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Microplastics in Dutch marine sediments

KWR

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Project manager
A.A. Reus

Client
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Quality Assurance
A. van Wezel

Author(s)
P.S. Bäuerlein, M. Erich, R. van Doorn

With contributions from B. Koelmans (WUR) and S. Mintenig (UU)

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W. van Loon

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More information
P.S. Bäuerlein
T +313 606 97 02
E patrick.bauerlein@kwrwater.nl

Postbus 1072
3430 BB Nieuwegein
The Netherlands

T +31 (0)30 60 69 511
F +31 (0)30 60 61 165
E info@kwrwater.nl
I www.kwrwater.nl

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Summary

To gain an indication on the amount of plastic in Dutch marine sediments, for this research project 4 marine sediment locations monitored by Rijkswaterstaat (Vlissingen, Noordwijk2, Kornwerderzand and Bocht van Watum) were chosen. At each location 3 samples were taken. In total 24 samples were analysed. These samples were analysed for the presence of microplastics in the size ranges of 100-300 µm, 300-1000 and 1000-5000 µm (according to OSPAR technical specifications). The plastics that were identified were polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), polyurethane (PU), polyvinylchloride (PVC), and polyester.

In all 24 samples microplastics were found. For the size fraction from 100-300 µm the particle numbers stretched over the various locations from 479±386 p/kg dry weight (d.w.) to 13931±4872 p/kg d.w. Various types of plastic (PE, PS, PP, PU, PA, PVC and polyester) were found during this study. However, PU was only found at one location and polyester at two out of eight. All other polymers were found at every location. By far the largest particle number was found for PE followed by PP. The lowest particle numbers were found for PU and PET. In the size fraction from 300-1000 the particle numbers are lower compared to the more tiny size fraction and vary between 134±190 and 619±316 p/kg d.w. In this size fraction no polyester was detected and PU was merely found at one location. Also in this larger fraction PE is the most abundant plastic followed by PP. There were no particles found larger than 1000 µm in any of the locations. The smallest particle number over the whole range from 100-5000 µm is 882±732 and largest number is 13931±4872 p/kg d.w. It can been seen that the particle number drops with the particle size mounting.

On average, in all size fractions the most abundant polymer with regard to particle number is PE (75%) followed by PP (19%). The other polymers have a mean abundance between nearly 0 and 4%.

When the mean particle number of all samples (5558 p/kg d.w.) is translated into mass of plastic per kg, a mean total polymer mass of about 4.5 mg/kg d.w. is found in these samples. The amount of plastic ranges from 0.04 to 22 mg/kg d.w.

Contents

1 Introduction

1.1 General

Over the last decade plastics have been a concern to the public, policy and the scientific community [1-6]. In 2016 the world-wide plastic production has reach 335 million ton per year of which 60 million tons are produced in Europe and global production is still increasing. About 99% of this plastic is petroleum-based and the packaging industry alone uses ca. 38% of these plastics [1]. Plastic produced for packaging will outlive its usefulness quickly after production to be become waste that is often neither fully collected nor recycled. It is estimated that in 2010 4.8 to 12.7 million ton of the produced plastics were released into the oceans by 192 coastal countries. Currently it is estimated that the amount of plastic ending up the oceans will have increased by an order of magnitude in 2025 if we continue to produce and handle plastic waste like this [2]. This means the total amount of plastic in the ocean will rise even further [3]. Apart from that, the plastic will not only litter the oceans, but it might also effect animals and plants in the aquatic environment [4]. Additionally, plastic particles will break down into smaller particles until they are as small as a few millimetres. From the moment that these plastic particles are smaller than 5 mm, they are considered to be microplastics [5]. Plastic macro-litter in general has shown to have harmful effects on various lifeforms [6]. Furthermore, it is possible that microplastics have the potential to cause severe damage in an organism as they can disrupt *e.g.* the function of the guts [7]. More and more it becomes clear now that these particles are ubiquitous in the environment and that it cannot be ruled out that these particles might pose a threat to flora and fauna in the future [7-10]. It is apparent now that the plastic problem is not confined to the ocean [11, 12], because plastic litter is introduced almost everywhere in the environment (*e.g.* rivers, lakes, waste water treatment plants) [7, 13-15].

