**Occurrence and size distribution of microplastics in mudflat sediments of the Cowichan/Koksilah Estuary, Canada: A baseline for plastic particles contamination in an anthropogenic-influenced estuary**

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## Abstract

Documenting the prevalence of microplastics in marine-coastal ecosystems serves as a first step towards understanding their impacts and risks presented to higher trophic levels. Estuaries exist at the interface between freshwater and marine systems, and provide habitats for a diverse suite of species, including shellfish, fish, and birds. We provide baseline values for estuarine mudflats usingsediment samples collected at Cowichan-Koksilah Estuary in British Columbia, Canada, a biologically-rich estuary. The estuary also contains a marine shipping terminal, forestry log sort area, and input of contaminants from nearby residential and agricultural areas. Microplastics, both fragments and fibers, occurred in 93% (13/14) of sediment samples. A mean of 6.8 microfibers/kg dw (range: 0-12 microfibers/kg dw) and 7.9 microfragments/kg (range: 0-19 fragments/kg dw) occurred in individual samples, and counts of fibers and fragments were strongly correlated ($r = 0.78$, $p = 0.008$, $n = 14$). The abundance of microplastics tended to be higher on the north side of the estuary that receives greater inputs from upland sources relative to the south side. Size distributions of microplastic fragments and fibers were similar to sediment grain size distribution with size categories 0.063 to 0.25 mm and 0.25 to 0.6 mm being the most common for plastics and sediment, indicating the occurrence of microplastics likely followed existing depositional processes within the estuary. Microplastics in sediments were composed of a variety of polymers, including high density polyethylene (HDPE), Nylon 6/6 (polyhexamethylene adipamide), and polyethylene terephthalate-PETE (poly(1,4-cyclohexylene dimethylene terephthalate)). This study indicates that microplastics occur throughout most of the Cowichan-Koksilah Estuary, and future studies should focus on the exposure risk and potential for bioaccumulation for wildlife species that feed on the surface of intertidal mudflats.

**Keywords:** microfibers; microfragments; polyethylene; mudflat sediments; sediment deposition patterns, Cowichan Estuary, British Columbia.

## Introduction

Globally, large plastics and microplastics are ubiquitous pollutants in marine environments and coastal areas (Bergmann et al., 2015; Jambeck et al., 2015; Lebreton et al., 2017), and have reached unprecedented levels in the oceans (Bergmann et al., 2017; Eriksen et al., 2014; Kane et al., 2020; Lebreton et al., 2018). Coastal and estuarine environments in particular often contain the highest levels of microplastics because of their proximity to human settlements, and estuaries experience conditions such as wave action that facilitates the breakdown of macroplastics into smaller microfragments, with the exception of microfibers which occur mostly as fibers. As our understanding of the prevalence of microplastics and their impacts on marine-coastal ecosystems and the species that inhabit them grows it is becoming increasingly important to document baseline levels in areas with unique hydrological processes to assess risks to local wildlife (Browne et al., 2007; Browne et al., 2010; Andrady, 2011; Ross and Morales-Caselles, 2015; Bergmann et al., 2015).

Microplastics are defined as plastic particles that are < 5 mm (i.e., < 5000 μm) in size, and can be separated into two categories: 1) primary microplastics that are deliberately manufactured (e.g., microbeads in cosmetics, industrial cleaners or virgin resin pellets for manufacturing and nurdles); and 2) secondary microplastics that are by-products, or break-down products, of larger plastics (i.e., polymers > 5mm), such as clothing, ropes, bags and bottles (Moore, 2008, GESAMP, 2010, Browne et al., 2007, Andrady, 2011, Duis and Coors, 2016). Most microplastics originate from terrestrial sources (e.g., household and industrial waste and wastewater), although these pollutants can also be released from marine activities (e.g., fishing, shipping) (Browne et al., 2007, Duis and Coors, 2016).

Along with other anthropogenic pollutants, microplastics have become an emerging contaminant of concern in the marine environment and sensitive ecosystems of British Columbia (BC) (Alava, 2019). In a large-scale spatial analysis of water column microplastics in coastal to offshore waters of BC, Desforges et al. (2014) revealed high microplastic concentrations (up to ~ 10,000 particles/$m^3$), with the highest levels occurring between the Strait of Georgia and Queen Charlotte Sound. The zooplankton, shellfish, and fish from nearshore waters using Baynes Sound and Lambert Channel/Denmann Island reflect these levels and have been found to contain notable abundances of microplastics (Cluzard et al., 2015; Kazmiruk et al., 2018; Collicutt et al., 2019; Covernton et al., 2019; Mahara et al., 2021). A recent study found microplastic incidence in sediments (i.e., maximum concentrations, ~50 microfibers/kg of sediment) and water column (i.e., maximum concentrations, ~1200 microfibers/$m^3$) in the Cowichan Bay, Cowichan Estuary (Collicutt et al., 2019), situated south of Baynes Sound. This occurrence is concerning because, when ingested, the impacts of microplastics on marine organisms are not well understood, especially for shellfish, Pacific herring (*Clupea pallasii*), and Pacific salmon (*Oncorhynchus* spp.), which are fundamental species in regional food webs and important traditional seafoods of First Nation coastal communities.

Recently microplastics were reportedly found in juvenile Chinook salmon, *Oncorhynchus tshawytscha* (i.e., maximum concentrations of microplastic fibers was > 2 fibers/g of fish), using the Cowichan Bay (Collicutt et al., 2019), raising concerns over the prevalence of microplastics in the industrialized estuary feeding into the bay. The Cowichan-Koksilah Estuary is designated as an Important Bird Area because of the globally significant numbers of Iceland Gull (*Larus glaucoides*) and Trumpeter Swan (*Cygnus buccinator*) that use this site during the winter. Furthermore, it is situated within the Pacific Flyway and is used by shorebirds during migration

(O'Reilly and Wingfield, 1995; Franks et al., 2014). Migratory shorebirds may be particularly susceptible to ingesting microplastics in the spring when they forage on intertidal biofilm on the surface of mudflats. Microplastics are one form of emerging pollutant that may be prevalent in the estuary and have the potential to affect environmental and wildlife health. Microplastics in particular can be ingested by filter-feeding species such as resident shellfish, and species directly feeding on mudflats such as migrating shorebirds (Western Sandpiper, *Calidris mauri*, and Dunlin *Calidris alpina*) that use the estuary during stopover or year-round. To date, however, a detailed assessment of the prevalence and distribution of microplastics in the estuary and associated mudflats has not been conducted.

While questions remain on the impact of microplastic pollution and associated pollution risks in the estuary and other marine biota and coastal wildlife, a detailed analysis of microplastic types and size distribution in mudflat sediment has yet to be conducted. Here, we investigate the presence of microplastics in intertidal mudflat sediments, which can act as an abiotic matrix (i.e., microplastics sink), by assessing whether the microplastics particle size distribution mirrors the particle size distribution of mudflat sediments. First, we determine the distribution of microplastics across the Cowichan-Koksilah Estuary. Then, by comparing microplastics concentrations found in sediments from the north and south parts of the estuary, we infer possible environmental or anthropogenic sources. Specifically, the seasonal and spatial occurrence of size-specific plastic particles in estuarine intertidal mudflat biofilm from the intertidal zones of stopover sites used for foraging by shorebirds in the Cowichan Estuary are characterized, aiming to contribute with baseline data to inform policy and decision makings to address plastic/microplastics pollution and improve solid waste and wastewater management in the region.

