Seagrass beds reveal high abundance of microplastic in sediments: a case study in the Baltic Sea

By Randel Kreitsberg[1,2], Merilin Raudna-Kristoffersen[1], Margit Heinlaan[3], Raymond Ward[2,4], Meeri Visnapuu[5], Vambola Kisand[5], Richard Meitern[1], Jonne Kotta[6] and Arvo Tuvikene[2].

[1] - Department of Zoology, Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51020 Tartu, Estonia;

[2] - Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 5, 51014 Tartu, Estonia;

[3] - National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia;

[4] - Centre for Aquatic Environments, University of Brighton, Moulsecoomb, Brighton, BN2 4GJ, United Kingdom;

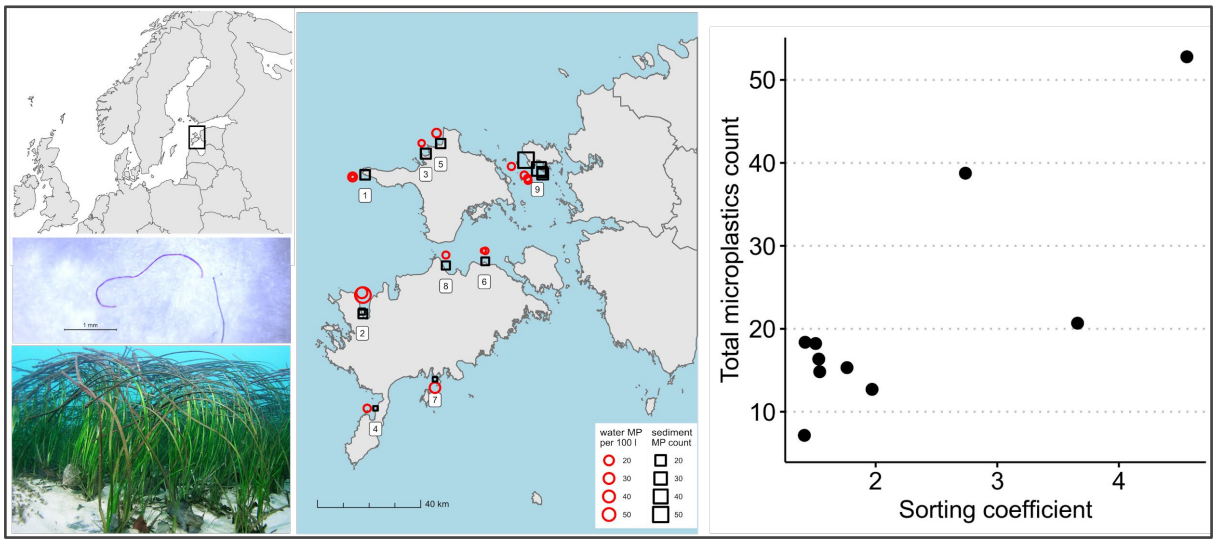
[5] - Institute of Physics, University of Tartu, W. Ostwaldi 1, 51014 Tartu, Estonia;

[6] - Estonian Marine Institute, University of Tartu, Mäealuse 14, 12618 Tallinn, Estonia.

Highlights

- First microplastic abundance study in seagrass beds in the Baltic Sea.
- Study revealed high abundance of microplastic in seagrass beds.
- Blue fibers were the prevalent microplastic in both water and sediments.
- More poorly sorted sediments had the highest microplastic concentrations.
- Study highlights heterogeneity of microplastic abundance within sediment samples and microplastic counts.

Graphical abstract



Abstract

Microplastic (MPL) contamination in the marine environment is extensively studied yet little is known about the extent of MPL abundance in seagrass beds.

The aim of this study was to evaluate MPL accumulation in seagrass beds. Water and sediment samples were collected from coastal *Zostera marina* beds in the Baltic Sea, Estonia.

Surface water was sampled by pumping using 40 µm plankton net, and sediments by trowel. MPL was extracted with NaCl, identified by microscopy and ATR-FTIR on selected samples.

Surface water in the seagrass beds had 0.04-1.2 (median 0.14) MPL/L, similar to other areas of the Baltic Sea. Sediments had 0-1817 (median 208) MPL/kg (dwt), much higher than previously recorded from adjacent unvegetated and offshore sediments, thereby suggesting a strong ability of the sediments in seagrass beds to retain MPL. Of identified MPL, blue fibres were dominant in both the sampled media. Sediment characterization showed a correlation between MPL counts with poorly sorted sediments.

Keywords: marine pollution, microlitter, sediment, water, *Zostera marina,* blue fibre

Introduction

Microplastics (MPL) are an emerging contaminant globally and as a result there is an increasing interest in monitoring their abundance and distribution in different ecosystems, including different marine ecosystems. To date, the primary focus of MPL in marine environments has been in open sea areas, and only recently have benthic ecosystems, including seagrass beds, gained attention as potential hotspots for MPL pollution (Goss et al., 2018; Huang et al., 2020; Jones et al., 2020; Seng et al., 2020).

Seagrass beds are among the most productive ecosystems in the world and offer a multitude of ecosystem services, including as nursery and spawning habitats, carbon sequestration and storage, nutrient cycling, erosion control, and improving water quality (Lamb et al., 2017). However, the levels of MPL pollution as well as the impacts on seagrass beds are poorly understood.

Recent studies show that seagrasses could act as sinks for MPL potentially due to a number of specific conditions, such as reduced water flow, increased settling rates, and trapping by blades that could favour retention of MPL (Huang et al., 2020). MPL readily adsorb to seagrass blades, and a trend towards higher MPL sorption on >50% of epibiont covered *T. testudinum* blades has been observed (Goss et al., 2018). This may facilitate MPL uptake into the food chain, since grazers prefer seagrasses with a higher density of epibionts (Goss et al., 2018), and have been shown not to distinguish between MPL-contaminated and clean algae (Gutow et al., 2016).

The retention potential of MPL by seagrass bed sediments has been less studied than MPL sorption on seagrasses. Both, significantly higher (Huang et al., 2020; Jones et al., 2020) and comparable (Cozzolino et al., 2020) MPL content compared to reference unvegetated areas have been shown and MPL numbers are expected to be higher in more eutrophic areas (Huang et al., 2020).

In the Baltic Sea region, MPL research has received relatively little attention, especially in coastal ecosystems. The common eelgrass *Zostera marina* L. is the most widespread seagrass species in the northern hemisphere and is the only seagrass species in the Baltic Sea. Seagrasses extend over 21,000 km^2 in the Baltic and are particularly common in the shallow West Estonian Archipelago in the eastern Baltic Sea (HELCOM HOLAS II Dataset: *Zostera marina* distribution (2018)). Increasing eelgrass density has been shown to support an elevated diversity and abundance of associated macroalgae and invertebrates (Möller et al., 2014), which could be at risk due to increased MPL exposure (Rani-Borges et al., 2021; Barboza & Gimenes, 2015).

The few available studies on MPL in seagrass beds highlight the heterogeneity of the results, plausibly reflecting a diversity of site-specific drivers (Cozzolino et al., 2020). One of the

generalizations that can currently be made is that fibres are the prevalent recorded MPL type both on seagrass blades (Goss et al., 2018; Cozzolino et al., 2020; Seng et al., 2020) as well as in seagrass sediments (Jones et al., 2020). In more than 75% of the reviewed MPL abundance studies (n=13) from open sea areas of the Baltic found fibres were the prevalent MPL type in water (Bagaev et al., 2017; 2018; Tamminga et al., 2018; Schönlau et al., 2020) sediments (Graca et al., 2017), and fish (Beer et al., 2018).

