



# Microplastics in Baltic bottom sediments: Quantification procedures and first results

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## ABSTRACT

Microplastics in the marine environment are known as a global ecological problem but there are still no standardized analysis procedures for their quantification. The first breakthrough in this direction was the NOAA Laboratory Methods for quantifying synthetic particles in water and sediments, but fibers numbers have been found to be underestimated with this approach. We propose modifications for these methods that will allow us to analyze microplastics in bottom sediments, including small fibers. Addition of an internal standard to sediment samples and occasional empty runs are advised for analysis quality control. The microplastics extraction efficiency using the proposed modifications is  $92 \pm 7\%$ . Distribution of microplastics in bottom sediments of the Russian part of the Baltic Sea is presented. Microplastic particles were found in all of the samples with an average concentration of  $34 \pm 10$  items/kg DW and have the same order of magnitude as neighbor studies reported.

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## 1. Introduction

At present plastic is the one of the most demanded industrial materials. Its physical and chemical properties provide durability, lightness, and longevity coupled with low prime cost. So plastic becomes an inherent part of most household goods, structures, and machinery. Contemporary worldwide plastic production is about 275–299 Mt per year (Rilling, 2012; Plastics Europe, 2014), while volumes of recycling and using plastic waste for energy production are incommensurably lower. Most of plastic waste is disposed to landfills (Barnes et al., 2009) and still a huge amount of it appears in the environment.

In Europe, annual plastic production in 2013 was 57 Mt, and approximately 25.2 Mt of the post-consumer plastic waste was disposed. About 62% of plastics waste was recycled but residual 9.6 Mt went to landfills (Plastics Europe, 2014). In Russia, plastic waste recycling rate is only 3–4% and most of plastic litter is buried to landfills or is thrown to illegal dumps (Kasyanenko and Filimonov, 2013).

When plastic waste is exposed to environmental conditions, it begins to degrade slowly (Tosin et al., 2012). A huge number of macro-, micro-, and nano-particles of plastic are generated in this process. These particles are called microplastics and potentially are the most dangerous for the environment (Andrady, 2011; Kershaw, 2015). Plastic waste and its particles carried out by runoff and wind reach water bodies. The density of the majority of plastics is close to that of water, and due to this plastic waste is transported along rivers, lakes, seas, and even the World Ocean (Rilling, 2012). Shipping, fishing and other

human activities in oceans and seas are also known as potential sources of plastic pollution (UNEP, 2016). Raw pellets and granules from post-consumer products and other plastics that come into environment in intact form are called primary microplastics (Andrady, 2011; UNEP, 2016).

Nowadays, there is still no clear definition of size ranges of microplastic particles, but in most cases microplastics are regarded as polymer particles less than five mm long (Arthur et al., 2009) and we use the same classification in our study.

Although plastic particles of different polymer types could be heavier or lighter than water and might be able to sink or float over its surface, many of them become negatively buoyant and sink to the sea floor as a result of fouling by organisms or adherence of denser particles (Lobelle and Cunliffe, 2011; Morét-Ferguson et al., 2010). Recent studies concluded that coastal submarine canyons act as conductors for transporting marine debris into the deep-sea areas (Ioakeimidis et al., 2014). Overall, the seabed becomes the ultimate repository for microplastic particles (Barnes et al., 2009). There is evidence that deep-sea sediments are likely to be the major sink for microplastic in the form of fibers (Woodall et al., 2014).

However, two challenges were revealed during our studies of Baltic seafloor sediments near Russian coasts. On the one hand, testing and implementing of the most elaborated analytical method into laboratory workflow was essential. On the other hand, comparison of the obtained results with other studies of the Baltic Sea was required. Both of the two challenges will be addressed further.

The Baltic Sea lacks a permanent system of currents and tides, so there might be hot spots of sea-bed litter (MONAS, 2014). Along with macro litter, there could also be local accumulation areas of microplastics all over the sea bottom. The fibers are found to be the

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most abundant fraction of microplastics in the Baltic Sea (MONAS, 2014). Studies of microplastic in the sediments of the Baltic Sea are rare. Spatial data are only available for its concentrations in beach sediments along German coast (Stolte et al., 2015; Stolte, 2015) and Kaliningrad region (Esiukova, 2016). There were publications reporting investigations of effluent water from sewage treatment plants as pathways for microscopic anthropogenic particles to marine systems in the Swedish and Finnish waters and some associated bottom sediments in hot spots (Magnusson et al., 2016; Talvitie et al., 2015). Microplastic studies in the North Sea are systematic and widely spread (Claessens et al., 2011; Dekiff et al., 2014; Norén, 2007; Leslie et al., 2013; Strand and Tairova, 2016; Löder et al., 2015) but quantitative comparison with most of them becomes problematic due to different analysis procedures and measuring units (Hidalgo-Ruz et al., 2012).

Sample analysis of the beach and bottom sediments is slightly different, but commonly consists of several steps: sieving, drying, density separation, organic matter oxidation, filtration, and visual sorting (Masura et al., 2015; Hidalgo-Ruz et al., 2012). The majority of studies use a method introduced by Thompson et al. (2004), with different improvements. The method is based on the fact that bottom sediments have significantly higher specific density ( $2.65 \text{ g/cm}^3$ ) than most of plastics ( $0.05\text{--}1.70 \text{ g/cm}^3$  (Chubarenko et al., 2016)) and this difference is used to separate comparatively light plastics from heavy sediments. Most frequently used separating liquids are saturated NaCl solution, sodium polytungstate, lithium metatungstate,  $\text{ZnCl}_2$ ,  $\text{CaCl}_2$ , NaI and tap or sea water in some cases (Hidalgo-Ruz et al., 2012; Masura et al., 2015; Claessens et al., 2013). Organic matter oxidation is frequently used to remove organic material from the separated extract and to clean microplastic particles from biofouling material. Biofouling of plastic particles makes them hard to distinguish under microscope observation. Acid, alkali, hydrogen peroxide, enzymes, and other oxidizers are also frequently used to digest organic material (Claessens et al., 2013; Cole et al., 2014; Nuelle et al., 2014).

