

# Microplastics in water and sediment of artificial sedimentation ponds and natural lakes in the municipality of Bø i Telemark (Norway)

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## ABBREVIATURES

ANOVA: Analysis Of Variance	PAH: Polycyclic Aromatic Hydrocarbons
ATR-FTIR: Attenuated Total Reflection- FTIR	PBDE: Polybrominated Diphenyl Ethers
Bø: Bø i Telemark	PCB: Polychlorinated Biphenyl
D: average depth	PERMANOVA: Permutational analysis of variance
d.w.: dry weight	PET: polyethylene terephthalate
EC: Electrical conductivity	PP: polypropylene
e.g: <i>exempli gratia</i>	PS: polystyrene
et al.: <i>et alia</i>	P <sub>T</sub> : Total phosphorous
etc.: etcetera	PTFE: polytetrafluoroethylene
EVA: ethylene-vinyl acetate	PVC: polyvinyl chloride
FTIR: Fourier Transform Infrared Spectroscopy	Q: flow rate
HCA: Hierarchical cluster	RO: Reverse osmosis
HPDE: high-density polyethylene	S: richness
HOC: Hydrophobic Organic Chemicals	T: Temperature
i.e: <i>id est</i>	TOC: Total Organic Carbon
L.: lake	UK: United Kingdom
LDPE: low-density polyethylene	US: United States
max: maximum	USN: University of South-Eastern Norway
min: minimum	UV: ultra-violet
n: sampling size	V: average speed
NIVA: Norsk Institutt for Vannforskning (Norwegian Institute for Water Research)	W: average width
NMDS: Non-metric multidimensional scaling	w.w.: wet weight
N <sub>T</sub> : Total nitrogen	WWTP: Wastewater treatment plant



## UNITS AND SYMBOLS

>: higher than, above	$\mu\text{g/L}$ : micrograms per litre
<: lower than, below	$\mu\text{m}$ : micrometres
=: equals to	$\mu\text{S/cm}$ : micro siemens per centimetre
x: multiplication sign	M: molar
-: minus	m: metres
%: percentage	$\text{m}^2$ : square metre
$\Sigma$ : sigma, summation symbol	m/s: metres per second
$^{\circ}\text{C}$ : Celsius degrees	$\text{m}^3/\text{s}$ : cubic metres per second
$\text{Fe}(\text{OH})_3$ : ferric oxyhydroxide	mg/L: milligrams per litre
g: gram	mg Pt/L: milligrams of platinum per litre
$\text{g/cm}^3$ : gram per cubic centimetre	mL: millilitre
h: hours	mm: millimetres
$H'$ : Shannon index	min: minutes
$\text{H}_2\text{O}_2$ : hydrogen peroxide	$n^{\circ}$ : number of
ha: hectarea	NaCl: sodium chloride
items/kg: items per kilogram	NaI: sodium iodide
items/L: items per litre	nm: nanometre
items/s: items per second	NTU: Nephelometric Turbidity Unit
kg: kilogram	$p$ : p-value
km: kilometre	r: Pearson correlation coefficient
$\text{km}^2$ : square kilometre	s: second
L: litre	v/v: volume per volume
L/s: litres per second	X: magnification
$\text{L}/(\text{s}\cdot\text{km}^2)$ : litres per second per square kilometre	$\text{ZnCl}_2$ : Zinc chloride
Log: Logarithm	



## ABSTRACT

Microplastics (plastic particles between 1  $\mu\text{m}$  and 5 mm) are emerging contaminants of relatively new concern due to their ubiquitous occurrence and their ability to interact with other environmental pollutants and biological elements. Rivers and other freshwater systems are believed to be the major sources of microplastics to the oceans, however, less research has been conducted in these environments. The main goal of this Master's thesis has been addressed to study the microplastic pollution in some sedimentation ponds built in the municipality of Bø i Telemark (Norway). These constructed wetlands were previously made to stop the nutrient diffuse pollution of the area, although they can be proposed as an alternative to prevent further plastic urban and agricultural pollution of freshwater ecosystems. Additionally, two natural lakes nearby Bø were selected as reference sites to compare with the sedimentation ponds. Results showed high concentrations of microplastics in both sediment (4784-37232 items/kg) and water compartments (18-45 items/L). Microplastic abundances in the sediment of the lakes were higher, possibly due to the frequent clothing airborne contamination. Additionally, it was demonstrated that these constructed sedimentation ponds are not enough to stop either microplastic nor nutrient pollution, and thus, becoming a source of pollution for the following aquatic systems. Fibres and fragments were the most abundant microplastics in both sediment and water samples. Fibres are likely to derive from wastewater treatment plants because of their small size, agricultural runoff after fibre-containing sludge application and wind disposal. Otherwise, fragments probably come from urban littering, road and storm runoff and in situ microplastic breakdown. Finally, due to the lack of standard methods on microplastic research, this study proposes a method based on sieving, organic matter removal (Fenton's reagent) and density separation (both filtered distilled water and  $\text{ZnCl}_2$  solutions) for sediment analysis and filtration for water analysis.

**Keywords:** microplastics, sediment, water, streams, lakes, sedimentation ponds, Norway



## 1. INTRODUCTION

The term *plastics* refers to a wide range of synthetic or semi-synthetic materials that are used for a huge number of applications. Plastics are organic materials which come from natural products such as cellulose, coal, natural gas, salt, and crude oil. The crude oil is processed in the oil refineries to produce plastics through two main methods: polymerization and polycondensation (PlasticsEurope, 2019). There are approximately 30,000 different polymers registered in the European Union, each with its own physical-chemical properties (i.e density, plasticity, etc.) (Horton et al., 2017b).

Plastics are a common material used in packaging (39.5 % total plastic production), building and construction (20.1%), mobility and transport (8.6%), electronic components (5.7%) agricultural materials (3.4%) and others such as household appliances, clothes, sporting equipment, healthcare inventions and energy industry (Horton et al. 2017b; PlasticsEurope 2019). Nowadays, 280 million tonnes of plastic are produced annually, and the forecasts predict the plastic production will increase to 33 billion tonnes by 2050 (Rochman et al., 2013). Plastic wastes constitute up to 54% by mass of the total human wastes (Hoellein et al., 2014) and, unfortunately, 10% of discarded plastics end up in the ocean either by being intentionally or unintentionally (Cole et al., 2011) posing a threat to the ecosystems.

Plastic debris is an environmentally persistent complex and ubiquitous contaminant (Bergmann et al., 2019; Horton et al., 2017b). Characteristics such as impermeability, durability and resistance make plastics a pervasive pollutant in the environment (Barnes et al., 2009, Imhof et al., 2013). In fact, their high molecular weight, hydrophobicity and cross-linked structure prevent plastics from biodegradation (Gautam et al., 2007; Shah et al., 2008). However, and despite being a slow process, plastics can breakdown in smaller pieces due to the biological activity and weathering. The exposure to UV radiation, mechanic abrasion, waves, wind and temperature fluctuation are the main reasons that cause plastic fragmentation (Horton et al., 2017b; Lin et al., 2018; Nor and Obbard, 2014) while their fate in the environment will be mainly regulated by their density.

The density will determine whether plastic debris float or sink in both freshwater and marine environments (Horton et al., 2017b). However, changes in the density through aging, weathering or biofouling can make buoyant plastics sink or make the high-density plastics float. Hydrological factors and strong currents can also perturb the bottom and mobilize the particle which had already sunk (Van Cauwenberghe et al., 2015). Some common buoyant plastics in freshwater ecosystems (density < 1 g/cm<sup>3</sup>) are polystyrene (PS), polypropylene (PP), low-density polyethylene (LDPE), ethylene-vinyl acetate (EVA) or high-density polyethylene (HDPE). Other plastics such as polytetrafluoroethylene (PTFE), polyvinyl chloride (PVC) or polyethylene terephthalate (PET) tends to sink due to the high density (1.38-2.30 g/cm<sup>3</sup>) (Frias et al. 2018).

Plastic wastes found in the environment can be classified by size. They can be distinguished as megaplastics (> 100 mm), macroplastics (> 20 mm), mesoplastics (20-5 mm) and microplastics (< 5 mm). Microplastic particles were

observed at the first time in the marine environment in the early 1970s (Buchanan, 1971; Carpenter and Smith, 1972), but it was not until thirty years after, in 2004, when the term *microplastics* became accepted (Thompson et al. 2004). Microplastics have been defined as plastic debris less than 5 mm in the largest dimension (Cole et al., 2011, Eerkes-Medrano et al., 2015). However, microplastics can still break into smaller particles hence, called *nanoplastics* (plastic particles lower than 1  $\mu\text{m}$ ) (Horton et al. 2017b).

Moreover, microplastics can be differentiated between primary and secondary microplastics (Horton et al., 2017a). Primary microplastics are those manufactured with a small size to use as plastic pellets, the virgin material for plastic manufacturing (*nurdles*), in cosmetics (e.g. microbeads of exfoliant products and glitter) and abrasive products (air blasting) (Prata et al., 2019). However, secondary microplastics come from the fragmentation of larger plastics due to photodegradation, physical (i.e. wind, waves, sand, etc.) and chemical weathering and biodegradation (Lin et al., 2019). Secondary microplastics also derived from the loss of synthetic textile fibres from the laundry systems of fabrics and houses that pass unchanged through the wastewater treatment plants (WWTPs) (Horton et al., 2017a). Secondary microplastics can be divided into two categories: large microplastic particles, from 1 to 5 mm and small microplastic particles, with a size lower than 1 mm (Andrady, 2011; Koelmans et al., 2015; Lambert and Wagner, 2016).

Research on the occurrence of microplastics have been mostly addressed to the marine environments, although their pervasive occurrence is equally in terrestrial and freshwater ecosystems (Eerkes-Medrano et al., 2015; Vaughan et al., 2017;). Microplastics have been reported from the shores of isolated uninhabited islands, sea surface, water column and deep ocean floor (Bergmann et al., 2017a). They have also been found in rivers and lakes close to urban sites (Horton et al., 2017a; Lin et al., 2018; Vaughan et al., 2017) and remote areas (Free et al., 2014). They have also reached polar areas as well including their beaches (Bergmann et al., 2017b), the sea ice (Obbard et al., 2014), the water column (Grøsvik et al., 2018), the sea surface and the seafloor (Lusher et al., 2015). Recently, microplastics were discovered in the Arctic snow and in some European mountains too (Bergmann et al., 2019). In addition, microplastics have been detected in the atmospheric fallout (Cai et al., 2017; Dehghani et al. 2017) and even in the rain (Wetherbee et al., 2019). This suggests that microplastics can travel and spread across the Earth through aerial transport, ocean currents, rivers and other waterways (Horton et al., 2017b; Bergmann et al. 2019).

The presence of microplastics and plastics, in general, in the aquatic environment suppose a risk to the organisms (Vaughan et al. 2017). On the one hand, the entanglement of the animals in drifting fishing nets, plastic bags or other mega- and macroplastic debris can produce external and internal lacerations or injuries, suffocation and death (Vaughan et al., 2017). Microplastics are known to reduce the reproduction, growth, fitness, feeding and the energy intake since animals confuse microplastics with food (Horton et al., 2017b). On the other hand, microplastics can release the toxic additives (e.g. Bisphenol A, phthalates, metals) (Nor and Obbard, 2014) that were added during manufacturing or can attach pollutants from the environment and transfer through the food

web (Lin et al., 2018). Microplastics can bind hydrophobic organic chemicals (HOCs) such as organochlorine pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dioxins, metals (Horton et al., 2017b) and brominated flame retardants (e.g PBDEs) (Zarfl and Matthies, 2010). Plastics can also behave as a vector of invasive and pathogen species as they are novel habitats for colonizing (Zettler et al., 2013).

Unfortunately, scientists must deal with the lack of universal and validated methods to analyse microplastics. There is a large variety of microplastic collectors (e.g nets, pumps, dredges, sediment corers), extraction methods (e.g digestion, density separation, organic matter removal), quantifying and identifying methods (e.g visual, Fourier Transform Infrared Spectroscopy (FTIR), Raman, dyes). Each research group must decide which methods to use and which modifications to make, therefore, compromises the interpretation, comparability and reproducibility of the results. Thus, there is an urgent need for creating a standard methodology which also establishes which units to use to report the findings (Prata et al., 2019).

Sources of microplastics are wide and numerous. Primary microplastics can enter in the environment directly through the accidental spillage of nurdles, or indirectly, through the sewage treatment works via effluents and sludge application in terrestrial systems. Due to their small size, most of microfibrils cannot be eliminated in the WWTPs and so are released to the environment (Horton et al., 2017b). Microfibrils and microbeads deposit in the sludge due to their high density. Sludge is commonly used as fertilizer in agricultural land in Europe (DEFRA, 2012). On average, four or five million tonnes of dry weight of sewage sludge are applied to land annually in Europe (Cieřlik et al., 2015), and thus, applying between 63,000 and 430,000 tonnes of microplastics to land per year (Nizzetto et al., 2016). Although there exist regulations of harmful substances within sludge applied to land, they do not apply for microplastics as they are not considered as such (Horton et al., 2017b). The soil erosion, runoff and wind dispersal action make microplastics reach marine and freshwater ecosystems (Horton et al., 2017a, b). Actually, Zubris and Richards (2005) found that soils with a long history of sludge application contained a significantly higher number of fibrils than soils without receiving sludge treatment, even after 15 years of the last sewage sludge application.

Sources of other secondary microplastics vary from releases during municipal solid waste collection and transport either accidentally or intentionally, landfills, wind action transporting light plastic items, airborne particles and fallout, fragmentation of typical agricultural plastics (e.g plastic mulches, polytunnels, seed polymer coating) sewage overflows, and runoff from drainage ditches in agricultural areas and roads (Horton et al., 2017b). Plastics from roads include litter, tyre wear particles, vehicle-derived fragments and road-marking paints (Horton et al., 2017a, b). A study carried out in the Thames river in UK (Horton et al., 2017a) proved that sewage, road and *in situ* degradation of litter were the main source of microplastics in urban areas.

The municipality of Bø i Telemark (Telemark, Norway) has built several sedimentation-pond constructed wetlands to reduce the diffuse pollution, mainly caused by the agricultural and road runoff. The initial purpose was to reduce the nutrient and organic content water enrichment of the main streams that flow throughout the town.

Constructed wetlands are ecological and technological solutions to remove contaminants from non-point pollution sources and water recycling (Li et al., 2019, Sun et al., 2017). Artificial sedimentation ponds are a specific type of constructed wetlands which Biggs et al. (2005) defined as natural and engineered water masses between 1 m<sup>2</sup> and 2 ha, permanent or temporary, that may clean the water before discharges in a natural water body. They are thought to function as part of the urban drainage system reducing the pollution from wastewater effluents, agricultural runoff or storm drain and preventing from flooding (Sun et al., 2017). Some studies (Karlsson et al., 2010; Sun et al., 2017; Vollertsen et al., 2007) have demonstrated that sedimentation ponds can remove nutrients and accumulate high levels of pollution since they retain metals and organic pollutants. Consequently, sedimentation ponds are proposed as a natural microplastic removal method, thus, preventing from their input in aquatic ecosystems.

Based on this assumption, it is hypothesized that the sedimentation ponds are retaining microplastics as well as nutrients. If microplastics are present in both water and sediment, most of them should be fibres, microbeads and fragments, as a consequence of their pollution sources such as urban wastewater, and agricultural and road runoff. Indeed, it is expected to find low-density microplastics in the water and high-density microplastics in the sediment. Additionally, it is also expected that these sedimentation ponds, immersed in a village, contain a larger number of microplastics than remote places far from urban areas. Therefore, two forest lakes close to Bø i Telemark and far from human presence were studied in the same way to confirm this hypothesis.

The objectives of this master project are 1) to assess the efficiency of the sedimentation ponds built at Bø i Telemark to retain microplastics analysing the sediment and water 2) to report the occurrence, abundance and diversity of microplastics at both water and sediment compared with two reference lakes 3) to study the water chemistry parameters to look for possible relationships with the presence/abundance of microplastics and pollution sources, and finally 4) assess the efficiency of the laboratory method used to extract microplastics.

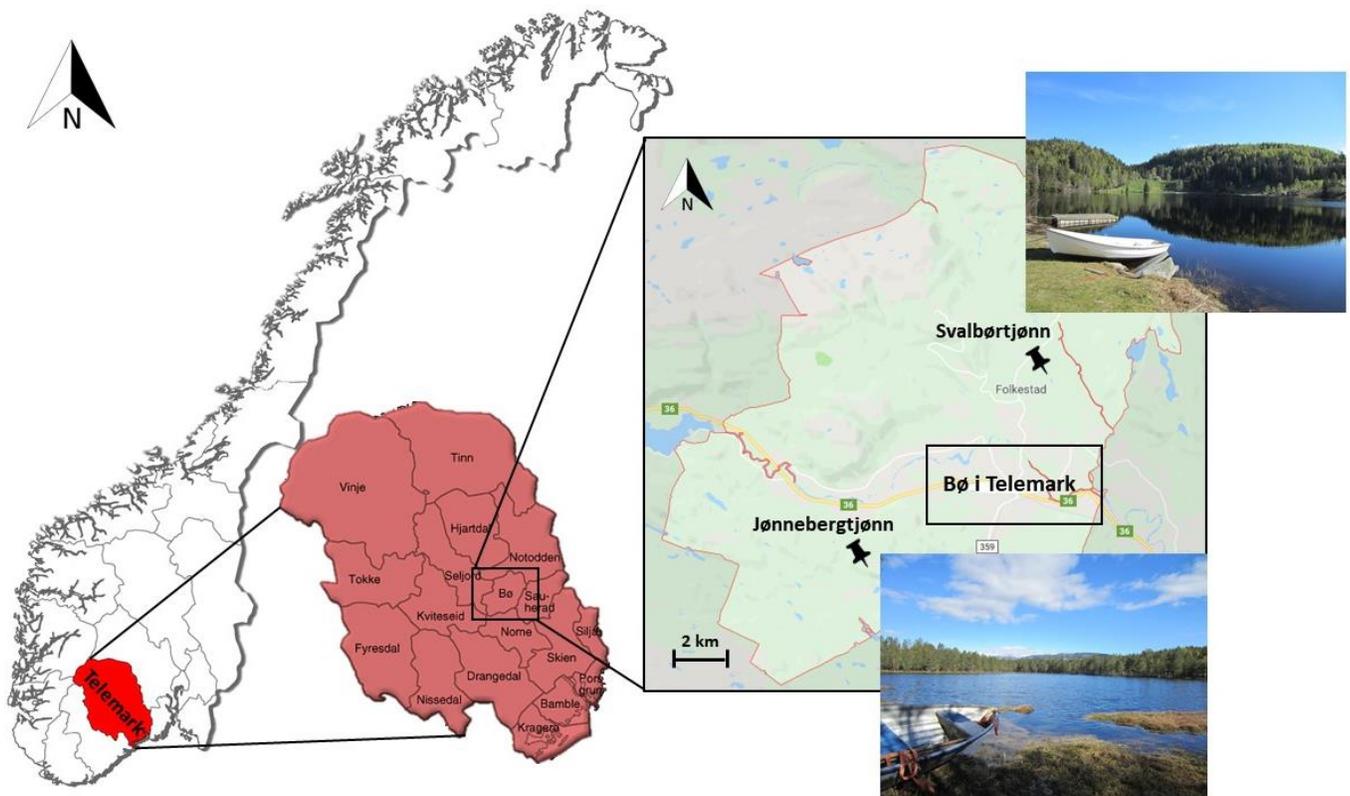
## 2. MATERIALS AND METHODS

### 2.1 Description of the area

The study was conducted in the South-Eastern region of Norway, specifically in the municipality of Bø i Telemark (here and after Bø) which belongs to the county of Telemark (figure 1). The area of Bø municipality is around 263 km<sup>2</sup> and from those, 258 km<sup>2</sup> correspond to land. Currently, the size of the population is about 6460 inhabitants (Citypopulation, 2019). Bø is an independent municipality since 1838 and its main economic activities are agriculture, forestry, tourism and education with one of the campuses of the University of South-Eastern Norway (USN).

The research was focused on occurrence of microplastics in two natural lakes (Lake Jønnebergjønn and Lake Svalbjørtjønn) and several artificial ponds built in two different streams, Presteevju and Borgjaevju, that go across

the village of Bø. Both lakes are small forest lakes located in remote areas (figure 1) far from human activities, so they were used as reference sites for microplastics. L. Jønnebergjønn (UTM 32 N 6583294, 497780) is located around 10 km west far from Bø, 365 m above the sea level (Mapcarta, 2019) with an area of approximately 3 ha and an average depth of 7 m (max: 17m). There is a narrow road close to L. Jønnebergjønn. Conversely, L. Svalbjørtjønn (UTM 32 N 0509554, 6594405) is situated around 16 km north of Bø, having an area of 5.6 ha, and 5 m of average depth (max: 10 m) (Mapcarta, 2019). Moreover, there are some groundwater springs in this lake. Even this lake is relatively isolated in a forest area, there were some houses surrounding the lake and moorings for boats. Both bathymetric maps of L. Jønnebergjønn and L. Svalbjørtjønn can be found in annex 1.



**Figure 1.** Study area with the location of Bø i Telemark and lakes Jønnebergjønn and Svalbjørtjønn (pushpins) in the county of Telemark in the Southeastern Norway (maps taken from alamy. com and Google Maps. Photos by Ariadna García-Astillero Honrado).

The streams Presteevju and Borgjaevju are immersed in the Bøelva watercourse (Vassdragsnr: 016.CA4). The streamflow of Bøelva watercourses is about  $8.8 \text{ L}/(\text{s}\cdot\text{km}^2)$ . The climate of this latter river basin is characterized with an annual precipitation of 887 mm, with summer and winter precipitation of 430-457 mm respectively, and an annual temperature of  $4.3 \text{ }^\circ\text{C}$ , with summer and winter temperatures of  $12.2$  and  $-1.3 \text{ }^\circ\text{C}$ . The area of this river basin measures  $18 \text{ km}^2$  and the main composition of the land area is forest (62.3 %), agriculture (25.2%), urban (4.9%) and swamp (0.1%) (NVE, 2019). Additional data is present in table 1.

**Table 1. Summary of information about Bøelva drainage area (climate, flow and field parameters) (NVE, 2019).**

Bøelva drainage area			
Climate		Flow and field parameters	
Region	South	Streamflow	8.8 L/(s · km <sup>2</sup> )
Annual precipitation	887 mm	Baseflow	3.9 L/(s · km <sup>2</sup> )
Summer precipitation	430 mm	Area	18 km <sup>2</sup>
Winter precipitation	457 mm	River length	6.8 km
Annual temperature	4.3 °C	Field length	4.4 km
Summer temperature	12.2 °C	Agriculture	25.2 %
Winter temperature	-1.3 °C	Marsh	0.1%
Temperature July	14.7 °C	Forest	62.3 %
Temperature August	14.1°C	Urban	4.9 %

These streams are under the influence of human activities, and therefore, their ecological status is moderate. The main impacts reported for both Presteevju and Borgjaevju streams are the agriculture and the cattle that produce diffuse pollution, increasing the amount of nutrients and organic matter in the water, and modifications in the morphology of the river. Other impacts are the lack of connection of some houses to the WWTPs upstream, the influence of the roads nearby and the existence of unknown discharge points (Vann-nett, 2019).

Moreover, a great part of the Scandinavian peninsula was submerged under the ocean during the Quaternary period due to the sea-level increase after glaciation. Due to post-accumulation of dead planktonic organisms and deposition of marine sediments in the seafloor, and the latter arising of the continent because of isostatic movements, the current sediments of the South of Norway are rich in nutrients and naturally lead to eutrophication in aquatic ecosystems (Low and Walker, 1997; NGU, 2019).

Due to the cattle pollution and the already nutrient-rich-nature of the sediments of the South area of Norway, some measures were taken to reduce the eutrophication downstream. Consequently, a purification park was built in the Presteevju stream in the late 1990s nearby Bø. This purification park consists of three artificial sedimentation ponds (A, B and C) (figure 2) where the stream can flood and the water is cleaned by passing through the ponds. In these sedimentation ponds, the water increases its residence time and thus the organic and inorganic particles can increase its sedimentation rates (Bakke et al., 1997). In addition, nutrients can be absorbed by the plants that grow in the shores of the riverbed. Conversely to expected, the system did not work at all as a three-year-investigation study demonstrated in 2004 (Kleiven, 2005).

A new purification park was built in the Borgjaevju stream in 2014. This park also consists of three artificial sedimentation ponds (A, B and C) which are larger than those at Presteevju stream, and can probably be more efficient trapping organic matter and nutrients. The ponds were also considered to stop the erosion of the riverbed, and thus, the eutrophication. These sedimentation ponds are close to the USN University and receive the water from the Presteevju river before the last pond (Borgjaevju C) (figure 2).

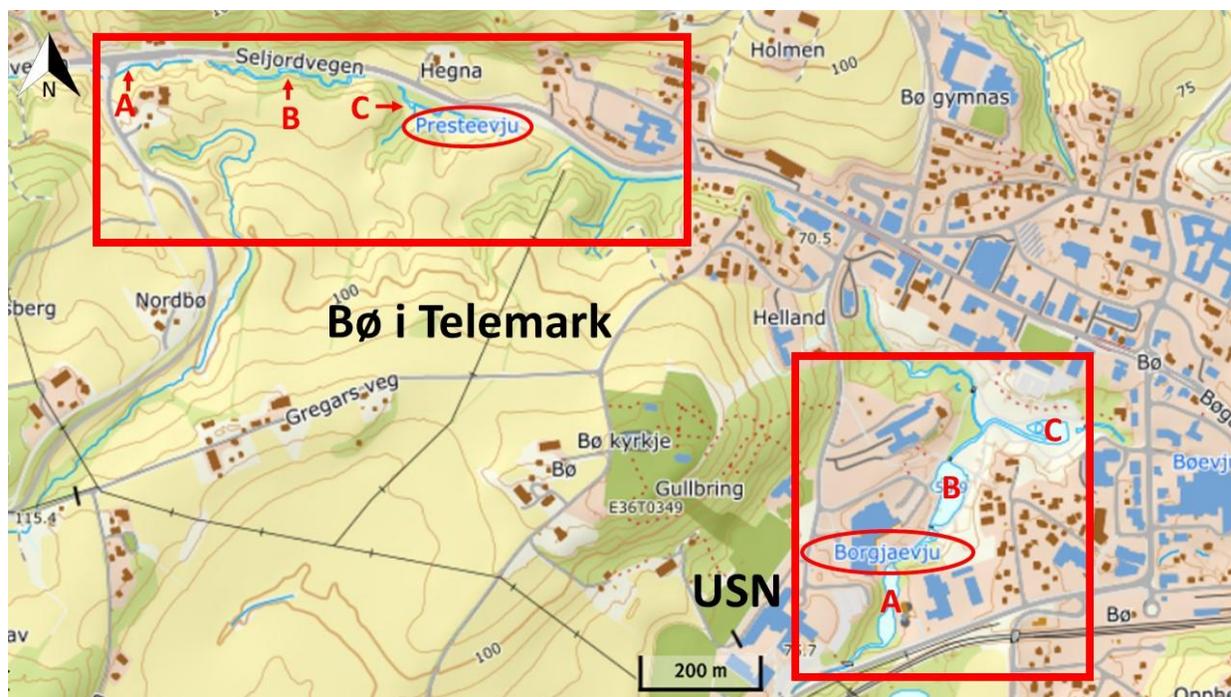


Figure 2. Sedimentation ponds at Borgjaevju and Presteevju streams (source map: Norgeskart, 2019). Letters A, B, C represent the ponds at each stream. A is always upstream and C downstream. The red square points out the area where they were built.

## 2.2 Experimental design and sampling

The sampling, the sample processing and the data collection occurred between May and June of 2019. Water and sediment samples were recovered from the field to study the occurrence of microplastic in the water mass and sediments. Additional measures were taken to characterize and describe the systems such as physical-chemical data of the water and water flow. The sampling of the sediments occurred only once, between May 7<sup>th</sup> and 8<sup>th</sup>, but the water mass was sampled three times during these months (May 7<sup>th</sup>, 8<sup>th</sup>, 21<sup>st</sup> and June 6<sup>th</sup>). The lakes were sampled only once between May 13<sup>th</sup> and 14<sup>th</sup> because of tight time schedule (table 2).

Table 2. Chronogram of the sampling days with the kind and number of samples taken. Water samples were taken for microplastics and water chemistry.

Date	Place	Samples	Number of samples
07/05/2019	Borgjaevju	Sediment and water	Sediment = 18 Water = 4 + 4
08/05/2019	Presteevju	Sediment and water	Sediment = 6 Water = 3 + 3
13/05/2019	Jønnebergjønn	Sediment and water	Sediment = 6 Water = 1 + 3
14/05/2019	Svalbjørtjønn	Sediment and water	Sediment = 6 Water = 1 + 3
21/05/2019	Borgjaevju and Presteevju	Water	7 + 7
04/06/2019	Borgjaevju and Presteevju	Water	7 + 7

### 2.2.1 Sampling of water

Water samples were taken for analysing physical-chemical parameters and the quantity of microplastics. 1L water sample was taken for both water quality parameters and microplastics. Water for chemical analysis were kept in plastic bottles and water for microplastics in metal cans. All the bottles and cans were previously cleaned with distilled water and latter rinsed three times with water from the river or lake.

In the case of the lakes, a water sampler (type Ruttner) was used to get a sample of the water column. Three different samples were recovered at three different depths. The first sample was taken at 1 m depth, the second one was at the Secchi disk depth and the last was at the double of the Secchi disk depth representing the compensation depth. Before starting the sampling, the deepest point of the lake had to be found. The depth was measured with a Sm-5 depthmate portable sounder. In L. Jønnebergtjønn the deepest point was found at 14 m and the water was sampled at 1, 3 and 6 m depth. In L. Svalbjørtjønn the deepest point was met at 10.4 m (32N 0509554, 6594405) and the water was recovered at 1, 2.5 and 5 m depth.

The sampling of the water in the sedimentation ponds was done differently because it was not possible to use the water sampler due to the low water depth of the section. In this case, the method consisted of collecting the water directly with the bottle or can close to the surface and always facing the current. The sampling points were established at the beginning, at the end and between ponds where the water was running. The only exception was after the Presteevju C sedimentation pond where water was not recovered as this stream ends at Borgjaevju C sedimentation pond. Table 3 shows the sampling coordinates for water samples:

**Table 3. Coordinates of water sampling points at Presteevju and Borgjaevju streams**

Water sampling coordinates		
Sample	Coordinate X	Coordinate Y
Presteevju A	32N 0502684	6586372
Presteevju B	32N 0502003	6586331
Presteevju C	32N 0501432	6586331
Borgjaevju A	32N 0503495	6585531
Borgjaevju B	32N 0503609	6585750
Borgjaevju C	32N 0503832	6585921
Borgjaevju C outlet	32N 0503832	6585921

### 2.2.2 Sampling of the sediment

The sediment was collected using a van Veen grab and later stored in 1L metal cans previously rinsed. The samples were frozen and stored at -20°C until further processing.

The sampling of the sediment and water in the lakes occurred during the same day. In the lakes, the sediment was recovered six times at different depths following a linear transect from the shore to the deepest part of the lake. The coordinates (not for Jønnebergtjønn because the GPS could not be used) and depths of each sample at

each lake are displayed in the table 4. Coordinates and depth were measured with a GPS and a Sm-5 depthmate portable sounder, respectively.

**Table 4. Coordinates of sediment sampling points at Lake Jønnebergjønn and Lake Svalbjørtjønn**

Water sampling coordinates			
Sample	Coordinate X	Coordinate Y	Depth (m)
Jønnebergjønn 1	-	-	16.3
Jønnebergjønn 2	-	-	17.3
Jønnebergjønn 3	-	-	10.7
Jønnebergjønn 4	-	-	3.9
Jønnebergjønn 5	-	-	7
Jønnebergjønn 6	-	-	9.4
Svalbjørtjønn 1	32N 0509556	6594392	10.4
Svalbjørtjønn 2	32N 0509543	6594312	7.2
Svalbjørtjønn 3	32N 0509542	6594278	3.5
Svalbjørtjønn 4	32N 0509526	6594352	8.1
Svalbjørtjønn 5	32N 0509512	6594283	6.3
Svalbjørtjønn 6	32N 0509527	6594252	2.7

"-": information not available.

The sampling of the sediment in the sedimentation ponds at Borgjaevju stream was carried out in a rubber boat from downstream to upstream. A view of the sedimentation ponds at Borgjaevju is displayed in figure 3. A total of six samples of sediment were recovered in each sedimentation pond following a linear transect as it shown in figure 4. Coordinates and depths of each sampling point were measured at the same time with the same devices used in the lakes (table 5). In annex 2 are shown schemes of the sedimentation ponds at Borgjaevju stream.



**Figure 3. View of the sedimentation ponds Borgjaevju A, B and C (by Ariadna García-Astillero Honrado).**



Figure 4. Images of the sedimentation ponds Borgjaevju A, B and C from the satellite view (source: Google Maps). The numbers in the labels represent the positions of the six sediment samples recovered in each linear transect. The sample 1 is close to the inlet and sample 6 is next to the outlet of the pond.

Table 5. Coordinates and depth of the sediment sampling points at both sedimentation ponds A, B and C at Borgjaevju stream.

Sampling site	Sample	Coordinate X	Coordinate Y	Depth (m)
Borgjaevju A	1	32N 503559	6585587	0.9
Borgjaevju A	2	32N 503558	6585593	0.7
Borgjaevju A	3	32N 503550	6585611	0.8
Borgjaevju A	4	32N 503550	6585625	1.1
Borgjaevju A	5	32N 503548	6585647	1.3
Borgjaevju A	6	32N 503550	6585668	0.8
Borgjaevju B	1	32N 503606	6585749	0.9
Borgjaevju B	2	32N 503629	6585769	0.9
Borgjaevju B	3	32N 503640	6585787	0.7
Borgjaevju B	4	32N 503633	6585801	0.7
Borgjaevju B	5	32N 503624	6585819	0.9
Borgjaevju B	6	32N 503628	6585834	1.2
Borgjaevju C	1	32N 503731	6585922	0.7
Borgjaevju C	2	32N 503749	6585918	0.7
Borgjaevju C	3	32N 503755	6585915	0.9
Borgjaevju C	4	32N 503769	6585923	0.8
Borgjaevju C	5	32N 503794	6585920	1.1
Borgjaevju C	6	32N 503804	6585921	1

Two samples of sediment were recovered from each pond at the Presteevju stream. The distance from each sedimentation pond to the main road was also measured because it may be a possible source of microplastics. Sedimentation ponds and sampling points are shown in figure 5 and table 6.

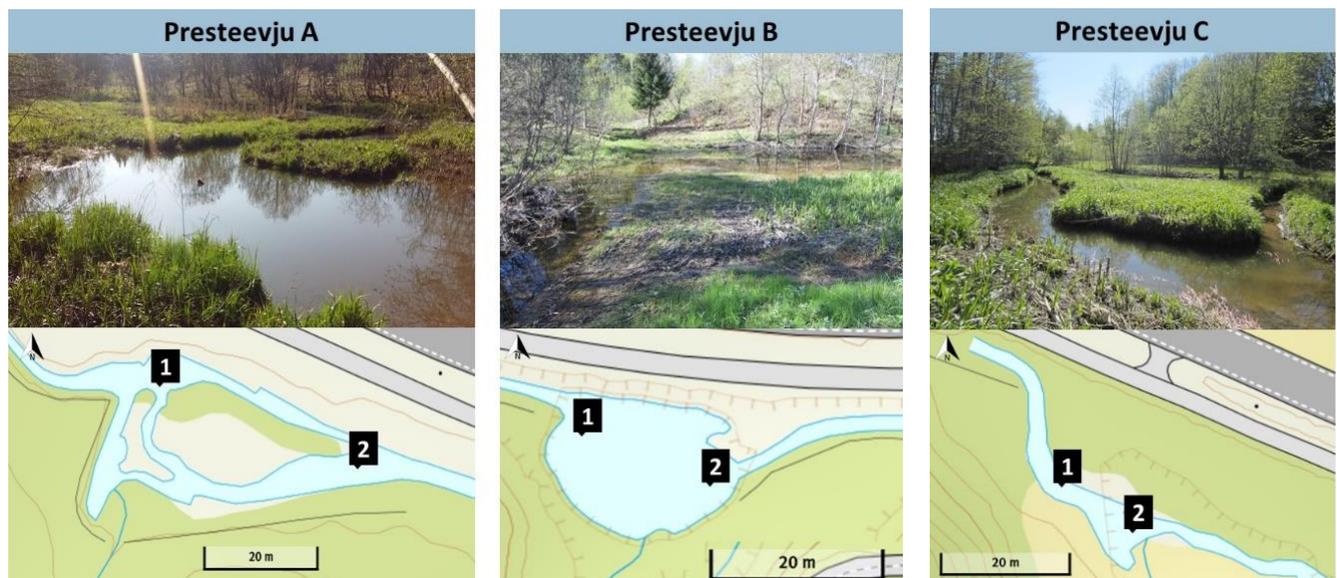


Figure 5. View (photos by Ariadna García-Astillero Honrado) and maps (source map: Norgeskart, 2019) of the sedimentation ponds A, B and C at Presteevju stream. The numbers in the labels represent the places where the sediment was recovered, in the inlet (1) and the outlet (2) of the pond.

