Microplastic distribution and composition in mudflat sediments and Varnish clams (*Nuttallia obscurata*) at two estuaries of British Columbia, Canada: an assessment of potential anthropogenic sources

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Abstract

Widespread microplastic contamination affects the marine-coastal ecosystems in British Columbia, Canada. To understand the characteristics and spatial distribution of of microplastics (MPs), we compared the MPs in sediments (n = 159) and Varnish clams (*Nuttallia obscurata*; n = 160) collected from two estuarine ecosystems (Cowichan and K'ómoks) experiencing different anthropogenic impacts; primarily resource extraction (i.e., logging) at Cowichan and urban development at K'omoks. Our objective wasto determine the MP abundance levels in sediments and clams and infer possible sources of MPs at the two estuaries. Microplastic polymer type was confirmed through FTIR spectrometry. The average abundance of MPs in sediments were 14.37 ± 11.57 particles/kg in the Cowichan Estuary and 30.96 ± 14.58 particles/kg in the K'ómoks Estuary. Varnish clam samples contained average abundance of 3.62±2.58 particles/g and 2.24±1.96 particles/g in Cowichan and K'ómoks estuaries, respectively. The Cowichan Estuary's marine terminal and K'ómoks Marina were found to be hotspots for MPs, likely due to a combination of industrial and local sources. Fibers were the most common type of MPs found in both sediment (53.34%) and clam samples (53.5%) from Cowichan, as well as in clam samples in% K’ómoks, indicating a potential link to textile sources contributing to the widespread presence of MPs in the marine environment. There was no clear signal based on the primary use of the estuary. Polyethylene was the predominant polymer type of MPs found in sediment and clam samples at Cowichan, whereas Polyester was most common at K’ómoks. Our study revealed the ubiquitous nature of these emerging pollutants in the sensitive estuarine environments of BC, with implications for plastic waste management and the reduction of plastic pollution at the regional level.

Keywords: Microplastics, Sediment, Varnish clam, K’ómoks, Cowichan, Fiber, British Columbia, Polyester, Polyethylene

Introduction

Over the past two decades, our understanding of plastic pollution in marine and estuarine ecosystems has grown significantly (Bendell et al., 2020). The mismanagement of plastic waste and other anthropogenic activities can introduce numerous chemical contaminants into aquatic ecosystems worldwide, both directly and indirectly (Alimba & Faggio, 2019); (Zoveidadianpour et al., 2023); (Lee et al., 2023); (Islam et al., 2024). These contaminants include macroplastics, microplastics, and nanoplastics (Hidalgo-Ruz et al., 2012), appearing across all levels of aquatic ecosystems and posing serious threats to aquatic life (Ma et al., 2020); (Bagheri et al., 2020). Discarded plastics can break down into smaller fragments, known as secondary microplastics, through physical, chemical, and biological processes. In estuarine environments like Cowichan and K'ómoks, potential sources of secondary microplastics include the breakdown of industrial debris, tire wear particles transported by urban runoff, and fibers released from sewage effluent. These secondary microplastics can accumulate in sediments and aquatic food webs, deteriorating ecosystems by affecting the health of marine organisms and disrupting ecological functions. For example, ingested microplastics can block digestive tracts, reduce feeding efficiency, and lower reproduction rates in aquatic species, while toxic chemicals adsorbed onto microplastics, such as PCBs and pesticides, can bioaccumulate and harm higher trophic levels. Additionally, the accumulation of microplastics in sediments can alter sediment properties, impacting benthic organisms that rely on these habitats.(Sajjad et al., 2022); (Zhang et al., 2023); (Alava et al., 2023); (Gull et al., 2025).

While estuaries are typically sheltered environments with low wave energy, localized wave action and tidal forces can still contribute to the fragmentation of plastics into smaller particles. For example, tidal currents and sediment abrasion in estuarine habitats can enhance the physical degradation of plastics over time. Synthetic fibers from sources such as textiles and industrial materials can also degrade into microfibers (<5 mm in size), which are a significant contributor to microplastic pollution in estuarine sediments (Nunez et al., 2021); (Zhang, 2017); (Pinheiro et al., 2021);(Malli et al., 2022). . As our understanding of the negative impacts of microplastics on benthic and pelagic ocean environments, marine-coastal ecosystems, and aquatic continues to grow, the need to establish spatial comparisons and identify sources of microplastics in coastal areas with distinct hydrological processes has become increasingly important. Such efforts are crucial for evaluating the risks microplastics pose to local marine and coastal wildlife (Browne et al., 2011); (Lebreton et al., 2017); (Lyu et al., 2024), and aquatic species (López-Martínez et al., 2021); (Kühn et al., 2015); (Kühn & Van Franeker, 2020) (Browne, 2015); (Meijer et al., 2021); (Bendell et al., 2020); (Kane et al., 2020); (Lebreton et al., 2018); (Alava et al., 2021).

An estimated 4.9 billion tons of plastics, accounting for ~80% of all plastics produced up to 2015, were disposed of in landfills or the natural environment (Gavigan et al., 2020); (Lam et al., 2023). Once in the oceans, plastics can float and spread via marine currents, or they can sink and accumulate in coastal marine and seabed sediments, gradually breaking down into smaller fragments through physical, chemical, and biological mechanisms (Auta et al., 2017); (Meyers et al., 2024). Consequently, there is a chronic accumulation of plastics in marine ecosystems, raising concerns about their potential risks to both ecosystems and human health (Lam et al., 2023). Most microplastics originate from terrestrial sources such as household waste, industrial waste, and wastewater, or from marine activities, including fishing, aquaculture and shipping that also contribute to their release (Moore, 2008); (Alencastro, 2012); (Browne, 2007); (Duis & Coors, 2016); (Alava et al., 2023); (

In British Columbia, Canada, microplastics have emerged as a concerning contaminant in the marine environment and sensitive ecosystems (Bendell, 2019); (Alava, 2019). Studies in BC have revealed significant concentrations of microplastics on ocean waters, with particularly high levels along the coast (Desforges et al., 2014); (Mahara et al., 2022) .Zooplankton species can ingest microplastic particles, and adult salmon in coastal BC may consume a substantial amount of microplastics daily. Effects and bioaccumulation potential of microplastics when consumed by marine organisms are not thoroughly understood (Alava et al., 2020). This lack of understanding is particularly concerning for shellfish species, Pacific herring (Clupea pallasii), and Pacific salmon (Oncorhynchus spp.), which are exposed to microplastic contamination and play essential roles in regional food webs as well as commercial and First Nations' traditional seafood harvests (Mahara et al., 2022). Recent research on BC coastal food webs suggests that species-specific interactions, rather than trophic position, are more significant for microplastic bioaccumulation (Covernton et al., 2022).