## Materials and Methods

### *Study Area: The Cowichan-Koksilah Estuary*

The Cowichan-Koksilah Estuary is situated on the east coast of Vancouver Island between the cities of Victoria and Nanaimo. It is formed by the Cowichan and Koksilah rivers draining into the Strait of Georgia (Fig. 1). The Koksilah River originates from Waterloo Mountain, south of the Cowichan Valley and the Cowichan River from Cowichan Lake, approximately 40 km upstream from the estuary. The two rivers with a combined watershed of close to 8000 sq. km are the major freshwater tributaries to the estuary and Cowichan Bay. This estuary is BC's eighth largest estuary (Ryder et al., 2007).  The Cowichan Estuary is part of the traditional territory of the Coast Salish People; for instance, the Cowichan-Koksilah Estuary embraces the traditional territory of Cowichan Tribes and supports the largest Indigenous community on Vancouver Island prior to European settlement in Cowichan Bay in the mid-1800s. This highly productive estuary has provided ecological services and benefits to the Hul’q’umi’num people by offering a rich and diverse sustainable harvest of shellfish, salmon, herring roe and seaweed for centuries. Because of its mild micro-climate and naturally abundant food/seafood sources, the estuary and associated adjacent lands had always been considered a preferred settlement area by the Coast Salish people (Schuerholz, 2006).

With the construction of the Esquimalt and Nanaimo Railway in 1886 and increasing settlements in Cowichan Bay and the adjacent hinterland, the Cowichan Estuary experienced major environmental changes. Salt marshes were diked, converted into agricultural fields, and the intertidal estuarine zone used for log sorting, storage, and processing by a rapidly expanding forestry industry in the area. In the late 1920s, the railroad line was extended into the center of the estuary providing access to a deep-water seaport, constructed by infilling over 40 acres of prime salt-marsh and inter-tidal areas. The Cowichan Estuary Environmental Management Plan (CEEMP), developed in the late 1970s and ratified by Order in Council in 1987, sets the framework for management of the estuary and aims to address anthropogenic stressors and environmental concerns for the conservation and sustainable use of key species inhabiting the estuary (Lambertson, 1987; Schuerholz, 2006). Land use in the estuary includes agriculture, forestry, dredging, waste management, as well as commercial and residential development. The Village of Cowichan Bay, situated near the head end of the bay on the south shore, supports approximately 2,394 people (Statistics Canada, 2016). Several farms, including at least one dairy operation, are also located on converted salt marsh habitat. Two main industrial operations are situated within the estuary: a terminal on the southern side of the intertidal zone is situated on a man-made causeway that extends 1.4 km into the mudflats; a sawmill operates to the north of the terminal, and south of the terminus of the main stem of the Cowichan River (Bell and Kallman, 1976).

As a result of these land uses and localized industrial developments, several kinds of anthropogenic pollution are affecting the estuary. The industrial footprint in the estuary includes: i) run-off from creosoted and sap stain-treated lumber and timber from the Westcan Terminal; ii) contaminated-material falling into the estuary and deep sea from a deteriorating dock; iii) mill pond contamination from hydrogen-sulfide, discarded used oil and lubricants from the previously dismantled sawmill stored at the Western Forest Product Mill site; iv) secondary treatment effluent from the Duncan/North Cowichan sewage treatment plant located about 5 km upstream of the estuary; and, v) contamination and nutrient loading from fertilizer and liquid manure originating from the farms located in the Cowichan/Koksilah floodplain and adjacent to the river channels (Schuerholz, 2006).

*Mudflat sediment sampling and collection*

No standardized protocol currently exists for sediment sampling for microplastics due to differences in positioning of sample locations (beach, intertidal zone), sampling techniques, and sample quantities. While most approaches from sampling to identification of microplastics in beach/intertidal sedimentary environments are lacking standardized methods (Frias et al., 2018; Hidalgo-Ruz et al., 2012; Stock, et al., 2019), a modified existing protocol (Frias, et al., 2018; Kazmiruk et al., 2018; MSFD TSG, 2011) for sampling and processing was applied for the collection of beach/intertidal sediments of the estuary.

A series of bulk sediment samples was used for quantitative and qualitative assessment of microplastics and sediment properties in the Cowichan Estuary (Fig. 1). A targeted sampling design was implemented, consisting of 42 sediment samples (14 sites x 3 replicates =42 samples) collected during low tide from August 2020 to October 2020 (Table 1). Sampling sites were chosen to capture the spatial variation in microplastics deposition on both sides of Westcan Terminal Rd., a causeway which extends through the intertidal saltmarsh and mudflats to Western Stevedoring's Cowichan Bay Terminal and effectively divides the estuary into two sections (Schuerholz, 2006**)**. Three samples (S1, S2, S3) were collected on the south side of the causeway where the Koksilah River is the main source of freshwater, eight samples (N2, N3, C1-C6) were collected from the north side of the causeway which is fed by the north and south forks of the Cowichan River, and three samples (T1, T3, T5) were collected from the perimeter of the Cowichan Bay Terminal. The distribution of sampling sites covered the tidal inundation gradient on both sides of the causeway (low, mid, high), as well as the intertidal areas influenced by the Western Forest Products Mill, the intertidal log booming area, and the artificial log transport channel which connects the mill pond to the open ocean. We used handheld GPS to mark the location and time information for sediment sampling sites at the study area. The period of sampling was determined based on the optimal meteorological conditions (e.g., absence of inclement weather) during the period of low tides.

Triplicate samples were collected from the top 5 cm of the oxygenated layer of the mudflat sediments (Browne et al., 2010) from separated 0.25 m x 0.25 (0.0625 $m^2$) quadrats (Gray et al., 2018), situated 3-5 m apart in undisturbed areas using a clean stainless-steel spatula (Claessens et al., 2011). The sediment samples were packed into pre-labelled, previously unused, sealed bioplastic bags, transferred to the laboratory and stored in the freezer at -20°C until further analysis. For background contamination control, a clean borosilicate Petri dish prepared with Vaseline (petroleum jelly) was placed beside each field sampling station and opened during sediment sample collection.

***Laboratory analysis of sediments***

*Grain size analysis*

A wet sieving technique was used to determine grain size (GS) distribution of sediment samples (Batley et al., 2016; Mudroch et al., 1997). Sediment particles were separated into six different size factions: gravel (GS > 5.0 mm; 5.0 mm > GS > 1.70 mm), coarse sand (1.70 mm > GS > 0.60 mm; 0.60 mm > GS > 0.25 mm), fine sand (0.25 mm > GS > 0.063 mm), and mud or fine sediments (silt, clay: GS < 0.063 mm). Sediment particle size analysis by wet sieving was undertaken on the thoroughly homogenised wet sediment subsamples, using standard high quality nylon and stainless steel sieves. Wet sieving was achieved by using de-ionised water ($dH_2O$) to wash the wet sediment subsamples three times through 5000 µm (5 mm), 1700 µm (1.7 mm), 600 µm (0.60 mm), 250 µm (0.25 mm), and 63 µm (0.063 mm) sieves. The sediment particles passing through the finest sieve and the sediment particles retained on each sieve were quantitatively collected and the relative amounts determined by drying and weighing the respective sediment size fractions.

*Organic content*

Organic matter concentration in the sediment samples was determined by "loss-on-ignition" (LOI) method, the most appropriate method for the total organic carbon (TOC) content analysis that involves the heated destruction of all organic matter in the sediment (Mudroch et al., 1997; ASTM, 2000a; ASTM, 2000b; ASTM, 2000c; Schumacher, 2002). The dried sediment subsamples weighing approximately 1.5 g – 2.0 g each were burned at 400°C – 440°C (to avoid the destruction of any inorganic carbonates in the sediments) for 5-10 hours. Organic content was determined as the difference between the initial and final (ashed) subsample weights, and expressed as a percentage.

*Extraction and analysis of microplastics in sediment samples*

For analyses of microplastics in sediment samples we used the following steps: subsample preparation, sieving, microplastics extraction, filtration, visual inspection, microscope exam, and FTIR spectroscopy analysis of microplastic particles for the chemical composition of polymeric materials.