Table 6. Coordinates, depth and distance to the road of the sediment sampling points at Presteevju stream.

Presteevju				
Sample	Coordinate X	Coordinate Y	Depth (m)	Distance to the road (m)
Presteevju A1	32N 501462	6586370	<0.5	16.1
Presteevju A2	32N 501509	6586363	<0.5	22.8
Presteevju B1	32N 502004	6586321	<0.5	17.7
Presteevju B2	32N 502022	6586321	<0.5	11.6
Presteevju C1	32N 502711	6586348	<0.5	26.4
Presteevju C2	32N 503368	6585578	<0.5	22.4

### 2.3 Water flow

Water flow was measured through different methods at both streams following water sampling. The water flow was measured with the salt method (Elosegui and Sabater, 2009) in the inlet and outlet of the Borgjaevju stream sedimentation ponds and in the outlet of Presteevju stream. Conversely, the water flow was measured in the inlet of the Presteevju stream sedimentation ponds using the float method (Hauer and Lamberti, 1996)

A research group from the USN had fixed some rulers in the riverbed of Borgjaevju (USN and Bøsenderet) and Presteevju streams, as figure 6 shows, to study the fluctuations in water level. This group measured the water flow several times during May-June of 2019 in different water flow events (e.g drought, flooding) using the salt method. The results were used to develop streamflow curves that allow calculation of the water flow easily only by interpolating the depth measures recorded from the rulers. The water flow measures of the inlet and outlet of Borgjaevju stream sedimentation ponds and the outlet of Presteevju stream presented in this master thesis were calculated based on the data borrowed from proff. Espen Lydersen (USN) (see annex 3).

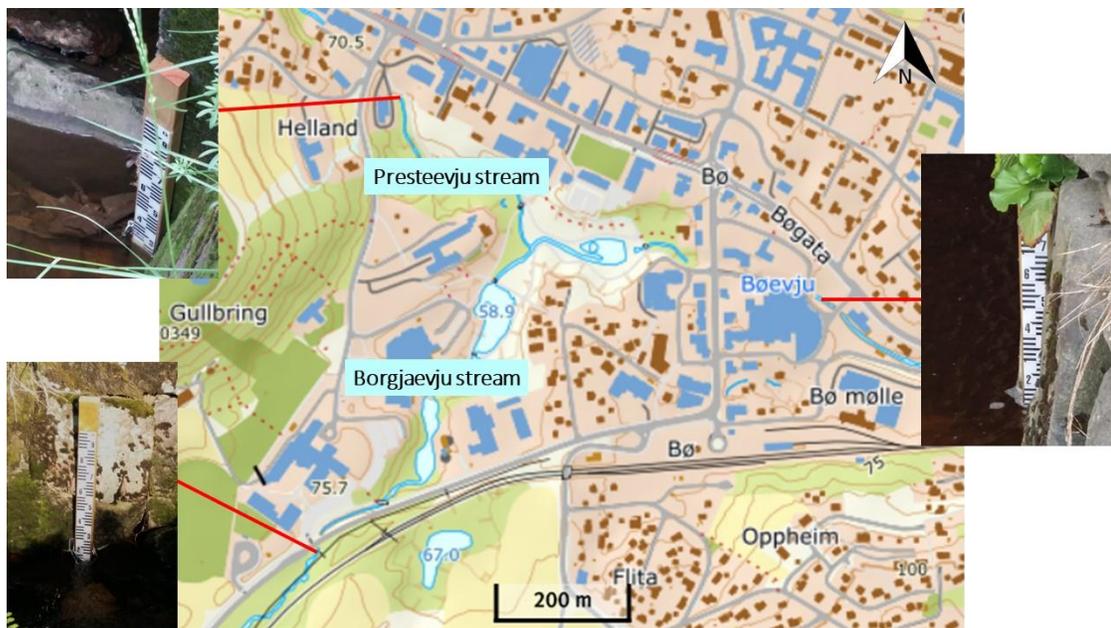


Figure 6. Location of the rulers to measure the water level at Borgjaevju (USN = left down corner, Bøsenderet = right) and Presteevju (left upper corner) streams. Red lines indicate the position of the rulers (photos by Ariadna García-Astillero Honrado, map from Norgeskart, 2019).

The measures of the flow rate in the inlet of Presteevju stream sedimentation ponds were carried out using the float method. The float (a leaf) was thrown three times on a 30 m stretch of the river and the time it took to sail it was recorded. Additional measures of the width (only where the water was present) and the depth of the riverbed were written down. Afterwards, the water flow was calculated according to the following formula:

$$^{(1)} \quad Q = V \cdot W \cdot D$$

Where Q is the flow rate in m<sup>3</sup>/s, V is the average speed in m/s, W is the average width (m) and D is the average depth (m). The average speed is calculated dividing the length of the stretch (m) by the average time (s). Finally, the flow rate was transformed into L/s.

## 2.4 Water chemistry

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Water samples were also taken in the lakes and the sedimentation ponds from both streams. Water was monitored for physical-chemical analysis such as temperature, pH, electrical conductivity (EC), turbidity, total organic carbon (TOC), total nitrogen and phosphorous.

Temperature (°C) was recorded *in situ* using a thermometer. Once in the laboratory, EC (µS/cm) was measured with the electrical conductivity meter WTW LF91 and consecutively, the pH using the pH-meter Mettler Toledo seven compact to avoid the pH electrode to modify the EC. Turbidity (FTU) was also measured with a Turbiquant 1100 IR kit according to NS-EN ISO 7027-1 (2016) protocol. Finally, water samples were stored in a cool room at 6°C until further chemical analysis.

For total nitrogen (µg/L) and phosphorous (µg/L) analysis, sub-samples of approximately 100 mL were saved from the initial 1L water sample, prefixed with 1 mL of 4M H<sub>2</sub>SO<sub>4</sub> and stored at 6°C prior to analysis. The total nitrogen analysis was conducted following the DIONEX ICS 1100 protocol and using the flow injection analyzer FIAlyzer 1000. The total phosphorous was measured according to NS-EN ISO 6878 (2004) protocol and using the Lambda 25 spectrophotometer at 880 nm.

The TOC was calculated from the analysis of the water colour number (NS-EN ISO 7887, 2011). The water colour number measures the colour of the water which is due to the presence of metal ions, organic matter, fulvic acids and others. It is expressed in mg Pt/L and the result divided by 10 is equal to the measure of TOC in mg/L (personal communication). To find out the real colour the turbidity of the water is needed to be eliminated. For that reason, the water was previously vacuum filtrated through 0.45 µm Whatman Non-Sterile Cellulose Nitrate Membranes. Once the water had been filtrated, the colour was measured in the Lambda 25 spectrophotometer at 410 nm.

## 2.5 Microplastics

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Due to the lack of standard methods for collecting, extracting, quantifying and identifying microplastics, the following protocol was developed according to findings in several publications from different research groups (Frias et al., 2018; Horton et al., 2017a; Hurley et al., 2018; NIVA, 2017; NIVA, 2018; Nor and Obbard; 2014)

### 2.5.1 Cross-contamination risk reduction measures

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Cross-contamination risk reduction measures were considered when sampling and analysis were carried out. These measures, as the Norwegian Institute for Water Research (NIVA) (2017, 2018) recommends, mainly consisted of avoiding the use of synthetic clothes, recording the colour of the worn clothes, decontaminating of all material used, running daily controls and filtering all the reagents, previously to use.

The daily controls were 1.2 µm Whatman GF/C glass microfiber filter inserted in an open Petri dish covered with aluminium foil to monitor airborne particles. Daily controls were used in each sampling in the field, in the fume hood and in the laboratories. The laboratory where the samples were processed and the fume hood were cleaned every day with 70% ethanol solution (Prata et al., 2019), and then, rinsed with distilled water. All the material used in the laboratory (beakers, spoons, glasses, etc.) was made of glass or metal and was again cleaned following the previous steps. The doors and windows stayed closed during the working hours, only one or as maximum two people (very few cases) were working in the area at the same time and moved slowly to avoid the air circulation (Prata et al., 2019).

Lab coat was worn in the laboratory in every moment to prevent the contamination of the samples with textile fibres from the clothes. Apart of writing down the colour of the clothes underneath the lab coat (Frias et al., 2018), some textile fibres were collected and stored in a labelled Petri dish to check later under the stereomicroscope and compare with those fibres found in the samples and the controls to enhance the reliability of the findings.

### 2.5.2 Microplastics in sediment

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#### 2.5.2.1 Sediment fractions and pre-treatment

After melting the samples, the 1L wet sediment was sieved to get the size fraction of 1 to 5 mm. Sediment was rinsed through a couple of sieves with mesh sizes of 5 and 1 mm to obtain large microplastic particles (Horton et al., 2017a). The sediment was cleaned with distilled water while passing through the sieves. Finally, a test sieve shaker Endecott was used during 15 min to enhance the sieving and reduce the content of water.

Afterwards, a sub-sample of 10 g of the previously homogenized wet sediment (except for the lakes which have less than 10 g for that sediment fraction, see annex 4) was placed in a glass beaker of 250 mL and covered with aluminium foil (NIVA, 2017). The glass beaker was set in the oven at 60 °C. Once the sediment was dry, the sediment was weight and the pre-treatment could start (figure 7).

The pre-treatment was focused on the removal of organic matter. The organic matter has a density of approximately 1.4 g/cm<sup>3</sup> and can give problems when extracting the microplastics because their density ranges overlap between 0.9-1.8 g/cm<sup>3</sup> (NIVA, 2017). Part of the organic matter was eliminated using the Fenton's reagent. The Fenton's reagent, consisting of 20 mL of 30% (v/v) H<sub>2</sub>O<sub>2</sub> and 10 mL of ferrous sulfate catalyst solution was added to the sample (NIVA, 2017). The ferrous sulfate catalyst solution was made in a 250 mL volumetric flask incorporating 5 g of iron (II) sulfate heptahydrate in 250 mL of filtered distilled water (Hurley et al., 2018). The

Fenton's reaction is an exothermal reaction that creates some bubbles, foam and gases. The reaction took around 20 minutes.

### 2.5.2.2 Density separation

Microplastics can be separated from the sediment using a density separation method (figure 7). This method used reagents with different densities depending on the purposes. Following the organic matter removal step, microplastics were extracted using two density solutions (NIVA, 2017). The first solution was filtered distilled water whose density is  $1 \text{ g/cm}^3$  and the microplastics extracted with it are those known as low-density microplastics because they float in freshwater systems (NIVA, 2017). The second solution was  $\text{ZnCl}_2$  whose density is  $1.8 \text{ g/cm}^3$  and can extract the high-density microplastics, those which sink in the freshwater ecosystems (Horton et al. 2017a; NIVA, 2017). Next steps are a combination of protocols from Horton et al. (2017a) and NIVA (2017) with some modifications.

The glass beaker with the sediment was filled with filtered distilled water until the brim. It was gently stirred with a metal stick and let settle for one hour. It was placed in a large metal vessel and covered with aluminium foil to avoid contamination. After one hour, the aluminium foil was removed, and the beaker was made overflow adding more filtered distilled water to get the particles that were floating in the surface. This step should be done carefully to not mix with the particles of the bottom. Additionally, the walls of the beaker were rinsed to get the attached particles. The buoyant particles that were already in the vessel were vacuum filtered through a  $1.2 \text{ }\mu\text{m}$  Whatman GF/C glass microfiber filter (NIVA, 2017). The filter was placed in a Petri dish covered by aluminium foil and was labelled as "water overflow" together with the name of the sample. The rest of the water in the beaker was also vacuum filtered through another  $1.2 \text{ }\mu\text{m}$  Whatman GF/C glass microfiber filter carefully to not lose the sediment that was in the bottom part. This step was added with the purpose to get the particle that could be in the middle of the beaker and extract the water before going on with the density separation method. The filter was also inserted in a Petri dish covered with aluminium foil and labelled as "water middle" and the name of the sample.

The next step was repeating the process but using the  $\text{ZnCl}_2$  high-density solution (Horton et al., 2017a). The  $\text{ZnCl}_2$  solution was made dissolving 180 g of  $\text{ZnCl}_2$  in 100 mL of distilled water. The beaker with the sediment was placed in another metal vessel, was filled with the high-density solution until the brim of the beaker and was well mixed with a metal stick. The beaker and the vessel were covered with aluminium foil and let to settle for two hours. After this time, the aluminium foil was removed and the beaker was made overflow in the large vessel by pouring more  $\text{ZnCl}_2$  solution, gently. Again, the walls of the beaker were also rinsed with  $\text{ZnCl}_2$  solution to get all the attached particles. The buoyant particles of the vessel were also vacuum filtered through a  $1.2 \text{ }\mu\text{m}$  Whatman GF/C glass microfibre filter. Then, the filter was flushed in another vacuum pump with distilled water to remove all traces of  $\text{ZnCl}_2$  thoroughly. The filter was kept in a Petri dish covered with aluminium foil and labelled as " $\text{ZnCl}_2$  overflow" and the name of the sample. The  $\text{ZnCl}_2$  solution was reused during the whole working period as it is a polluting and expensive substance.

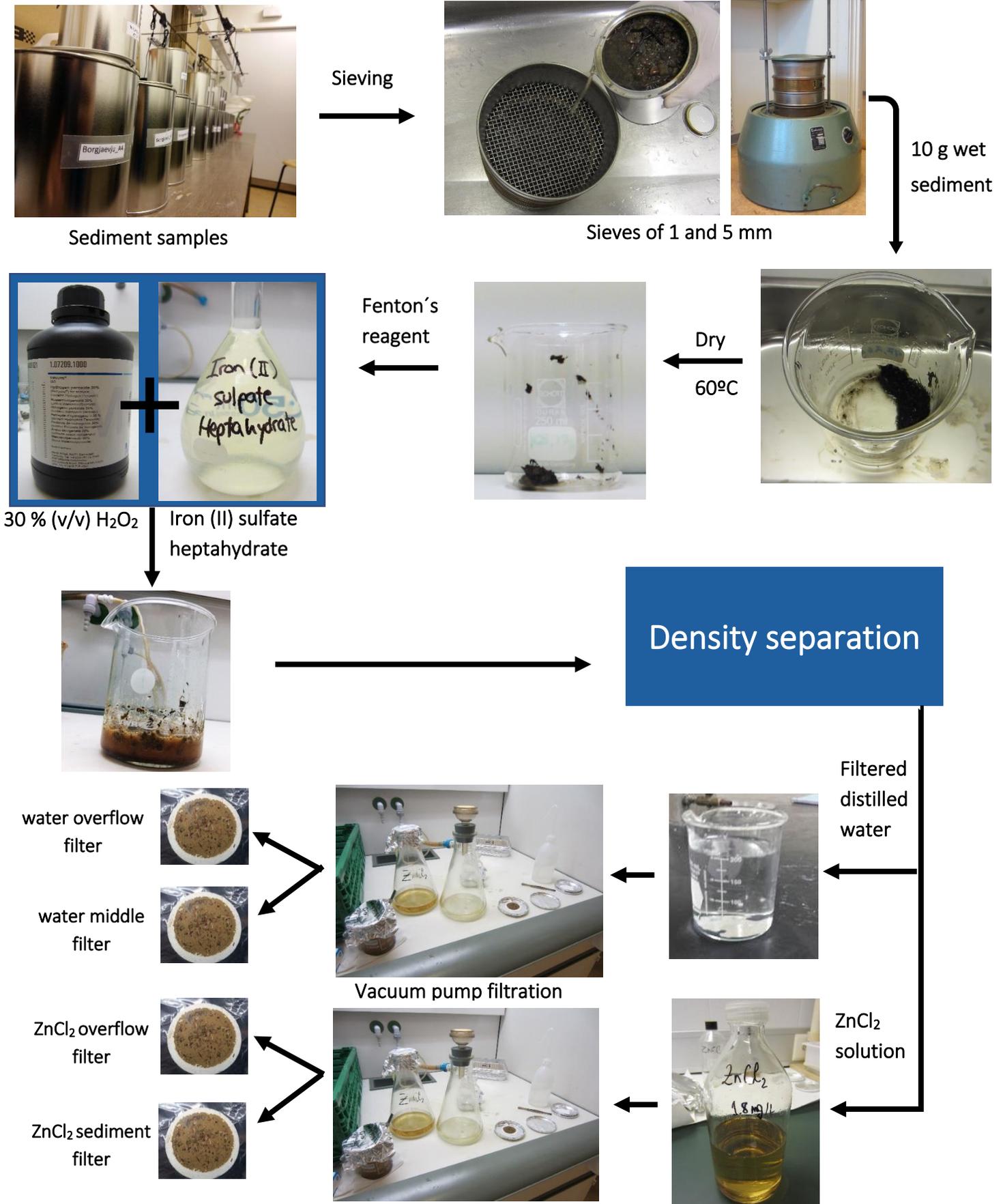


Figure 7. Scheme of the method used to extract the microplastics from the sediment (photos by Ariadna García-Astillero Honrado).

The final step was to vacuum filter the remaining  $\text{ZnCl}_2$  with the non-floating sediment through one or several 1.2  $\mu\text{m}$  Whatman GF/C glass microfibre to make sure there were not more microplastics and recover the  $\text{ZnCl}_2$  solution. The filter or filters were also saved in different Petri dishes covered with aluminium foil and labelled as “ $\text{ZnCl}_2$  sediment” and the name of the sample.

All the filters were placed in the oven at 60 °C until they were dry (Horton et al., 2017a; Munno et al., 2018). Then they were moved to a room at 6 °C until further identification.

### 2.5.3 Microplastics in water

One litre of water was vacuum filtered through 1.2  $\mu\text{m}$  Whatman GF/C glass microfibre filters. The vacuum pump was covered with aluminium foil during the filtration to prevent contamination with airborne microplastics. In the samples of Presteevju A, B and C from the first sampling day, on May 8<sup>th</sup>, only 865, 940 and 920 mL were filtered, respectively. Later, the number of particles were corrected to 1L. In the case of the lakes, the water of the three different depths was mixed in a 3L glass bottle and only one integrative sample of 1L was analysed.

Subsequently, filters were kept in labelled Petri dishes previously covered with aluminium foil and dry in the oven at 60 °C to avoid degrading microplastics (Munno et al., 2018). Once, the filters were dry, they were stored at 6 °C until further identification. Microplastics in water are expressed as items/L.

### 2.6 Identification of microplastics

The identification of microplastic was carried out under the stereomicroscope Zeiss SteREO Discovery.V20. Each filter was inspected for maximum 20 minutes (Horton et al., 2017a), being enough time to visualize the whole filter. At the first time, the filter was visualized at 10X magnification to have an overview (Nor and Obbard, 2014). Finally, microplastics were identified and counted at 30X magnification according to NIVA (2017).

The filter was transferred from the Petri dish to a glass plate and read from up to down and from side to side (Hidalgo-Ruz et al., 2012) as it is shown in figure 8. During the visualization, the colour, the type and the number of microplastics were recorded. The size of ten microplastic particles of each type was measured in the water samples, contrary to the sediment samples that were already sieved (1-5 mm). Some samples had less than ten microplastic particles per type so all of them were measured. Microplastics were measured in order of appearance.

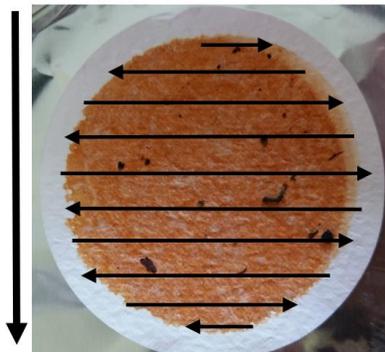


Figure 8. Procedure to read the microplastics of the filter (photo by Ariadna García-Astillero Honrado).

In order to collect all microplastic particles the following criteria have been established by Nor and Obbard (2014): 1) no visible cellular or organic structure, 2) particles/fibres are not segmented, 3) fibres are equally thick throughout their entire length and should not be tapered at the end.

The particles also had to fulfil at least two of the following criteria (Nor and Obbard, 2014): 1) unnaturally coloured compared to most of the other particles in the sample and appear to be homogenous material or texture, 2) unnaturally brightly coloured coating on another particle, 3) unnatural shape (e.g perfectly spherical), 4) fibre remains intact with a firm tug/poke with tweezers, 5) shiny/glassy, 6) flexible/can be compressed without being brittle.

To make sure that the observed particles were microplastics, tweezers and hooks were used to poke and scratch (Hidalgo-Ruz et al., 2012). In addition, the hot needle method (De Witte et al., 2014) was used with the same purpose, as plastic can degrade with the application of temperature.

### 2.6.1 Classification of microplastics

Microplastics were classified according to the recommendations of some peer-reviewed publications and recovered by Frias et al. (2018). They were classified according their physical properties such as type and colour (table 7).

**Table 7. Classification of microplastics according to the type and colour (based on Frias et al., 2018)**

Type		
Pellet	Film	Sponge/foam
Fragment	Rope and filaments	Rubber
Fibre	Microbeads	
Colour		
Black ■	Transparent □	Multicolour
Blue ■	Red ■	Others ■■■■
White □	Green ■	

The multicolour category includes those microplastics which have different colours within the particles. In addition, the category “others” refers to microplastics of different colours than the most typical ones like grey, pink, yellow or brown among others. Finally, the difference between white and transparent is the opacity meaning that light can pass through the transparent microplastics (Frias et al., 2018).

### 2.7 Statistical analysis

Normal distribution of the data and homogeneity of the variance (homoscedasticity) was checked using a Shapiro-Wilk normality test and a Levene’s test, respectively. Those normal and homoscedastic variables were analysed through parametric tests such as one-way ANOVA (ANalysis Of VAriance). On the opposite, those non-normal distribution data were analysed using non-parametric tests such as Kruskal-Wallis test. For both tests the significance acceptance level was  $p < 0.05$ . These tests were applied using the Rcmdr 2.5-3 package (Fox and

Bouchet-Valat, 2019) of R program (version 3.6.1. R Core Team, 2019). Following the application of these models and where significant differences were matched, a post-hoc Tukey test ( $p < 0.05$ ) was used to identify significant differences between data pairs in R studio using the function *kruskalmc* of *pgirmess* 1.6.9 R package (Giraudoux, 2018).

A one-way ANOVA test was applied to the Shannon index ( $H'$ ) variable using site for sediment samples, and, site and date, for water samples, as fixed factors. The Shannon index (Shannon, 1948) is a quite common species diversity index used in Ecology, but in this work microplastic types were studied instead of species. It can be calculated as:

$$(2) H' = - \sum_{i=1}^S (P_i \cdot \text{Log}_2 \cdot P_i)$$

where,  $S$  is the microplastic richness and  $P_i$  is the relative abundance of each microplastic type. This index was calculated in R studio using the *diversity* function of *vegan* 2.5-5 R package (Oksanen et al. 2019).

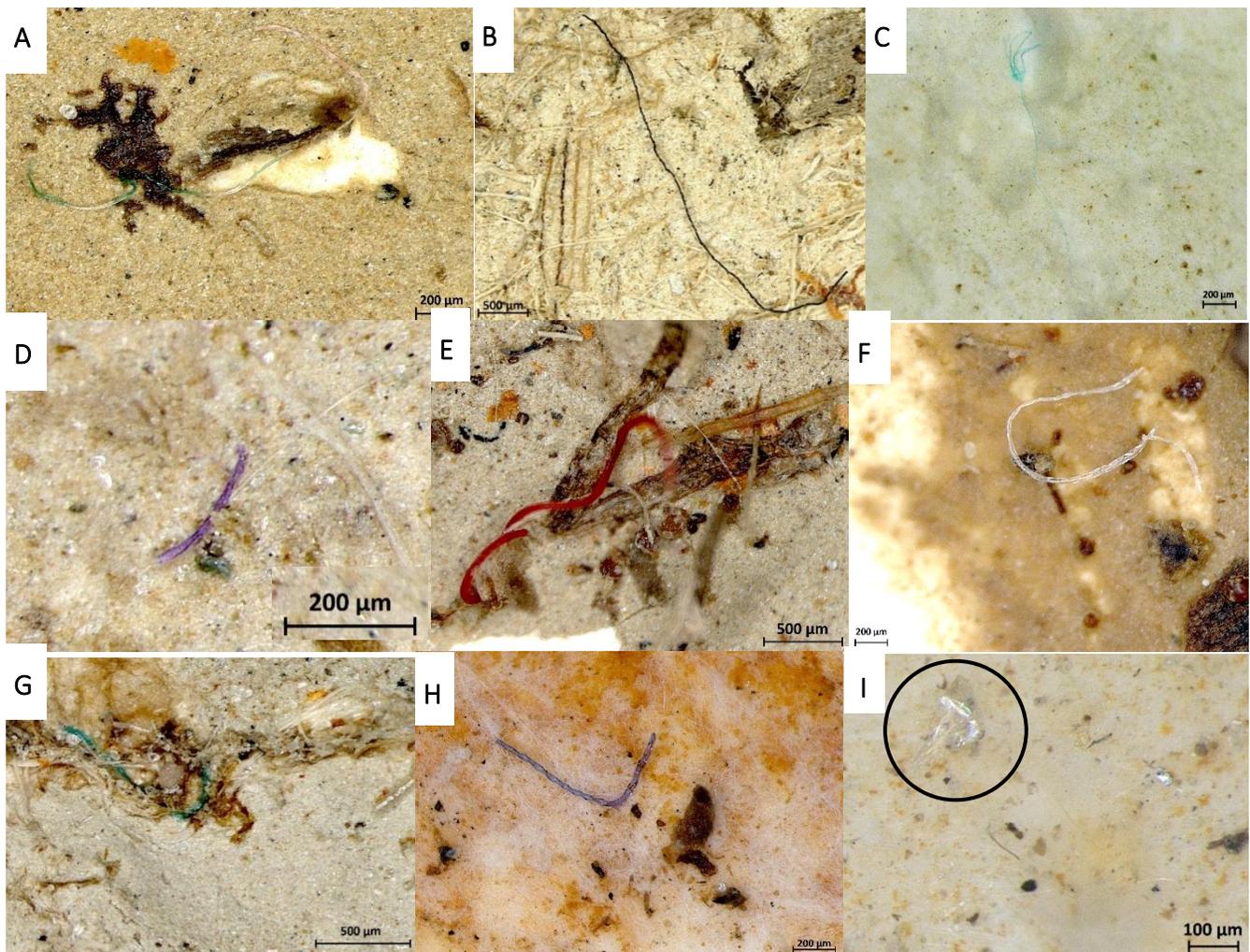
To standarize between sites for comparability, the number of microplastics found in the sediment samples were transformed to number of items per kilogram of dry sediment weight (d.w). A Kruskal-Wallis test was used to analyse the number of items/Kg across the sediment of all sites, the number of items/L across the water of all sites and dates and the microplastic sizes according type, colour, site and date.

To test whether the factors sampling site and sampling date had a significant effect ( $p < 0.05$ ) on the microplastic composition of sediment and water samples, a permutational multivariate analysis of variance (PERMANOVA) and pairwise PERMANOVA (*adonis* function in *vegan* R package, 999 permutations) were done. A requisite for correct interpretation of PERMANOVA results is to check for homogeneity of the data, therefore, an ANOVA test was carried out. If the significance value was  $p > 0.05$  the homogeneity assumption of the data was correct. A two-dimensional non-metric multidimensional scaling (NMDS) plot based on the Bray-Curtis similarity matrix was created to visualize the samples according to the sampling site and date. The NMDS was created using the *metaMDS* function of *vegan* 2.5-5 R package (Oksanen et al. 2019) in R studio. Complementary to these results, a hierarchical cluster (HCA) was also made using the Bray-Curtis similarities and the average method. The HCA was also performed in R studio. All graphs, except the HCA, and some descriptive statistics (i.e average, standard deviation, median, etc.) were carried out in Microsoft Excel program, version 2016.

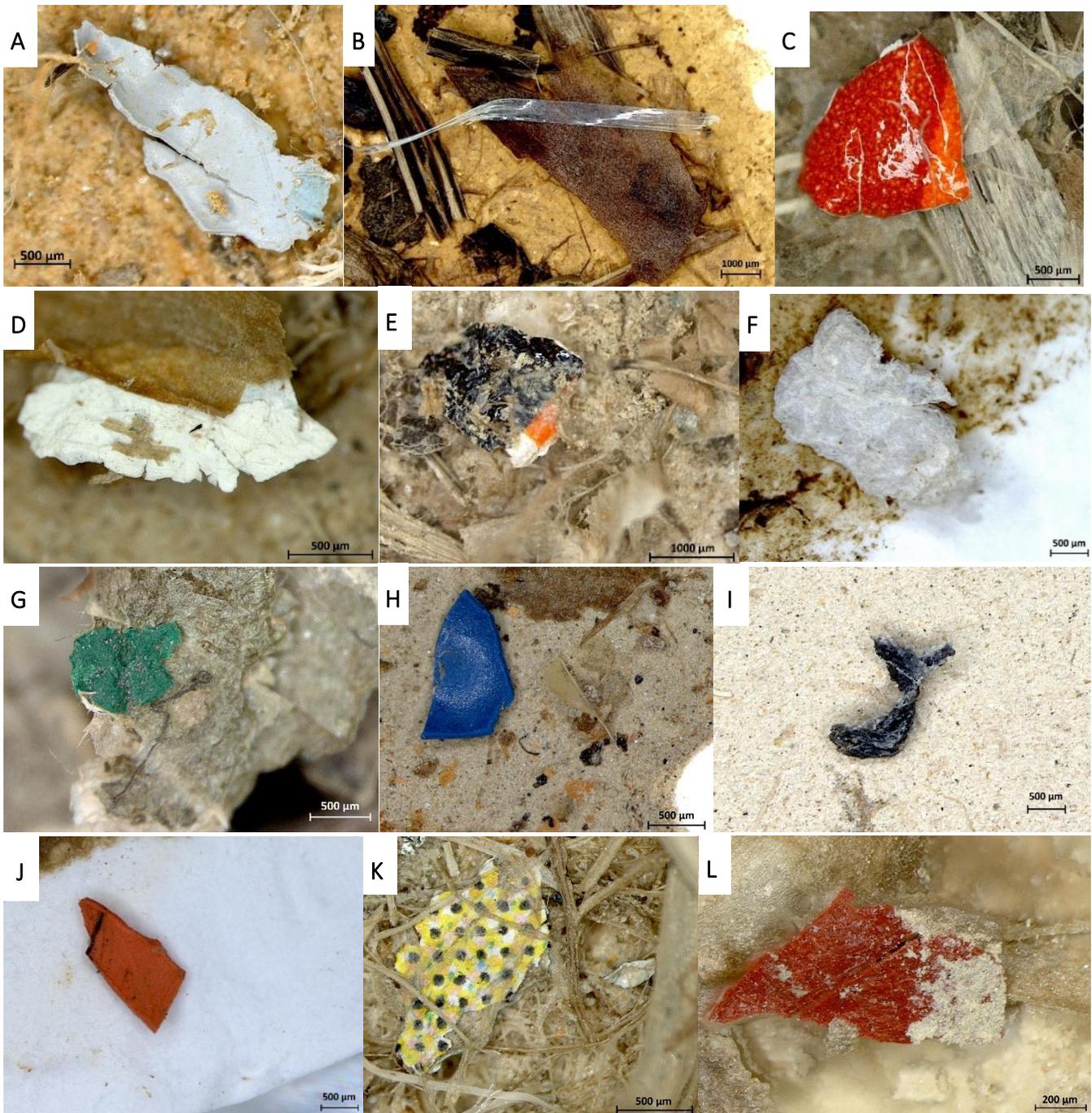
Finally, to study the relationship among quantitative variables such as the physical-chemical parameters (temperature, pH, turbidity, EC, TOC, total phosphorous and nitrogen), the number of microplastic particles/L and the flow rate, several correlation tests were done. Pearson coefficient ( $r$ ) was studied in all cases and accepted when its absolute value was higher than 0.8 and the  $p$ -value was lower than 0.05. These correlation tests were also made using the *Rcmdr* 2.5-3 R package (Fox and Bouchet-Valat, 2019).

### 3. RESULTS

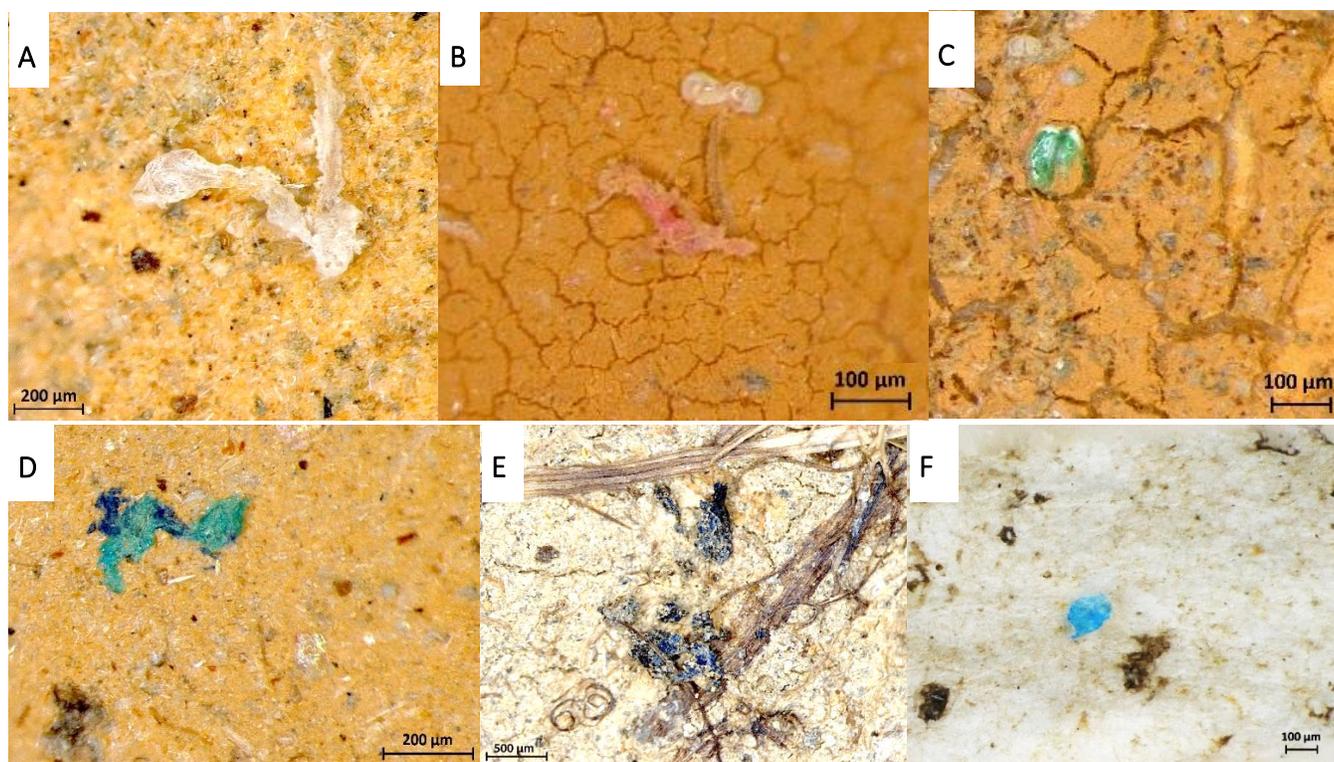
Microplastics were found in all water and sediment samples. The most common type of microplastic was the fibre (77 %), followed by the fragment (22.7 %). In some cases, films (0.1 %) were also found, and other category, called unknown (0.2 %), was established to include those findings which could be plastic but did not match with some of the criteria mentioned in methods. Fibres were black and blue in most of the cases, but there were other typical colours such as transparent, violet, red or green. Fragments were mainly blue, followed by yellow, green and white among others. Film was always transparent. Some examples of microplastics found in the sediments and the water mass of the sedimentation ponds can be seen in figure 9 and 10 and microplastics found in L. Svalbjørtjønn and L. Jønnebergjønn are shown in figure 11.



**Figure 9.** Microplastics found in water and sediment samples of the sedimentation ponds at both Borgjaevju and Presteevju streams in 2019 (photos by Ariadna García-Astillero Honrado). **A)** Multicolour fibre (green, pink and white) at Borgjaevju C4. 89X magnification. **B)** Black fibre at Borgjaevju C4. **C)** Blue fibre in the water sample at Presteevju A (04/06/2019). 1350  $\mu\text{m}$ . 84X magnification. **D)** Purple fibre at Borgjaevju C3. 230  $\mu\text{m}$ . 114X magnification. **E)** Red fibre at Borgjaevju C2. 52X magnification. **F)** Transparent fibre at Borgjaevju A5. 90X. **G)** Green fibre at Borgjaevju B2. 64X magnification. **H)** Blue fibre at Borgjaevju A4. 83X magnification. **I)** Transparent film in the water sample at Presteevju C (08/05/2019). 209  $\mu\text{m}$ . 150 X.



