that obtained average values represent actual conditions, corresponding average values of pH, EC, oxygen saturation level and temperature were calculated in the same way, compared with manual recordings, and found to be similar. These comparisons are displayed in Appendix C.

2.6 Statistical analysis and figures

All statistical analyses were performed using RStudio 2023.12.1+402 "Ocean Storm" Release (4da58325ffcff29d157d9264087d4b1ab27f7204, 2024-01-28) for Windows. All figures, except those for the Principal Component Analysis (PCA), were created using the ggplot2 package. Trend lines were added to some figures using generalised linear models included in the ggplot2 package.

2.6.1 Principal Component Analysis

Multivariate statistics in the form of PCAs were performed to explore trends in the data material, using the change from raw tunnel wash water to biofiltered tunnel wash water. Different fractions were excluded in the different PCAs to explore how the trends changed depending on the size fractions. Hence, PCAs were performed on the change in total concentrations, change in particulate, colloidal, and LMM fractions, change in colloidal and LMM fractions, and change in LMM-fraction, respectively. Data from all the hydraulic retention times were included in all PCAs.

The PCAs were conducted using the factoextra package in RStudio. The PCAs were conducted on the correlation matrix of the data, which involved centring and scaling of the data before analysis. This was achieved by specifying center = TRUE and scale = TRUE within the prcomp() function.

Centring the data means that the mean of each variable is subtracted from each data point of that variable. Thereby, every variable is expressed relative to the mean for that column. Scaling the data means that each data point within a variable is divided by the standard deviation of all the data points within the variable. Centring and scaling the data prevents the PCA from being biased by different units and ranges in included variables.

From the PCAs, score plots and loading plots were created using fviz_pca_ind and fviz_pca_var from the *factoextra* package.

2.6.2 Kruskal Wallis and Dunn's post-hoc test

A Kruskal Wallis test (KW), called a One-way ANOVA on ranks, is a non-parametric method for testing whether samples originate from the same distribution (Ostertagova et al., 2014). A KW test was performed ($\alpha = 0.05$) to explore statistical differences between metal and metalloid concentrations in raw tunnel wash water and tunnel wash water after biofiltration. KW tests were also performed to explore statistical differences between the hydraulic retention times.

A KW test was used instead of the more common ANOVA since the data violated the assumptions of parametric tests regarding the assumption of normal distribution and the assumption of homogeneity of variance. Any attempt to normalise the data failed.

A KW will only show if some groups are different, but not which ones. Therefore, Dunns Post-hoc tests were performed on the significant variables in the KW tests to explore which groups were statistically different. The KW analyses and the Dunns posthoc tests were performed in RStudio using the R base function kruskal.test() and the function dunnTest() from the FSA package. Method "Holm" was used to adjust the p-values of the Dunns test. Padjusted was used to compare significant results.

2.7 Modelling of sulphide precipitation

VisualMINTEQ, version 3.1, was used to model the potential precipitation of metals and metalloids in the biofilter. To simulate how tunnel wash water would behave when obtaining reducing conditions in the biofilter, average values of element concentrations obtained from ICP-MS and IC of raw tunnel wash water < 0.45μ m were added to the analysis, as well as average values of ORP and pH conditions obtained from tunnel wash water after biofiltration. Metal sulphide precipitation of Cu (Covellite), Ni (Nickel sulphide NiS) and Zn (Sphalerite) was added as possible solid phases in the model. Solid phases of As, Cr and Sb were unavailable in the program and, therefore, not included. SO_4^{2-} and HS⁻ were added as redox couples in the analysis.

3 Results and discussion

3.1 Precision and accuracy of measurements

The accuracy of the methodology was tested using CRMs for ICP-MS and ion chromatography. A recovery of within ± 15 % from the *certified concentrations* ± *expanded uncertainty* was considered within good agreement and is used as a limit to evaluate the CRM results. The CRMs results for the ICP-MS analyses are shown in Table 2 and Table 3.

CRMs for dissolved elements (water) were analysed with and without digestion (Table 2). The non-digested sample of CRM was within good agreement with the certified concentrations, showing that the instrument's accuracy was good. The digested CRM was in good agreement (± five per cent) with certified concentrations for most elements, except Aluminium (Al) and Cr, which had recoveries of 124 % and 118 %, respectively. Since these metals and metalloids agreed with the certified concentrations of non-digested CRM, it seems like contamination occurred during preparation before or during digestion. The contamination also affected method blanks, where concentrations above LOD of Al were detected. No contamination was found in the method blanks of Cr.

CRMs for geologic material (soil) were used to obtain information about method accuracy for particles (Table 3). The CRMs were in good agreement with certified concentrations for most elements. However, concentrations of Na, Sb, Mg, and Ca had low (< 85 %) recovery in some or all soil CRMs. Especially Sb stands out with only 1.4 and 1.86 % recovery. No elements had above 115 % recovery, only below 85 % recovery. The low recoveries indicates the presence of particles that were not completely digested by using HNO₃. Complete digestion could have been obtained using parallel digestion with hydrogen fluoride (HF) instead of HNO₃ or HF and HNO₃ combined (Dean, 2003).

Hence, the soil CRMs show that the particulate bound fractions of some elements, especially Ca, Mg, Na and Sb, tend to be underestimated regarding total particle content in the present study. However, the main interest of this study is the fraction of elements that can potentially dissolve in water, which do not include elements bound within the lattice structure of geological particles.

Table 4 shows the CRM results of the IC analysis on size-fractionated samples. All recorded concentrations were in good agreement with certified concentrations, with recoveries of 85 to 115 %.

Table 2: Displays results of ICP-MS analysis of CRMs for dissolved elements (water), for CRMs with and without digestion. u = expanded uncertainty (95% confidence interval). Percentage recovery <100% mean that measured concentration < (certified conc-u). Percentage recovery >100% mean that measured concentration > (certified conc+u).

			CRM NIST1643F	CRM NIST1643F			
			Not digested	Digested			
	Unit	Measured conc.	Cert. Conc. ± u	Recovery	Measured conc.	Cert. Conc. ± u	Recovery
Са	mg/L	28.2	29.43 ± 0.33	95.8%	28.3	29.43 ± 0.33	97.3%
К	mg/L	1.94	1.9326 ± 0.094	100%	1.92	1.9326 ± 0.094	100%
Mg	mg/L	7.38	7.454 ± 0.6	100%	7.24	7.454 ± 0.6	100%
Na	mg/L	19.2	18.83 ± 0.25	101%	18	18.83 ± 0.25	96.9%
Ρ	µg/L	<lod< td=""><td>-</td><td></td><td><lod< td=""><td>-</td><td></td></lod<></td></lod<>	-		<lod< td=""><td>-</td><td></td></lod<>	-	
S	mg/L	0.02	-		<lod< td=""><td>-</td><td></td></lod<>	-	
Al	µg/L	141	133.8 ± 1.2	105%	167	133.8 ± 1.2	124%
Fe	µg/L	95.3	93.44 ± 0.78	102%	<145.7	93.44 ± 0.78	
Mn	µg/L	37.1	37.14 ± 0.6	100%	35.9	37.14 ± 0.6	98.3%
As	µg/L	55.4	57.42 ± 0.38	96.5%	55.7	57.42 ± 0.38	97.7%
Cr	µg/L	18.5	18.5 ± 0.1	100%	22	18.5 ± 0.1	118%
Cu	µg/L	21.7	21.66 ± 0.71	100%	22.8	21.66 ± 0.71	102%
Ni	µg/L	59.7	59.8 ± 1.4	100%	60.1	59.8 ± 1.4	100%
Sb	µg/L	53.7	55.45 ± 0.4	96.9%	52.5	55.45 ± 0.4	95.4%
Zn	µg/L	78.2	74.4 ± 1.7	105%	79.4	74.4 ± 1.7	104%
		1			1		

	NCS ZC73007					NCS DC73325			NCS DC73324a		
	Unit	Measured conc.	Cert. Conc. ± u	Recovery	Measured conc.	Cert. Conc. ± u	Recovery	Measured conc.	Cert. Conc.± u	Recover y	
Ca*	g/kg	2.78	2.9 ± 0.11	100%	0.869	1.14 ± 0.023	77.6%	0.696	0.93 ± 0.019	76.4%	
К*	g/kg	17.1	20.8 ± 0.83	85.8%	1.48	1.66 ± 0.03	91%	3.24	3.65 ± 0.073	90.5%	
Mg*	g/kg	4.87	5.1 ± 0.25	100%	1.12	1.57 ± 0.047	73.6%	1.15	1.21 ± 0.024	97.3%	
Na*	g/kg	0.63	2.45 ± 0.049	26.3%	0.236	0.59 ± 0.012	40.6%	0.162	-		
Ρ	mg/kg	<1077	972 ± 34		1250	1150 ± 39	105.1%	287	240 ± 40	102.5%	
S	g/kg	0.299	0.261 ± 0.026	104.2%	0.254	0.25 ± 0.036	100%	0.595	0.534 ± 0.073	100%	
Al*	g/kg	95.7	94 ± 11.3	100%	168	155 ± 52.7	100%	155	141 ± 19.7	100%	
Fe*	g/kg	38.5	38 ± 1.9	100%	130	131 ± 4.3	100%	91.5	87 ± 14.7	100%	
Mn	g/kg	0.428	0.441 ± 0.020	100%	1.83	1.78 ± 0.113	100%	2.55	2.30 ± 0.10	106%	
As	mg/kg	20.1	18 ± 2	100.5%	4.2	4.8 ± 1.3	100%	93.5	88 ± 5	100.5%	
Cr	mg/kg	64.9	67 ± 3	100%	447	410 ± 23	103.2%	86.6	86 ± 8	100%	
Cu	mg/kg	33.4	32 ± 2	100%	109	97 ± 6	105.8%	418	358 ± 18	111.2%	
Ni	mg/kg	28.3	27.4 ± 0.9	100%	327	276 ± 15	112.4%	87.4	75 ± 6	107.9%	
Sb	mg/kg	0.0204	1.7 ± 0.2	1.4%	<lod< td=""><td>0.42 ± 0.09</td><td>-</td><td>0.223</td><td>14 ± 2</td><td>1.86%</td></lod<>	0.42 ± 0.09	-	0.223	14 ± 2	1.86%	
Zn	mg/kg	107	100 ± 8	100%	151	142 ± 11	100%	1770	1529 ± 79	110.1%	

Table 3: Displays results of ICP-MS analysis of CRMs for geologic material (soil) in unfiltered samples. u = expanded uncertainty (95% confidence interval). Percentage recovery <100% mean that measured concentration < (certified conc-u). Percentage recovery >100% mean that measured concentration > (certified conc-u).

* Values for certified concentrations ± u were initially specified as oxides (MgO, Al2O3, K2O, CaO, Fe2O3) and have been recalculated to concentrations for the element only.

Table 4: Displays results of ion chromatography analysis of CRMs in fractionated samples. u = expanded uncertainty (95% confidence interval). A percentage recovery below 100% means the measured concentration was below (certified conc-u). A percentage recovery above 100% means the measured concentration was above (certified conc+u).

			RM1 ion 96.4		RM2 Sangamon 03			
	Unit	Measured conc.	Measured conc. Cert. conc. ± u Recovery			Cert. conc. ± u	Recovery	
Cl	mg/L	78.7	74 ± 3.8	101%	17.6	16.6 ± 1.5	100%	
F	mg/L	0.112	0.122 ± 0.035	100%	0.195	0.19 ± 0.045	100%	
SO4 ²⁻	mg/L	83.2	76.3 ± 4.2	103%	19.0	18 ± 1.4	100%	

		QC3060				
	Unit	Measured conc.	Cert. conc. ± u	Recovery		
Cl	mg/L	169	159 ± 1	105.62%		
F [.]	mg/L	0.837	0.85 ± 0.017	100%		
SO4 ²⁻	mg/L	86.3	77 ± 0.6	111.2%		

3.2 Tunnel wash water

3.2.1 Chemical composition of tunnel wash water

Chemical parameters recorded in the sedimentation pond the day after the tunnel wash event and in the raw tunnel wash water after five to 19 days of sedimentation are displayed in Table 5.

The maximum amount of dissolved oxygen in water is dependent on, among others, temperature and ionic strength. Therefore, the oxygen saturation level indicates the percentage content of oxygen in relation to the natural total content at the current temperature and ionic strength. The day after the tunnel wash event, 3.57 mg/L of dissolved oxygen, corresponding to an oxygen saturation level of 8.3 %, was recorded in the sedimentation pond, showing that the tunnel wash water was undersaturated regarding dissolved oxygen. Low concentrations of dissolved oxygen (down to one mg/L) in tunnel wash water have been reported by Meland et al. (2010a) in the outlet of a sedimentation pond at the end of a tunnel wash. Garshol et al. (2015) recorded dissolved oxygen of 10 mg/L in tunnel wash water directly in the pumphouse, indicating that processes in the sedimentation pond decrease dissolved oxygen levels rather than the tunnel wash water itself. Low dissolved oxygen concentrations, down to anaerobic conditions, can occur in sedimentation ponds following oxidation of organic carbon in the pond and sediments (Rathnaweera et al., 2023).

Dissolved oxygen levels of raw tunnel wash water increased over time from about zero mg/L on day five to 10 mg/L on day 19 (Figure 6). The dissolved oxygen saturation level also increased,

showing an increase unexplained by temperature changes. This implies that oxygen-consuming respiration was not a dominating process in the tanks. Growth of algae on the tank sides was observed during the experiment, indicating that increasing dissolved oxygen levels might come from photosynthesis and the absorption of gaseous oxygen from the atmosphere (Boyd, 2020). The results of the present study are the opposite of recordings reported by Eivik Karlsen (2021), who recorded declining dissolved oxygen concentrations during 19 days of sedimentation of tunnel wash water. This contradiction might come from different methodological approaches since the study of Eivik Karlsen (2021) was conducted in a laboratory and hence diminished variables such as sunlight and wind.

Due to equipment failure, ORP was not recorded directly in the sedimentation pond. ORP in raw tunnel wash water displayed in Table 5 is a result of automatic recordings in a separate tank after about three weeks of sedimentation. The Mean of dissolved oxygen (7.6 mg/L), oxygen saturation levels (63 %) and ORP recorded by the automatic meter were close to recordings in raw tunnel wash water on day 19, showing that the ORP from the automatic meter is representative of the raw tunnel wash water. The recordings of ORP show slightly oxidising conditions (mean of +142 mV). The reported ORP of tunnel wash water in the literature differs from +30 mV recorded in the outlet of an open sedimentation pond (Meland et al., 2010a), to +190 mV recorded in a closed sedimentation tank (Rathnaweera et al., 2023). Both studies performed recordings directly after a tunnel wash event. Rathnaweera et al. (2023) and Garshol et al. (2015) recorded decreases in ORP in tunnel wash water during sedimentation from +190 mV to +97 mV and from +270 mV to -250 mV during four weeks in the field and 10 days in the lab, respectively.

Rathnaweera et al. (2023) also discusses the presence of sediments in the tanks and its possible impact on the ORP since lower ORP was recorded during sedimentation in a tank with sediments, than in a tank without sediments. This might indicate that the lack of sediments in the tanks of the present study might explain the increasing oxygen levels and high ORP in the raw tunnel-wash water.

pH recorded in the sedimentation pond was 7.6, which is in agreement with pH recorded in the same sedimentation pond by Meland et al. (2010a) (pH = 7.6 to 7.7) and in other tunnels by Rathnaweera et al. (2023) (pH = 7.8) and Vistnes et al. (2024) (pH = 7.4 to 8.9). The pH of the present study is lower than recordings of tunnel wash water from the same tunnel but in the pump house by Garshol et al. (2015) (pH = 8 to 8.9), and some of the recordings from another tunnel by Vistnes et al. (2024) (pH = 8.9). Differences in pH might come from methodological differences between the washes, like the type of detergent and detergent concentrations used (Aasum, 2014), but also from other factors, such as dilution and degradation in the ponds, in contrast to recordings done in pump houses. However, the recordings of the present study are clearly within the range of Norwegian tunnel wash water values as reported by Meland and Rødland (2018) of a median pH of 7.7 in tunnel wash water collected from 34 different tunnels in Norway. pH was rather stable over time; only small changes were recorded without a clear trend, aligning with recordings by Garshol et al. (2015) and Eivik Karlsen (2021).

Recorded EC in the sedimentation pond was 1160 μ S/cm. Garshol et al. (2015) recorded EC of 1500 to 3000 μ S/cm under an ongoing summer half wash of both tubes in the Nordby tunnel, slightly higher than in the present study. Meland and Rødland (2018) reported a median EC for

tunnel wash water of 1400 μS/cm (range between 170 to 13000 μS/cm) for 34 tunnels in Norway. In comparison, mean recordings of EC from Årungselva were 195 μS/cm in 2015-2019 (Miljødirektoratet, n.d.-a). Hence, tunnel wash water collected in this study got a typically high EC compared to natural waters. The EC was not impacted by sedimentation, which is in accordance with observations done by Eivik Karlsen (2021).

Table 5: Displays recorded chemical parameters from one measurement conducted in the sedimentation pond the day after the tunnel wash event, right before tunnel wash water was collected to the tanks. The table also displays the mean, median and SD of chemical parameters recorded in the raw tunnel wash water in the tanks from day five to day 19 of sedimentation. DO = Dissolved Oxygen.

		Sedimentation pond	F	Raw tunnel wash wate	er
	Unit		Median	Mean	SD
DO	%	8.3	34.5	34.4	21.4
DO	mg/L	3.57	3.53	4	2.8
Temperature	°C	13.5	12.6	12	3.6
ORP	mV	-	149	142	23.9
pH*		7.6	7.6	7.6	7.4-7.8
EC	µS/cm	1160	1226	1223	7.6

* pH is calculated from [H+]. SD for pH is displayed as "(mean+SD)-(mean-SD)", calculated from [H+].



Figure 6: Displays the change in oxygen saturation level (blue), dissolved oxygen (mg/L) (green) and temperature (°C) (red) over the number of days since tunnel wash water was collected in the inlet tanks. Trend lines from generalised linear regression models are displayed for each variable.

As indicated by the high EC, the tunnel wash water contained high concentrations of major ions like Na, K, Ca, and Mg (Table 6). Concentrations of Ca, Mg, Na and K were within the range of 0.91 to 2700 mg/L that Meland and Rødland (2018) recorded in 34 tunnels in Norway, and similar to reported concentrations by Meland et al. (2010a) of K, Mg and Ca (12.6 \pm 0.4, 10.3 \pm 0.4 and 46 \pm 0.5 mg/L, respectively) in the outlet of a sedimentation pond.

Concentrations of Na and Cl⁻ reported by Meland et al. (2010a) was about 2.5 and 3.5 times higher than the recorded concentrations in the present study. Meland et al. (2010a) argues that these high concentrations of Na and Cl⁻ come primarily from road salts and an excess of Na from the wear of brakes. However, the high concentrations of Na and Cl⁻ reported by Meland et al. (2010a) cannot be seen in the present study. The reason is probably seasonal since the present study was carried out during autumn, when road salts had not yet been applied to roads, whereas the study of Meland et al. (2010a) was conducted during April, after the winter season. Recorded concentrations of Na and Cl⁻ in the present study were about 10 times and seven times higher than concentrations reported in Årungselva (Miljødirektoratet, n.d.-b). Concentrations of SO₄²⁻ were on the same levels as reported by Meland et al. (2010a). SO₄²⁻ concentrations were not impacted by sedimentation.

The ICP-MS instrument was contaminated with P during the analysis of total concentrations, causing a high LOD of 300 μ g P/L (Appendix I). Therefore, all total concentrations of P were below LOD.