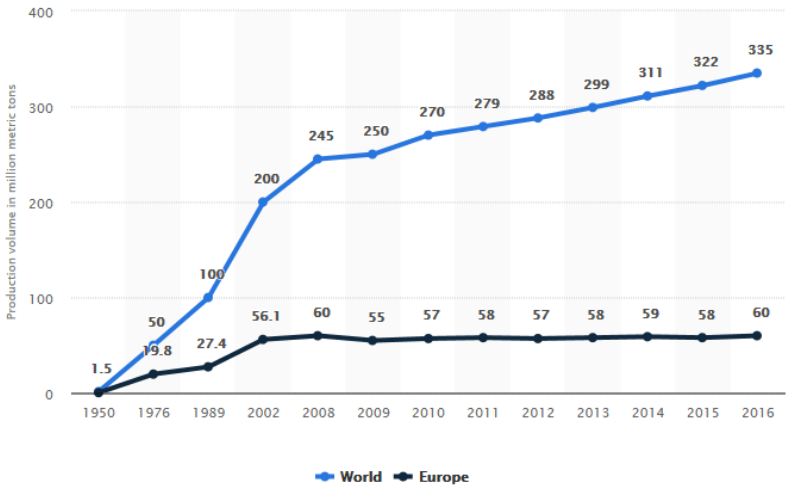
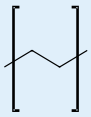
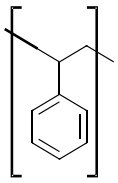
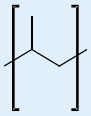
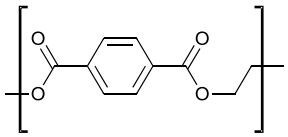
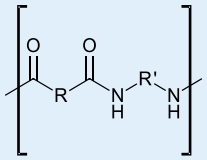
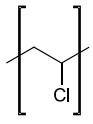
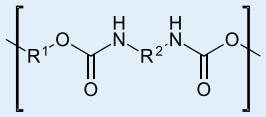


Figure 1: Global and European plastic production. Source: https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/

1.2 This research

To gain an indication on the amount of plastic in Dutch marine sediments, for this research 4 different marine sediment locations were chosen. At each location 3 samples were taken. In total 24 samples were analysed. These samples were analysed for the presence of microplastics in the size ranges of 100-300 µm, 300-1000 and 1000-5000 µm (according to the OSPAR)[1] . The plastics that were identified were polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), polyurethane (PU), polyvinylchloride (PVC), and polyester (Table 1).

Table 1: Polymers that were searched for and their most common application.

Plastic (Polymer)	Chemical structure	Most common use
Polyethylene (PE)		Packaging, furniture, bags, beads
Polystyrene (PS)		Tableware, foam peanuts, packaging
Polypropylene (PP)		Pipes, caps, containers, straws
Polyester: Polyethylene terephthalate (PET)		Bottles, Fibres
Nylon (PA)		toothbrush, fishing nets, clothes
PVC (Polyvinyl chloride)		Wraps, pipes, floor
PU (Polyurethane)		Foam

[1] Convention for the Protection of the Marine Environment of the North-East Atlantic, www.ospar.org

2 Materials and Methods

2.1 Method

The method developed and used for this project is based on the method developed by Wageningen University & Research, the University of Utrecht and KWR Water B.V. in the framework of the STW project TRAMP (www.stwtramp.nl).

2.2 Chemicals and materials

Perdrogen™ 30% hydrogen peroxide (H_2O_2) (w/w) and technical grade zinc chloride ($ZnCl_2$) were purchased from Boom (The Netherlands). Potassium hydroxide (KOH) was purchased from Merck (Germany). MilliQ water with a resistivity > 18 MΩ was used for the sample treatment and to make a $ZnCl_2$ solution with a density of 1.6 g/cm^3 as well as a 12.5% KOH solution. All chemical solutions were filtered over a 20 µm metal mesh prior to usage. Anodisc filter 25 mm with a pore size of 200 nm were purchased from Whatman®. Fluorescent Green Polyethylene Microspheres 90 – 106 µm were purchased from Cospheric (USA).

2.3 Dry weight

The following table shows the dry weight determined for each sample. It was determined by RWS for a subsample taken after homogenisation.

Table 2: Dry and wet weight of the different samples.

RWS sample code	RWS LIMS code	Crucible empty	Crucible wet	Crucible dry	Wet weight (g)	dry weight (g)	Dry weight %
VLISSGBISSVH [2017008163-01/VBC]	2017008163	123.36	145.71	141.38	22.35	18.02	81
VLISSGBISSVH [2017008164-01/VBC]	2017008164	121.96	157.73	149.79	35.77	27.83	78
VLISSGBISSVH [2017008165-01/VBC]	2017008165	122.64	144.69	140.94	22.05	18.3	83
VLISSGBISSVH [2017008166-01/VBC]	2017008166	123.54	150.2	145.83	26.66	22.29	84
VLISSGBISSVH [2017008167-01/VBC]	2017008167	124.04	161.96	153.86	37.92	29.82	79
VLISSGBISSVH [2017008168-01/VBC]	2017008168	123.69	148.42	143.14	24.73	19.45	79
VLISSGBISSVH [2017008169-01/VBC]	2017008169	124.94	151.72	147.39	26.78	22.45	84
VLISSGBISSVH [2017008170-01/VBC]	2017008170	123.87	143.98	140.53	20.11	16.66	83
VLISSGBISSVH [2017008171-01/VBC]	2017008171	123.01	151.09	145.6	28.08	22.59	80
NOORDWK2 [2017008172-01/VBC]	2017008172	123.58	149.4	144.5	25.82	20.92	81
NOORDWK2 [2017008173-01/VBC]	2017008173	124.37	142.97	139.75	18.6	15.38	83
NOORDWK2 [2017008174-01/VBC]	2017008174	124.88	164.32	156.71	39.44	31.83	81
NOORDWK2 [2017008175-01/VBC]	2017008175	124.05	153.72	148.19	29.67	24.14	81
NOORDWK2 [2017008176-01/VBC]	2017008176	124.71	157.31	151.56	32.6	26.85	82