The aim of the current study was to map microplastic (MPL) abundance in the coastal seagrass beds of West Estonian Archipelago Sea. Further, on the basis of literature data, the study evaluated whether the abundance of MPL in the seagrass habitat is comparable to other habitats (e.g. the open sea areas of the Baltic Sea). To our best knowledge, this is the first study on MPL abundance in seagrass ecosystems of the Baltic Sea.

Materials and Methods

Study area

MPL samples from surface water as well as bottom sediments were collected from a research vessel during July 2018. All-together 15 sampling stations were chosen in the Western-Estonian archipelago in the Baltic Sea, (Figure 1). Each area was described using a set of parameters, including depth, pH, oxygen concentration and water temperature (Table 5 in SI).

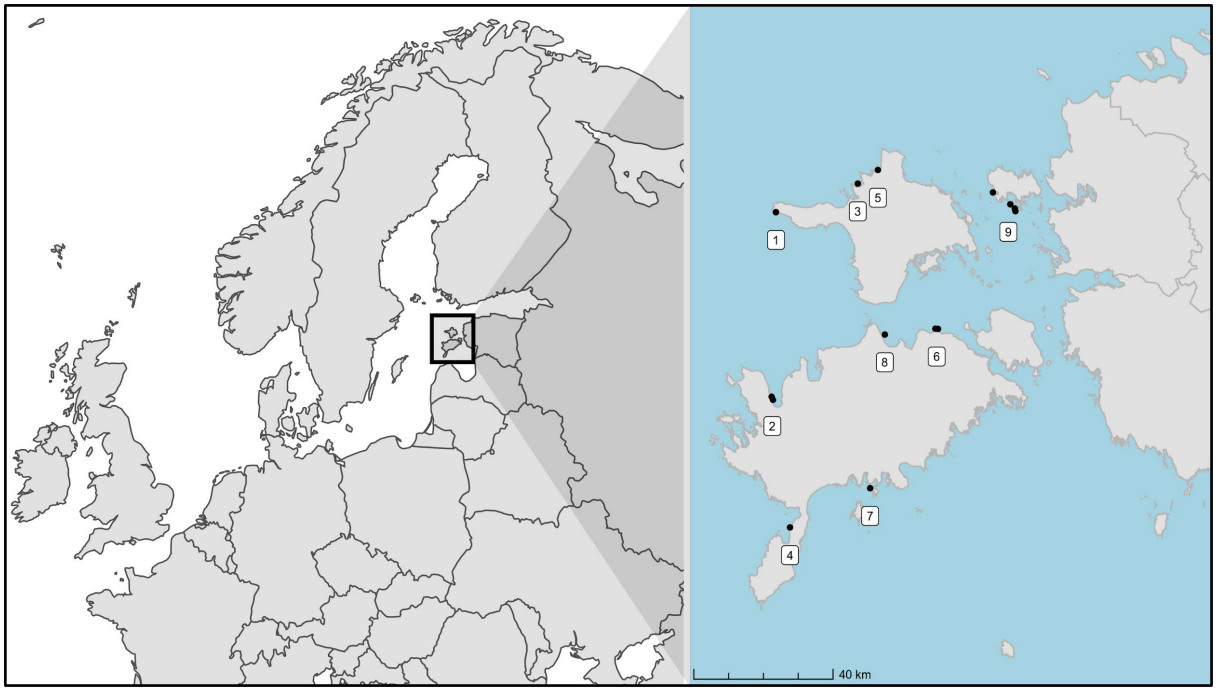


Figure 1. Location of study sites in NE Baltic Sea. Numbers represent main sampling areas; altogether 15 locations were studied: (1) Kalana (2 areas), (2) Kehila (2 areas), (3) Kõrgessaare, (4) Lõu, (5) Mangu, (6) Rannaküla (2 areas), (7) Sepamaa, (8) Soela, (9) Vormsi (4 areas).

Characterization of seagrass beds

The studied seagrass beds were situated in shallow coastal waters at 2.4-4.4 m depth. The ecosystems were predominantly covered with 50-100% *Zostera marina*, with infrequent *Zannichellia palustris* and *Potamogeton perfoliatus*, as well as some rhodophytes, phaeophytes and chlorophytes present where finer sediment was topped with interspersed boulders and cobbles. Sediment was predominantly sand with some sites characterised with gravel fractions and others characterised by substantial silt fractions. The selected sites reflected the current distribution of seagrass habitats in which seagrass had at least 75% cover within the last 5 years. There were typically few epiphytes on the leaves and above ground blades ranged between 20 cm and 1.2 m long. Average grain size (d50), sorting coefficient

(So), soil organic matter (%SOM), seagrass, other higher plants and algae cover (%), dry bulk density (g/cm^3), exposure class and exposure to waves was measured and calculated to characterize the seabed (Table 5; SI). No sites had substantial evidence of anthropogenic disturbance (anchoring, prop damage, litter).

Sampling of water and sediments

Two different sampling techniques were used to determine concentrations and characterize MPL within the upper 10 cm of surface water and similarly, in the upper 10 cm of the bottom sediments:

Surface water was pumped through a 40 micron mesh nylon plankton net, and the water pump was situated on the bow, which was pointing headwind to minimize possible contamination from the vessel. On average, 201 litres of water per site was filtered to yield final water samples of 50 ml. Altogether 15 water samples were collected, one from each study area.

Sediment samples of approximately 1 kg were collected by hand using scuba equipment. Sediments were removed using a 10 cm wide semi-circular stainless steel trowel (providing a 10cm diameter core). Samples were carefully transferred to a container and sealed in-situ prior to transportation to the surface. Duplicate samples were collected from each of the 15 study sites, within a few metres of each other.

Sample extraction and filtration

For density separation, 50 ml water samples were diluted in 250 ml of saturated sodium chloride solution (NaCl, Sigma Aldrich, min 1.2 g cm-3), and allowed to settle in separating

funnels for 30 minutes. The density of solution obtained after mixing water sample and sodium chloride solution was 1.17 g cm-3. The method is both an economical and reliable method that allows polymers to float, thus facilitating their separation in separatory funnels during the 30-minute settling period. However, this method will not allow separation of denser polymers such as polyvinyl chloride (PVC) (Gago et al., 2018). Upon removal of any residues of sodium chloride and organic matter, the supernatant was vacuum-filtered through 47 mm-diameter Whatman glass fibre filter (GF/D, with particle retention size of 2.7 μm). After filtration, filters were removed with clean forceps and stored in a covered glass Petri dishes for subsequent microscopic analysis. To minimise contamination, prior to use and inbetween samples, all glassware was first rinsed with water and then in triplicate with Milli-Q (ultrapure) water.

For density separation of sediment samples, a 50 ml subsample of sediment was diluted in 250 ml of saturated sodium chloride solution, stirred amply in glass beaker, and supernatant poured in separating funnels for density separation – followed by the same filtration process as water samples. Since the sediment did not contain a remarkable amount of water, the density of sodium chloride solution was not decreased in case of sediment experiments. Each sediment sample was manipulated twice continuously and two separate supernatants were filtered and counted, using a microscope, separately to segregate results from the two flushes, and determine any possible residue that might be left after the first dilution.

Microscopy

The filters were examined under a stereo microscope (Leica M165 FC) equipped with a camera (Leica DFC 450C). Potential MPL were confirmed by noting unnatural pigmentation, flexibility and the lack of cellular structures at a magnification of 20-100x. Each MPL

particle was photographed, measured using a Leica Application Suite program, and described by colour (black, blue, red, transparent, multicolour, other (incl. green, pink, beige, violet etc.)) and shape (angular, fiber, film, other (incl. round, oval etc.)).