Bivalves such as varnish clams (Nuttallia obscurata) are valuable for studying microplastic pollution in marine ecosystems due to their role in bioaccumulating contaminants, which poses potential health risks for humans who consume these clams. Varnish clams are typically found at depths of up to 30 cm in sediments (Bendell et al., 2020). Unlike epifaunal bivalves like oysters and mussels, which respond to increased levels of phytoplankton and detritus in the water by increasing their filtration capacity and producing pseudofaeces, varnish clams adjust their clearance rates (Frias et al., 2014). Additionally, unlike oysters and mussels, which are highly selective in filtering particles from the water, varnish clams are deposit feeders that consume various food sources, including phytoplankton, benthic diatoms, organic matter from surface sediments, seagrasses, and seaweeds, and thus provide a good representative sample of the overall environment (Bendell eta al, 2019). Substantial evidence indicates that the gills and gut of bivalves can capture and accumulate microplastics. (Corami et al., 2020(Moreschi, 2020 #115). Moreover, certain types of bivalves, particularly non-selective feeders like Varnish clams exhibit widespread distribution, accessibility, sedentary behavior, and high tolerance to environmental stressors, making them ideal candidates for assessing the microplastic pollution levels in their habitats by quantifying microplastic content within their bodies (Ding et al., 2021). Consequently, an increasing number of studies have employed bivalves to monitor microplastic pollution at regional (Ding et al., 2021); (Dowarah et al., 2020); (Bendell et al., 2020) and national scales (Cho et al., 2024). Importantly, bivalves serve as a significant source of human nutrition The consumption of microplastic-contaminated whole bivalves, without removing their viscera and gills, constitutes the primary route for human exposure to microplastics (Ding et al., 2021), with potentially higher exposure levels compared to consuming seafood that lacks a digestive tract, such as gutted finfish (Lam et al., 2018).(Ding et al., 2021), (Lam et al., 2018). The presence of microplastics has been found in both market-sold (Jang et al., 2020); (Zhou et al., 2021) and wild-caught bivalves (Cho et al., 2021); (Jeyasanta et al., 2020), yet their health effects of contaminated bivalve consumption remains limited due to the nascent nature of this research field (Barboza et al., 2018). Despite this, understanding prevalence of microplastics in edible bivalves across different geographical regions, as well as their physical characteristics and polymeric composition, represents a crucial step toward a comprehensive assessment of human health risks associated with microplastics (Rokomatu et al., 2021); (Jankauskas et al., 2024).

This research had three primary objectives. Firstly, it aimed to employe a unique twinned approach to compare spatial differences and infer the sources and behavior of microplastics within ecologically and biologically sensitive ecosystems and the intertidal mudflats of the Cowichan-Koksilah and K'ómoks estuaries on the coast of Vancouver Island, British Columbia, Canada. Secondly, it sought to examine the distribution of microplastics found in varnish clam samples collected from both estuaries. Lastly, we compared the results of this study with those obtained during an initial phase study on the Cowichan estuary (Alava et al., 2021) to assess changes in distribution or trends over timeBy integrating spatial, temporal, and source analyses, this study provides a comprehensive understanding of microplastic contamination in biologically and ecologically critical estuaries, addressing knowledge gaps in coastal ecosystems.

2. Materials and Methods

2.1. Study Area

British Columbia's estuaries serve as vital ecosystems, playing crucial roles in supporting diverse marine life and providing essential habitats for a wide variety of wildlife. These estuaries face threats such as habitat degradation due to urbanization, pollution from industrial and agricultural activities (Ross & Morales-Caselles, 2015); (Alava et al., 2021); (Bendell, 2019), and impacts from climate change, including sea level rise and changes in water temperatures (Okey et al., 2015).

The Cowichan-Koksilah Estuary is situated on the east coast of Vancouver Island between Victoria and Nanaimo, and is formed by the Cowichan and Koksilah rivers draining into the Strait of Georgia. The Koksilah River originates from Waterloo Mountain, south of the Cowichan Valley, while the Cowichan River flows from Cowichan Lake, approximately 40 km upstream from the estuary. Together, these rivers, with a combined watershed of nearly 8000 km², are the primary freshwater tributaries to the estuary and Cowichan Bay, making this estuary the eighth largest in British Columbia. The Cowichan Estuary forms part of the Salish People's territory, particularly the Cowichan Tribes. It has sustained the largest indigenous community on Vancouver Island for centuries, offering abundant resources like shellfish, salmon, herring roe, some commercially and ecologically significant species such as Pacific salmon (Oncorhynchus spp.), Dungeness crab (Metacarcinus magister), and varnish clams (Nuttallia obscurata) and seaweed (Schuerholz et al., 2006). The Village of Cowichan Bay, located near the head of the bay, supports a population of approximately 2,394 people. Industrial operations within the estuary include a metal manufacturing plant, a sawmill, and a marine shipping terminal in the center of the estuary, which may contribute to various forms of anthropogenic pollution (Alava et al., 2021).These industrial activities include untreated storm-water run-off draining into the estuary from the marine terminal, effluent discharge into the mill pond from a forestry mill, effluent from local sewage treatment plans, and nutrient loading from agricultural runoff (Schuerholz et al., 2006); (Alava et al., 2021).

The K’ómoks Estuary (formerly the Courtenay River Estuary) is situated seaward of the confluence of the Puntledge and Tsolum Rivers, is also important for bird fish species. Human activities like shoreline development and pollution pose significant challenges to the health and sustainability of these estuarine ecosystems (Walters et al., 2019). Both estuaries heavily rely on the health of the rivers that feed into them, with the Cowichan River providing vital freshwater input to the Cowichan Estuary, and the Courtenay River serving as the primary sources of freshwater inflow for the K’ómoks Estuary (Mackas et al., 2001).

The K’ómoks Estuary is the second most important estuary in British Columbia (second to the Fraser River Estuary), and provides habitat for 145 bird species (more than 70,000 birds), 218 plant species, 29 fish species (including all five species of Pacific salmon), and innumerable species of intertidal animals (e.g., clams, worms, bacteria, viruses). The K’ómoks Estuary has been severely impacted by industrial and urbanization activities, the building of hospitals and assisted living centers as well as resorts along the sides of the estuary.

2.2. Sample Collection

Comprehensive sampling was conducted at the Cowichan and K’ómoks estuaries to assess microplastics and sediment properties using a series of bulk sediment samples collected during low tide in July 2023. A targeted sampling approach was employed, consisting of 156 sediment samples collected from 26 sites for Cowichan and 81 sediment samples from 9 sites for K’ómoks.

For the Cowichan estuary, sampling sites were strategically selected to encompass the spatial distribution of microplastic deposition on both sides of the Westcan Terminal causeway that stretches across the intertidal saltmarsh and mudflats to the de-commissioned Government dock. This causeway effectively separates the estuary into two distinct sections, allowing for comprehensive examination of microplastic distribution across the area. Four samples (S1–S4) were obtained from the south side of the causeway, where the primary source of freshwater is the Koksilah River. On the north side of the causeway, which is fed by both the north and south forks of the Cowichan River, ten samples (N1–N4, C1–C6) were collected. Additionally, three samples (T1, T3, T5) were taken from the the Westcan Terminal sample sites three above the sewage outfall of the Cowichan River (CO1–CO3), two from the north fork (CO4-A, CO4-B), two from the south fork (CO5, CO6), and two from the Koksilah River (K1-A, K1-B).

Sample sites for K’ómoks included the Trent River (TR), K’ómoks Marina (COM), and Goose Spit Park (Zhu et al.). Sites were chosen to represent the north and south sides of the estuary (Fig. 1), At each sample site, 3 replicates (two sediment bags were taken from each replicate) of sediment were collected from a 25 x 25 cm square quadrant to a depth of 5 cm resulting in a total of six bags per station. Sediment samples were carefully packed into labeled, sealed bioplastic bags and stored at -20°C in the laboratory until further analysis.