**Figure 10.** Microplastic fragments found in the sediment samples of the sedimentation ponds at both Borgjaevju and Presteevju streams on 2019 (photos by Ariadna García-Astillero Honrdo). **A)** Grey fragment at Presteevju C1. 2747  $\mu\text{m}$ . 52X magnification. **B)** Transparent fragment at Presteevju A2. 9898  $\mu\text{m}$ . 16X magnification. **C)** Orange fragment from Presteevju A1. 1861  $\mu\text{m}$ . 46X magnification. **D)** White fragment at Presteevju A1. 1841  $\mu\text{m}$ . 79X magnification. **E)** Multicolour fragment at Presteevju A1. 1879  $\mu\text{m}$ . 35X magnification. **F)** White fragment at Borgjaevju C5. 3323  $\mu\text{m}$ . 38X magnification. **G)** Green fragment at Borgjaevju C3. 941  $\mu\text{m}$ . 50X magnification. **H)** Blue fragment at Borgjaevju C2. 1306  $\mu\text{m}$ . 49X magnification. **I)** Black fragment at Borgjaevju C2. 1467  $\mu\text{m}$ . 10X magnification. **J)** Orange fragment at Borgjaevju C1. 1909  $\mu\text{m}$ . 10X magnification. **K)** Multicolour fragment at Borgjaevju B6. 1658  $\mu\text{m}$ . 64X magnification. **L)** Red fragment at Borgjaevju B6. 1156  $\mu\text{m}$ . 113X magnification.



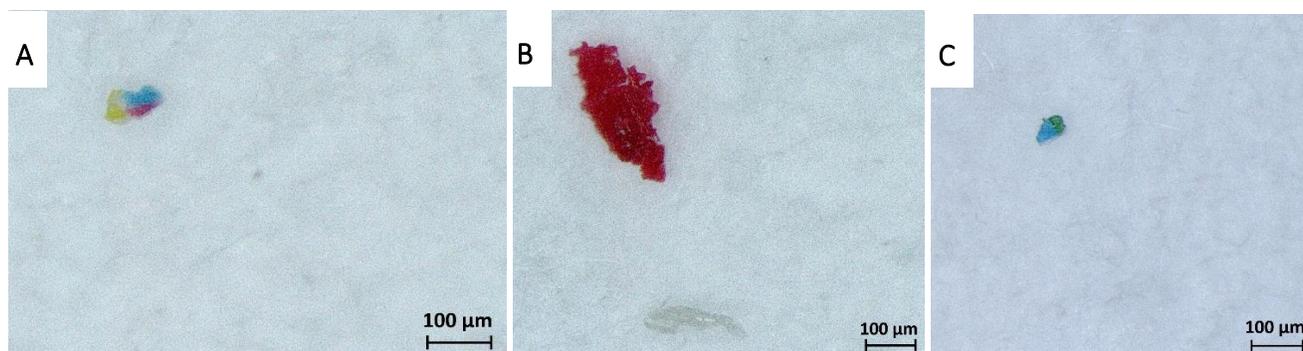
**Figure 11.** Microplastics found in the reference lakes Svalbjørtjønn and Jønnebergjønn in 2019 (photos by Ariadna García-Astillero Honrado). **A)** White fragment from L. Svalbjørtjønn 6. 110X magnification. **B)** Red fragment at L. Svalbjørtjønn 5. 213 µm. 150X magnification. **C)** Green fagment at L. Svalbjørtjønn 5. 122 µm. 150X magnification. **D)** Multicolour fragment at L. Svalbjørtjønn 3. 336 µm. 150X magnification. **E)** Multicolour fragment at L. Svalbjørtjønn 1. 56X magnification. **F)** Blue fragment at L. Jønnebergjønn water sample. 163. µm. 150X magnification.

### 3.1 Sorting method

A total of 31 filters for 23 water samples, 183 filters for 36 sediment samples and 64 control filters (including sampling, room, fume hood and laboratory) were examined under the stereomicroscope. Microplastics were detected in most of the controls assuming the 16% of microplastics found in the whole study. Indeed, there were found fibres (74.2 %) and fragments (25.8 %). Some examples can be seen in figure 12. The largest number of microplastics occurred in the control fibres from the fume hood (microplastic particles) as can be seen in table 8. The total number of microplastics classified per type and colour are placed in annex 5.

**Table 8.** Number of controls, percentage and average ( $\pm$  SD) number of microplastic particles per control type.

Control type	Number of control filters	% of microplastic particles	Average n <sup>o</sup> of microplastic items
Field sampling	11	8.3	2.17 $\pm$ 3.13
Room	16	26.1	4.82 $\pm$ 3.96
Fume hood	18	44.9	7.42 $\pm$ 5.40
Stereomicroscope laboratory	19	20.7	3.25 $\pm$ 2.34.
<b>Total</b>	<b>64</b>	<b>100</b>	<b>4.62 <math>\pm</math> 4.31</b>



**Figure 12.** Microplastic particles found in some controls 2019 (photos by Ariadna García-Astillero Honrado). **A)** Multicolour fragment at control hood 18/05/2019. 100 µm. 150X magnification. **B)** Red fragment at control hood 21/05/2019. 318 µm. 150X magnification. **C)** Multicolour fragment at Jønnebergtjønn sampling control. 64 µm. 150X magnification.

Moreover, some spoiled fibres were found in the samples, as figure 13 shows. This could be due to environmental conditions or the sampling processing method.



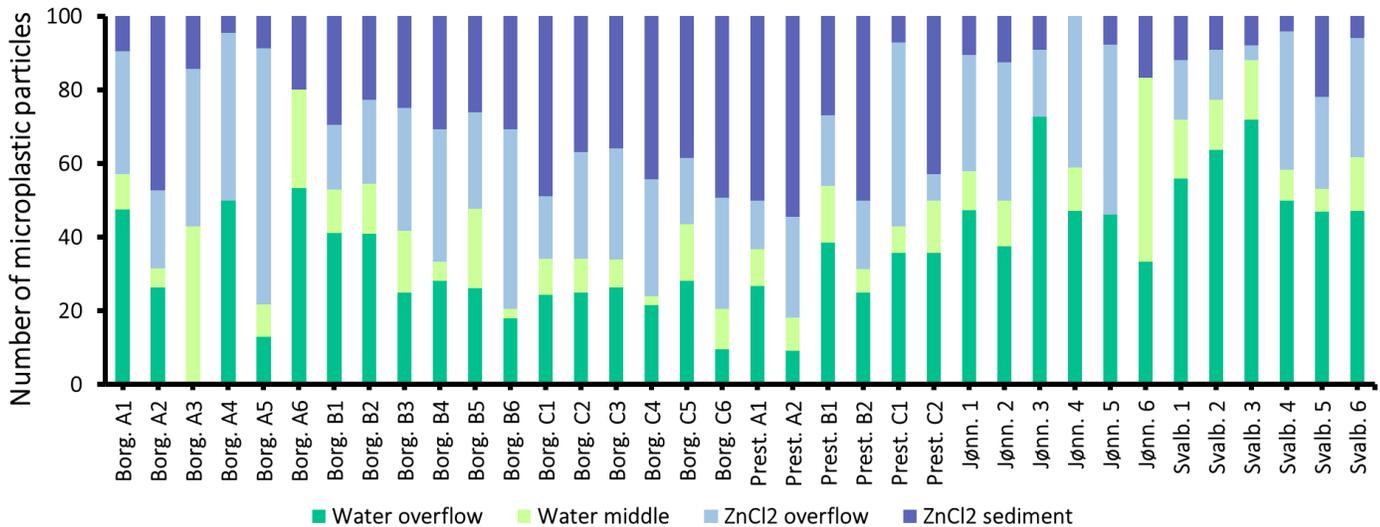
**Figure 13.** Some spoiled fibres found in the sediment samples (photos by Ariadna García-Astillero Honrado). **A)** Blue fibre at Jønnebergtjønn 4. **B)** Detail of a spoiled blue fibre at Borgjaevju B1. **C)** Multicolour or degraded fibre at Borgjaevju B1.

In order to assess the effectiveness of the density separation method to extract microplastics from the sediment, the percentage of particle removal of each step of the density separation method has been compared. Both filtered distilled water and  $\text{ZnCl}_2$  density solutions extracted about half of the microplastic particles each. Percentages of extraction of all steps are presented in table 9.

**Table 9.** Average percentage of extraction ( $\pm$  SD) of microplastics per extraction step.

Extraction step	Density solution	Average percentage of extraction	Standard deviation
Water overflow	Filtered distilled water	36.0 %	$\pm$ 17.0
Water middle	Filtered distilled water	12.3 %	$\pm$ 10.2
$\text{ZnCl}_2$ overflow	$\text{ZnCl}_2$	27.5 %	$\pm$ 15.0
$\text{ZnCl}_2$ sediment	$\text{ZnCl}_2$	24.2 %	$\pm$ 16.5

The percentages of microplastic removal by the extraction steps in each sample can be seen in the figure 14. All the steps brought out microplastics, but in some samples (e.g Borgjævu A4 or Borgjævu A6) there were steps that did not extract any microplastic particles.



**Figure 14. Percentage of microplastic removal from sediment samples for each step of the microplastic density separation method of the sediment samples.** Borg. corresponds to the samples belonging to Borgjævu river, Prest. corresponds to the Presteevju river, Jønn. to L. Jønnebergtjønn and Svalb. to L. Svalbjørtjønn.

### 3.2 Microplastics in the sediment

Microplastics were found in all sediment samples in the size fraction from 1 to 5 mm from 8 sampling sites (3 at Borgjævu, 3 at Presteevju and 2 lakes). All the raw data is shown in annex 5. The average concentration of microplastics in the sediment was 41757 ( $\pm$  65111) items/Kg and ranged from 4784 ( $\pm$  3857) to 129859 ( $\pm$  123965) items/Kg. The concentration of microplastics in the sediment of the sedimentation ponds was 17434 ( $\pm$  15271) items/Kg and in the lakes 90401 ( $\pm$  95547) items/Kg. Figure 15 shows the average concentration of microplastics per kilogram of dry sediment at both Borgjævu and Presteevju streams and both L. Jønnebergtjønn and L. Svalbjørtjønn.

The average number of items/Kg seems to increase throughout the ponds at Borgjævu stream (A < B < C), and decreases along the three sedimentation ponds at Presteevju stream (A > B > C). The last pond at Borgjævu stream and the first pond at Presteevju stream contain the highest amount of microplastics of all sedimentation ponds (37232  $\pm$  15438 and 15208  $\pm$  2062 items/Kg, respectively) according to table 10. However, L. Svalbjørtjønn and L. Jønnebergtjønn present the highest concentration with 129859 ( $\pm$  123965) and 50944 ( $\pm$  31315) items/Kg, respectively.

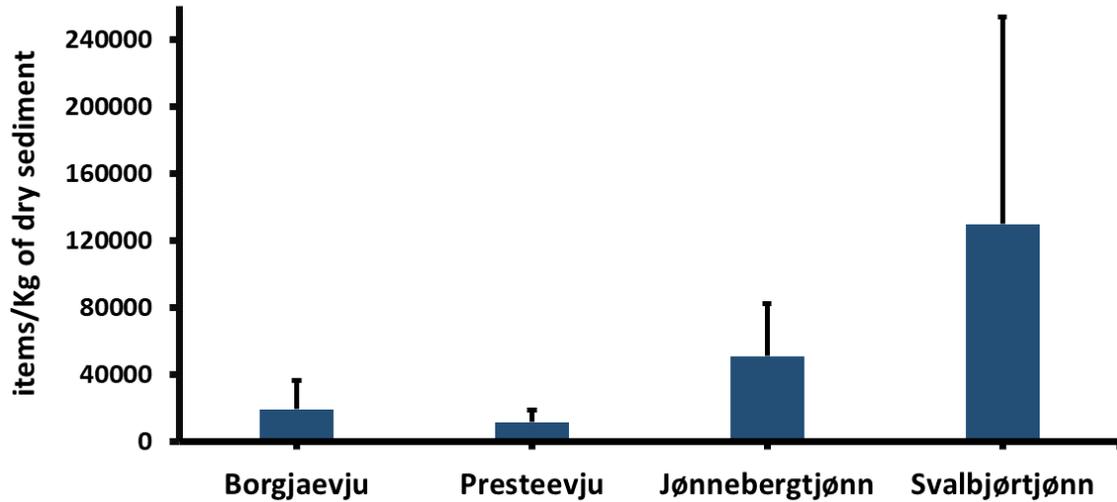


Figure 15. Average number of items per kilogram of dry sediments at Borgjaevju and Presteevju streams and L. Jønnebergjtjønn and L. Svalbjørtjønn. Error bars represent standard deviation.

There were significant differences in the concentration of microplastics in the sediment among the sampling sites (Kruskal-Wallis test,  $p < 0.001$ ). In particular, Borgjaevju A was significantly different from Borgjaevju C (Tukey,  $p < 0.05$ ), L. Jønnebergjtjønn (Tukey,  $p < 0.05$ ) and L. Svalbjørtjønn (Tukey,  $p < 0.05$ ) because it registered the lowest abundance of microplastics ( $4784 \pm 3857$  items/kg of dry sediment).

Table 10. The average ( $\pm$  SD) dry weight from 10 g of wet sediment weight (w.w) proceeding from 1L volume of sediment. The average number ( $\pm$  SD) of microplastic particles found in the dry sediment that come from 10 g w.w. The average number ( $\pm$  SD) of microplastic particles calculated per kilogram of dry sediment weight (d.w.).

Sampling site	Average dry weight (g)	Average n° of items/10 g w.w	Average n° of items/kg d.w.
Borgjaevju A	$5.5 \pm 3.6$	$18 \pm 6$	$4784 \pm 3857$
Borgjaevju B	$2.2 \pm 1.6$	$28 \pm 9$	$16126 \pm 7895$
Borgjaevju C	$1.7 \pm 0.5$	$61 \pm 18$	$37232 \pm 15438$
Presteevju A	$1.7 \pm 0.1$	$26 \pm 5$	$15208 \pm 2062$
Presteevju B	$2.3 \pm 1.5$	$21 \pm 7$	$13258 \pm 11892$
Presteevju C	$3.4 \pm 2.8$	$14 \pm 0$	$63201 \pm 5203$
Jønnebergjtjønn*	$0.3 \pm 0.2$	$12 \pm 5$	$50944 \pm 31315$
Svalbjørtjønn*	$0.4 \pm 0.4$	$27 \pm 4$	$129859 \pm 123965$

\*: It was not used 10 g of wet sediment weight. Wet and dry sediment weights are detailed in annex 4.

Fibres and fragments were the dominant microplastic types (82.5 % and 17.2 %, respectively) in the sediment samples although films (0.1%) and unknown fragments (0.2 %) were found in one sampling site. The highest number of fibres was found at Borgjaevju C ( $49 \pm 17$  fibres/10 g w.w) while the lowest number occurred at Presteevju A ( $11 \pm 3$  fibres/10 g w.w). The highest number of fragments was found at Presteevju A ( $15 \pm 9$  microplastic fragments /10 g w.w) whereas the lowest number occurred at L. Jønnebergjtjønn ( $0.7 \pm 0.8$  microplastic fragments per 8.8 g of wet sediment on average, since some samples did not have 10 g of 1-5-mm-size sediment) (table 11).

**Table 11. Average number ( $\pm$  SD) of fibres, fragments, films and unknown fragments of all sampling sites per 10 g w.w of sediment from the initial 1L volume.**

Sampling site	Microplastic types per 10 g w.w			
	Average number of fibres	Average number of fragments	Average number of films	Average number of unknown
Borgjaevju A	$16 \pm 7$	$2 \pm 3$	0	0
Borgjaevju B	$23 \pm 6$	$5 \pm 4$	0	0
Borgjaevju C	$49 \pm 17$	$11 \pm 5$	0	$0.3 \pm 0.8$
Presteevju A	$11 \pm 3$	$15 \pm 9$	0	0
Presteevju B	$16 \pm 5$	$5 \pm 3$	$0.5 \pm 0.7$	0
Presteevju C	$12 \pm 1$	$2 \pm 1$	0	0
Jønnebergjtjønn*	$12 \pm 6$	$0.7 \pm 0.8$	0	0
Svalbjørtjønn*	$25 \pm 4$	$2 \pm 2$	0	0

\*: It was not used 10 g of wet sediment weight. Wet and dry sediment weights are detailed in annex 4.

Predominantly, fibres were black (average value of  $10 \pm 10$  fibres/10 g w.w) and blue ( $4 \pm 3$  fibres/10 g w.w), but they were also found transparent ( $4 \pm 4$  fibres/10 g w.w), violet (including violet and purple) ( $2 \pm 2$  fibres/10 g w.w), green ( $2 \pm 2$  fibres/10 g w.w), red ( $1 \pm 1$  fibres/ 10 g w.w), pink ( $0.3 \pm 0.6$  fibres/10 g w.w), multicolour (e.g blue and white, green, white and pink, green and white) ( $0.2 \pm 0.5$  fibres/10 g w.w) and from other colours (i.e white, orange, fluorescent yellow, brown and grey) ( $0.2 \pm 0.5$  fibres/10 g w.w).

In addition, microplastic fragments were mostly blue (average value of  $1 \pm 1$  fragments/10 g w.w). There were also found fragments of other colours such as green ( $0.9 \pm 2.1$  fragments/10 g w.w), yellow ( $0.8 \pm 1.2$  fragments/10 g w.w), black ( $0.5 \pm 1.3$  fragments/10 g w.w), white ( $0.5 \pm 1.5$  fragments/10 g w.w), transparent ( $0.4 \pm 1.1$  fragments/10 g w.w), multicolour ( $0.3 \pm 0.8$  fragments/10 g w.w), red ( $0.2 \pm 0.5$  fragments/10 g w.w), orange ( $0.1 \pm 0.5$  fragments/10 g w.w) and other colours (i.e pink, purple, grey) ( $0.1 \pm 0.4$  fragments/10 g w.w). The figure 16 shows a bar chart with the average number of fibres and microplastic fragments according to the colour found per 10 g w.w of sediment in the sedimentation ponds of both Borgjaevju and Presteevju streams and both L. Svalbjørtjønn and L. Jønnebergjtjønn.

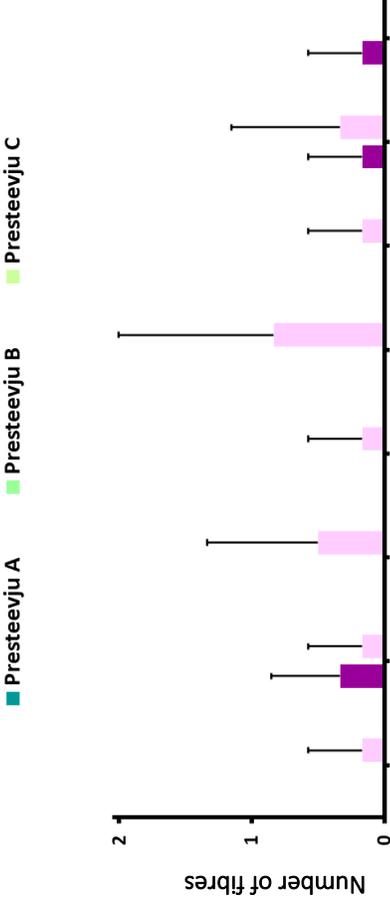
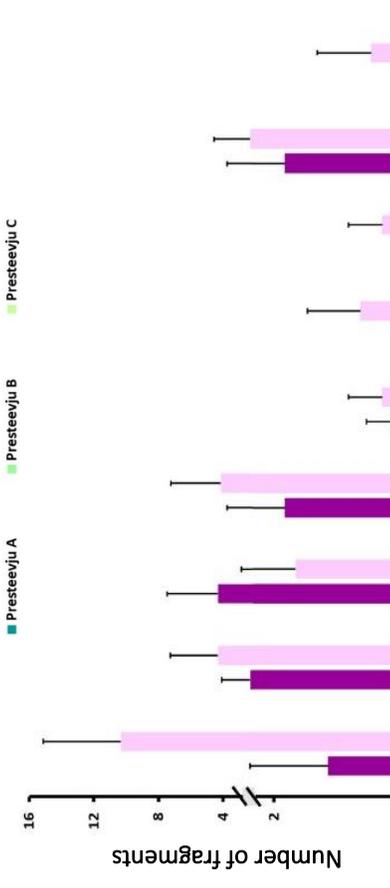
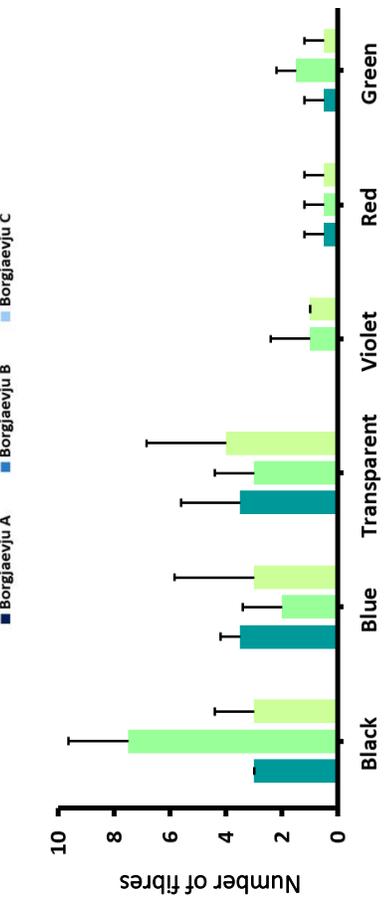
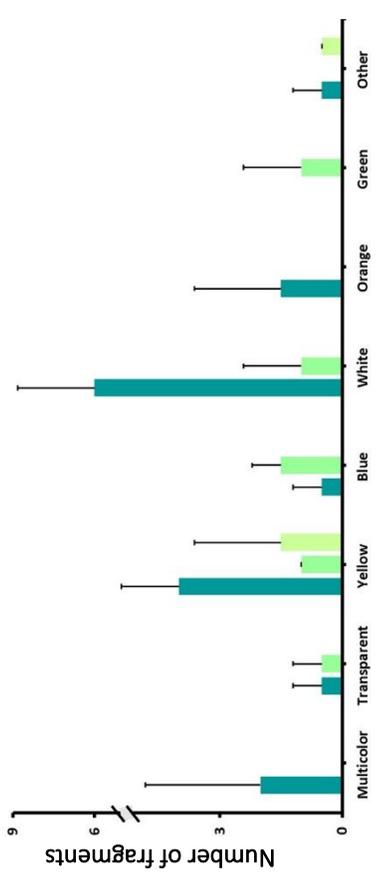
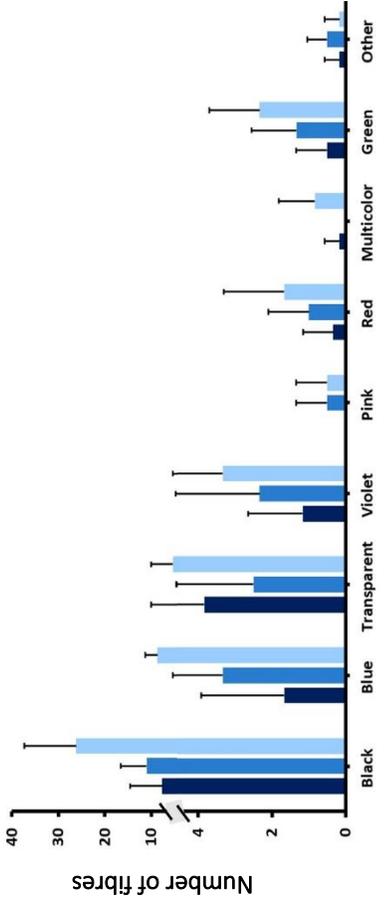
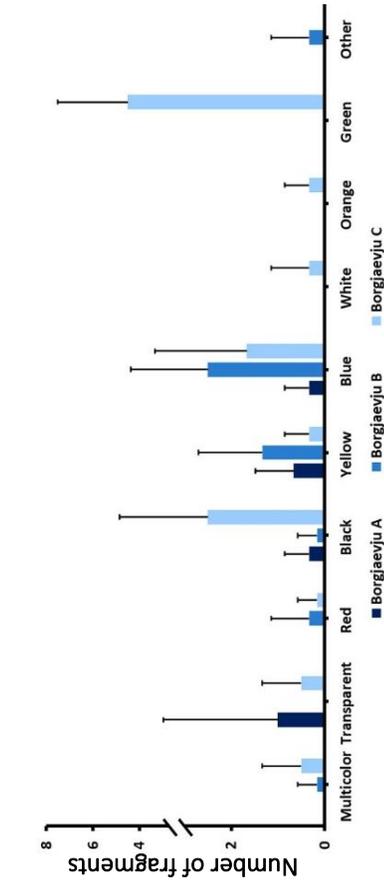


Figure 16. Average number ( $\pm$  SD) of fibres and fragments per 10 g w.w of sediment per sampling site (Borgjaevju and Presteevju streams and L. Svalbjørtjønn and L. Jønnebergstjønn).

The Shannon index allows to understand the diversity of microplastics in the sediment, linking the richness and the abundances of microplastics within. Presteevju A ( $H' = 1.97$ ), Presteevju B ( $H' = 1.94$ ) and Borgjaevju C ( $H' = 1.72$ ) showed the highest median values of the Shannon index (table 12). Sediment samples from this sedimentation ponds contain more variety of microplastics and higher abundances of each type. Borgjaevju A showed the lowest value ( $H' = 1.03$ ). The site was a significant factor determining the diversity of microplastics in the sediment samples (ANOVA,  $p < 0.001$ ). The most significant difference was found between Borgjaevju A and Borgjaevju C (Tukey,  $p < 0.01$ ). Borgjaevju A was also significantly different from Borgjaevju B, Presteevju A and Presteevju B (Tukey,  $p < 0.05$ ). Although less significant, L. Svalbjørtjønn and L. Jønnebergtjønn were slightly different from Borgjaevju A (Tukey,  $p = 0.05$ ) and Borgjaevju C (Tukey,  $p = 0.07$ ), respectively, showing  $p$ -values lower than 0.1.

**Table 12. Shannon Index median values of the microplastic composition of the sediment from both sedimentation ponds at Borgjaevju and Presteevju streams and both L.Jønnebergtjønn and L. Svalbjørtjønn.**

Shannon Index	Borgjaevju stream			Presteevju stream			Lakes	
	A	B	C	A	B	C	Jønnebergtjønn	Svalbjørtjønn
	1.03	1.70	1.72	1.97	1.94	1.55	1.38	1.59

A NMDS (figure 17) and a HCA (figure 18) were performed to classify the sampling sites based on the Bray-Curtis similarities of the composition of the microplastic debris found in the sediment. The studied variables correspond to the abundances of each type and colour of the microplastics. The sampling points of Borgjaevju A are quite far from the central cloud of points. This agrees with the information provided by the other statistical tests (Kruskal-Wallis and ANOVA tests) as Borgjaevju A has less abundance of microplastics and less diversity. The rest of points are closer, so they are more similar, even though, some slightly differences can be appreciated.

The sedimentation ponds at Borgjaevju stream seems to have a higher variability within microplastics. The samples from Borgjaevju C sedimentation pond are more similar than those at Borgjaevju A which are divided in two groups. Some samples from Borgjaevju B are more similar to those from Borgjaevju C while other samples are more similar to the previous pond (Borgjaevju A) or even the reference lakes. The reference lakes show a similar microplastic debris composition to the sedimentation ponds. L. Jønnebergtjønn is more homogenous than L. Svalbjørtjønn, as the samples are located in the same area of the figure (figure 17). Sediment samples from Presteevju stream seems to be similar among them and to some of Borgjaevju stream. The sampling point Presteevju A1 stands out from the rest as there were found much more and different microplastics than the rest sedimentation ponds of the same river (see annex 6).

A PERMANOVA test ( $p < 0.05$ ) was performed to check the differences between the groups of the NMDS. The sampling site resulted to be a determinant factor as the obtained  $p$ -value was 0.001, thus, meaning the groups are significantly different. A one-way ANOVA test was made to see whether the homogeneity of the variances of the groups was met. The obtained  $p$ -value was 0.052, so the assumption of the homogeneity of the variances is correct.

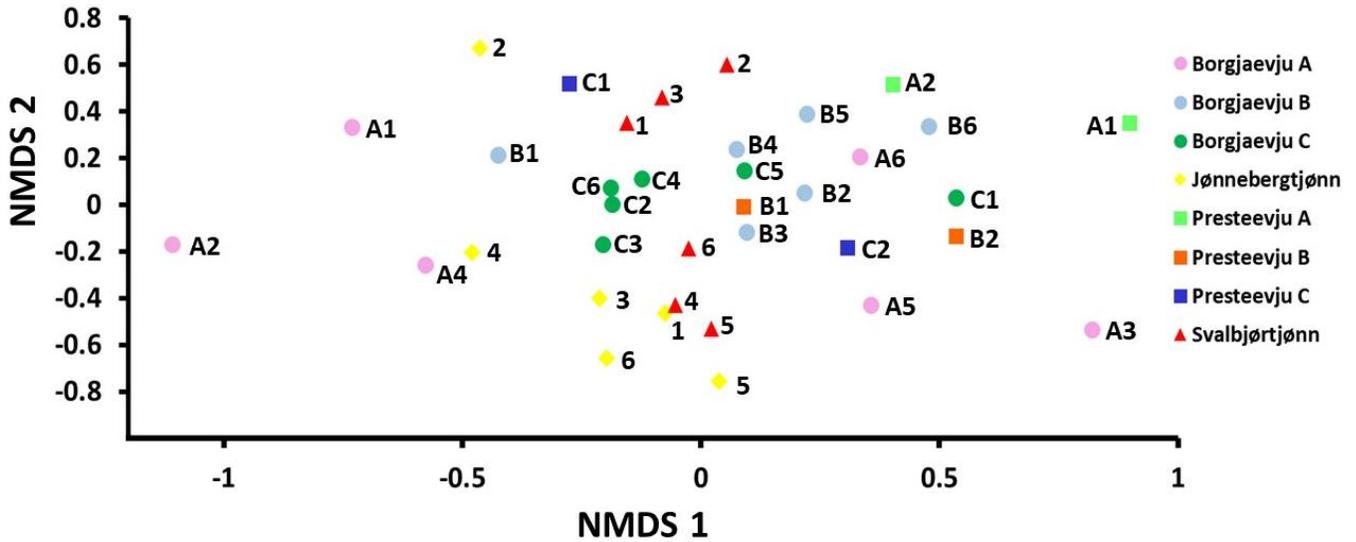


Figure 17. Non-metric multidimensional scaling (NMDS) ordination visualizing Bray-Curtis similarities of the microplastic composition debris of the sediment among the sampling sites. Symbols and colours represent different sampling sites and numbers represent the different samples.

The HCA displays the sampling places based on the Bray-Curtis similarities. It can be seen that the sedimentation pond Borgjaevju C is different from the rest of places as it appears out of the cluster. Borgjaevju C contained more microplastics of each type and more types of microplastics within the sediment. Presteevju A seems to be also different from the rest of sampling places as they also had a high number of plastics but the dominant microplastic type was fragments instead of fibres as at f Borgjaevju C (see table 11). The microplastic composition of the rest of the sampling places seemed to be more similar.

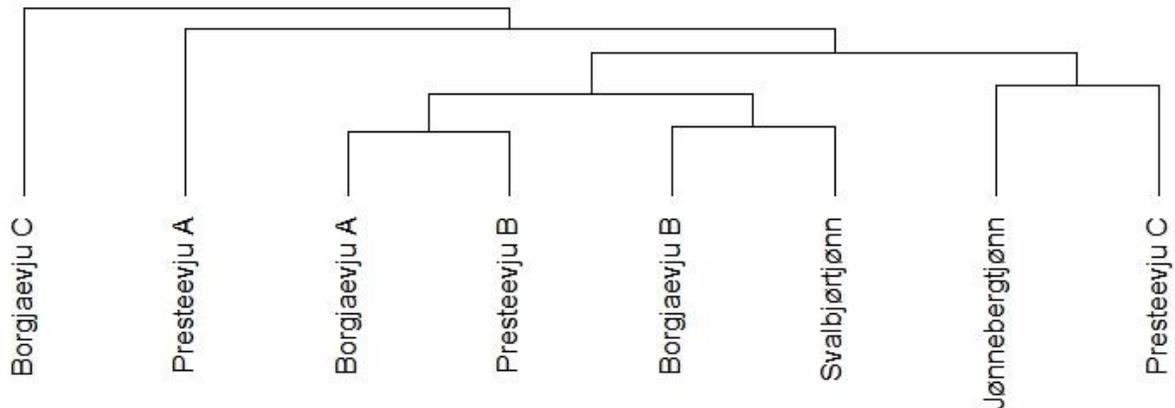


Figure 18. The hierarchical cluster (HCA) based on the Bray-Curtis similarities of the microplastic debris composition of the sediment at each sampling place. Letters correspond to the different sedimentation ponds at each stream. It was used the average method to build it.

### 3.3 Microplastics in water

Microplastics were also found in the water samples at all sampling sites and over the whole sampling period (see annex 7). Table 13 summarizes the information of average number of microplastic particles per litre of water, the Shannon index calculated to compare the diversity of microplastics between sampling points and the average

number of the different microplastic types (fibres, fragments, films and unknown) per litre of water. The average corresponds to the arithmetic mean of the samples recovered at three different weeks (May 7<sup>th</sup> and 8<sup>th</sup>, May 21<sup>st</sup> and June 4<sup>th</sup>) for the streams and the value for one sampling location for the two lakes.

**Table 13. The median of the Shannon index calculated per each sampling point. The average ( $\pm$  SD) number of microplastic particles per litre of water and the correspondent average number ( $\pm$  SD) of each type of microplastic (fibres, fragments, films and unknown). The averages were calculated based on three different sampling days.**

Sample	Median Shannon Index	Average n <sup>o</sup> of items/L	Microplastic types per 1L of water			
			Average n <sup>o</sup> of fibres	Average n <sup>o</sup> of fragments	Average n <sup>o</sup> of films	Average n <sup>o</sup> of unknown
Borgjaevju A	1.73	25 $\pm$ 16	21 $\pm$ 15	4 $\pm$ 1	0	0
Borgjaevju B	1.89	29 $\pm$ 20	21 $\pm$ 16	7 $\pm$ 5	0	0.3 $\pm$ 0.6
Borgjaevju C	1.54	18 $\pm$ 10	10 $\pm$ 7	8 $\pm$ 5	0	0
Borgjaevju C outlet	1.43	23 $\pm$ 17	16 $\pm$ 17	7 $\pm$ 6	0	0
Presteevju A	1.77	45 $\pm$ 55	29 $\pm$ 35	11 $\pm$ 12	0	0
Presteevju B	1.69	35 $\pm$ 40	22 $\pm$ 24	11 $\pm$ 14	0	0
Presteevju C	1.68	35 $\pm$ 29	24 $\pm$ 19	9 $\pm$ 7	0.3 $\pm$ 0.6	0
Jønnebergjtjønn*	1.04	25	9	16	0	0
Svalbjørtjønn *	1.51	21	16	5	0	0

“\*”: Only one composite sample from three different depths.

Water from Presteevju stream contained more microplastics (35-45 items/L) than water from Borgjaevju stream and lakes which showed a similar abundance, between 20 and 30 microplastics per one litre of water (see table 13). By contrast, that differences in numbers were not significantly different (Kruskal-Wallis,  $p > 0.05$ ). Water from Presteevju A got the highest number of microplastics with an average number of 45 ( $\pm$  55) items/L. Presteevju B and C showed a high number of microplastics as well (35  $\pm$  40 and 35  $\pm$  29 items/L, respectively). The lowest abundance of microplastics was registered at Borgjaevju C sedimentation pond with an average value of 18  $\pm$  10 items/L. Moreover, microplastics were found in larger abundances in the first sampling date than in the rest of dates. The number of microplastics per litre resulted to be significantly different between the sampling days June 6<sup>th</sup> and May 8<sup>th</sup> (Kruskal-Wallis,  $p < 0.05$ ; Tukey,  $p < 0.05$ ).

The figures 19 and 20 compare the number of microplastics (items/L) and the water flow at both Borgjaevju and Presteevju streams over the three different sampling dates. At a glance, it is difficult to see any clear relationship between both variables. Moreover, the Pearson correlation coefficient which was 0.09 ( $p > 0.05$ ) proved that both variables are not well correlated. The variability on the abundances of microplastics was higher at Borgjaevju stream. Microplastics were more abundant in the outlet than in the inlet, except in the first sampling day. On May 7<sup>th</sup> the water from the outlet of Borgjaevju sedimentation ponds recorded the lowest amount of microplastics (9 items/L) matching with the lowest water flow at this point. However, the highest peak of microplastics over the whole period

occurred at Borgjaevju B in the same day (52 items/L). At Presteevu stream, microplastic abundance seems to decrease or keep constant in the water through the sedimentation ponds. Again, the first sampling day (May 8<sup>th</sup>) registered the highest number of microplastics found in these sedimentation ponds.

The water flow experienced increases and decreases over the sampling period at both streams. The general trend of the water flow at Borgjaevju stream was characterized to be higher before the sedimentation ponds than after. On the contrary, the water flow at Presteevu stream was higher before the sedimentation ponds than after, although the trend changed it on June 4<sup>th</sup> when the water flow was lower at the beginning. Nevertheless, the measures taken in the inlet of the sedimentation ponds at Presteevu stream are not accurate at all since the method used was not carried out properly (it will be discussed further in the discussion).