Table 2: Continued

RWS sample code	RWS LIMS code	Crucible empty	Crucible wet	Crucible dry	Wet weight (g)	dry weight (g)	Dry weight %
NOORDWK2 [2017008177-01/VBC]	2017008177	122.96	149.44	144.47	26.48	21.51	81
NOORDWK2 [2017008178-01/VBC]	2017008178	123.71	149.68	144.56	25.97	20.85	80
NOORDWK2 [2017008179-01/VBC]	2017008179	121.95	152.43	146.92	30.48	24.97	82
NOORDWK2 [2017008180-01/VBC]	2017008180	120.72	149.68	144.5	28.96	23.78	82
BOCHTVWTDVVA [2017010214-01/VBC]	2017010214	121.98	157.01	138.92	35.03	16.94	48
BOCHTVWTDVVA [2017010215-01/VBC]	2017010215	122.79	141.96	132.03	19.17	9.24	48
BOCHTVWTDVVA [2017010216-01/VBC]	2017010216	123.1	153.54	137.45	30.44	14.35	47
KORNWDZBTSKM [2017010217-01/VBC]	2017010217	122.63	143.32	129.18	20.69	6.55	32
KORNWDZBTSKM [2017010218-01/VBC]	2017010218	123.64	142.37	129.64	18.73	6	32
KORNWDZBTSKM [2017010219-01/VBC]	2017010219	123.73	146.15	130.79	22.42	7.06	31

2.4 Sample preparation

The sample was homogenised and 150 g of this sample was suspended in MilliQ water. This suspension was subjected to filtration through a 500 and a 60 µm metal mesh cascade, as has been used previously for surface water samples[2]. The remaining solids on both filters were treated differently.

The solids from the 500 µm filter were re-suspended in Milli-Q water and placed in a Bogorov chamber (PMMA, 70 mL, HydroBios, Germany) and examined under a stereomicroscope (Nikon Stereo SMZ2T). All particles potentially made from plastics were sorted visually, photographed and identified using an attenuated total reflection-Fourier-transform infrared spectroscopy (ATR-FT-IR).

- The solid retained by the 60 µm filter was transferred into a glass beaker and 600 mL of $ZnCl_2$ solutions (density 1.6 g/cm^3) was added.
- The beakers were placed on a stir plate and stirred for 15 min using a magnetic stirrer with a glass surface (60x8 mm, 120 rpm).
- After 10 min the magnetic stirrer was stopped and denser solids settled while lighter particles were floating on the surface.
- Using a vacuum pump and a filtration setup with a Teflon tube the supernatant was filtered over a 20 µm stainless steel filter.
- This procedure was repeated with another 600 mL of $ZnCl_2$ solution.
- The filter with the retained material is then transferred into a beaker with 75 mL KOH (12.5%), covered with aluminium foil and left standing for 5 days at 35˚C.
- Subsequently, this suspension was filtered again through the same 20 µm metal filter. In the next step the remaining solid was transferred into a beaker with 50 mL H_2O_2 (30%) and left standing for one day at 35 ˚C.

[2] S.M. Mintenig, M. Kooi, M.W. Erich, S. Primpke, P.E. Redondo- Hasselerharm, S.C. Dekker, A.A. Koelmans, A.P. van Wezel. 2020. A systems approach to understand microplastic occurrences in Dutch riverine surface waters. Water Research, in press.

- The sample was filtered again through the same metal filter, and the remaining solids transferred into a separation funnel using a 100 mL $ZnCl_2$ solution.
- The funnels were shaken and left to enable settling of denser materials. The settled material is discarded by continuously turning the valve of the funnel to prevent clogging, re-suspension and loss of plastics.
- About 10 mL of liquid were allowed to remain in the funnel. These 10 mL were filtered again over the metal filter.
- Using Milli-Q water the retained materials were removed from the filter and filtered through an Anodisc filter. In case of a high load of remaining sample residues, the residue were split over two Anodisc filters as a thick layer of solid would interfere with the analysis using the micro-FTIR.
- The filters were then placed in glass petri dishes and dried in a half opened dish in a stove for 48 hours at 35˚C.