The TOC recorded in the present study was in accordance with the reported TOC in tunnel wash water by Meland and Rødland (2018) and Rathnaweera et al. (2023). They found a median of 34 mg/L TOC in discharge from 34 tunnels in Norway (Meland & Rødland, 2018) and 39 mg/L recorded in a closed sedimentation tank (Rathnaweera et al., 2023), respectively. The TOC and DOC of the present study were about three times higher and two times higher, respectively than the TOC and DOC concentrations reported by Meland et al. (2010a). The variations between the present study and Meland et al. (2010a) might be attributed to differences in detergent concentrations, seasonality (degraded plant material and traffic density) or occurrence of oil contaminants (Du et al., 2022; Markiewicz et al., 2017). The mean values of TOC concentrations recorded in Årungselva 2015-2019 were about one-third of the concentrations recorded in tunnel wash water of the present study (Miljødirektoratet, n.d.-a).

		5-8 days of sedimentation (Inlet			16-19 days	Removal			
			45h n=3)			11h n=2)			
	Unit	Median	Mean	SD	Median	Mean	SD	%	
Ca	mg/L	38.3	40.5	4.94	35.2	35.2	5.72	18.6	
К	mg/L	12	12.9	1.77	11.4	11.4	1.56	14.2	
Mg	mg/L	5.22	5.54	0.794	4.68	4.68	0.693	19.7	
Na	mg/L	240	260	39.1	238	238	33.2	10.8	
Р	µg/L	<lod< td=""><td><lod< td=""><td>-</td><td><lod< td=""><td><lod< td=""><td>-</td><td>-</td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td>-</td><td><lod< td=""><td><lod< td=""><td>-</td><td>-</td></lod<></td></lod<></td></lod<>	-	<lod< td=""><td><lod< td=""><td>-</td><td>-</td></lod<></td></lod<>	<lod< td=""><td>-</td><td>-</td></lod<>	-	-	
S	mg/L	27.4	29.1	3.63	25.9	25.9	3.82	15.3	
Cl-*	mg/L	225	225	5.36	216	216	11.1	5.1	
F-*	mg/L	0.389	0.415	0.0483	0.345	0.345	0.0477	33.8	
SO4 ²⁻ *	mg/L	63.5	63.7	0.921	61.7	61.7	2.09	4.2	
TOC**	mg/L	35	35	-	29	29	-	17.1	
DOC***	mg/L	21.3	21.3	2	22.3	22.3	1.41	7.7	

Table 6: Displays the mean, median and SD of total concentrations measured in raw tunnel wash water at the start and end of the field trial. Percentage removal is calculated as percentage change from first to last inlet sample. n = the number of observations.

* = 0,45 um filtered samples. ** = TOC was recorded in n=1 sample analysed by Eurofins. *** = Concentrations measured in the LMM-fraction.

3.2.2 Concentrations of metals and metalloids in tunnel wash water

Total concentrations of metals and metalloids recorded in the raw tunnel wash water are displayed in Table 7, along with limit values from the Norwegian Environment Agency and the County Governor.

The total concentrations of metals and metalloids (Al, Fe, Cr, Cu, Ni, Sb and Zn) in raw tunnel wash water of the present study are placed in the lower range of concentrations reported from 34 tunnels by Meland and Rødland (2018). However, concentrations reported by Meland and Rødland (2018) are from tunnel wash water collected directly from the tunnels, thereby not affected by five days of sedimentation or dilution from pond water, like in the present study.

Total concentrations of Zn, As and Fe after five to eight days of sedimentation were lower than reported in other studies of tunnel wash water (Meland et al., 2010a; Rathnaweera et al., 2023), and Zn was lower than reported in urban runoff (Birch et al., 2011). However, Zn and As concentrations were higher than reported in road runoff (Andradóttir & Vollertsen, 2015), and Zn was 500 times higher than the mean value of 2022 recorded in Årungselva (Miljødirektoratet, n.d.-b). Hence, the tunnel wash water of the present study show higher pollution levels than natural water and road runoff after five to eight days of sedimentation.

Measured total concentrations of Mn and Ni after five to eight days of sedimentation were on the same levels as recorded in tunnel wash water (Meland et al., 2010a), and higher than reported in urban runoff and road runoff (Andradóttir & Vollertsen, 2015; Swadener et al., 2014). As discussed before, Cr might be slightly overestimated in this study (about 18 %) according to the CRM, but are on similar levels as recorded by Meland et al. (2010a) also if taking into account the uncertainty. Concentrations of Cu were slightly higher than reported by Meland et al. (2010a), but much lower than reported in tunnel wash water by Rathnaweera et al. (2023). Total concentrations of both Cr and Cu were higher than reported in road runoff (Andradóttir & Vollertsen, 2015).

Indications of changes in the tunnel wash water concentrations of all metals and metalloids were observed in the tanks during the four weeks of field tests of the biofilter (Table 7), showing that an initial sedimentation of five days was not enough to achieve relatively constant concentrations in the raw tunnel wash water. However, these changes were not statistically significant when concentrations from the first and last days of sedimentation were compared (Appendix H).

Limit values set by the Norwegian Environment Agency AA-EQS were exceeded by As Cr, Cu, Ni and Zn after five to eight days of sedimentation (Table 7). After 16 to 19 days of sedimentation, AA-EQS was still exceeded by As, Cu, Ni and Zn in the tunnel wash water, and concentrations of Zn exceeded the diluted 10 × AA-EQS with more than two times the diluted limit value in the tunnel wash water. However, a dilution factor of 10 × AA-EQS is generalised and is used in the present study to display how concentrations compare to a generalised dilution factor of 10. In practice, the dilution factor will vary from recipient to recipient. Hence, the dilution factor for Årungselva might be higher or lower.

Cu (barely) and Zn exceeded the limit values for some Oslo tunnels set by the County governor after five to eight days of sedimentation. Concentrations of Zn were after 16 to 19 days of

sedimentation close to the limit value, reaching 104.1 ± 25 µg/L, while the limit value from the County Governor is 110 µg/L. Concluded, Zn exceeded AA-EQS, MAC-EQS and 10 × AA-EQS as well as limit values from the County Governor also after 16 to 19 days of sedimentation, showing that longer sedimentation times are needed to decrease concentrations of Zn in tunnel wash water to below limit values. In comparison, the Nordby tunnel is cleaned about every three to seven weeks, which roughly corresponds to the sedimentation time in the Vassum cleaning basin (personal communication, Kai Gundersen, 2024-04-29). Concentrations of Zn are not sufficiently removed after 16 to 19 days of sedimentation, and further cleaning is needed.
Table 7: Displays the mean, median and SD of total concentrations measured in the inlet tanks at the start and end of the field trial, compared to set limit values for contaminated water by the Norwegian environment agency and the County Governor. Values in parentheses are for the fraction < 0.45 µm. Limit values marked in red are exceeded in the tunnel wash water (TWW) also at the end of the experiment, after 19 days of sedimentation. Note that the concentrations have different units.

		5-8 da	ays of sedime	ntation	16-19 days of sedimentation		Removal efficiency in the inlet tank	Norwegian Environment Agency: limit values in the recipient			County governor; TWW after sedimenta tion	
	Unit	Mean	Median	SD	Mean	Median	SD	%	AA-EQS	MAC- EQS	10×AA- EQS	Oslo area
Al	µg/L	1367	1437	243	81.3	81.3	25.0	95.9				
Fe	µg/L	2747	2640	362	916	916	12.7	65.6				
Mn	µg/L	191	184	16.1	56.4	56.4	2.33	68.5				
As	µg/L	2.01	2.55	0.994	2.21	2.21	0.750	43.2	0.5	8.5	50	6
Cr	µg/L	6.26	6.64	0.676	1.86	1.86	0.248	74.7	3.4	3.4	34	10
Cu	µg/L	51.1	51.8	3.50	16.7	16.7	2.83	72.8	7.8	7.8	78	50
Ni	µg/L	11.4 (7.80)	11 (7.79)	1.35 (0.04)	9.28 (7.62)	9.28 (7.62)	1.31 (0.12)	24.1	(4)	(34)	40	40
Sb	µg/L	6.29	8.55	4.73	7.70	7.70	3.06	32.6				
Zn	µg/L	246	254	17.4	104	104	25.5	66.1	11	11	110	110

3.2.3 Size fractions of metals and metalloids in tunnel wash water

The samples of raw tunnel wash water were size fractionated to explore the distribution of metals and metalloids between the particulate, colloidal and LMM-fraction. The size fraction distribution in raw tunnel wash water over time is shown in Figure 7.

The observation on day nine of the particulate fraction is especially high for all metals, suggesting that this observation might be an outlier. Plastic syringes with applied filters were used to determine the particulate fraction. Such manual filters are prone to errors such as uneven flow rate and clogging, which affects the amount of retained elements in the filter (Morrison & Benoit, 2001). However, despite uncertainties in the exact concentrations of day nine, trends can still be seen in the data material.

At the start of sampling on day five, Cr, Cu and Zn were mainly present in raw tunnel wash water as sorbed to particles (76 %, 80 % and 53 %, respectively). Meland et al. (2010a) used size fractionation on tunnel wash water in the outlet of the sedimentation pond of the Nordby tunnel and reported concentrations of metals and metalloids in the size fraction above 10 kDa (Particulate+Colloidal fraction as defined in the present study) for Cr, Cu and Zn at 80 %, 75 % and 26 % respectively. Hence, the particulate fraction of Zn in the present study was higher than recorded by Meland et al. (2010a). Eivik Karlsen (2021) recorded particulate fractions of Cu and Zn in tunnel wash water from two tunnels of ~71 to 88 % and ~47 to 66 %, respectively, which is in agreement with the present study. This shows that the particulate fraction of Zn varies significantly between tunnels and washing occasions, while Cu has lower variations between the studies.

After about 12 days of sedimentation, the particulate fraction of As, Cr, Cu and Zn decreased and became lower or equal to the LMM and colloidal fraction. The decrease results from sedimentation of particles since no increase in LMM- or colloidal fraction was recorded. The observations are in line with Eivik Karlsen (2021), who recorded a gradual decline in Zn and Cu from day four until day 11 of sedimentation. After day 11, only small changes in the particulate fraction of about five µg/L of Cu and 25 µg/L of Zn were recorded by Eivik Karlsen (2021).

Sb and Ni distinguish from the other metals and metalloids by a lower particulate fraction than the respective LMM fraction. Ni and Sb are both known for being mainly present as LMM species in tunnel wash water, as recorded by Meland et al. (2010a), who recorded an LMM fraction of 71 % of Ni and 100 % of Sb in the outlet of a sedimentation pond receiving tunnel-wash water. Eivik Karlsen (2021) recorded a higher particulate fraction of Ni than the "truly dissolved" fraction (< 3kDa) up until day four of sedimentation. After four days, the "truly dissolved" fraction dominated, which aligns with the present study.

Looking at the trend lines shown in Figure 7, no or very small changes can be noted in the LMMand colloidal fraction of Cr, Cu, Ni and Zn over time. This is in accordance with LMM- and colloidal fraction reported by Eivik Karlsen (2021) for As, Cu and Zn during sedimentation. The trend line for the LMM fraction is more evident for As and Sb, showing some decrease over time.

The results suggest that 19 days of sedimentation did not largely impact the LMM and colloidal fraction of the studied metals and metalloids in the tunnel wash water.



Figure 7: Displays the size fractions of metals plotted over the number of days since tunnel wash water was collected in the inlet tanks. Trend lines are from generalised linear regression models for each size fraction.

3.2.4 Charge fractions of metals and metalloids in tunnel wash water

To explore the distribution of metals and metalloids in the LMM-fraction between anionic, cationic, and neutral species, the tunnel wash water samples were fractionated by charge. The percentage distributions of charge fractions and the distribution of charge fractions plotted over time are shown in Table 8 and Figure 8 respectively.

The cationic fraction was the dominating fraction for Mg (100 %), Ca (100 %), and Mn (100 %) in the tunnel wash water. Mn is in good compliance with the results of Meland et al. (2010a), who found that almost all Mn was in the cationic fraction in samples collected from the outlet of a sedimentation pond. However, Meland et al. (2010a) recorded that Mg and Ca were present as almost 100 % in the neutral fraction. Meland et al. (2010a) argues that the high neutral fractions of Ca and Mg might result from formation binding with Cl⁻ since high Cl⁻ concentrations were recorded in the study. The present study did not find high Cl⁻ concentrations in tunnel wash water, probably due to samples collected during fall before the road salt season. Hence, the lower availability of ligands, such as Cl⁻, that bind stronger to Mg and Ca than the cation exchange resin might explain the present study's high cationic fraction in these elements.

For P, K, Sb, and As, the neutral fraction was dominant in tunnel wash water, which is in agreement with results recorded by Meland et al. (2010a). Sb is known to usually exist in anionic or neutral fractions as Sb(V) or Sb(III), in natural waters under oxic conditions (Filella et al., 2002). Arsenic (As) indicates an increase in anionic fraction over time; between 16 to 19 days of sedimentation, more than 50% of As relies as anionic species, which is partly a result of actual increasing concentrations in the anionic fraction, and partly a result of a decreasing LMM fraction (Figure 8).

High anionic and cations fractions of Zn were recorded, resulting in these fractions combined exceeds the concentrations of the total LMM fraction by 160 % (Table 8). Charge fractions obtained from the difference between ion-exchanged and ultrafiltered samples have a combined uncertainty from the individual samples and the method blanks, possibly explaining the total ions exceeding the total LMM fraction. Meland et al. (2010a) recorded that the anionic fraction of Zn amounted to 20 % of the total LMM fraction in a sedimentation pond after a tunnel wash event. At the end of the tunnel wash, Meland et al. (2010a) reported that almost no Zn relied in the anionic fraction, divided evenly between the neutral and cationic fractions. A study in river water downstream of an old shooting range recorded that 89 ± 8 % of Zn was present as positively charged species (Sørlie Heier et al., 2010). High cationic and anionic fractions of Zn in the present study indicate bonding to, for example, a ligand that allows binding to both the cationic and anionic resin, doubling the contribution to the total ions. However, such results were not found in the presented literature.

Al, S, Fe and Cr were mainly present as anions (91, 100, 90, 90, 57 and 76 %, respectively). This is higher than reported by Meland et al. (2010a), who investigated water from the outlet of a sedimentation pond, where most metals were identified as neutral species. 100 % of sample concentrations of Cu and Ni were below LOD for anion-exchanged samples (LOD = 0.6 and 3 μ g/L respectively, (Appendix I)), resulting in the anionic fraction being equal to the LMM fraction, as seen in Figure 8. However, if assuming that the amount of Cu and Ni in anion exchanged samples was equal to LOD, the lowest possible anionic fractions would be 76 % and 57 %, respectively, and the highest being 94 % and 73 %, respectively, if using the lowest and highest recorded LMM fractions. Hence, including uncertainty from samples below LOD, Cu and Ni are still dominated by the anionic fraction. Meland et al. (2010a) recorded even distributions between cationic, and neutral species for Cu, and the neutral fraction as dominating for Ni. Sørlie Heier et al. (2010) recorded that Cu species < 0.45 μ m relied as 30 ± 10 % as cationic species, and 61 ± 14 % as neutral species in river water downstream a shooting range. Hence, anionic fractions of Al, S, Fe, Cr, Cu and Ni of the present study were high compared to earlier reported fractions in tunnel wash water and polluted river water.

As discussed in Chapter 3.2.1, about two-thirds of the TOC was present as LMM-fraction DOC in the raw tunnel-wash water of the present study. DOC can include compounds such as organic acids from degradation, oil contaminants, or detergents (Du et al., 2022; Markiewicz et al., 2017). DOC can be an important metal ligand (Tipping & Hurley, 1992). Therefore, one can hypothesise that high anionic fractions recorded for metals in the present study may result from anionic metal complexes formed with organic compounds. For example, according to the contractor, the detergent used in the tunnel wash preceding the present study contains anionic surfactants (SLES and benzenesulfonic acid). The conjugate base of benzenesulfonic acid; benzene sulfonate, has a high affinity for the anion exchange resin used in the present study (Bio-Rad, 2000). Additionally, the anion exchange resin used also has a high affinity for SO₄²⁻ species (Bio-Rad, 2000). For example, thiosulphate, being a part of the anoxic S cycle, can form anionic species with Cu and Zn. (Jørgensen, 1990). Hence, the high anionic fractions of some metals and metalloids might result from complexation with anionic surfactants, SO₄²⁻, or their metabolites in the tunnel-wash water.

Since humic matter and clays often have a negative surface charge in soils and water, anions are generally considered more mobile in the environment than cations (VanLoon & Duffy, 2017). Hence, as indicated in the present study, high anionic fractions of metals and metalloids in tunnel wash water might affect the efficiency of sedimentation ponds since anionic species, to a lesser extent, will bind to particles. However, further investigation is needed to elucidate if high anionic fractions are commonly found in tunnel-wash water or similar waters such as general road runoff.

The LMM-fraction in tunnel wash water of the present study does not seem to be impacted by sedimentation over time. The charge fractions of some elements change slightly over time, but, except for As, most elements show no indications of redistribution of charge fractions.

Table 8: Displays percentages of charge fractions for the raw tunnel wash water between five to eight days and 16 to 19 days of sedimentation. Percentages are calculated from average concentrations of each fraction, n=3 samples in each group. Total ions = anions + cations + neutral.

	5		16-19 days o	fsedimenta	tion			
	Anions	Cations	Neutral	Total ions	Anions	Cations	Neutral	Total ions
Са	1%	100%	0%	101%	1%	100%	0%	101%
К	0%	46%	54%	100%	2%	58%	40%	100%
Mg	0%	100%	0%	100%	2%	100%	0%	102%
Na	0%	-	-	-	1%	-	-	-
Р	23%	5%	72%	100%	-	-	-	-
S	100%	2%	0%	102%	99%	4%	0%	103%
Al	91%	4%	5%	100%	83%	35%	0%	118%
Fe	90%	15%	0%	105%	98%	20%	0%	118%
Mn	2%	100%	0%	102%	8%	98%	0%	106%
As	14%	1%	85%	100%	54%	4%	42%	100%
Cr	90%	8%	2%	100%	93%	11%	0%	104%
Cu	100%*	25%	0%	125%	100%*	21%	0%	121%
Ni	100%*	25%	0%	125%	100%*	15%	0%	115%
Sb	44%	5%	51%	100%	29%	2%	70%	101%
Zn	92%	78%	0%	170%	93%	75%	0%	168%

* 100% of samples <LOD



Figure 8: Shows stacked charge fractions of metals and metalloids relative to the total LMM fraction over days since tunnel wash water was collected. Non-coloured space beneath the line for LMM-fraction in some metals and metalloids is assigned as the neutral fraction. All anion-exchanged samples had concentrations below LOD of Cu and Ni. Because of this, the anionic fraction of Cu and Ni becomes equal to the LMM fraction when charge fractions are calculated.

3.3 Impact of biofiltration on tunnel wash water

3.3.1 Change of tunnel wash-water characteristics in the biofilter

Chemical parameters of temperature, dissolved oxygen, ORP, EC, pH, DOC and major cat- and anions were recorded in the tunnel wash water after biofiltration. The results, compared to the same parameters recorded in tunnel wash water before biofiltration, are displayed in Figure 9 and Figure 10.

Biofiltration of tunnel-wash water decreased ORP from about +150 to -400 mV, showing that the biofilter developed strongly reducing conditions. Dissolved oxygen remained close to zero. Low dissolved oxygen and ORP indicate anaerobic conditions in the biofilter.

The LMM- and colloidal fraction of DOC was higher in tunnel wash water after biofiltration than before, indicating that the biofilter was a source of DOC. Increasing concentrations of DOC in the form of Tannin-Lignin have earlier been recorded by Lepine et al. (2021) from chip wood biofilters under strongly reducing conditions. Lepine et al. (2021) also recorded higher concentrations of Tannin-Lignin from tests with strongly reducing conditions than tests under aerobic conditions, suggesting that reducing conditions increase DOC concentrations.

The concentrations of some major ions in the tunnel wash water changed from before to after biofiltration. Generally, the concentrations of Na, K and SO_4^{2} decreased from before to after biofiltration, while Ca, Mg and Cl⁻ increased from before to after biofiltration (Figure 10). Taking

the size fractions into account, results show that all size fractions of K, Na and S decreased in the biofilter and contributed to the decrease in total concentrations of these elements. Other elements showed decreases in only one or some size fractions, such as the LMM- and colloidal fraction of Cl⁻, colloidal fraction of F⁻, colloidal- and particulate fraction of Mg, and LMM fraction of SO₄²⁻. Size fractions that increased in the biofilter were all size fractions of Ca, the LMM fraction of Mg and F⁻, the LMM and colloidal fraction of P and the colloidal fraction of Cl⁻ and SO₄²⁻.