Varnish clams (*Nuttallia obscurata*) were collected by shoveling at 5–10 cm depth within the sediment. Upon collection, all bivalves were securely fastened with a thick rubber band around their shells to prevent any loss of particle contents. The clams were covered with aluminum foil, and put in brand-new, unopened, polyethylene Ziploc® bags labeled according to their respective collection sites for convenient transportation. These bags were then placed in a cooler with ice packs to maintain a temperature of -20°C during transportation to the Ecotoxicology Lab at Simon Fraser University. The clams remained stored in the freezer until they were ready for analysis. The collection of bivalves was conducted under Scientific Licenses obtained from the Department of Fisheries and Oceans, Canada, ensuring compliance with regulations and ethical standards to minimize contamination from previous handling, all tools used during collection were initially rinsed with seawater at the collection site. However, we recognize that seawater may contain microplastics, so tools were subsequently rinsed with distilled water to further reduce the risk of contamination Moreover, the color of any synthetic clothing worn during collection was recorded to account for potential sources of contamination during handling and transportation.

2.3. Laboratory Analyses of Sediments and Clams

2.3.1. Extraction and Analysis of Microplastics in Sediment Samples

Once in the laboratory, sediment samples underwent sieving to isolate particles smaller than 5 mm using CE Tyler Canadian Standard 4.75 mm sieves.Cross-contamination between samples was minimized by rinsing the sieve with filtered de-ionized water and drying it with a paper towel between each use. Sieving was conducted in a controlled laboratory environment to reduce airborne contamination. The sieved sediment was then subdivided into sub-samples for each study site, homogenized, and weighed while wet. Subsequently, the trays containing the wet sediment sub-samples were placed into a Precision® Gravity Convection Incubator set at 60°C for 48 hours or until completely dried. The lower temperature of 60°C was chosen to avoid potential melting of microplastics in the samples, as opposed to the 110°C recommended by NOAA’s Marine Debris Program. Once dried, the sub-samples were weighed to obtain their dry weights and then set aside for particle extraction.

Following the general protocol outlined by (Maes et al., 2017), a stock solution of zinc chloride ($ZnCl_2$) was prepared gravimetrically at room temperature. This solution, with a density of 1.38 g/mL, was used to suspend the sediment samples along with a stock solution of Nile Red dye to facilitate particle tagging.

Approximately 20 g of dry sediment was suspended in 30 mL of the ZnCl2 stock solution and 2 mL of the Nile Red stock solution within a 50 mL centrifuge tube. The samples were then shaken for 30 minutes in a Precision® Scientific Dubnoff Metabolic Shaking Incubator at 100 rpm. This suspension and staining process was repeated for all sediment sub-samples.

To extract particles from the sediment samples, the procedure outlined by Maes ,2017 (Maes et al., 2017) was followed with minor modifications. The sediment mixture in each centrifuge tube was centrifuged at 3900 g for 8 minutes in a Beckman Allegra 64R centrifuge with a braking speed of 9. The supernatant containing the stained particles was collected using a glass Pasteur pipette and decanted through a Whatman™ 1001-090 Grade 1, Pore Size, 9 μm qualitative cellulose nitrate filter paper under vacuum filtration. This process was repeated for each sub-sample. The dried filter papers containing the collected particles were placed into labeled petri dishes and sealed with Parafilm® M all-purpose laboratory tape until visual inspection could be conducted

2.3.2. Extraction and Analysis of Microplastics in Varnish Clam Samples

In the laboratory, biometric measurements were taken of each bivalve, including total weight, internal tissue weight, and shell dimensions (i.e., length, height, width). To preserve their internal tissues until further analysis, the bivalves were stored frozen at -4°C. The tissues were carefully removed from the clams' shells using sterilized stainless-steel scoopulas and forceps, and then immediately placed into separate 100 mL glass Erlenmeyer flasks. To minimize the risk of cross-contamination, the dissection instruments were thoroughly cleaned with 70% ethanol and wiped with a clean paper towel between each bivalve tissue removal.

Since ouraim was to quantify and identify microplastic particles per individual bivalve, no pooling of the samples was conducted. Tissue digestion was performed by incubating the removed tissue samples in freshly prepared 10% potassium hydroxide (KOH) solution (4 times the tissue volume) for 48 hours at60°C within a Precision® Gravity Convection Incubator. At the 24-hour mark, Rit Dye More® was added to the digestion mixture to stain any synthetic polymer particles to a periwinkle color for easier identification (Gao et al. (2022). After the incubation period, each sample was vacuum filtered through Whatman™ 1001-090 Grade 1, Pore Size, 9 μm qualitative cellulose nitrate filter paper. The filter paper was rinsed with 30% hydrogen peroxide (H_2O_2) to remove any biofilm or fat membrane remnants that could interfere with particle identification. Any remaining particles within the flask were rinsed with de-ionized water and transferred onto the filter paper using a rubber policeman. Finally, the dried filter papers containing the particles were placed into labeled petri dishes and sealed with Parafilm® M all-purpose laboratory tape until visual inspection could be conducted

2.4. Microplastics Identification and FTIR Analysis

Visual identification and photography of each filter paper were conducted using a Cole-Parmer Cordless Stereo Microscope connected to Motic image software. Visual identification was used to distinguish plastics from non-plastics, with studies showing that this method can achieve an accuracy of over 90% (Ory et al., 2017).To minimize the risk of contamination, the lids of the petri dishes were not removed during this process. For each suspected microplastic particle (SMP) observed, characteristics such as length, width (in millimeters), color, shape, and texture were recorded. Each SMP was photographed to document these features, aiding in the identification of synthetic particles. Subsequently, each suspected particle was carefully transferred using tweezers to a glass slide coated with 2% dextrose for adhesion in preparation for Fourier-Transform Infrared (FTIR) analysis.

Polymeric material types of SMPs were identified using a Fourier-Transform Infrared Spectrometer (Corami et al., 2020) with an attenuated total reflectance accessory (Perkin Elmer Inc. Spectrum FTIR). The polymer type associated with examined particle was determined by comparing the sample spectra with a spectral library of reference polymers. Particles with a match score of 0.7 or higher out of 1 were confirmed to be polymeric, while those failing to meet the threshold or originating from natural sources (e.g., cellulose particles) were dismissed and excluded from subsequent analyses. This rigorous analysis process ensured the accurate identification and characterization of microplastic particles in the samples.

2.5. Quality Assurance and Quality Control

Field blanks were employed to account for potential background or external contamination originating either from the nearby atmosphere or the shedding of fibers from attire and field gear. At each sample site, a clean Petridish prepared with Vaseline was placed beside the mudflat sediment collection location and opened any time sample were begin collected. Each batch of sediment or clams undergoing analysis necessitated the collection of one background blank. This procedure involved the placement of a 20 μm filter paper in a cleaned petri dish. The filter paper was subsequently moistened with filtered water and positioned within 30 cm of the sample whenever it was exposed to air. For the lab controls, all the procedures were conducted in a designated clean room, using a laminar flow hood. The clean room attire was limited to clothing made entirely of 100% cotton to prevent synthetic fiber contamination of the samples. Each batch of field samples was accompanied by three lab procedural blanks. For each blank, 100 mL of Milli-Q water was filtered through a 10 μm, 47 mm polycarbonate filter and stored in a clean Petri Slide dish. Final microplastic abundance were adjusted by excluding any microplastics found in the same size, color, and type categories present in the procedural blanks.