Sample contamination occurring from air dust, wearing and laboratory glassware may lead to overestimation of microplastic concentrations during analysis (Hidalgo-Ruz et al., 2012; Cauwenberghe et al., 2013; Browne et al., 2011). Different mesh sizes and filter pores used for filtering of solutions also introduce uncertainties into the comparison process.

That is why one of the most significant problems during microplastic pollution assessment in marine environment is lack of standardized procedures for sample collection, preparation, and microplastic identification. Elaborating of those standardized procedures is vital for microplastic concentrations assessment and data comparison between different studies.

One of the first breakthroughs in this regard is the National Oceanic and Atmospheric Administration Laboratory Methods for quantifying synthetic particles in water and sediments (hereafter – NOAA laboratory methods) (Masura et al., 2015). In brief, the method consists of two sequential density separation steps and wet oxidation with hydrogen peroxide between them. Microplastic particles are detected with stereomicroscope. In this paper, we make an attempt to implement this method into laboratory workflow with the Baltic bottom sediments. Although this routine is thoroughly elaborated, during the analysis some procedures were found not entirely applicable to our samples.

The aim of this study is to propose an improvement of the NOAA method, in order to extract not only the particles but also the fibers, to integrate the quality control analysis into the laboratory workflow, and to facilitate the extraction procedures. The proposed improvements were put in practice and tested while analyzing samples of the Baltic Sea shoreface and offshore sediments.

The first evaluations of microplastic concentrations of bottom sediments for the Russian part of the Baltic Sea region are presented. Thanks to similar extraction technology and the same units reported, we are able to provide quantitative comparison with the North Sea data by Claessens et al. (2011).

## 2. Materials and methods

### 2.1. Study area and sample collection

Bottom sediment samples were collected on 9 October 2015 in the South-Eastern part of the Baltic Sea near the Baltiysk Strait inlet (Fig. 1). Permanent water exchange between the Vistula Lagoon and the Baltic Sea exist via the Baltiysk Strait (400 m wide and 6 to 12 m deep). The Vistula Lagoon is one of the largest lagoons of the Baltic Sea. Discharge of lagoon waters into the sea is about  $20.7 \text{ km}^3$  per year (Chubarenko et al., 2012). The main river of the lagoon is the Pregolya, with its discharge of about  $2.7 \text{ km}^3$  per year, total watershed area of  $14,783 \text{ km}^2$  and population of 1.28 million people (Nilsson, 2006). Lots of potential contamination point sources are located upstream and along the lagoon coast including businesses, factories, wastewater treatment plants, fish-processing facilities, and ports belonging to the towns of Kaliningrad, Baltiysk and Svetliy. The Kaliningrad Marine Canal is the navigation pass from the Baltiysk Strait to the city of Kaliningrad. The lagoon is intensively used for shipping and fishing. This is the reason why the Baltiysk Strait region is expected to be highly contaminated with microplastics.

The calm and warm weather with light Eastern wind ( $<5 \text{ m/s}$ ) prevailed during one week before sampling.

Sediment samples were collected at seven stations under calm water conditions from the shore face out to 13 km offshore from the board of a small vessel (Fig. 1). Sampling depth varied from 3 m to 30 m. At each station (named GbXXm, meaning “the Gdansk Bay, depth XX m”), the samples (10 l in volume each) were taken by means of a rectangular hand-operated dredge with mouth size of  $200 \times 100 \text{ mm}$  outfitted with replaceable cotton sample bags.

Samples were transported in cotton bags and buckets and later were transferred into aluminum cans in a laboratory. Before the analysis, samples were homogenized at the laboratory by intensive stirring with a stainless steel spoon. After stirring, the subsamples were taken. The same stirring procedure was applied by Leslie et al. (2013). A 400 g-subsample was used for microplastic analysis and a second 400 g-subsample was used for determination of sediment dry weight and grain size distribution.

### 2.2. The analysis procedure: An improved NOAA method

The microplastic analysis in the sediments was performed using modified NOAA laboratory methods. The analysis consisted of five main steps: extraction, wet peroxide oxidation, calcite fraction digestion, density separation, and microplastics detection.

#### 2.2.1. Extraction

The extraction step was aimed to separate microplastic particles, organic matter and other lighter fractions (total solids) from heavier sediments. It was modified from section 3.5 of the NOAA method (Masura et al., 2015). In detail (Fig. 2), 300 ml of  $\text{ZnCl}_2$  separation solution (55%; specific density  $1.6 \text{ g/ml}$ ) were added into a 800 ml glass beaker containing 400 g of wet sediment and then stirred for 10 min with a stainless steel spoon. After 1 h of settling, the supernatant was filtered through a filter funnel. The filter funnel was made from 150 mm glass funnel and a  $20 \times 20 \text{ cm}$  polyamide net with  $0.174 \text{ mm}$  mesh. The net was coned, placed into the glass funnel and fastened with a paper clip. All floating solids were poured on to the net by inclination of the beaker. The beaker walls were rinsed by zinc chloride solution twice using the squirt bottle. The neutrally buoyant material that remained in the beaker above the sediment was separated in the same way, and was analyzed simultaneously with the supernatant. The net was rinsed with distilled water to wash out the separation solution. The net was removed from the filter funnel and total solids from the net surface were rinsed with distilled water into a 600 ml glass beaker.