The EC of tunnel wash water in the biofilter increased from about 1200 μ S/cm to 1450 μ S/cm, demonstrating that the contribution of ions was higher than the sink in the biofilter. A minor decrease was observed in pH from 7.6 to 7.1. Increased concentrations of LMM-fraction Ca species after biofiltration indicate pH buffering, for example, by calcium carbonates (CaCO₃) from the shell sand. As a comparison, tests with a woodchip biofilter without shell sand recorded a decrease in pH from 7.8 before biofiltration to 6.4 after biofiltration in urban stormwater (Ashoori et al., 2019). Decreasing pH can occur in anaerobic reactors due to the production of organic fatty acids from microbial activity (Ali et al., 2019; Liu et al., 2008). Such processes might explain the increase in DOC in the present study, together with solubilisation of wood chip material.

A smell of rotten eggs was noted from the outlet end of the biofilter, which is characteristic of the presence of gaseous hydrogen sulphide (H_2S). Decreasing LMM-fraction S and $SO_4^{2^-}$ concentrations and low recorded ORP in tunnel wash water after biofiltration further indicate sulphur reduction in the biofilter.

Overall, the main changes in most elements are in either the LMM- or the particulate fraction, while the biofilter seem to have a generally low impact on the colloidal fraction.



Figure 9: Displays chemical parameters recorded in the tunnel wash water before (inlet) and after (outlet) biofiltration. The horizontal lines mark the median of each group's observations. The number of observations was n=3 for all groups, except for the ORP inlet, which is displayed as the average of 6 days of measurements in another tank.



Figure 10: Displays the change from before to after biofiltration in LMM-, colloidal- and particulate fractions. The horizontal lines mark the median of each group's observations. The number of observations n=3 for all groups. A positive change indicates a lower concentration after biofiltration than in the raw tunnel-wash water. A negative change indicates a higher concentration after biofiltration than in the raw tunnel-wash water. * Variables do not contain data for particulate fractions, since IC and TOC analysis was not performed on total samples.

3.3.2 Impact of biofiltration on metal and metalloid concentrations in tunnel wash water

Total concentrations of metals and metalloids in tunnel wash water before and after biofiltration are displayed in Table 9. The change in size fractions of Al, Fe and Mn from biofiltration are shown in Figure 11.

The total concentrations of Al, Fe and Mn in tunnel wash water were lower after biofiltration compared to before (Table 9). Considering the size fractions, results show that the biofilter was a sink of all size fractions of Al and the particulate and colloidal fractions of Fe. The biofilter was a source of LMM fractions of Fe and Mn. However, the increase in LMM fraction Fe was very small, ranging from 16 to 38 μ g/L. Increasing levels of Fe and Mn in the LMM fraction from biofiltration might come from microbial use of Fe and Mn as electron acceptors, reducing them to more water-soluble species during oxidation of other compounds (McMahon & Chapelle, 2008). The presence of H₂S detected in the outlet of the biofilter suggests that SO₄²⁻ at some point, was the energetically most favourable available electron acceptor. Fe- and Mn, being

more favourable than $SO_4^{2^2}$ as electron acceptors, was probably used earlier in the biofilter (McMahon & Chapelle, 2008).



Figure 11: Displays the change from before to after biofiltration in size fractions of tunnel wash water. The horizontal lines mark the median of each group's observations. A positive change indicates lower concentrations after biofiltration than in the raw tunnel wash water. A negative change indicates a higher concentration after biofiltration than in the raw tunnel. The number of observations n=3 for all groups.

The total concentrations of Ni and Sb in tunnel wash water after biofiltration were significantly lower (p < 0.05) than the concentrations before biofiltration (Table 9). If using a 90 % confidence interval (p < 0.1), also concentrations of Cr and Zn were statistically significantly decreased after biofiltration compared to before (P = 0.06 and P = 0.08, respectively). However, more data points would be needed for using a 90 % confidence interval. The calculated decrease in Zn concentrations in tunnel wash water after biofiltration was 95%, suggesting that the low number of observations in each group might increase the risk of type two errors (risk of not rejecting an incorrect null hypothesis) in the KW analysis, hence not coming out as statistically significant. Although not statistically significant, decreases in concentrations from before to after biofiltration calculated was 76 % for As and 91 % for Cu (Table 9). More measurements are needed to identify if As, Cr, Cu or Zn are significantly decreased from biofiltration.

Table 9: Displays mean, median and SD of metals and metalloid concentrations in tunnel wash water before and after biofiltration, the removal efficiency in element concentrations from before to after biofiltration, and the result of the KW-analysis with the following posthoc test (Padjusted). Statistically significant results (Padj < 0.05) are marked in green.

		Raw tunnel wash water			Tunnel wash water after biofiltration			Removal efficiency*	
	Unit	Median	Mean	SD	Median	Mean	SD	%	Padj
Al	µg/L	1437	1367	243	29.9	34.3	11.4		
Fe	µg/L	2640	2747	362	159	164	44.7		
Mn	µg/L	184	191	16.1	137	143	12.7		
As	µg/L	2.55	2.01	0.994	0.721	0.707	0.091	75.6	0.12
Cr	µg/L	6.64	6.26	0.676	0.72	1.05	0.60	81.7	0.063
Cu	µg/L	51.8	51.1	3.50	3.80	3.82	0.35	91.2	0.17
Ni	µg/L	11	11.4	1.35	4.40	4.52	0.541	60.1	0.017
Sb	µg/L	8.55	6.29	4.73	0.807	0.807	0.021	90.5	0.015
Zn	µg/L	254	246	17.4	12.4	11.9	1.11	94.6	0.083

* removal efficiency is calculated as the average decrease of three paired samples

Impact of biofiltration on size fractions of metals and metalloids in tunnel wash water

After biofiltration of tunnel wash water, the concentration of the LMM and colloidal fraction of Zn was significantly lower (P < 0.05) than in the raw tunnel wash water (P = 0.03 and P = 0.04 respectively, Figure 12). The decrease in LMM-fraction of Sb from before to after biofiltration was significant on a 90 % confidence level (P = 0.06). However, more data points would be needed for using a 90 % confidence interval. All results from the KW analysis are reported in Appendix D. The results are in agreement with removal efficiencies recorded by Ashoori et al. (2019), who reported removal efficiencies above 80 % for Ni and Cu from urban stormwater using a woodchip bioreactor under reducing conditions. However Ashoori et al. (2019) recorded a removal efficiency of 20 % for Zn, which is lower than the present study.

Although not statistically significant, there are indications of decreasing concentrations of metals and metalloids in tunnel wash water after biofiltration in all size fractions of As, the particulate fraction of Cr, Cu, Ni, Sb and Zn, and the LMM fraction of Ni, as seen by the lower concentrations in the outlet than the inlet in Figure 12.



Size fraction 📥 LMM 💽 Colloidal 💻 Particulate

Figure 12: Displays concentrations for size fractions of metals and metalloids in tunnel wash water before biofiltration (inlet) and after biofiltration (outlet). The horizontal lines mark the median of each group's observations. Statistically significant results from Dunn's posthoc test following KW-analysis (P adjusted <0.05) between the raw tunnel wash water and tunnel wash water after biofiltration for each size fraction are displayed as p-values. P-value close to significant is displayed for Sb. Groups without displayed p-values did not have statistically significant concentration changes. LMM-fraction of Ni and Zn was below LOQ after biofiltration.

Impact of biofiltration on charge fractions of metals and metalloids in tunnel wash water

To study if biofiltration caused the charge fractions to change, ion exchange chromatography was used on tunnel-wash water before and after biofiltration. The results are shown in Figure 13, and in Table 10.

A statistically significant (p < 0.05) decrease in anionic and cationic fractions of Zn in tunnel wash water after biofiltration compared to before biofiltration was found (Figure 13). However, as discussed in chapter 3.2.4, extra uncertainty is connected to anionic and cationic fractions of Zn in the raw tunnel wash water. Although not statistically significant, indications of decreases can be seen in both anionic and cationic fractions of Cr and Ni, as well as the anionic fraction of Sb. Indications of decreases in LMM fractions of Ni and Sb were seen in chapter 0, indicating that the decreases in charge fractions are not due to redistribution between the fractions for these metals and metalloids, but a result of a possible decrease in the LMM fraction.

After biofiltration, the concentrations of Cr, Cu and Ni in anion-exchanged samples were lower than their respective LOQ and/or LOD (LOQ = 0.046, LOD = 0.6 and LOD = $3 \mu g/L$ for Cr, Cu and Ni, respectively). Concentrations of Zn were lower than LOQ in cation-exchanged samples (LOQ = $1 \mu g/L$). However, similar as shown in the chapter 3.2.4, the lowest possible anionic fractions of Cr and Cu calculated from LOD and LOQ were 95 and 80%, respectively. Hence, high anionic fractions for Cr, Cu and Sb recorded in the raw tunnel wash water do not seem to be impacted by biofiltration (Table 10). Ni and Zn had LMM-fraction samples below LOQ, making the uncertainty higher for the charge fractions of these metals. Arsenic (As) stands out from the other metals by indications of an increase in the anionic fraction, which might be assigned to a redistribution from the neutral fraction to the anionic fraction during biofiltration (Table 10). As shown in Appendix D, the decrease in As LMM fraction was not found to be statistically significant.

The results show that the anionic fractions, which were found to be high in raw tunnel wash water, continue to be high after biofiltration. This could also have an effect on the removal efficiency of the biofilter since anionic species won't participate in processes like cation exchange and cationic binding to negative surfaces (VanLoon & Duffy, 2017).

In conclusion, the decrease in total concentrations in tunnel wash water from before to after biofiltration was statistically significant for Ni and Sb (P < 0.05). If a 90 % confidence interval (P < 0.1) could be used, biofiltration significantly decreased Cr and Zn. Total concentrations of Ni were statistically significantly decreased from biofiltration, but significant effects were not found in any of the size or charge fractions. This indicates that the decrease in Ni concentrations was not assigned to a certain fraction. Decreases in LMM and colloidal fractions from biofiltration were significant for Zn, showing that, statistically, the LMM fraction of Zn contributed most to the decrease through biofiltration. The decrease in anions and cations was significant for Zn, although high uncertainty is connected to this result.

The present study used size and charge fractionation techniques on strongly reduced water after biofiltration. Extra care was taken during fractionation to remove any air from the filtration system before samples were collected. Because of equipment failure of the ORP electrode, the ORP in sample water could not be monitored during fractionation. Oxidation might cause, for example, Cu and Fe to change their oxidation state, possibly changing their interactions with other species in the water and hence the fractions. However, this uncertainty is probably limited since high anionic fractions were recorded both before biofiltration, in water with low dissolved oxygen levels, and after reoxygenation, as shown in the next chapter.



Size fraction 🗠 Anions_LMM 🔄 Cations_LMM

Figure 13: Displays concentrations for anionic and cationic fractions of metals and metalloids in tunnel wash water before (Inlet) and after (Outlet) biofiltration. The horizontal lines mark the median of each group's observations. Statistically significant results from Dunn's post hoc test following KW analysis (P adjusted <0.05) between raw tunnel wash water and biofiltered tunnel wash water are displayed as p-values. Groups without displayed p-values were not statistically significant.

Table 10: Displays the size and charge fractions as percentage distributions in raw tunnel wash water compared to tunnel wash water after biofiltration.

	Tunne	Tunnel wash water before biofiltration				Tunnel wash water after biofiltration			
	Anions	Cations	Neutral	Total ions	Anions	Cations	Neutral	Total ions	
Ca	1%	100%	0%	101%	1%	100%	0%	100%	
К	0%	46%	54%	100%	0%	66%	34%	100%	
Mg	0%	100%	0%	100%	1%	100%	0%	101%	
Na	0%	-	-	-	-	-	-	-	
Ρ	23%	5%	72%	100%	34%	7%	59%	100%	
S	100%	2%	0%	102%	99%	0%	1%	100%	
Al	91%	4%	5%	100%	82%	16%	2%	100%	
Fe	90%	15%	0%	105%	97%	26%	0%	123%	
Mn	2%	100%	0%	102%	2%	99%	0%	101%	
As	14%	1%	85%	100%	60%	5%	35%	100%	
Cr	90%	8%	2%	100%	91%*	12%	0%	103%	
Cu	100%*	25%	0%	125%	100%*	6%	0%	106%	
Ni	100%*	25%	0%	125%	100%*	10%	0%	110%	
Sb	44%	5%	51%	100%	33%	0%	67%	100%	
Zn	92%	78%	0%	170%	100%	24%	0%	124%	

* Ni and Cu had 100% of anion-exchanged samples <LOQ. Cr had >50% of anion-exchanged samples <LOQ.

3.3.3 Impact of reoxygenation on metals and metalloids in biofiltered tunnel wash water

Since low-ORP tunnel wash water treated through the biofilter would be discharged into a natural recipient, the changes in species distribution in tunnel wash water after biofiltration, when the water underwent oxygenation was examined. No statistically significant differences were observed in total, size, or charge fractions between tunnel wash water after biofiltration and reoxygenated tunnel wash water (Appendix E).

The reoxygenated tunnel wash water samples were expected to contain the same total concentrations of metals and metalloids as the samples collected after biofiltration. However, as seen in Figure 14, the variation in total concentrations in some samples were higher in oxygenated samples than in samples collected directly after biofiltration, indicating contamination.

Regarding size fractions, there are indications of an increase after oxygenation in Zn for colloidal fractions, as seen in Figure 15, as well as an increase in anionic and cationic fraction, as seen in Figure 16. LMM fraction samples of Zn was below LOD and LOQ, affecting the anionic, cationic and colloidal fractions. However, the high anionic fraction recorded in several metals and metalloids in the raw tunnel wash water is not impacted by either sedimentation, biofiltration or following reoxygenation, as indicated by Figure 16. Hence, these anions appear to have low sorption to wood and are minimally affected by variations in redox conditions.



Figure 14: Displays total concentrations of metals after biofiltration (Outlet) and oxygenated samples. The horizontal lines mark the median of each group's observations.



Fraction 📥 LMM 📥 Colloidal 💻 Particulate

Figure 15: Displays concentrations for size fractions of metals after biofiltration (Outlet) and reoxygenated (Ox) samples. The horizontal lines mark the median of each group's observations. LMM-fraction for Ni and Zn of reoxygenated samples is uncertain since the concentrations of all three samples were below LOQ.



Charge fraction 📥 Anions_LMM 🔁 Cations_LMM

Figure 16: Displays concentrations for charge fractions of metals after biofiltration (Outlet) and oxygenated samples. The horizontal lines mark the median of each group's observations. Negative values indicate that concentrations after charge fractionation were higher than recorded LMM concentrations.

3.3.4 Change of metals and metalloid concentrations across all hydraulic retention times

To identify the impact of hydraulic retention time on the biofilter, it was tested with a doubled and quadrupled pumping rate in addition to the standard pumping rate. Only results from biofiltration using a hydraulic retention time of 45 hours have been examined in earlier chapters. Results from the other hydraulic retention times are displayed and discussed in the next chapter. To examine how the results changed when more data was added, KW analyses were performed on all data across the different hydraulic retention times (Table 11).

The 45-hour hydraulic retention time results, presented in the chapter 3.3.2 indicated decreases in metal and metalloid concentrations but were only statistically significant (p < 0.05) for Ni and Sb. KW analyses work best on data with five or more observations in each group. A very small sample size, like in the results presented thus far of three observations in each group, increases the risk of a type two error and might lead to incorrect p-values. Therefore, KW analyses are usually not recommended on sample sizes below 5 (Bjørndal & Hofoss, 2004; Minitab, 2024).

When adding the hydraulic retention times together in the KW analysis, biofiltration resulted in statistically significant (p < 0.05) decreases in the total concentrations of As, Cr, Cu, Ni, Sb and Zn in the tunnel wash water. Statistically significant decreases in particulate fraction from biofiltration were also found for As, Cr, Cu, Sb and Zn, and in LMM fraction for As, Cu, Ni, Sb and Zn. No statistically significant changes were recorded in the colloidal fraction of any metals or metalloids. However, statistically significant changes in the total, particulate and LMM fractions

show that some of the insignificant results of the KW analyses presented for biofiltration using a 45-hour hydraulic retention time might result from type two errors due to small sample sizes.

Table 11: Displays statistically significant (Padjusted < 0.05) results from KW-analyses with the following Dunns posthoc test for different size fractions, where metal and metalloid concentrations in raw tunnel wash water were compared with metal and metalloid concentrations in tunnel wash water after biofiltration. The z-value indicates how the average rank for each group compares to the average rank of all observations; the higher the absolute value, the further a group's average rank is from the overall average rank. P adjusted (P.adj) is used for the discussion of significance levels in the present study.

Fraction	Metal	Comparison	Z	P.unadj	P.adj
total fraction	As	Inlet - Outlet	3.7	0.00026	0.00078
total fraction	Cr	Inlet - Outlet	2.9	0.0037	0.011
total fraction	Cu	Inlet - Outlet	3.9	0.000087	0.00026
total fraction	Ni	Inlet - Outlet	2.9	0.0029	0.0088
total fraction	Sb	Inlet - Outlet	3.3	0.00082	0.0025
total fraction	Zn	Inlet - Outlet	3.9	0.000073	0.00022
particulate fraction	As	Inlet - Outlet	3.3	0.00096	0.0029
particulate fraction	Cr	Inlet - Outlet	2.4	0.014	0.043
particulate fraction	Cu	Inlet - Outlet	3.1	0.0019	0.0057
particulate fraction	Sb	Inlet - Outlet	2.7	0.0069	0.014
particulate fraction	Zn	Inlet - Outlet	3.3	0.0010	0.0030
lmm fraction	As	Inlet - Outlet	3.0	0.0024	0.0071
lmm fraction	Cu	Inlet - Outlet	3.9	0.000080	0.00024
lmm fraction	Ni	Inlet - Outlet	2.6	0.0097	0.019
lmm fraction	Sb	Inlet - Outlet	3.5	0.00054	0.0016
lmm fraction	Zn	Inlet - Outlet	3.5	0.00039	0.0012

3.3.5 Impact of hydraulic retention time on metal and metalloid concentrations

Chapter 3.2.3 discussed how sedimentation in raw tunnel wash water caused the particulate content to decrease with time. Therefore, there were indications, although not statistical differences (Appendix H), between the inlet concentrations of especially particles between the tests of different hydraulic retention times. To adjust for the indicated concentration differences, Figure 17 displays the calculated change in metal and metalloid concentrations in tunnel wash water from before to after biofiltration.

Statistically significant (p < 0.05) decreases from before to after biofiltration was found in the 45-hour hydraulic retention time for Sb and Ni (chapter 3.3.2). For the 23-hour and 11-hour hydraulic retention times, no statistically significant results were found in total concentrations of any metals or metalloid concentrations from before to after biofiltration (Appendix G). This shows that, statistically, the biofilter only removes metals and metalloids from tunnel-wash water with a hydraulic retention time of 45 hours.

Regarding size fractions, statistically significant decreases were found for colloidal and LMM fractions for Zn with a hydraulic retention time of 45 hours in the biofilter. No statistically significant metals or metalloid results were found in the 23-hour or 11-hour hydraulic retention time tests.

Moreover, significant decreases in metal and metalloid concentrations were detected when data from all hydraulic retention times were analysed (chapter 3.3.4), showing that a low number of observations might have resulted in statistically insignificant results for metals and metalloids in the 45-hour, 23-hour and 11-hour hydraulic retention times. Hence, further tests using more observations on both the 45-hour retention time and shorter retention times could be conducted to elucidate the actual impact on the removal efficiency of metals and metalloids in the biofilter. However, the statistically significant results presented only in the 45-hour hydraulic retention time reveal differences between the removal efficiencies.