Subsample preparation included drying, weighting, and labeling. Polymeric microparticles were separated from sediments using sieves. Dried sediment samples were weighted and sieved using sieves with different mesh sizes of 5000 µm, 1700 µm, 600 µm, 250 µm, and 63 µm to allow dividing the polymeric particles into the following fractions: >5000 µm, 5000 - 1700 µm, 1700 - 600 µm, 600 – 250 µm, 250 - 63 µm, and < 63 µm.

In general, to extract microplastics from sediment samples we used density separation. First, the microplastics particles were extracted from sediments using the flotation method (Thompson et al., 2004) with our modifications (Kazmiruk et al., 2018). The saturated saline (NaCl and sea salt) solutions with a density of approximately 1.60 $g/cm^3$ (360 g NaCl/l $H_2O$- solubility of NaCl in the water at $25^0C$) were used as density separators.

Dry-sieved subsamples were transferred directly to Erlenmeyer flasks used for density separation, and 250-900 ml (depending on the volume of the flask) of NaCl / sea salt solution was added. Using a magnetic stirrer, each subsample was stirred vigorously for 20–25 min and sediments were allowed to settle overnight or during the next 24 hours, depending on the observed clearance rate of the sediments from the suspension (Thompson et al., 2004).

For the filtration, the surface supernatant of each subsample in the Erlenmeyer flask was then extracted from the solution surface using a 30 or 50 ml glass pipette, and expelled from the pipette onto a glass fibre filter (Whatman GF/A 47 mm diameter, GE Healthcare Whatman). The top 250-500 ml of the remaining supernatant was decanted and filtered through a separate glass microfiber filter using a vacuum filtration unit to analyse residual polymeric material in the water column above the sediments. The same procedure of filtration was applied to the 1700 - 600 µm; 600 - 250 µm, 250 - 63 µm, and < 63 µm grain size fractions. In some cases, the density separation method was not applied to the fractions of > 5000 µm and 5000 - 1700µm in size.

Instead, these sediment subsamples were visually inspected under the dissecting microscope without further processing.

*Microplastics identification*

Microplastics were recovered after extraction of microplastic particles from sediment subsamples (Kazmiruk et al., 2018). Recovered microplastics were enumerated (in polymeric particles/kg of sediment dry weight (dw) and categorized based on size, shape, colour, and surface structure. Positive identification was done using digital microscope with 40X -1000X magnification and dissecting stereo microscope (Olympus SZ51 or similar) with high magnification, melting point analysis (e.g., polymeric materials melts at 60-135° C), and FTIR spectroscopy analysis.

*FTIR Analysis*

A subset of 10 filters containing suspected microplastics particles from mudflat sediment samples was analyzed using a Fourier-Transform Infrared Spectrometer with attenuated total reflectance (ATR) accessory (Perkin Elmer Inc. Spectrum FTIR) to identify the type (polymeric materials) of microplastics particles. The Perkin- Elmer ATR of Polymers Library and Atlas of Plastics Additives Analysis by Spectrometric Methods (Hummel, 2002) was used for identification of microplastics' chemical composition.

***Quality Assurance / Quality Control***

*Field controls*. Each field sampling station had a paired background contamination control. At each sample site, a clean PetriSlide prepared with Vaseline (jelly) was placed beside the mudflat sediment collection location and opened any time sample were begin collected.

*Lab controls*. All activity and lab procedures that expose samples to the environment were performed in a designated clean room with a laminar flow hood when appropriate. Clothing in the clean room was restricted to clothing composed of 100% natural fibres (100% cotton) to avoid synthetic fibre contamination of samples. Three lab procedural blanks were conducted with each batch of samples from the field. For each procedural blank, 100 mL of Milli-Q water was filtered through a 10 μm 47mm polycarbonate filter and stored in a clean PetriSlide dish. Final microplastic concentrations were blank-corrected by excluding any microplastics occurring in the same size/colour/type category found in procedural blanks.

**Statistical data analyses**

*Data analyses*

We used generalized linear models (GLM) to test for spatial variations in organic content and numbers of fibers and fragments in sediment samples among the four parts of the estuary (south, terminal, central, north), and along the tidemark spectrum (low, medium, high; Fig. 1). Each model was fit using the *glm* procedure in R that estimated the average difference in percent organic content or counts of fibers/fragments, with location and tidemark as explanatory variables. The significance of individual terms was determined with a likelihood ratio test (LRT), and non-significant terms ($P > 0.10$) were removed from the model for final inference. We tested for differences using a multiple comparisons approach based on a Tukey test, using the *multcomp* package in R. We assessed assumptions of normality and homoscedasticity of residuals through residual plots of the final models, and found no major departures from those assumptions.

*Mapping: Interpolation by Empirical Bayesian Kriging*

To provide a visual characterization of microplastic distribution throughout the estuary, sediment microplastic concentrations were predicted and mapped using Empirical Bayesian Kriging (EBK) interpolation with the Geostatistical Toolbox in ArcGIS Pro (ESRI, 2020). Empirical Bayesian Kriging (EBK) is a geostatistical interpolation method that automates many of the parameter adjustments necessary to construct a valid kriging model, implementing numerous semi-variogram models to calculate parameters through a process of sub-setting and simulations (Chilès and Delfiner, 2012). The EBK method can handle non-stationary input data and compensates for error introduced by estimating the underlying semi-variogram through repeated simulations (Finzgar et al., 2014). Assessment of model accuracy was performed with a cross-validation technique (Supplementary Material) which removes each data location one at a time and predicts the associated data value data at the rest of the locations (Krivoruchko, 2011).

## Results

*Microplastic distribution and abundance*

A total of 86% (12/14) of samples from the Cowichan-Koksilah Estuary contained microfibers and 93% (13/14) contained microfragments. A mean of 6.8 microfibers/kg dw (range: 0-12 microfibers/kg dw) and 7.9 microfragments/kg (range: 0-19 fragments/kg dw) occurred in individual samples, and counts of fibers and fragments were strongly correlated ($r = 0.78$, $P = 0.008$, $n = 14$). The abundance of microplastics varied over the estuary (Fig. 2). The mean abundance of fibers varied among the four locations (LRT: $X^2 = 123.2$, $df = 3$, $p < 0.001$), but did not vary significantly among tidemarks. Similarly, the mean abundance of fragments varied among the four locations (LRT: $X^2$ = 142.3, df = 3, p = 0.04), but did not vary significantly among tidemarks. Mean abundances of both types of microplastics were highest at the north side and central locations and near the terminal, and lowest at the south end of the estuary (Fig. 2).

*Sediment and microplastics size distribution*

Sediment grains varied in size, and most commonly fell into 0.063 to 0.25 mm (average 36.3% of volume) and 0.25 to 0.6 mm (29.0 % of volume) size categories. The plastic particle size distributions mirrored the particle size distribution in the sediment (Fig. 3), and the most common size categories of plastic fibers and fragments were 0.063 to 0.25 mm and 0.25 to 0.6 mm (Fig. 3).

*Sediment TOC*

The average total organic content of sediment samples was 4.5% (range: 2.6-8.1%). Organic content was weakly negatively correlated with counts of plastic fibers (r = -0.40, P = 0.11, n = 14), and counts of plastic fragments (r = -0.30, P = 0.29, n = 14), indicating a trend of higher counts of plastics in sites with sites with lower organic content. Organic content did not vary among locations, but varied across the depth of tidal spectrum (LRT: $X^2$ = 24.7, df = 2, p = 0.017), with organic content at mid- and high-tidemark locations being ~1.7x the values observed at low-tidemarks (Fig. 4).

*Microplastic polymer composition and identification*

Only four of the 10 selected samples (40%) analyzed with FTIR provided clear composition (83-98%) of polymeric materials. The FTIR analysis for polymer composition of these samples revealed that microfragments within the range of 1.70 to 0.60 mm in site NI were high density polyethylene (HDPE), i.e., search score composition: 98% (infrared spectrum shown in Figure

5a), while microplastics of the same size range in site C1 were identified as Nylon 6/6 (polyhexamethylene adipamide), i.e., search score composition: 95% (Figure 5c). Plastic particles including either microfibers or microfragments < 0.063 mm in site T3, and also microplastics ranging 0.60 to 0.25 mm in site C3 were identified as polyethylene terephthalate-PETE (poly(1,4-cyclohexylene dimethylene terephthalate)); search score compositions: 84 and 83%, respectively (Figure 5b). These are acceptable results as most of the polymeric macro- and microparticles found in sedimentary environment are aged or degraded, making difficult the identification of their polymeric composition by using the Standard Library of Polymeric Materials.