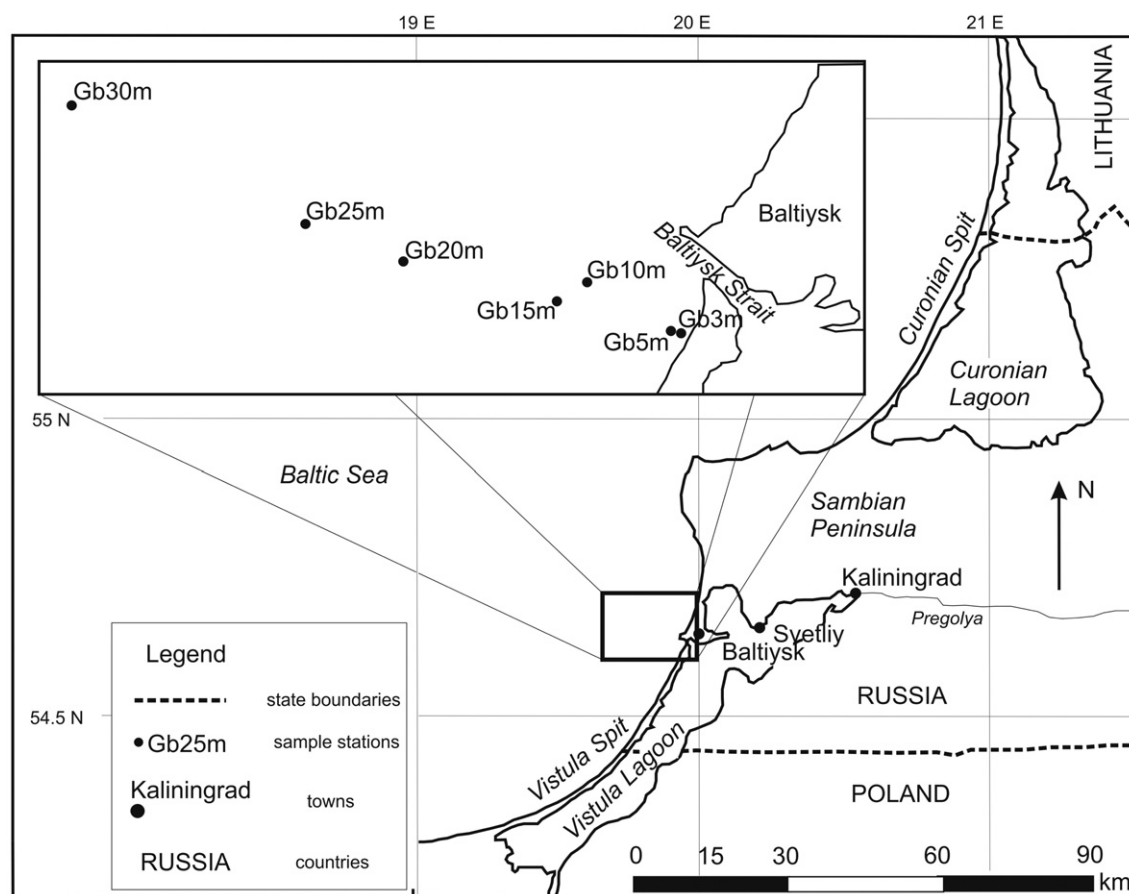


Fig. 1. Locations of sampling sites.

### 2.2.2. Wet peroxide oxidation

The wet peroxide oxidation step was performed in order to remove natural organic material. The step followed the procedures of Section 3.7 of the NOAA method (Masura et al., 2015), but a water bath was used instead of a hot plate for safety reasons. 25 ml of 30% hydrogen peroxide and 25 ml of Fe(II) catalyst solution were added into the 600 ml glass beaker containing total solids. The beaker was placed onto a water bath and heated up to 75 °C. Fe(II) catalyst solution was prepared according to (Masura et al., 2015). An additional portion of hydrogen peroxide was added into the beaker if undissolved organic matter remained. After all of the organic matter was dissolved, the beaker was removed from the water bath. The beaker was covered with aluminum foil and left for 15 h. All the solids were filtered through the filter funnel and rinsed with distilled water to wash out reagents.

### 2.2.3. Calcite fraction digestion

Acidity of  $\text{ZnCl}_2$  solution was found to be insufficient to digest mussel shells and their fragments that had been found in great numbers in our sediment samples. To digest them completely, a new step was implemented. It consisted of adding 25 ml of 4.5% HCl solution into the beaker immediately after the wet peroxide oxidation step.

### 2.2.4. Density separation

In this step, microplastic particles were separated from mineralized organic matter using density separator. It was made from a glass funnel with a segment of latex tubing on the bottom of the stem and a pinch clamp attached to control liquid flow from the funnel (Masura et al., 2015). The step generally followed the procedures

of Section 3.8 NOAA method (Masura et al., 2015).  $\text{ZnCl}_2$  solution, same as in the first separation step, was applied for microplastic extraction. After 1 h of settling, the sediment and the lower half of the solution were drained off. Settled solids were discarded. Floating solids were drained into the filter funnel. To replace floated particles that were adhered to separator walls, they were rinsed twice with 0.5 l of distilled water. The filter net containing the floated solids was rolled up, tied and placed into a Petri dish. The filter was dried at room temperature for 24 h.