Fourier Transform Infrared (FTIR) analysis

A subsample of 76 particles, identified as MPLs by microscopy analysis, and large enough to be manipulated by tweezers, were analysed using Fourier transform infrared spectroscopy (FTIR) with an attenuated total reflection (ATR) accessory. ATR-FTIR spectra were measured in the 400–4000 cm−1 range with 2 cm−1 resolution using Bruker VERTEX 70 spectrometer. ATR-FTIR spectra were normalized to the background scans. For identification, measured spectra were compared to the reference spectra of pure materials.

Procedural blanks

Multiple procedural blanks for laboratory manipulation of samples were performed: (i) by filtering the same amount of ultrapure Milli-Q water and (ii) sodium chloride solution separately, using the same filtering equipment as used for sampling; (iii) by following all the steps of sample extraction, separation and filtration using saturated sodium chloride solution. Measures taken to minimise contamination included wearing natural fibre clothes while working with the samples, keeping filters in glass petri dishes or stainless steel containers, and keeping the filters covered whenever possible. Finally, precipitation of plastic dust particles in laboratory work space was tested by exposure of filters next to the equipment during the working process during sample preparation and in the microscopy room.

Statistical analysis

In order to quantify site MPL abundance, raw microscope counts were blank corrected. All the sites that displayed less (or equal) MPL content than in (to) blanks (i.e. the sum of mean sampling blanks and mean sample analysis blanks) were removed. For all other samples, MPL particles removed were proportional to the distribution of plastic in the blanks regarding shape, size and colour. Only colours that were present in blanks were removed (i.e. all fibre pieces of different colour than transparent, black, mixed or blue were kept even if total fibre count was below the mean of blanks). Prior to statistical analyses the seawater counts were adjusted to 100 L of water and the sediment counts for 50 ml of sediment. Statistical analyses were performed in R version 3.6.3 and standard summary statistics (mean, median, quartiles etc) and multiple linear regressions were computed. Multiple linear regression was used when comparing MPL size distribution among sites and types, size was log transformed prior to analyses to meet model assumptions. The model included MPL size as the dependent variable and area, sample type (water or sediment), colour and shape as predictors. For comparing the means for the levels of a factor in the above-mentioned linear regression, Tukey honest significant differences were computed. Summary tables and graphs were generated using packages ggplot2, ggpubr, GGally, sf and their dependencies (> 70 packages).

Results

Microplastic counts and characteristics

A total of 955 particles (626 from the surface water samples and 329 from sediment samples) from 15 sites (Figure 1) were identified using microscopy. Based on procedural blanks (contamination blanks) we expected the sample processing to yield 25 pieces of fibrous MPL per sample and subsequently, 198 observations from water and 81 from sediment datasets were removed. Remaining microplastic counts by area are shown in Figure 2 for shape (A) and for colour (B). Counts by sampling stations are given in Table 4, SI. As a microscopy

control, FTIR analysis confirmed visually identified MPL and assigned them to different polymer types (Table 3 in SI) with nylon being the dominant one.

Blue fibrous MPL were prevalent in both the water and sediment (Tables 1 and 2). On average, 65.5±36.5% of all plastics in water and 79.2±14.6% sediments were blue and 69.4±35.5% of all plastics in water and 97.1±5.1% in sediments were classified as fibres.

Sediment samples were flushed twice for MPL. The duplicates, used to calculate the total count, were correlated ($R = 0.48$, $p = 0.016$) if an outlier (Vormsi 1) was removed (otherwise the correlation was on the borderline, $p = 0.054$). Mean±SD values for MPL counts in duplicate flushes were 37.1±39.5 in first and 7.9±5.3 in second flush.

Duplicate sediment samples taken within one site did not show a similarity in the total count of MPL (even if the flushes are combined all correlations $p > 0.5$), suggesting there is a considerable amount of heterogeneity within each site. There was no correlation between water and sediment MPL count even after excluding an outlier (Kehila 1, $R = -0.35$, $p = 0.24$; Figure 1).

Within all sites, the majority of observed MPL were small (mean = 1351 µm, SD =1804 µm, median 913 µm, 1st quartile 524 µm, 3rd quartile 1593 µm, min = 72 µm, max = 27635 µm; Figure 3). However, multiple linear regression revealed that the size of MPL differed between water and sediment ($F_{1,516}=19.8$, $p<0.001$), with larger MPL identified in the water, but the difference in plastic size between sites was not significant ($F_{8,516}=0.8$, $p=0.5$). The size of the MPL was different depending on colour ($F_{5,516}= 6.8$, $p <0.001$), with transparent pieces being larger than blue pieces (Tukey post-hoc comparisons $p < 0.001$). Size and shape

were also related $F_{3,516}$= 16.5, $p < 0.001$), angular plastics were smaller than other types (all Tukey post-hoc comparisons $p < 0.02$). Adding or removing model predictors did not change the observed patterns.

Site physico-chemical properties

Correlating sediment MPL counts with exposure class (R=-0.02, p=0.9), sorting coefficient (R = 0.83, p = 0.002), seagrass cover (R = -0.38, p= 0.17) and bulk density (R = -0.30, p = 0.36) revealed a strong correlation only with sorting coefficient (Figure 4; Table 5 in SI).