*Mapping of microplastic distribution*

The EBK interpolation model used to estimate the distribution of total microplastics concentration throughout the Cowichan-Koksilah Estuary indicated that the two sides of the estuary relative to the Westcan Terminals causeway had opposing spatial distributional patterns, with a gradual decrease in microplastics occurrence from the north to south shores (Fig. 6). However, the small number of samples used for interpolation resulted in a high degree of uncertainty (MSE = - 0.0095; RMSE = 6.43) and thus the prediction map should only be used to visually assess the likely patterns of microplastics distribution rather than to quantitatively predict microplastics at any given point in the estuary. In general, sample locations on the north side had the highest microplastics counts compared to the south side; within the north side of the estuary, the highest microplastics abundance was found nearest to the sawmill and mouth of North Arm of the Cowichan River, decreasing seaward from the sawmill to the low tide mark. In contrast, the south side of the estuary tended to have lower abundances near the high tide mark, increasing seaward along the southmost branch of the Koksilah River towards the village of

Cowichan Bay. No microplastics hotspots were observed near the mouth of the Koksilah River nor the mouth of the South Arm of the Cowichan River. Intermediate microplastics concentrations (10.1 – 15 plastic particles [pp] /kg dw) were observed on perimeter of Westcan terminals and predicted to be distributed at similar concentrations along the low tide mark on the south side of the estuary.

**Discussion**

Sediments are fairly stable abiotic matrices which serving as a sink or source  allows for the investigation of the depositional patterns of microplastics incidence, and  exposure in coastal-marine and estuarine ecosystems (Browne et al., 2010; Claessens et al., 2011; Kazmiruk et al., 2018; Brandon et al., 2019). Microplastics are sediment-associated contaminants that tend to accumulate in the depositional areas, such as intertidal zones, with large amount of small fine-grained particles and high concentrations of organic matter. We report a widespread microplastics prevalence in the Cowichan-Koksilah Estuary with significant distributional variation unique to the estuary.

Overall, concentrations of sediment micro-fibers and -fragments in the Cowichan Estuary fell within the range reported in Lambert Channel and Baynes Sound sediments (Kazmiruk et al., 2018), but the highest concentrations of micro-fibers and -fragments (12 to 19/kg dw) were well below the maximum concentrations reported in sediments (i.e.100 to 300/kg dw) from these other coastal locations (Kazmiruk et al., 2018). Similarly, the maximum microfibers concentration (12 microfibers/kg dw) in the estuary was lower than those that reported (~50 microfibers/kg dw) in Cowichan Bay (Collicutt et al., 2019), and the average sediment concentration (60 microfibers/kg dw) from several locations on the east coast of Vancouver Island (see Collicutt et al., 2019).

The hydrodynamic processes and specific-particle deposition (sedimentation and burial) rates of the Cowichan River and floodplain in tandem with the local or regional sources of microplastics pollution, storm water outfalls and tidal/wave regime are likely the primary drivers of the occurrence and size distribution of sediment particles and microplastics (Figure 6).For instance, the high flow and water volume of the north forks of the Cowichan River (upriver and agricultural areas) may influence the deposition of more sediments and occurrence of high microplastic densities in the north side relative to the lower flow and sedimentation rates in the south region of the Cowichan Estuary (Figure 6). The moderate microplastics concentrations observed in the central sampling locations of the estuary mudflats close to the sawmill, terminal, and residential areas indicate these may be local contributors to the trends we observed.

The EBK interpolation of the microplastics distribution provided a better spatial visualization of the site-specific accumulation and deposition pattern of microfibers and fragments. There was a higher abundance of fragments compared to fibers on the north transect despite the fragments and fibers have generally similar spatial distributions. Based on this projection, the north arm of the Cowichan River appears to be an important source of microplastics pollution. Across the study site the highest microplastics concentrations (fibers and fragments) were found at the site next to the saw mill. While this concentration represents one sampling location it suggests the saw mill may be altering or contributing to the distribution of microplastics we report. The prediction and mapping of sediment microplastic concentrations support the notion of a concentration gradient related to potential or putative contamination sources, as well as the plausible identification of contamination "hot spots" in the Cowichan Estuary.

We found that the plastic particle size distribution mirrored the particle size distribution observed in sediments grains, indicating that microplastics are deposited on the sediment following existing depositional patterns of other fine particles. The partitioning of polymeric particles in abiotic compartments of the environment (e.g., water, bottom sediments, suspended solids, air) dictates the distribution and redistribution of microplastics, which is density-dependent in aquatic ecosystems. Marine and estuarine sediments are complex matrices (for example, vary by size) and microplastic particles (measuring <5 mm in size) tend to accumulate in depositional areas with high proportion of fine-grained particles which have a very high surface area and tendency for higher concentration of organic matter, related to the *in situ* biogeochemical cycling and the local estuarine food webs. Modern sediments (surficial sediment layers) are able to contain the largest proportion of microplastics (Brandon et al., 2019); and, it seems that the pore spaces in the sediments with a high proportion of fine-grained particles functions as “catchment” compartments for plastic particles, depending on the density of the polymer.

The prediction and mapping of sediment microplastic concentrations support the notion of a concentration gradient related to potential or putative contamination sources, as well as the plausible identification of contamination "hot spots" in the Cowichan Estuary.

The polymer composition of a small subset of suspected microplastic particles and fiber identified by FTIR spectroscopy indicates that HDPE is a common microplastic in the north side (i.e. N1) of the causeway of the Cowichan Estuary, while the specific detection of Nylon 6/6 in site C1 warrants further investigation as this polymer is commonly used in textile and plastic industries. The finding of PETE (i.e., poly(1,4-cyclohexylene dimethylene terephthalate)) in sampling sites C3 and T3 may well be an indication that this type of thermoplastic engineering material has been used in or released from anthropogenic activities released further up river and within the estuary. The treated waters released from the secondary treatment effluent from the Duncan and North Cowichan sewage treatment plant upstream of the estuary may also be a potential source of microplastics. As an example, Gies et al. (2018) estimated that municipal wastewater treatment plants (WWTP) in Vancouver released 30 billion microplastics (most of which were fibers made of plastic polymers), following conventional wastewater treatment into the receiving aquatic environment.

Identifying rates of exposure and ingestion of microplastics by higher trophic level species is emerging as a conservation priority in many areas. The Cowichan-Koksilah Estuary serves as a stopover for migratory shorebirds, which depend on estuarine intertidal mudflats to support the marine invertebrate and biofilm prey that provide energy and essential nutrients for long-distance migration (Schnurr et al. 2019, Schnurr et al., 2020). Worldwide, ~51% of *Calidris* sandpipers (the genus in which Dunlin and Western Sandpiper fall within) have been found to contain some form of plastics pollution (Flemming et al. unpublished). Foraging on mudflats and coastal environments may make them particularly susceptible to plastics ingestion (Flemming et al., unpublished). To date, while no studies have investigated consequences of plastics ingestion on this genus, lethal effects of ingesting larger plastics pieces than reported here have been demonstrated in Red Phalarope (*Phalaropus fulicarius*; Drever et al., 2018), a closely related shorebird species, and numerous other marine wildlife (Gall and Thompson, 2015).

In this context, the efficient conservation of migrating shorebirds and their habitats relies on the protection of important stopover sites (Senner et al., 2016). Biofilm tends to have higher abundances in the upper intertidal sections of estuarine mudflats (Schnurr et al., 2020), consistent with our findings of higher organic content in the mid- and high tide water marks (Figure 4).