This higher efficiency might be partly attributed to an extended hydraulic retention time, affording more opportunity for metal removal processes such as microbial sulphur reduction, facilitating the precipitation of metal and metalloids within the biofilter. Similar effects regarding the influence of hydraulic retention time on NO_3^- removal have been documented in wood chip bioreactors designed for denitrification (Rivas et al., 2020).

Moreover, increased concentration of LMM-fraction DOC was recorded after biofiltration. The increases were particularly evident with a 45-hour hydraulic retention time and present in the 23-hour hydraulic retention time in the biofilter. Conversely, the 11-hour hydraulic retention time showed decreased LMM-fraction DOC in tunnel wash water after biofiltration compared to the raw tunnel wash water. Higher DOC concentrations after biofiltration on 45-hour hydraulic retention time might suggest that a longer hydraulic retention time fosters enhanced opportunities for the oxidation and dissolution of organic material in the biofilter (Rivas et al., 2020).

However, the methodological approach used in this study may have influenced the outcomes. The different hydraulic retention times were tested in the same biofilter: 45 hours, then 23 hours, and finally 11 hours. A relatively new filter material during the 45-hour residence time containing high amounts of readily available organic carbon might have stimulated higher microbial activity and, hence, a higher removal efficiency. Consequently, the easily available organic carbon might be diminished for the 23-hour and 11-hour hydraulic retention times. Other studies have reported the correlation between the elevated discharge of DOC and increased NO₃⁻ removal efficiencies in woodchip bioreactors, often connected to the startup period of the biofilter (Rivas et al., 2020; Robertson et al., 2005; Robertson, 2010; Schipper et al., 2010).

Tests using parallel biofilters should be conducted to elucidate if the lower removal efficiency in the shorter hydraulic retention times might be assigned to the ageing or washout of the biofilter rather than the hydraulic retention time itself. Additionally, only shorter retention times were tested in this study to explore the opportunities for treating higher volumes of water in a shorter time. Even higher removal efficiencies than recorded in the present study might be achieved if the hydraulic retention time is extended above 45 hours.



Fraction 🔄 LMM 💽 Colloidal 💽 Particulate

Figure 17: Displays the change in size fractions from before to after biofiltration for the three hydraulic retention times. The horizontal lines mark the median of each group's observations. A positive change indicates lower concentrations after biofiltration than in the raw tunnel wash water. A negative change indicates higher concentrations after biofiltration than in the raw tunnel wash water.

3.3.6 Concentrations of metals and metalloids in tunnel wash water after biofiltration compared to environmental requirements

All metals and metalloids recorded in this study exceeded the limit values of AA-EQS in the raw tunnel wash water after five to eight days of sedimentation (Table 7). If diluting EQS values with a factor of 10, the limit values were exceeded only by Zn in tunnel wash water after five to eight days of sedimentation. However, Zn alone vastly exceeded the 10 × AA-EQS with total concentrations of two times the corresponding limit value in the raw tunnel wash water.

Biofiltration of tunnel wash water led to statistically significant (P < 0.05) total concentrations of all metals and metalloids across the hydraulic retention times, as shown in chapter 3.3.5. After biofiltration, concentrations of Cr, Cu and Ni were below their corresponding limit values of AA-EQS from the Norwegian Environment Agency (Table 12). Concentrations of all metals and metalloids were below the corresponding 10 × AA-EQS, as well as the limit values from the County Governor. The total concentration of Zn was, after biofiltration, decreased to about 1/10th of the corresponding diluted 10 × AA-EQS. However, concentrations of Zn was right at the limit, and As was still above limit value AA-EQS after biofiltration.

Table 12: Displays median and SD of total concentrations recorded in tunnel wash water (TWW) after biofiltration on 45h hydraulic retention time, compared to limit values for contaminated water by the Norwegian Environment Agency and the County Governor. Mean and median values marked with red exceeds at least one of the limit values. Concentrations in parenthesis are for the fraction <0.45 µm.

							County		
	45-hour hydraulic retention time after			Norwegian	gency: limit	governor,			
	45-nour nyc	hiofiltration	in time after	valı	les in the recir	ngency, anne nient	TWW, after		
		Diolitti attori			values in the recipient				
							ion		
	Median	Mean	SD	AA-EQS	MAC-EQS	10×AA-EQS	Oslo area		
As (µg/L)	0.72	0.707	0.09	0.5	8.5	50	6		
Cr (µg/L)	0.72	1.05	0.60	3.4	3.4	34	10		
Cu (µg/L)	3.80	3.82	0.35	7.8	7.8	78	50		
Ni (µg/L)	4.40 (3.67)	4.52 (3.69)	0.54 (0.076)	(4)	(34)	40	40		
Sb (µg/L)	0.81	0.807	0.02						
Zn (µg/L)	12.4	11.9	1.10	11	11	110	110		

3.3.7 Processes for removal of metals and metalloids in the biofilter

Principal Component Analysis

To explore trends in the dataset and the processes within the biofilter, Principal Component Analyses (PCAs) were used on calculated changes in tunnel wash water from before to after biofiltration. This chapter uses correlations in the PCAs to identify which processes might be important for metal and metalloid removal within the biofilter. PCAs maximise the differences between variables in a dataset and create Principal Components, also called Dimensions (Dim), that explain different amounts of variation in the data. The number of dimensions chosen to include in further analysis is usually based on the amount of variation explained by the dimensions. A commonly used cut-off point is to include as many dimensions as needed to reach 70 % of the total variation in the data explained (Jolliffe & Cadima, 2016). Therefore, the dimensions forwarded in the interpretation might show only the most evident correlations and exclude weaker ones.

Figure 18 shows the PCA for change in size-fractionated samples in the particulate, colloidal and LMM fractions. Dim 1 and Dim 2 together explain 72 % of the variation in the dataset. The arrows of Al, Fe, As, Cr and Cu point straight towards the observations of particulate fraction for the 45-hour hydraulic retention time test. This means that change in the particulate fraction was important for Al, Fe, Cr and Cu in the 45-hour retention time test. Zn is also correlated to these observations, but not very strongly. Al, Fe, Cr and Cu showed removal efficiencies > 50 % during sedimentation (Table 7), although not statistically significant. Arsenic (As) showed 43 % removal efficiency and a clear decreasing trend line in Figure 7. Hence, the correlation between Al, Fe, As, Cr and Cu and the change in the particulate fraction of the 45-hour hydraulic retention time test suggests that particle exclusion by filtration and/or sedimentation seems to have been an important process for removal in the 45-hour hydraulic retention time test. As shown in chapter

3.2.3, the particulate content in raw tunnel wash water was also higher during the 45-hour hydraulic retention time test than during testing of the other hydraulic retention times.

Sb, S, Ni and Zn were correlated to change in both the particulate and the LMM metal-fraction, shown by their arrows pointing between these observations in Figure 18. However, Sb and Ni do not reach the circumference in the loading plot (Figure 19), meaning that these variables do not strongly influence the PCA.



Figure 18: Biplot for PCA on change for size-fractionated samples in the particulate, colloidal and LMM fraction. Red arrows mark the present study's metals and metalloids of particulate interest.



Figure 19: Loading plot for PCA on change for size-fractionated samples in the particulate, colloidal and LMM fractions. Red arrows mark the present study's metals and metalloids of particulate interest.

Figure 20 displays a biplot for the change in the colloidal and LMM fractions from before to after biofiltration. Dim 1 and Dim 2 together explain 62.6 % of the variation in the dataset. Therefore,

Dim 3 was also considered but showed no valuable correlations. The biplot for Dim 3 is, therefore, displayed in Appendix L.

When taking only the dissolved fraction into account (excluding the particulate fraction from the PCA), all metals and metalloids are correlated with Dim 1. Dim 1 explains the differences between the size fractions (Figure 20), seen by the clustering of LMM and colloidal fraction observations along the Dim 1-axis. Dim 1 explains 46.4 % of the variation in the dataset. Cu does not strongly influence the PCA when the particulate fraction is excluded, suggesting that changes in the particulate fraction during biofiltration were most important for removing Cu across the different hydraulic retention times.

Al, Fe, Mn, As, Cr and F^- are all mainly impacted by changes in the colloidal fraction, and Sb, Zn, Ni, S and SO4 are mainly impacted by changes in the LMM-fraction (Figure 20). Ni and Zn, F^- and Al got short lines (Figure 21), indicating that these variables do not strongly influence the principal component.



Figure 20: Biplot for PCA on change for size-fractionated samples in the colloidal and LMM fraction. Red arrows mark the present study's metals and metalloids of particulate interest.



Figure 21: Loading plot for PCA on change for size-fractionated samples in the colloidal and LMM fractions. Red arrows mark the present study's metals and metalloids of particulate interest.

Figure 22 displays a biplot for the change in the LMM fraction from raw tunnel wash water to tunnel wash water after biofiltration. Dim 1 and Dim 2 together explain 66.8 % of the total variation. Therefore, Dim 3 was also included in the interpretation but did not show any clear correlations and is shown in Appendix L.

If only looking at the LMM-fraction, Al, Fe, As, Cr, Cu, Sb, and Zn got shorter lines, indicating that these variables do not strongly influence the principal component. For Al, Fe and Cr, this supports the PCA for Particulate, colloidal and LMM fraction, and the PCA for colloidal and LMM fraction; that these elements are mostly correlated to changes in the particulate fraction. Additionally, these metals (Al, Fe and Cr) are more correlated to changes in the colloidal fraction than the LMM fraction when particles are excluded from the analyses.

The changes in other elements like P, Mg, and Ca have a strong influence on the PCA for the LMM fraction (Figure 23). Changes in SO_4^{2-} , S, Ni and As are positively correlated. This might suggest that As and Ni in the LMM fraction might be removed by the precipitation of sulphides. Zn and Sb are also positively correlated to S and SO_4^{2-} but are not very strongly correlated. As and Ni are also negatively correlated to Mg and Ca, which might indicate ion exchange processes. However, both As and Ni were mainly in the anionic fraction before and after biofiltration, suggesting that the extent of retention through cation exchange processes may be limited.



Figure 22: Biplot for PCA on change for size-fractionated samples in the LMM fraction. Red arrows mark the present study's metals and metalloids of particulate interest.



Figure 23: Loading plot for PCA on change for size-fractionated samples in the LMM-fraction. Red arrows mark the present study's metals and metalloids of particulate interest.

Cr, Cu and Al seem to be mostly removed through filtration of particles and colloids.

Ni and Sb were correlated to changes in the LMM fraction in all the PCAs. Sb was only weakly correlated to $SO_4^{2^\circ}$, which might indicate that other processes than sulphide precipitation are important for Sb removal, such as adsorption to surfaces in the filter material. However, Sb show correlations with changes in pH. Sb was mostly recorded in neutral fraction both before and after biofiltration

Zn was is not strongly correlated with either the particulate, colloidal or LMM fraction in any of the PCAs. This might suggest that Zn removal is controlled by variables that are not included in the PCA. Or, that the removal of Zn is attributed to a mix of several processes, resulting in Zn not showing strong correlations in any of the PCAs. Such processes can be filtration, adsorption processes in the biofilter, ion exchange and sulphide precipitation,

The liability of the PCAs should also be assessed. The dataset of the present study contains censored data, which can affect the results of PCA analyses, dependent on which censoring has been performed (Farnham et al., 2002; Hopke et al., 2001). Farnham et al. (2002) found that replacing data below the detection limit with half of the detection limit led to underestimations of the variance for those variables. In the present study, 33 % of Zn and 66 % of Ni were below LOQ or LOD in the LMM fraction and hence replaced using (LOD+LOQ)/2 or LOD/2, respectively (Appendix I). This might explain the low impact of Zn and Ni on several of the PCAs. Hopke et al. (2001) recommended that variables with over 30 % censored data should be excluded from the PCA. However, the results of a PCA can change radically depending on which variables are included or excluded (Reimann et al., 2002). Hence, it was decided to include these metals in the analyses.

Sulphide precipitation

Due to the observation of H_2S in the outlet of the biofilter, the possibilities of sulphide precipitation was investigated further. On a molar basis, the amount of sulphide possibly formed from SO_4^{2-} in the LMM fraction exceeds the total molar change in the metals and

metalloids (Σ As+Cu+Cr+Ni+Sb+Zn). Hence, the decrease in the LMM fraction of all these metals and metalloids combined could theoretically be assigned only to metal-sulphide precipitates. Metal sulphide precipitation of Cu (Covellite), Ni (Nickel sulphide NiS) and Zn (Sphalerite) was predicted using Visual MINTEQ. Solid metal sulphide species were unavailable in Visual MINTEQ for As, Cr and Sb. Concentrations of metals in samples filtered for <0.45 µm were used. The model showed a saturation index at equilibrium for Cu, Ni and Zn, with > 99 % precipitation (Appendix J).

However, > 99 % precipitation of the colloidal and LMM fraction is not in accordance with the percentage decrease recorded in the present study. Hence, the model overestimates sulphide precipitation. Such an overestimation might come from the model ignoring some of the variables competing with sulphide in binding with the metals in the biofilter. Such variables can be sorption sites on the wood chips, complexation with DOC, ion exchange and other precipitation reactions (Ashoori et al., 2019; Kovacova et al., 2020). However, the model supports the idea that sulphide precipitation removes metals from tunnel-wash water in the biofilter. Sulphide precipitation of metals in woodchip and sawdust under reducing conditions has been reported in literature (Ashoori et al., 2019; Lepine et al., 2021).

It would be interesting to test out the performance of the biofilter material during oxidising and reducing conditions simultaneously, to further investigate how much of metal and metalloid removal by biofiltration that could be assigned to reducing conditions.

3.4 Tracer test

A NaCl tracer test using the pumping rate corresponding to the 23-hour calculated hydraulic retention time was conducted to explore the biofilter properties regarding hydraulic retention time and residence time distribution.

Tracer test results are shown in Figure 24. The residence time distribution ranged from 15 hours to over 45 hours, which includes the calculated hydraulic retention time of 23 hours. The highest increase in EC occurred after about 19 hours, and the mean residence time of NaCl in the biofilter was 27 hours (the EC reached 50 % of the total EC increase).

NaCl was used as a tracer due to its low cost and ease of obtaining the components needed. However, NaCl has the disadvantage that high concentrations makes the tracer water heavier than the raw water in the biofilter. This might lead to the tracer travelling along the bottom of the biofilter, pushing the raw water upwards and resulting in a long, slacking EC curve in the outlet due to mixing of the tracer and raw water.

Another disadvantage of using NaCl as a tracer is the possibility of interactions between the ions of the tracer and the biofilter material. Cl⁻ is conservative and moves quite undisturbed throughout the system, but Na can participate in ion exchange with other ions (Singha et al., 2011). For example, Na can participate in ion exchange processes with Ca from the shell sand, thereby altering concentrations in solution (Singha et al., 2011). As a result, the measured EC may reflect not only solute transport but also reactive processes in the biofilter. Column experiments have shown that the mean arrival time of NaCl tracer in the outlet was lowered in the presence of ion exchange processes (Singha et al., 2011). Since the tracer used contains more ions that possibly participate in ion exchange processes than tunnel wash water, the

mean hydraulic retention time based on NaCl tracer tests might be underestimated compared to tunnel wash water.

Hence, the breakthrough of the tracer might be delayed compared to the breakthrough for tunnel-wash water, but the mean retention time of NaCl might be shorter than the hydraulic retention time of tunnel-wash water. It is, therefore, uncertain how the mean retention time of NaCl compares to the actual hydraulic retention time of tunnel wash water. Concluded, the hydraulic retention time probably lies somewhere in between 15 and 45 hours, but further tests are needed to find the true hydraulic retention time of the biofilter.

If a new tracer test would be conducted, it would be recommended to use lower concentrations of NaCl in the tracer than in the present study to reduce the weight difference between the tracer and raw water. It would also be recommended to determine the changes in concentrations of Cl in the outflow of the biofilter rather than the EC, since EC is affected by other ions in the solution. Other tracers could also be evaluated, such as for example Br⁻, which is a widely used conservative tracer (Levy & Chambers, 1987).



Figure 24: Figure 6: Linechart showing the EC over time in the outlet of the biofilter during the tracer test. The line is coloured after the increase in EC/minute, with red as the highest increase and blue as the lowest.

3.5 General discussion around the biofilter

3.5.1 Future for biofiltration of tunnel wash water

The results of the present study show that total concentrations of As, Cr, Cu, Ni, Sb and Zn in tunnel wash water were statistically significantly decreased (P < 0.05) from biofiltration when data from all hydraulic retention times was used in the analysis. However, as shown by Meland and Rødland (2018), tunnel wash water is heterogeneous in terms of pollution levels. The performance of the biofilter should, therefore, be investigated under different conditions with higher and lower pollution levels, as well as the presence of road salt and detergents in the tunnel wash water.

The biofilter used in the present study was small and could only filter comparably low volumes of water compared to discharges after a tunnel wash. In the present study, 88 m³ of wash water was used during tunnel washing when one tube was washed. If both tubes of the same tunnel are washed, one can assume that at least 170 m³ of water would be used during the wash.

When using biofiltration as a secondary treatment after sedimentation, the volume of the biofilter can be regulated by operating the filter over several days. Hence, for one or 10 days of operation, a biofilter of 541 m³ or 57 m³ filter material, respectively, would be needed to filtrate 170 m³ of tunnel wash water, if using a hydraulic retention time of 45 hours. A shorter hydraulic retention time would decrease the volume of biofilter material needed. If using the lowest hydraulic retention time of 11 hours, the size of the biofilter could be reduced to 120 or 12 m³ filter material for one and 10 days of operation, respectively. Calculations are shown in Appendix K. Hence, if using a higher hydraulic retention time and/or a short operation time, the biofilter would probably need to be stationary. Such a stationary solution could be built similarly to some denitrification biofilters used for cleaning NO₃⁻ from polluted water (Hoffmann et al., 2019; Roseth et al., 2023)

However, a possible solution could be a mobile treatment system. A mobile treatment system could also be used for smaller tunnels with less water used during tunnel wash, a possible solution could be a mobile treatment system. Such a solution could be moved around to the tunnels needed. Mobile treatment solutions for polluted water exist today using coagulation and other methods, as discussed by (Arctander Vik et al., 2016).

After the tunnel wash water has been treated using a biofilter, the water would probably follow the normal route of tunnel wash water: out into the local water treatment system or a natural recipient. The present study found anoxic conditions in the outlet water. Hence, the receiving recipient should be evaluated in terms of the risk of organisms in the recipient suffering from suffocation from discharge water. Hence, biofiltered tunnel wash water might need to be reoxygenated before being discharged to a natural recipient. However, an eventual need for reoxygenation will be heavily dependent on the actual recipient. For example, it could be expected that anoxic water would have limited to no effect in rivers with turbulent flow.

3.5.2 Opportunities for improvement of the biofilter

Alteration of pH

The results of the present study suggest that metal removal by sulphide precipitation might be a property of the biofilter, indicated by the observation of gaseous H₂S in the biofilter outlet. H₂S formation is very pH-sensitive; at a pH below seven, there is mostly gaseous H₂S formed, and at a pH above seven, there is mostly HS⁻ formed (Stumm & Morgan, 2012). This is because the solubility product (pK) for H₂S is 7.1 (Stumm & Morgan, 2012), the same pH as recorded in tunnel wash water after biofiltration. Hence, increasing the pH in the biofilter might promote higher production of HS⁻, potentially increasing the amount of sulphide precipitation further and limiting the emissions of H₂S, a toxic gas (Beauchamp et al., 1984). However, a pH between five and six has been shown to optimise adsorption capacity in sawdust (Rahman & Islam, 2009). Hence, it's uncertain how increasing the pH would affect the total removal capacity of the biofilter, and more tests could be done, altering the pH to investigate the impact on metal and metalloid removal efficiency.