2.6. Statistical Analyses

Statistical analysis was conducted in the R statistical environment (R version 4.3.3). Results for sediment were expressed in MPs kg^{-1} dry weight sediment and, for clams, MPs/g wet weight (ww). A two-way ANOVA was calculated for MP types (i.e., fiber, fragment, pellet, and film) recovered in samples of sediments and clam samples, with a pairwise t-test as post hoc analysis to identify samples that differed from each other where the ANOVA was significant. A one-way ANOVA was performed to assess differences in the number of microplastic items among the studied sites/stations within Cowichan followed by a Tukey post-hoc test to identify specific differences between stations. . This analysis evaluated whether microplastic abundance varied significantly across locations. Average counts of the three sampling events(for both clam and sediment samples) at each station were treated as replicates for statistical analyses. Shapiro-Wilk tests and Levene's tests were used to test for normality and homogeneity of variance, respectively. Moreover, a two-tailed Spearman's rank correlation test was performed to detect the relationships between the physical characteristics of the clams and the mean abundance or size of the microplastics. Statistical significance was accepted at the p-level of <0.05. As data were normally distributed (Normality Test; Shapiro-Wilk; $P > 0.05$), no transformations were required. A one-way ANOVA was applied to determine differences in weight, length, and height of clams collected from the two regions. In addition, the health of Varnish clams was estimated using the condition index (CI) , which compares the dry weight of the clam's soft tissue to its shell weight or total wet weight. A higher condition index value typically indicates better overall health and nutritional status of the clams

3. Results

The sediment samples revealed substantial microplastic contamination in both study sites, with higher counts observed in Cowichan compared to K'ómoks. In Cowichan ($n = 78$), 1,121 suspected plastic particles were identified, of which 74.4% (835 particles) were confirmed as microplastics. In contrast, K’ómoks (n = 27) had a total of 836 suspected plastic particles, with 52.08% (483 particles) confirmed as microplastics. Similarly, varnish clams from both regions showed notable levels of microplastic contamination. Clams from Cowichan (n = 57) contained a total of 112 suspected particles, with 67.40% (75 particles) confirmed as microplastics. Clams from K’ómoks (n = 103) had a total of 231 suspected particles, with 60.17% (139 particles) confirmed as microplastics.

Additionally, the condition index values indicated a potential difference in the overall health of varnish clams between the two locations. Clams from K’ómoks exhibited a higher average condition index (38.79 g/cm^3) compared to those from Cowichan (29.10 g/cm^3), suggesting that K’ómoks clams may be in better health (Table 2).

3.1. Microplastic abundance in sediments

Microplastics were identified in all sediment samples across both estuaries. K’ómoks Estuary had approximately twice the concentration of microplastics in sediment compared to Cowichan, with an overall average of 30.96 ± 14.58 particles/kg (range: 8 to 55 particles/kg) in K’ómoks versus 14.37 ± 11.57 particles/kg (range: 2 to 153 particles/kg) in Cowichan.The characteristics of MPs, including quantity, size, and color in the sediments of each estuary, are listed in Figure 2.

Within Cowichan locations, the concentration of MP in sediments was higher near the terminal (22.11 ± 16.99 particles particles/kg) and at central (20.17± 13.52 particles/kg) sites, and the lowest values came from site K1.A (7 ±2.898 particles particles/kg) in Koksilah River, although this difference was not statistically significant. . According to one-way ANOVA analysis, no significant difference was observed in the number of MPs items among studied areas in Cowichan samples ($p= 0.2068$). Tukey test showed a significantly higher MPs items in stations T3 and compared to the other stations ($p < 0.05$). Within K’ómoks, the abundance of MPs varied widely among sampling locations ($p < 0.05$). Average concentrations of MPs in the surface sediments were in descending order: CM > GP > TR. K’ómoks Marina had the highest MP concentration in this area, with 44 ± 9.65 particles/kg.The locations characterized by the highest population density, touristy spots, and therefore, with the greatest anthropogenic influences, exhibited the highest concentrations of MPs.

3.2 Microplastic shape, size and color

A variety of shapes of MPs were observed in the sediments at Cowichan and K'ómoks, including fiber, fragments, pellet, and film (Fig. 2B). In the Cowichan Estuary, fibers were the most common MPs (53.34%), followed by fragments, and pellets and films were the least abundant. Fibers were detected in all stations with the dominant proportion in the central part 30.26%), followed by the terminal site, i.e., 20.90% (Fig. 3b). For MP sizes, the particles were within range classes of 1-5 mm, 0.5-1 mm, 0.1-0.5 mm, and < 0.1 mm (Fig. 2c). The dominant particle size within stations was in the range of 0.1-0.5 mm (45.58%). The characterization of the plastic particle color at all sampling stations, showed that the majority of MPs were black, transparent (clear), and blue, followed by red, brown, and white (Fig.2C).

Conversely, MPs detected from K'ómoks exhibited different shapes, sizes, and colors. The highest proportion was represented by fragments 53.70% (16.6±13.1), followed by fibers 41.50%, films 3.70%, and pellets 1.07%. Fibers and fragments were found at all examined locations, and films and pellets were identified at all K'ómoks sampling locations except for Trent River where film was not detected. There were clear differences in the mean abundance of MP color, in the following dominant order: clear (13.78) > black (7.6) > blue (5.7) > red (1.85) > brown (1) > white (0.5). Similar to Cowichan, microplastics in the size category of 0.1–0.5 mm (56.22%) was most abundant in the K'ómoks sediment samples at all sampling locations, followed by MP sizes of 1.0–5.0 mm (mean ±SD: mean ±SD: 17.4±10.2), 0.5–1.0 mm (mean ±SD: 8.4 ±4.5), 1-5 (mean ±SD: 4.2 ±3), and < 0.1 mm (mean ±SD: 0.8±1).

3.3. Polymer Identification

FTIR identification positively identified 67.61% and 49.88% of the analyzed MPs of sediment as plastic for Cowichan and K'ómoks, respectively. For Cowichan, Polyethylene (PE, 23.82%), Polyethylene terephthalate (PET, 19.30%), Polypropylene (PP, 15%), Polyester (PES, 6.25%), and Polyamide (PA, 3.30%) were the most numerous polymers. As for K'ómoks, Polyester (PES, 14.47%), Polypropylene (PP, 13.39%), Polyethylene terephthalate (PET, 12.4%), Polyethylene (PE, 6.9%), and Polyamide (PA, 2.6%) were identified (Fig. 2E).

3.2. Microplastic abundance in Varnish clams

Microplastics of varying shapes and sizes were found in the Varnish Clams from Cowichan and K'ómoks with an overall average concentration of 3.62±2.58 and 2.24 ±1.96 particles/g, respectively. Fibers were the most abundant category, accounting for 53.5% and 57.57% of the total count, followed by fragments (38% and 36.79%), pellets (1% and 2.1%), and films (7.5% and 3.4%) for Cowichan and K'ómoks, respectively (Fig. 2B). Overall, microplastics in the size class of 0.1–0.5 mm was dominant in clams from K'ómoks (39.82%), while microplastics in the size class of 0.5 -1 mm were abundant in Cowichan (37%). Particle sizes of 1 to 5.0 mm in K'ómoks and of 0.1 to 0.5 mm in Cowichan were the least common. A particle size <0.1 was detected less than other sizes in Varnish clam across stations for both estuaries. Microplastics had the same color composition in the clams collected, with the highest percentage of black MPs in clams from K'ómoks (53.6%) and Cowichan (36%) (Fig. 2C). Among all suspected microplastics identified in clams, 67.40% in Cowichan and 60.17% in K'ómoks were confirmed as plastics. Across all samples, this corresponds to an average confirmation rate of 90.6%, indicating a high accuracy in visual identification methods. Polyester (PES, 24.23%) and Polyethylene (PE, 20.77%) were the most dominant plastic types in K'ómoks Varnish. The varnish clams collected from K'ómoks Marina and site 216 were notably larger than those harvested from other locations in K'ómoks and Cowichan (Table 1). The abundances polymer type in Varnish in Cowichan estuary were Polyethylene (PE, 34.25%), Polyethylene terephthalate (PET, 28.72%), followed by Polyester (PES, 22.65%), as shown in Fig2E.