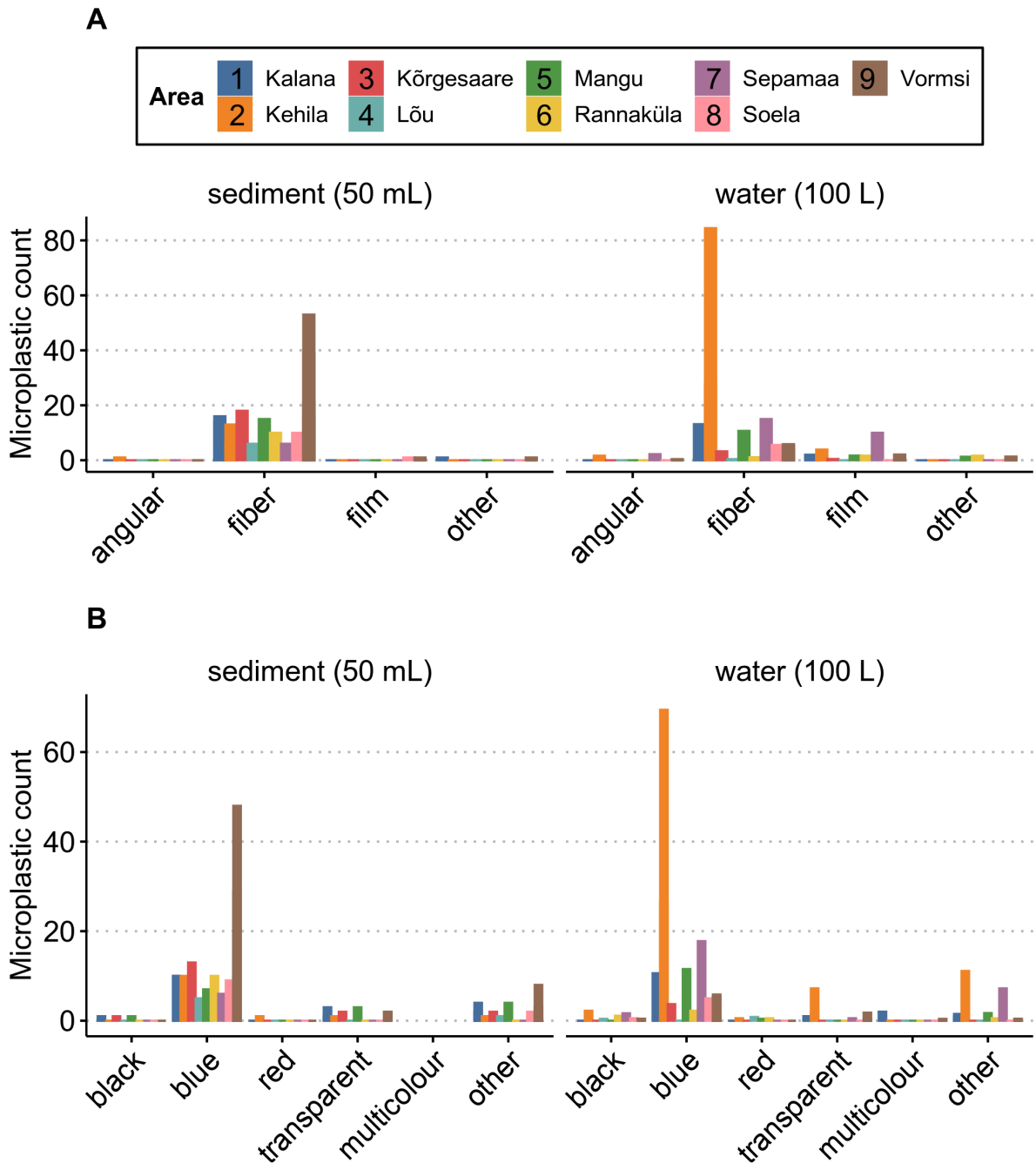


Figure 2. Volume adjusted microplastic counts by site in water and sediment samples by shape (A) and by colour (B). For numeric values see supplementary materials (Table 4).

Table 1. Total microplastic counts in water and sediments by colour.

Colour	Water	Sediment
Black	13	3
Blue	322	196
Red	5	1
Transparent	24	14
Multicolour	5	0
Other	45	34
Uncharacterized	14	0
Total	**428**	**248**

Table 2. Total microplastic counts in water and sediments by shape.

Shape	Water	Sediment
Angular	9	1
Fibre	346	243
Film	47	2
Other	10	2
Uncharacterized	16	0
Total	**428**	**248**

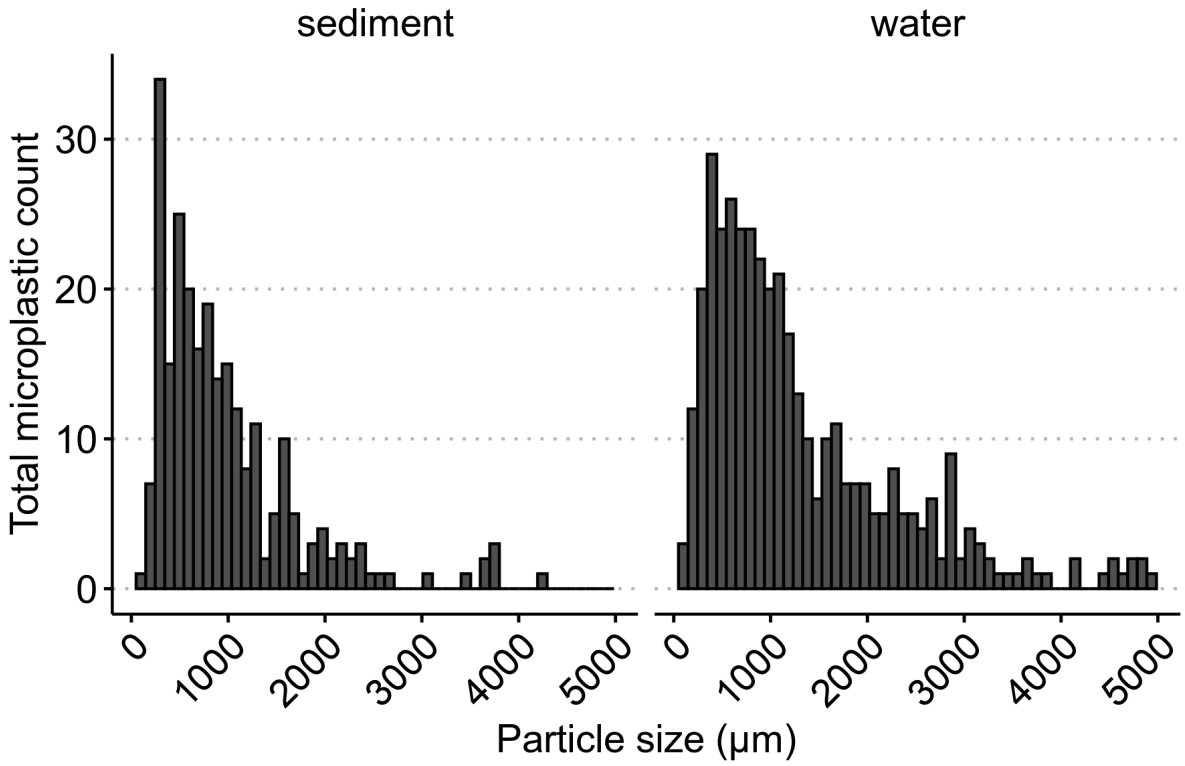


Figure 3. Size distribution of microplastic in sediment and water samples.

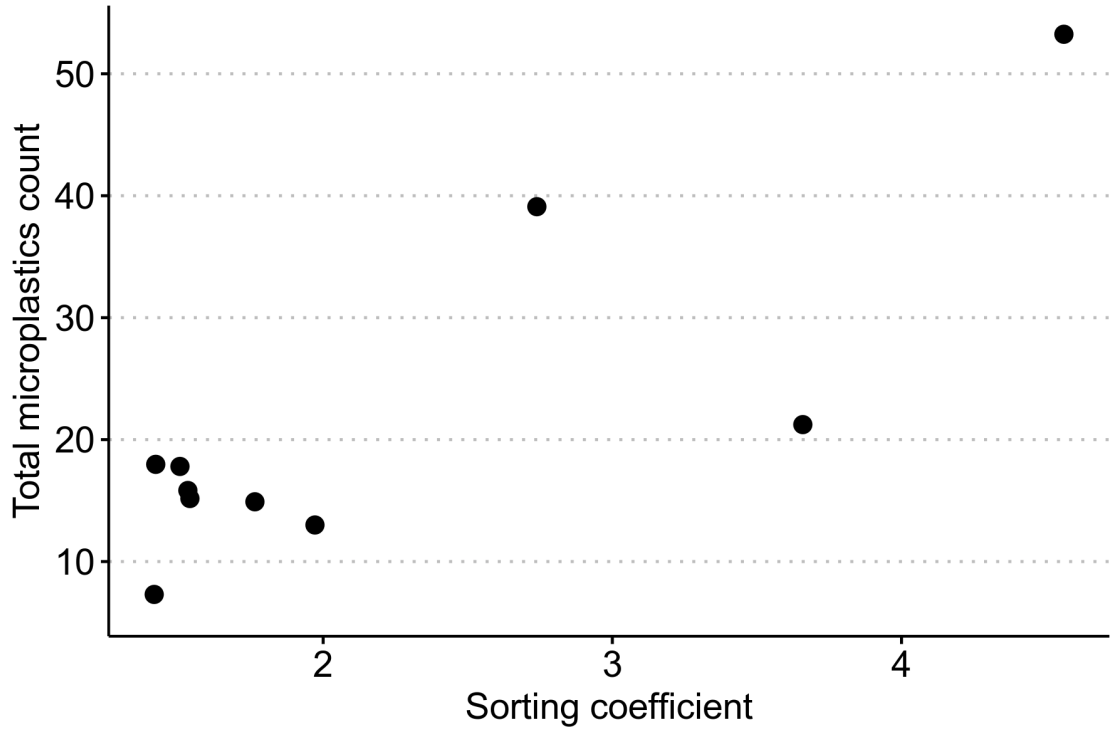


Figure 4. Correlation between total sediment microplastic counts and sorting coefficient by site.