Alteration of temperature

Another consideration is the influence of temperature on biofilter efficiency. The present study was conducted during fall (September to October), and the temperature in tunnel wash water after biofiltration was recorded as 12°C. In general, biological reaction rates increase with increasing temperature. For example, studies of NO₃⁻ removal through denitrification, where

NO₃⁻ was not limited, support a positive relationship between NO₃⁻ removal and annual temperature (Robertson et al., 2005; Schipper et al., 2010). Hence, isolating the biofilter or elevating the temperature might help maintain the efficiency during fall. A mobile solution would allow for warming in between biofiltration occasions. A stationary solution would require a below-ground solution like the ones used in denitrification facilities, with an isolating top layer on ground level.

However, testing of NO_3^- removal reactors has shown that NO_3^- removal continues down to + 1.5°C (Robertson & Merkley, 2009). Hence, the biofilter might also be able to operate during colder conditions but with reduced efficiency.

Different wood chips

Several studies have also tested different types of alterations of sawdust to increase desired properties. For example, alkalisation has proved to increase the adsorption capacity in sawdust (Asadi et al., 2008). The result of the present study suggests that the anionic fraction of metals and metalloids can be elevated in the tunnel-wash water. Hence, a treatment that increases the cationic binding sites of wooden sawdust could be tested.

3.5.3 Durability and lifetime of the biofilter

The lifetime of the biofilter will depend on factors like the size of the biofilter, the amount of water treated at each tunnel wash, the concentrations of contaminants in the tunnel wash water and the actual sorption behaviour of the biofilter material.

Tunnel wash water contains SO_4^{2-} and essential nutrients like Nitrogen and Phosphorus (Meland et al., 2010a), possibly maintaining the needs of the microbial community in the biofilter. Hence, sulphide precipitation would be maintained over time if favourable conditions are upheld for the SO_4^{2-} -reducing microbial community.

The total amount of metals and metalloids that the woodchips can potentially sorb might also be limiting due to the saturation of binding sites in the woodchips. Tests of different types of chip wood and sawdust for metal sorption have shown that Cu follows a sorption model where saturation was not reached, indicating that new binding sites on the sawdust were created during sorption. Other metals, like Zn, reached a sorption maximum, indicating that active sites on the sawdust surface were limited (Kovacova et al., 2020). Hence, the biofilter might be able to sorb higher amounts of some metals than others before saturation occurs. Maximum sorption capacities found in the studies were variable for different species of trees (grey alder not included) but ranging from 1.46-10.9mg/g of sawdust for Zn (Kovacova et al., 2020; Rafatullah et al., 2009; Shukla & Pai, 2005). A simple calculation using the volume of chip wood in the present study, a density of grey alder woodchips of 0.208 kg/L (grey alder density = 0.52g/cm3 and an estimated solid wood percentage of 40 % (Belbo & Gjølsjø, 2008; Foslie & Grindeland, 2023)), and the most modest sorption capacity reported for Zn of 1.46 mg/g shows that it would take 13 years to reach sorption of Zn in the biofilter, using a hydraulic retention time of 45 hours. However, a higher surface area in sawdust than in chipwood suggests that chipwood might reach sorption maximum faster, suggesting that the actual time before maximum sorption would be lower than 13 years. The whole calculation can be viewed in Appendix K. Other factors, like decay and compaction of the biofilter material, can also limit the biofilter lifetime. There are examples of sawdust denitrification biofilters that have been successfully operated for seven years (Robertson et al., 2000).

The present study was performed over a limited period of three weeks. Hence, the long-term properties of the biofilter remain unknown. Earlier studies of tunnel wash water during sedimentation have recorded remobilisation of Cu after two and three weeks of sedimentation (Garshol et al., 2015; Rathnaweera et al., 2023). Even though the study of Garshol et al. (2015) and Rathnaweera et al. (2023) was sedimentation studies, there are similarities in the processes encountered in sediments and woodchips regarding the presence of organic material, sulphides and reducing conditions. Hence, additional experiments should be conducted to investigate the long-term removal of metals and metalloids by the biofilter.

4 Conclusion

The hypotheses of the present study were:

1) The biofilter would decrease the LMM fraction concentrations of As, Cr, Cu, Ni, Sb and Zn in tunnel wash water

2) Changing the hydraulic retention time in the biofilter would affect the removal efficiency of metals and metalloids.

3) Reoxygenation of biofiltered tunnel wash water would push the size fraction of metals and metalloids towards a higher particulate fraction.

The results of the present study show that the total concentrations of all investigated metals and metalloids (As, Cr, Cu, Ni, Sb and Zn) were significantly (p < 0.05) decreased from biofiltration. The changes were mainly attributed to changes in the particulate and LMM fraction since significant decreases were found in the particulate fraction of As, Cr, Cu, Sb and Zn, and in the LMM fraction of As, Cu, Ni, Sb and Zn. No significant decreases were recorded from biofiltration in the colloidal fraction of any metals or metalloids. Sulphide precipitation occurred in the biofilter, detected from the smell of H_2S , and was also indicated by modelling for Ni, Cu and Zn. The results show that biofiltration decreased the LMM fraction of As, Cu, Ni, Sb and Zn, therefore supporting hypothesis one.

When studying the hydraulic retention times separately, statistically significant decreases in total concentrations of metals and metalloids were found only in the 45-hour hydraulic retention time, showing that longer hydraulic retention times increased the metal removal efficiency of the biofilter. Hence, the results support that hydraulic retention time does affect the cleaning efficiency of the biofilter (hypothesis 2), but more tests are needed to elucidate the actual impact.

Reoxygenation showed no statistically significant differences from the outlet samples regarding size or charge distribution. Therefore, reoxygenation does not seem to impact the species distribution after biofiltration. Hence, the results do not support hypothesis three.

The work also identified that a large part of the metals and metalloids such as Cr, Cu and Ni were present as mainly negatively charged species in tunnel wash water, possibly due to complexation with detergents. As anionic species have lower sorption to particles, this can be one of the reasons why some metals and metalloids are mobile, and concentrations are not efficiently decreased in the sedimentation ponds. More research should be done to investigate these findings.

The results of the present study show that biofiltration, in combination with reducing conditions, can decrease concentrations of metals and metalloids in tunnel-wash water after sedimentation.

5 Reference list

- Aasum, J.-H. (2014). Effekter av vaskemiddel (TK601) på mobilitet av metaller ved sedimentering av tunnelvaskevann fra Nordbytunnelen (E6), Ås kommune, Akershus: et laboratorieforsøk: Norwegian University of Life Sciences, Ås.
- Abdel-Tawwab, M., El-Sayed, G. O. & Shady, S. H. (2016). Growth, biochemical variables, and zinc bioaccumulation in Nile tilapia, Oreochromis niloticus (L.) as affected by water-born zinc toxicity and exposure period. *International Aquatic Research*, 8 (3): 197-206.
- Ali, S., Hua, B., Huang, J. J., Droste, R. L., Zhou, Q., Zhao, W. & Chen, L. (2019). Effect of different initial low pH conditions on biogas production, composition, and shift in the aceticlastic methanogenic population. *Bioresource technology*, 289: 121579.
- Allan, I. J., O'Connell, S. G., Meland, S., Bæk, K., Grung, M., Anderson, K. A. & Ranneklev, S. B. (2016). PAH accessibility in particulate matter from road-impacted environments. *Environmental science & technology*, 50 (15): 7964-7972.
- Andersen, S. & Vethe, Ø. (1994). Mobilisation of heavy metals during tunnel maintenance. *Science of the total environment*, 146: 479-483.
- Andradóttir, H. Ó. & Vollertsen, G. E. G. (2015). Temporal variability of heavy metals in suburban road runoff in a rainy cold climate. *Journal of Environmental Engineering*, 141 (3): 04014068.
- Arctander Vik, E., Sahu, A. & Garshol, F. (2016). Litteraturundersøkelse-mobile renseløsninger: For vaskevann fra veitunneler.
- Arenas, J. P. (2008). Potential problems with environmental sound barriers when used in mitigating surface transportation noise. *Science of the total environment*, 405 (1-3): 173-179.
- Asadi, F., Shariatmadari, H. & Mirghaffari, N. (2008). Modification of rice hull and sawdust sorptive characteristics for remove heavy metals from synthetic solutions and wastewater. *Journal of hazardous materials*, 154 (1-3): 451-458.
- Ashoori, N., Teixido, M., Spahr, S., LeFevre, G. H., Sedlak, D. L. & Luthy, R. G. (2019). Evaluation of pilot-scale biochar-amended woodchip bioreactors to remove nitrate, metals, and trace organic contaminants from urban stormwater runoff. *Water research*, 154: 1-11.
- Audet, J., Jéglot, A., Elsgaard, L., Maagaard, A. L., Sørensen, S. R., Zak, D. & Hoffmann, C. C. (2021).
 Nitrogen removal and nitrous oxide emissions from woodchip bioreactors treating agricultural drainage waters. *Ecological Engineering*, 169: 106328.
- Beauchamp, R., Bus, J. S., Popp, J. A., Boreiko, C. J., Andjelkovich, D. A. & Leber, P. (1984). A critical review of the literature on hydrogen sulfide toxicity. *CRC critical reviews in toxicology*, 13 (1): 25-97.
- Belbo, H. & Gjølsjø, S. (2008). *Trevirke-brennverdier og energitetthet*: Norsk institutt for skog og landskap.
- Bio-Rad. (2000). AG® 1, AG MP-1 and AG 2 Strong Anion Exchange Resin Instruction Manual.
- Birch, H., Mikkelsen, P. S., Jensen, J. & Lützhøft, H.-C. H. (2011). Micropollutants in stormwater runoff and combined sewer overflow in the Copenhagen area, Denmark. *Water Science and technology*, 64 (2): 485-493.
- Bjørndal, A. & Hofoss, D. (2004). *Statistikk for helse- og sosialfagene*. 2. utg. ed. Oslo: Gyldendal akademisk.
- Boyd, C. E. (2020). Dissolved oxygen and other gases. *Water quality: An introduction*: 135-162.
- Božić, D., Stanković, V., Gorgievski, M., Bogdanović, G. & Kovačević, R. (2009). Adsorption of heavy metal ions by sawdust of deciduous trees. *Journal of hazardous materials*, 171 (1-3): 684-692.
- Bruun, J., Hoffmann, C. C. & Kjaergaard, C. (2016). Nitrogen removal in permeable woodchip filters affected by hydraulic loading rate and woodchip ratio. *Journal of Environmental Quality*, 45 (5): 1688-1695.
- Bulut, Y. (2007). Removal of heavy metals from aqueous solution by sawdust adsorption. *Journal of environmental sciences*, 19 (2): 160-166.

- Canivet, V., Chambon, P. & Gibert, J. (2001). Toxicity and bioaccumulation of arsenic and chromium in epigean and hypogean freshwater macroinvertebrates. *Archives of Environmental Contamination and Toxicology*, 40: 345-354.
- Croghan, C. W. (2003). Methods of dealing with values below the limit of detection using SAS. *Southern SAS User Group*, 22 (24): 22-24.
- Damsgård, M. B. (2011). Accumulation of heavy metals in benthic invertebrates and frogs from sedimentation ponds receiving runoff from a four lane motorway (E6). Master thesis. Oslo: M.B. Damsgård.
- Datta, S., Ghosh, D., Saha, D. R., Bhattacharaya, S. & Mazumder, S. (2009). Chronic exposure to low concentration of arsenic is immunotoxic to fish: role of head kidney macrophages as biomarkers of arsenic toxicity to Clarias batrachus. *Aquatic toxicology*, 92 (2): 86-94.
- Dean, J. R. (2003). Methods for environmental trace analysis, vol. 12: John Wiley and Sons.
- Direktoratsgruppen vanndirektivet. (2018). Veileder 02:2018 Klassifisering av miljøtilstand i vann.
- Du, X., Chi, Z., Chen, M., Yu, Z. & Zhu, Y. (2022). Characteristics of dissolved organic matter in urban road runoff under different traffic densities in Beijing, China. *Environmental Science and Pollution Research*, 29 (60): 90520-90529.
- Eivik Karlsen, S. (2021). *Evolution of tunnel wash water quality during sedimentation*: NTNU: Norwegian University of Science and Technology.

Endr. i vannforskriften. (2012, Vedlegg VIIIA). *Forskrift om endring i forskrift om rammer for vannforvaltningen*. (FOR-2012-03-27-321). Lovdata. Available at:

https://lovdata.no/LTI/forskrift/2012-03-27-321 (accessed: 2024-05-10).

- Farm, C. (2002). Evaluation of the accumulation of sediment and heavy metals in a storm-water detention pond. *Water Sci Technol*, 45 (7): 105-112. doi: 10.2166/wst.2002.0122.
- Farnham, I. M., Singh, A. K., Stetzenbach, K. J. & Johannesson, K. H. (2002). Treatment of nondetects in multivariate analysis of groundwater geochemistry data. *Chemometrics and Intelligent Laboratory Systems*, 60 (1-2): 265-281.
- Filella, M., Belzile, N. & Chen, Y.-W. (2002). Antimony in the environment: a review focused on natural waters: II. Relevant solution chemistry. *Earth-Science Reviews*, 59 (1-4): 265-285.
- Forurensningsloven. (1983). *Lov om vern mot forurensninger og om avfall*. (LOV-1981-03-13-6). Lovdata. Available at: <u>https://lovdata.no/lov/1981-03-13-6</u> (accessed: 2023-12-15).
- Forurensningsloven. (1983, §16). Lov om vern mot forurensninger og om avfall. (LOV-1981-03-13-6). Lovdata. Available at: <u>https://lovdata.no/lov/1981-03-13-6/</u>§16 (accessed: 2023-12-15).
- Foslie, M. & Grindeland, J. M. (2023). *Or*: Store norske leksikon. Available at: <u>https://snl.no/or</u> (accessed: 2024-05-03).
- Furunes-Olsen, J. (2023). Removal of metals and particles from tunnel wash water: NTNU.
- Gadd, G. (2009). Heavy metal pollutants: environmental and biotechnological aspects. In *Encyclopedia of microbiology*, pp. 321-334: Academic Press.
- Garshol, F., Estevez, M., Eftekhar Dadkhah, M., Stang, P., Rathnaweera, S., Sahu, A. & Vik, E. (2015). *Laboratory tests - treatment of tunnel wash water from the Nordby tunnel*. NORWAT, editor. Report, 15-032.
- Grung, M., Meland, S., Ruus, A., Ranneklev, S., Fjeld, E., Kringstad, A., Rundberget, J. T., Dela Cruz, M.
 & Christensen, J. H. (2021). Occurrence and trophic transport of organic compounds in sedimentation ponds for road runoff. *Science of the Total Environment*, 751: 141808.
- Hallberg, M., Renman, G., Byman, L., Svenstam, G. & Norling, M. (2014). Treatment of tunnel wash water and implications for its disposal. *Water science and technology*, 69 (10): 2029-2035.
- Haygarth, P. M. & Sharpley, A. (2000). Terminology for phosphorus transfer. *Journal of environmental quality*, 29 (1): 10-15.
- Helsel, D. R. (1990). Less than obvious-statistical treatment of data below the detection limit. *Environmental science & technology*, 24 (12): 1766-1774.
- Hoffmann, C. C., Larsen, S. E. & Kjaergaard, C. (2019). Nitrogen removal in woodchip-based biofilters of variable designs treating agricultural drainage discharges. *Journal of Environmental Quality*, 48 (6): 1881-1889.

- Hopke, P. K., Liu, C. & Rubin, D. B. (2001). Multiple imputation for multivariate data with missing and below-threshold measurements: time-series concentrations of pollutants in the Arctic. *Biometrics*, 57 (1): 22-33.
- ION-96.4. (n.d.). *lot 0316; A natural river water from the Grand River, Ontario*; Environment and Climate Change Canada. 867 Laneshore Road, Burlington, ON L7S1A1 CANADA (15 Match 2016).
- Jarslett, Y. (2024). Nordbytunnelen. snl.no. Available at: <u>https://snl.no/Nordbytunnelen</u> (accessed: 2023-10-26).
- Jolliffe, I. T. & Cadima, J. (2016). Principal component analysis: a review and recent developments. Philosophical transactions of the royal society A: Mathematical, Physical and Engineering Sciences, 374 (2065): 20150202.
- Jong, T. & Parry, D. L. (2003). Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. *Water research*, 37 (14): 3379-3389.
- Jørgensen, B. B. (1990). The sulfur cycle of freshwater sediments: role of thiosulfate. *Limnology and Oceanography*, 35 (6): 1329-1342.
- Kovacova, Z., Demcak, S., Balintova, M., Pla, C. & Zinicovscaia, I. (2020). Influence of wooden sawdust treatments on Cu (II) and Zn (II) removal from water. *Materials*, 13 (16): 3575.
- Lepine, C., Christianson, L., Soucek, D., McIsaac, G. & Summerfelt, S. (2021). Metal leaching and toxicity of denitrifying woodchip bioreactor outflow—Potential reuse application. *Aquacultural engineering*, 93: 102129.
- Levy, B. & Chambers, R. (1987). Bromide as a conservative tracer for soil-water studies. *Hydrological Processes*, 1 (4): 385-389.
- Liane, A. S. (2018). Effects of road runoff on tadpoles of the common frog (Rana temporaria).
- Liu, C.-f., Yuan, X.-z., Zeng, G.-m., Li, W.-w. & Li, J. (2008). Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresource technology*, 99 (4): 882-888.
- Markiewicz, A., Björklund, K., Eriksson, E., Kalmykova, Y., Strömvall, A.-M. & Siopi, A. (2017). Emissions of organic pollutants from traffic and roads: Priority pollutants selection and substance flow analysis. *Science of the Total Environment*, 580: 1162-1174.
- McMahon, P. & Chapelle, F. (2008). Redox processes and water quality of selected principal aquifer systems. *Groundwater*, 46 (2): 259-271.
- Meez, E., Rahdar, A. & Kyzas, G. Z. (2021). Sawdust for the removal of heavy metals from water: a review. *Molecules*, 26 (14): 4318.
- Meland, S. (2010). *Ecotoxicological effects of highway and tunnel wash water runoff*: Norwegian University of Life Sciences, Ås.
- Meland, S., Borgstrøm, R., Sørlie Heier, L., Rosseland, B. O., Lindholm, O. & Salbu, B. (2010a). Chemical and ecological effects of contaminated tunnel wash water runoff to a small Norwegian stream. *Science of the Total Environment*, 408 (19): 4107-4117.
- Meland, S., Sørlie Heier, L., Salbu, B., Tollefsen, K. E., Farmen, E. & Rosseland, B. O. (2010b). Exposure of brown trout (Salmo trutta L.) to tunnel wash water runoff—chemical characterisation and biological impact. *Science of the Total Environment*, 408 (13): 2646-2656.
- Meland, S., Farmen, E., Heier, L. S., Rosseland, B. O., Salbu, B., Song, Y. & Tollefsen, K. E. (2011).
 Hepatic gene expression profile in brown trout (Salmo trutta) exposed to traffic related contaminants. *Sci Total Environ*, 409 (8): 1430-1443. doi: 10.1016/j.scitotenv.2011.01.013.
- Meland, S. (2012). Tunnelvaskevann–En kilde til vannforurensning. Vegvesen.no.
- Meland, S. & Rødland, E. S. (2018). Forurensning i tunnelvaskevann en studie av 34 veitunneler i Norge.
- Meland, S., Gomes, T., Petersen, K., Håll, J., Lund, E., Kringstad, A. & Grung, M. (2019). Road related pollutants induced DNA damage in dragonfly nymphs (Odonata, Anisoptera) living in highway sedimentation ponds. *Scientific reports*, 9 (1): 16002.