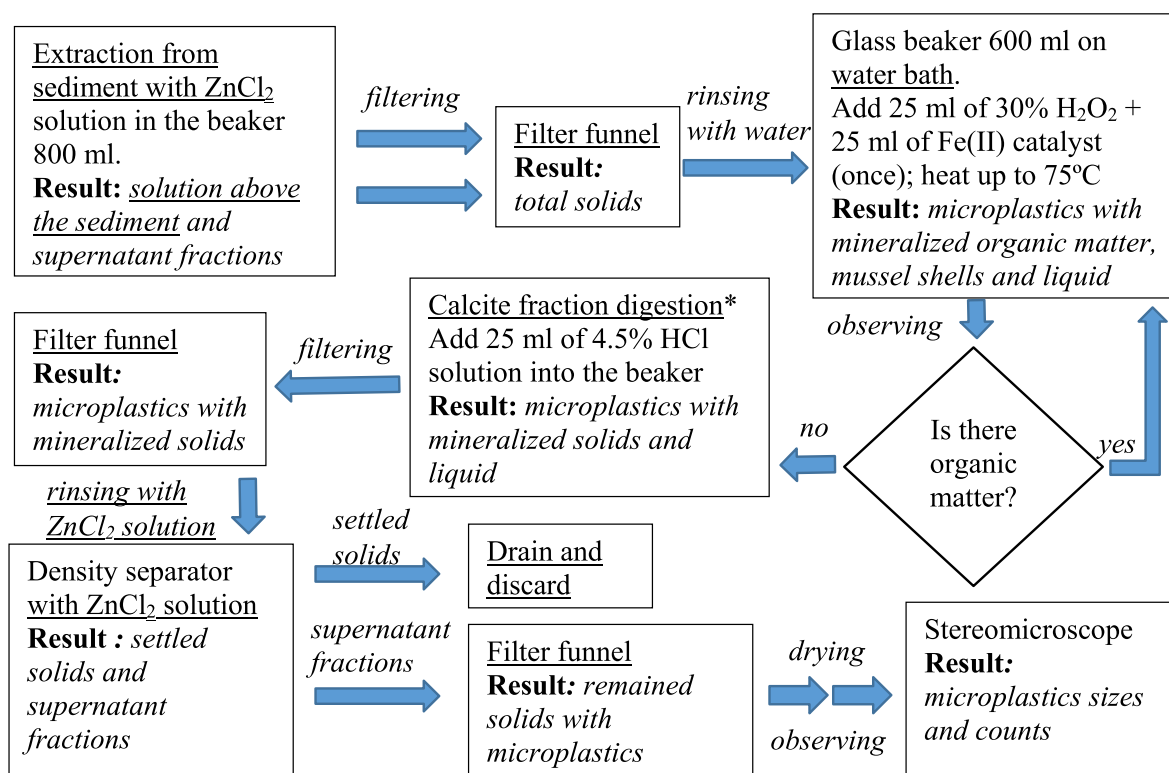
### 2.2.5. Microplastics detection

Microplastic particles were detected under stereomicroscope (Micromed MC2 Zoom Digital) with magnification from 10× to 40× directly on the net surface according to recommendations for microscopic determination (Norén, 2007).

The extracted microplastic particles were classified into three groups: fragments, films, and fibers. Fragments were pieces of thick plastic with all three size dimensions comparable. Films were sheets of plastic bags and other similar stuff, with their thickness significantly lower than other two dimensions. Fibers were identified as thin elongated particles with one dimension significantly greater than the other two.

Sediment grain size determination was performed using the sieve cascade with mesh sizes 2.5, 2.0, 1.5, 1.0, 0.5, 0.175 mm followed by weighting sediment in each size fraction. Grain size statistics was calculated using logarithmic method of moments (Blott and Pye, 2001).

We validated the modified method through determination of extraction efficiency and our results were then compared with the results of the original approach.



**Fig. 2.** The method for microplastic sizes and counts determination in sediment samples. The main steps involved: extraction, wet peroxide oxidation, calcite fraction digestion, density separation, and microplastics detection. Apparatus and materials: stereo microscope, water bath, glass beakers 800 ml and 600 ml, 150 mm glass funnel, Petri dish, stainless steel spoon, squirt bottle (2 pieces), density separator, polyamide nets (3 pieces), aluminum foil. Reagents: ZnCl<sub>2</sub> separation solution, Fe(II) catalyst solution, 30% hydrogen peroxide, 4.5% HCl solution, distilled water. Modifications made to NOAA methods are underlined. \* - calcite fraction digestion was involved later and was not applied during samples procession, presented in this paper.

### 2.3. Method validation and contamination sources

In order to assess recovery rates of the proposed method, we used artificial reference particles (ARPs) that were added as internal standard to the known mass of natural sediment before the first extraction step. ARPs were made from a sheet of fluorescent polyethylene terephthalate (PET) bottle with thickness of  $0.46 \pm 0.02$  mm ( $p = 0.05$ ;  $n = 40$ ). The shape of the ARPs was rectangular, with each side of  $0.90 \pm 0.39$  mm ( $p = 0.05$ ;  $n = 40$ ). Fluorescence nature of particles and their obviously artificial shape allowed us to be confident that they would not be confused with microplastics from natural sediments. It also allowed finding possible ways of losing analytical material during analysis. This could be done with the help of a UV-lamp, because fluorescence particles even in micro size range were clearly distinguishable with the naked eye from any other material under the UV-light. We used the UV-lamp Philips TL 8 W/08 BLB FAM to find the ways of possible loss of particles on the glass beaker surfaces and other laboratory glassware.

Even though no clean room was available for this study, all clothes in the lab were made of cotton and the materials used for analysis were made of glass or stainless steel where possible.

To assess potential contamination rates and sources, two approaches were used: empty run and lab air contamination evaluation. During the empty run, the analysis was implemented according to foregoing procedures, but using a separating solution only, without adding of sediment sample. The extract was analyzed under stereomicroscope to evaluate possible sample contamination.

The lab air is one of the most probable sources of sample contamination. To assess its rates, we implemented the technique proposed by A. Stolte (2015). The technique consisted of drawing of laboratory air through a preliminarily cleaned paper filter by means of a vacuum filtering system for 2 h and then its consequent analysis under a

stereomicroscope. Diaphragm vacuum pump (capacity 20 l/min, power 160 W) was used for these purposes. The filtering system was equipped with three separate filters.

### 2.4. Data analysis

To understand patterns of microplastics spatial variations, the correlation analysis was performed. The relation between concentrations of microplastics types (items/kg DW) and sediment grain size characteristics (grain size and sorting) was obtained for this means.

Relative confidence intervals were calculated for extraction efficiency and rate of sunken fibers in separation solution where possible. The rate of sunken fibers was calculated as a ratio of number of particles sunken in separation solution to a total number of fibers extracted from the sample. Then the relative confidence interval for different samples was calculated.