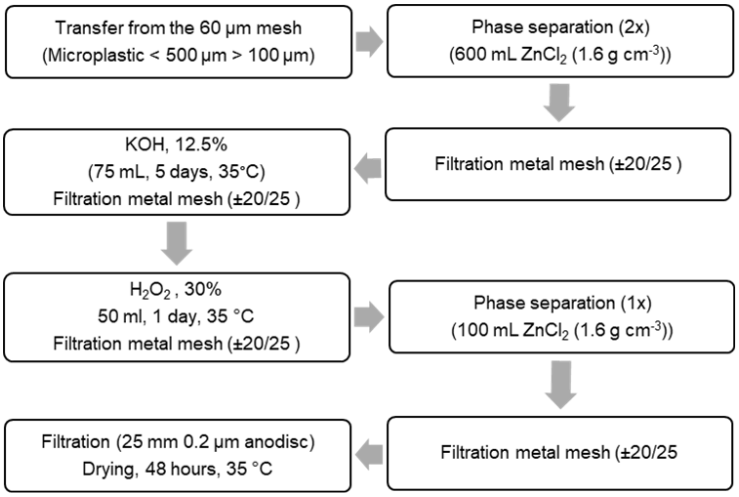


Figure 1: Scheme illustrating the extraction of microplastics >60 um < 500 µm from an aqueous environmental sample.

2.5 Quality assurance

Potentially occurring contamination during sample handling needs to be assessed and mitigated as good as possible. Therefore, before starting, all laboratory surfaces were whipped with ethanol, equipment was rinsed and covered immediately with aluminium foil, and a cotton lab coat was worn at all times. Next to this, solutions of chemicals were filtered prior to use. Equipment chosen was not made from plastic (*e.g.* a metal filter setup with a Teflon tube, the separation funnels made of glass). Negative controls were treated in parallel to each batch of actual samples to determine the degree of contamination (Table 3). The limit of quantification (LOQ) was calculated from the number of particles found in the procedural blanks. The LOQ was determined for each type of plastic particle. For the particle numbers below the LOQ the sample was regarded as void of this type of plastic. LOQ is the mean of all blanks per particle for the respective size range.

Table 3: Number of plastic particles found in the procedural blanks

	Blank 1 100-300 µm	Blank 1 300-1000 µm	Blank 2 100-300 µm	Blank 2 300-1000 µm	Blank 3 100-300 µm	Blank 3 300-1000 µm
PE	71	53	0	0	240	40
PP	0	0	20	0	0	0
PS	0	0	0	0	0	0
PA	0	0	0	0	0	0
PU	0	0	0	0	0	0
PVC	0	0	0	0	0	0
Polyester	0	0	0	0	0	0
Total	71	53	20	0	240	40
	Blank 4 100-300 µm	Blank 4 300-1000 µm	Blank 5 100-300 µm	Blank 5 300-1000 µm	Blank 6 100-300 µm	Blank 6 300-1000 µm
PE	76	76	99	60	158	59
PP	0	0	20	0	0	0
PS	0	0	0	0	0	0
PA	0	0	0	0	0	0
PU	0	0	0	0	0	0
PVC	0	19	0	0	0	0
Polyester	0	0	0	0	0	0
Total	76	95	119	60	158	59

2.6 Analysis particles larger than 500 µm

Particles larger than 500 µm were sorted individually. Particle characterisation was achieved by means of ATR-FTIR spectroscopy (Biorad).

2.7 Analysis particles larger smaller than 500 µm

Particles smaller than 500 µm were analysed on an Anodisc filter using a µFT-IR (Nicolet iN10 FT-IR microscope, ThermoFisher) in the laboratories of Wageningen University and Research. The data files were converted to further be analysed using MPHunter (Arhus Aalborg University, Denmark and Alfred Wegener Institute, Helgoland). The particle spectra were compared to reference spectra. The software reports the amount and size for each type of particle (minimum and maximum dimension). For the analysis an area of ca. 6 x 15 mm is scanned that represents between 40-46% of the total filter area. The amount of plastic is then extrapolated to the filter as a whole. For 6 samples (2017008176, 2017008177-, 2017010216, 2017010217, 2017010218 and 2017010219) the total amount of plastic per type was received, but the sample matrix made size estimation for the particles impossible. The reason for this is unclear. For these samples it was assumed that they only contained particles in the size range of 100-300 µm and not the bigger size ranges.

2.8 Data evaluation

To determine the amount of plastic in each sample, the 3 samples for each location were measured and the mean particle number for each type of plastic was calculated. This number was corrected for the mean recovery rate of 73% (see also Table 4) that was determined for this project using fluorescent green polyethylene microspheres (90–106 µm).

Table 4: Recovery rate for six different batches.

Batch No.	No. of particles	Recovered particles	Recovery
1	119	67	56%
2	107	95	89%
3	187	116	62%
4	136	130	96%
5	129	80	62%
6	119	85	71%
		mean	73%
		SD	16%

The plastic particle amount is expressed in number of particles per kg dry weight of sediment (particles/kg d.w.). Furthermore, the mean mass of plastic found in 18 of the 24 samples was estimated by the MPHunter software. The mass was based on the polymer density, the two-dimensional information received for each particle (major dimension and minor dimension), and an estimated third dimension (g/kg dry weight). The third dimension was estimated as follows: The identified plastic particles were analysed for the longest distance between pixels of the particle, yielding the major dimension of the particle. The minor dimension was found by assuming the particle shape is an ellipse and knowing the area of the particle in the scan. The third dimension, the thickness, was assumed as being 0.67 times the minor dimension. The volume was calculated assuming the particle is an ellipsoid. The mass was calculated from the volume and the density of the identified plastic material[3].