Discussion

Microplastic abundance in the surface water around seagrass beds

Only recently specific marine ecosystems, including seagrass beds, have started to gain attention as potential hotspots for MPL pollution (Huang et al., 2020). In our study, surface water in seagrass beds had 0.04-1.2 (median 0.14) MPL/L. The closest areas to our sampling stations that have been monitored for MPL are within Estonian public sector studies (Lind et al., 2019; Lips, 2020). Compared to these reports from the area, current results are 200-6000-fold higher than previously recorded in the surface waters of the Gulf of Riga and the West Estonian archipelago (0.0002 MPL/L; Lips, 2020) and the central part of the Gulf of Finland

(0.0004 MPL/L; Lind et al., 2019). The differences could be due to i) different MPL sampling and identification methods, ii) anthropogenic pressure and/or iii) trapping effect of the seagrass beds.

Here, pumping using a 40 µm mesh size plankton net was applied for surface water sampling. Schönlau et al. (2020) have shown pump sampling to yield significantly higher MPL counts than trawl sampling, which was applied in the both cited Estonian public sector projects. This difference is potentially due to uncertainties regarding the sampled water volume since, depending on the wave action, the trawl may not be consistently submerged. In addition, smaller mesh sizes yield higher MPL counts, especially fibres (Setälä et al., 2016; Schönlau et al., 2020). Indeed, here, 69.4±35.5% of the recorded MPL in the surface water sampled was fibrous (Table 2). Comparison with peer-reviewed data from other areas in the Baltic Sea supports the linkage between higher MPL abundance and pump sampling using filters with smaller mesh sizes. Our surface water data are in line with 0.5-9.4 MPL/L from the Helsinki archipelago surface water (20 µm pump) (Talvitie et al., 2015), 0.01-0.07 MPL/L from Northern Baltic Proper surface water (50 µm pump) (Schönlau et al., 2020) and 0.1-10 MPL/L from Baltic Proper (90 µm vertical trawl) (Gorokhova et al., 2015). Significantly lower MPL abundance in the Baltic Sea has been recorded using bigger mesh sizes in surface water sampling: 0.0001 MPL/L (≥ 300 µm pump) and ≤ 0.00004 MPL/L (333 µm trawl) in Northern Baltic Proper (Schönlau et al., 2020), 0.0001 MPL/L in the South Funen Archipelago (300 µm trawl) (Tamminga et al., 2018). Therefore, based on our results and comparison with the literature data, we support pump sampling method using filters with smaller mesh sizes for MPL abundance studies.

Land-based sources, including wastewater treatment plants (WWTPs) are one of the main routes for MPL to reach the sea. WWTPs do capture a significant proportion of MPL, but due

to large processing volumes, treated wastewater still makes a considerable contribution to aquatic MPL pollution (as recently reviewed by Freeman et al., 2020). In the vicinity of the current sampling area, WWTPs (Pärnu, Kuressaare, Haapsalu) effluents contained 0.4-0.9 MPL/L. Pärnu and Kasari rivers that drain into the Gulf of Riga and West Estonian Archipelago Sea were determined to have 0.1-0.5 MPL/L (Lips, 2020). Of the studied rivers in Estonia, the Kasari river was determined to be the most contaminated with MPL. MPL levels in effluents and river water were ≥500-fold higher than in the unvegetated areas of the receiving water bodies of the West Estonian Archipelago and the Gulf of Riga, yet comparable to the current surface water results of the seagrass beds. Importantly, for sampling effluents and rivers, a similar technique to the current study was used, only the filter mesh size was larger (333 μm Manta net).

MPL abundance in the sampled seagrass surface water is comparable to data from other areas in the Baltic Sea that have been sampled using similar techniques (pumping with small mesh size filters). The concentration of MPL fibers in the surface water of Estonian seagrass beds is similar to the majority of the recorded MPL contamination profiles in the Baltic Sea. The fact that MPL counts in surface water and sediment samples were not correlated, implies that it is a long-term process for MPL to reach to the bottom sediments from surface waters. MPL counts in sediments describe the accumulation over the longer period of time (Pinheiro et al. 2021). Blue fibres have been shown to dominate MPL occurrence within all samples (biota, water, sediment) collected from *Z. marina* beds in Scotland (Jones et al., 2020), *E. acodoides* beds' sediments in China (Huang et al., 2020) and vegetated coastal ecosystems in Portugal (Cozzolino et al., 2020). Shipping ropes have been hypothesized to be a source of fibers in MPL abundance studies (Gewert et al., 2017). In Welden and Cowie (2017), in the marine environment, polymer ropes were shown to lose 0.39-1.02% of their mass/month, yielding an average of 0.427 g MPL/meter/month.

Microplastic abundance in the sediments of the seagrass beds

The sediments of the seagrass beds had 0-131 (median 16.5) MPL per 50 ml sediment-water suspension. According to the average calculated dry weight (dwt) (72.1±9.1 g per 50 ml), this results in 0-1817 (median 208) MPL/kg dwt.

In *Z. marina* sediments in Scotland MPL contamination was 300 ± 30 MPL/kg dwt (Jones et al., 2020) and in *E. acodoides* sediments in China, values were 80 - 885 MPL/kg dwt (Huang et al., 2020). In both these cited cases, sediment MPL contamination was significantly higher than in unvegetated reference sites. In the current study, unvegetated sites in the area were not sampled, but earlier studies have also shown much lower densities at 74 and 133 MPL/kg dwt in the sediments of the Gulf of Riga and the West Estonian Archipelago, respectively (Lips, 2020), and 0-27 MPL/kg sediment (dwt) (Graca et al., 2017) and 34 ± 10 MPL/kg sediment (dwt) (Zobkov and Esiukova, 2017) in offshore ecosystems of the Baltic Sea.

Sediments are heterogeneous environments due to biophysical influences on sedimentation processes, which causes sediment stratification and irregular seafloor patterns. Despite the duplicate sediment samples taken in this study (within few meters), significant heterogeneity in the total count of MPL within each site was shown. MPL concentration often showed high variance among replicate samples, especially in heterogeneous matrices such as the sediments (Jones et al., 2020).