There were no significant correlations between MP counts in K'ómoks ($p = 0.7124$, $r = -0.07437$) and Cowichan ($p = 0.3570$, $r = 0.1304$) clam and sediments. This indicates that sites with high microplastic abundance in sediments did not necessarily have high microplastic abundance in clams. Additionally there were no significant differences in microplastic abundances among the sites where Varnish clams were collected in Cowichan and K'ómoks, respectively (p= 0.1092, p= 0.4766).

3.3. Temporal Dynamics of Microplastic Pollution in the Cowichan Estuary

When comparing to a previous assessment conducted in August 2020 (Alava et al., 2021), this study, conducted in July 2023, revealed various advancements and alterations in site selections, microplastic detection, and polymer composition identification. Alava et al. (2021) deployed sediment sampling spanning from August to October 2020, involving the collection of 42 sediment samples during low tide from various stations across the Cowichan Estuary. These stations encompassed regions categorized as North (N2, N3), Central (C1-C6), Terminal (T1, T3, T5), and South (S1-S3) (see map in Figure 1 from Alava et al. 2021). In contrast, this study witnessed an expansion in sampling points, with 78 sediment samples collected from three sets of 26 locations each. This expansion included an increase in North and South stations to four stations, alongside the incorporation of two new subsites: CO, obtained from Above Sewage Outfall (CO1, CO2, CO3), Cowichan River South (CO5, CO6), and North Forks (CO4-A, CO4-B). Microplastic analysis revealed an obvious shift between the two study phases regarding the dominant shape of microplastics.

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4. Discussion

The objective of this research was to examine the prevalence, spatial distribution, and diversity of microplastics within the sediments of the Cowichan and K'ómoks estuaries, as well as to assess microplastic content within clams to understand their presence in biota. The expansion of agriculture, forestry, dredging, waste management, and urban and industrial development in these estuarine regions has led to increased environmental degradation over recent years. Consequently, various forms of anthropogenic pollution such as industrial discharge, agricultural runoff, plastic waste, untreated sewage, and chemical contaminants have adversely affected these estuaries, contributing to the current state of environmental deterioration (Alava eta al, 2021). Given the widespread presence of MPs in the environment and the potential concerns regarding their impact on environmental health, it is imperative to document baseline levels in areas with unique hydrological processes to evaluate risks to local wildlife and their role in supporting human livelihoods and food resources. Since estuaries are highly susceptible to contamination, assessing microplastic levels in these environments can provide insights into local plastic waste accumulation and distribution patterns, helping to identify contamination hotspots (Browne et al., 2011); (Li et al., 2018). This approach may inform future studies on environmental health trends in these areas.

Compared to the initial study conducted by Alava et al. (2021) in the Cowichan estuary, the expansion of sampling stations from 14 to 26 in the current study has significant implications for the assessment of MP contamination. By increasing the spatial coverage of sampling points, our study captured a more comprehensive representation of microplastic distribution within the Cowichan Estuary. This broader sampling coverage likely increased the likelihood of encountering microplastic particles, leading to a higher observed abundance in the current phase. Additionally, the inclusion of new subsites such as CO1, CO2, CO3, CO5, CO6, CO4-A, and CO4-B provided insights into previously unexplored areas, potentially revealing additional sources of microplastic pollution such as urban runoff, wastewater discharge, recreational boating, and nearby industrial activities.

While Alava et al. (2021) predominantly identified fragments, our data demonstrated a dominance of fiber shapes, particularly evident in the Central part of the estuary (C3). Additionally, the size distribution of microplastics differed between the two collection periods. While Alava et al. (2021) reported microplastics primarily within the size range of 0.063-0.25 mm, we found microplastics predominantly in the size range of 0.1-0.5 mm. Polymer composition analysis revealed variations between the study phases. Nylon and PET were prominent in the data reported by Alava et al. (2021), with Nylon prevalent in the North side and central part (C3) of the estuary's causeway, and PET detected in C3 and T3. Conversely, here we identified PE, PET, and PP as the most common polymers across different locations. PE predominated in the Central and Terminal parts, significantly in C6 and T1, while PET and PP were abundant in the Central region and above sewage outfall areas.

Another contributing factor to the higher number of MPs specifically for fibers, observed in the current study could be the prevailing environmental conditions during data collection. The assessment by Alava et al. (2021) was conducted during the COVID-19 pandemic, a period marked by reductions in certain human activities that contribute to plastic pollution, such as tourism, recreational boating, and vehicle traffic (leading to fewer tire-wear particles and road-based microplastics in runoff). Studies have shown that reduced vehicle use correlates with lower emissions of black fragments and microplastics from tires (Benson et al., 2021; Peng et al., 2021). However, the pandemic also led to an increase in single-use plastics, such as masks, gloves, and takeout containers, contributing to a surge in plastic waste worldwide (Benson et al., 2021). The current study, conducted post-pandemic, may reflect a shift in plastic pollution sources, with increased human activity and residual effects from heightened single-use plastic consumption potentially contributing to the higher microplastic abundance observed in sediment samples. activities and human emissions on the environment. Conversely, this study occurred during a period when societal activities returned to normal or possibly even increased beyond pre-pandemic levels. This resurgence in anthropogenic activities may have led to higher inputs of plastic waste into the Cowichan Estuary through sources such as industrial discharge, urban runoff, and recreational activities. The resumption of normal activities could have intensified the anthropogenic footprint, resulting in elevated microplastic concentrations observed in the current phase (Cordova et al., 2021).; (Ardusso et al., 2021)

In addition, the microplastic levels in varnish clams sampled from these two estuaries were assessed. On the west coast of British Columbia, these clams are invasive, originating from Asia, and more recently introduced through ballast waters (Larsen & Baker, 2003; Larson et al., 2003). They possess characteristics necessary for biomonitoring, including being sessile, residing in sediment, being opportunistic feeders, being abundant and widely distributed, and serving as important food sources for tertiary consumers, including humans, thus incorporating a human food safety component into biomonitoring efforts (Bendell et al., 2020); (Renkers, 2022). As a result, these species of bivalve may be suitable as bio monitors and could prove to be useful tools for determining whether reduction policies for plastics use are having a positive effect on their release into marine environments.

Overall, the precise toxicological impacts of microplastic particles on marine organisms are not fully understood. However, some evidence suggests that ingested microplastic particles may serve as carriers for toxic substances like trace metals and persistent organic pollutants (POPs). These substances have the potential to bioaccumulate in apex predators and may lead to adverse health effects (Moore et al., 2020). Studies have shown that microplastics can lead to physical and physiological stress in bivalves, such as reduced feeding rates, compromised energy reserves, and altered immune responses (Huvet et al., 2016) (Green et al., 2019). In this study, MP concentrations were higher in clams than those reported by (Renkers & Bendell, 2024); (Bendell et al ,2020), which suggests that the current levels might pose potential risks to shellfish health and, indirectly, to human consumers. The elevated abundance levels found here highlight the need for further research to assess potential health implications for both marine organisms and humans.