[3] Source: http://simple-plastics.eu/about.html (accessed March 11th 2020). siMPle includes the MPHunter interface.

3 Results and discussion

3.1 Results

In all 24 samples microplastics were found. For the size fraction from 100-300 µm (see Table 5 and graphical representations in Appendix II) the particle numbers stretched over the various locations from 479±386 p/kg d.w. to 13931±4872 p/kg d.w. However, the large particle number from location 201701017/18/19 has to be handled with care, as no exact particle size information was received. Therefore, it is possible that particles larger than 300 µm and smaller than 100 µm are included. All types of plastic were found during this study. However, PU was only found at one location and polyester at two out of eight. All other polymers were found at every location. By far the largest particle number was found for PE followed by PP. The lowest particle numbers were found for PU and PET. In the size fraction from 300-1000 µm (see Table 6 and graphical representations in Appendix III) the particle numbers are lower compared to the more tiny size fraction and vary between 134±190 and 619±316 p/kg d.w. In this size fraction no polyester was detected and PU was merely found at one location. Also in this larger fraction PE is the most abundant plastic followed by PP. There were no particles found larger than 1000 µm in any of the locations. The smallest particle number over the whole range from 100-5000 µm is 882±732 and largest number is 13931±4872 p/kg d.w (see Table 7). For all individual polymers the highest particle number per kg sediment are found in the smallest size fraction. On mean in all size fractions the most abundants polymer with regard to particle number is PE (75%) followed by PP (19%). The other polymers have a mean abundance between nearly zero and 4% (Table 7).

When the mean particle number of all samples (5558 p/kg d.w.) is translated into mass of plastic per kg, a mean total polymer mass of about 4.5 mg/kg d.w. is found in these samples.

Table 5: Mean of plastic particle concentration (10 300 µm) expressed in p/kg d.w. in the eight marine sediment samples. The number in brackets signifies the LOQ.

	Vlissingen 20170081 63/64/65		Vlissingen 20170081 66/67/68		Vlissingen 20170081 69/70/71		Noordwijk 20170081 72/73/74		Noordwijk 20170081 75/76/77 [a]		Noordwijk 20170081 79/80/81		Bocht van Watum 20170102 14/15/16 [a]		Kornwerder-zand 20170102 17/18/19 [a]	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
PE (107)	702	438	920	492	689	65	1024	264	1534	181	450	367	1692	1822	8213	5490
PP (7)	112	90	97	103	93	19	102	23	345	130	22	18	1418	1751	5509	4366
PS	40	41	8	11	8	11	16	11	171	225	0	0	162	229	149	211
PA	16	23	8	11	0	0	0	0	8	11	0	0	20	14	30	42
PU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	14
PVC	24	34	16	23	0	0	0	0	83	25	8	11	223	273	10	14
Polyester	0	0	8	11	8	11	0	0	0	0	0	0	0	0	10	14
Total (114) [b]	893	624	1057	599	798	68	809	629	1636	1192	479	386	3516	4075	13931	4872

[a] See Materials and Methods section. [b] The total amount of plastic that on mean is found in the triplicates. This is not the sum of the individual plastics in this table.

Table 6: Plastic particle concentration (300-1000 µm) expressed in p/kg d.w. in the eight marine sediment samples. The number in brackets signifies the LOQ.

	Vlissingen 20170081 63/64/65		Vlissingen 20170081 66/67/68		Vlissingen 20170081 69/70/71		Noordwijk 20170081 72/73/74		Noordwijk 20170081 75/76/77 [a]		Noordwijk 20170081 79/80/81		Bocht van Watum 20170102 14/15/16 [a]		Kornwerder-zand 20170102 17/18/19 [a]	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
PE (48)	289	172	538	260	154	56	213	108	126	178	274	201	81	115	-	-
PP	80	63	65	42	0	0	31	11	8	11	86	76	51	72	-	-
PS	24	34	0	0	0	0	0	0	0	0	36	50	10	14	-	-
PA	8	11	8	11	0	0	0	0	0	0	7	10	0	0	-	-
PU	8	11	0	0	0	0	0	0	0	0	0	0	0	0	-	-
PVC (3)	0	0	8	11	0	0	0	0	0	0	0	0	0	0	-	-
Polyester	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Total (51) [b]	409	287	619	316	154	56	173	169	134	190	403	337	143	202	-	-

[a] See Materials and Methods section, [b] The total amount of plastic that on mean is found in the triplicates. This is not the sum of the individual plastics in this table.

Table 7: Total plastic particle concentration (100-5000 µm) expressed in p/kg d.w. in the eight marine sediment samples. The number in brackets signifies the LOQ.