Given the variable spatial distribution of plastics across the estuary (Figure 6), our findings suggest plastic deposition may not always occur in areas of high value for shorebird foraging, and will depend on the particular sources of pollution within estuaries. This has important implications for trophic transfer in foodwebs because if the exposure and distribution patterns of microplastic particles in abiotic and biotic compartments (partitioning coefficients, biota-sediment accumulation factor-BSAF) are known, then the application of bioaccumulation modeling can be used to predict how these particles are likely to be accumulated by biota. This study also represents a baseline for coastal sediments upon which future work can build upon. Thus, future studies should pursue field-based and foodweb modelling research to understand the bioaccumulation behavior of microplastic as a function of intake, net accumulation and elimination by shorebirds such as Western Sandpiper during migration at the Cowichan-Koksilah Estuary.

## Conclusion

Our findings complement the study of Collicutt et al. (2019) by using FTIR to identify the composition of microplastic polymers (Figure 5) and establishing the link between abundance of microplastics and depositional processes in intertidal sediments (Figure 3). Ultimately, the first preliminary application of EBK interpolation modelling to mapping and projecting the regional distribution of predicted concentrations for total abundance of microfragments and microfibers (Figure 6) in the Cowichan-Koksilah Estuary is developed here. Doing so, this work contributes with the development of new baseline reference data to determine current risk by microplastics in the Cowichan- Koksilah Estuary and provided a reference point to assess if proactive polices aimed at reducing plastics within our environment are actually working.

**CRediT authorship contribution statement**

**Juan José Alava:** Conceptualization; Laboratory methodology, Investigation, Writing – Original Draft, Data processing, Review & editing, Supervision; **Tamara Kazmiruk:** Fieldwork Designing, Laboratory methodology, and analysis, Writing; **Tristan Douglas:** Investigation, Fieldwork Designing, Methodology, Data processing, Geospatial Analysis, Writing. **Goetz Schuerholz**: Conceptualization, Project administration, Supervision, Fieldwork, Resources Writing. **Bill Heath:** Conceptualization, Fieldwork, Methodology, Resources, Writing – Review & Editing; **Scott Flemming:** Writing – Review & editing; **Leah Bendell:** Supervision, Laboratory space & equipment, – Review & editing; **Mark Drever:** Supervision, Formal analysis, Data processing and statistical analyses, Writing – Review & editing.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1.** Sampling station locations, coordinates, and sampling dates of sediment samples taken from the Cowichan/Koksilah Estuary, British Columbia.

| Station | Subsite | Longitude (W) | Latitude (N) | Sampling date |
|---|---|---|---|---|
| N2 | North | -123.6208300 | 48.7638900 | August 27, 2020 |
| N3 | North | -123.6208300 | 48.7624400 | August 27, 2020 |
| C1 | Central | -123.6313900 | 48.7608100 | August 27, 2020 |
| C2 | Central | -123.6262780 | 48.7586110 | August 27, 2020 |
| C3 | Central | -123.6232500 | 48.7577780 | August 27, 2020 |
| C4 | Central | -123.6333610 | 48.7587780 | October 21, 2020 |
| C5 | Central | -123.6283060 | 48.7568890 | October 21, 2020 |
| C6 | Central | -123.6263610 | 48.7553890 | October 21, 2020 |
| T1 | Terminal | -123.6338610 | 48.7528890 | August 28, 2020 |
| T3 | Terminal | -123.6267780 | 48.7512220 | August 28, 2020 |
| T5 | Terminal | -123.6323060 | 48.7498610 | August 28, 2020 |
| S1 | South | -123.6406110 | 48.7465000 | August 29, 2020 |
| S2 | South | -123.6347220 | 48.7444440 | August 29, 2020 |
| S3 | South | -123.6258610 | 48.7432500 | August 30, 2020 |

## Figures

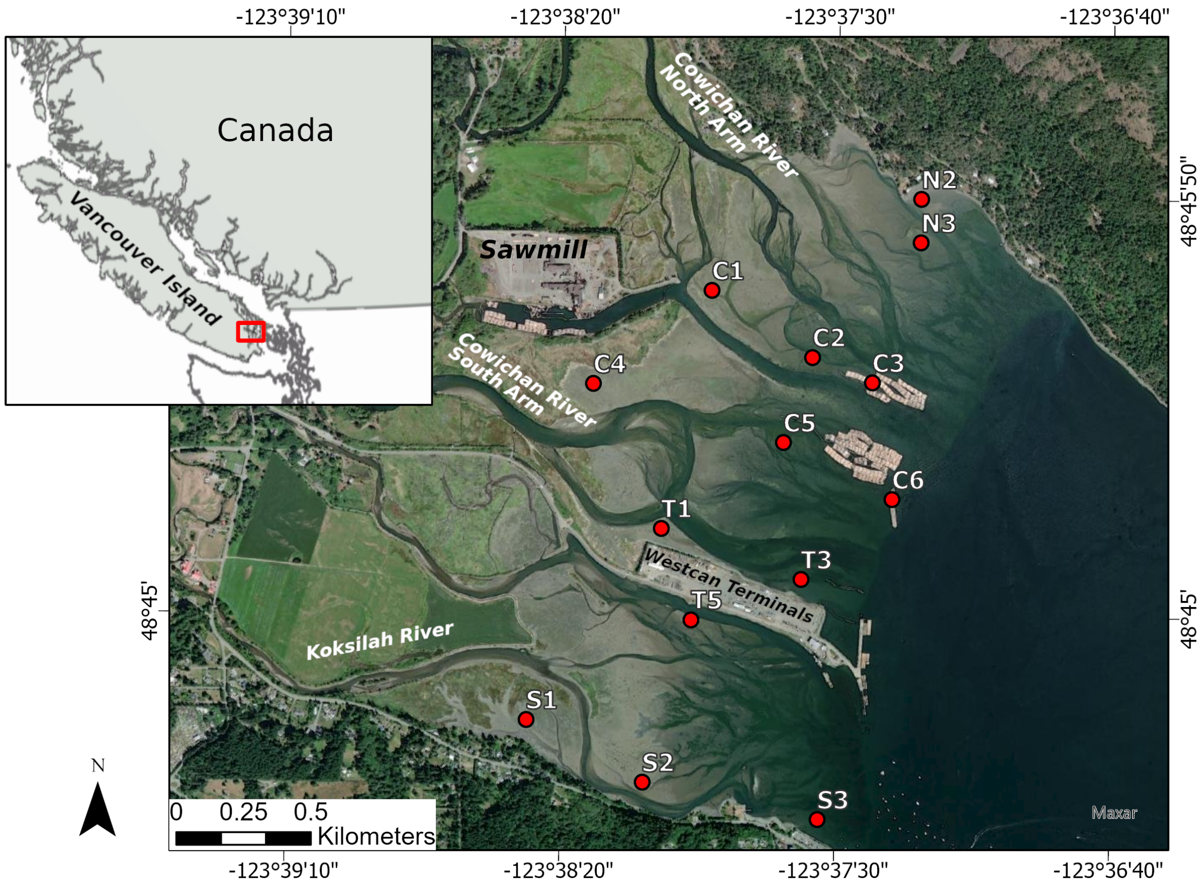



Figure 1. Map of the Cowichan-Koksilah Estuary, British Columbia, Canada. Alphanumeric codes indicate locations of sampling sites. The road to the forestry-shipping terminal (near the sites T1-T6) divides the estuary into north and south sections that have different hydrodynamic regimes. Sites are divided into 4 locations denoted by station codes (N = north, C = central, T = terminal, and S = south).