The results of this study showed that there was a strong correlation between the coefficient of sorting and MPL abundance in the sediments. Sorting coefficient is based on distribution of grain sizes and is a good indicator of depositional energy of the hydrodynamic regime. Well sorted sediments will have larger interstitial spaces and lower depositional energy. Poorly

sorted the opposite – these are mixed sediments with small particles filling the interstitial space between larger ones. In this study, we found that the sites with the most poorly sorted sediments were those located to the south and west of Vormsi Island (Fig. 1) and these had also the highest concentrations of MPL. This could be related to either greater deposition occurring in sites in the lee of the island most impacted by Baltic Sea storms (e.g. areas impacted by extreme weather events such as storms or rapid runoff from land during extreme precipitation events) or that there is greater retention of MPL in poorly sorted sediments due to smaller interstitial spaces and lower exchange between pore water and surrounding seawater. It was also noted that these sites had relatively high incident wave energy, although other sites with a higher incident wave energy, notably Mangu, Kõrgessaare, and the Kalana sites (Fig. 1), had more well sorted sediments and lower abundance of MPL, although these sites are unlikely to receive large volumes of eroded terrestrial sediments due to their location. Importantly, the Vormsi sites are the only sites with poorly sorted sediments that are proximal to the mainland. Therefore, the authors suggest that abundance of MPL in the sediments is likely to be linked to the proximity to the mainland (MPL source), high volume of terrestrial sediments, and the ability of the sediments to retain the MPL (e.g. those with smaller interstitial spaces).

Seagrasses strongly influence sedimentation by slowing water velocity as a result of drag from the canopy, thus increasing settling and promoting vertical accretion. This is principally influenced by available sediment from either terrestrial or marine sources, incident wave energy, and tidal currents (negligible in Estonia). The rapid accumulation of sediments in vegetated coastal systems provides an important ecosystem service by trapping and storing environmental contaminants and organic matter (Celis Hernandez et al. 2020; Lima et al. 2020; Veetil et al 2020), particularly MPL, where they may be more rapidly deposited due to changes in relative density as a result of changes in salinity, combined with biofouling and

decreases in water velocity associated to seagrass canopy density (Pinheiro et al. 2021). Cozzolino et al. (2020) reported macroplastics in vegetated coastal areas, but not in unvegetated ones, however, similar to the current research and Jones et al. (2020) no correlation between the percentage of seagrass cover and MPL counts was found.

Measures taken to avoid over- and underestimation of microplastic abundance

Although multiple official guidelines and standardized protocols (e. g. Gago et al. 2018; Frias et al., 2018) on MPL sampling have been issued, there still are no consistent methods in use (as reviewed by Hermsen et al., 2018 and Pinheiro et al. 2021). Recent quality assurance criteria, according to Koehlmans et al. (2019), requiring improvement are: sample treatment, polymer identification, laboratory preparation, clean air conditions and blank sampling.

In the current study, measures to avoid/minimize sample contamination were taken both, during the sampling as well as the laboratory analysis. To assess potential contamination of the samples during all steps of the laboratory manipulation, a series of repetitive procedural blanks was performed. Laboratory salt (NaCl ≥99.5%; Sigma Aldrich) and airborne contamination in the microscopy facility were determined the highest contamination sources in this study (contributing an average of 13 and 11 pieces of internal contamination, respectively), whereas ultrapure water was lower (average of 3 pieces). Consequently, for statistical analyses, 25 pieces of MPL was subtracted from each sample. Previous studies indicate that the dominance of microfibres may in fact be largely due to aerial contamination in laboratories (Wesch et al., 2017), but at the same time these also dominate in the marine environment (Browne et al., 2011). In our case, microfibres dominated both water and the sediment samples, being however too numerous to originate solely from laboratory air. In

agreement with other studies (Koelmans et al., 2019; Wesch et al., 2017), our results emphasize the need for an objective and earnest set of procedural blanks in the MPL studies. In addition to overestimation due to contamination, potential underestimation of MPL may occur if very small particles become trapped within the sediment and/or adhere to laboratory glassware (Erni-Cassola et al., 2017). As a countermeasure, we flushed and extracted each sediment sample twice and thoroughly rinsed the glassware with ultrapure water before and inbetween different steps of extraction and filtering. Assuming that contamination subtraction was efficient, MPL abundance correlation between the two flushes showed that using the methods described in the current study (density separation of 50 ml of sediment with 250 ml of NaCl), it is possible that not all MPL are collected from the first flush. It was however a borderline correlation and needs further data, therefore we encourage analysis of multiple sediment flushes to clarify this matter in future studies. Quinn et al., (2017) found that in the case of separation with NaCl solution, three flushes may be necessary for successful recovery. Spiking samples to evaluate MPL recovery rates and applying UV-light to detect possible loss of MPL on the glass beaker surfaces (Zobkov and Esiukova, 2017) could be used as additional control measures.

In addition to procedural blanks and double flushing of the sediments, ATR-FTIR analysis was performed to assess the efficiency of microscopy. Of the 76 particles analysed by ATR-FTIR, 20 samples were assigned to a specific polymer type on the basis of Jung et al. (2018). The rest either contained too little material to produce good spectra or the spectra consisted of a mixture of signals from multiple materials (including organic matter) and therefore no polymer types could be assigned. Nylon was assigned the most often (8/20 samples) however, due to the limited number of analyses we will refrain from further discussion on dominant polymer types.

It is highly likely that background contamination remains an underestimated factor in MPL research unless the analyses are performed in clean room and/or clean-air devices are installed (Hermsen et al., 2018; Wesch et al., 2017) or, alternatively, a conservative approach towards considering the contamination in MPL data analyses is taken as in the current study.

Conclusions

The results of the current study support the conclusions of research in other areas, that there is a potential threat of MPL accumulation in seagrass beds to a greater extent than in unvegetated marine ecosystems. However, factors, potentially leading to over-or underestimation of MPL in the samples should be carefully considered. High MPL abundance was determined in the surface water and in most of the sediments of the seagrass beds. The identified MPLs were predominantly blue and fibrous, in line with the bulk of MPL research in the marine environment. More poorly sorted sediments had the highest concentrations of MPL thus the authors suggest that abundance of MPL in the mapped seagrass fields is likely to be linked to the ability of the sediments to retain the MPL, as well as proximity and connectivity with terrestrial environments.

Acknowledgements

Funding sources

This work was supported by the Basic Financing Project of Estonian University of Life Sciences P180272 (Arvo Tuvikene and Randel Kreitsberg) and the Estonian Research Council grant PUT1512 (Margit Heinlaan). This work was also supported by the Estonian Research Council grant MOBJD243 (Raymond Ward). Richard Meitern was supported by

the Estonian Research Council grants IUT34-8, PUT653 and PSG458. Jonne Kotta was supported by the JPI Oceans project Hotmic “Horizontal and vertical oceanic distribution, transport, and impact of microplastics”. Vambola Kisand and Meeri Visnapuu were supported by the the Estonian Centre of Excellence in Research project “Advanced materials and high technology devices for sustainable energetics, sensorics and nanoelectronics” TK141 (2014-2020.4.01.15-0011).

Author statement

Randel Kreitsberg: Conceptualization, Investigation; Methodology, Writing - original draft, review & editing; **Merilin Raudna-Kristoffersen:** Methodology, Writing - original draft; **Margit Heinlaan:** Funding acquisition; Project administration; Writing - original draft, review & editing; **Raymond Ward:** Funding acquisition; Investigation; Methodology, Writing - original draft; review & editing; **Meeri Visnapuu:** Methodology, Writing - original draft; **Vambola Kisand:** Methodology, Writing - original draft; **Richard Meitern**: Formal analysis; Visualization; Writing - original draft, review & editing; **Jonne Kotta**: Writing - original draft, review & editing; **Arvo Tuvikene**: Funding acquisition; Investigation; Methodology; Project administration; Resources.

References

- Barboza, L., G., A., Gimenes, B., C., G. 2015. Microplastics in the marine environment: Current trends and future perspectives. Marine Pollution Bulletin 97, 5-12.
- Bagaev, A., Khatmullina, L., Chubarenko, I. 2018. Anthropogenic microlitter in the Baltic Sea water column. Marine Pollution Bulletin 129(2), 918-923.