	Vlissingen 20170081 63/64/65		Vlissingen 20170081 66/67/68		Vlissingen 20170081 69/70/71		Noordwijk 20170081 72/73/74		Noordwijk 20170081 75/76/77 [a]		Noordwijk 20170081 79/80/81		Bocht van Watum 20170102 14/15/16 [a]		Kornwerder-zand 20170102 17/18/19 [a]	
PE (155)	991	610	1458	752	844	121	1236	371	1458	461	724	567	1774	1938	8213	5490
PP (7)	191	153	162	145	93	19	134	34	337	162	108	93	1469	1823	5509	4366
PS	64	74	8	11	8	11	16	11	171	225	36	50	172	243	149	211
PA	24	34	16	23	0	0	0	0	8	11	7	10	20	14	30	42
PU	8	11	0	0	0	0	0	0	0	0	0	0	0	0	10	14
PVC (3)	24	34	24	34	0	0	0	0	67	48	8	11	223	273	10	14
Polyester	0	0	8	11	8	11	0	0	0	0	0	0	0	0	10	14
Total (165)	1302	916	1676	977	952	162	1386	416	2040	907	882	732	3658	4291	13931	4872

[a] see method section

Table 8: Relative amount (in percentage of total amount of plastic particles) found in the samples

	Vlissingen 20170081 63/64/65	Vlissingen 20170081 66/67/68	Vlissingen 20170081 69/70/71	Noordwijk 20170081 72/73/74	Noordwijk 20170081 75/76/77 [a]	Noordwijk 20170081 79/80/81	Bocht van Watum 20170102 14/15/16 [a]	Kornwerder-zand 20170102 17/18/19 [a]	All locations
PE	76	87	89	89	71	82	48	59	75
PP	15	10	10	10	17	12	40	40	19
PS	5	0	1	1	8	4	5	1	3
PA	2	1	0	0	0	1	1	0	1
PU	1	0	0	0	0	0	0	0	0
PVC	2	1	0	0	3	1	6	0	2
Polyester	0	0	1	0	0	0	0	0	0

3.2 Discussion

The particle numbers from this research are in accordance with findings of plastic in other sediments. In riverine sediment from the rivers Rhine and Main Klein *et al.* have found between 300 and 12000 particles/kg d.w in the size fraction between 63–5000 µm. In this particular research, however, also particles larger than 1000 µm were found. These larger particles were detected in all samples in the two rivers in contrast to the marine sediment samples. A possible explanation for this could be that the researchers have taken a larger amount (3-4 kg) of sampling material compared to 150 g for this research, which will increase the chance to find particles. Also the finding from this research coincide with the findings from Leslie *et al.* [14]. Particle numbers in the size range from 10-5000 µm in sediment from the Dutch coast range from about 100 to 3600 p/kg d.w.

In Amsterdam in an urban canal the sediment contained 2071±4061 p/kg d.w in the size range from 10–5000 µm [14]. In lake sediment from Edgbaston pool 250–300 p/kg d.w. were found [16].

Comparing the relative abundance of each type of plastic reveals that there is a strong coincidence (Table 9). In almost all studies, except for the rivers Rhine/Main study, PE was the largest contributor to particle numbers, followed by PP. It is noticeable that the relative abundance in the Dutch marine environment and on the Brazilian coast are almost identical. The plastic particles found in Brazil were plastic resin pellets, used as a raw material for industrial purposes [17] and these pellets found their way into the marine environment [18]. As these pellets are omnipresent [11, 17], such particles could be the source of the microplastic particles in the Dutch marine sediment as a consequence of abrasion.

Table 9: Type of plastics found in various water samples. Size ranges differ for each research.

Type of water	**Most abundant plastic in terms of particle number (%)**
Rhine/Main [19]	PS(54), PE(23), PP(15), PA(2)
Largo Maggiore [20]	PE(48), PP(17), PS(15)
Largo Garda [20]	PE(45), PS(24), PP(22)
Largo Iseo [20]	PE(41), PS(27), PET(9), PP(5)
River Antuã [21]	PE(29), PP(29), PS(9), PET(9)
Brasilian coast [18]	PE(78), PP(18)
Marine sediment (this research)	**PE (75), PP (19), PS(3), PVC (2), PA(1)**

As in the current report, also in the research by Klein *et al.* it can been seen that the particle number drops with the particle size mounting. In terms of particle number PS is the largest contributor to the amount of plastic in the river samples followed by PE and PP (Table 9) [19]. Here, the finding differs from the findings in marine sediment, but also from other research. Two publications deal with the presence of plastic in surface water. The researchers find that PE is the largest fraction in terms of particle numbers, either followed by PP or PS. In lakes in Italy about 45% of the plastic particles are PE [20] and in the river Antuã 29% are PE [21].

The amount of plastic in the size range from 100-1000 µm found in the Dutch marine sediment ranges from 0.04 to 22 mg/kg d.w. (mean value 4.5 mg/kg d.w.) and is comparable to the amount of plastic in the size range from 63–630 µm found in the samples from the river Rhine and Main (6–100 mg/kg d.w.). The deviation can be explained by the different size range and by the fact that the particle weight for the Dutch marine sediment was an estimation based on the particle number and two-dimensional size, whereas the mass in case of the Rhine/Main samples was determined by weighing.