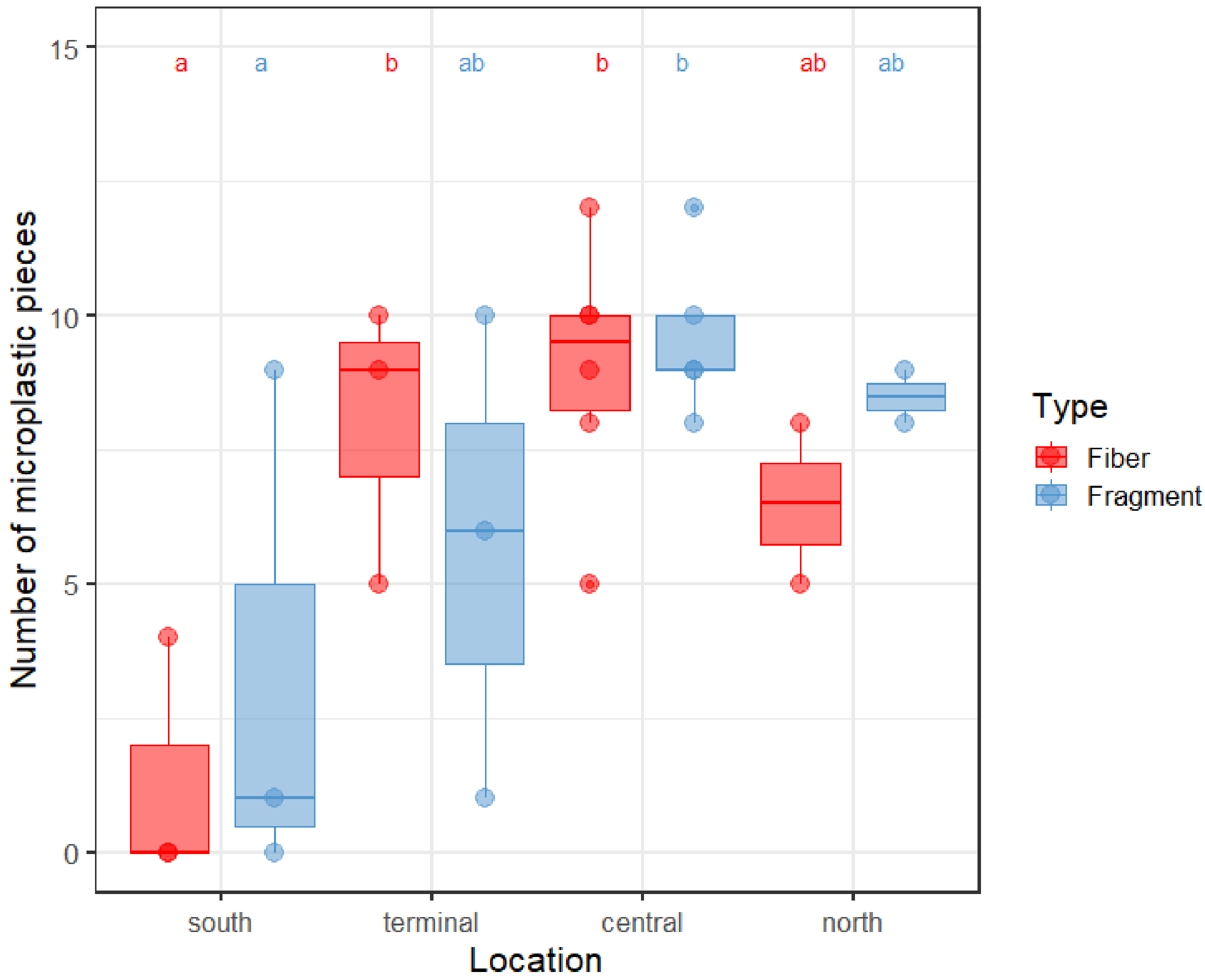



Figure 2. Abundance of microplastics at sampling locations, including south (S), terminal (T), central (C) and north (N) sites, in the Cowichan-Koksilah Estuary, British Columbia, August to October 2020. Box plots represent the distribution of observed values, where midline is the median, with the upper and lower limits of the box being 75th and 25th percentiles. Whiskers extend up to 1.5× the interquartile range, and outliers are depicted as points. Locations with different letters above the histogram have different means, according to Tukey multiple

comparisons test. Not shown is one data point (Location = central, Type = Fragment, value = 19) to avoid compression of axes.

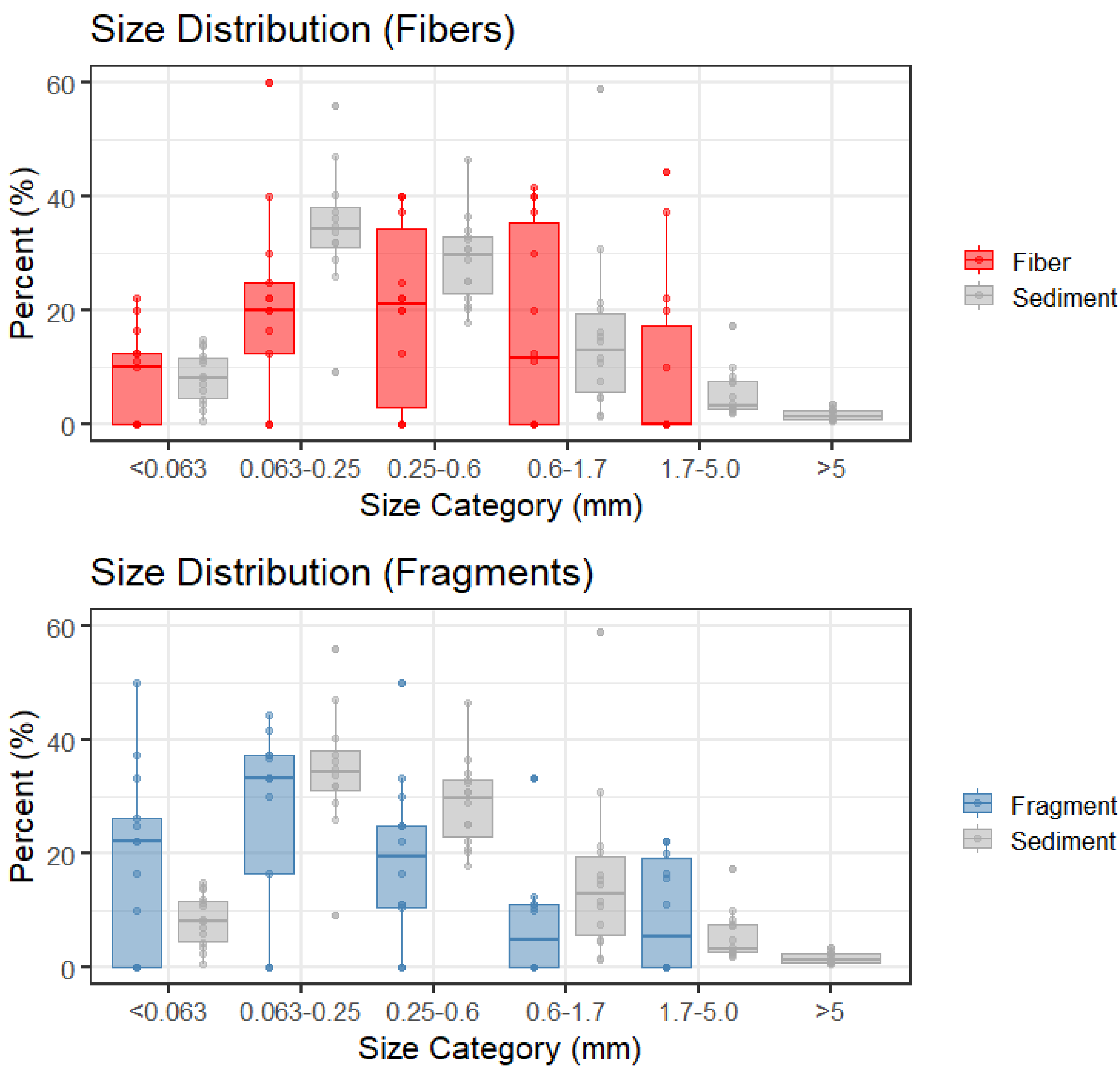



Figure 3. Size distribution of microplastic fibers, fragments, and sediment grains in samples from the Cowichan-Koksilah Estuary, British Columbia, August to October 2020. Box plots represent the distribution of observed values, where midline is the median, with the upper and lower limits of the box being 75th and 25th percentiles. Whiskers extend up to 1.5× the interquartile range, and outliers are depicted as points.