The arithmetic mean and standard deviation of the mean was calculated for the near shore area (3–10 m deep) and the off shore location (20–30 m deep). The microplastic concentration at 15 m deep was not used for these values calculation because it differed considerably from the observed in these two locations. The arithmetic mean and the confidence interval calculated over all the samples data were presented as the average microplastic concentration at the studied area.

It was difficult to compare the results from different studies because different measuring units were reported. To convert data of (Talvitie et al., 2015) into dry weight units, the average wet sediment/dry sediment ratio 1.25 (Claessens et al., 2011) was used. The data of (Strand et al., 2013), reported in items/10 g DW, was converted into items/kg DW and average values for similar locations were calculated. The first value was obtained for Arcona and Hjelm Bugt locations, and the second – for Fehmarn Bael, Syd for Hven, Vejro and Koge Bugt locations. Standard



deviation of the mean was given where no confidence intervals were available.

### 3. Results and discussion

#### 3.1. Modifications to NOAA procedures

Although the NOAA laboratory methods are thoroughly elaborated, some procedures were found not entirely applicable during the analysis of our samples. Some uncertainties and methodological faults concerning microplastic analysis in beach and bottom sediments were also found.

Fibers were found to be the most abundant fraction of microplastic (MONAS, 2014). But due to multiple sievings with 0.3 mm meshes most of fibers and particles <0.3 mm were lost during the analysis procedure (Sections 3.3, 3.5 and 3.8 of NOAA methods). Instead of sieves with 0.3 mm mesh size, we used a filter funnel with mesh size of 0.174 mm. This mesh size was chosen for two reasons: it is quite enough to prevent losing the majority of fibers and provide fast filtration speed under gravity force. Although particles with sizes less than the mesh size are still lost, they give low detection confidence for microscopic detection in any case (Song et al., 2015). So we considered the loss of particles <0.2 mm to be insignificant for our study. Our test showed that when a cascade of two 0.174 mm filter nets was used, the second net filter would remain empty in most cases (with the exception of a few very small fibers).

One more limitation to the NOAA laboratory methods was found in flotation procedure steps. Some plastic particles did not pass the first separation step (Section 2.3 of NOAA methods) due to sand adsorption or biofouling. The method advised to collect them with forceps, using naked eye detection, but since most of microplastic particles have dimensions <1 mm (Duis and Coors, 2016; Hidalgo-Ruz et al., 2012; Song et al., 2015), they were hardly distinguishable on a sand surface. It was nearly impossible to find fibers this way. Hence, naked eye detection brought ambiguity into the analysis process. In our study this problem was solved by including a separate analysis procedure of the solution remaining in the beaker above the sediment. The similar technique was applied in (Stolte, 2015).

An analogous situation was observed with the second separation step. Here, the NOAA method suggested using NaCl solution with specific density of 1.15 g/ml instead of lithium metatungstate solution with specific density of 1.6 g/ml (Hidalgo-Ruz et al., 2012), that was applied in the first separation step (Sections 1.5, 1.6, 2.5, 2.6, 3.7 and 3.8 of NOAA methods). As a result, the settled solids had to be inspected with naked eye again, because the particles that had been successfully separated on the first separation step, sank. We solved this problem by using the same separation liquid as in the first separation step.

Another issue was discovered in the rinsing procedure of the beaker, when solids were transferred from it (Sections 1.6, 2.3, 2.6, 3.5 and 3.8 of NOAA methods). The method advised to use distilled water to rinse particles from the beaker walls, but this decreased the density of the separation liquid and resulted in sinking of particles that had already been afloat. We substituted distilled water with the same separation liquid and used a squirt-bottle for rinsing.

The NOAA laboratory methods required weighing sediment or beach samples with mass of 400 g as well as microplastic extract with an accuracy of 0.1 mg (Sections 2.2 and 3.2 of NOAA methods). This precision might be necessary only to determine dry weight in some cases and weight of total solids (floated microplastic and organic matter together). In our opinion, weighing sediment samples with this precision was excessive in this case and required special analytical balances with the upper limit of 500 g or more, that are rare and very expensive. Besides, this determination of total solids weight was not essential and might be required only in some specific studies. We supposed that using the laboratory balance with an accuracy of 0.1–0.01 g was quite enough to weigh sediments. In addition, it sped up the analysis procedure. In

this case, the precision of 0.1 g obtained for the mass of 100 g during sediment weighing would be equivalent to precision of 0.1 mg obtained for the mass of 100 mg during microplastic weighing. Related error value obtained during concentration calculations would be of the same order of magnitude.

Results of microplastics weighing are not provided in this paper because of the very small nature of fibers and fragments observed. Moreover, these particles are hard to transfer into the vial for weighing. However, our revisions do not exclude conducting of those measurements, if necessary, according to the NOAA laboratory methods.

Since the mesh size during extraction was decreased, it was impossible to compare the received results with those for water samples collected by means of neuston net of the most widespread mesh size (0.300–0.335 mm). To make this comparison possible, nets with lower mesh size (0.175–0.200 mm) might be used for sample collection. Small mesh sizes (0.100 mm) were also recommended for zooplankton assessment in the Baltic Sea (HELCOM, 2008). Another way is to use a 0.300–0.335 mm filter net placed over a 0.174 filter net during the last step of filtration and to analyze filters under stereomicroscope separately. Applying this approach in this study revealed that most of the fragments and films remained on the first 0.333 mm net, but in the case of fibers approximately one half of them was found on the 0.174 mm filter net.

During samples analysis it was discovered that mussel shells appeared in great numbers in sea samples and hampered microplastic determination with microscope. Adding an extra calcite digestion step (Fig. 2) permitted to remove mussel shells completely and sped up microscopic observation. This step was involved later and was not applied during the samples examination presented in this paper.