- Bagaev, A., Mizyuk, A., Khatmullina, L., Isachenko, I., Chubarenko, I., 2017. Anthropogenic Fibres in the Baltic Sea Water Column: Field Data, Laboratory and Numerical Testing of Their Motion. Science of the Total Environment, 599-600, 560-571.
- Beer, S., Garm, A., Huwer, B., Dierking, J., Nielsen, T.G. 2018. No increase in marine microplastic concentration over the last three decades – A case study from the Baltic Sea. Science of The Total Environment, 621, 1272-1279.
- Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R. 2011. Accumulation of Microplastic on Shorelines Worldwide: Sources and Sinks. Environmental Science and Technology, 45, 21, 9175–9179.
- Celis-Hernandez, O., Giron-Garcia, P.M. Ontiveros-Cuadras, J., Canales-Delgadillo, J., Pérez-Ceballos, R., Ward, R.D., Acevedo-Gonzales, O., Merino-Ibarra, M. 2020. Environmental risk of heavy metals in mangrove ecosystems: An assessment of natural vs oil and urban inputs. Science of the Total Environment, 730, 138643.
- Cozzolino, L., Nicastro, K.L., Zardi, G.I., de los Santos, C.B. 2020. Species-specific plastic accumulation in the sediment and canopy of coastal vegetated habitats. Science of The Total Environment, 723, 138018.
- Erni-Cassola, G., Gibson, M.I., Thompson, R.C., Christie-Oleza, J.A. 2017. Lost, but found with nile red: a Novel method for detecting and quantifying small microplastics (1 mm to 20 mum) in environmental samples. Environmental Science and Technology, 51, 13641-13648.
- Freeman, S., Booth, A.M., Sabbah, I., Tiller, R., Dierking, J., Klun, K., Rotter, A., Ben-David, E., Javidpour, J., Angel, D.L. 2020. Between source and sea: The role of wastewater treatment in reducing marine microplastics. Journal of Environmental Management, 266, 110642.

- Frias, J., Pagter, E., Nash, R., O'Connor, I., Carretero, O., Filgueiras, A., Viñas, L., Gago, J., Antunes, J., Bessa, F., Sobral, P., Goruppi, A., Tirelli, V., Pedrotti, M.L., Suaria, G., Aliani, S., Lopes, C., Raimundo, J., Caetano, M., Palazzo, L., de Lucia, G.A., Camedda, A., Muniategui, S., Grueiro, G., Fernandez, V., Andrade, J., Dris, R., Laforsch, C., Scholz-Böttcher, B.M., Gerdts, G. 2018. Standardised protocol for monitoring microplastics in sediments. JPI-Oceans BASEMAN project.
- Gago, J., Filgueiras, A., Pedrotti, M.L., Suaria, G., Tirelli, V., Andrade, J., Frias, J. Nash, R., O'Connor, I., Lopes, C., Caetano, M., Raimundo, J., Carretero, O., Viñas, L., Antunes, J., Bessa, F., Sobral, P., Goruppi, A., Aliani, S., Palazzo, L., de Lucia, G.A., Camedda, A., Muniategui, S., Grueiro, G., Fernandez, V., Gerdts, G. 2018. Standardised protocol for monitoring microplastics in seawater. JPI-Oceans BASEMANproject.
- Gewert, B., Ogonowski, M., Barth, A., MacLeod, M. 2017. Abundance and composition of near surface microplastics and plastic debris in the Stockholm Archipelago, Baltic Sea. Marine Pollution Bulletin, 120 (1-2), 292-302.
- Gorokhova, E. 2015. Screening for microplastic particles in plankton samples: How to integrate marine litter assessment into existing monitoring programs? Marine Pollution Bulletin, 99, 271-275.
- Goss, H., Jaskiela, J., Rotjan, R. 2018. Thalassia testudinum as a potential vector for incorporating microplastics into benthic marine food webs. Marine Pollution Bulletin, 135, 1085-1089.
- Graca, B., Szewc, K., Zakrzewska, D., Dołęga, A., Szczerbowska-Boruchowska, M. 2017. Sources and fate of microplastics in marine and beach sediments of the Southern Baltic Sea—a preliminary study. Environmental Science and Pollution Research, 24, 7650–7661.

- Gutow, L., Eckerlebe, A., Giménez, L., Saborowski, R. 2016. Experimental Evaluation of Seaweeds as a Vector for Microplastics into Marine Food Webs. Environmental Science and Technology, 50, 915−923.
- Hermsen, E., Mintenig, S.M., Besseling, E., Koelmans, A.A. 2018. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. Environmental Science and Technology, 52(18), 10230–10240.
- Huang, Y., Xiao, X., Xu, C., Perianen, Y.D., Hu, J., Holmer, M. 2020. Seagrass beds acting as a trap of microplastics - Emerging hotspot in the coastal region? Environmental Pollution, 113450.
- Jones, K.L., Hartl, M.G.J., Bell, M.C., Capper, A. 2020. Microplastic accumulation in a Zostera marina L. bed at Deerness Sound, Orkney, Scotland. Marine Pollution Bulletin, 152, 110883.
- Jung, M.R., Horgen, F.D., Orski, S.V., Rodriguez, C.V., Beers, K.L., Balazs, G.H., Jones, T.T., Work, T.M., Brignac, K.C., Royer, S.-J., Hyrenbach, K.D., Jensen, B.A., Lynch, J.M. 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. Marine Pollution Bulletin, 127, 704-716.
- Koelmans, A.A., Nor, N.H.M., Hermsen, E., Kooi, M., Mintenig, S.M., De Franced, J. 2019. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Research, 155, 410–422.
- Lamb, J.B., van de Water, J.A.J.M., Bourne, D.G., Altier, C., Hein, M.Y., Fiorenza, E.A., Abu, N., Jompa, J., Harvell, C.D. 2017. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. Science, 355, 731–733.
- Lima, M., Ward, R., Joyce, C. 2020. Environmental drivers of carbon stocks in temperate seagrass meadows. Hydrobiologia, 847, 1773–1792.

- Lind, K., Turov, P., Lips, I. 2019. Public sector (Estonia) project “Mikroprügi sisalduste uuring merevee pinnakihist ja mere põhjasetetest 2018. aastal”, Tallinn University of Technology, pp. 51.
- Lips, I. 2020. Public sector (Estonia)" project LMIN18023 “Sources and pathways of microplastic in the Estonian coastal sea, potential impact to pelagic and benthic organisms” (23.03.2018−4.05.2020), Tallinn University of Technology.
- Möller, T., Kotta, J., Martin, G. 2014. Spatiotemporal variability in the eelgrass Zostera marina L. in the north-eastern Baltic Sea: canopy structure and associated macrophyte and invertebrate communities. Estonian Journal of Ecology 63(2), 90-108.
- Pinheiro, L.M., Agostini, V.O., Lima, A.R.A., Ward, R.D., Pinho, G.L.L. (2021). The fate of plastic litter within estuarine compartments: knowledge gaps for the transboundary issue and guidelines for future assessments. Water Research. In Press.
- Quinn, B., Murphy, F., Ewins, C. 2017. Validation of density separation for the rapid recovery of microplastics from sediment. Analytical Methods, 9, 1491.
- Rani-Borges, B., Moschini-Carlos, V., Pompêo, M. 2021. Microplastics and freshwater microalgae: what do we know so far? Aquatic Ecology, https://doi.org/10.1007/s10452-021-09834-9
- Schönlau, C., Karlsson, T.M., Rotander, A., Nilsson, H., Engwall, M., van Bavel, B., Kärrman, A. 2020. Microplastics in sea-surface waters surrounding Sweden sampled by manta trawl and in-situ pump. Marine Pollution Bulletin, 153, 111019.
- Seng, N., Lai, S., Fong, J., Saleh, M.F., Cheng, C., Cheok, Z. Y., Todd, P.A. 2020. Early evidence of microplastics on seagrass and macroalgae. Marine and Freshwater Research, https://doi.org/10.1071/MF19177.