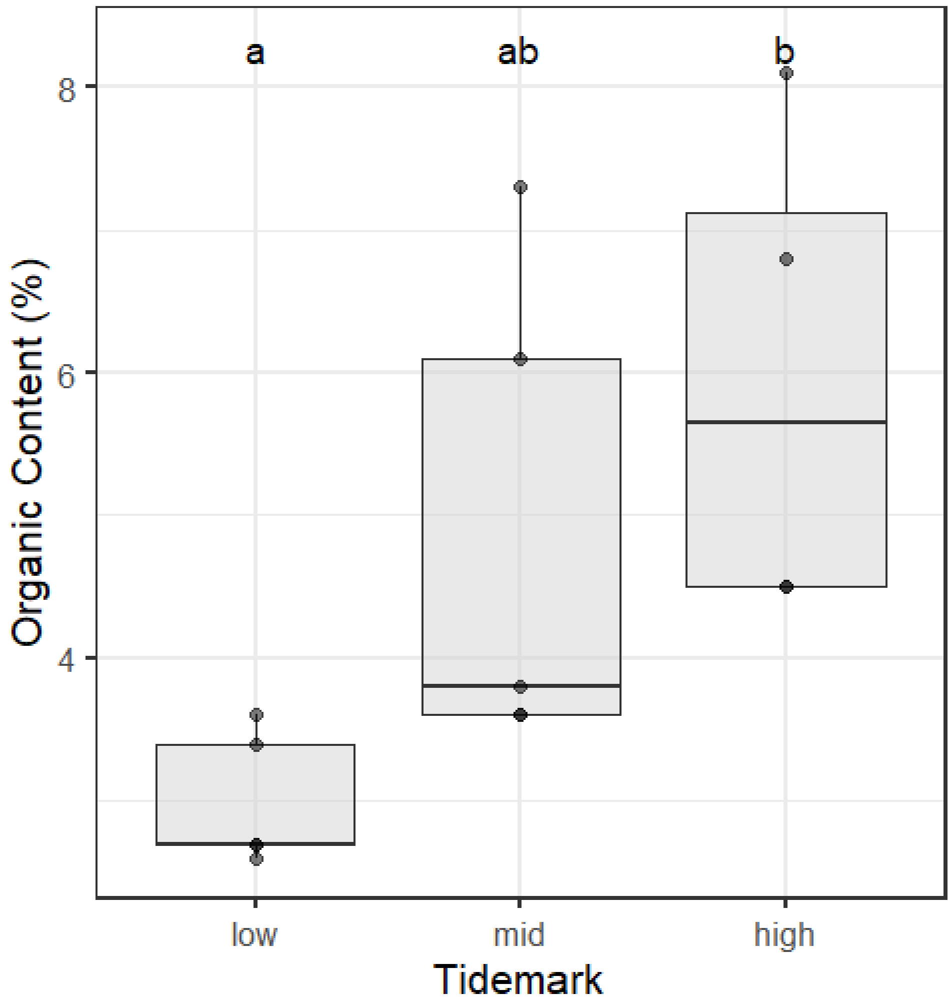



Figure 4. Organic content (percent) in sediment samples collected at three tidal regions of the Cowichan-Koksilah Estuary, British Columbia, August to October 2020. Box plots represent the distribution of observed values, where midline is the median, with the upper and lower limits of the box being 75th and 25th percentiles. Whiskers extend up to 1.5× the interquartile range, and outliers are depicted as points. Tidemarks with different letters above the histogram have different means, according to Tukey multiple comparisons test.

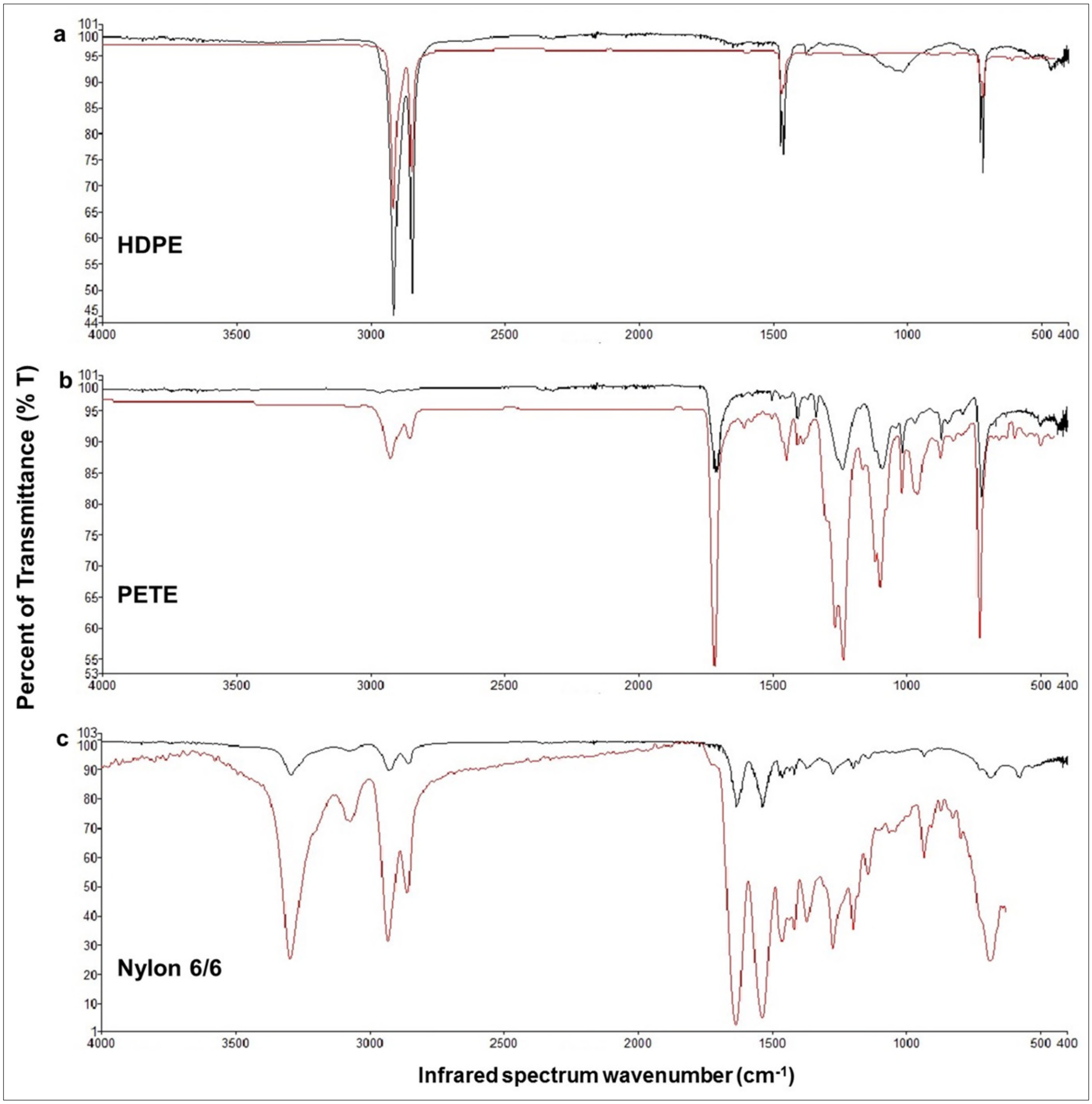



Figure 5. FTIR analysis results showing the infrared spectra represented by the frequency of infrared light transmitted (% T) versus the absorbance band, including the group bond [4000-1500 cm$^{-1}$] and fingerprint frequency [1500-600 cm$^{-1}$] regions, of selected microplastic samples as indicated by the mid-IR spectrum wavelength (i.e., 4000 to 400 cm$^{-1}$). The infrared spectrum for identified polymers is shown here for a) HDPE (sample site N1); b) PETE (samples sites C3 and T3); and c) Nylon 6/6 (sample site C1).