#### 3.2. Validation results

Adding 20 ARPs into sediment sample before the analysis was used to assess extraction efficiency. It was evaluated separately for the supernatant fraction only and using the supernatant and the solution above the sediment together. Five samples with different grain size fractions (Gb3m, Gb5m, Gb10m, Gb20m, and Gb30m) were analyzed with this internal standard. Average extraction efficiency only for supernatant was  $53 \pm 16\%$  (min 30%, max 80%,  $p = 0.05$ ,  $n = 5$ ), but then, using the supernatant and the solution above the sediment fraction together, the average value of  $92 \pm 7\%$  (min 80%, max 100%;  $p = 0.05$ ,  $n = 5$ ) was achieved. No ARPs were found on the glassware and they all seemed to be buried in the sediment. The minimal extraction efficiency (80%) using the supernatant and the solution above the sediment was observed in sample Gb20m with the largest mean grain size. Sequential extractions (up to 3 times) enabled us to extract all of the lost ARPs.

For tested samples Gb3m, Gb5m, Gb10m, Gb20m, and Gb30m microplastics concentrations were corrected using individual extraction efficiency values for all of the microplastics types. For samples Gb15m and Gb25m, the results were corrected using the average extraction efficiency value.

A sample contamination test was conducted. Six separate empty runs were performed for this purpose. Contamination with fibers was found in two of them, 1 to 2 fibers 0.28–1.10 mm long were found. Average sample contamination was about 0.5 fibers/sample or 1.4 fibers per kilogram of dry weight (hereinafter abbreviated as kg DW). For all of the samples the fibers concentration was corrected according to this value.

A laboratory air test was performed twice. It was discovered that two of the six filters contain contamination, and 2 to 4 particles of natural origin 0.18 to 0.33 mm long were found. Knowing the pump capacity and running time, it was possible to calculate dust concentration in the air. It turned out to be about  $1.25 \text{ items/m}^3$ . Taking into account that the sample exposition to the laboratory air is significantly shorter than running time, sample contamination from laboratory air with microplastics was assumed to be negligible.

NOAA laboratory methods were checked by adding a polyethylene microplastic pellets to natural sediments and recovery rate of 81% was obtained. The polyethylene pellets have specific density of about 0.95 g/ml against 1.40 for PET (Chubarenko et al., 2016) and significantly larger size than the ARPs we used. Our test demonstrated that handling the solution above the sediment together with the supernatant significantly increases recovery rates with no need for inspection of settled solids with naked eye. Using  $\text{ZnCl}_2$  solution (55%; specific density of solution 1.6 g/ml) at all the separating steps allowed the researcher to extract the higher density plastics with recovery rates >90%. In our opinion this revision boosts the analysis process without quality loss.

Although during samples analysis most of the plastic particles were found in the supernatant filtrate, significant part of them was observed in the separating solution above the sediment. The relation between sunken and afloat particles depended on their type. Only one fragment did not float (20% of total fragments content in the sample). Numbers of sunken fibers were significantly higher ( $34 \pm 23\%$  of total fibers content in the sample;  $p = 0.05$ ;  $n = 7$ ) and almost all of the films (75–100% of total sample content) did not float in the separating solution. It was impossible to obtain microplastics from the bottom layer of the separating solution of sample Gb15m because of the high calcite fraction content. It might be due to this particular problem that this sample had the lowest total microplastic concentration.

### 3.3. Microplastics in Baltic Sea sediments

Microplastics were observed in all of the samples (Table 1). An average total microplastic concentration was  $34 \pm 10$  items/kg DW ( $p = 0.05$ ,  $n = 7$ ). Fragments, films, and fibers content varied significantly at different stations. Primary microplastic particles originating from post consumer products or raw pellets were not found in this study.

Fragments were observed only near the coastline (Fig. 3), while films primarily occurred far from the shore. Fibers distribution was different: the maximum concentration was observed near the shore with unsteady decrease while moving away from a coast. We think it could be explained by different physical properties of those microplastic types and different near-bottom current velocities in those coastal zones. Since sediment grain size characteristics are the result of a function of currents velocities (McLaren and Bowles, 1985), it is possible to use those characteristics to explain the obtained microplastics spatial patterns. In order to do this, we determined relationships between microplastic concentrations (Table 1) and sediment grain size characteristics (Table 2).

Although there was no clear correlation of microplastic concentrations with mean grain size of sand ( $R^2 = 0.20$ ,  $n = 7$ ), there was some relation with its sorting characteristics: microplastic particles concentrations (fragments + films) increased with sediment sorting (decreased with  $\sigma_{\phi}$ ;  $R^2 = 0.64$ ,  $n = 7$ ). Sorting is usually defined by value of standard deviation ( $\sigma_{\phi}$ ) in  $\Phi$ -scale (Blott and Pye, 2001). Higher  $\sigma_{\phi}$  means lower sorting and vice versa. Well-sorted sands concentrate in areas with high hydrodynamic energy, while poorly sorted sediments are associated with areas of hydraulic volatility (Schwartz, 2005; Rasul and Stewart, 2015). So, it is possible to suggest that microplastic particles with certain hydrodynamic characteristics are likely to settle in a similar way to sands of a specific grain size and

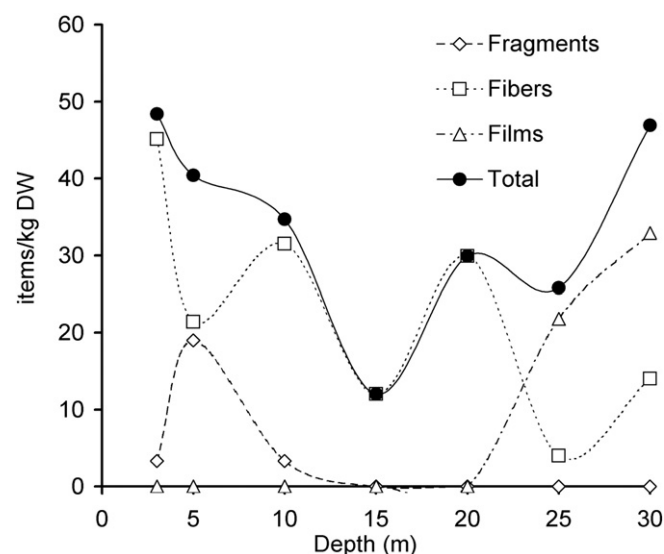


Fig. 3. Variation of microplastics concentration in bottom sediments with the location depth.

thus generally concentrate in areas with well-sorted sands. Meanwhile, in the areas with poorly sorted sands, current velocity changes frequently and microplastic is re-suspended with a higher probability.