- Miljødirektoratet. (n.d.-a). *Vannmiljø vannlokalitet 005-29663 Årungen* Available at: <u>https://vannmiljofaktaark.miljodirektoratet.no/Home/Details/29663?datofra=2014-03-</u>06&datotil=2024-05-03 (accessed: 2024-05-03).
- Miljødirektoratet. (n.d.-b). *Vannmiljø vannlokalitet 005-42449 Årungenelva*. Available at: <u>https://vannmiljofaktaark.miljodirektoratet.no/Home/Details/42449?param=CL&medium=V</u> <u>F</u> (accessed: 2024-05-14).
- Minitab. (2024). *Data considerations for Kruskal-Wallis Test*. Available at: <u>https://support.minitab.com/en-us/minitab/help-and-how-</u> <u>to/statistics/nonparametrics/how-to/kruskal-wallis-test/before-you-start/data-</u> <u>considerations/</u> (accessed: 2024-05-12).
- Morrison, M. A. & Benoit, G. (2001). Filtration artifacts caused by overloading membrane filters. *Environmental science & technology*, 35 (18): 3774-3779.
- NCS DC 73324a. (n.d.). Soil; NCS Testing Technology Co,. Ltd. Beijing, China (2020).
- NCS DC 73325. (n.d.). Soil; China National Analysis Center for Iron and Steel. Beijing, China (2004).
- NCS ZC 73007. (n.d.). Soil; China National Analysis Center for Iron and Steel. Beijing, China (2003).
- Nersten, G. (2016). *En studie om forbedring av metode for rensing av tunnelvaskevann*: Norwegian University of Life Sciences, Ås.
- NIST 1643f. (n.d.). *Trace Elements in Water*; National Institute of Standards & Technology. U.S. Department of Commerce: Gaithersburg MD (18 August 2015).
- Normann, A. A. (2017). *Electrocoagulation for treatment of tunnel wash water : a proof of concept:* Norwegian University of Life Sciences, Ås.
- Ostertagova, E., Ostertag, O. & Kováč, J. (2014). Methodology and application of the Kruskal-Wallis test. *Applied mechanics and materials*, 611: 115-120.
- Paquin, P. R., Gorsuch, J. W., Apte, S., Batley, G. E., Bowles, K. C., Campbell, P. G., Delos, C. G., Di Toro, D. M., Dwyer, R. L. & Galvez, F. (2002). The biotic ligand model: a historical overview. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 133 (1-2): 3-35.
- Paruch, A. M. & Roseth, R. (2008). Treatment of tunnel wash waters—experiments with organic sorbent materials. Part II: removal of toxic metals. *Journal of Environmental Sciences*, 20 (9): 1042-1045.
- Pyle, G. & Couture, P. (2011). Nickel. In vol. 31 *Fish physiology*, pp. 253-289: Elsevier.
- QC3060. (n.d.). *LOT no: LRAD1054 Anions Whole Volume*; Sigma-Aldrich RTC. The life science business of Merck KGaA, Darmstadt, Germany (29-November-2021).
- Rafatullah, M., Sulaiman, O., Hashim, R. & Ahmad, A. (2009). Adsorption of copper (II), chromium (III), nickel (II) and lead (II) ions from aqueous solutions by meranti sawdust. *Journal of hazardous materials*, 170 (2-3): 969-977.
- Rahman, M. S. & Islam, M. R. (2009). Effects of pH on isotherms modeling for Cu (II) ions adsorption using maple wood sawdust. *Chemical Engineering Journal*, 149 (1-3): 273-280.
- Rathnaweera, S. S., Vik, E. A., Garshol, F. K., Åstebøl, S. O. & Frost, K. (2019). Investigation of technologies for road tunnel wash water treatment. *Vann*, 3: 222–233.
- Rathnaweera, S. S., Vik, E. A., Manamperuma, L. D., Åstebøl, S. O., Vollertsen, J., Heier, L. S. & Kronvall, K. W. (2023). Study of traffic-related pollution and its treatment with a particular focus on microplastics in tunnel wash and road runoff water. *Water science and technology*, 88 (4): 874-884. doi: 10.2166/wst.2023.232.
- REHIRUP. (2022). *Rehirup (Reducing highway runoff pollution) Final report*, 2022-01.
- Reimann, C., Filzmoser, P. & Garrett, R. G. (2002). Factor analysis applied to regional geochemical data: problems and possibilities. *Applied geochemistry*, 17 (3): 185-206.
- Rivas, A., Barkle, G., Stenger, R., Moorhead, B. & Clague, J. (2020). Nitrate removal and secondary effects of a woodchip bioreactor for the treatment of subsurface drainage with dynamic flows under pastoral agriculture. *Ecological engineering*, 148: 105786.
- Robertson, W., Blowes, D., Ptacek, C. & Cherry, J. (2000). Long-term performance of in situ reactive barriers for nitrate remediation. *Groundwater*, 38 (5): 689-695.

- Robertson, W., Ford, G. & Lombardo, P. (2005). Wood-based filter for nitrate removal in septic systems. *Transactions of the ASAE*, 48 (1): 121-128.
- Robertson, W. & Merkley, L. (2009). In-stream bioreactor for agricultural nitrate treatment. *Journal* of Environmental Quality, 38 (1): 230-237.
- Robertson, W. (2010). Nitrate removal rates in woodchip media of varying age. *Ecological Engineering*, 36 (11): 1581-1587.
- Roseth, R., Skrutvold, J., Vartdal, I. V., Fjermestad, H. & Barland, T. (2023). *E16 Bjørum–Skaret. Resultater for renseanlegg for nitrogen i 2022*. NIBIO Rapport.
- Roseth, R., Myhre Sverdrup, E. & Kozera, R. (2024). *Nitrogen i tunneldrivevann–en pilotstudie av rensefilter*. NIBIO Rapport, 8217034389.
- Sahinkaya, E., Kilic, A., Altun, M., Komnitsas, K. & Lens, P. N. (2012). Hexavalent chromium reduction in a sulfur reducing packed-bed bioreactor. *Journal of hazardous materials*, 219: 253-259.
- SANGAMON-03. (n.d.). *lot 0618; A River Water Sample*; Environment and Climate Change Canada. 867 Laneshore Road, Burlington, ON L7S1A1 CANADA (26-June-2019).
- Schipper, L. A., Robertson, W. D., Gold, A. J., Jaynes, D. B. & Cameron, S. C. (2010). Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. *Ecological engineering*, 36 (11): 1532-1543.
- Shukla, S. & Pai, R. S. (2005). Adsorption of Cu (II), Ni (II) and Zn (II) on dye loaded groundnut shells and sawdust. *Separation and purification Technology*, 43 (1): 1-8.
- Singha, K., Li, L., Day-Lewis, F. D. & Regberg, A. B. (2011). Quantifying solute transport processes: Are chemically "conservative" tracers electrically conservative? *Geophysics*, 76 (1): F53-F63.
- Statens Vegvesen. (2014). *Standard for drift og vedlikehold av riksveger*. [Rev. utg.]. ed. Håndbok R610. Oslo: Statens vegvesen.
- Statens Vegvesen. (n.d.). *Nordbytunnelen*. Trafikkdata: Statens Vegvesen. Available at: <u>https://trafikkdata.atlas.vegvesen.no/#/utforsk?datatype=averageDailyYearVolume&display</u> <u>=chart&from=2023-10-26&trpids=52685V444218</u> (accessed: 2023-10-26).
- Statistisk sentralbyrå. (2021). *Flere utsatt for støy ved boligen*. Available at: <u>https://www.ssb.no/natur-og-miljo/artikler-og-publikasjoner/flere-utsatt-for-stoy-ved-boligen</u> (accessed: 2024-05-05).
- Statistisk sentralbyrå. (2023). *Tabell 10781: Fysisk nedbygd areal (km²) (K) 2011 2023*. Available at: https://www.ssb.no/statbank/table/10781/ (accessed: 2024-05-05).
- Statistisk sentralbyrå. (2024a). 12577: Kjørelengder, etter statistikkvariabel, kjøretøytype, drivstofftype og år. Available at:

https://www.ssb.no/statbank/table/12577/tableViewLayout1/ (accessed: 2024-05-05).

- Statistisk sentralbyrå. (2024b). *Fakta om bil og bilkjøring*. Available at: <u>https://www.ssb.no/transport-og-reiseliv/faktaside/bil-og-transport</u> (accessed: 2024-05-05).
- Steinnes, E. & Salbu, B. (1995). *Trace elements in natural waters*. Boca Raton, Fla: CRC Press.
- Sternbeck, J., Sjödin, Å. & Andréasson, K. (2002). Metal emissions from road traffic and the influence of resuspension—results from two tunnel studies. *Atmospheric environment*, 36 (30): 4735-4744.
- Stumm, W. & Morgan, J. J. (2012). *Aquatic chemistry: chemical equilibria and rates in natural waters*: John Wiley & Sons.
- Swadener, L. M., Javeherian, M. H., Jones, E. G. & Bartelt-Hunt, S. L. (2014). Chemical characterization and toxicity of bridge deck runoff and impacts to receiving water quality. *Journal of Environmental Engineering*, 140 (4): 04014010.
- Sørlie Heier, L., Meland, S., Ljønes, M., Salbu, B. & Einride Strømseng, A. (2010). Short-term temporal variations in speciation of Pb, Cu, Zn and Sb in a shooting range runoff stream. *Science of the total environment*, 408 (11): 2409-2417.
- Teclu, D., Tivchev, G., Laing, M. & Wallis, M. (2008). Bioremoval of arsenic species from contaminated waters by sulphate-reducing bacteria. *Water research*, 42 (19): 4885-4893.
- Tipping, E. & Hurley, M. A. (1992). A unifying model of cation binding by humic substances. *Geochimica et Cosmochimica Acta*, 56 (10): 3627-3641.

- van Bohemen, H. & Janssen Van De Laak, W. (2003). The influence of road infrastructure and traffic on soil, water, and air quality. *Environmental management*, 31: 0050-0068.
- VanLoon, G. W. & Duffy, S. J. (2017). *Environmental chemistry: a global perspective*. Fourth ed.: Oxford University Press.
- Vistnes, H., Sossalla, N. A., Asimakopoulos, A. G. & Meyn, T. (2024). Occurrence of traffic related trace elements and organic micropollutants in tunnel wash water. *Journal of Hazardous Materials*, 465: 133498.
- Yu, B., Zhang, Y., Shukla, A., Shukla, S. S. & Dorris, K. L. (2000). The removal of heavy metal from aqueous solutions by sawdust adsorption—removal of copper. *Journal of hazardous materials*, 80 (1-3): 33-42.
- Zeghnoun, A., Pascal, M., Fréry, N., Sarter, H., Falq, G., Focant, J.-F. & Eppe, G. (2007). Dealing with the non-detected and non-quantified data. The example of the serum dioxin data in the French dioxin and incinerators study. *Organohalogen Compounds*, 69.
- Åstebøl, S., Kjølholt, J., Hvitved-Jacobsen, T., Berg, G. & Saunes, H. (2012). *Beregning av forurensning fra overvann*. Oslo: COWI.

Appendices

Appendix A

Calculation of nominal volume of water-saturated filter material

Length of biofilter = 3m. End caps were not included in this calculation since they did not contain filter material.

The tube was filled with water up to 2/3 of the tube diameter.

 $V_{tube} = length * \pi * radius^2$

 $V_{tube} = 300 cm * \pi * 15 cm^2 = 211950 cm^2 = 211.95 L$

Volume of water saturated filter material = $211.95 L * \frac{2}{3} = 141.3 L$

Appendix **B**

Table 13: Concentrations of F-, Cl- and SO42- used as calibration standards

Standard	F ⁻	Cl	SO 4 ²⁻	
	(mg/L)	(mg/L)	(mg/L)	
Std1	0,20	0,20	0,20	
Std2	2,00	2,00	2,00	
Std3	20	20	20	

Table 14: Standards used as a reference in TOC analysis

Standard	DOC
	(mg/L)
K1	1.17
K5	5.82
Appendix C

Table 15: Table showing recordings from the automatic meter in the tank after the field experiment.

Outlets. Time in wintertime												
Date	04-okt	05-okt	06-okt	07-okt	08-okt	09-okt	24-sep	25-sep	27-sep	29-sep	01-okt	02-okt
Time manual recording	11:06	11:06	11:06	11:06	11:06	11:06	11:08	11:06	11:06	12:00	11:06	11:06
Time automatic recording	11:30	11:30	-	11:30	11:30	11:30	11:30	11:30	11:30	11:30	11:30	11:30
EC (µS/cm) manual	1380	1359	1345	1302	1295	1280	1146	1335	1433	1449	1461	1457
EC (mS) automatic	1.353	1.344	-	1.290	1.278	1.266	1.130	1.298	1.416	1.450	1.453	1.434
DO (mg/L) manual	0.051	0.198	0.162	0.141	0.323	0.342	0.384	0.164	0.005	0.185	0.274	0.096
DO (mg/L) automatic	0.287	0.292	-	0.305	0.345	0.3625	0.7835	0.257	0.242	0.246	0.2865	0.2595
DO (%) manual	0.4	1.7	1.5	1.2	2.4	2.5	3.8	1.8	0.1	1.8	2.4	0.9
DO (%) automatic	2.783	2.804	-	2.8395	2.9495	2.9955	7.5235	2.7195	2.625	2.638	2.775	2.694
pH manual	7.26	7.31	7.38	7.49	7.56	7.62	6.92	6.98	7.12	7.1	7.24	7.16
pH automatic	7.47	7.48	-	7.56	7.67	7.73	7.15	7.17	7.32	7.40	7.45	7.39
ORP (mV) manual	-	-	-	-	-	-	-	-	-	-	-	-
ORP (mV) automatic	-393	-425	-	-426	-430	-296	-139	-219	-346	-358	-407	-416
Temp (°C) manual	9.1	9	9.3	8	4.9	3	9.7	13.5	15.2	14.5	9.5	12.5
Temp (°C) automatic	9.9	9.4	-	8.2	4.8	3.6	10.3	13.7	14.9	14.2	9.8	12.8

Appendix D

Table 16: Table showing results from Dunns post-hoc test for 45-hour hydraulic retention time. Fractions of metals that are not shown in the table were not significant in the previous Kruskal Wallis analysis.

Fraction	Metal	comparison	Z	P.unadj	P.adj
total fraction	As	45h_Inlet - 45h_Outlet	2.761300515	0.005757168	0.11514335
particulate fraction	As	45h_Inlet - 45h_Outlet	2.900456964	0.00372619	0.0745238
lmm fraction	As	45h_Inlet - 45h_Outlet	1.726462479	0.084264244	1
colloidal fraction	As	45h_Inlet - 45h_Outlet	2.347105438	0.0189199	0.321638299
Anions	As	45h_Inlet - 45h_Outlet	-2.830333028	0.004649958	0.09299915
total fraction	Cr	45h_Inlet - 45h_Outlet	2.968398054	0.002993564	0.062864841
colloidal fraction	Cr	45h_Inlet - 45h_Outlet	2.416137951	0.015686117	0.298036226
total fraction	Cu	45h_Inlet - 45h_Outlet	2.62323549	0.008709905	0.16548819
particulate fraction	Cu	45h_Inlet - 45h_Outlet	1.587747796	0.11234338	1
lmm fraction	Cu	45h_Inlet - 45h_Outlet	2.209871973	0.027114049	0.460938831
colloidal fraction	Cu	45h_Inlet - 45h_Outlet	2.209040412	0.02717183	0.489092942
Cations	Cu	45h_Inlet - 45h_Outlet	1.795520978	0.072570733	0.943419535
Anions	Cu	45h_Inlet - 45h_Outlet	2.209871973	0.027114049	0.460938831
total fraction	Ni	45h_Inlet - 45h_Outlet	3.349337209	0.000810051	0.01701108
particulate fraction	Ni	45h_Inlet - 45h_Outlet	2.278072925	0.022722233	0.477166894
lmm fraction	Ni	45h_Inlet - 45h_Outlet	2.750847902	0.005944124	0.124826594
Anions	Ni	45h_Inlet - 45h_Outlet	2.750847902	0.005944124	0.124826594
total fraction	Sb	45h_Inlet - 45h_Outlet	3.382593131	0.000718049	0.015079035
particulate fraction	Sb	45h_Inlet - 45h_Outlet	2.830333028	0.004649958	0.09299915
lmm fraction	Sb	45h_Inlet - 45h_Outlet	2.968398054	0.002993564	0.062864841
colloidal fraction	Sb	45h_Inlet - 45h_Outlet	2.454350521	0.014113933	0.268164727
Cations	Sb	45h_Inlet - 45h_Outlet	2.831398465	0.004634495	0.097324385
Anions	Sb	45h_Inlet - 45h_Outlet	2.693281467	0.007075251	0.134429771
total fraction	Zn	45h_Inlet - 45h_Outlet	2.865927715	0.004157892	0.083157835
particulate fraction	Zn	45h_Inlet - 45h_Outlet	1.656780309	0.097563899	1
lmm fraction	Zn	45h_Inlet - 45h_Outlet	3.184405075	0.001450519	0.030460894
colloidal fraction	Zn	45h_Inlet - 45h_Outlet	3.10646308	0.0018934	0.03976139
Cations	Zn	45h_Inlet - 45h_Outlet	3.10646308	0.0018934	0.03976139
Anions	Zn	45h_Inlet - 45h_Outlet	3.340054386	0.00083762	0.017590017

Appendix E

Table 17: shows results from Dunns post-hoc test for reoxygenated samples. Fractions of metals that are not shown in the table were not significant in the previous Kruskal Wallis analysis.

data	Metal	comparison Z P.unadj		P.unadj	P.adj
total fraction	As	45h_Outlet - 45h_Oxygenated	-0.48323	0.628934	1
particulate fraction	As	45h_Outlet - 45h_Oxygenated	-0.13812	0.890148	1
lmm fraction	As	45h_Outlet - 45h_Oxygenated	-0.55247	0.580628	1
colloidal fraction	As	45h_Outlet - 45h_Oxygenated	-1.10452	0.269368	1
Anions	As	45h_Outlet - 45h_Oxygenated	-0.48323	0.628934	1
total fraction	Cr	45h_Outlet - 45h_Oxygenated	-1.10452	0.269368	1
colloidal fraction	Cr	45h_Outlet - 45h_Oxygenated	-0.4142	0.678731	1
total fraction	Cu	45h_Outlet - 45h_Oxygenated	-0.82839	0.40745	1
particulate fraction	Cu	45h_Outlet - 45h_Oxygenated	1.518715	0.128834	1
lmm fraction	Cu	45h_Outlet - 45h_Oxygenated	-1.48476	0.137608	1
colloidal fraction	Cu	45h_Outlet - 45h_Oxygenated	-1.51872	0.128834	1
Cations	Cu	45h_Outlet - 45h_Oxygenated	-1.10494	0.269187	1
Anions	Cu	45h_Outlet - 45h_Oxygenated	-1.48476	0.137608	1
total fraction	Ni	45h_Outlet - 45h_Oxygenated	-1.13947	0.254509	1
particulate fraction	Ni	45h_Outlet - 45h_Oxygenated	-0.89742	0.369493	1
lmm fraction	Ni	45h_Outlet - 45h_Oxygenated	0	1	1
Anions	Ni	45h_Outlet - 45h_Oxygenated	0	1	1
total fraction	Sb	45h_Outlet - 45h_Oxygenated	-0.55226	0.58077	1
particulate fraction	Sb	45h_Outlet - 45h_Oxygenated	0.414195	0.678731	1
lmm fraction	Sb	45h_Outlet - 45h_Oxygenated	-0.48323	0.628934	1
colloidal fraction	Sb	45h_Outlet - 45h_Oxygenated	-0.38025	0.703759	1
Cations	Sb	45h_Outlet - 45h_Oxygenated	-1.20852	0.226846	1
Anions	Sb	45h_Outlet - 45h_Oxygenated	0.138117	0.890148	1
total fraction	Zn	45h_Outlet - 45h_Oxygenated	-1.13947	0.254509	1
particulate fraction	Zn	45h_Outlet - 45h_Oxygenated	0.690325	0.48999	1
lmm fraction	Zn	45h_Outlet - 45h_Oxygenated	-0.80485	0.420906	1
colloidal fraction	Zn	45h_Outlet - 45h_Oxygenated	-1.65678	0.097564	1
Cations	Zn	45h_Outlet - 45h_Oxygenated	-1.17355	0.240574	1
Anions	Zn	45h_Outlet - 45h_Oxygenated	-1.04377	0.296593	1

Appendix F

Table 18: Results from Dunns post-hoc test comparing outlet concentrations between hydraulic retention times are shown. Fractions of metals that are not shown in the table were not significant in the previous Kruskal Wallis analysis.