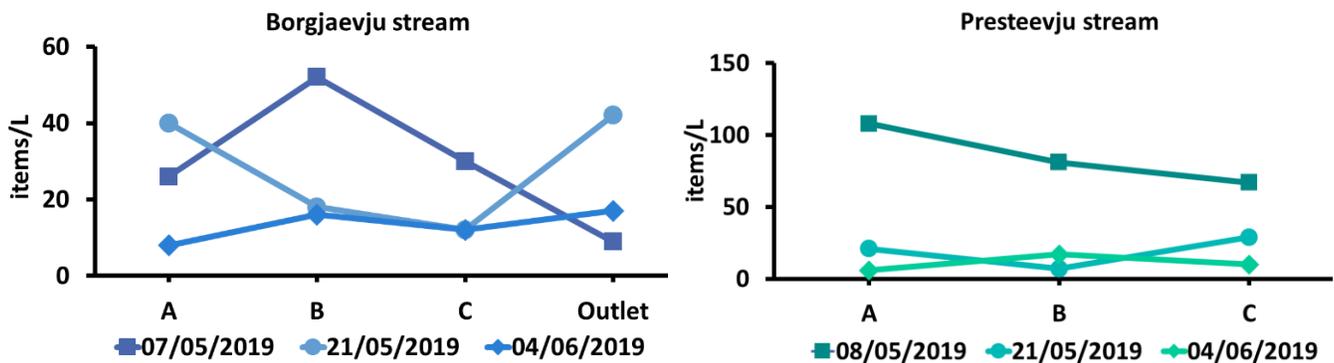


Figure 19. Microplastics (items/L) in the water of the sedimentation ponds at both Borgjaevju and Presteevu streams over the three sampling days.

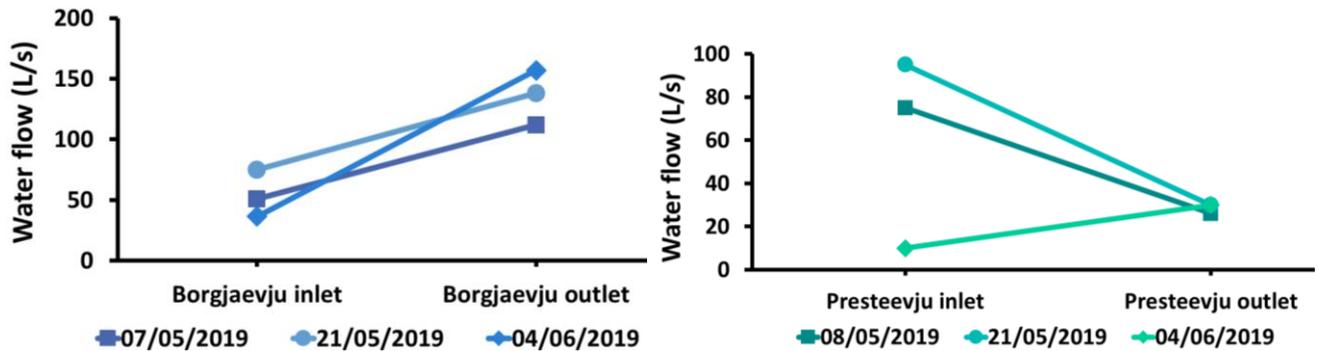


Figure 20. Water flow in L/s calculated for both inlet and outlet of Borgjaevju and Presteevu streams at the different sampling days. The flow rate was measured at the same places where water samples were recovered except at Presteevu outlet.

The number of microplastics that were entering and going out from the ponds was calculated according to the water flow (L/s) (table 14). In summary, the average number of microplastics that were entering in the Borgjaevju stream sedimentation ponds was 1541 (± 13645) items/s while 31578 (± 2431.12) items/s were released to Borgjaevju stream again between May and June.

The number of microplastics that entered in the Presteevju stream sedimentation ponds, on average was higher, being 3385 ( $\pm$  4196) items/s. It could not be estimated the number of items/s after the sedimentation ponds at Presteevju stream because no water samples were taken. However and based on the graph of Presteevju stream displayed on figure 20, the sedimentation ponds do not retain microplastics at all, so likely, some microplastic items may be released from Presteevju to Borgjaevju stream as well.

**Table 14. Number of microplastics (items /L), flow rate (L/s) and calculated number of microplastic items per second at both sedimentation ponds of Borgjaevju and Presteevju streams over the whole sampling period.**

Sample	Date	n° of items/L	Flow rate (L/s)	n° of items/s
Borgjaevju A inlet	07/05/2019	26	51	1326
Borgjaevju C outlet	07/05/2019	9	112	1008
Borgjaevju A inlet	21/05/2019	40	75	3000
Borgjaevju C outlet	21/05/2019	42	138	5796
Borgjaevju A inlet	04/06/2019	8	37	296
Borgjaevju C outlet	04/06/2019	17	157	2669
Presteevju A inlet	08/05/2019	108	75	8100
Presteevju A inlet	21/05/2019	21	95	1995
Presteevju A inlet	04/06/2019	6	10	60

As regards the microplastic composition of the water, fibres (70 %) followed by fragments (29.7 %) were the most common microplastic types. Films were seldom recovered (0.2 %), only at Presteevju C, and one unknown fragment was found at Borgjaevju B. The maximum average number of fibres was detected at Presteevju A ( $29 \pm 35$ ) and the maximum average number of fragments was discovered at Presteevju B and A ( $B = 11 \pm 14$ ,  $A = 11 \pm 12$ ).

Figure 21 and 22 displays the average abundances per litre of water of the different microplastics found in the water samples from the different sampling sites during the sampling period. On average, black, blue and transparent fibres occurred often in larger numbers ( $10 \pm 11$ ,  $5 \pm 5$ ,  $2 \pm 3$  fibres/L, respectively) than fibres of colours such as green ( $0.8 \pm 1.2$  fibres/L), purple ( $0.6 \pm 0.8$  fibres/L), red ( $0.6 \pm 0.8$  fibres/L) or multicolour ( $0.2 \pm 0.6$  fibres/L). Otherwise, blue fragments (average value of  $5 \pm 5$  fragments/L) were the most dominant fragments. Colours such as transparent ( $1 \pm 2$  fragments/L), black ( $0.8 \pm 1.1$  fragments/L) or green ( $0.7 \pm 1.2$  fragments/L) were also frequent, and red ( $0.3 \pm 0.8$  fragments/L), multicolour ( $0.2 \pm 0.5$  fragments/L), white ( $0.1 \pm 0.3$  fragments/L) and other colours (i.e pink, grey, purple, orange) ( $0.2 \pm 0.5$  fragments/L) appeared sporadically.

In L. Jønnebergtjønn and L. Svalbjørtjønn, black fibres and blue fragments were the most common microplastics (see figure 21). The microplastic types and abundances discovered at both Borgjaevju and Presteevju streams were quite similar. The diversity of microplastics in the water samples were studied through calculating the Shannon index as well as in the sediments (see table 13). The pond with the highest average of microplastic diversity was Borgjaevju B followed by Presteevju A whose median Shannon index were 1.89 and 1.77 separately, whereas L.

Jønnebergtjønn and L. Svalbjørtjønn had the lowest diversity ( $H' = 1.04$ ,  $H' = 1.51$  respectively). However, there were not significant differences for this variable either sampling site nor sampling date (ANOVA,  $p > 0.05$ ).

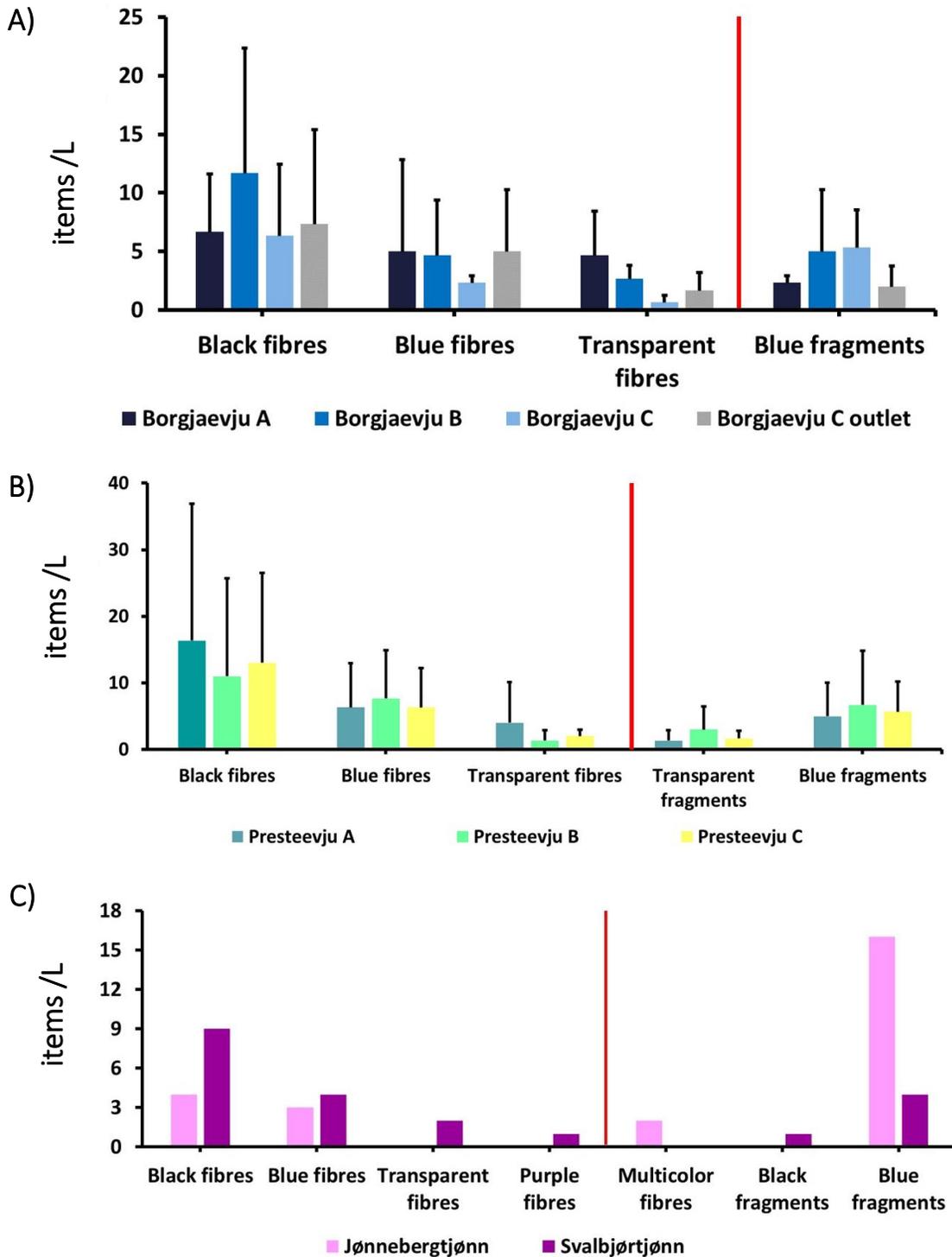


Figure 21. Average numbers of the most abundant types of microplastics found in each sedimentation pond of Borgjaevju and Presteevju streams and L. Jønnebergtjønn and L. Svalbjørtjønn over the whole sampling period. A) Sedimentation ponds at Borgjaevju stream. B) Sedimentation ponds at Presteevju stream. C) L. Jønnebergtjønn and L. Svalbjørtjønn. The error bars mean the standard deviation. There was only one sample of L. Jønnebergtjønn and L. Svalbjørtjønn. The red line separate fibres from fragments.

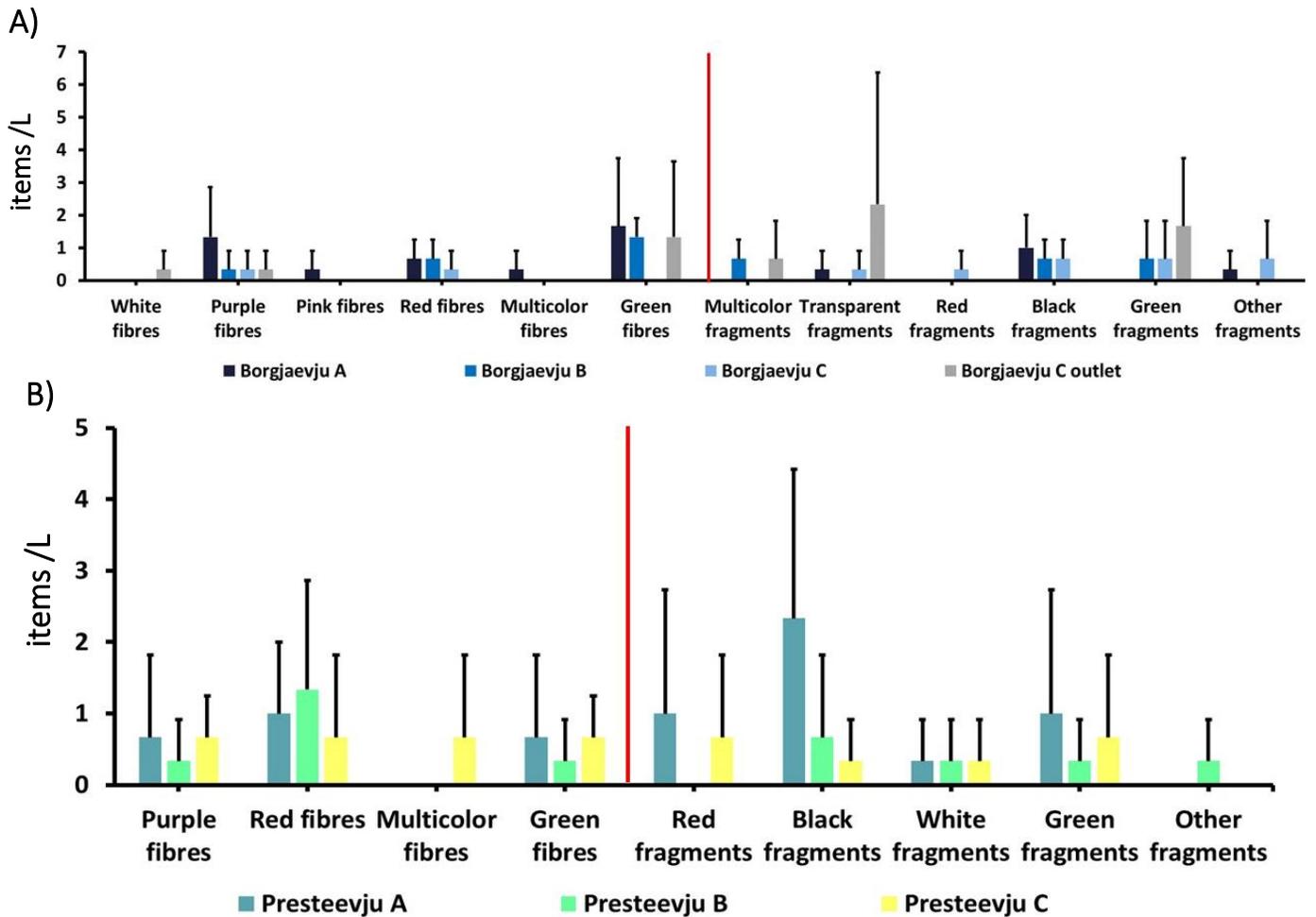


Figure 22. Average numbers of the least abundant types of microplastics found in each sedimentation pond of both Borgjaevju and Presteevju streams over the whole sampling period. A) Sedimentation ponds at Borgjaevju stream. B) Sedimentation ponds at Presteevju stream. The error bars mean the standard deviation. The category “other fragments” includes pink, grey, purple and orange fragments. The red line separate fibres from fragments.

Following the sediment statistical analysis, a NMDS (figure 23) and two latter HCAs (figure 24) were again performed to classify the water samples based on the Bray-Curtis similarities of the composition of the microplastic debris found in the sampling points and sampling dates. The variables used to build this model correspond to the abundances of each microplastic type and colour.

It is not possible to see clear groups in the NMDS plot, but most of the samples corresponding to the same sampling date seem to be close. A PERMANOVA test ( $p < 0.05$ ) was performed to check the differences between the groups of the NMDS. The sampling site was not a significant factor as the obtained  $p$ -value was 0.98 ( $p > 0.05$ ), but the sampling date was a significant factor with a  $p$ -value of 0.002. Consequently, the composition of microplastic debris varies according to the dates instead of the sites. A one-way ANOVA test was made to see whether the homogeneity of the variances of the data and the groups had met. The obtained  $p$ -value was 0.23 for sampling site and 0.09 for sampling date, so the assumption of the homogeneity of the variances is correct in both cases.

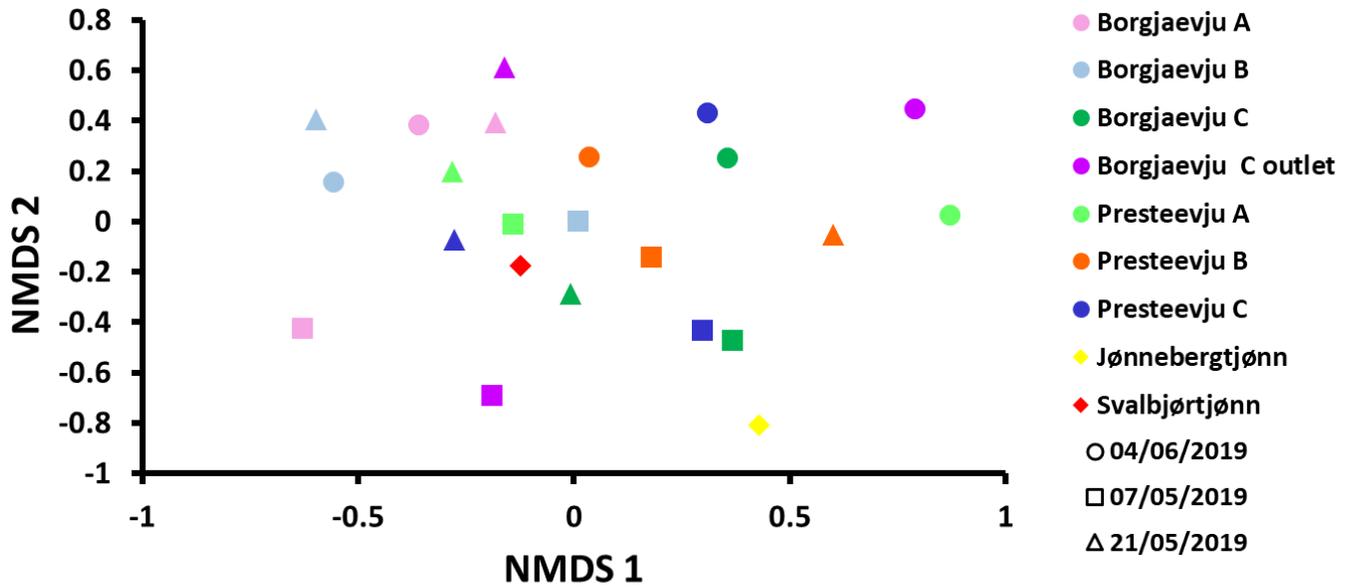


Figure 23. Non-metric multidimensional scaling (NMDS) ordination visualizing Bray-Curtis similarities of the microplastic composition debris of the water samples among the sampling sites at and dates. Symbols represent the different sampling dates (May 7<sup>th</sup>, 8<sup>th</sup>, 21<sup>st</sup> and June 4<sup>th</sup>) and colours represent the sampling sites (Borgjaevju and Presteevju streams and Svalbjørtjønn and Jønnebergjtjønn lakes).

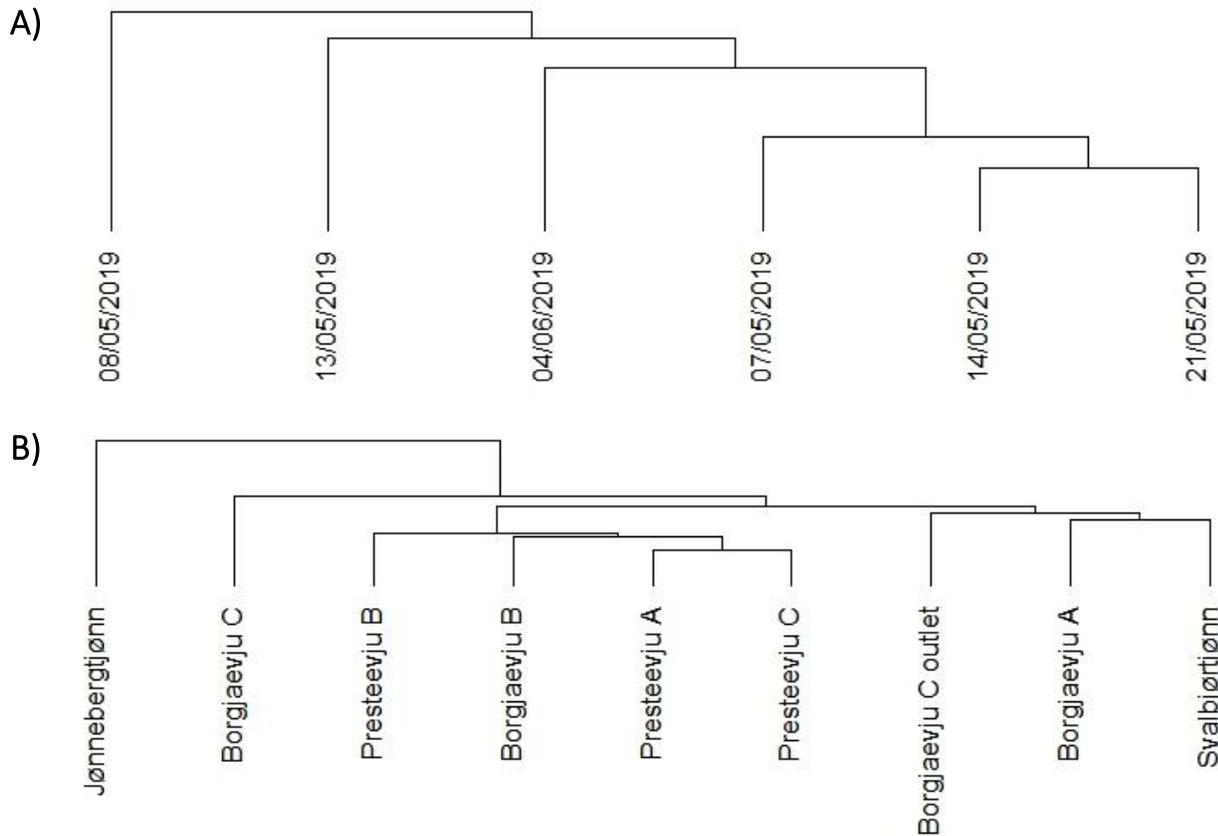


Figure 24. The hierarchical cluster (HCA) based on the Bray-Curtis similarities of the microplastic debris composition of the water samples at A) each sampling date (May 7<sup>th</sup>, 8<sup>th</sup>, 21<sup>st</sup> and June 4<sup>th</sup>) and B) site (Svalbjørtjønn and Jønnebergjtjønn lakes). HCAs were built using the average method in both cases.

The HCAs (figure 24) classify the sampling sites and dates according to the Bray-Curtis similarities. Only clusters of the HCA based on the sampling date are significantly different, so, sampling of May 8<sup>th</sup> and 13<sup>th</sup> and June 4<sup>th</sup> seems to be more different from the rest. Looking at the places, L. Jønnebergtjønn seems to be more different, but that difference is not significant, hence, it is not possible at all to assume it is very different from the others. On the other hand, microplastic composition of the water from L. Svalbjørtjønn appear to be similar to the microplastic composition of the water of some sedimentation ponds.

The water samples were not sieved and, the range of sizes of the microplastics recovered is wider than in the sediment, being 1.2  $\mu\text{m}$  the lower limit as it was the pore size of the filter. To provide an approach of the sizes ten microplastics of each type were measured in each sample. Thus, figures 25 and 27 show the range of sizes of fibres and microplastic fragments found among the different sampling sites. Fibres were significantly larger than fragments (Kruskal-Wallis,  $p < 2.2 \times 10^{-16}$ ). The average size for fibres was  $916.9 \pm 982.0 \mu\text{m}$  (min = 83.6  $\mu\text{m}$ , max = 10849.6  $\mu\text{m}$ ) and for fragments was  $191.3 \pm 274.9 \mu\text{m}$  (min = 191.3  $\mu\text{m}$ , max = 2402.6  $\mu\text{m}$ ). Fibre sizes were not significantly different among the sampling sites (Kruskal-Wallis,  $p > 0.05$ ), but was significantly different among the sampling dates (Kruskal-Wallis,  $p < 0.001$ ). In particular, the size of the fibres observed June 4<sup>th</sup> were smaller than those recovered on May 7<sup>th</sup> and 8<sup>th</sup> (Tukey,  $p < 0.05$ ) (figure 26). Conversely, there were not significant differences either for sampling site nor for sampling date for microplastic fragments (Kruskal-Wallis,  $p > 0.05$ ).

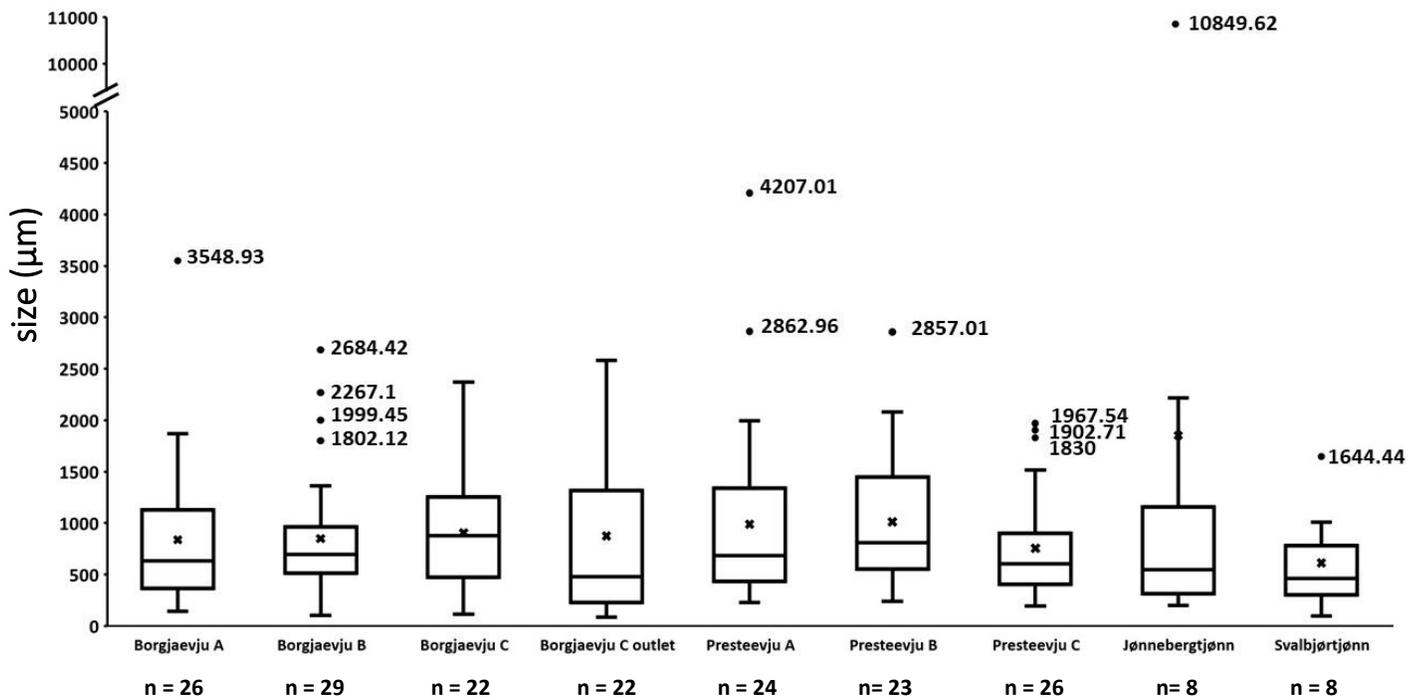


Figure 25. Boxplot of fibre sizes ( $\mu\text{m}$ ) according to the sampling sites. Crosses represent the mean and n is equal to the total number of fibres measured. Points represent outliers.

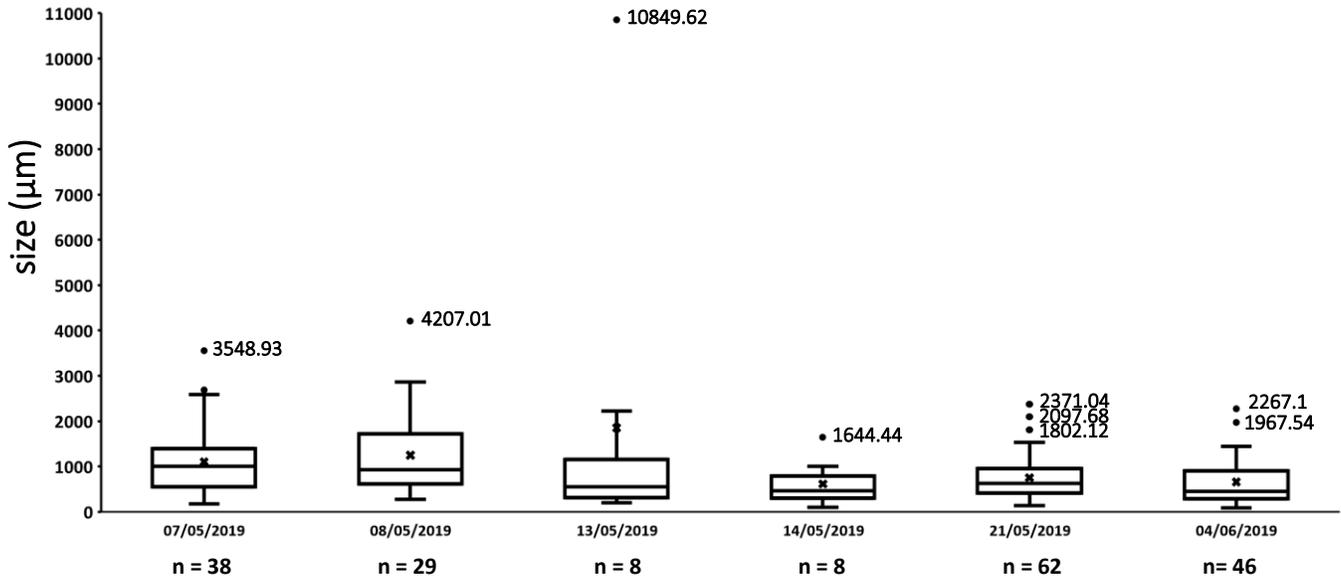


Figure 26. Boxplot of fibre sizes (µm) according to the sampling dates. Crosses represent the mean and n is equal to the total number of fibres was measured. Points represent outliers.

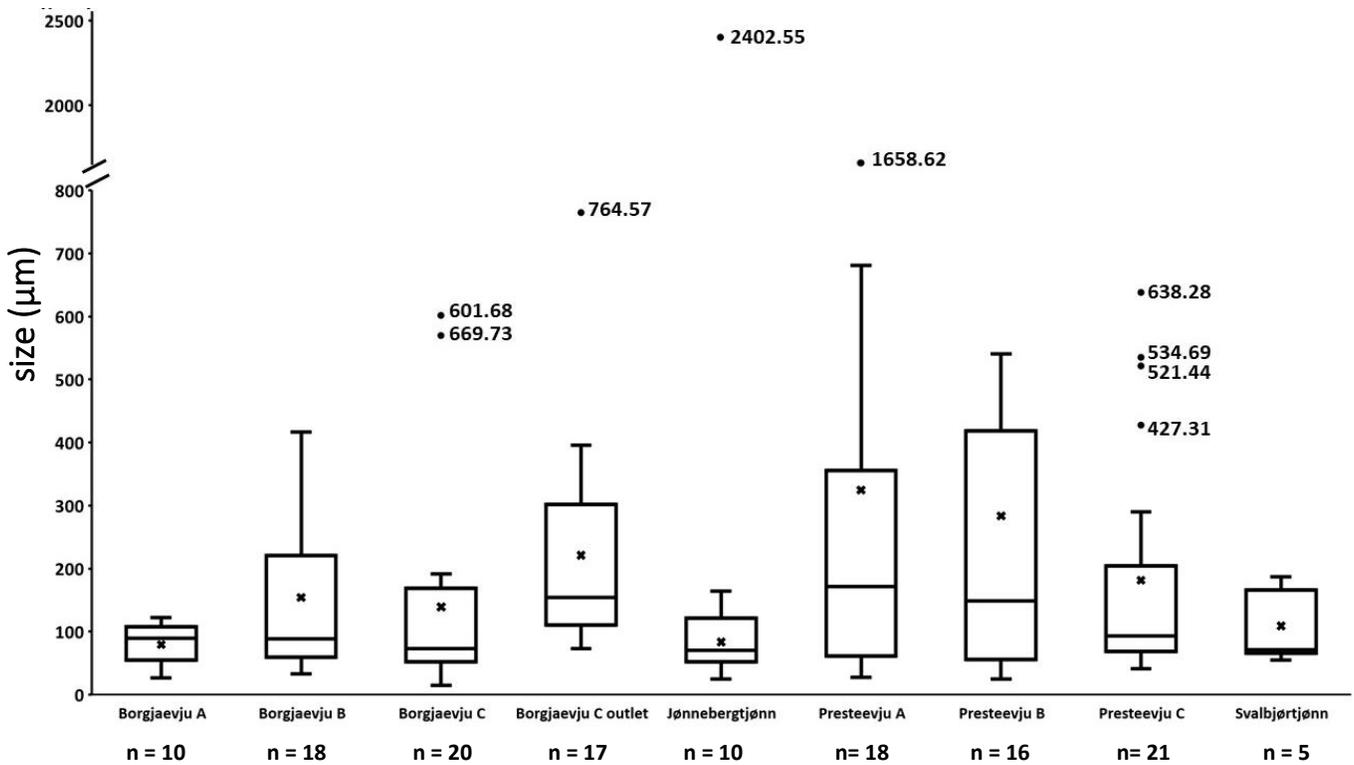


Figure 27. Boxplot of microplastic fragment sizes (µm) according to the sampling sites. Crosses represent the mean and n is equal to the total number of fibres was measured. Points represent outliers.

The size of the microplastics according to their colour was more variable among the fragments than among the fibres (see annex 8). In fact, there were significant differences for the size of microplastic fragments (Kruskal-Wallis,  $p < 0.001$ ) between black and blue fragments and blue (Tukey,  $p < 0.05$ ), green and red with transparent fragments (Tukey,  $p < 0.05$ ) (figure 28). Transparent fragments presented a higher variability within the data including large

sizes, and black, blue, green and red were predominantly small (<500 μm). Norwithstanding, there were not significant differences in the size of fibres according to the colours (Kruskal-Wallis,  $p > 0.05$ ). Most of them show a similar distribution of sizes as can be seen in figure 29.

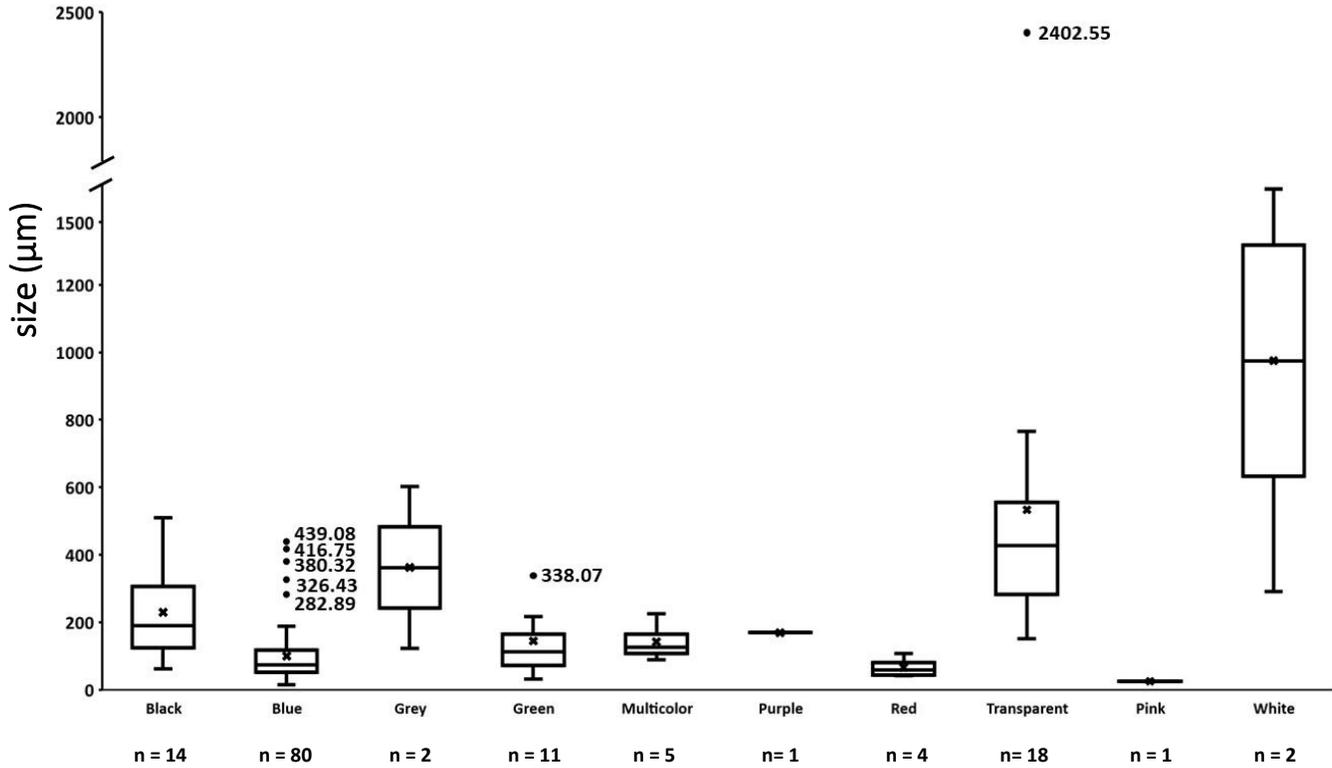


Figure 28. Boxplot of microplastic fragment sizes (μm) according to the colours. Crosses represent the mean and n is equal to the total number of fibres was measured. Points represent the outliers.