4.1. Characteristics of MPs

In Cowichan sediments and clams, fibers were the dominant MP type (53.34% and 53.50%, respectively), while in the K'ómoks estuary, fragments predominated in the sediment (53.70%), and fibers were more prevalent in the clams collected from this area (57.57%). The source of fragments is mostly related to the breakdown of larger plastic debris. The conclusion for this statement is supported by the irregular shapes, rough edges, and weathered surfaces observed in the fragments, which are consistent with physical degradation processes (Andrady, 2011);(Cole et al., 2011). The polymer types identified, such as polyethylene (PE) and polypropylene (PP), are also frequently associated with larger consumer plastics, suggesting that these fragments originated from such sources (Duis & Coors, 2016); (Erni-Cassola et al., 2017). The higher quantities of fragments found in K'ómoks sediments may result from the ongoing mechanical degradation of high-density polyethylene (HDPE) materials over time, leading to their gradual breakdown and subsequent accumulation within intertidal sediments Research indicates that HDPE, commonly used in items such as bottles, bags, and containers, is one of the most prevalent polymers found in marine debris due to its durability and resistance to environmental degradation (Andrady, 2011); (Horton et al., 2017). While Kazmiruk et al. (2018) speculated that HDPE contributes to microplastic fragments in K'ómoks, further polymer-specific analysis would be needed to confirm HDPE as the primary source of these fragments.

The overall averages of 14.37 ± 11.75 and 30.96 ± 14.58 particles per kilogram for Cowichan and K'ómoks, respectively, fall within the range of sediment samples collected previously along the British Columbia Coast(Noël et al., 2022), Lake Erie nearshore sediments, (Dean et al., 2018), Ottawa River sediments (Vermaire et al., 2017), Lake Ontario and Lake Huron nearshore sediments (Athey et al., 2020), Lake Ontario nearshore sediments (Ballent et al., 2016), the Baltic Sea(Hengstmann et al., 2021), San Francisco Bay(Maes et al., 2017), the Vanuatu and Solomon Islands in the South Pacific Ocean (Bakir et al., 2020), the English Channel(Huntington et al., 2020), the Dutch Coast (Adams et al., 2021), as well as the Arctic(Klasios et al., 2021).

The prevalence of fibers where in the sediment and clam samples can be attributed to their smaller size relative to other types of microplastics as observed in this study, where the average fiber size ranged from 0.1 -0.5 mm, compared to the size of the fragments. (Hidalgo-Ruz et al., 2012). Their smaller size allows them to easily migrate through pore spaces and become trapped in sediments, leading to their accumulation (Woodall et al., 2014); (Browne et al., 2011). In this study, sediments in the sampling areas included both fine-grained and coarse-grained types, allowing for an analysis of how sediment characteristics influence microplastic distribution. Our observations support the notion that smaller microplastic particles, like fibers, are more likely to settle within fine-grained sediments, as they can penetrate deeper into the sediment matrix. Conversely, larger particles such as fragments and pellets were more often observed on the surface of course-grained sediments, where they are susceptible to dispersion by ocean currents. These findings align with the relationship between sediment grain size and microplastic distribution as described by Flores-Ocampo & Armstrong-Altrin (2023).

In line with previous studies, fibers were the most common microplastic type in clams from both estuaries, underscoring the potential contributions of textiles (such as clothing and upholstery) and fisheries (including lines and nets) to microplastic contamination in wild clam populations (Sait et al., 2021); (Lam et al., 2023). The higher number of fibers in our study may be attributed to two factors. Firstly, the living environments of the clams could be heavily contaminated with plastic fibers. Our sediment analysis showed that fibers were prevalent in the sampled sediments, suggesting that the clams are exposed to a high level of fiber contamination within their habitats. This environmental exposure likely contributes to the accumulation of fibers observed in the clams. Secondly, fibers, particularly those exceeding 0.5 mm in length, are more likely to remain in the digestive tract for longer periods as they can intertwine and form clusters within the gut potentially increasing their availability to benthic organisms like clams. (Joyce & Falkenberg, 2022); (Ward et al., 2019). Additional origins of microfibers may involve synthetic grass, the release of fibers from household furnishings like sofas and rugs, as well as the spreading of sewage treatment residue, which contains microfibers, onto farmland (Dris et al., 2016); (Magnusson et al., 2016); (Noël et al., 2022). In coastal and estuarine areas, sewage treatment outflows are often documented contributors to microfiber pollution, particularly in regions with urban runoff. While synthetic grass and household textiles are common sources of fiber release, confirming their direct contribution to microfiber levels in these estuaries would require further local investigation. Studies in similar environments have highlighted sewage effluent as a significant pathway for microfiber contamination in sediments and biota (Henry et al., 2019).

The microplastic abundance of 3.64 particles/gram in varnish clams from the Cowichan Estuarywas lower than the reported range found in razor clams (*Siliqua patula*) gathered from various locations, including the coast of Oregon (Baechler et al., 2020), Daya Bay in China (Li et al., 2022), the Persian Gulf (Naji et al, 2018), and Yantai in China (Liu et al., 2021). However, it was higher than the concentrations observed in wild clams from the French English Channel (Doyen et al, 2019), South Korea (Cho et al., 2021), and

Puducherry in India (Dowarah et al., 2020), as well as Baynes Sound in BC (Bendell et al., 2020), South Brazil (Jankauskas et al., 2024), and other bivalves from BC (Bendell et al., 2020), Oregon (Baechler et al., 2020);, and Thailand (Chinfak et al., 2021). Microplastic abundance in varnish clams from the K'ómoks Estuary (2.92 n/g) was higher than the 0.9 n/g reported in BC clams ((Davidson, 2016 #208)and wild clams from Hong Kong, China (Lam et al., 2023).In addition, it was within the range reported in bivalves in Covernton et al. (2022) for other marine species along the BC coast (1.84 to 60.05 particles/gram) (Covernton et al., 2022). Nevertheless, it is important to interpret these findings in this research with caution. Direct comparisons relying solely on particle abundance have several limitations and only provide a broad overview of the prevalence of microplastic pollution in wild clam populations worldwide. These limitations include variability in sampling and processing methods, differences in local environmental conditions, species-specific feeding behaviors, temporal differences in sampling, and lack of standardization in particle size detection. Together, these factors can introduce significant variability in particle counts across studies, making it difficult to draw precise conclusions about pollution levels without a more standardized approach (Bom & Sá, 2021); (Ding et al., 2022).

Considering the relationship between microplastic size, specific surface area, and interactions with contaminants like PCBs is crucial in understanding potential ecological impacts in the Cowichan and K'ómoks estuaries. As microplastic particles decrease in size, their surface area increases, enhancing their capacity to adsorb toxic substances (Issac & Kandasubramanian, 2021); (Rodrigues et al., 2019). In our study, smaller microplastics, including fibers, may have settled into deeper, fine-grained sediment layers within these estuarine environments, consistent with findings from other studies (Zhou et al., 2021); (Flores-Ocampo & Armstrong-Altrin, 2023). This distribution pattern suggests that fine sediments in these estuaries could act as long-term sinks for smaller, potentially contaminant-laden microplastics, posing risks to benthic organisms. Additionally, the higher abundance of microplastics observed in certain sampling locations might relate to hydrodynamic factors, where changes in water currents facilitate the resuspension and eventual trapping of microplastics within sediment layers (Martin et al., 2022); (Muthuvairavasamy, 2022); (Al Nabhani et al., 2022).