data	Metal	comparison	Z	P.unadj	P.adj
total fraction	Cr	11h_Outlet - 23h_Outlet	1.10452	0.269368	1
total fraction	Cu	11h_Outlet - 23h_Outlet	1.10452	0.269368	1
total fraction	Zn	11h_Outlet - 23h_Outlet	0.725114	0.468382	1
total fraction	Ni	11h_Outlet - 23h_Outlet	0.828702	0.407273	1
total fraction	As	11h_Outlet - 23h_Outlet	1.725813	0.084381	1
total fraction	Sb	11h_Outlet - 23h_Outlet	0.82839	0.40745	1
particulate fraction	Zn	11h_Outlet - 23h_Outlet	0.138065	0.890189	0.890189
particulate fraction	Cu	11h_Outlet - 23h_Outlet	-0.06903	0.944964	0.944964
particulate fraction	Ni	11h_Outlet - 23h_Outlet	0.966455	0.333816	1
particulate fraction	As	11h_Outlet - 23h_Outlet	1.173994	0.240397	1
particulate fraction	Sb	11h_Outlet - 23h_Outlet	1.035488	0.300441	1
lmm fraction	Cu	11h_Outlet - 23h_Outlet	-0.86323	0.38801	1
lmm fraction	Zn	11h_Outlet - 23h_Outlet	0.524902	0.599651	1
lmm fraction	Ni	11h_Outlet - 23h_Outlet	-0.28214	0.777838	1
lmm fraction	As	11h_Outlet - 23h_Outlet	0	1	1
lmm fraction	Sb	11h_Outlet - 23h_Outlet	1.311618	0.189649	1
colloidal fraction	Cr	11h_Outlet - 23h_Outlet	1.38065	0.167387	1
colloidal fraction	Cu	11h_Outlet - 23h_Outlet	1.173553	0.240574	1
colloidal fraction	Zn	11h_Outlet - 23h_Outlet	-0.27613	0.782448	1
colloidal fraction	As	11h_Outlet - 23h_Outlet	1.311618	0.189649	1
colloidal fraction	Sb	11h_Outlet - 23h_Outlet	0.933345	0.350642	1
Cations	Zn	11h_Outlet - 23h_Outlet	-0.2071	0.835934	0.835934
Cations	Cu	11h_Outlet - 23h_Outlet	0.276234	0.782368	1
Cations	Sb	11h_Outlet - 23h_Outlet	0.310763	0.755981	1
Anions	Cu	11h_Outlet - 23h_Outlet	-0.86323	0.38801	1
Anions	Zn	11h_Outlet - 23h_Outlet	0.487091	0.626194	1
Anions	Ni	11h_Outlet - 23h_Outlet	-0.28214	0.777838	1
Anions	As	11h_Outlet - 23h_Outlet	-0.96646	0.333816	1
Anions	Sb	11h_Outlet - 23h_Outlet	0.552468	0.580628	1
total fraction	Cr	11h_Outlet - 45h_Outlet	1.173553	0.240574	1
total fraction	Cu	11h_Outlet - 45h_Outlet	0.207098	0.835934	1
total fraction	Zn	11h_Outlet - 45h_Outlet	0.345292	0.729874	1
total fraction	Ni	11h_Outlet - 45h_Outlet	1.726462	0.084264	1
total fraction	As	11h_Outlet - 45h_Outlet	0.966455	0.333816	1
total fraction	Sb	11h_Outlet - 45h_Outlet	1.725813	0.084381	1
particulate fraction	Cu	11h_Outlet - 45h_Outlet	-1.24259	0.214021	1
particulate fraction	Zn	11h_Outlet - 45h_Outlet	-1.31162	0.189649	1
particulate fraction	Ni	11h_Outlet - 45h_Outlet	1.38065	0.167387	1
particulate fraction	As	11h_Outlet - 45h_Outlet	1.173994	0.240397	1
particulate fraction	Sb	11h_Outlet - 45h_Outlet	1.38065	0.167387	1
lmm fraction	Cu	11h_Outlet - 45h_Outlet	0.138117	0.890148	1
lmm fraction	Zn	11h_Outlet - 45h_Outlet	1.399738	0.161592	1
lmm fraction	Ni	11h Outlet - 45h Outlet	1.199088	0.230494	1

lmm fraction	As	11h_Outlet - 45h_Outlet	-1.48476	0.137608	1
lmm fraction	Sb	11h_Outlet - 45h_Outlet	1.449683	0.147147	1
colloidal fraction	As	11h_Outlet - 45h_Outlet	1.863878	0.062339	0.872744
colloidal fraction	Cr	11h_Outlet - 45h_Outlet	0.82839	0.40745	1
colloidal fraction	Cu	11h_Outlet - 45h_Outlet	1.10452	0.269368	1
colloidal fraction	Zn	11h_Outlet - 45h_Outlet	0.621293	0.534407	1
colloidal fraction	Sb	11h_Outlet - 45h_Outlet	1.693848	0.090294	1
Cations	Cu	11h_Outlet - 45h_Outlet	-0.06906	0.944943	1
Cations	Zn	11h_Outlet - 45h_Outlet	0.621293	0.534407	1
Cations	Sb	11h_Outlet - 45h_Outlet	0.44888	0.653518	1
Anions	As	11h_Outlet - 45h_Outlet	-2.00194	0.045291	0.815236
Anions	Cu	11h_Outlet - 45h_Outlet	0.138117	0.890148	1
Anions	Zn	11h_Outlet - 45h_Outlet	1.600443	0.1095	1
Anions	Ni	11h_Outlet - 45h_Outlet	1.199088	0.230494	1
Anions	Sb	11h_Outlet - 45h_Outlet	0.552468	0.580628	1
total fraction	Cr	23h_Outlet - 45h_Outlet	0.069033	0.944964	0.944964
total fraction	Cu	23h_Outlet - 45h_Outlet	-0.89742	0.369493	1
total fraction	Zn	23h_Outlet - 45h_Outlet	-0.37982	0.704078	1
total fraction	Ni	23h_Outlet - 45h_Outlet	0.89776	0.369313	1
total fraction	As	23h_Outlet - 45h_Outlet	-0.75936	0.447639	1
total fraction	Sb	23h_Outlet - 45h_Outlet	0.897423	0.369493	1
particulate fraction	Cu	23h_Outlet - 45h_Outlet	-1.17355	0.240574	1
particulate fraction	Zn	23h_Outlet - 45h_Outlet	-1.44968	0.147147	1
particulate fraction	Ni	23h_Outlet - 45h_Outlet	0.414195	0.678731	1
particulate fraction	As	23h_Outlet - 45h_Outlet	0	1	1
particulate fraction	Sb	23h_Outlet - 45h_Outlet	0.345163	0.729972	1
lmm fraction	Sb	23h_Outlet - 45h_Outlet	0.138065	0.890189	0.890189
lmm fraction	Cu	23h_Outlet - 45h_Outlet	1.001348	0.316658	1
lmm fraction	Zn	23h_Outlet - 45h_Outlet	0.874837	0.381663	1
lmm fraction	Ni	23h_Outlet - 45h_Outlet	1.481226	0.138546	1
lmm fraction	As	23h_Outlet - 45h_Outlet	-1.48476	0.137608	1
colloidal fraction	Cu	23h_Outlet - 45h_Outlet	-0.06903	0.944964	0.944964
colloidal fraction	Cr	23h_Outlet - 45h_Outlet	-0.55226	0.58077	1
colloidal fraction	Zn	23h_Outlet - 45h_Outlet	0.897423	0.369493	1
colloidal fraction	As	23h_Outlet - 45h_Outlet	0.55226	0.58077	1
colloidal fraction	Sb	23h_Outlet - 45h_Outlet	0.760503	0.446954	1
Cations	Sb	23h_Outlet - 45h_Outlet	0.138117	0.890148	0.890148
Cations	Cu	23h_Outlet - 45h_Outlet	-0.34529	0.729874	1
Cations	Zn	23h_Outlet - 45h_Outlet	0.82839	0.40745	1
Anions	Cu	23h_Outlet - 45h_Outlet	1.001348	0.316658	1
Anions	Zn	23h_Outlet - 45h_Outlet	1.113351	0.265558	1
Anions	Ni	23h_Outlet - 45h_Outlet	1.481226	0.138546	1
Anions	As	23h_Outlet - 45h_Outlet	-1.03549	0.300441	1
Anions	Sb	23h_Outlet - 45h_Outlet	0	1	1

Appendix G

Table 19: Results from Dunns post-hoc test comparing inlet and outlet concentrations within hydraulic retention times are shown. Fractions of metals that are not shown in the table were not significant in the previous Kruskal Wallis analysis.

data	Met	comparison	Z	P.unadj	P.adj
Anions	Cu	11h_Inlet - 11h_Outlet	2.640573425	0.008276586	0.148978541
Anions	Zn	11h_Inlet - 11h_Outlet	0.855775784	0.392121813	1
Anions	Ni	 11h_Inlet - 11h_Outlet	0.86746041	0.385689795	1
Anions	As	11h_Inlet - 11h_Outlet	0.694626262	0.48728955	1
Anions	Sb	11h_Inlet - 11h_Outlet	1.266239888	0.205427211	1
Cations	Cu	11h_Inlet - 11h_Outlet	2.300850528	0.021400081	0.363801382
Cations	Zn	11h_Inlet - 11h_Outlet	1.373816384	0.169498693	1
Cations	Sb	11h_Inlet - 11h_Outlet	1.497869136	0.134167254	1
colloidal fraction	Zn	11h_Inlet - 11h_Outlet	1.867772837	0.061793741	0.926906118
colloidal fraction	Cr	11h_Inlet - 11h_Outlet	1.589922332	0.111852313	1
colloidal fraction	Cu	11h_Inlet - 11h_Outlet	1.497305497	0.134313785	1
colloidal fraction	As	11h_Inlet - 11h_Outlet	1.373816384	0.169498693	1
colloidal fraction	Sb	11h_Inlet - 11h_Outlet	1.236753714	0.216178531	1
lmm fraction	Cu	11h_Inlet - 11h_Outlet	2.640573425	0.008276586	0.148978541
lmm fraction	Zn	11h_Inlet - 11h_Outlet	0.892024471	0.372379816	1
lmm fraction	Ni	11h_Inlet - 11h_Outlet	0.86746041	0.385689795	1
lmm fraction	As	11h_Inlet - 11h_Outlet	1.436101336	0.150973508	1
lmm fraction	Sb	11h_Inlet - 11h_Outlet	1.157710436	0.246982224	1
particulate fraction	Sb	11h_Inlet - 11h_Outlet	0.046308417	0.96306443	0.96306443
particulate fraction	Cu	11h_Inlet - 11h_Outlet	1.559050054	0.118984534	1
particulate fraction	Zn	11h_Inlet - 11h_Outlet	1.497305497	0.134313785	1
particulate fraction	Ni	11h_Inlet - 11h_Outlet	-0.571137148	0.567906679	1
particulate fraction	As	11h_Inlet - 11h_Outlet	0.664003844	0.506687851	1
total fraction	Cr	11h_Inlet - 11h_Outlet	0.169797531	0.865169369	1
total fraction	Cu	11h_Inlet - 11h_Outlet	1.373816384	0.169498693	1
total fraction	Zn	11h_Inlet - 11h_Outlet	1.466985236	0.142380072	1
total fraction	Ni	11h_Inlet - 11h_Outlet	0.416932646	0.676727674	1
total fraction	As	11h_Inlet - 11h_Outlet	0.818115375	0.413291311	1
total fraction	Sb	11h_Inlet - 11h_Outlet	0.972476766	0.330813418	1
Anions	Cu	23h_Inlet - 23h_Outlet	2.002696475	0.045209877	0.67814816
Anions	Zn	23h_Inlet - 23h_Outlet	1.913572825	0.05567476	0.8351214
Anions	As	23h_Inlet - 23h_Outlet	0.103548769	0.917527445	0.917527445
Anions	Ni	23h_Inlet - 23h_Outlet	0.811147458	0.417280995	1
Anions	Sb	23h_Inlet - 23h_Outlet	1.795520978	0.072570733	1
Cations	Cu	23h_Inlet - 23h_Outlet	2.865927715	0.004157892	0.087315727
Cations	Zn	23h_Inlet - 23h_Outlet	1.898394104	0.057644186	0.807018599
Cations	Sb	23h_Inlet - 23h_Outlet	1.415699233	0.156863583	1
colloidal fraction	As	23h_Inlet - 23h_Outlet	2.588719233	0.00963336	0.183033848
colloidal fraction	Cr	23h_Inlet - 23h_Outlet	2.381621695	0.017236595	0.310258701
colloidal fraction	Sb	23h_Inlet - 23h_Outlet	2.039530714	0.041397089	0.579559247

colloidal fraction	Cu	23h_Inlet - 23h_Outlet	1.967426617	0.049134048	0.83527882
colloidal fraction	Zn	23h_Inlet - 23h_Outlet	1.829361592	0.067345457	0.942836404
lmm fraction	Cu	23h_Inlet - 23h_Outlet	2.002696475	0.045209877	0.67814816
lmm fraction	Sb	23h_Inlet - 23h_Outlet	1.967426617	0.049134048	0.687876675
lmm fraction	Zn	23h_Inlet - 23h_Outlet	1.994627354	0.046083538	0.691253068
lmm fraction	As	23h_Inlet - 23h_Outlet	1.864579477	0.062240372	0.995845948
lmm fraction	Ni	23h_Inlet - 23h_Outlet	0.811147458	0.417280995	1
particulate fraction	Zn	23h_Inlet - 23h_Outlet	2.312589182	0.020745237	0.373414264
particulate fraction	As	23h_Inlet - 23h_Outlet	2.106284224	0.035179671	0.633234075
particulate fraction	Cu	23h_Inlet - 23h_Outlet	2.03645913	0.041704273	0.667268369
particulate fraction	Ni	23h_Inlet - 23h_Outlet	0.793873898	0.427268846	1
particulate fraction	Sb	23h_Inlet - 23h_Outlet	1.691296566	0.090780176	1
total fraction	As	23h_Inlet - 23h_Outlet	2.726784259	0.006395484	0.121514195
total fraction	Cu	23h_Inlet - 23h_Outlet	2.726784259	0.006395484	0.127909679
total fraction	Zn	23h_Inlet - 23h_Outlet	2.45157672	0.014223186	0.256017353
total fraction	Cr	23h_Inlet - 23h_Outlet	1.760329079	0.078352027	1
total fraction	Ni	23h_Inlet - 23h_Outlet	1.346640733	0.178095969	1
total fraction	Sb	23h_Inlet - 23h_Outlet	1.484199027	0.137756128	1
Anions	Zn	45h_Inlet - 45h_Outlet	3.340054386	0.00083762	0.017590017
Anions	As	45h_Inlet - 45h_Outlet	-2.830333028	0.004649958	0.09299915
Anions	Ni	45h_Inlet - 45h_Outlet	2.750847902	0.005944124	0.124826594
Anions	Sb	45h_Inlet - 45h_Outlet	2.693281467	0.007075251	0.134429771
Anions	Cu	45h_Inlet - 45h_Outlet	2.209871973	0.027114049	0.460938831
Cations	Zn	45h_Inlet - 45h_Outlet	3.10646308	0.0018934	0.03976139
Cations	Sb	45h_Inlet - 45h_Outlet	2.831398465	0.004634495	0.097324385
Cations	Cu	45h_Inlet - 45h_Outlet	1.795520978	0.072570733	0.943419535
colloidal fraction	Zn	45h_Inlet - 45h_Outlet	3.10646308	0.0018934	0.03976139
colloidal fraction	Sb	45h_Inlet - 45h_Outlet	2.454350521	0.014113933	0.268164727
colloidal fraction	Cr	45h_Inlet - 45h_Outlet	2.416137951	0.015686117	0.298036226
colloidal fraction	As	45h_Inlet - 45h_Outlet	2.347105438	0.0189199	0.321638299
colloidal fraction	Cu	45h_Inlet - 45h_Outlet	2.209040412	0.02717183	0.489092942
lmm fraction	Zn	45h_Inlet - 45h_Outlet	3.184405075	0.001450519	0.030460894
lmm fraction	Sb	45h_Inlet - 45h_Outlet	2.968398054	0.002993564	0.062864841
lmm fraction	Ni	45h_Inlet - 45h_Outlet	2.750847902	0.005944124	0.124826594
lmm fraction	Cu	45h_Inlet - 45h_Outlet	2.209871973	0.027114049	0.460938831
lmm fraction	As	45h_Inlet - 45h_Outlet	1.726462479	0.084264244	1
particulate fraction	As	45h_Inlet - 45h_Outlet	2.900456964	0.00372619	0.0745238
particulate fraction	Sb	45h_Inlet - 45h_Outlet	2.830333028	0.004649958	0.09299915
particulate fraction	Ni	45h_Inlet - 45h_Outlet	2.278072925	0.022722233	0.477166894
particulate fraction	Cu	45h_Inlet - 45h_Outlet	1.587747796	0.11234338	1
particulate fraction	Zn	45h_Inlet - 45h_Outlet	1.656780309	0.097563899	1
total fraction	Sb	45h_Inlet - 45h_Outlet	3.382593131	0.000718049	0.015079035
total fraction	Ni	45h_Inlet - 45h_Outlet	3.349337209	0.000810051	0.01701108
total fraction	Cr	45h_Inlet - 45h_Outlet	2.968398054	0.002993564	0.062864841
total fraction	Zn	45h_Inlet - 45h_Outlet	2.865927715	0.004157892	0.083157835
total fraction	As	45h_Inlet - 45h_Outlet	2.761300515	0.005757168	0.11514335
total fraction	Cu	45h_Inlet - 45h_Outlet	2.62323549	0.008709905	0.16548819

Appendix H

Table 20: Results from Dunns post-hoc test comparing concentrations in tunnel wash water before biofiltration between the hydraulic retention times. Fractions of metals that are not shown in the table were not significant in the previous Kruskal Wallis analysis.