Using sediment grain characteristics permitted to define two local sedimentation zones at the studied area. The first one was located 5 m deep where fine, very well-sorted sands with negatively skewed size distribution (Table 2) were deposited. The highest fragments concentration was observed at this location. The second sedimentation zone was situated 25 m deep, where sands became finer, better sorted and grain size distribution was more negatively skewed than for sediments upslope (20 m deep). The ancient submerged wave-cut cliffs (Dorokhov and Dorokhova, 2014) located near the studied region also support the idea about the sedimentation zone situated deeper than the 20 m isobath. The highest films concentrations were observed in this zone. Meanwhile it is important to emphasize that most of films in our samples did not pass the flotation procedure and sink in separation solution as a result of biofouling and (or) adhering sand grains.

Recently it was found that the shape of microplastic particles affects considerably their settling velocity (Khatmullina and Isachenko, 2016) and fate in marine environment (Chubarenko et al., 2016). Once the seabed deposits are highly interchangeable in the studied region (Babakov, 2010) it seems that microplastics concentrations are also alternating according the sediment transport. Currents with speeds > 10 cm/s were observed in shallow (5–8 m) areas of the studied region more frequently (in 15.5–29.0% of cases) than in deeper areas (11–15 m, 8.0–9.6% of cases) (Babakov, 2010). Considering that, let us suppose that the current velocities required for fragments transportation are higher than for films. Shear stress value was earlier obtained for near spherical raw pellets (Ballent et al., 2013), similar to natural sediments in shape, however, for films and fibers it was not yet evaluated. Hence, it is quite hard to obtain quantitative description of the observed phenomenon.

### 3.4. Comparison with other studies

When comparing the obtained results with other Baltic Sea and North Sea studies (Table 3), it is clear that concentrations of fragments and fibers in our study have the same order of magnitude as the data of Claessens et al. (2011) for the North Sea and are close to particles and fibers concentrations in the Baltic beach sediments (Stolte, 2015). Claessens et al. (2011) data show slight decrease in fibers concentrations from the beach to the offshore. The concentration of films reported by Claessens et al. (2011) was one order of magnitude lower than ours

Table 1  
Concentrations of different types of plastic particles, items/kg DW.

Sample	Sampling depth, m	Fragments	Films	Fibers	Total
Gb3m	3	3.3	0.0	45.1	48.4
Gb5m	5	19.0	0.0	21.4	40.4
Gb10m	10	3.3	0.0	31.5	34.7
Gb15m	15	0.0	0.0	12.0	12.0
Gb20m	20	0.0	0.0	29.9	29.9
Gb25m	25	0.0	21.8	4.0	25.8
Gb30m	30	0.0	32.9	14.0	46.9

**Table 2**  
Sediment grain size characteristics.

Sample	$\bar{d}$ , mm	$\bar{d}_{\phi}$	$\sigma_{\phi}$	$Sk_{\phi}$	$K_{\phi}$	$V_{\phi}$ , %
Gb3m	0.23	2.12	0.76	0.36	0.02	36
Gb5m	0.15	2.69	0.83	−1.32	1.71	31
Gb10m	0.43	1.23	0.73	0.08	−0.23	59
Gb15m	0.28	1.86	0.93	0.08	−0.53	50
Gb20m	1.20	−0.26	0.93	1.38	0.97	−36
Gb25m	0.21	2.26	0.77	0.19	−0.32	34
Gb30m	0.28	1.82	0.38	2.27	12.43	21

$\bar{d}$  – mean grain size;  $\phi$  – values in a  $\phi$ -scale;  $\sigma_{\phi}$  – standard deviation (inversed sorting);  $Sk_{\phi}$  – skewness;  $K_{\phi}$  – kurtosis;  $V_{\phi}$  – variation coefficient;  $\bar{d}_{\phi} = -\log_2 \bar{d}$ .

for the offshore location. The most possible reason of these differences was neglecting of films content in the separating solution above the sediment during extraction in the work of Claessens et al. (2011). The presence of strong tidal currents in the North Sea is another reason for lower films content in the bottom deposits.

At the same time, our concentrations are more than two times higher than in Talvitie et al. (2015) reported for the Finnish part of the Baltic Sea and more than ten times lower than the data of Strand et al. (2013) for Arcona Basin and Danish straits and bays. Without getting into details, one might notice a trend of rising microplastic concentration from North to South. But a more thorough examination reveals that the average microplastic concentration in the central part of the Baltic Sea seems to be 20 times higher than in the area near the Helsinki wastewater discharge. Taken into account that wastewater plants are known as point sources of primary microplastics (Andrady, 2011; Rilling, 2012), one could get disappointed with the result. However, when comparing extraction and detection techniques applied in those studies (Table 3), it becomes obvious that they differ considerably. So it is presently impossible to equalize them. For example, concentrations provided by Strand et al. (2013) are significantly higher than in other works, especially when it comes to particles. These high concentrations may be explained by small sample mass taken for analysis (100 g of wet sediment) and significantly different analysis procedures. Yet another possible reason is that samples were taken in sludge basins where microplastic concentrations, especially fibers, are expected to be high (Woodall et al., 2014). One more challenge appears during a broad-

scale comparison of results: most of the microplastics studies in the Baltic Sea do not distinguish fragments from films and assume them as particles, in spite of their differing physical properties (Chubarenko et al., 2016). Despite the different analysis procedures, most of these studies (Claessens et al., 2011; Stolte, 2015; Strand et al., 2013), as well as ours, report fibers as the dominant item fraction of microplastics.