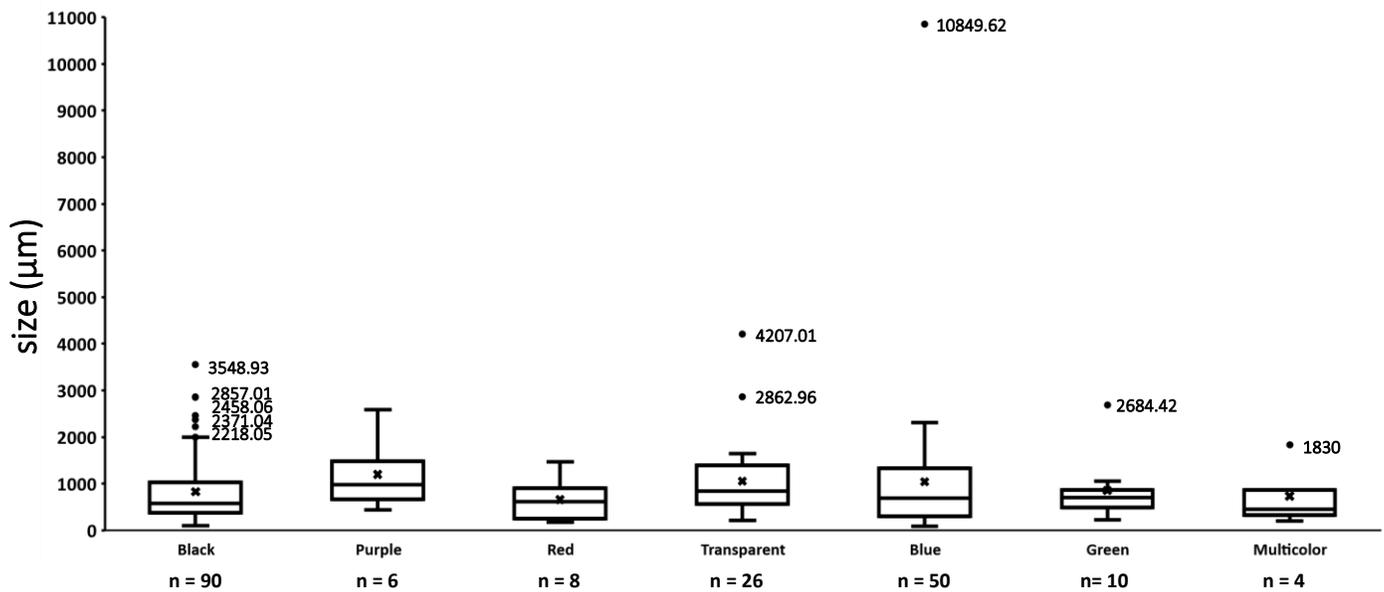


Figure 29. Boxplot of microplastic fragment sizes (μm) according to the sampling sites. Crosses represent the mean and n is equal to the total number of fibres was measured. Points represent outliers.

### 3.4 Water chemistry

On average, the water temperature for Jønnebergjønn was  $11 (\pm 0.6) ^\circ\text{C}$  over the first 6 m depth and  $8 (\pm 2.5) ^\circ\text{C}$  over the first 5 m depth for Svalbjørtjønn. The pH was a bit acid for Jønnebergjønn with a value of  $5.5 (\pm 0.1)$  while for Svalbjørtjønn was slightly higher,  $6.6 (\pm 0.3)$ . Turbidity was quite low for both L. Jønnebergjønn and L.Svalbjørtjønn (average values of  $0.9 \pm 0.2$  NTU and  $0.74 \pm 0.1$  NTU, respectively). EC and TOC were low as well, although the EC was slightly higher in Svalbjørtjønn, (average value of  $24.0 \pm 2.7 \mu\text{S}/\text{cm}$ ) than at Jønnebergjønn (average value of  $15.8 \pm 0.5 \mu\text{S}/\text{cm}$ ). On the opposite, TOC was higher at Jønnebergjønn than at Svalbjørtjønn whose values were  $5 (\pm 0.1)$  and  $4.2 (\pm 0.1) \text{ mg}/\text{L}$ , respectively. Additionally, total nitrogen was pretty much higher than total phosphorous in both lakes. The average total nitrogen was  $499.6 (\pm 3.6)$  and  $350.0 (\pm 27.9) \mu\text{g}/\text{L}$  and the average total phosphorous was  $13.5 (\pm 8.0)$  and  $11.1 (\pm 1.6) \mu\text{g}/\text{L}$  for Jønnebergjønn and Svalbjørtjønn, respectively. Overall, the total nitrogen was higher at Jønnebergjønn and the total phosphorous was higher at Svalbjørtjønn (table 15, annex 9).

Values of physical-chemical parameters at Borgjaevju and Presteevju streams were higher than those of the reference lakes (table 15, annex 9). The average temperature of the water at Borgjaevju sedimentation ponds over the sampling period was  $11.4 (\pm 3.4) ^\circ\text{C}$  and  $11.3 (\pm 3.9) ^\circ\text{C}$  for Presteevju sedimentation ponds, so the temperature was practically the same. pH was also similar at both rivers with values of  $7.1 (\pm 0.1)$  for Borgjaevju and  $7.4 (\pm 0.1)$  for Presteevju streams. However, the rest of parameters differed between the two streams. EC and total nitrogen were lower at Borgjaevju whereas turbidity, TOC and total nitrogen was lower at Presteevju stream. EC was one order of magnitude higher at Presteevju than at Borgjaevju stream. At Presteevju stream the EC average was  $199.1 (\pm 21.5) \mu\text{S}/\text{cm}$  while at Borgjaevju river was  $80.7 (\pm 26.5) \mu\text{S}/\text{cm}$ . Turbidity at Borgjaevju stream was around the double of the Presteevju stream value,  $7.2 (\pm 5.1)$  and  $3.2 (\pm 1.1)$  NTU, respectively. The same occurred with the TOC values which were  $6.6 (\pm 0.9) \text{ mg}/\text{L}$  for Borgjaevju stream and  $2.5 (\pm 0.4) \text{ mg}/\text{L}$  for Presteevju stream. Moreover, the total nitrogen decreased from the  $2304.6 (\pm 404.8) \mu\text{g}/\text{L}$  of Presteevju stream to the  $1075.4 (\pm 298.7) \mu\text{g}/\text{L}$  of Borgjaevju stream, but the total phosphorous was higher at Borgjaevju stream with  $26.7 (\pm 6.7) \mu\text{g}/\text{L}$  than at Presteevju stream whose value was  $18.2 (\pm 4.0) \mu\text{g}/\text{L}$ .

**Table 15. Summary of the physical-chemical parameters of the water (average  $\pm$  SD) at Borgjaevju and Presteevju streams and L. Jønnebergjønn and L. Svalbjørtjønn over the whole sampling period.**

Site	T water ( $^\circ\text{C}$ )	pH	EC ( $\mu\text{S}/\text{cm}$ )	Turbidity (NTU)	TOC (mg/L)	$\text{N}_\text{T}$ ( $\mu\text{g}/\text{L}$ )	$\text{P}_\text{T}$ ( $\mu\text{g}/\text{L}$ )
Borgjaevju	$11.4 \pm 3.4$	$7.1 \pm 0.1$	$80.7 \pm 26.5$	$7.2 \pm 5.1$	$6.6 \pm 0.9$	$1075.4 \pm 298.7$	$26.7 \pm 6.7$
Presteevju	$11.3 \pm 3.9$	$7.4 \pm 0.1$	$199.1 \pm 21.5$	$3.2 \pm 1.1$	$2.5 \pm 0.4$	$2304.6 \pm 404.8$	$18.2 \pm 4.0$
Jønnebergjønn	$11 \pm 0.6$	$5.5 \pm 0.1$	$15.8 \pm 0.5$	$0.9 \pm 0.2$	$5.0 \pm 0.1$	$499.6 \pm 23.6$	$13.5 \pm 8.0$
Svalbjørtjønn	$8 \pm 2.5$	$6.6 \pm 0.3$	$24.0 \pm 2.7$	$0.7 \pm 0.1$	$4.2 \pm 0.1$	$350.0 \pm 27.9$	$11.1 \pm 1.6$

NOTE: The average values at Borgjaevju and Presteevju streams come from the samples taken at the different sampling dates. The average values for lakes come from the samples taken at different depths.

Figure 30 shows the variation of the physical-chemical parameters at both Borgjaevju and Presteevju streams according to sampling sites and dates. Roughly, it can be seen that in spite of changing the physical-chemical parameter values according to the sampling date, the same trend of the data is maintained throughout the sampling sites. The variability of the values of physical-chemical parameters at Presteevju stream was larger than at Borgjaevju stream, where the differences of the values were smaller among the sampling dates.

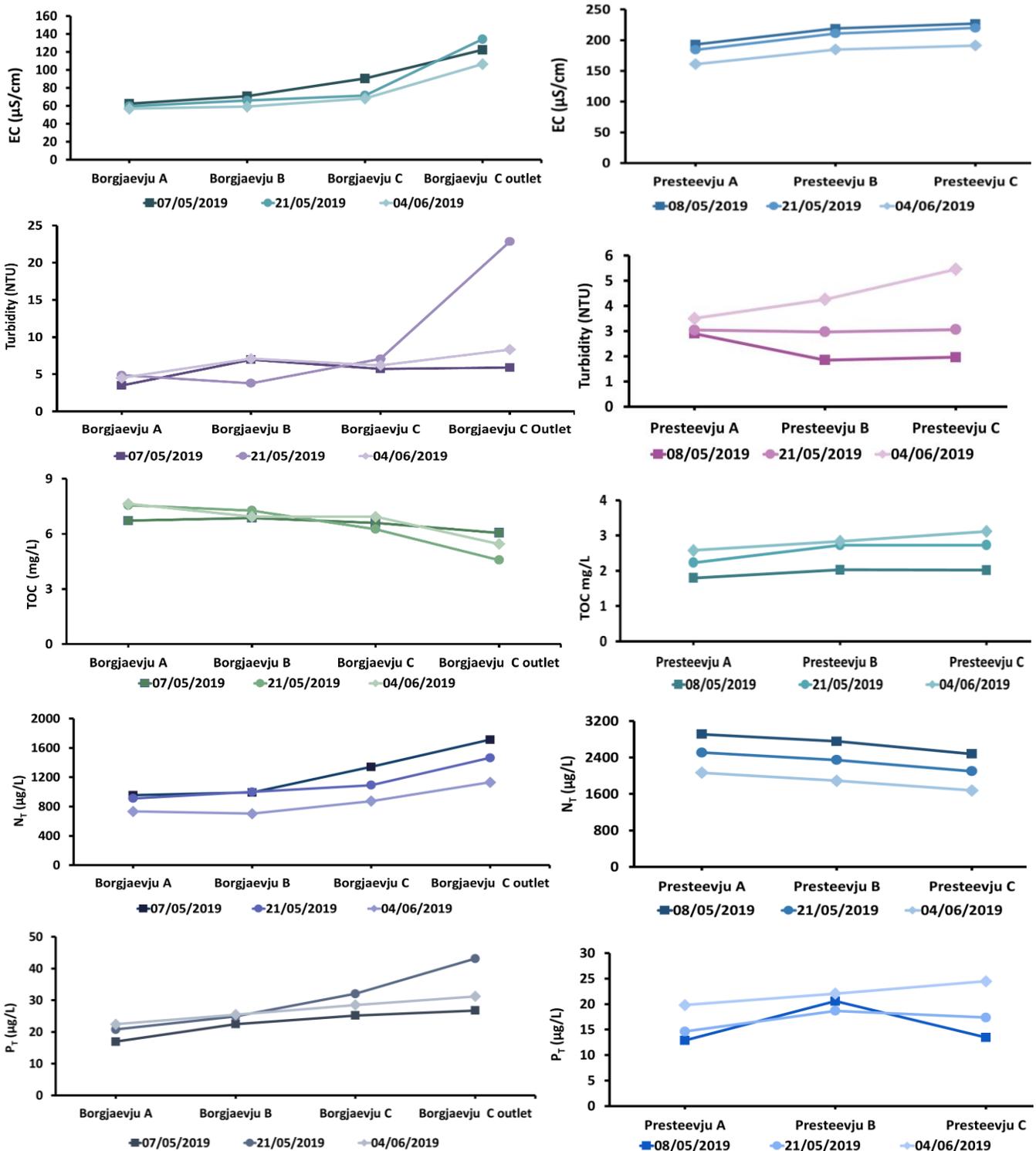


Figure 30. Physical-chemical parameter evolution of the sampling points at Borgjaevju and Presteevju streams according to the sampling dates.

EC, turbidity and total nitrogen and phosphorous tended to increase along the sedimentation ponds at Borgjaevju stream, but the TOC showed a decreasing trend over the sampling period. Therefore, the water that was going out of the system of sedimentation ponds had higher EC, turbidity, nitrogen and phosphorous and lower organic matter than the water was entering in the system. Overall, the values seemed to increase/decrease gradually through the ponds, although there was a drastically change in the last pond. The increasing and decreasing trend of the parameters is gradually, in most of the sampling dates, however, the EC, turbidity and total phosphorous showed a dramatic raise out of the sedimentation ponds in the last sampling date. On the other hand, physical-chemical parameters at Preteevju stream, although they experienced slightly variations, they showed a constant (e.g TOC, total phosphorous) or a decreasing trend (e.g total nitrogen) along the three sedimentation ponds. EC increased through the sedimentation ponds and turbidity raised at Presteevju C in the last sampling date.

From the correlation matrix performed with the physical-chemical parameters, the following significant ( $p < 0.05$ ) relationships were extracted. The strongest negative relationship with a Pearson coefficient of  $-0.97$  ( $p = 0.0006$ ) was between TOC and the EC, so these variables are inversely proportional. The TOC and the total nitrogen were also high inversely proportional with a Pearson coefficient of  $-0.94$  ( $p = 0.009$ ). The strongest positive relationships were between the total nitrogen and EC with a Pearson coefficient  $0.96$  ( $p = 0.001$ ) and between the total phosphorous and turbidity with a Pearson coefficient of  $0.92$  ( $p = 0.02$ ).

## 4. DISCUSSION

### 4.1 Suitability of the sorting method

The lack of standard, validated and universal methods for detecting microplastics was the first handicap that had to be overcome. The method described in this thesis was the summary of several tests made before treating the real samples and still, there are some considerations. On the one hand, there is a large variety of existing methods to deal with within the studies, from sampling to identifying microplastics and some authors have also agreed and reported such inconvenient (e.g Han et al. 2019; Horton et al. 2017b; NIVA, 2017; Prata et al. 2019, Van Cauwenberghe et al., 2015). On the other hand, there is also an absence of details (e.g. units per dry or wet sediment, weight or volume of the subsample, time that controls are opened) on reporting the steps of the methods used in most of the studies which makes the replication and understanding of the method difficult.

Given the amount of methods available the first task was focused on developing the method was going to be used. Different tests were performed before choosing the most appropriate one to the kind of samples of this study. It was necessary, at least in general trends, to discuss about the protocols and explain the suitability of the method chosen to better understand the results.

Most of the research on microplastics have been conducted on coastal or marine systems, and so sampling design have been developed accordingly. Unfortunately, the least amount of research carried out in freshwater

ecosystems and the general lack of standard methods lead to the application of those used in the seaside. Protocols to monitor microplastics on beaches recommend collecting a minimum of three samples in a 100 m stretch parallel to the sea edge (Frias et al., 2018). Due to the heterogenous distribution of the microplastics in the sediment some authors recommend a better sampling strategy consisting of analysing composite samples from several discrete samples than larger volume of only one or few samples (Han et al. 2019). According to these suggestions, it was concluded to take six sediment samples along the longitudinal axis of the Borgjaevju sedimentation ponds drawing an imaginary linear transect. No samples were recovered from the regions close to the shore and these areas could hold larger numbers of microplastics as they are more protected from the influence of the main water current. This is the first study on microplastics carried out in these sedimentation ponds so the results presented here can be understood as the starting point for future research. Although, a study performed in an urban lake at Birmingham (UK) recovered samples from several linear transects perpendicular to the edges of the lake and from the shores (Vaughan et al. 2017), they did not find significant differences among the samples regarding the kind of environment.

In contrast, only two samples were recovered at Presteevju sedimentation ponds since the ponds were smaller and there was more vegetation. Samples were recovered at the inlet and outlet of the sedimentation pond where the current can be stronger and microplastics may be drifting. Consequently, it is proposed to recover an additional sample from the intermediate area of the pond where the water remains stagnant.

The way of collecting the sediment samples using dredges seems to be commonly accepted in the research world, although other instruments such as grab samplers (Frias et al., 2018), box corers (Prata et al., 2019), stainless steel spatulas (Nor and Obbard, 2014) or scoops (Horton et al., 2017a) can be used depending on the purpose of the work. However, there is a higher variability of possibilities on how to treat the samples afterwards.

Some studies have worked with dry or wet sediment and sieving it or not before the density separation step (Prata et al. 2019). Here, the decision of following a protocol combining size- and density separation was chosen according to the aim of working only with large size microplastics (between 1 and 5 mm). In addition, the sediment was wet-sieved due to the drying process of the sediment took too much time during the trials (around five days to dry 500 mL of sediment sample at 60 °C). Publications such as Horton et al. (2017a) proposed to dry the sediment at 80 °C but this idea was discarded since Munno et al. (2018) determined the maximum temperature limit of 60 °C without being a risk to the integrity of microplastics. When wet sediment is dry, it forms aggregates which cannot be sieved properly, so they must be crushed (Lin et al., 2018; Nor and Obbard, 2014) to not mislead particles. At the same time the crushing step could break microplastics in smaller particles and change the initial microplastic composition. To speed up the process sediment was wet sieved following Horton et al. (2017a).

Notwithstanding, the wet-sieving was an arduous task since it came down with some difficulties. In general, all sediment samples mainly consisted of clay, silt and vegetation fragments. The vegetation created a kind of net trapping the compacted sediment making it difficult to sieve. The sediment was gently rinsed with distilled water

to ensure the correct sieving of the sediment, but likely, introducing some bias since the original content of water of the sediment may have been modified.

Consistent with previous literature, most of the methods applied the density separation to subsamples containing all size fractions (e.g Lin et al., 2018; NIVA, 2017; NIVA 2018) or the already sieved whole fractions (Vaughan et al. 2017). Horton et al. (2017a) did it with subsamples but they did not mention the weight of each one. The protocol developed for this Master's thesis included standard homogenous subsamples of 10 g of 1-5 mm-size-fraction- wet sediment (except for some special cases such as L. Jønnebergtjønn and L. Svalbjørtjønn) to make sure the density separation worked and reduce bias. Han et al. (2019) have suggested that there are two important factors that govern the success of the extraction method: the sample mass and the ratio of sample mass and the applied volume of flotation solution, requiring more flotation solution when using more sample mass.

There are many variations in the density separation method. The initial density separation method proposed by Thompson et al. (2014) used a concentrated sodium chloride ( $1.2 \text{ g/cm}^3$ ) (NaCl) solution as it was designed to extract buoyant microplastics in seawater. Unfortunately, this solution can only separate low-density microplastics and not high-density microplastics such as PVC or PET which comprise the 17% of the worldwide plastic demand (PlasticsEurope, 2013). To extract high-density microplastics, Imhof et al. (2012) started applying  $\text{ZnCl}_2$  solution and Claessens et al. (2013) made up the two-step extraction using tap water and NaI solution. Lately, other solutions such as NaBr or  $\text{ZnBr}_2$  among others (Frias et al., 2018) have also been investigated. NIVA (2017) modified the protocol proposed by Claessens et al. (2013) for extracting microplastics from the sediment of freshwater ecosystems. The tap water was replaced by filtered reverse osmosis (RO) water since tap water contains dissolved salts which increase the density of the water, and included the second step with NaI.

This work modified again NIVA's protocol (2017) using filtered distilled water instead of RO water and  $\text{ZnCl}_2$  instead of NaI, due to the reported high recovery rates (Horton et al. 2017a; Prata et al., 2019) and the lower price (Frias et al., 2018). The recovery rate of both filtered distilled water and  $\text{ZnCl}_2$  was high enough to recover most of the microplastics from the sediment. Almost of the 25 % of microplastics remain in the sediment after the density separation, similar percentage to that observed by Horton et al. (2017a).

Based on observations, there were no differences between the microplastics extracted with each density solution. Fibres and fragments of different colours were isolated in both steps and found in the remaining sediment, thus, it is difficult to assert that microplastics found at the non-floating sediment were high-dense plastics (e.g PVC, PET, PTFE as said by Frias et al. (2018)). The  $\text{ZnCl}_2$  whose initial density was  $1.8 \text{ g/cm}^3$  was reused during the whole sample processing period, so it might have been mixed with water decreasing its density and becoming less effective extracting microplastics. NIVA et al. (2017) suggested that the repetition of the second step can enhance the retrieval of microplastics, however, it was not done due to the time schedule constraints. A recent study (Han et al., 2019) has created an optimized density-based extraction approach for microplastics in soil and sediment samples through enhancement and standardization of the extraction process. They proposed to use NaCl-NaI (1.5

g/cm<sup>3</sup>) as flotation solution allowing the extraction of the densest plastic particles and can be reused at least 5 times. NaI is more environmentally friendly than ZnCl<sub>2</sub> and can be recycled until 10 times having similar costs than NaCl solution. It is recommended not to use NaI with cellulose fibres as turn the filter black and hinder the visual identification (Prata et al., 2019).

This thesis supports the idea of incorporating an organic matter removal step to improve the density separation and the visual identification, independently of the organic matter content of the samples. Environmental samples usually contain biological material which can easily be confused with plastics (e.g algae) (Prata et al., 2019). There are a wide range of options with different pros and cons including from acid digestion, alkali digestion or oxidizing agents to the most expensive and less hazardous, enzymatic digestion. Most of the studies, which have applied an organic matter removal step, have used oxidizing agents such as 30% (v/v) H<sub>2</sub>O<sub>2</sub> or Fenton's reagent (Prata et al., 2019). During the tests performed in the trial period both H<sub>2</sub>O<sub>2</sub> and Fenton's reagent was applied to subsamples of the same sample to decide which was the most appropriate.

Preliminary tests showed that samples without receiving any treatment were harder to filter and due to the organic content, the filter pore clogged quicker and more filters were needed, increasing the workload and effort. The 30% (v/v) H<sub>2</sub>O<sub>2</sub> was the most efficient method to remove organic matter, but it was quite time-consuming because the subsample must be treated 24 h with the H<sub>2</sub>O<sub>2</sub>. The subsample looked bleached and so, microplastics might be affected. Hurley et al. (2018) detected that H<sub>2</sub>O<sub>2</sub> could degrade some plastics such as nylon (PA-6,6) or polypropylene (PP) bleaching their surfaces as well. They also observed that this degradation was exacerbated when the temperature was above 60 °C. Consequently, due to the degradation potential of plastics and the long-lasting process, this chemical was discarded. Fenton's reagent was quick and efficient removing the organic matter from the subsamples, so it was chosen as the most suitable method. Fenton's reagent cause the production of foam and elevated temperature. The raise of the temperature could degrade the plastic materials, although Hurley et al. (2018) did not observe any degradation, they recommend doing the Fenton's reaction in an ice bath to keep the temperature lower than 40 °C. However, in this study the Fenton's reagent was added at room temperature. The foam can lead to a reduction of the retrieved material, if it overflows from the recipient. In addition, if the pH is higher than 4 the reaction can give precipitation of Fe(OH)<sub>3</sub> which can turn the filter red. The foam from the Fenton's reaction only gave overflow in three samples (i.e L. Svalbjørtjønn 4, Presteevju A1 and Presteevju B1) during the whole process and some filters acquired a light red colour, but there are no evidences that the reagents did affect the microplastic integrity.

Although Fenton's reagent allowed to eliminate a great proportion of organic matter, items such as stiff vegetation parts (e.g sticks, vegetal bark) or animal structures among other particulate organic matter could not be oxidized and remained in the sediment. Due to their similar density as microplastics they arise during the density separation, spoiling the process and hindering the visual identification in many samples.

Water samples have also been proposed to be sieved, pre- treated and subjected to density separation by different authors (e.g reported in Prata et al., 2018) but none of that was done in this study to reduce bias and save time and reagents.

The visual identification of microplastics is tricky and confusing and the lack of experience could have resulted in some bias. Only those particles were sure they were microplastics were considered to avoid overestimation. The large amount of organic matter in many of the filters impeded the correct inspection of the whole filter. This organic matter had to be removed or displaced for a better examination of the filter. Fibres and fragments were the clearest microplastic items, however, films, microbeads or pellets were more difficult to identify.

According to the method described in this thesis, filters should have been moved from the Petri dish to a glass plate before looking in the stereomicroscope. This could not be done in many occasions since the filter was completely stick to the aluminium foil, therefore, they were visualized in the Petri dish. It occurred often in the filters with  $ZnCl_2$  traces. Conversely, when the filters were placed at the glass plate, they curved or folded making difficulties for the identification therefore, it was even more difficult to prod and poke the doubtful microplastics under the stereomicroscope when the filter moved, sank or blurred. Hidalgo-Ruz et al. (2012) recommend sticking the filters with glue to the glass plate. The hot needle method did not work either, partly because of the same reasons. The time spent looking through the stereomicroscope to find the position of the doubtful microplastic and to bring the needle closer to it, was larger than the time the needle was hot after stop heating.

As said by Nor and Obbard (2014) shiny items usually are microplastics (films or transparent fragments), however, the shiny things were found in these samples were not microplastics. Some of them were minerals and others, which also were rather fragile, finally were shines from the dry  $ZnCl_2$  or other substances. Minerals were also colourful (e.g red, green, black, white, translucent) with different shapes (e.g, angular, spherical, sharp edges, laminar), and thus, easy to confuse with plastics. They were discarded as plastics because of their high abundances and common occurrence among the samples. Yellow, black, brown and green fragments could have been confused with vegetation material. Some objects looked degraded, bleached or darkened, probably due to the chemicals and so, complicating the search for cellular structures. They were discarded in such cases.

Following the same criteria (Nor and Obbard, 2014), fibres are supposed to be equally thick through their length, should not be tapered at the end or should remain intact with a firm tug/poke with tweezers or be compressed without being brittle. By contrast, some fibres were found with clear evidences of degradation, some of those were discoloured or bleached, splitted or frayed and wrinkled. There are some uncertainties about the reasons of the degradation. They could have been in that state because of the applied treatments (e.g sieving, temperature, Fenton's reagent) or for weathering and mechanical action of environmental phenomena (Horton et al., 2017b; Lin et al., 2018; Nor and Obbard, 2014). Moreover, some blue and black fibres broke when they were poked while they look like plastic and not cotton or other natural textile. Transparent fibres could also be confused with animals such as insect larvae or worms. These animals appeared really harmed, likely because of the sieving and organic matter

removal step, so most of them had lost the keratinized structures and it was really difficult to differentiate tissues or organs. Consequently, the criteria used was not really accurate at all, and many items required a special attention. Transparent fibres could also easily be confused with glass fibres.

Uncertain whether items were or not microplastics, they were not taken into account. Due to such uncertainties, it is quite recommendable to do chemical characterization of the microplastics (e.g FTIR, attenuated total reflection FTIR (ATR-FTIR), Raman spectroscopy) to ensure they are plastics and which family of plastics they belong to (Prata et al., 2019). These analyses were not carried out in this thesis since their high cost and the availability of specialized equipment. However, this analysis provides information that can be useful to identify the sources of the microplastics and understand their fate in the environment.

Despite the cross- contamination risk reduction measures, there were found more microplastics in the controls of this thesis than in other studies (e.g less than two fibres per control in Lin et al., 2019, an average of two fibres per control in Horton et al, 2017a). Controls remained opened during the whole workday (approximately 8h), however, samples were covered most of the time and were exposed to the air few minutes while working with them, hence, minimizing the risk of contamination. Actually, covering samples during handling them can reduce more than the 90% of the total contamination (Prata et al., 2019).

All the material used was always cleaned with 70 % ethanol and water before and after using. Although, the lab was deeply cleaned every day, the door-opening and the entry and exit of people (although minimum) could be a source of airborne microplastics. Some microplastics found in the controls were not retrieved in the samples (e.g khaki fibres or some multicolour fragments). Controls were also used during sampling but some of them needed to be replaced since the wind took them or fell to the water. For following studies, filters are proposed to be stick to the Petri dish and prevent from losing them. Studies such as Prata et al. (2019) have reported that using fume hoods can reduce 50% of the contamination, but this work got the most contaminated controls from there.

Clothes were an important source of fibre contamination as well. The clearest evidence is the high presence of pink and violet (including purple) fibres in the samples since their occurrence in the ecosystems has not been report widely (Frias et al., 2018). Likely, most of these fibres might proceed from the clothes used in the sampling (no lab coat was worn) and sample processing despite of wearing the lab coat. Violet fibres were compared with those extracted from the jacket and sweatshirt used while working and, based on visual observations, they were concluded to be pretty similar. Clothes of other colours were also worn but the relationship between them and those find in the samples are not so clear. Further recommendations include a higher consideration and reduction of possible sources of contamination and more exhaustive cleaning measures.

#### 4.2 Occurrence of microplastics in sediment and water compartments

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This thesis has reported evidences of microplastic pollution in all sampling sites. Comparing these results with other made in other freshwater ecosystems over the world, abundances of microplastics were relatively high in

both water and sediment compartments. It should be advised that interstudy comparison must be made with caution due to the differences in the protocols and reported units of each study.

Microplastic concentrations of water samples showed high numbers (even the lowest concentrations) although not so high as Dris et al. (2015) who found in the Seine river between 260-320 items/L. However, concentrations at Presteevju stream (35-45 items/L) were similar to those detected in heavy polluted seawater in Germany and Australia with above 50 items/L. Borgjaevju stream, L. Jønnebergjønn and L. Svalbjørtjønn which showed lower concentrations (18-29 items/L), they far exceeded the lower concentrations reported by other researchers. Studies that have analysed the occurrence of microplastics in freshwater ecosystems close to urban areas discovered concentrations between 0.38 and 7.92 items /L in the Pearl river along the Guanzhou city in China (Lin et al., 2018), between 0.1 and 4.1 items/L in three urban Chinese estuaries (Zhao et al., 2015), between 1.66 and 8.92 items/L in the surface waters of Wuhan (China) (Wang et al. 2017b), an average of 10.9 items/L in the Yangtze River Estuary (Zhao et al., 2014), between 0.05 and 1.26 items/L in Antuã river of Portugal (Rodrigues et al., 2018), between 0.08 and 7.4 items/L in different freshwater ecosystems nearby Shanghai and between 3.67 and 10.7 items/L in the Wei river of China (Ding et al., 2019). Nevertheless, Baldwin et al. (2016), Hoellin et al. (2017) and McCormick et al. (2016) reported even lower microplastic abundances in the Great Lakes tributaries (0.001-0.032 items/L), in the North Shore Channel (0.001-0.01 items/L) and in the Higgen's creek (0.002-0.018 items/L).

The differences in the protocols can lead to some misinterpretation among studies. The lower limit of detection of microplastics in this thesis is 1.2 µm due to the mesh size of the filter pore, whereas in Rodrigues et al. (2018) the detection limit was 55 µm as they used a 55 µm mesh size net. Hence, they likely lost smaller microplastic particles driving to an underestimation of microplastics in the system. In this study there could be microplastics smaller than 1.2 µm that the filter did not recover. Particle size of 1.2 µm is approached to 1 µm and below this size microplastics must be considered nanoplastics (Horton et al. 2017b), so the lost information might be minimum. The above-mentioned studies had used different density separation substances or none of them, therefore, this diversity of methods may head to different microplastic recovery rates and some possible bias when comparing the results. Since that, it is highlighted the need for creating a standard method that allows the interstudy comparison.

Sediment samples registered more microplastic particles in this thesis than in other research works. Unexpectedly, sediment from the reference lakes had more microplastics than that of the sedimentation ponds which are enclosed in the city. These results are quite unreasonable as L. Jønnebergjønn had almost double of the number of microplastics compared to the sedimentation ponds and L. Svalbjørtjønn exceed that number of microplastics in one order of magnitude. Most of the microplastics discovered in the lakes were textile fibres, so likely, these fibres come from the contamination of the clothes. In particular, the presence of green fibres in the samples is reliable that they proceed from the green plastic rope of the Van Veen dredge. However, the presence of humans was greater in L. Svalbjørtjønn where there were several houses surrounding the lake, several boats tied

to berths and even some plastic debris (e.g blue ropes) was perceived in the shores of the lake. Hence, the presence of some microplastics in this lake could be possible (e.g ropes, fragments, boat painting).

Despite the uncertainties caused by the methods used in the different studies, it is done an attempt of comparing the number of microplastics found in this work with those of other works. The highest numbers of 1-5 mm-size-microplastic particles found in the sedimentation ponds (4784-37232 items/Kg of dry sediment) resembles to those found in sewage sludge while the lower numbers were more similar to those found in some natural ecosystems.

NIVA (2017) analysed the sludge of eight different Norwegian WWTPs and the average number of microplastics consisted of 6077 items/Kg of dry sludge and ranged from 1701 to 19837 items/Kg of dry sludge. Furthermore, a Chinese study carried out in 28 WWTPs recovered an average of 22700 items/Kg of dry sludge in a range from 1600 to 56400 items/Kg of dry sludge. Overall, studies performed in natural ecosystems discovered lower numbers of microplastic within the sediment, although Hoellein et al. (2017) estimated between 3600 and 1610000 items/Kg in the sediments of North Shore Channel. Other reports have discovered between 80 and 9597 items/Kg in the Pearl river (China) (Lin et al., 2018), between 178 and 544 items/Kg in Beijiang River (China) (Wang et al. 2017a), between 6.3 and 160.1 items/Kg in Bloukrans river (South Africa) (Nel et al., 2018), between 18 and 629 items/Kg in the Antuã river of Portugal (Rodrigues et al., 2018) or between 360 and 1320 items/Kg in the Wei river of China (Ding et al., 2019). NIVA (2018) assess the microplastic concentration in two lakes of Norway. They found between 40 and 7310 items/Kg in the sediment of lake Mjøsa which is affected by more potential pollution sources (i.e five big cities, road drainage, industry, landfills, agriculture, WWTPs) than the lake Femunden which is in a rural area, where they found a maximum of 690 items/Kg of dry sediment. Horton et al., (2017a) found an average of 91.5-330 items/Kg of dry sediment in the size of 1-4 mm (1-2 mm = 102-419 items/Kg, 2-4 mm = 81-241 items/Kg) in the River Thames (UK) and Vaughan et al. (2017) detected maximum concentrations of 250-230 items/Kg in an urban lake of Birmingham (UK).

Limitations must be taken into account when contrasting microplastic concentrations. The main responsible of the bias is the size of particles which the studies are dealing with. Hence, there is a huge variability in the results, for example, Lin et al, (2018) did not previously sieve the sediment, although the lower limit was 20 µm since it was the pore size of the membrane filter. Other studies worked with the sediment size fractions of 5-0.055 mm (Rodrigues et al., 2018), 1 mm and 500 µm (Vaughan et al., 2017) or 1 mm as Ding et al. (2019).

Many of the studies could be underestimating the number of microplastics since there is a lack of means to get the smallest microplastics. Moreover, this thesis is potentially underestimating microplastics as no microplastics lower than 1 mm were considered. There is a great probability of encountering small microplastics may come from the *in-situ* fragmentation of larger microplastics or from the water. According to the measures of some microplastics from the water samples, the mean size was below 1 mm (mean size for fibres = 916.9 µm and mean

size for fragments = 191.3  $\mu\text{m}$ ). Likely some of them might sediment, and so, expect similar amounts of microplastics to those from 1 to 5 mm as well. Future research should also consider these smaller microplastics.

Additionally, important factors such as the density separation solutions used in each study, the organic removal method and the drying temperature of sediments should be also considered. These variations in the methods severely affect the comparisons among studies, and even can hamper the approaches of microplastic global impacts and the understanding of their fate in the environment. It is recommended to develop an universal standard method that overwhelm these limitations (e.g particle size and density) and allow the interstudy comparison.