data	Metal	comparison	Z	P.unadj	P.adj
total fraction	Zn	11h_Inlet - 23h_Inlet	-0.077209749	0.938456686	0.938456686
total fraction	Cu	11h_Inlet - 23h_Inlet	-0.077180696	0.938479798	0.938479798
total fraction	As	11h_Inlet - 23h_Inlet	-0.077180696	0.938479798	0.938479798
total fraction	Ni	11h_Inlet - 23h_Inlet	-0.04632585	0.963050536	0.963050536
total fraction	Cr	11h_Inlet - 23h_Inlet	-0.416775757	0.676842436	1
total fraction	Sb	11h_Inlet - 23h_Inlet	0.385903479	0.699568154	1
total fraction	Cr	11h_Inlet - 45h_Inlet	-1.435560941	0.151127316	1
total fraction	Cu	11h_Inlet - 45h_Inlet	-0.787243097	0.431139573	1
total fraction	Zn	11h_Inlet - 45h_Inlet	-0.787539443	0.430966149	1
total fraction	Ni	11h_Inlet - 45h_Inlet	-1.03461064	0.300850783	1
total fraction	As	11h_Inlet - 45h_Inlet	-0.787243097	0.431139573	1
total fraction	Sb	11h_Inlet - 45h_Inlet	-0.509392592	0.610477068	1
total fraction	Cr	23h_Inlet - 45h_Inlet	-1.139036463	0.254687946	1
total fraction	Cu	23h_Inlet - 45h_Inlet	-0.793873898	0.427268846	1
total fraction	Zn	23h_Inlet - 45h_Inlet	-0.79417274	0.427094876	1
total fraction	Ni	23h_Inlet - 45h_Inlet	-1.104935986	0.269187328	1
total fraction	As	23h_Inlet - 45h_Inlet	-0.793873898	0.427268846	1
total fraction	Sb	23h_Inlet - 45h_Inlet	-1.000971437	0.316840618	1
particulate fraction	Cu	11h_Inlet - 23h_Inlet	-0.324158922	0.74581772	1
particulate fraction	Zn	11h_Inlet - 23h_Inlet	-0.447648035	0.65440723	1
particulate fraction	Ni	11h_Inlet - 23h_Inlet	-0.416775757	0.676842436	1
particulate fraction	As	11h_Inlet - 23h_Inlet	-0.169861448	0.8651191	1
particulate fraction	Sb	11h_Inlet - 23h_Inlet	-0.54026487	0.589014382	1
particulate fraction	Cu	11h_Inlet - 45h_Inlet	-0.972476766	0.330813418	1
particulate fraction	Zn	11h_Inlet - 45h_Inlet	-1.157710436	0.246982224	1
particulate fraction	Ni	11h_Inlet - 45h_Inlet	-1.373816384	0.169498693	1
particulate fraction	As	11h_Inlet - 45h_Inlet	-0.880191142	0.378755772	1
particulate fraction	Sb	11h_Inlet - 45h_Inlet	-1.250327271	0.21118002	1
particulate fraction	Cu	23h_Inlet - 45h_Inlet	-0.724841385	0.468549343	1
particulate fraction	Zn	23h_Inlet - 45h_Inlet	-0.793873898	0.427268846	1
particulate fraction	Ni	23h_Inlet - 45h_Inlet	-1.07000395	0.284617531	1
particulate fraction	As	23h_Inlet - 45h_Inlet	-0.79417274	0.427094876	1
particulate fraction	Sb	23h_Inlet - 45h_Inlet	-0.793873898	0.427268846	1
lmm fraction	Cu	11h Inlet-23h Inlet	0.077209749	0.938456686	0.938456686

lmm fraction	Zn	11h_Inlet - 23h_Inlet	-0.422537907	0.672632438	1
lmm fraction	Ni	11h_Inlet - 23h_Inlet	-0.110404052	0.91208894	1
lmm fraction	As	11h_Inlet - 23h_Inlet	-0.231629248	0.816825989	1
lmm fraction	Sb	11h_Inlet - 23h_Inlet	0.571137148	0.567906679	1
lmm fraction	Cu	11h_Inlet - 45h_Inlet	0.787539443	0.430966149	1
lmm fraction	Zn	11h_Inlet - 45h_Inlet	-0.704229845	0.481289647	1
lmm fraction	Ni	11h_Inlet - 45h_Inlet	-0.520476246	0.60273168	1
lmm fraction	As	11h_Inlet - 45h_Inlet	-1.436101336	0.150973508	1
lmm fraction	Sb	11h_Inlet - 45h_Inlet	-0.200669809	0.840956768	1
lmm fraction	Cu	23h_Inlet - 45h_Inlet	0.79417274	0.427094876	1
lmm fraction	Zn	23h_Inlet - 45h_Inlet	-0.314941161	0.752806329	1
lmm fraction	Ni	23h_Inlet - 45h_Inlet	-0.45847465	0.646611471	1
lmm fraction	As	23h_Inlet - 45h_Inlet	-1.346640733	0.178095969	1
lmm fraction	Sb	23h_Inlet - 45h_Inlet	-0.862906411	0.388188925	1
colloidal fraction	Sb	11h_Inlet - 23h_Inlet	0.247350743	0.804636791	0.804636791
colloidal fraction	As	11h_Inlet - 23h_Inlet	0.231542087	0.816893693	0.816893693
colloidal fraction	Zn	11h_Inlet - 23h_Inlet	-0.015436139	0.987684232	0.987684232
colloidal fraction	Cr	11h_Inlet - 23h_Inlet	0.694626262	0.48728955	1
colloidal fraction	Cu	11h_Inlet - 23h_Inlet	0.787243097	0.431139573	1
colloidal fraction	Cr	11h_Inlet - 45h_Inlet	0.169797531	0.865169369	0.865169369
colloidal fraction	Cu	11h_Inlet - 45h_Inlet	0.509392592	0.610477068	1
colloidal fraction	Zn	11h_Inlet - 45h_Inlet	-0.3550312	0.722566217	1
colloidal fraction	As	11h_Inlet - 45h_Inlet	0.941604488	0.346395171	1
colloidal fraction	Sb	11h_Inlet - 45h_Inlet	0.556539171	0.577842324	1
colloidal fraction	Cr	23h_Inlet - 45h_Inlet	-0.58677636	0.557353916	1
colloidal fraction	Cu	23h_Inlet - 45h_Inlet	-0.310646308	0.756069519	1
colloidal fraction	Zn	23h_Inlet - 45h_Inlet	-0.379678821	0.704183843	1
colloidal fraction	As	23h_Inlet - 45h_Inlet	0.793873898	0.427268846	1
colloidal fraction	Sb	23h_Inlet - 45h_Inlet	0.345683172	0.729580832	1
Cations	Cu	11h_Inlet - 23h_Inlet	-0.01544195	0.987679596	0.987679596
Cations	Zn	11h_Inlet - 23h_Inlet	-0.509392592	0.610477068	1
Cations	Sb	11h_Inlet - 23h_Inlet	0.509584345	0.610342694	1
Cations	Cu	11h_Inlet - 45h_Inlet	0.633119944	0.526655319	1
Cations	Zn	11h_Inlet - 45h_Inlet	-0.848987653	0.395888163	1
Cations	Sb	11h_Inlet - 45h_Inlet	-0.633119944	0.526655319	1
Cations	Cu	23h_Inlet - 45h_Inlet	0.725114241	0.468381948	1
Cations	Zn	23h_Inlet - 45h_Inlet	-0.379678821	0.704183843	1
Cations	Sb	23h_Inlet - 45h_Inlet	-1.277582234	0.201396768	1
Anions	Cu	11h_Inlet - 23h_Inlet	0.077209749	0.938456686	0.938456686
Anions	Zn	11h_Inlet - 23h_Inlet	-0.420108112	0.674406477	1
Anions	Ni	11h_Inlet - 23h_Inlet	-0.110404052	0.91208894	1

Anic	ons As	11h_Inlet - 23h_Inle	et -0.262414366	0.793001999	1
Anic	ons St	11h_Inlet - 23h_Inle	et 0.154419499	0.87727898	1
Anic	ons Cu	11h_Inlet - 45h_Inle	et 0.787539443	0.430966149	1
Anic	ons Zn	11h_Inlet - 45h_Inle	et -0.700180187	0.483814784	1
Anic	ons Ni	11h_Inlet - 45h_Inle	et -0.520476246	0.60273168	1
Anic	ons As	11h_Inlet - 45h_Inle	et 1.435560941	0.151127316	1
Anic	ons St	11h_Inlet - 45h_Inle	et -0.648561894	0.516621593	1
Anic	ons As	23h_Inlet - 45h_Inle	et 1.898394104	0.057644186	0.979951157
Anic	ons Cu	23h_Inlet - 45h_Inle	et 0.79417274	0.427094876	1
Anic	ons Zn	23h_Inlet - 45h_Inle	et -0.313130099	0.754181823	1
Anic	ons Ni	23h_Inlet - 45h_Inle	et -0.45847465	0.646611471	1
Anic	ons St	23h_Inlet - 45h_Inle	et -0.897760489	0.369313253	1

Appendix I

Table 21: Shows the percentage of samples below LOD and LOQ for digested (total) samples. The table includes samples from all hydraulic retention times.

			Raw tunnel wash		Biofilter	Biofiltered tunnel		ed tunnel
			W	ater	wash	water	wash	water
	LOD in (w/V)	LOQ in (w/V)	% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<></td></loq<>	% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<>	% <loq< td=""><td>% <lod< td=""></lod<></td></loq<>	% <lod< td=""></lod<>
Samples	Total	Total	Total	Total	Total	Total	0.45um- filtered	0.45um- filtered
Na (mg/L)	0.03	0.11	0%	0%	0%	0%	0%	0%
Mg (mg/L)	0.001	0.0047	0%	0%	0%	0%	0%	0%
Al (µg/L)	1	4	0%	0%	0%	0%	0%	0%
P (µg/L)	300	980	100%	100%	100%	100%	100%	100%
S (mg/L)	0.03	0.089	0%	0%	0%	0%	0%	0%
K (mg/L)	0.04	0.13	0%	0%	0%	0%	0%	0%
Ca (mg/L)	0.007	0.022	0%	0%	0%	0%	0%	0%
Cr (µg/L)	0.3	0.96	0%	0%	0%	0%	0%	0%
Mn (µg/L)	0.04	0.15	0%	0%	0%	0%	0%	0%
Fe (µg/L)	7	22	0%	0%	0%	0%	0%	0%
Ni (µg/L)	0.1	0.34	0%	0%	0%	0%	0%	0%
Cu (µg/L)	2	7.3	0%	0%	0%	0%	0%	0%
Zn (µg/L)	0.2	0.8	0%	0%	0%	0%	0%	0%
As (µg/L)	0.004	0.013	0%	0%	0%	0%	0%	0%
Sb (µg/L)	0.005	0.016	0%	0%	0%	0%	0%	0%

Samples filtered by 0.45 $\mu m.$ Includes samples from all hydraulic retention time-tests.

					Biofiltered	tunnel wash	Oxygenated tunnel		
			wash wat	er	water		wash wat	er	
	LOD in	LOQ in	% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<></td></loq<>	% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""></lod<></td></loq<></td></lod<>	% <loq< td=""><td>% <lod< td=""></lod<></td></loq<>	% <lod< td=""></lod<>	
	w/V	w/V	-		-		-		
Na (mg/L)	0.002	0.0055	0%	0%	0%	0%	0%	0%	
Mg (mg/L)	0.0004	0.0013	0%	0%	0%	0%	0%	0%	
Al (µg/L)	1	3.2	0%	0%	0%	0%	0%	0%	
Ρ (µg/L)	0.4	1.2	0%	0%	0%	0%	0%	0%	
S (mg/L)	0.001	0.004	0%	0%	0%	0%	0%	0%	
K (mg/L)	0.002	0.0077	0%	0%	0%	0%	0%	0%	
Ca (mg/L)	0.05	0.18	0%	0%	0%	0%	0%	0%	
Cr (µg/L)	0.02	0.053	0%	0%	0%	0%	0%	0%	
Mn (µg/L)	0.005	0.017	0%	0%	0%	0%	0%	0%	
Fe (µg/L)	0.1	0.33	0%	0%	0%	0%	0%	0%	
Ni (µg/L)	0.07	0.23	0%	0%	0%	0%	0%	0%	
Cu (µg/L)	0.1	0.37	0%	0%	0%	0%	0%	0%	
Zn (µg/L)	0.06	0.18	0%	0%	0%	0%	0%	0%	
As (µg/L)	0.0002	0.00077	0%	0%	0%	0%	0%	0%	
Sb (µg/L)	0.0009	0.0029	0%	0%	0%	0%	0%	0%	

<10kDa	LOD	LOQ	% <loq< td=""><td>%<lod< td=""></lod<></td></loq<>	% <lod< td=""></lod<>					
Na (mg/L)	0.002	0.0059	0%	0%					
Mg (mg/L)	0.001	0.0032	0%	0%					
Al (µg/L)	1	4.8	0%	0%					
Ρ (μg/L)	0.3	1.1	0%	0%					
S (mg/L)	0.002	0.0074	0%	0%					
K (mg/L)	0.003	0.0096	0%	0%					
Ca (mg/L)	0.03	0.12	0%	0%					
Cr (µg/L)	0.008	0.027	0%	0%					
Mn (µg/L)	0.01	0.032	0%	0%					
Fe (µg/L)	0.7	2.3	0%	0%					
Ni (µg/L)	2	5.4	0%	0%					
Cu (µg/L)	0.02	0.08	0%	0%					
Zn (µg/L)	2	5.6	0%	0%					
As (µg/L)	0.002	0.0051	0%	0%					
Sb (µg/L)	0.0005	0.0016	0%	0%					

			45h outlet		23h outlet		11h outlet	
	LOD	LOQ	% <loq< td=""><td>% <lod< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""></loq<></td></lod<></td></loq<></td></lod<></td></lod<></td></loq<>	% <lod< td=""><td>% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""></loq<></td></lod<></td></loq<></td></lod<></td></lod<>	% <lod< td=""><td>% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""></loq<></td></lod<></td></loq<></td></lod<>	% <loq< td=""><td>% <lod< td=""><td>% <loq< td=""></loq<></td></lod<></td></loq<>	% <lod< td=""><td>% <loq< td=""></loq<></td></lod<>	% <loq< td=""></loq<>
Na (mg/L)	0.002	0.0059	0%	0%	0%	0%	0%	0%
Mg (mg/L)	0.001	0.0032	0%	0%	0%	0%	0%	0%
Al (µg/L)	1	4.8	0%	0%	0%	0%	0%	0%
P (µg/L)	0.3	1.1	0%	0%	0%	0%	0%	0%
S (mg/L)	0.002	0.0074	0%	0%	0%	0%	0%	0%
K (mg/L)	0.003	0.0096	0%	0%	0%	0%	0%	0%
Ca (mg/L)	0.03	0.12	0%	0%	0%	0%	0%	0%
Cr (µg/L)	0.008	0.027	0%	0%	0%	0%	0%	0%
Mn (µg/L)	0.01	0.032	0%	0%	0%	0%	0%	0%
Fe (µg/L)	0.7	2.3	0%	0%	0%	0%	0%	0%
Ni (µg/L)	2	5.4	100%	0%	0%	0%	0%	100%
Cu (µg/L)	0.02	0.08	0%	0%	0%	0%	0%	0%
Zn (µg/L)	2	5.6	33%	66%	0%	33%	0%	66%
As (µg/L)	0.002	0.0051	0%	0%	0%	0%	0%	0%
Sb (µg/L)	0.0005	0.0016	0%	0%	0%	0%	0%	0%

LOD LOQ		Inlets all 45h outlet		23h outlet		11h outlet		Oxygenated samples				
			%	%	%	%	%	%	%	%	%	%
			<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""></lod<></td></loq<>	<lod< td=""></lod<>
Na (mg/L)	0.09	0.3	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Mg (mg/L)	0.006	0.02	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Al (µg/L)	0.9	3.1	14%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Ρ (µg/L)	0.6	2	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
S (mg/L)	0.007	0.024	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
K (mg/L)	0.02	0.059	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Ca (mg/L)	0.07	0.23	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Cr (µg/L)	0.01	0.046	14%	0%	0%	67%	33%	0%	0%	0%	33%	33%
Mn (µg/L)	0.05	0.16	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Fe (µg/L)	0.4	1.4	43%	29%	0%	0%	0%	0%	33%	0%	66%	0%
Ni (µg/L)	6	19	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
Cu (µg/L)	4	13	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
Zn* (µg/L)	0.1	0.42	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
As (µg/L)	0.003	0.011	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Sb (µg/L)	0.001	0.004 6	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Anion-exchanged samples. "Inlets all" = raw tunnel wash water. "Outlets" = Tunnel wash water samples after biofiltration are divided by hydraulic retention time.

*LOD and LOQ of Zn was only based on two method blanks

					Inlets 11h	45h	45h outlet		23h outlet		Oxyg	genated
	LOD	LOQ	%	%	%	%	%	%	%	%	%	%
			<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<></td></loq<>	<lod< td=""><td><loq< td=""><td><lod< td=""></lod<></td></loq<></td></lod<>	<loq< td=""><td><lod< td=""></lod<></td></loq<>	<lod< td=""></lod<>
Na	8	26	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(mg/L)												
Mg	0.002	0.006	66%	0%	33%	0%	100%	0%	33%	0%	33%	0%
(mg/L)												
Al	1	4.5	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)												
P	0.5	1.6	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)	0.005	0.045	00/	00/	00/	00/	00/	00/	00/	00/	00/	00/
5 (mg/l.)	0.005	0.015	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(IIIg/L) K	0 1	0.30	0%	1106	0%	330%	0%	3306	0%	230/	0%	0%
(mg/l)	0.1	0.55	0.70	1170	070	3370	070	5570	070	55%	070	070
(116/E) Ca	0.05	0.16	33%	0%	33%	0%	66%	0%	0%	0%	33%	0%
(mg/L)	0.00		0070	0,0	0070	0,0	0070	0,0	0,0	0,0	00,0	0,0
Cr	0.02	0.066	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)												
Mn	0.009	0.031	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)												
Fe	0.1	0.47	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)												
Ni	0.02	0.081	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)												
Cu	0.04	0.15	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)												
Zn	0.3	1	0%	0%	33%	0%	0%	0%	0%	0%	0%	0%
(µg/L)												
As	0.002	0.005	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)	0.000	9	00/	00/	00/	00/	00/	00/	00/	00/	00/	00/
SD	0.002	0.005	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(µg/L)		2			1		1					

Cation-exchanged samples. "Inlets" = raw tunnel wash water, divided by corresponding hydraulic retention time. "Outlets" = Tunnel wash water samples after biofiltration are divided by hydraulic retention time.

Appendix J

Table 22: Shows results from modelling for sulphide precipitation done in visual MINTEQ.

Equili	brated mass distri	bution				
Component	Total dissolved	% dissolved	Total sorbed	% sorbed	Total precipitated	% precipitated
Al+3	8.4132E-07	100	0	0	0	0
AsO4-3	1.0294E-08	100	0	0	0	0
Ca+2	0.00073103	100	0	0	0	0
Cr(OH)2+1	2.8079E-08	100	0	0	0	0
Cu+2	2.1432E-26	0	0	0	1.1645E-07	100
Fe+3	6.3298E-06	100	0	0	0	0
H+1	0.00031415	100	0	0	0	0
HS-1	0.00065402	99.725	0	0	1.8066E-06	0.275
K+1	0.00025574	100	0	0	0	0
Mg+2	0.00016864	100	0	0	0	0
Mn+3	2.1478E-06	100	0	0	0	0
Na+1	0.009265	100	0	0	0	0
Ni+2	4.4979E-15	0	0	0	1.3286E-07	100
PO4-3	6.7476E-07	100	0	0	0	0
Sb(OH)6-1	4.5914E-08	100	0	0	0	0
SO4-2	2.3884E-29	100	0	0	0	0
Zn+2	3.3355E-13	0	0	0	1.5573E-06	100

- Solid Equilibrium amount (mol/l)
- Covellite 1.1645E-07
- NiS (gamma) 1.3286E-07
- Spharelite 1.5573E-06

Appendix K

The volume of filter material needed for a full-scale facility

Pumping rate (45-hour HRT) = $50L/d = 0.05 \text{ m}^3/d$ Pumping rate (11-hour HRT) = $200L/d = 0.2 \text{ m}^3/d$ Volume biofilter material used in pilot = $141L = 0.141\text{ m}^3$ The volume of water in a tunnel wash = 170m^3 V = Volume of filter material needed to the treatment of all tunnel wash water in one day

> V = Volume of water in TW ((pumping rate)/(volume of filtermat in pilot))

Sorption capacity

Volume of woodchips used in pilot = 0.8*141.3L = 113 L (Appendix A).

Assuming a dry weight of wood chips of 208kg/m3 = 208g/L, the biofilter in the present study contained about 23504g = 23.5kg of dry-weight chip wood. Hence, using the lowest reported qmax for Zn of 1.46 mg/g sawdust, the biofilter would be able to sorb a total of about 23504mg of Zn before saturation would occur. The biofilter removed about 90 μ g/L of LMM+colloids of Zn in the 45h residence time test = 4320ug/day = 4.32mg/day. Hence, the biofilter could operate for about (23504/4.92)/365 = 13 years.

Appendix L

Colloidal and LMM fraction:



Figure 25: Biplot for PCA on change for size-fractionated samples in the colloidal and LMM fraction. Red arrows mark the present study's metals and metalloids of particulate interest.



Figure 26: Loading plot for PCA on change for size-fractionated samples in the colloidal and LMM fraction. Red arrows mark the present study's metals and metalloids of particulate interest.

LMM fraction only:



Figure 27: Biplot for PCA on change for size-fractionated samples in the LMM fraction only. Red arrows mark the present study's metals and metalloids of particulate interest.



Figure 28: Biplot for PCA on change for size-fractionated samples in the LMM fraction only. Red arrows mark the present study's metals and metalloids of particulate interest.



Norges miljø- og biovitenskapelige universitet Noregs miljø- og biovitskapelege universitet Norwegian University of Life Sciences Postboks 5003 NO-1432 Ås Norway