In this study, microplastic sizes in sediments for both estuaries ranged from 0.1 to 0.5 mm. While these particles may not be small enough to cross cellular membranes, their relatively small size makes them more accessible for ingestion by fish and other aquatic organisms, which can lead to accumulation in digestive systems and potential adverse health effects. Additionally, microplastics of this size behave similarly to suspended particles in water bodies, resulting in higher concentrations in areas with changing water currents, where they may remain in the water column or settle in sediments (Al Nabhani et al., 2022). Particle size is a critical factor contributing to the availability of microplastics to biota (Wright et al., 2013). According to Wright et al. (2013), smaller microplastics are more likely to be ingested by a range of marine organisms, including zooplankton, filter-feeding bivalves, and juvenile fish, due to their accessibility and prevalence in the environment. In our study, microplastic sizes in sediments for both estuaries ranged from 0.1 to 0.5 mm, which suggests that these particles could be readily ingested and accumulated by local aquatic biota, potentially posing ecological risks. Microplastics sized 0.1–1 mm constituted the largest proportion observed in this study. Typically, the predominant size class of microplastics accumulated in bivalves is around 1 remains in the gut rather than other tissues. In some cases, smaller particles, especially those under 100 µm, may translocate into other tissues, though this is less frequently observed and depends on factors like species and exposure conditions (Ben-Haddad et al., 2022); (Klasios et al., 2021);)Phuong et al., 2018);(Ribeiro et al., 2023);(Webb et al., 2020);(Von Moos et al., 2012). This prevalence in size may be related to the microplastic size selectivity of clams, as mini-microplastics (typically defined as particles in the 0.1–1 mm range) share a similar size range with their food items such as diatoms and microplankton (Li et al., 2022), however, this preference may not solely be due to size effects.

Biofouling of microplastics can promote their ingestion, as the presence of biofilms can lead biota to mistakenly uptake microplastics as nutritious food. Recent laboratory studies have supported this notion, showing that biofilm-coated microplastics were nearly three times more attractive to deposit feeders in terms of the number of items ingested compared to virgin microplastics (Fabra et al., 2021). Since biofouling occurs more rapidly on smaller microplastics due to their higher surface-to-volume ratio (Fischer et al., 2021), it is reasonable to assume that the prevalence of mini-microplastics in varnish clams observed in this study may partly resulted from biofouling, as biofouling increases the density of microplastics, causing them to sink and accumulate in sediments where clams feed. However, the high prevalence may also be due to the general abundance of microplastics in the surrounding water and sediment. Both factors likely contribute to the increased ingestion of microplastics by varnish clams.

The dominance of black and transparent microplastics in the sediments of Cowichan and K'ómoks estuaries, respectively, warrants attention for their potentially higher negative impact on aquatic organisms, due to their potential origins from industrial sources or tire wear, which may introduce heavy metals and other contaminants In our study, 10% of the microplastics in the sediments of Cowichan and 13% in K'ómoks estuaries were transparent, which could pose a significant risk to aquatic organisms. Transparent microplastics are more challenging to identify and easily ingested by aquatic organisms (Wen et al., 2018), and have been reported to absorb greater quantities of toxic compounds like dioxins, pesticides, flame retardants, and PCBs compared to colored microplastics (Alimi et al., 2018); (Rochman, 2018).Furthermore, over 20% of the microplastics identified in our samples were , polyester (Jankauskas et al., 2024) generally exhibits a greater capacity to absorb contaminants than other polymers (Llorca et al., 2020); (Wang et al., 2021). The presence of both transparent and black microplastics in these estuaries suggests that microplastics in this region may have elevated contaminant loads, posing additional risks to the aquatic organisms that inhabit these environments.

The dominance of black-colored microplastics in the clam samples from both estuaries raises important considerations about potential contamination sources and environmental persistence. Although clams do not visually select particles based on color, black microplastics can have heightened environmental risks. These particles often originate from sources like industrial and automotive waste, which can carry heavy metals and other contaminants. (Roch et al., 2020); (N. C. Ory et al., 2018). Black microplastics can include primary particles <5 mm used in cosmetics and secondary microplastics resulting from the breakdown of larger black plastic items. Black plastics pose unique challenges due to their high demand in various sectors, such as food packaging, cooking utensils, trays, toys, electronic household goods, electronics, and automobile components, and the difficulty in sorting them efficiently and cost-effectively. Consequently, a large portion of black plastics end up in landfills, incinerators, rivers, and oceans after single use due to the lack of affordable and efficient sorting technologies and their relatively low market value (Nguyen et al., 2019); (Huang et al., 2022). The accumulation of black microplastics in estuarine sediments and their ingestion by clams in this study suggest that these plastics could introduce additional contaminants into the food web, posing potential risks to aquatic organisms and higher trophic levels.

4.2. Polymer Types

In K'ómoks sediments, the prevalent polymers were polyester, polypropylene (Jafarabadi et al., 2019), and polyethylene (PE), while polyethylene (PE) and polyethylene terephthalate were predominant in Cowichan sediments. In varnish clams, polyester and polypropylene were the primary polymers in K'ómoks and Cowichan, respectively. The prevalence of PES can be attributed to its extensive use in the textile industry, clothing, and woolen goods (Vetrimurugan et al., 2020), which are known sources of microplastic pollution. Given the proximity of sewage effluent sources to the estuarine areas, it is likely that PES fibers are entering the environment through wastewater discharge, which often carries fibers released during washing of synthetic textiles. Noël et al. (2022) reported polyester dominance in sediment and mussels along the BC coasts. This polymer's prevalence suggests that wastewater effluent discharge may be a significant source of microplastics in coastal BC. (Gies et al., 2018) identified polyester as the dominant polymer in wastewater from a Vancouver treatment plant, further supporting this notion. (Browne et al., 2011) also noted similar polymer compositions in sediment and wastewater effluent worldwide.

As mentioned previously, in the K'ómoks estuary, the average number of fragments in sediments exceeded that of fibers, and polyethylene and polyester were in the same range found in clams. (Bendell et al., 2020) noted that micro fragments recovered from clams sampled from Baynes Sound could be directly matched to polymers HDPE and a PP composite commonly used by the aquaculture shellfish industry. It is speculated that the high numbers of fragments observed may result from inputs from the Courtney-K’ómoks Estuary, which receives effluent from multiple sources, including municipalities, assisted living facilities, and historically, a hospital.

The distribution of microplastics (MPs) in the water column depends largely on their density, which influences whether they float or sink, affecting their availability to different species. For instance, low-density MPs like polypropylene and polyethylene tend to float, making them accessible to pelagic species, while denser MPs, such as polyester and polyvinyl chloride, tend to sink and are more likely to affect benthic organisms. In this study, the prevalence of denser microplastics, particularly PES, suggests potential exposure risks for benthic and deposit-feeding species in the estuary. Additionally, environmental factors like biofilm growth can alter MP density, contributing to the accumulation patterns observed in this region (Hidalgo-Ruz et al., 2012); (Crawford et al., 2016); (Ding et al., 2019); (Flores-Ocampo & Armstrong-Altrin, 2023).

4.3. Potential Sources of Microplastics in K’ómoks and Cowichan Estuaries

Identifying potential sources of microplastics entering the estuaries is crucial for understanding the observed results and devising effective management strategies. Both estuaries receive wastewater effluent from treatment plants, although the extent of the catchment area and volume of effluent remain unknown. Despite their distinct anthropogenic impacts, the Cowichan Estuary, characterized by two major tributaries and a history of intertidal log transport and storage, stands in contrast to the K’ómoks/Courtenay estuary, which lacks industrial activity but has heavily populated shorelines (14806) and uplands. The suspected microplastics found in both estuaries exhibited various colors, including black, transparent, blue, red, and white, with fibers displaying the most diverse range of colors, followed by fragments. The diverse colors of microplastics suggest a wide array of potential sources. While the diverse colors suggest a wide array of potential sources, it is important to note that the dyeing process may have altered or intensified the visibility of certain colors. Efforts were made to account for any influence of staining on color observation during analysis.