Regarding the often large variation between the triplet samples and the consequential high SD it has to be said that this is most certainly a consequence of a) the difficulty to properly homogenise suspension and b) the fact that only parts of the filter have been measured with the FT-IR. As the particles were not evenly distributed on the filters (see Figure 2 and Appendix I), there is a realistic chance that the part of the filter measured is not an exact representation of the whole of the filter. To avoid this in the future the whole of the filter should be measured if possible. However, for these samples this method had to be applied as otherwise the time to acquire the data, would have been too long.

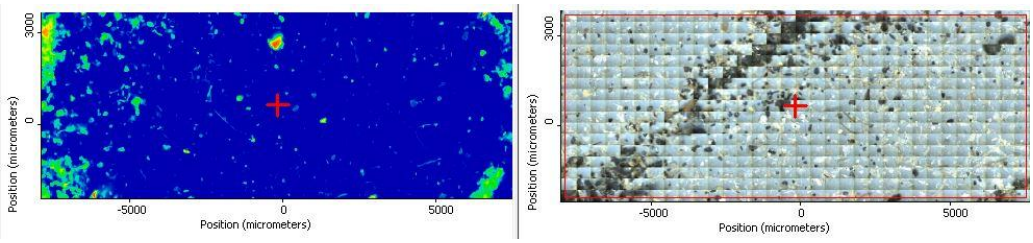


Figure 2: Example of the particle distribution on the scanned filter area. On the left the heat map of the infrared scan is shown. A deviation from blue indicates that an infrared spectrum was received. This does not necessarliy mean that a plastic particle has been found. The right-hand picture is a phtopgraphy of the area. Here no information regarding the nature of particles can be received from.

4 Conclusion and recommendations

4.1 Conclusion

There are three main conclusions from this report. The first one is, as already shown in earlier research, that microplastic are present in the Dutch marine sediment. For the size fraction from 100-300 µm the particle numbers stretched over the various locations from 479±386 p/kg d.w. to 13931±4872 p/kg d.w. In the size fraction from 300-1000 µm the particle numbers are lower compared to the more tiny size fraction and vary between 134±190 and 619±316 p/kg d.w. All types of plastic (PE, PS, PP, PU, PA, PVC and polyester) were found during this study. The findings are in good agreement with earlier research by other scientists.

The second conclusion concerns the sample collection/measurement. It has been shown that there is a great variance between the triplicate samples from each of the locations. As possible reasons for this a) the difficulty to homogenise sediment samples and b) the sample volume have been identified. If the samples are not homogeneous, the consequence will be different results for each triplicate. Additionally, it is important to realise that a sediment sample containing microplastic particles cannot be compared to a homogenous solution and as a consequence triplicates of a sediment sample will always display a larger variance than triplicates of a homogenous sample would do. An increase in sample volume could be a way forward to increase the quality of the data, however, a work-up of larger volumes, will be extremely laborious and time-consuming in case of marine sediment. In contrast to homogenous systems, in which each subsample regardless of their size can be considered representative of the whole system, sampling particles in sediment matrix (or aqueous suspension) taking representative samples is more complicated, and should be evaluated.

The last point that needs to be addressed is that only parts of the filter is being scanned with the FT-IR. This will increase the inaccuracy of the quantification further. However, it was necessary for this study due to the data acquisition time. In future research it will be attempted to measure the entire to reduce additional measurement variation if this is feasible. In the section below, several possible changes to the protocol are presented along with their (possible) advantages and disadvantages.

4.2 Recommendations

Table 10 presents possible method improvements with regards to data quality and work-up time for future research. Based on these assessments a recommendation is given whether these should be implemented in the next stage of this project. In this table it is indicated on which part of the sample protocol a change will have an effect: Sample preparation (SP), Recovery rate (RR) or Analysis (A).

Table 10: Recommendations for future research

Less sample material could be used (SP)	
Benefit Less sample material would be beneficial as work-up time will be reduced. Furthermore, less sample material could reduce the risk of a contamination of the Anodisc filter with (in)organic material that can impede measurement of the plastic particles.	**Detriment** Given the inhomogeneous nature of a sediment sample, a reduced amount of sample material will result in a larger error when extrapolating from this subsample to the actual sample.
Recommendation: Currently, 150 g of sample are being treated and analysed. The sample amount should be reduced to 75 g	
More sample material could be used (SP)	
Benefit: This could increase the data quality as a larger sample volume will result in more accurate data.	**Detriment** The currently handled 150 g of sample material already proved to be very time-consuming. A further increase in sample volume might make the work-up disproportionally laborious.
Recommendation: It is not recommended to increase the sample volume due to the time requirement. Especially when this method will be employed for routine measurement, this will be detrimental.	
The samples should be homogenised further (SP)	
Benefit Further homogenisation of the sample will make subsamples more representative.	**Detriment** The samples are sediment samples with a high water content. Quickly after homogenisation, separation will occur, regardless of how long the sample has been stirred.
Recommendation: It is unlikely that a better homogenisation can be achieved. RWS has tested and evaluated several other homogenization techniques, and came to the conclusion that a careful manual homogenization seems to be the only practical method. Therefore, here no improvement is expected and such a step is not recommended.	