### 4.3 Microplastic pollution sources

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Fibres were the most dominant type of microplastics in all water and sediment samples of all sampling sites in this work. This is consistent with findings of practically all research works where synthetic fibres have been reported as the most abundant microplastic type in freshwater, terrestrial and marine environments (Horton et al., 2017b). The fibre presence in the reference lakes are likely to be contamination from clothes. Conversely, the high amount of fibres encountered in the sedimentation ponds is far from being only clothing contamination. The presence of fibres in the sedimentation ponds is likely linked to wastewater effluents and sewage sludge application on land (Horton et al., 2017b).

Yang et al. (2019) reviewed several studies about domestic washing as microfibre source pollution (Browne et al., 2011; Hernández et al., 2017; Napper and Thompson, 2016; Pric et al., 2016). The washing of a single clothe garment can release up to 1900 fibres and 6 kg of clothes made of different synthetic textiles can discharge between  $1.4 \times 10^5$ - $7 \times 10^5$  fibres per wash. Additionally, the use of detergents and softeners can increase the loss of fibres when washing. For instance, 75% more fibres are released after using detergent and the use of tumble-drying cycle boost a 3.5-fold increase the release of fibres. Furthermore, the Norwegian Environmental Agency reported that around 100-600 of fibres are annually discharged in the environment from laundries and domestic washing systems, respectively, in Norway.

Norway has also become one of the leading countries in the world concerning both the quantity and quality of sewage sludge applied to agriculture. The current limit for agricultural applications of sludge is 20 tons per hectare per 10 years (Whipps and Tornes, 2018). With these figures and knowing that a great proportion of land use at the municipality of Bø is dedicated to agricultural use, it is highly possible that the application of sewage sludge turns into one of the main sources of microplastics in the surrounding water bodies, apart of the WWTP effluents and unknown point discharges (Vann-nett, 2019).

Sewage sludge commonly contains microbeads and synthetic microfibres since they cannot be eliminated through the treatment process in WWTPs due to their small size and density. Fibres have at least a very small dimension and they usually pass through the mesh size of the filter used during treatment, even when the length

of the fibre exceed this size (Horton et al., 2017b). Synthetic fibres are typically made of PET (Rodrigues et al., 2018) which has a high density (Frias et al., 2018), and so can deposit in the sludge when the water movement is minimum. Microbeads whose density is higher than  $1 \text{ g/cm}^3$  can easily sediment in the sludge (Lin et al., 2018). If microplastics are not efficiently removed by sedimentation in the sludge they can be eliminated through the effluent and be released to the aquatic ecosystems (Horton et al., 2017b).

Consequently, fibres could have reached the sedimentation ponds via erosion and runoff from agricultural areas to which sludge have been applied or through wastewater effluents (Horton et al., 2017b), storm water (Luo et al., 2018) and unknown discharges. Dris et al. (2015) determined that fibres could easily be transport by the wind and so can also reach the aquatic ecosystems through fallout and aerial deposition. Fibres were mostly black, blue and transparent which is in accordance with the typical reported colours, although other colours appeared. In contrast other studies (Lin et al., 2018; Rodrigues et al. 2018) also have reported higher occurrences of fibres of green and red colours, hence, colours of the particles could be used as an aid to identify the pollution sources (e.g washing machines, fishing) (Luo et al., 2018).

In contrast, no microbeads were found in the samples. It could have happened that microbeads were in the sediment, but they were not encountered due to their typical size lower than 1 mm and the lack of experience identifying microplastics. In such case they may proceed from the erosion of the agricultural soil. However, microbeads could not be present due to their settlement and retention in the WWTs (Horton et al., 2017b).

Fragments are likely to be the result of the fragmentation of larger plastics found nearby the sedimentation ponds of both streams and within them. There were found some macroplastics (e.g bags, wrappers, glasses, cutlery) and another kind of rubbish (e.g a hubcap, a bike, a map, glass bottles). Although low, the degradation of plastics can be possible through physical, chemical and biological action. No signs of biodegradation were found so it is more probable that the fragmentation was due to mechanical mechanisms than to biofouling.

Some microorganisms can degrade microplastics while those can also be colonized by new species and behave as a vector of pathogen and other species (Van Cauwenberghe et al., 2015). Biofouling dynamics depends on many factors such as polymer characteristics, nutrient availability, water turbulence, light, etc. (Vaughan et al. 2017). Wright et al. (2013) found that biofilm could colonize a plastic bag in the marine environment in only one week.

Vaughan et al. (2017) highlighted that small urban lakes are less dynamic than oceans, and thus there are no strong currents or wave action that potentially favour the plastic decomposition. They also mentioned that photodegradation caused by the UV radiation seems to be the main reason of decomposition. However, such characteristics differ in the present study. Upstream the sedimentation ponds at Borgjaevju stream, the water falls through a waterfall increasing the mechanical action to which plastic debris is exposed. Although the sediment of these ponds were generally thin, there were also pebbles and big stones (especially Borgjaevju A and B) and the current within was medium-high based on observations (when the dredge was thrown from the boat, it quickly drifted before touching the bottom). In this particular study, the weather conditions can also be important. The

freezing-melting events according to summer-winter seasons and the floods during the spring-autumn can increase the fragmentation of plastics.

Fragments and other secondary microplastics can also derive from littering, wind-disposal and the closest roads (Horton et al., 2017a). Neither tyre wear particles nor road painting were found in the pond sediment. Samples of the road marking paint were taken (annex 10) to check suspected yellow and white fragments, but there was not any similarity. The non-detection of these particles can be due to the lack of experience, the presence of these particles but smaller than 1 mm size or the non-influence of the roads nearby.

Rodrigues et al. (2018) found that blue fragments tend to appear more often than other colours as in this study. Blue fragments could be an indication of wastewater effluents as usually the tanks where the water is stored in the WWTPs, are blue painted (personal communication). However, colours can differ according to the pollution sources. Moreover, the appearance of coloured microplastics can increase the risk of being ingested by aquatic organisms (Wang et al. 2017b). For instance, blue particles were found in the stomachs of bluegill (*Lepomis macrochirus*) and longear sunfish (*Lepomis megalotis*) (Peters and Bratton, 2016). Besides, white and transparent particles were found in the Asian clam (*Corbicula fluminea*) (Su et al., 2016).

#### 4.4 Assessment of the sedimentation ponds' efficiency

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Several studies have demonstrated that rivers are important sources of microplastics to the marine ecosystems (Van Cauwenberghe et al., 2015). Actually, rivers can transport between 70 and 80% of marine plastic debris through different waterways (Bowmer and Kershaw, 2010). Rivers are also the link between terrestrial and oceanic environments, although rivers and sea can deposit the plastics in the land again by tidal deposition or floods. The appearance and behaviour of plastics in the environment will depend on several factors including those anthropogenic such as littering, spillages or recycling, the polymer characteristics such as size, shape or density and environmental factors such as weather (i.e wind, precipitation, temperature, floods), topography/bathymetry and hydrology. In summary, the same factors that also rule the sediment transport and deposition (Horton et al. 2017b).

Low flow velocities may lead to a higher deposition of microplastics and particulate organic matter whereas high flow velocities and erosion lead to mobilization and resuspension of the deposited particles (Horton et al. 2017b). Source discharge is also involved in microplastic transportation (Luo et al., 2018). There were not found any significant relationship between microplastic concentrations and flow velocity in this study.

Flow velocity calculated here, can only be used as a general approach. Both streams counted with an important number of unknown discharge point sources which increase the water flow and could be supplying microplastics and other pollutants (e.g chemicals, nutrients, organic matter, solids) to the sedimentation ponds and streams. In addition, the measures taken at Presteevju stream inlet was recovered using a different method since the water flow was really slow due to their narrow riverbed, low depth and the presence of algae, aquatic plants and small

dams created by different vegetal structures. Despite the method was not accurate at all, measures can be used to make a raw approach of the input of microplastics.

The existence of discharge points, the changes of the level of water and the floods caused by the rainfall of the season can explain the lack of connection between microplastic numbers and flow velocity. The first sampling day matches with the end of the ice and snow melting period and absence of rainfall. Plastics could be reaching the streams and sedimentation ponds after running through the land and the low flow may have allowed the settlement of plastics in the sediments, especially in the last sedimentation pond where the water flows slower after staying in the previous ponds at Borgjaevju and Presteevju sedimentation ponds. These reasons could explain the numbers of microplastics in the sediment and the water. Conversely, the following samplings took place after heavy rainfall days, hence, the surrounding lands have been already washed through the following runoff and thus, the water samples might have fewer microplastics than in the first sampling day.

Additionally, and looking at the numbers of microplastics that are entering and going out from the sedimentation pond system it can be said that they are not efficient enough to remove microplastics from the streams in which they were built. Presteevju sedimentation ponds suppose a source of microplastic to the Borgjaevju stream and this latter to the Bøelva river. Given that the number of microplastics going out from the systems, overall, exceeds the number that is entering it is highly likely that the differences in numbers come from additional sources of pollution such as the discharges points, runoff or the mobilization of microplastic particles in the sediment during flooding periods (Rodrigues et al., 2018).

Both Borgjaevju and Presteevju streams can be classified as eutrophic systems according to the high mean values of total nitrogen and phosphorous. Total nitrogen values fluctuated in the range of 1000-2000  $\mu\text{g/L}$  and total phosphorous values varied in the range of 10-30  $\mu\text{g/L}$ , which according to Richardson et al. (2007) signify eutrophic and mesotrophic conditions, respectively. However, Presteevju stream can even be considered hypereutrophic depending of the date due to the higher concentration of nitrogen ( $\text{N}_T > 2000 \mu\text{g/L}$ ). The significant strong relationship between the total nitrogen and the electrical conductivity suggest a possible pollution focus in the Presteevju stream as both parameters are rather high. Both sedimentation pond systems so far did not help to reduce the amount of nutrient of the streams. Consequently, the increasing trend of both total and phosphorous throughout the sedimentation ponds indicate that the system does not accomplish the purpose they were built for and comprises a source of nutrients to the Bøelva river as well.

Li et al. (2019) show how ponds in constructed wetlands (no deeper than 1.5 m) with a plant coverage between 60 and 80% allows to improve the efficiency of nutrient removal (particularly nitrogen). The average depth of the sedimentation ponds in this study was shallow (mean depth for Borgjaevju sedimentation ponds = 0.9 m and for Presteevju sedimentation ponds < 0.5 m) and the plant coverage was generally low. Deeper sedimentation ponds could also favour the sedimentation of microplastics and prevent their mobilization from the sediment since hydrologic processes and erosion might be minimized (Luo et al., 2018).

Strand et al. (2013) discovered the existence of a strong relationship between microplastic abundance and both organic content (TOC) and sediment size fraction ( $< 63 \mu\text{m}$ ). Sedimentation ponds at Prestevju and Borgjaevju streams seemed to work for retaining organic pollution, although is not so high and is lower than the recommended value of 10 mg/L for natural waters (Directive 2000/60/EC, 2000). Turbidity showed an increasing trend down the streams and a strong relationship with the total phosphorous. The turbidity variations could be due to an increase in the total concentration of phosphorous as mineral particles and not the total organic carbon as it would be expected (Wetzel, 2001).

## 5. CONCLUSIONS

Abundances of microplastics in both water and sediments were, on average, higher than in other studies. Microplastic sedimentation concentrations were sometimes like those concentrations found in WWTPs and to other polluted places and microplastics water concentrations were also similar to those of highly polluted waters. Assessment of L. Jønnebergjønn and L. Svalbjørtjønn could not be made properly as most of the microplastics proceeded from airborne contamination.

Fibres and fragments were the most typical findings. Presence of fibres points at wastewater effluents and agricultural runoff after sewage sludge application and wind disposal as main sources. Fragments are likely to derive from mechanical break-up of larger plastics coming from littering, road and urban runoff, and *in situ* fragmentation. Black, blue and transparent were the most abundant microplastic colours, however, the presence of microplastic of other colours was also remarkable. The presence of coloured microplastics involves a higher risk to the aquatic organisms since they are more visible, are more likely to be ingested.

Overall, the sedimentation ponds have not accomplished the objective for they were designed to. It is quite clear that both systems are affected by nutrient and microplastic pollution. However, these sedimentation ponds cannot prevent the further pollution of the following natural water currents (Borgjaevju stream and Bøelva river). The government of the city should raise awareness and take some measures to stop and prevent pollution. According to the literature these measures could be addressed to increase the depth of the ponds and increase the vegetation cover to extend the water residence time, enhance the sedimentation of microplastics, avoid mobilization of the sediment and allow the nutrient uptake by aquatic plants. Later on, the sediment cleaning and vegetation harvest could help to maintain the sedimentation ponds 'efficiency.

Finally, this study highlights the necessity of creating a universal, validated and standard method which can allow the interstudy comparison. Firstly, it should define the range size of microplastics and secondly, establish how to collect the samples, the volume /mass needed and how to treat the samples as regards to the organic matter removal and the density separation steps. This method should also determine the units to report the findings in water and sediments. More cross-contamination preventing measures should be considered when working with

microplastics. However, as long as this absence of protocols continues, future studies should report all the details of the method used to avoid mistakes when comparing results.

Further research should be conducted to explore the possible seasonal variations of microplastics in water and sediments, to identify and characterize unknown discharges, to study the occurrence of microplastics in areas closer to the land (e.g shores) and its connection with the aquatic environment, to assess the effects of microplastics in the local biota, to improve the methods analysing microplastic and include chemical characterization of microplastics for a better comprehension of the results.

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## Personal communication

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Professor Espen Lydersen. (2019). University of South-Eastern Norway.

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## ANNEXES

**Annex 1.** Bathymetric maps of L. Jønnebergjøonna and L. Svalbjørtjønn.

**Annex 2.** Schemes of sedimentation ponds at Borgjævju stream (A, B, C, in this order).

**Annex 3.** Graphs of streamflow curves performed by Proff. Lydersen from USN.

**Annex 4.** Weight of wet and dry sediment and number of microplastics per sampling site.

**Annex 5.** Abundance of microplastic types in the control filters (sampling, fume hood, room and stereomicroscope laboratory)

**Annex 6.** Abundances of microplastic types in all the sediment samples.

**Annex 7.** Abundances of microplastic types in all the water samples.

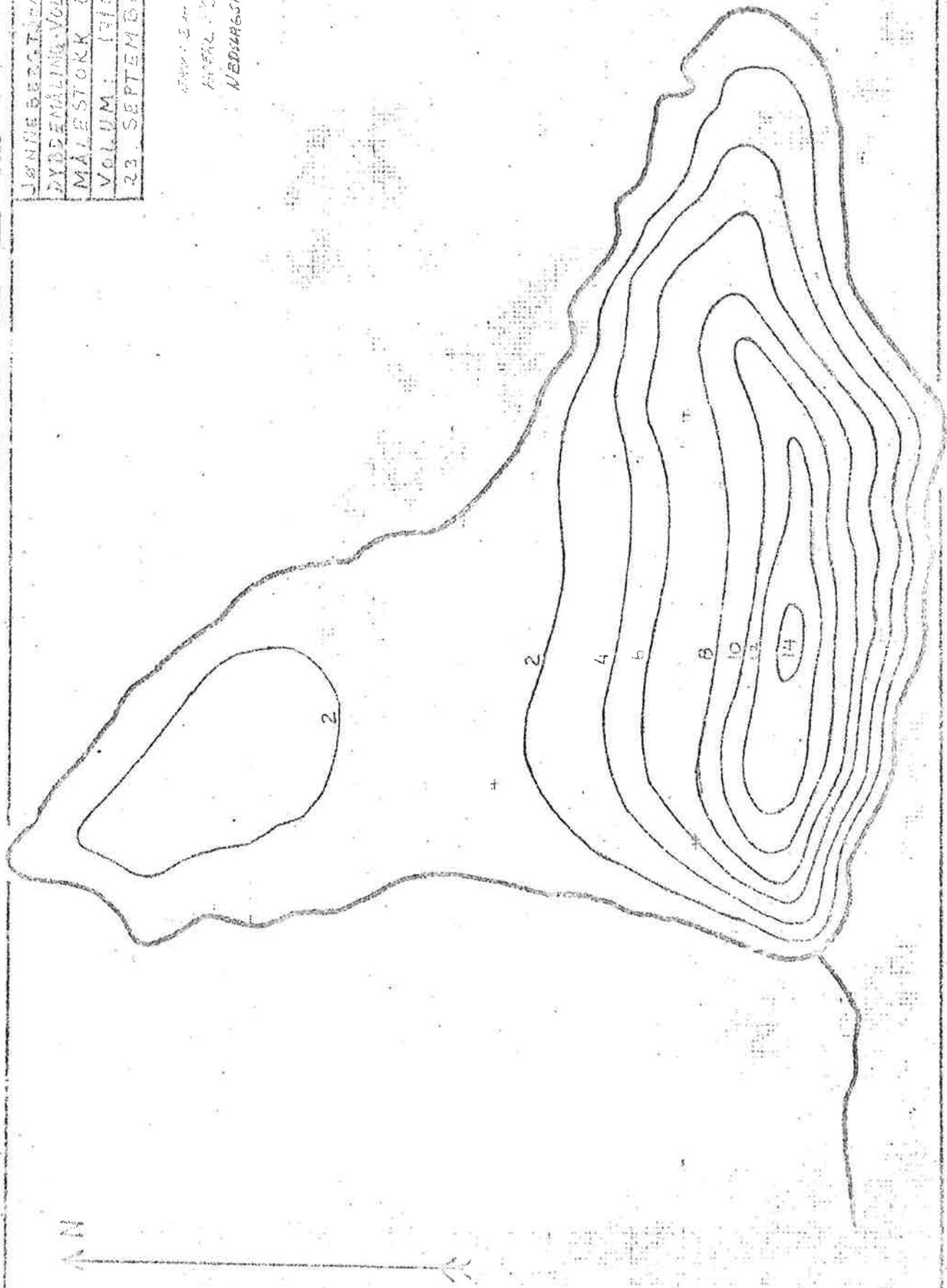
**Annex 8.** Measures and colours of some microplastics from the water samples.

**Annex 9.** Physical-chemical parameters of the water samples.

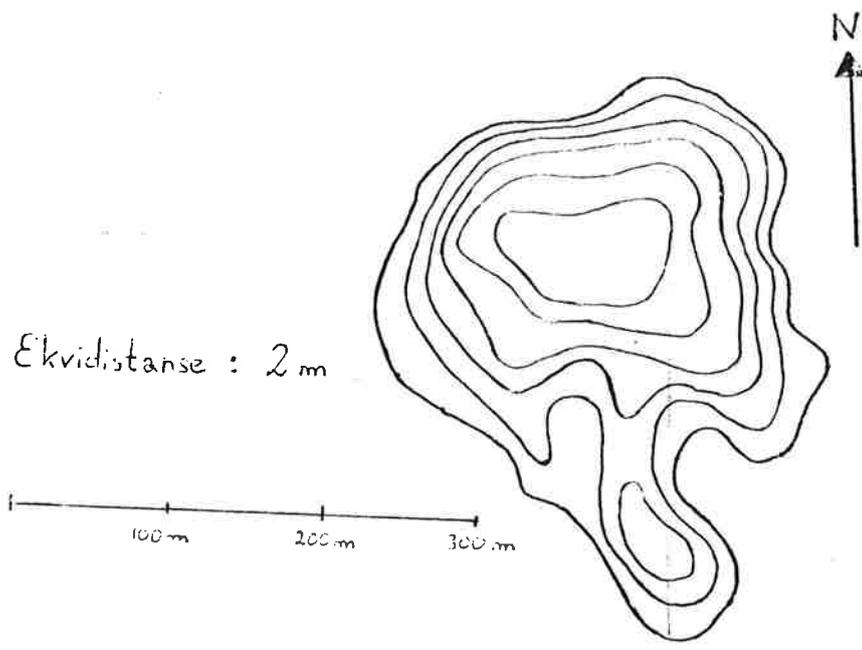
**Annex 10.** Pictures of road painting samples.

LØNNEBERGSTEIN  
DYDENMÅLING VØLTHORSTEN  
MÅLESTOK 1 : 1500  
VOLUM 171000 M<sup>3</sup>  
23. SEPTEMBER 1911

Skala 1 : 500  
Høfde 3615 dm  
NEDSÆTTELSE 2734



Bathygrafisk kart over Svalbjertjønnen



- Areal :  $0,056 \text{ km}^2$
- Volum :  $112100 \text{ m}^3$  ?
- Middeldyp :  $4,75 \text{ m}$
- Avrenning :  $1,67 \text{ l/sek.}$  ?
- Største dyp :  $10,75 \text{ m}$

Field course

freshwater ecology

1980.

FORKLARINGER:

SONE	MATERIALE	TYKKELSE
①	SANDIG-GRUS	0 - 30mm
②	SANDIG-GRUS	0 - 200mm
③	SANDIG-GRUS	0 - 50mm
④	PLASTRING, BLOKK	0,40m < D < 0,50m

ANVISNINGER:

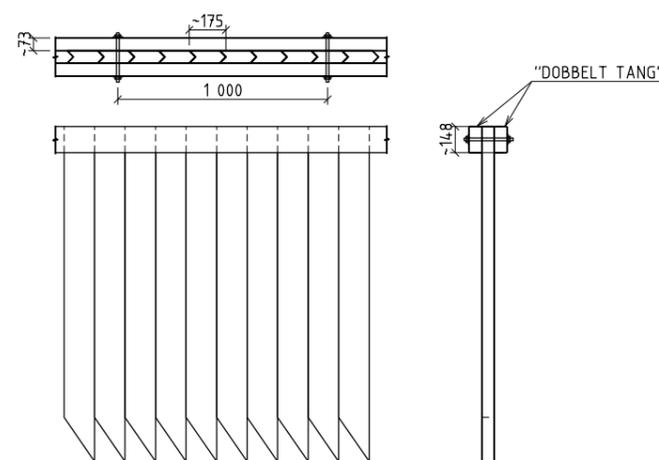
- Forbiledning av vann i byggetida  
Før tapperør er etablert ledes tilsiget forbi damstedet i grøft. Tøpperør bør etableres med tilstrekkelig lengde oppstrøms dammen, slik at det kan bygges en fangdam med høyde ~1 meter for forbiledning av vann i byggetida.
- Fundamentarbeider  
Rester av gammel dam fjernes og det graves ned i jomfruelige masser. Underlag for sone 1 avsluttes i tilstrekkelige fette masser. Kum plasseres i dypløp. For å unngå erosjon i damtå på nedstrøms side graves det her en grøft tilpasset en fåstein med volum > 0,5 m<sup>3</sup>. Elveleiet på nedstrøms side av fåsteinen skal være mest mulig urørt.  
Vist gravelinje under dammen er lagt 0,5 meter lavere enn terrengoverfalten på kart oversendt fra Bø kommune.
- Dammasser og innbygging i dam  
Grus og støtrefylling legges ut i 0,3 meter tykke lag, komprimeres ved 6 overfarer, valsevekt minimum 2 tonn. Se Teknisk beskrivelse for flere detaljer.
- Plastring  
Terskelens overflate skal beskyttes med grov-fraksjonert stein, D > 0,4 m i ytterlag og d > 0,1 m i indre lag.
- Trespunt  
Som trespunt skal det benyttes trykkimpregneret plank med tykkelse minst 70 mm. Planken skal være skråningskjært i enden slik at den ved ramming blir presset mot nabospunten, og den skal være utformet slik at den gir føring ved ramming. For å styre linjeføringen langs toppen av spunten, skal det benyttes "dobbel tang" som vist på tegning. Den skal forbindes med spuntnålene for hver meter med en gjennomgående bolt. Spunten føres over hele dammen og ut til fette masser i vederlagene. Rundt rørgjennomføringen tilpassens enden av spunten til overflaten på betongen.
- Fiberduk  
Bruksklasse 4, minimum overlapp 0,5 meter. Skjøter legges slik at vannstrømmen glir over skjøtene og IKKE inn i skjøtene. Mot spunt legges dukken med minimum 0,15 m oppstikk.
- Erosjonssikring  
Sideferreng langs dammens vederlag erosjonsbeskyttes med stein D>0,2 m. Erosjonsbeskyttelse skal føres en meter over damhøyden.

Tegningsnummer	Revisjon
110	H02

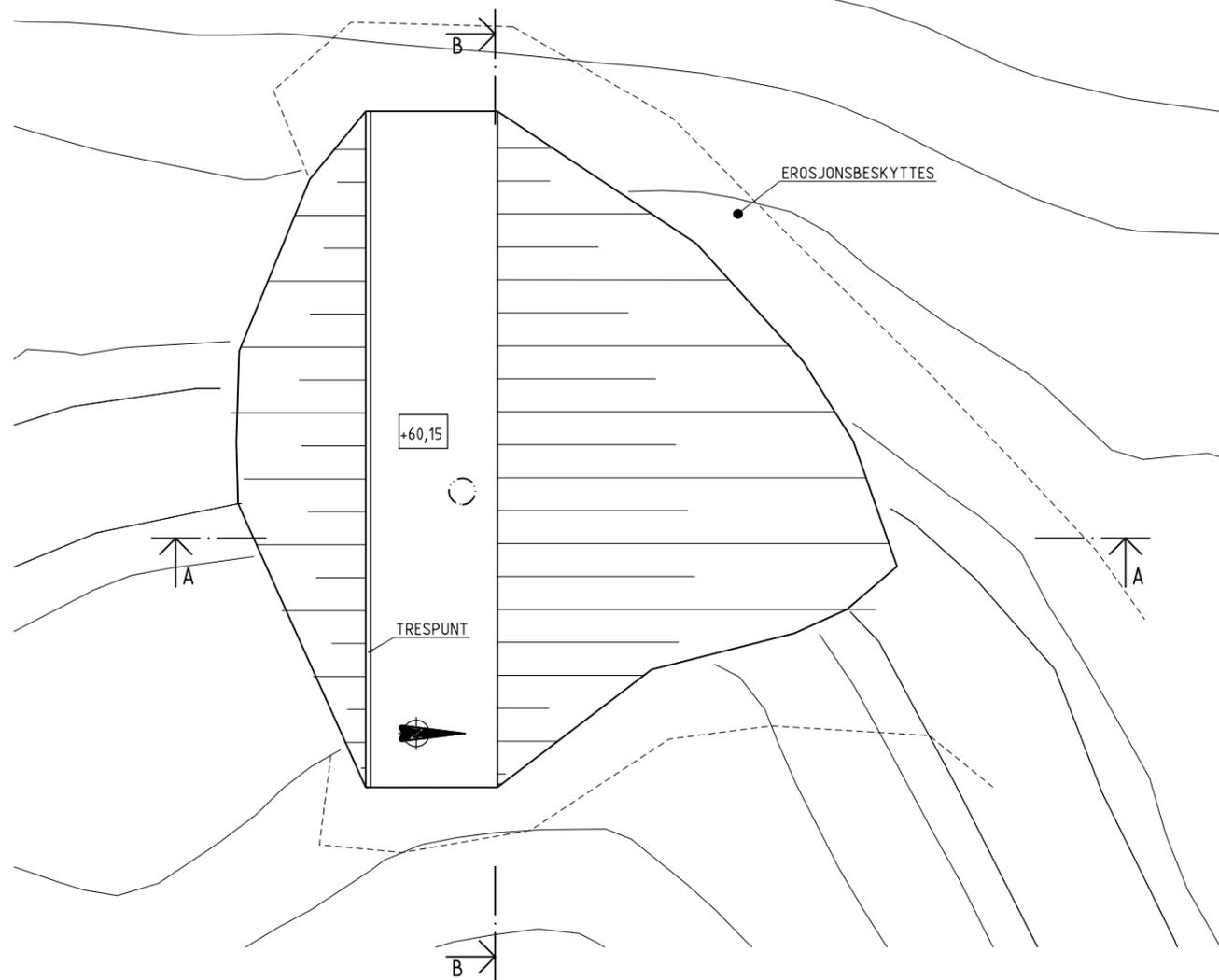
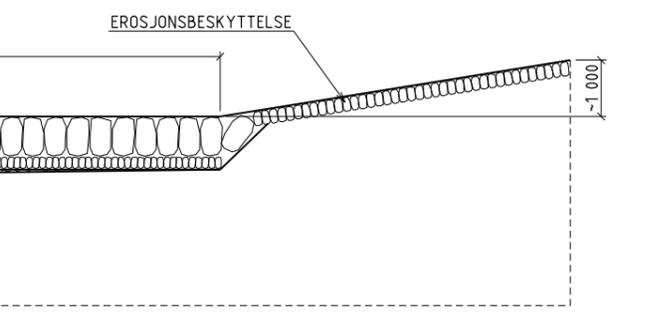
HENVISNINGER:

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- Teknisk beskrivelse - se "Terskler i Evjua, Teknisk underlag for bygging"

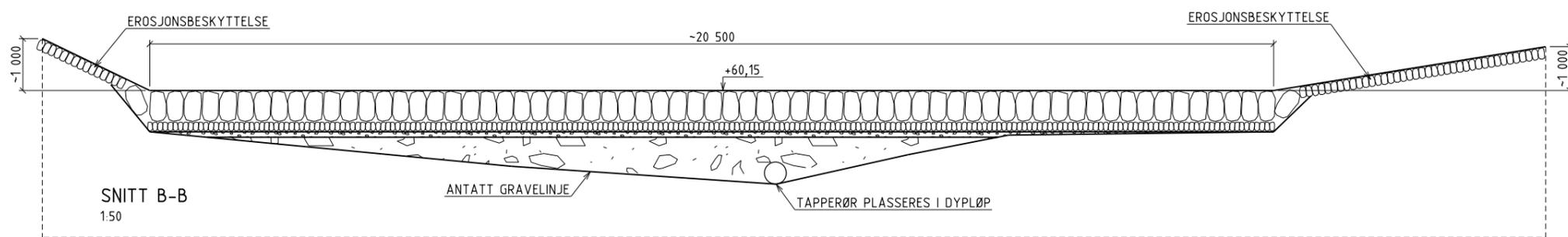
PLAN - OVERSIKT  
1:1000



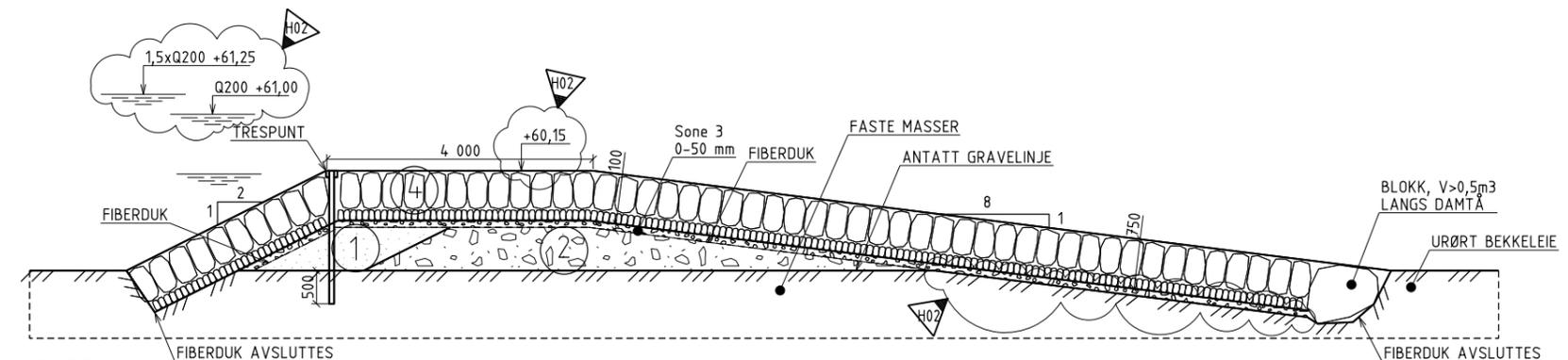
DETALJER SPUNT  
1:20



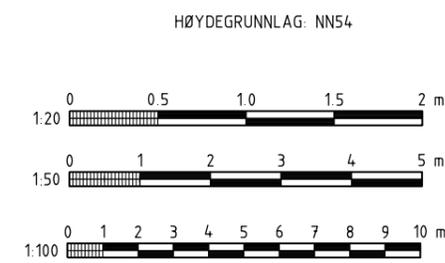
PLAN - ØVRE TERSKEL  
1:100



SNITT B-B  
1:50



SNITT A-A  
1:50



H02	2014-08-12	Endret damhøyde	LKNeb	EØ	EØ
H01	2014-07-11	For utførelse	LKNeb	EØ	EØ
Revisjon	Dato	Beskrivelse	Utarbeidet	Fagkontroll	Godkjent

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**BØ KOMMUNE** Må bestekket igjelder for A1 formål SOM VIST

EVJUDALEN TERSKLER  
ØVRE TERSKEL  
PLAN, OPPRISS OG SNITT

Norconsult	Oppdragsnummer	Tegningsnummer	Revisjon
	5133159	110	H02

Oppdrag - H:\ADAK\Byggeteknikk\Arkiv\110.dgn - Innb - 15.08.14 - 15:13:58 - Mod - Ark - Ref: 110.dgn; Terskler.dgn; Kart.dgn; Innmålinger - punkter juli 2014.dgn; 130.dgn

FORKLARINGER:

SONE	MATERIALE
①	SANDIG-GRUS 0 - 30mm
②	SANDIG-GRUS 0 - 200mm
③	SANDIG-GRUS 0 - 50mm
④	PLASTRING, BLOKK 0,40m < D < 0,50m

ANVISNINGER:

- Forbileding av vann i byggetida  
Før tapperør er etablert ledes tilsiget forbi damstedet i grøft. Tøpperørret bør etableres med tilstrekkelig lengde oppstrøms dammen, slik at det kan bygges en fangdam med høyde ~1 meter for forbileding av vann i byggetida.
- Fundamentarbeider  
Rester av gammel dam fjernes og det graves ned i jomfruelige masser. Underlag for sone 1 avsluttes i tilstrekkelige tette masser. Kum plasseres i dypløp.  
For å unngå erosjon i damtå på nedstrøms side graves det her en grøft tilpasset en tåstein med volum > 0,5 m<sup>3</sup>. Elveleiet på nedstrøms side av tåsteinen skal være mest mulig urørt.  
Vist gravelinje under dammen er lagt 0,5 meter lavere enn terrengoverfalten på kart oversendt fra Bø kommune.
- Dammasse og innbygging i dam  
Grus og støttestoff fylling legges ut i 0,3 meter tykke lag, komprimeres ved 6 overfarer, valsevekt minimum 2 tonn.  
Se Teknisk beskrivelse for flere detaljer.
- Plastring  
Terskelens overflate skal beskyttes med grov-fraksjonert stein, D > 0,4 m i ytterlag og d > 0,1 m i indre lag.
- Trespunt  
Som trespunt skal det benyttes trykkimpregneret plank med tykkelse minst 70 mm. Planken skal være skråningskjært i enden slik at den ved ramming blir presset mot nabospunten, og den skal være utformet slik at den gir føring ved ramming. For å styre linjeføringen langs toppen av spunten, skal det benyttes "dobbelte tang" som vist på tegning. Den skal forbindes med spuntfålene for hver meter med en gjennomgående bolt. Spunten føres over hele dammen og ut til tette masser i vederlagene. Rundt rørgjennomføringen tilpassens enden av spunten til overflaten på betongen.
- Fiberduk  
Bruksklasse 4, minimum overlapp 0,5 meter. Skjøter legges slik at vannstrømmen glir over skjøtene og IKKE inn i skjøtene. Mot spunt legges dukken med minimum 0,15 m oppstikk.
- Erosjonssikring  
Siderenget langs dammens vederlag erosjonsbeskyttes med stein D>0,2 m. Erosjonsbeskyttelse skal føres en meter over damhøyden.