Wind and tidal currents play significant roles in transporting microplastics to marine and terrestrial environments. Less dense microplastics can be actively transported by atmospheric and oceanic currents. Rather than simply sinking, these microplastics may follow two potential pathways: remaining suspended in the water column or, through processes such as biofouling or aggregation with organic matter, eventually settling and becoming incorporated into sediments. Rivers, estuaries, and lagoons can act as temporary reservoirs for microplastics, where particles accumulate before eventually being transported to the ocean, especially during the rainy season when elevated water levels increase flow. The movement and ultimate destination of microplastics depend on factors such as the distance between source and reservoir. Atmospheric currents further facilitate the rapid, long-distance transport of microplastics, contributing to their accumulation in coastal environments. Furthermore, microplastics are likely transported from surface waters to benthic habitats through both biotic and abiotic mechanisms, similar to those responsible for plankton transport and benthic-pelagic coupling (Díaz-Mendoza et al., 2020); (Flores-Ocampo & Armstrong-Altrin, 2023). Within this process, bioturbation mechanisms play a dominant role in benthic-pelagic exchange pathways.

5. Conclusions

This study validates the presence of microplastics in the sediments and varnish clams sampled from two estuaries, K’ómoks and Cowichan, highlighting concerns regarding their occurrence in marine food webs and potential seafood contamination. The analysis revealed a abundance of fibers in the estuary, indicating that they may be accumulating as a result of environmental degradation processes

Five distinct types of polymers were identified, including polyester, polyethylene, polypropylene, polyethylene terephthalate, and polyamide, with PES and PE being the most prevalent. This dominance is attributed to intense anthropogenic activity in the estuarine areas. This dominance is attributed to intense anthropogenic activity in the estuarine areas, consistent with findings by Sharma et al. (2023), who highlight the substantial contribution of human activities to microplastic pollution in aquatic ecosystems. The diversity in polymer types suggests various sources, such as domestic and industrial sewage discharge, tourism, and fishing activities. Comparative analysis between study phases reveals dynamic changes in microplastic composition, size distribution, and polymer types, highlighting the need for continued research and mitigation efforts to address this environmental concern effectively (Jankauskas et al., 2024).

Given these findings, varnish clams could serve as effective biomonitoring tools for tracking trends in microplastic abundance in environmental media, particularly as policies aimed at reducing plastic usage are implemented. Further exploration of additional ecological factors such as seasonal variations, food availability, and age would enhance our understanding of the suitability of these clams as bio monitors for microplastics.

Figure 1. Map showing sampling locations for the collection of sediment and Varnish clam samples in the Cowichan-Koksilah and Comox estuaries, including salt marshes and mudflats from the eastern coast of Vancouver Island (British Columbia, Canada) in July 2023 For the Cowichan estuary, the sampling sites are labelled as North (N1, N2, N3 and N4), Central (C1, C2, C3, C4, C5, AND C6), Terminal (T1, T2, T3, and T4), and South (S1-S4), as well as the sewage outfall of the Cowichan River (CO1, CO2, CO3), the north fork (CO4-A, CO4-B), the south fork (CO5, CO6), and two from the Koksilah River (K1-A, K1-B). For the K'ómoks estuary, the labels represent samples points for the Trent River (TR: CO1-1, CO1-2, and CO1-3), K'ómoks Marina (CM: CO2-1; CO2-2, and CO2-3), and Goose Spit Park (GP: CO3-1; CO3-2, and CO3-3).

(A)

(B)

(C)

(D)

(E)

(F)

Figure 2. Spatial distribution and physical-chemical characteristics of microplastics (MPs) in sediment and varnish clam samples from the Cowichan and K'ómoks estuaries: (A) Abundance of MPs across sampling sites, (B) Distribution of MPs by shape (e.g., fragments, fibers, etc.), (C) Distribution of MPs by color categories, (D) Size distribution of MPs in different matrices, (E) Polymer types identified in MPs, categorized by material composition, (F) Comparative abundance of MPs between sediment and varnish clam samples across all samples.

Figure 3. Comparison of the sediment microplastic concentration data (microplastic particle/kg) by type of plastic particles (i.e., fiber and fragments) for Phase 1 (August 2020) and Phase 2 (July 2023) in sampling locations (i.e., N = North; C= Central; T = Terminal; S= South; CO = Cowichan River; K = Koksilah River) of the Cowichan Estuary. The number of replicates (n = 3).

Table 1. Average abundance of plastic particles recovered from sediments between the K’ómoks and Cowichan estuaries, British Columbia, Canada. Sampling sites for the K’ómoks Estuary are indicated by CM = K’ómoks Marina, TR = Trent River; and, GP = Goose Spit Park. For the Cowichan Estuary the samples sites are indicated by N = North; C= Central; T = Terminal; S= South; CO = Cowichan River; and, K = Koksilah River (Figure 1). P-value at base of table indicates results from XYZ statistical test.

Estuary	**Site**	**Number**	**Total Particle (kg/dw)**	
			Mean±SD	**(Min-Max)**
K’ómoks	CM	9	44 ±9.65	33-55
	TR	9	17.67± 7.28	8-31
	GP	9	31.22 ±12.44	11-49
Cowichan	N	13	23.85 ±40.79	2-155
	CO	21	11.33 ±8.58	2-35
	C	18	20.17 ±13.52	8-66
	T	9	22.11± 16.99	1-57
	S	12	10.33 ± 6.66	4-24
	K	6	7 ±2.89	4-11
p=0.0002				

Table 2. Field data, biometrics information and microplastic concentration data of Varnish clams sampled from the K’ómoks and Cowichan estuaries, British Columbia, Canada, in July 2023. Site location codes as in Figure 1. P-values at base of table indicates results from XYZ statistical test.

Estuary	**Site**	**Number**	**Whole weight (g)**	**Wet Weight (g)**	**Width (mm)**	**Length (mm)**	**Height (mm)**	**Total particles/g (ww)**	**CI (g/cm3)**
K’ómoks	COM	40	37.43±3.59	6.01±1.38	43.89±2.02	46±3.60	16.7±5.33	1.97± 1.93	35.8
	TR	40	31.88±4.61	7.21±1.65	41.50±5.26	35.8±8.84	16.75±4.49	2.48± 2.05	41.97
	GP	23	18.44±3.75	6.87±1.17	30.7±5.75	29.33±2.50	11.86±1.77	2.16± 1.7	43.03
Cowichan	216	8	24.92±3.44	9.06±1.42	42.29±2.36	37.44±8.57	16.56±4.24	2.77± 1.98	36.37
	C	10	19.74±8.64	7.19±3.05	40.2±8.28	30.14±2.61	10.7±1.25	2.52 ±1.84	36.42
	N	10	19.6±1.48	6.37±3.21	38.45±10.22	36.2±8.31	10.09±2.21	4.33 ±2.56	32.51
	217	11	12.72±4.34	4.55± 2.63	36.4 ±13.95	30.47± 4.86	15.47± 5.125	4.85 ± 3.53	32.59
	219	8	7.02± 1.51	2.94± 1.04	31.87 ±1.59	29.47 ±2.16	11.93 ±1.43	2.2 ±2.1	22.62
	220	5	5.02± 2.54	2.16 ±1.04	25.73 ±3.77	29.91± 4.80	10.64± 1.20	3.2. ±59	18.35
			p<0.0001	p<0.0001	p <0.0001	p<0.0001	p=0.7975	p=0.0038	p=0.0634

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