- Setälä, O., Magnusson, K., Lehtiniemi, M., Noren, F. 2016. Distribution and abundance of surface water microlitter in the Baltic Sea: A comparison of two sampling methods. Marine Pollution Bulletin 10(1), 177-183.
- Talvitie, J., Heinonen, M., Pääkkönen, J-P., Vahtera, E., Mikola, A., Setälä, O., Vahala, R. 2015. Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. Water Science & Technology, 72(9), 1495-1504.
- Tamminga, M., Hengstmann, E., Fischer E.K. 2018. Microplastic analysis in the South Funen Archipelago, Baltic Sea, implementing manta trawling and bulk sampling. Marine Pollution Bulletin 128, 601-608.
- Veettil, B., Ward, R.D., Lima, M., Stankovic, M., Quang, N. 2020. Opportunities for seagrass research derived from remote sensing: a review of current methods. Ecological Indicators, 117, 106560.
- Welden, N.A., Cowie, P.R. 2017. Degradation of common polymer ropes in a sublittoral marine environment. Marine Pollution Bulletin 118(1-2), 248-253.
- Wesch, C., Elert, A.M., Wörner, M., Braun, U., Klein, R., Paulus, M. 2017. Assuring quality in microplastic monitoring: About the value of clean-air devices as essentials for verified data. Scientific Reports, 7, 5424.
- Zobkov, M.B., Esiukova, E. 2017. Microplastics in Baltic bottom sediments: Quantification procedures and first results. Marine Pollution Bulletin, 114(2), 724-732.

Supplementary Information (SI):

Table 3. Plastic types in randomly selected samples and blanks analysed by FTIR

Plastic type	Blank	Sample
Ethylene vinyl acetate	0	2
Latex	0	1
Nylon	2	8
Polyethylene	0	1
Polyethylene terephthalate	0	3
Polypropylene	0	3

Table 4. Microplastics size and shape by area and sample type.

	water per 100 L												
	color							shape					
area	black	blue	red	transp	green	multicol	other	angular	fiber	film	other	uncharact	total
Soela	0.6	5	0	0	0	0	0	0	5.6	0	0	0	5.6
Kalana	0	10.6	0	1	0	2	2.2	0	13.1	2.7	0	0	15.9
Kehila	2.2	96.1	0.6	8.9	0	0	12.2	2.2	112.2	5.6	0	0	120
Kõrgesaare	0	3.7	0	0	0	0	0	0	3.2	0.5	0	0	3.7
Lõu	0.4	0	0.8	0	0	0	0	0	0.4	0	0	5.8	7.1
Mangu	0	11.5	0.4	0	0	0	1.7	0	10.7	1.7	1.3	0	13.7
Rannaküla	1.7	2.2	0.6	0	0	0	0.6	0	1.7	1.7	1.7	0	5
Sepamaa	1.7	17.8	0	0.6	0	0	7.2	2.2	15	10	0	0	27.2
Vormsi	0.5	21,0	0	2.2	0	0.4	0.5	0.4	19.9	2.5	1.8	0	24.6
	sediment per 50 mL												
	color							shape					
area	black	blue	red	transp	green	multicol	other	angular	fiber	film	other	uncharact	total
Soela	0	9	0	0	0	0	2	0	10	1	0	0	11
Kalana	1	20	0	4	0	0	6	0	30	0	1	0	31
Kehila	0	16	1	1	0	0	2	1	19	0	0	0	20
Kõrgesaare	1	13	0	2	0	0	2	0	18	0	0	0	18
Lõu	0	5	0	0	0	0	1	0	6	0	0	0	6
Mangu	1	7	0	3	0	0	4	0	15	0	0	0	15
Rannaküla													
Sepamaa	0	6	0	0	0	0	0	0	6	0	0	0	6
Vormsi	0	110	0	4	1	0	16	0	129	1	1	0	131

Table 5. Sediment data. Columns representing (starting from left): site name; site latitude; site longitude; pH; oxygen concentration (mg/L); temperature (°C); average grain size; sorting coefficient; soil organic matter (%); cover of seagrass (%); cover of other higher plants (%); cover of algae (%); dry bulk density; sediment composition: % of clay, silt, and sand; depth; exposure class and exposure to waves.

site	lat	lon	pH	O2_mg/l	temp	d50	S_0	%SOM	% cover seagrass	% cover other higher plants	%cover algae	Bulk density	%Clay	%Silt	% Sand	Depth	Exposure class	Exposure to waves
Rannakõla 1	58.6275	22.8798	8.36	9.79	17				60	10	5					3.3	52632	50000
Rannakõla 2	58.6279	22.8669	8.27	9.85	17.2	373.2	2.06	1.33	60	10	0	1.69	0.9801	6.6341	92.385	4	52488	50000
Soela	58.6104	22.6166	8.73	9.51	17.5	352.5	1.46	0.02999	19	0	29	1.33362	0	7.6946	92.305	5	45087	50000
Lõu	58.1057	22.1721	8.57	10.41	16.5				36	0	38					4.3	260754	260000
Sepamaa	58.2121	22.5599	8.58	9.41	17.4				14	5	3					3.3	60418	60000
Kehila 2	58.4422	22.0638	8.75	9.68	18.4	316.7	1.41	0.04199	86	0	6	1.44508	0.0152	0.4239	99.560	3.4	44485	40000
Kehila 1	58.4344	22.0709	8.75	9.94	19	1195.	1.97	0.06866	45	0	0	1.46180	0.1627	1.7472	98.090	3.5	44239	40000
Kalana 1	58.91971	22.05872	8.51	11.34	14.2	117.1	1.54	3.15075	95	0	4	1.44552	1.3157	11.732	86.951	2.3	453814	450000
Kalana 2	58.9199	22.05879	8.42	11.99	14.2	115.3	1.53	3.51	18	2	1	1.28	0.7905	12.084	87.125	2.9	453814	450000
Kõrgesaare	58.99923	22.4652	8.6	10.9	19.6	498.7	1.50	0.194	57	11	9	1.66195	0.0768	2.0301	97.892	2.9	191404	190000
Mangu	59.0356	22.56465	8.51	10.23	19.5	184.0	1.76	0.66030	62	3	3	1.55226	0	2.8440	97.155	3.1	188061	190000
Vormsi 1	58.98271	23.14388	8.21	10.93	5.7	319.9	4.56	2.74369	15	0	0	1.18845	7.4755	19.507	73.016	3.3	161439	160000
Vormsi 2	58.9525	23.231567	9.27	9.16	20.2	305.0	2.73	1.81014	17	0	57	1.56737	3.9645	18.697	77.337	4.2	49919	50000
Vormsi 3	58.94185	23.254533	9.31	9.32	21.5	572.2	1.42	0.58	32	0	1	1.65	0.0523	0.6983	99.249	2.6	52862	50000
Vormsi 4	58.93482	23.25764	9.31	10.03	18.9	341.6	3.66	2.96589	20	0	0	1.44688	5.6362	16.361	78.002	3.2	65984	70000