Tegningsnummer	Revisjon
120	H02

HENVISNINGER:

- Spunt - se tegning 110
- Teknisk beskrivelse - se "Terskler i Evjua, Teknisk underlag for bygging"

HØYDEGRUNNLAG: NN54



H02	2014-08-12	For utførelse	LKNeb	EØ	EØ
H01	2014-07-11	For utførelse	LKNeb	EØ	EØ
Revisjon	Dato	Beskrivelse	Utarbeidet	Fagkontroll	Godkjent

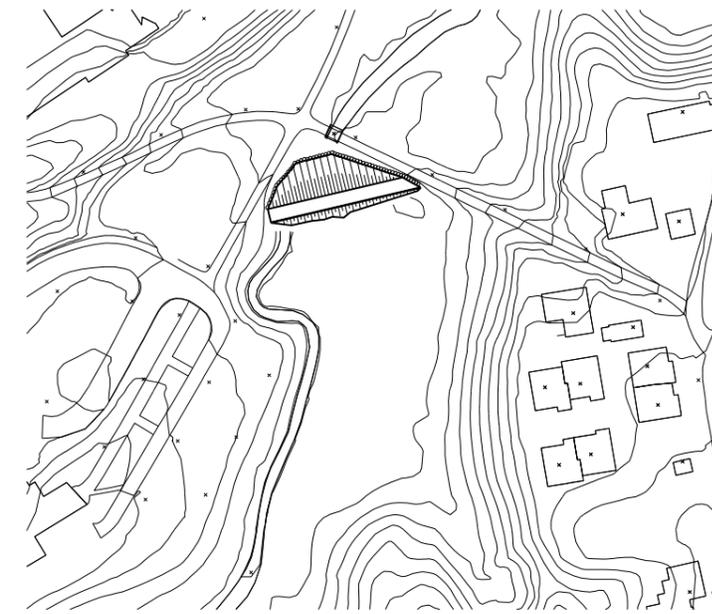
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BØ KOMMUNE

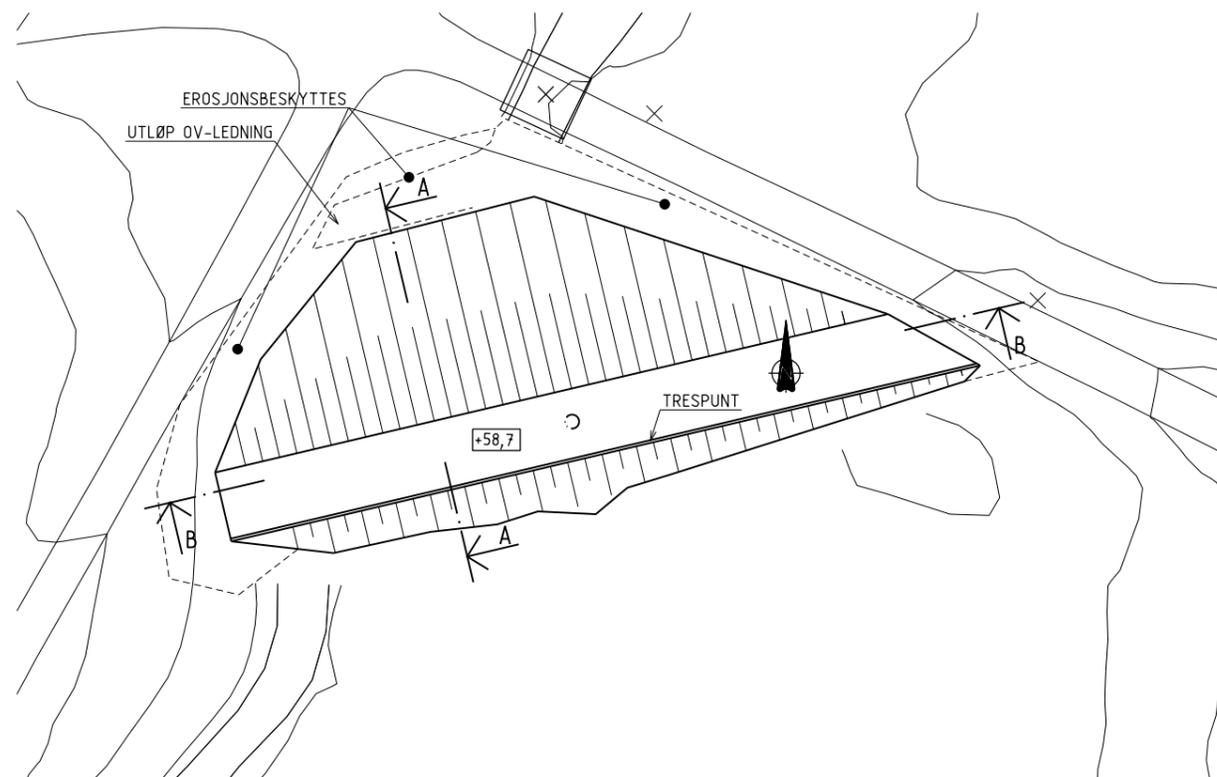
Må bestekkes igjeld for A1 format!  
SOM VIST

EVJUDALEN TERSKLER  
MIDTRE TERSKEL  
PLAN, OPPRISS OG SNITT

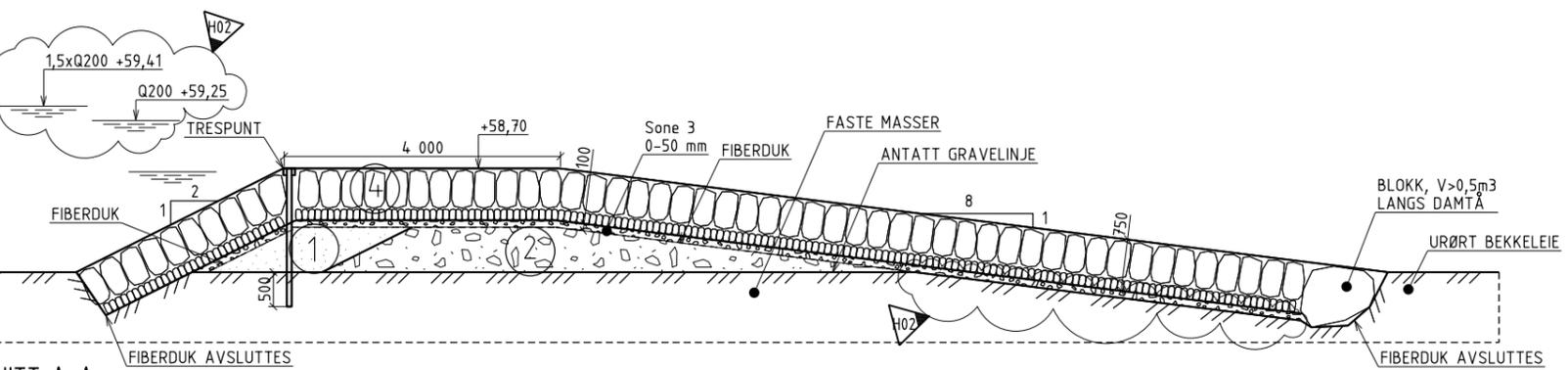
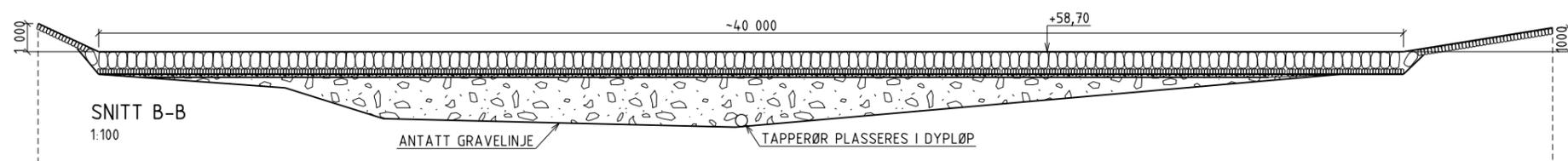
Norconsult	Oppdragsnummer	Tegningsnummer	Revisjon
	5133159	120	H02



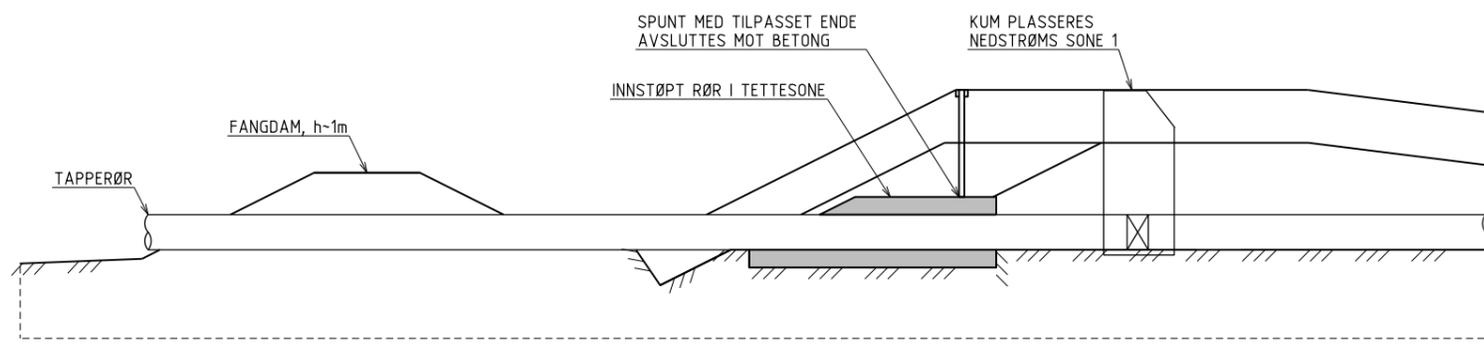
PLAN - OVERSIKT  
1:1000



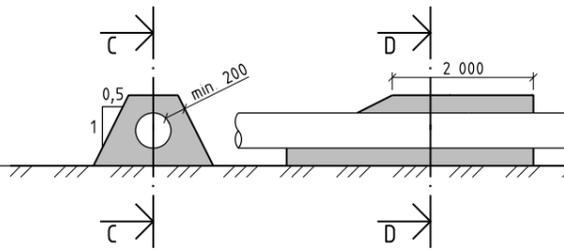
PLAN - MIDTRE TERSKEL  
1:200



SNITT A-A  
1:50



PRINSIPP FANGDAM OG PLASSERING AV RØRGJENNOMFØRING  
1:50



SNITT D-D  
1:50

SNITT C-C  
1:50

Oppdrag - H:\DOK\Byggeteknikk\Arkiv\120.dgn - llineb - 15.08.14 - 15:10:51 - Mod - Ark - Ref: 120.dgn; Terskler.dgn; Kart.dgn; innmålinger - punkter juli 2014.dgn; 130.dgn

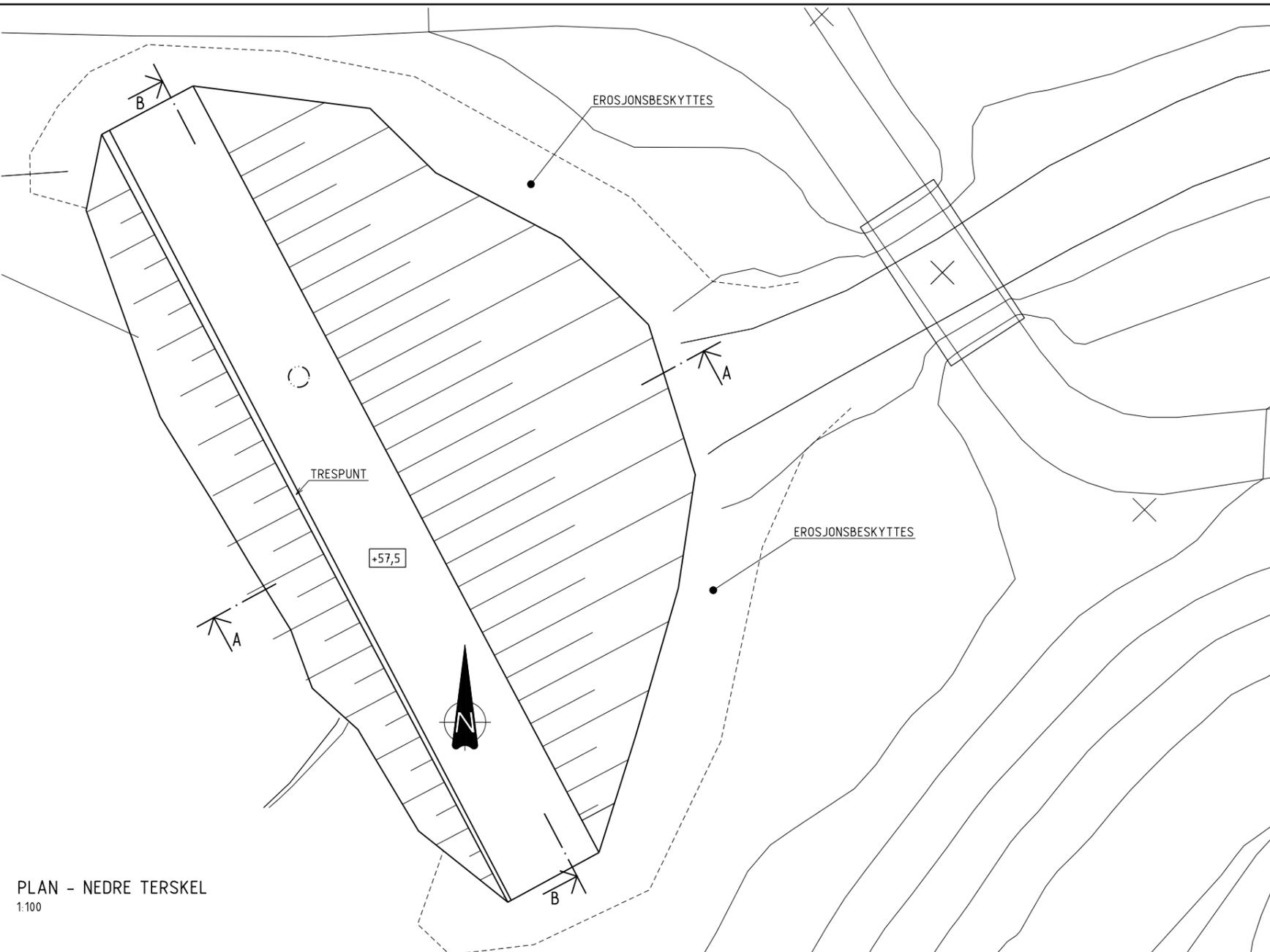
FORKLARINGER:

SONE	MATERIALE
①	SANDIG-GRUS 0 - 30mm
②	SANDIG-GRUS 0 - 200mm
③	SANDIG-GRUS 0 - 50mm
④	PLASTRING, BLOKK 0,40m < D < 0,50m

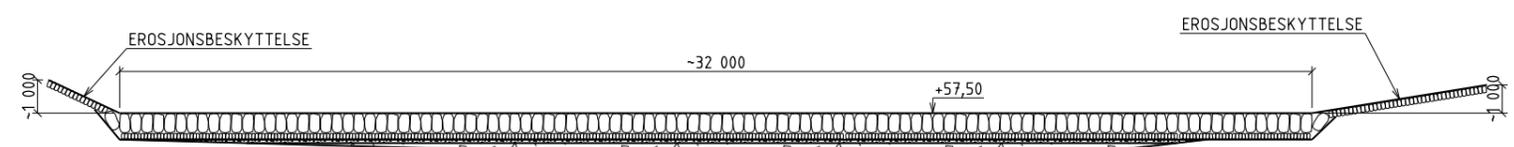
ANVISNINGER:

- Forbiledning av vann i byggetida  
Før tapperør er etablert ledes tilsiget forbi damstedet i grøft. Tapperøret bør etableres med tilstrekkelig lengde oppstrøms dammen, slik at det kan bygges en fangdam med høyde ~1 meter for forbiledning av vann i byggetida.
- Fundamentarbeider  
Rester av gammel dam fjernes og det graves ned i jomfruelige masser. Underlag for sone 1 avsluttes i tilstrekkelige tette masser. Kum plasseres i dypløp.  
For å unngå erosjon i damtå på nedstrøms side graves det her en grøft tilpasset en fåstein med volum > 0,5 m<sup>3</sup>. Elveleiet på nedstrøms side av fåsteinen skal være mest mulig urørt.  
Vist gravelinje under dammen er lagt 0,5 meter lavere enn terrengoverfalten på kart oversendt fra Bø kommune.
- Dammasser og innbygging i dam  
Grus og støttestoff legges ut i 0,3 meter tykke lag, komprimeres ved 6 overførter, valsevækt minimum 2 tonn.  
Se Teknisk beskrivelse for flere detaljer.
- Plastring  
Terskelens overflate skal beskyttes med grov-fraksjonert stein, D > 0,4 m i ytterlag og d > 0,1 m i indre lag.
- Trespunt  
Som trespunt skal det benyttes trykkimpregneret plank med tykkelse minst 70 mm. Planken skal være skråskjært i enden slik at den ved ramming blir presset mot nabospunten, og den skal være utformet slik at den gir føring ved ramming. For å styre linjeføringen langs toppen av spunten, skal det benyttes "dobbel tang" som vist på tegning. Den skal forbindes med spuntnålene for hver meter med en gjennomgående bolt. Spunten føres over hele dammen og ut til tette masser i vederlagene. Rundt rørgjennomføringen tilpassens enden av spunten til overflaten på betongen.
- Fiberduk  
Bruksklasse 4, minimum overlapp 0,5 meter. Skjøter legges slik at vannstrømmen glir over skjøtene og IKKE inn i skjøtene. Mot spunt legges dukken med minimum 0,15 m oppstikk.
- Erosjonssikring  
Siderenrenget langs dammens vederlag erosjonsbeskyttes med stein D>0,2 m. Erosjonsbeskyttelse skal føres en meter over damhøyden.

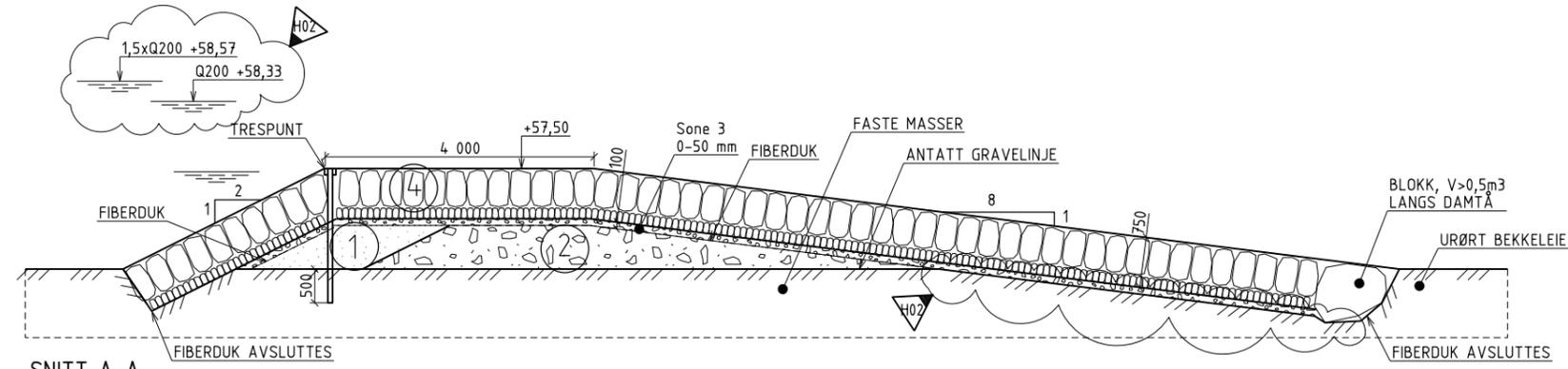
PLAN - OVERSIKT  
1:1000



PLAN - NEDRE TERSKEL  
1:100



SNITT B-B  
1:100



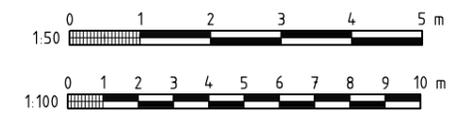
SNITT A-A  
1:50

HENVISNINGER:

- Rørgjennomføring - se tegning 120
- Spunt - se tegning 110
- Teknisk beskrivelse - se "Terskler i Evjuva, Teknisk underlag for bygging"

Tegningsnummer	Revisjon
130	H02

HØYDEGRUNNLAG: NN54



H02	2014-08-12	For utførelse	LKNeb	EØ	EØ
H01	2014-07-11	For utførelse	LKNeb	EØ	EØ
Revisjon	Dato	Beskrivelse	Utarbeidet	Fagkontroll	Godkjent

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Må bestekkes igjennom for A1 formål  
BØ KOMMUNE SOM VIST

EVJUDALEN TERSKLER  
NEDRE TERSKEL  
PLAN, OPPRISS OG SNITT

Norconsult	Oppdragsnummer	Tegningsnummer	Revisjon
	5133159	130	H02

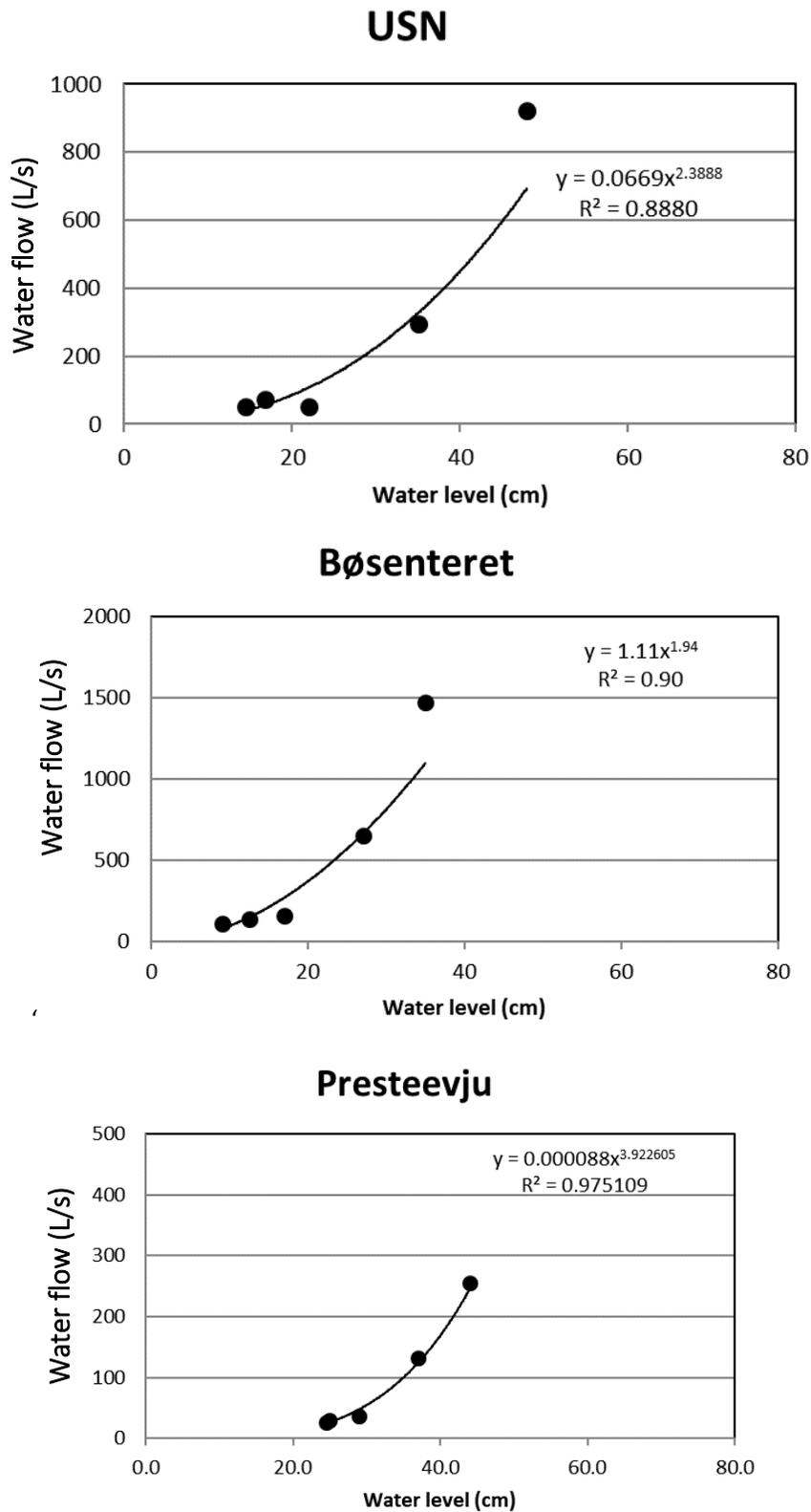


Figure 1. Streamflow curves calculated at both Borgjaevju and Presteevju streams at the different locations of the rulers (USN and Bøsenderet at Borgjaevju stream and Presteevju at Presteevju stream). Analysis were carried out on 2019.

## Annex 4

Table 1. Wet and dry sediment weight (g), number of microplastics (items/g dry sediment) and number of items/kg of dry sediment per sample of sediment (2019)

Sample	Date	Wet weight (g)	Dry weight (g)	items/g dry sediment	items/kg of dry sediment
Borgjaevju A1	07/05/2019	10	8.6	21	2441.9
Borgjaevju A2	07/05/2019	10	9	19	2111.1
Borgjaevju A3	07/05/2019	10	3	7	2333.3
Borgjaevju A4	07/05/2019	10	8.7	22	2528.7
Borgjaevju A5	07/05/2019	10	2.1	23	10952.4
Borgjaevju A6	07/05/2019	10	1.8	15	8333.3
Borgjaevju B1	07/05/2019	10	5.4	17	3148.1
Borgjaevju B2	07/05/2019	10	1.5	22	14666.7
Borgjaevju B3	07/05/2019	9	1.4	24	17142.9
Borgjaevju B4	07/05/2019	10	1.4	39	27857.1
Borgjaevju B5	07/05/2019	10	1.3	23	17692.3
Borgjaevju B6	07/05/2019	10	2.4	39	16250.0
Borgjaevju C1	07/05/2019	10	2.5	41	16400.0
Borgjaevju C2	07/05/2019	10	2.1	76	36190.5
Borgjaevju C3	07/05/2019	10	1.4	53	37857.1
Borgjaevju C4	07/05/2019	10	1.4	79	56428.6
Borgjaevju C5	07/05/2019	10	1.6	39	24375.0
Borgjaevju C6	07/05/2019	10	1.4	73	52142.9
Presteevju A1	08/05/2019	10	1.8	30	16666.7
Presteevju A2	08/05/2019	10	1.6	22	13750.0
Presteevju B1	08/05/2019	10	1.2	26	21666.7
Presteevju B2	08/05/2019	10	3.3	16	4848.5
Presteevju C1	08/05/2019	10	5.3	14	2641.5
Presteevju C2	08/05/2019	10	1.4	14	10000.0
Jønnebergjtjønn 1	13/05/2019	5.7	0.2	19	95000.0
Jønnebergjtjønn 2	13/05/2019	6.9	0.1	8	80000.0
Jønnebergjtjønn 3	13/05/2019	10	0.2	11	55000.0
Jønnebergjtjønn 4	13/05/2019	10	0.5	17	34000.0
Jønnebergjtjønn 5	13/05/2019	10	0.6	13	21666.7
Jønnebergjtjønn 6	13/05/2019	10	0.3	6	20000.0
Svalbjørtjtjønn 1	14/05/2019	12.7	0.5	25	50000.0
Svalbjørtjtjønn 2	14/05/2019	7	0.1	22	220000.0
Svalbjørtjtjønn 3	14/05/2019	10	0.3	25	83333.3
Svalbjørtjtjønn 4	14/05/2019	10	1.1	24	21818.2
Svalbjørtjtjønn 5	14/05/2019	5.3	0.5	32	64000.0
Svalbjørtjtjønn 6	14/05/2019	4.1	0.1	34	340000.0

## Annex 5

Table 2. Abundances of types of microplastics in the control filters (sampling, fume hood, room and stereomicroscope laboratory) over the whole period (2019)

Control	Black fibres	Blue fibres	White fibres	Transparent fibres	Purple fibres	Pink fibres	Red fibres	Multicolor fibres	Green fibres	Orange fibres	Brown fibres	Grey fibres
sampling Borgjaevju A 07/05	0	0	0	0	0	0	0	0	0	0	0	0
sampling Borgjaevju B 07/05	1	0	0	0	0	0	0	0	0	0	0	0
sampling Borgjaevju C 07/05	0	0	0	1	0	0	0	0	0	0	0	0
sampling Presteevju A 08/05	0	0	0	0	0	0	0	0	0	0	0	0
sampling Presteevju B 08/05	0	0	0	0	0	0	0	0	0	0	0	0
sampling Presteevju C 08/05	0	0	0	0	0	0	0	0	0	0	0	0
sampling Jønnebergtjønn	1	2	0	0	0	0	0	0	0	0	0	0
sampling Svalbjørtjønn	0	4	0	0	0	0	0	0	1	0	0	0
sampling Borgjaevju 21/05	1	0	0	0	0	0	0	0	0	0	0	0
sampling Presteevju 21/05	0	0	0	0	0	0	0	0	0	0	0	0
sampling Borgjaevju 04/06	1	1	0	0	0	0	0	0	0	0	0	0
sampling Presteevju 04/06	0	0	0	1	0	0	0	0	1	0	0	0
hood 07/05	2	0	0	2	1	0	0	0	0	0	0	0
hood 08/05	0	0	0	1	1	0	0	0	0	0	0	0
hood 13/05	2	1	0	0	0	0	0	0	0	0	0	0
hood 14/05	2	1	0	1	0	0	0	0	0	0	0	0
hood 15/05	0	0	0	0	0	0	0	0	0	0	0	0
hood 18/05	6	4	0	1	0	0	0	0	0	0	0	0
hood 20/05	3	2	0	0	0	0	0	0	0	0	0	0
hood 21/05	4	1	0	2	0	0	0	0	0	0	0	0
hood 22/05	5	5	0	2	2	0	0	0	2	0	0	0
hood 24/05	5	0	0	0	1	0	0	0	0	0	0	0
hood 26/05	0	1	0	0	0	0	0	0	0	0	0	0
hood 27/05	3	6	0	1	0	0	0	0	0	0	0	0
hood 28/05	3	0	0	0	2	0	0	0	0	0	0	0

hood 29/05	3	0	0	0	2	0	1	0	0	0	0	0
hood 30/05	5	0	0	0	0	0	0	0	0	0	0	0
hood 31/05	1	1	0	1	0	0	0	0	0	0	0	0
hood 01/06	4	1	0	0	0	0	0	0	0	0	0	0
hood 02/06	5	0	0	2	0	0	1	0	2	0	0	0
hood 04/06	0	1	0	0	0	0	0	0	0	0	0	0
Room 14/05	6	5	0	0	0	0	0	0	0	0	0	0
Room 15/05	1	0	0	0	0	0	1	0	0	0	0	0
Room 18/05	1	1	0	2	0	0	0	0	0	0	0	0
Room 19/05	0	0	0	1	0	0	0	0	0	0	0	0
Room 20/05	1	0	0	0	0	0	0	0	0	0	0	0
Room 21/05	4	2	0	1	0	0	0	0	0	0	0	0
Room 22/05	1	0	0	0	0	0	0	0	0	0	0	0
Room 23/05	0	0	0	0	0	0	0	0	1	0	0	0
Room 24/05	0	1	0	2	1	0	0	0	0	0	0	0
Room 25/05	0	1	0	2	0	0	0	0	0	0	0	0
Room 26/05	1	0	0	0	0	0	1	0	0	0	0	0
Room 27/05	1	1	0	1	0	0	0	0	0	0	0	0
Room 28/05	1	0	0	1	2	0	0	0	0	0	0	0
Room 29/05	3	0	0	1	0	0	0	0	0	0	0	0
Room 30/05	1	0	0	3	0	0	0	0	0	0	0	0
Room 01/06	0	0	0	1	0	0	0	0	0	0	0	0
Room 02/06	0	1	0	1	0	0	0	0	0	0	0	0
Lab 16/05	1	0	0	0	0	0	0	0	0	0	0	0
Lab 3/06	1	0	0	0	1	0	2	0	0	0	0	0
Lab 5/06	1	1	0	1	2	0	1	0	1	0	0	0
Lab 6/06	0	0	0	0	0	0	0	0	0	0	0	0
Lab 7/06	1	0	0	0	0	0	0	0	0	0	0	0
Lab 8/06	0	1	0	1	0	0	0	0	0	0	0	1
Lab 9/06	1	0	0	0	0	0	1	0	1	0	0	0









## Annex 6

**Table 3. Abundances of microplastics according to the type and colour and Shannon index of the sediment samples at Borgjaevju and Presteevju streams and L. Jønnebergjtjønn and L. Svalbjørtjønn over the whole experiment (2019).**

Sample	Abundances of fibres /10 g w.w.									n° of fibres/10 g w.w	Unknown	Film
	Black	Blue	Transparent	Violet	Pink	Red	Multicolor	Green	Other			
Borgjaevju A1	16	1	3	0	0	0	0	0	1	21	0	0
Borgjaevju A2	11	0	1	0	0	0	0	0	0	12	0	0
Borgjaevju A3	2	0	0	3	0	0	1	0	0	6	0	0
Borgjaevju A4	14	6	0	0	0	0	0	2	0	22	0	0
Borgjaevju A5	0	1	16	3	0	0	0	1	0	21	0	0
Borgjaevju A6	3	2	3	1	0	2	0	0	0	11	0	0
Borgjaevju B1	2	4	5	2	2	0	0	0	1	16	0	0
Borgjaevju B2	10	2	0	2	1	1	0	2	0	18	0	0
Borgjaevju B3	8	2	4	6	0	1	0	0	0	21	0	0
Borgjaevju B4	17	6	3	0	0	3	0	3	1	33	0	0
Borgjaevju B5	13	5	0	0	0	1	0	1	0	20	0	0
Borgjaevju B6	16	1	3	4	0	0	0	2	1	27	0	0
Borgjaevju C1	9	9	1	3	0	0	2	3	0	27	2	0
Borgjaevju C2	32	10	3	6	2	1	0	2	0	56	0	0
Borgjaevju C3	24	6	4	5	0	0	0	4	1	44	0	0
Borgjaevju C4	40	10	13	1	0	2	2	2	0	70	0	0
Borgjaevju C5	19	5	2	4	0	3	1	0	0	34	0	0
Borgjaevju C6	33	12	9	1	1	4	0	3	0	63	0	0
Jønnebergjtjønn 1	2	3	4	5	0	0	0	5	0	19	0	0
Jønnebergjtjønn 2	3	3	1	0	0	0	0	0	0	7	0	0
Jønnebergjtjønn 3	1	2	4	1	0	0	0	3	0	11	0	0
Jønnebergjtjønn 4	1	5	8	0	1	0	0	2	0	17	0	0
Jønnebergjtjønn 5	0	0	8	3	0	0	0	1	0	12	0	0
Jønnebergjtjønn 6	0	1	1	2	0	0	0	0	0	4	0	0
Presteevju A1	3	3	2	0	0	0	0	1	0	9	0	0
Presteevju A2	3	4	5	0	0	1	0	0	0	13	0	0
Presteevju B1	9	3	4	2	0	0	0	1	0	19	0	0
Presteevju B2	6	1	2	0	0	1	0	2	0	12	0	1
Presteevju C1	4	1	6	1	0	1	0	0	0	13	0	0
Presteevju C2	2	5	2	1	0	0	0	1	0	11	0	0
Svalbjørtjønn 1	9	6	3	2	1	0	1	0	1	23	0	0
Svalbjørtjønn 2	12	1	1	1	0	2	1	1	2	21	0	0
Svalbjørtjønn 3	8	9	0	2	1	1	0	0	0	21	0	0
Svalbjørtjønn 4	3	5	3	9	0	0	0	4	0	24	0	0
Svalbjørtjønn 5	13	2	1	6	0	0	0	5	0	27	0	0
Svalbjørtjønn 6	17	3	2	5	0	1	0	4	0	32	0	0

Sample	Abundances of fragments/10 g w.w.										n° of fragments/ 10 g w.w.	Shannon Index
	Multicolor	Transparent	Red	Black	Yellow	Blue	White	Orange	Green	Others		
Borgjaevju A1	0	0	0	0	0	0	0	0	0	0	0	0.78
Borgjaevju A2	0	6	0	1	0	0	0	0	0	0	7	0.99
Borgjaevju A3	0	0	0	0	1	0	0	0	0	0	1	1.28
Borgjaevju A4	0	0	0	0	0	0	0	0	0	0	0	0.86
Borgjaevju A5	0	0	0	0	1	1	0	0	0	0	2	1.06
Borgjaevju A6	0	0	0	1	2	1	0	0	0	0	4	1.99
Borgjaevju B1	0	0	0	0	0	1	0	0	0	0	1	1.79
Borgjaevju B2	0	0	0	0	1	3	0	0	0	0	4	1.71
Borgjaevju B3	0	0	0	1	0	2	0	0	0	0	3	1.69
Borgjaevju B4	0	0	0	0	1	5	0	0	0	0	6	1.69
Borgjaevju B5	0	0	0	0	3	0	0	0	0	0	3	1.19
Borgjaevju B6	1	0	2	0	3	4	0	0	0	2	12	1.97
Borgjaevju C1	2	0	0	0	1	5	0	1	3	0	12	2.21
Borgjaevju C2	0	0	1	6	0	3	0	1	9	0	20	1.90
Borgjaevju C3	0	0	0	3	0	0	0	0	6	0	9	1.70
Borgjaevju C4	1	2	0	4	0	1	0	0	1	0	9	1.65
Borgjaevju C5	0	0	0	0	1	0	2	0	2	0	5	1.69
Borgjaevju C6	0	1	0	2	0	1	0	0	6	0	10	1.74
Jønnebergtjønn 1	0	0	0	0	0	0	0	0	0	0	0	1.56
Jønnebergtjønn 2	0	0	0	0	0	0	0	0	0	1	1	1.26
Jønnebergtjønn 3	0	0	0	0	0	0	0	0	0	0	0	1.47
Jønnebergtjønn 4	0	0	0	0	0	0	0	0	0	0	0	1.30
Jønnebergtjønn 5	0	1	0	0	0	0	0	0	0	0	1	1.03
Jønnebergtjønn 6	0	1	0	0	0	0	0	0	1	0	2	1.56
Presteevju A1	4	0	0	0	5	1	8	3	0	0	21	2.02
Presteevju A2	0	1	0	0	3	0	4	0	0	1	9	1.92
Presteevju B1	0	0	0	0	1	2	2	0	2	0	7	1.94
Presteevju B2	0	1	0	0	1	1	0	0	0	0	3	1.93
Presteevju C1	0	0	0	0	0	0	0	0	0	1	1	1.48
Presteevju C2	0	0	0	0	3	0	0	0	0	0	3	1.63
Svalbjørtjønn 1	1	0	0	0	1	0	0	0	0	0	2	1.81
Svalbjørtjønn 2	0	0	0	0	0	1	0	0	0	0	1	1.61
Svalbjørtjønn 3	0	0	1	0	0	3	0	0	0	0	4	1.58
Svalbjørtjønn 4	0	0	0	0	0	0	0	0	0	0	0	1.51
Svalbjørtjønn 5	0	1	2	0	0	0	0	0	2	0	5	1.71
Svalbjørtjønn 6	0	0	0	0	0	1	1	0	0	0	2	1.57

## Annex 7

Table 4. Abundances of microplastics according to the type and colour and Shannon index of the water samples at Borgjaevju and Presteevju streams and L. Jønnebergtjønn and L. Svalbjørtjønn over the whole experiment (2019).