Since methodological aspects of all of observed studies are different, it is strongly required to elaborate standardized methods for microplastics sampling and analysis in different sea environments (Hidalgo-Ruz et al., 2012). The NOAA methods were assumed to be the first breakthrough in this direction, but they appeared to be in need of revision.

#### 4. Conclusions

Microplastics were found in all of the analyzed samples from the Gdansk Bay bottom sediments, with mean concentrations of  $34 \pm 10$  items/kg DW ( $p = 0.05$ ,  $n = 7$ ) and fibers were found to be the greatest in numbers. Since methodological aspects of all of observed studies in the Baltic Sea are different, it was impossible to define microplastic spatial patterns in the Baltic Sea, but the obtained results are comparable to the data from the studies of the North Sea, thanks to the similar analytical base.

Some spatial trends in microplastic concentrations were observed. Both fragments and films were found to sink in local sedimentation zones, but if the former sink near the coast at the depths of around

**Table 3**  
Mean concentrations of microplastics found in the North and the Baltic Seas sediments. Values in parentheses represent the standard deviation of the mean.

Location	Lower range	Observed concentrations, items/kg DW			Separation & detection method
		Fragments	Films	Fibers	
This study, near the coast (3–10 m)	175 $\mu$ m	8.5 (9.1)	0	32.7 (11.9)	Floatation in 55% ZnCl <sub>2</sub> solution, supernatant + residual extraction, H <sub>2</sub> O <sub>2</sub> digestion, microscope detection
This study, offshore (20–30 m)	175 $\mu$ m	0	18.2 (16.7)	16.0 (13.1)	
North Sea, Intertidal zone <sup>1</sup>	38 $\mu$ m	4.1 (1.8)	3.5 (2.6)	83.7 (25.5)	
North Sea, Subtidal zone <sup>1</sup>	38 $\mu$ m	5.9 (1.0)	0.9 (0.9)	46.0 (6.1)	Floatation in saturated NaCl solution, supernatant extraction only, microscope detection + FT-IR for some particles
North Sea, Belgian Coast <sup>1</sup>	38 $\mu$ m	29.3 (6.7)	2.8 (1.3)	59.8 (17.4)	
North Sea, Belgian offshore <sup>1</sup>	38 $\mu$ m	29.9 (5.4)	1.4 (1.8)	74.3 (4.0)	
Baltic Sea Beaches <sup>2</sup>	55 $\mu$ m	2.5 $\pm$ 2.5		140 $\pm$ 135	Floatation in saturated CaCl <sub>2</sub> solution, supernatant + residual extraction, H <sub>2</sub> O <sub>2</sub> digestion, microscope detection
Baltic Sea, Helsinki, Viikinmäki near wastewater discharge <sup>3</sup>	20 $\mu$ m	9.0 $\pm$ 6 <sup>a</sup>		2.1 $\pm$ 1.3 <sup>a</sup>	
Baltic Sea, Helsinki, Suomenoja near wastewater discharge <sup>3</sup>	20 $\mu$ m	23.8 $\pm$ 17.5 <sup>a</sup>		5.9 $\pm$ 4.4 <sup>a</sup>	
Baltic Sea, Helsinki, Reference, (offshore) <sup>3</sup>	20 $\mu$ m	0		2.2 $\pm$ 1.6 <sup>a</sup>	Oxidizers digestion, floatation in saturated NaCl solution, supernatant extraction only, microscope + FT-IR for some particles >0.5 mm
Baltic Sea, Arcona Basin (offshore) <sup>4</sup>	38 $\mu$ m	175 (35) <sup>b</sup>		480 (28) <sup>b</sup>	
Baltic Sea, Denmark straits and bays (offshore) <sup>4</sup>	38 $\mu$ m	100 (69) <sup>c</sup>		125 (106) <sup>c</sup>	

<sup>a</sup> Recalculated from wet weight using 1.25 as average wet sediment/dry sediment ratio (Claessens et al., 2011), confidence interval calculated with  $P < 0.01$ .

<sup>b</sup> Original unit (pcs/10 g DW), average calculated for Arcona & Hjelm Bught locations.

<sup>c</sup> Original unit (pcs/10 g DW), average calculated for Fehmarn Bael, Syd for Hven, Vejro, Koge Bugt locations.

<sup>1</sup> Claessens et al., 2011.

<sup>2</sup> Stolte, 2015.

<sup>3</sup> Talvitie et al., 2015.

<sup>4</sup> Strand et al., 2013.



5 m, the latter were observed far from the shore at the depths of 25–35 m. Fibers concentrations decrease slowly with moving from the coast to offshore. The current velocity required for transportation of fragments is likely to be relatively higher than for films.

Our modifications to the NOAA methods allow microplastic extraction numbers to be increased sufficiently, which was preconditioned by the use of the solution above the sediment surface as well as supernatant fractions of separating solution. Applying ZnCl<sub>2</sub> solution (55%; specific density of solution 1.6 g/ml) at all of the separating steps allows extracting higher density plastics with recovery rates >90%. It is important to make sure that analyte was not lost or contamination did not occur during the analysis process. For this reason, procedures of quality control are always introduced into laboratory workflow (Westgard and Barry, 1986). We proposed using fluorescent ARPs as the internal standard in all of the samples during microplastic analysis for this purpose. Occasional empty runs are advised in order to take into account sample contamination rates. The proposed modifications were tested and recommended for laboratory application. It is recommended in future to single out plastic films in a separate category during microscopic analysis due to their specific physical characteristics and biofouling behavior.

In summary, further data collection is required in order to establish microplastic migration and accumulation trends in the coastal zones and deep sea areas. Although microplastic films and fibers behavior in marine environment is still unknown, it is required to continue laboratory experiments to determine quantitative characteristics for sedimentation and suspension processes of microplastics of different shapes. It is essential to develop and implement consistent analytical methods that provide ground for quantitative data comparison between different studies.

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