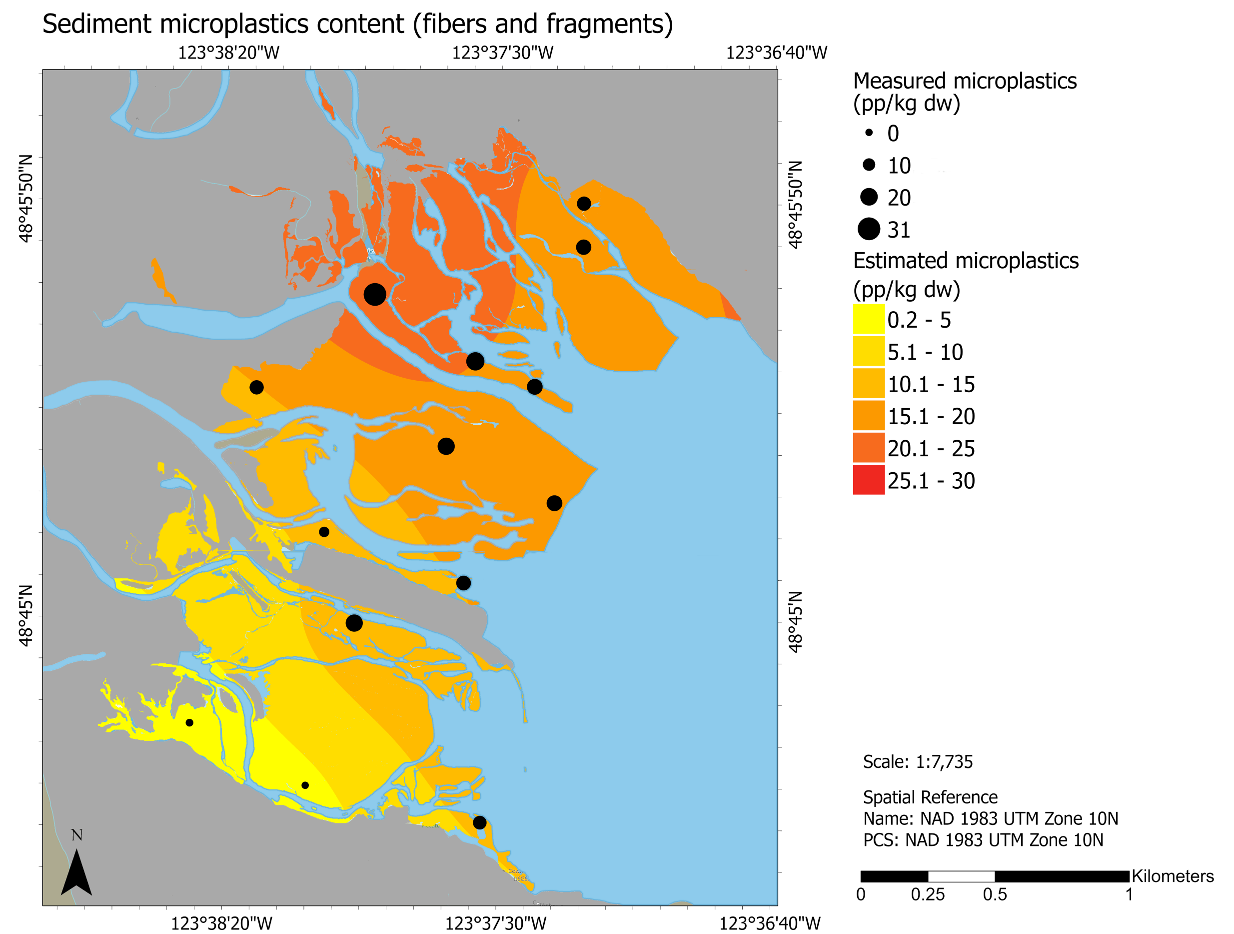


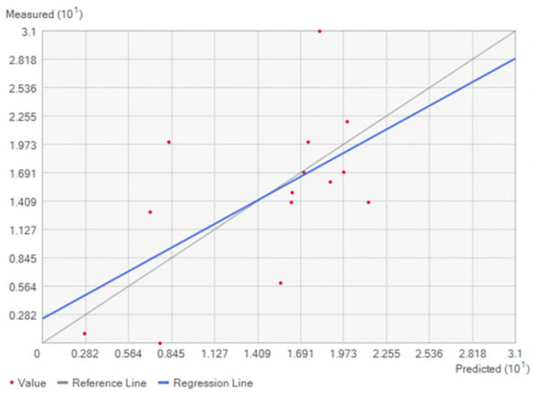

Figure 6. Empirical Bayesian Kriging interpolation modelling showing the regional distribution of predicted concentrations for total abundance of microplastic fragments and fibers in the Cowichan-Koksilah Estuary, British Columbia, August to October 2020, based on measured density or concentration of microplastics in units of plastic particle (pp)/kg dw on mudflat sediments.

**Supporting Information**

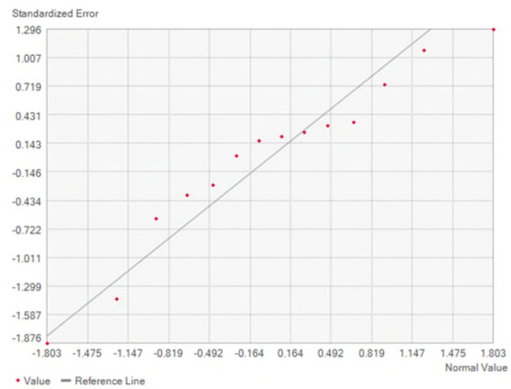
| **Description of Kriging cross validation statistics** | |
|---|---|
| Average CRPS | Should be as small as possible |
| Inside 90 Percent Interval | Should be close to 90 |
| Inside 95 Percent Interval | Should be close to 95 |
| Mean error | Should be close to zero |
| Root-Mean-Square | Should be close to Average Standard Error |
| Mean Standardized | Should be close to zero |
| Root-Mean-Square Standardized Error | Should be close to 1 |
| Average Standard Error | Should be close to Root-Mean-Square |

## Total Microplastics (fibers and fragments)

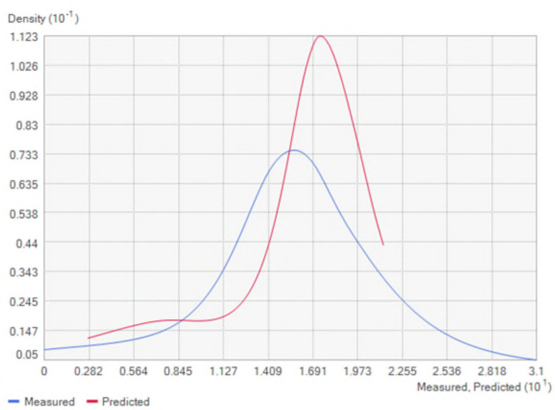
### Predicted




### Normal QQ plot




### Distribution




### Summary

| | |
|---|---|
| Count | 14 |
| Average CRPS | 3.63 |
| Inside 90 Percent Interval | 92.9 |
| Inside 95 Percent Interval | 100 |
| Mean | 0.026 |
| Root-Mean-Square | 6.42 |
| Mean Standardized | -0.0095 |

| | |
|---|---|
| Root-Mean-Square Standardized | 0.84 |
| Average Standard Error | 8.13 |