Sample	Site	Sampling date	n° of fibres/L	n° of fragments/L	Abundance of fibres/L								
					Black	Blue	White	Transparent	Purple	Pink	Red	Multicolor	Green
Borgjaevju A 07/05	Borgjaevju A	07/05/2019	22	4	9	0	0	9	3	0	1	0	0
Borgjaevju A 21/05	Borgjaevju A	21/05/2019	35	5	10	14	0	3	1	1	1	1	4
Borgjaevju A 04/06	Borgjaevju A	04/06/2019	5	3	1	1	0	2	0	0	0	0	1
Borgjaevju B 21/05	Borgjaevju B	21/05/2019	11	6	6	1	0	2	0	0	1	0	1
Borgjaevju B 04/06	Borgjaevju B	04/06/2019	13	3	5	3	0	2	1	0	0	0	2
Borgjaevju B 07/05	Borgjaevju B	07/05/2019	40	12	24	10	0	4	0	0	1	0	1
Borgjaevju C 04/06	Borgjaevju C	04/06/2019	4	8	1	3	0	0	0	0	0	0	0
Borgjaevju C 07/05	Borgjaevju C	07/05/2019	17	13	13	2	0	1	1	0	0	0	0
Borgjaevju C 21/05	Borgjaevju C	21/05/2019	9	3	5	2	0	1	0	0	1	0	0
Borgjaevju C outlet 21/05	Borgjaevju C outlet	21/05/2019	35	7	16	11	1	3	0	0	0	0	4
Borgjaevju C outlet 04/06	Borgjaevju C outlet	04/06/2019	5	12	0	3	0	2	0	0	0	0	0
Borgjaevju C outlet 07/05	Borgjaevju C outlet	07/05/2019	8	1	6	1	0	0	1	0	0	0	0
Jønnebergtjønn	Jønnebergtjønn	13/05/2019	9	16	4	3	0	0	0	0	0	2	0
Presteevju A 08/05	Presteevju A	07/05/2019	69	24	40	14	0	11	2	0	2	0	0
Presteevju A 21/05	Presteevju A	21/05/2019	13	8	6	3	0	1	0	0	1	0	2
Presteevju A 04/06	Presteevju A	04/06/2019	5	1	3	2	0	0	0	0	0	0	0
Presteevju B 08/05	Presteevju B	07/05/2019	49	27	28	16	0	1	1	0	3	0	0
Presteevju B 21/05	Presteevju B	21/05/2019	5	2	2	3	0	0	0	0	0	0	0
Presteevju B 04/06	Presteevju B	04/06/2019	12	5	3	4	0	3	0	0	1	0	1
Presteevju C 08/05	Presteevju C	07/05/2019	45	16	27	13	0	1	1	0	0	2	1
Presteevju C 21/05	Presteevju C	21/05/2019	20	9	12	2	0	2	1	0	2	0	1
Presteevju C 04/06	Presteevju C	04/06/2019	7	3	0	4	0	3	0	0	0	0	0
Svalbjørtjønn	Svalbjørtjønn	14/05/2019	16	5	9	4	0	2	1	0	0	0	0

Sample	Abundance of fragments/L								Film/L	Unknown/L	Shannon Index
	Multicolor	Transparent	Red	Black	Blue	White	Green	Others			
Borgjaevju A 07/05	0	0	0	0	3	0	0	1	0	0	1.483415
Borgjaevju A 21/05	0	1	0	2	2	0	0	0	0	0	1.899223
Borgjaevju A 04/06	0	0	0	1	2	0	0	0	0	0	1.732868
Borgjaevju B 21/05	0	0	0	1	3	0	2	0	0	1	1.955984
Borgjaevju B 04/06	1	0	0	1	1	0	0	0	0	0	1.890363
Borgjaevju B 07/05	1	0	0	0	11	0	0	0	0	0	1.42776
Borgjaevju C 04/06	0	1	0	1	4	0	2	0	0	0	1.632631
Borgjaevju C 07/05	0	0	1	1	9	0	0	2	0	0	1.538132
Borgjaevju C 21/05	0	0	0	0	3	0	0	0	0	0	1.42413
Borgjaevju C outlet 21/05	2	0	0	0	4	0	1	0	0	0	1.67789
Borgjaevju C outlet 04/06	0	7	0	0	1	0	4	0	0	0	1.43035
Borgjaevju C outlet 07/05	0	0	0	0	1	0	0	0	0	0	1.002718
Jønnebergtjønn	0	0	0	0	16	0	0	0	0	0	1.035327
Presteevju A 08/05	0	3	3	4	10	1	3	0	0	0	1.82174
Presteevju A 21/05	0	0	0	3	5	0	0	0	0	0	1.769488
Presteevju A 04/06	0	1	0	0	0	0	0	0	0	0	1.011404
Presteevju B 08/05	0	7	0	2	16	1	0	1	0	0	1.694838
Presteevju B 21/05	0	1	0	0	1	0	0	0	0	0	1.277034
Presteevju B 04/06	0	1	0	0	3	0	1	0	0	0	1.925408
Presteevju C 08/05	0	3	2	0	10	1	0	0	1	0	1.684776
Presteevju C 21/05	0	1	0	0	6	0	2	0	0	0	1.777137
Presteevju C 04/06	0	1	0	1	1	0	0	0	0	0	1.418484
Svalbjørtjønn	0	0	0	1	4	0	0	0	0	0	1.508729

## Annex 8

Table 5. Measures of microplastics from water samples at the different sampling sites at Borgjaevju and Presteevju streams and L. Jønnebergjtjønn and L. Svalbjørtjønn (2019).

Sample	Sampling date	Type	Colour	Measure ( $\mu\text{m}$ )
Borgjaevju A 07/05	07/05/2019	Fibre	Black	374.14
Borgjaevju A 07/05	07/05/2019	Fibre	Transparent	396.15
Borgjaevju A 07/05	07/05/2019	Fibre	Purple	1268.42
Borgjaevju A 07/05	07/05/2019	Fibre	Transparent	1403.08
Borgjaevju A 07/05	07/05/2019	Fibre	Black	1157
Borgjaevju A 07/05	07/05/2019	Fibre	Red	1086.23
Borgjaevju A 07/05	07/05/2019	Fibre	Black	335.57
Borgjaevju A 07/05	07/05/2019	Fibre	Transparent	405.07
Borgjaevju A 07/05	07/05/2019	Fibre	Black	3548.93
Borgjaevju A 07/05	07/05/2019	Fibre	Purple	696.77
Borgjaevju B 07/05	07/05/2019	Fibre	Green	2684.42
Borgjaevju B 07/05	07/05/2019	Fibre	Black	748.75
Borgjaevju B 07/05	07/05/2019	Fibre	Blue	904.95
Borgjaevju B 07/05	07/05/2019	Fibre	Black	888.45
Borgjaevju B 07/05	07/05/2019	Fibre	Black	1029.32
Borgjaevju B 07/05	07/05/2019	Fibre	Black	1999.45
Borgjaevju B 07/05	07/05/2019	Fibre	Blue	975.02
Borgjaevju B 07/05	07/05/2019	Fibre	Blue	1359.47
Borgjaevju B 07/05	07/05/2019	Fibre	Transparent	831.79
Borgjaevju B 07/05	07/05/2019	Fibre	Red	175.33
Borgjaevju C 07/05	07/05/2019	Fibre	Black	839.9
Borgjaevju C 07/05	07/05/2019	Fibre	Black	1344.1
Borgjaevju C 07/05	07/05/2019	Fibre	Blue	1194.14
Borgjaevju C 07/05	07/05/2019	Fibre	Black	571
Borgjaevju C 07/05	07/05/2019	Fibre	Black	340.24
Borgjaevju C 07/05	07/05/2019	Fibre	Black	546.47
Borgjaevju C 07/05	07/05/2019	Fibre	Black	1456.95
Borgjaevju C 07/05	07/05/2019	Fibre	Black	1140.8
Borgjaevju C 07/05	07/05/2019	Fibre	Purple	1556.2
Borgjaevju C 07/05	07/05/2019	Fibre	Transparent	1465.14
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Black	2458.06
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Black	260.02
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Black	1437.33
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Black	1246.51
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Blue	227.09
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Black	187.5
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Purple	2580.17
Borgjaevju C outlet 07/05	07/05/2019	Fibre	Black	727.48
Presteevju A 08/05	08/05/2019	Fibre	Purple	643.69
Presteevju A 08/05	08/05/2019	Fibre	Red	273.13
Presteevju A 08/05	08/05/2019	Fibre	Transparent	591.72
Presteevju A 08/05	08/05/2019	Fibre	Transparent	839.62
Presteevju A 08/05	08/05/2019	Fibre	Transparent	2862.96
Presteevju A 08/05	08/05/2019	Fibre	Black	773.07
Presteevju A 08/05	08/05/2019	Fibre	Black	994.87
Presteevju A 08/05	08/05/2019	Fibre	Transparent	4207.01
Presteevju A 08/05	08/05/2019	Fibre	Black	1648.39

Presteevju A 08/05	08/05/2019	Fibre	Blue	683.23
Presteevju B 08/05	08/05/2019	Fibre	Blue	599.53
Presteevju B 08/05	08/05/2019	Fibre	Blue	1198.32
Presteevju B 08/05	08/05/2019	Fibre	Black	712.92
Presteevju B 08/05	08/05/2019	Fibre	Black	2857.01
Presteevju B 08/05	08/05/2019	Fibre	Black	961.28
Presteevju B 08/05	08/05/2019	Fibre	Blue	1797.24
Presteevju B 08/05	08/05/2019	Fibre	Black	1741.49
Presteevju B 08/05	08/05/2019	Fibre	Transparent	1464.51
Presteevju B 08/05	08/05/2019	Fibre	Black	2076.85
Presteevju B 08/05	08/05/2019	Fibre	Black	844.89
Presteevju C 08/05	08/05/2019	Fibre	Blue	1142.14
Presteevju C 08/05	08/05/2019	Fibre	Blue	891.53
Presteevju C 08/05	08/05/2019	Fibre	Blue	485.82
Presteevju C 08/05	08/05/2019	Fibre	Black	325.55
Presteevju C 08/05	08/05/2019	Fibre	Transparent	1513.91
Presteevju C 08/05	08/05/2019	Fibre	Blue	1902.71
Presteevju C 08/05	08/05/2019	Fibre	Black	469.22
Presteevju C 08/05	08/05/2019	Fibre	Multicolor	1830
Presteevju C 08/05	08/05/2019	Fibre	Black	605.53
Presteevju C 08/05	08/05/2019	Fibre	Black	472.73
Borgjaevju A 21/05	21/05/2019	Fibre	Black	1049.83
Borgjaevju A 21/05	21/05/2019	Fibre	Blue	139.75
Borgjaevju A 21/05	21/05/2019	Fibre	Blue	1531.44
Borgjaevju A 21/05	21/05/2019	Fibre	Blue	1140.87
Borgjaevju A 21/05	21/05/2019	Fibre	Green	797.02
Borgjaevju A 21/05	21/05/2019	Fibre	Black	252.27
Borgjaevju A 21/05	21/05/2019	Fibre	Blue	1868.43
Borgjaevju A 21/05	21/05/2019	Fibre	Multicolor	360.96
Borgjaevju A 21/05	21/05/2019	Fibre	Blue	336.92
Borgjaevju A 21/05	21/05/2019	Fibre	Blue	526.84
Borgjaevju B 21/05	21/05/2019	Fibre	Black	1802.12
Borgjaevju B 21/05	21/05/2019	Fibre	Black	1091.49
Borgjaevju B 21/05	21/05/2019	Fibre	Black	827.85
Borgjaevju B 21/05	21/05/2019	Fibre	Red	642.42
Borgjaevju B 21/05	21/05/2019	Fibre	Black	342.58
Borgjaevju B 21/05	21/05/2019	Fibre	Green	829.43
Borgjaevju B 21/05	21/05/2019	Fibre	Black	521.17
Borgjaevju B 21/05	21/05/2019	Fibre	Transparent	516.23
Borgjaevju B 21/05	21/05/2019	Fibre	Black	923.62
Borgjaevju B 21/05	21/05/2019	Fibre	Black	455.97
Borgjaevju C 21/05	21/05/2019	Fibre	Black	2371.04
Borgjaevju C 21/05	21/05/2019	Fibre	Transparent	879.24
Borgjaevju C 21/05	21/05/2019	Fibre	Blue	399.31
Borgjaevju C 21/05	21/05/2019	Fibre	Black	398.43
Borgjaevju C 21/05	21/05/2019	Fibre	Black	1101.47
Borgjaevju C 21/05	21/05/2019	Fibre	Black	725.49
Borgjaevju C 21/05	21/05/2019	Fibre	Blue	1312.82
Borgjaevju C 21/05	21/05/2019	Fibre	Black	960.16
Borgjaevju C 21/05	21/05/2019	Fibre	Red	582.06
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Blue	2097.68
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Transparent	1386.88
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Green	225.29

Borgjaevju C outlet 21/05	21/05/2019	Fibre	Black	416.14
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Green	480.54
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Transparent	939.98
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Blue	1094.83
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Black	426.49
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Black	211.94
Borgjaevju C outlet 21/05	21/05/2019	Fibre	Black	413.75
Presteevju A 21/05	21/05/2019	Fibre	Blue	1336.58
Presteevju A 21/05	21/05/2019	Fibre	Black	286.91
Presteevju A 21/05	21/05/2019	Fibre	Black	686.92
Presteevju A 21/05	21/05/2019	Fibre	Black	440.06
Presteevju A 21/05	21/05/2019	Fibre	Green	430.62
Presteevju A 21/05	21/05/2019	Fibre	Red	1467.07
Presteevju A 21/05	21/05/2019	Fibre	Black	227.83
Presteevju A 21/05	21/05/2019	Fibre	Black	266.89
Presteevju A 21/05	21/05/2019	Fibre	Transparent	627.81
Presteevju A 21/05	21/05/2019	Fibre	Blue	493.56
Presteevju B 21/05	21/05/2019	Fibre	Black	772.41
Presteevju B 21/05	21/05/2019	Fibre	Black	713
Presteevju B 21/05	21/05/2019	Fibre	Blue	614.88
Presteevju B 21/05	21/05/2019	Fibre	Blue	1476.31
Presteevju C 21/05	21/05/2019	Fibre	Red	832.48
Presteevju C 21/05	21/05/2019	Fibre	Black	204.44
Presteevju C 21/05	21/05/2019	Fibre	Transparent	864.01
Presteevju C 21/05	21/05/2019	Fibre	Black	765.16
Presteevju C 21/05	21/05/2019	Fibre	Black	472.5
Presteevju C 21/05	21/05/2019	Fibre	Black	226.87
Presteevju C 21/05	21/05/2019	Fibre	Transparent	707.2
Presteevju C 21/05	21/05/2019	Fibre	Red	195.21
Presteevju C 21/05	21/05/2019	Fibre	Black	371.44
Presteevju C 21/05	21/05/2019	Fibre	Black	436.35
Borgjaevju A 04/06	04/06/2019	Fibre	Green	879.95
Borgjaevju A 04/06	04/06/2019	Fibre	Blue	291.93
Borgjaevju A 04/06	04/06/2019	Fibre	Transparent	601.5
Borgjaevju A 04/06	04/06/2019	Fibre	Transparent	657.48
Borgjaevju A 04/06	04/06/2019	Fibre	Black	385.63
Borgjaevju A 04/06	04/06/2019	Fibre	Black	285.81
Borgjaevju B 04/06	04/06/2019	Fibre	Purple	437.38
Borgjaevju B 04/06	04/06/2019	Fibre	Black	2267.1
Borgjaevju B 04/06	04/06/2019	Fibre	Green	519.66
Borgjaevju B 04/06	04/06/2019	Fibre	Blue	190.6
Borgjaevju B 04/06	04/06/2019	Fibre	Blue	172
Borgjaevju B 04/06	04/06/2019	Fibre	Black	590.98
Borgjaevju B 04/06	04/06/2019	Fibre	Black	508.46
Borgjaevju B 04/06	04/06/2019	Fibre	Blue	101.4
Borgjaevju B 04/06	04/06/2019	Fibre	Green	604.83
Borgjaevju B 04/06	04/06/2019	Fibre	Black	529.91
Borgjaevju C 04/06	04/06/2019	Fibre	Black	348.1
Borgjaevju C 04/06	04/06/2019	Fibre	Blue	111.45
Borgjaevju C 04/06	04/06/2019	Fibre	Blue	986.07
Borgjaevju C 04/06	04/06/2019	Fibre	Blue	233.72
Borgjaevju C outlet 04/06	04/06/2019	Fibre	Transparent	208.34
Borgjaevju C outlet 04/06	04/06/2019	Fibre	Transparent	528.2

Borgjaevju C outlet 04/06	04/06/2019	Fibre	Blue	2307.57
Borgjaevju C outlet 04/06	04/06/2019	Fibre	Blue	138.47
Borgjaevju C outlet 04/06	04/06/2019	Fibre	Blue	83.59
Presteevju A 04/06	04/06/2019	Fibre	Black	915.8
Presteevju A 04/06	04/06/2019	Fibre	Blue	298.18
Presteevju A 04/06	04/06/2019	Fibre	Black	1992.6
Presteevju A 04/06	04/06/2019	Fibre	Black	382.8
Presteevju A 04/06	04/06/2019	Fibre	Blue	1350.12
Presteevju B 04/06	04/06/2019	Fibre	Transparent	380.49
Presteevju B 04/06	04/06/2019	Fibre	Blue	236.57
Presteevju B 04/06	04/06/2019	Fibre	Transparent	1071.13
Presteevju B 04/06	04/06/2019	Fibre	Blue	418.96
Presteevju B 04/06	04/06/2019	Fibre	Black	413.89
Presteevju B 04/06	04/06/2019	Fibre	Black	329.01
Presteevju B 04/06	04/06/2019	Fibre	Black	728.61
Presteevju B 04/06	04/06/2019	Fibre	Blue	1442.75
Presteevju B 04/06	04/06/2019	Fibre	Green	1053.42
Presteevju B 04/06	04/06/2019	Fibre	Black	343.12
Presteevju C 04/09	04/06/2019	Fibre	Blue	1967.54
Presteevju C 04/10	04/06/2019	Fibre	Transparent	913.45
Presteevju C 04/11	04/06/2019	Fibre	Transparent	444.51
Presteevju C 04/12	04/06/2019	Fibre	Blue	1166.21
Presteevju C 04/13	04/06/2019	Fibre	Blue	244.79
Presteevju C 04/14	04/06/2019	Fibre	Blue	673.66
Presteevju C 04/06	04/06/2019	Fibre	Blue	236.84
Jønnebergtjønn 13/05	13/05/2019	Fibre	Black	1158.73
Jønnebergtjønn 13/05	13/05/2019	Fibre	Black	2218.05
Jønnebergtjønn 13/05	13/05/2019	Fibre	Multicolor	200.47
Jønnebergtjønn 13/05	13/05/2019	Fibre	Black	504.61
Jønnebergtjønn 13/05	13/05/2019	Fibre	Black	315.52
Jønnebergtjønn 13/05	13/05/2019	Fibre	Blue	303.67
Jønnebergtjønn 13/05	13/05/2019	Fibre	Blue	10849.62
Jønnebergtjønn 13/05	13/05/2019	Fibre	Multicolor	546.87
Jønnebergtjønn 13/05	13/05/2019	Fibre	Blue	568.56
Svalbjørtjønn 14/05	14/05/2019	Fibre	Transparent	1644.44
Svalbjørtjønn 14/05	14/05/2019	Fibre	Black	1006.59
Svalbjørtjønn 14/05	14/05/2019	Fibre	Black	533.12
Svalbjørtjønn 14/05	14/05/2019	Fibre	Black	407.15
Svalbjørtjønn 14/05	14/05/2019	Fibre	Black	460.2
Svalbjørtjønn 14/05	14/05/2019	Fibre	Black	303.66
Svalbjørtjønn 14/05	14/05/2019	Fibre	Black	272.98
Svalbjørtjønn 14/05	14/05/2019	Fibre	Blue	782.49
Svalbjørtjønn 14/05	14/05/2019	Fibre	Black	97.72
Borgjaevju A 07/05	07/05/2019	Fragment	Blue	26.09
Borgjaevju A 07/05	07/05/2019	Fragment	Blue	109.5
Borgjaevju A 07/05	07/05/2019	Fragment	Blue	28.63
Borgjaevju A 07/05	07/05/2019	Fragment	Grey	122.37
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	73.87
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	55.04
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	63.43
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	51.38
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	380.32
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	416.75

Borgjaevju B 07/05	07/05/2019	Fragment	Blue	33
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	157.81
Borgjaevju B 07/05	07/05/2019	Fragment	Blue	46.68
Borgjaevju B 07/05	07/05/2019	Fragment	Multicolor	88.85
Borgjaevju C 07/05	07/05/2019	Fragment	Blue	82.89
Borgjaevju C 07/05	07/05/2019	Fragment	Blue	69.71
Borgjaevju C 07/05	07/05/2019	Fragment	Blue	117.55
Borgjaevju C 07/05	07/05/2019	Fragment	Blue	25.39
Borgjaevju C 07/05	07/05/2019	Fragment	Black	191.57
Borgjaevju C 07/05	07/05/2019	Fragment	Blue	48.9
Borgjaevju C 07/05	07/05/2019	Fragment	Purple	168.76
Borgjaevju C 07/05	07/05/2019	Fragment	Red	72.14
Borgjaevju C 07/05	07/05/2019	Fragment	Blue	116.43
Borgjaevju C 07/05	07/05/2019	Fragment	Grey	601.68
Borgjaevju C outlet 07/05	07/05/2019	Fragment	Blue	81.39
Presteevju A 08/05	08/05/2019	Fragment	Blue	439.08
Presteevju A 08/05	08/05/2019	Fragment	Transparent	2402.55
Presteevju A 08/05	08/05/2019	Fragment	Black	509.1
Presteevju A 08/05	08/05/2019	Fragment	Green	348.4
Presteevju A 08/05	08/05/2019	Fragment	Blue	177.7
Presteevju A 08/05	08/05/2019	Fragment	Blue	27.54
Presteevju A 08/05	08/05/2019	Fragment	Blue	50.73
Presteevju A 08/05	08/05/2019	Fragment	Black	362.44
Presteevju A 08/05	08/05/2019	Fragment	Black	313.39
Presteevju A 08/05	08/05/2019	Fragment	Red	107.77
Presteevju B 08/05	08/05/2019	Fragment	Transparent	540.93
Presteevju B 08/05	08/05/2019	Fragment	Pink	24.94
Presteevju B 08/05	08/05/2019	Fragment	Blue	47.6
Presteevju B 08/05	08/05/2019	Fragment	Blue	282.89
Presteevju B 08/05	08/05/2019	Fragment	Black	469.39
Presteevju B 08/05	08/05/2019	Fragment	Blue	55.16
Presteevju B 08/05	08/05/2019	Fragment	Transparent	517.59
Presteevju B 08/05	08/05/2019	Fragment	Blue	142.39
Presteevju B 08/05	08/05/2019	Fragment	Blue	55.61
Presteevju B 08/05	08/05/2019	Fragment	White	1658.62
Presteevju C 08/05	08/05/2019	Fragment	Transparent	521.44
Presteevju C 08/05	08/05/2019	Fragment	White	290.25
Presteevju C 08/05	08/05/2019	Fragment	Blue	67.05
Presteevju C 08/05	08/05/2019	Fragment	Blue	67.08
Presteevju C 08/05	08/05/2019	Fragment	Blue	106.59
Presteevju C 08/05	08/05/2019	Fragment	Red	43.88
Presteevju C 08/05	08/05/2019	Fragment	Blue	90.16
Presteevju C 08/05	08/05/2019	Fragment	Transparent	534.69
Presteevju C 08/05	08/05/2019	Fragment	Blue	41.08
Presteevju C 08/05	08/05/2019	Fragment	Red	40.84
Presteevju C 08/05	08/05/2019	Fragment	Transparent	209.52
Borgjaevju A 21/05	21/05/2019	Fragment	Black	107.27
Borgjaevju A 21/05	21/05/2019	Fragment	Blue	106.93
Borgjaevju A 21/05	21/05/2019	Fragment	Blue	58.29
Borgjaevju A 21/05	21/05/2019	Fragment	Black	97.82
Borgjaevju B 21/05	21/05/2019	Fragment	Blue	78.27
Borgjaevju B 21/05	21/05/2019	Fragment	Black	280.84
Borgjaevju B 21/05	21/05/2019	Fragment	Blue	110.99

Borgjaevju B 21/05	21/05/2019	Fragment	Blue	78.79
Borgjaevju B 21/05	21/05/2019	Fragment	Green	216.85
Borgjaevju B 21/05	21/05/2019	Fragment	Green	338.07
Borgjaevju C 21/05	21/05/2019	Fragment	Blue	183.79
Borgjaevju C 21/05	21/05/2019	Fragment	Blue	72.89
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Borgjaevju C outlet 21/05	21/05/2019	Fragment	Green	123.01
Borgjaevju C outlet 21/05	21/05/2019	Fragment	Multicolor	125.51
Borgjaevju C outlet 21/05	21/05/2019	Fragment	Blue	72.63
Borgjaevju C outlet 21/05	21/05/2019	Fragment	Blue	161.58
Borgjaevju C outlet 21/05	21/05/2019	Fragment	Multicolor	106.49
Borgjaevju C outlet 21/05	21/05/2019	Fragment	Blue	186.71
Borgjaevju C outlet 21/05	21/05/2019	Fragment	Blue	326.43
Presteevju A 21/05	21/05/2019	Fragment	Blue	59.26
Presteevju A 21/05	21/05/2019	Fragment	Black	173.59
Presteevju A 21/05	21/05/2019	Fragment	Black	171.46
Presteevju A 21/05	21/05/2019	Fragment	Blue	125.74
Presteevju A 21/05	21/05/2019	Fragment	Blue	33.79
Presteevju A 21/05	21/05/2019	Fragment	Blue	85.82
Presteevju A 21/05	21/05/2019	Fragment	Blue	35.94
Presteevju A 21/05	21/05/2019	Fragment	Black	62.57
Presteevju B 21/05	21/05/2019	Fragment	Transparent	417.96
Presteevju B 21/05	21/05/2019	Fragment	Blue	39.63
Presteevju C 21/05	21/05/2019	Fragment	Green	89.63
Presteevju C 21/05	21/05/2019	Fragment	Green	73.05
Presteevju C 21/05	21/05/2019	Fragment	Blue	44.3
Presteevju C 21/05	21/05/2019	Fragment	Blue	75.86
Presteevju C 21/05	21/05/2019	Fragment	Blue	136.89
Presteevju C 21/05	21/05/2019	Fragment	Blue	188.59
Presteevju C 21/05	21/05/2019	Fragment	Blue	114.86
Presteevju C 21/05	21/05/2019	Fragment	Transparent	638.28
Borgjaevju A 04/06	04/06/2019	Fragment	Blue	80.9
Borgjaevju A 04/06	04/06/2019	Fragment	Blue	53.47
Borgjaevju B 04/06	04/06/2019	Fragment	Multicolor	225.01
Borgjaevju B 04/06	04/06/2019	Fragment	Black	192.26
Borgjaevju B 04/06	04/06/2019	Fragment	Blue	36.8
Borgjaevju C 04/06	04/06/2019	Fragment	Blue	71.1
Borgjaevju C 04/06	04/06/2019	Fragment	Transparent	569.73
Borgjaevju C 04/06	04/06/2019	Fragment	Blue	14.43
Borgjaevju C 04/06	04/06/2019	Fragment	Transparent	184.46
Borgjaevju C 04/06	04/06/2019	Fragment	Blue	47.92
Borgjaevju C 04/06	04/06/2019	Fragment	Green	32.16
Borgjaevju C 04/06	04/06/2019	Fragment	Green	66.72
Borgjaevju C 04/06	04/06/2019	Fragment	Blue	52.14
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Green	103.93
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Blue	94.35
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Transparent	227.03
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Transparent	213.95
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Transparent	395.39
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Transparent	764.57
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Transparent	339.09
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Green	122.29
Borgjaevju C outlet 04/06	04/06/2019	Fragment	Green	146.59

Borgjaevju C outlet 04/06	04/06/2019	Fragment	Transparent	389.82
Presteevju A 04/06	04/06/2019	Fragment	Transparent	680.89
Presteevju B 04/06	04/06/2019	Fragment	Blue	41.49
Presteevju B 04/06	04/06/2019	Fragment	Green	66.69
Presteevju B 04/06	04/06/2019	Fragment	Blue	159.02
Presteevju B 04/06	04/06/2019	Fragment	Transparent	150.87
Presteevju B 04/06	04/06/2019	Fragment	Blue	148.23
Presteevju C 04/06	04/06/2019	Fragment	Transparent	427.31
Presteevju C 04/07	04/06/2019	Fragment	Blue	89.87
Presteevju C 04/08	04/06/2019	Fragment	Black	95.97
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	83.91
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Multicolor	163.9
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	109.77
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	130.81
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	52.18
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	24.17
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	56.42
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	125.2
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	35.31
Jønnebergjtjøn 13/05	13/05/2019	Fragment	Blue	52.57
Svalbjørtjtjøn 14/05	14/05/2019	Fragment	Blue	165.82
Svalbjørtjtjøn 14/05	14/05/2019	Fragment	Blue	54.51
Svalbjørtjtjøn 14/05	14/05/2019	Fragment	Blue	71.12
Svalbjørtjtjøn 14/05	14/05/2019	Fragment	Blue	66.04
Svalbjørtjtjøn 14/05	14/05/2019	Fragment	Black	186.63

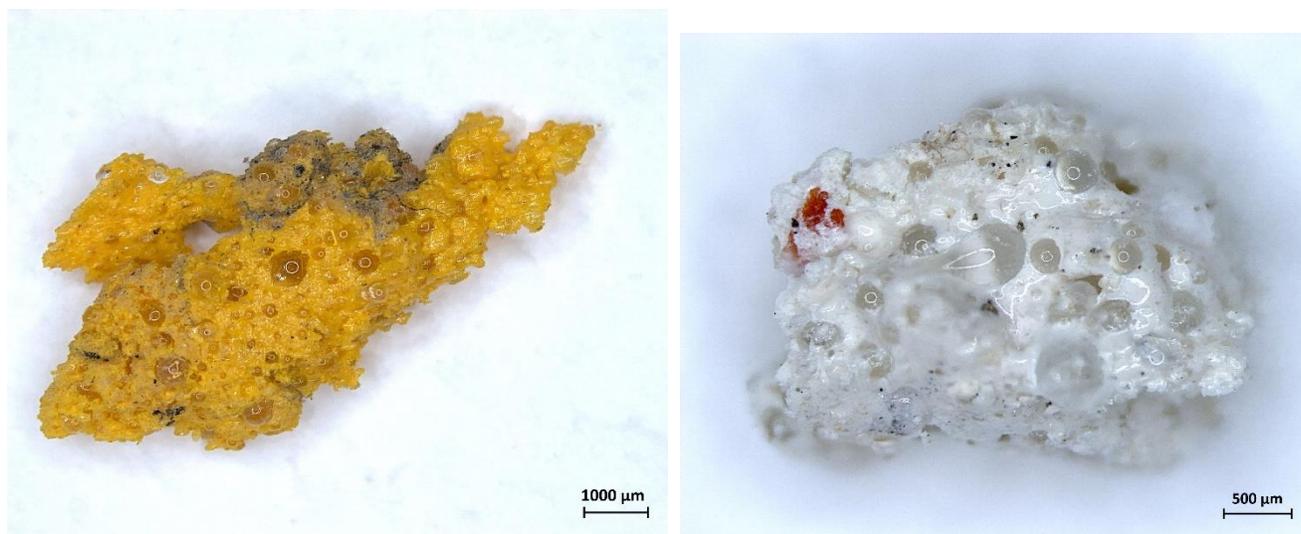
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## Annex 9

Table 6. Physical-chemical parameters of the water samples, the number of microplastics (items/L), the flow rate (L/s) and the number of microplastics per second (items/s).

Sample	Site	Date	T water (°C)	pH	EC (µS/cm)	Turbidity (NTU)	TOC (mg/L)	N <sub>T</sub> (µg/L)	P <sub>T</sub> (µg/L)	items/L	Flow rate (L/s)	items/s
Borgjaevju_A 07/05	Borgjaevju A	07/05/2019	7	7.31	62.40	3.52	6.72	954.67	16.96	26	51.00	1326.00
Borgjaevju_B 07/05	Borgjaevju B	07/05/2019	10	7.18	70.80	6.98	6.87	991.59	22.49	52	NA	NA
Borgjaevju_C 07/05	Borgjaevju C	07/05/2019	6	7.05	90.40	5.73	6.60	1341.10	25.18	30	NA	NA
Borgjaevju_C outlet 07/05	Borgjaevju C outlet	07/05/2019	6	7.10	122.40	5.90	6.06	1711.70	26.77	9	112.00	1008.00
Borgjaevju_A 21/05	Borgjaevju A	21/05/2019	14	7.05	59.60	4.89	7.56	910.76	20.79	40	75.00	3000.00
Borgjaevju_B 21/05	Borgjaevju B	21/05/2019	14	6.99	65.90	3.81	7.27	1000.29	24.88	18	NA	NA
Borgjaevju_C 21/05	Borgjaevju C	21/05/2019	10	6.89	71.50	7.07	6.26	1091.74	32.03	12	NA	NA
Borgjaevju_C outlet 21/05	Borgjaevju C outlet	21/05/2019	14	7.02	134.30	22.86	4.58	1464.02	43.14	42	138.00	5796.00
Borgjaevju_A 04/06	Borgjaevju A	04/06/2019	14	6.96	56.80	4.52	7.64	732.57	22.46	8	37.00	296.00
Borgjaevju_B 04/06	Borgjaevju B	04/06/2019	14	7.00	59.20	7.11	6.94	702.23	25.45	16	NA	NA
Borgjaevju_C 04/06	Borgjaevju C	04/06/2019	14	6.93	68.10	6.21	6.94	874.07	28.52	12	NA	NA
Borgjaevju_C outlet 04/06	Borgjaevju C outlet	04/06/2019	14	7.14	106.60	8.33	5.45	1130.35	31.23	17	157.00	2669.00
Jønnebergtjønn 1 m depth	Jønnebergtjønn	13/05/2019	11	5.5	16.3	0.613	5.19	512.66	9.57	25	NA	NA
Jønnebergtjønn 3 m depth	Jønnebergtjønn	13/05/2019	10	5.40	15.4	0.847	4.95	472.34	22.72	25	NA	NA
Jønnebergtjønn 6 m depth	Jønnebergtjønn	13/05/2019	11	5.47	15.8	1.083	4.95	513.82	8.16	25	NA	NA
Presteevju_A 08/05	Presteevju A	08/05/2019	7.4	7.47	193.20	2.90	1.80	2912.12	12.87	108	75.00	8100.00
Presteevju_B 08/05	Presteevju B	08/05/2019	5.3	7.41	219	1.85	2.03	2756.86	20.58	81	NA	NA
Presteevju_C 08/05	Presteevju C	08/05/2019	6	7.44	227	1.96	2.02	2479.12	13.45	67	NA	NA
Presteevju_A 21/05	Presteevju A	21/05/2019	13	7.28	220	3.04	2.23	2511.33	14.63	21	95.00	1995.00
Presteevju_B 21/05	Presteevju B	21/05/2019	15	7.40	211	2.97	2.73	2347.02	18.68	7	NA	NA
Presteevju_C 21/05	Presteevju C	21/05/2019	13	7.40	184.4	3.06	2.73	2099.35	17.36	29	NA	NA
Presteevju_A 04/06	Presteevju A	04/06/2019	14	7.53	161.1	3.50	2.58	2066.01	19.80	6	10.00	60.00
Presteevju_B 04/06	Presteevju B	04/06/2019	15	7.43	184.9	4.26	2.84	1890.88	22.07	17	NA	NA
Presteevju_C 04/06	Presteevju C	04/06/2019	13	7.38	191.3	5.46	3.12	1678.42	24.49	10	NA	NA
Svalbjørtjønn 1 m depth	Svalbjørtjønn	14/05/2019	10	6.87	22.8	0.63	4.23	329.3	9.79	21	NA	NA
Svalbjørtjønn 2 m depth	Svalbjørtjønn	14/05/2019	8	6.42	22.2	0.74	4.14	338.96	12.85	21	NA	NA
Svalbjørtjønn 5m depth	Svalbjørtjønn	14/05/2019	5	6.43	27.1	0.84	4.35	381.68	10.60	21	NA	NA

NA: Not available



**Figure 2. Yellow and white road painting of the roads nearby Bø (2019) (Photos by Ariadna García-Astillero Honrado). It can be appreciated the typical transparent microbeads.**