Table 10: Continued

Elimination of the work-up with KOH and merely use Fenton's reagent (H_2O_2 + Fe_2SO_4) to remove organic content (SP)	
Benefit Elimination of this step would reduce the amount of steps needed to prepare a sample. This will make the sample preparation less laborious (costly) and reduces the risks for errors.	**Detriment** The elimination of this step can result in incomplete removal of organic material, which then might impede the FT-IR analysis. Also, this will not reduce the overall time that is needed to prepare the sample. The reaction time of the KOH solution will be added to the Fenton's reagent work-up step. Additionally, working with Fenton's reagent requires to work at a controlled pH.
Recommendation: It is recommended not to use Fenton's reagent.	
(Freeze-)dry the samples prior to the density separation (SP)	
Benefit This might result in easier sample handling.	**Detriment** Plastic particles that are aggregated with inorganic material (hetero-aggregation) will be removed during the density separation
Recommendation: It is not recommended to dry samples prior to work-up.	
Eliminate the use of the 500 µm filter (SP)	
Benefit There are no benefits to this. Possible time-savings will not compensate for negative effects.	**Detriment** Removing this filtration step will have two major negative consequences. The first is that larger particles will clog the smaller filter that are later on used in the work-up process. The second consequence would be that particles larger than 500 µm will end up on the Anodisc filter for the FT-IR analysis. Because the FT-IR works in transmission mode, these particles will appear as black dots on the filter, as they are too thick to be penetrated by the IR-beam.
Recommendation: It is highly advised not to leave out the 500 µm filtering step.	

Table 10: Continued

Elimination of the 25 µm filtration step following the H_2O_2 treatment and filtrate directly over the Anodisc filters (SP)	
Benefit This would remove a work-up step and hence make the sample treatment less laborious	**Detriment** Doing this would increase the risk that more inorganic particles will end up on the Anodic filter. This will impede the measurement.
Recommendation: It is not recommended to remove the 25 µm filtration step. Although, Anodisc filter can withstand H_2O_2, the likelihood of larger inorganic particles impeding the measurement, does not justify the elimination of the filtration step. If the filter is contaminated with inorganic material, there is no remedy for this anymore.	
Use of a extraction solvent for the density separation (lower density) (SP)	
Benefits Working with a lower density solution might remove unwanted organic material during the density separation. The Anodisc filter could be cleaner which will be beneficial to the analysis.	**Detriment** When working with a lower density solution, one risks to loose also microplastics during the density separation, such as PS and PET.
Recommendation: It is not recommended to use a different solution. A loss of plastic particles during the work-up is undesirable.	
Measuring the whole filter instead of merely about 45% (A)	
Benefit This will give a more accurate picture of the sample as no extrapolation is needed anymore.	**Detriment** With the FT-IR used for the samples in this report, time was the defining factor for opting not to measure the whole filter. One measurement would have taken more than 14 hours. The new FT-IR that will be used for the next phase, will be significantly faster.
Recommendation: It is recommended to measure the whole filter, if it is reasonable.	
Use also 200 µm fluorescent plastic particles for the determination of the recovery efficiency (RR)	
Benefit This would improve the recovery efficiency as next to 100 µm recovery rate also the 200 µm recovery rate is determined.	**Detriments** No negative effects can be foreseen.
Recommendation: This step is recommended.	
Rinsing of glassware with a little ethanol when necessary and possible	
Benefit This might increase the recovery rate.	**Detriments** No negative effects can be foreseen.
Recommendation: This step is recommended.	

The recommendations that are going to be implemented for the next phase of the project are shown in Table 11.

Table 11: Changes proposed to the method based on the recommendations in Table 10.

Current method	Proposed new method	Expected effect
100 µm PE particles were used for the determination of the recovery rate	100 and 200 µm PE - particles will be used for the determination of the recovery rate.	A better recovery rate will be received reflecting the actual sample more accurately
150 g of sediment wet weight as sub- sample was used.	75 g of sediment wet weight. as sub-sample will be used.	The work-up of the sample should be faster and less time-consuming. Additionally, less undesirable (in)organic material might end up on the Anodisc filter
45 % of the Anodisc filter was analysed with an FT-IR microscope	100 % of the Anodisc filter will be analysed using an FT-IR microscope, if feasible.	The quality of the data will be improved.
Rinsing only with water	Use of water/ethanol	Better recovery rate

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Appendix I - Pictures of samples

The pictures show the section of the filter analysed using the FT-IR microscope. The left-hand picture is the heat map of this section and here the infrared signals can be seen. A deviation from blue indicates that an infrared spectrum was received. This does not necessarily mean that a plastic particle has been found. The right-hand picture is a photography of the area. Here no information regarding the nature of particles can be received from. Figures marked with an "*", show the pictures and heat map of samples that could not have been analysed properly (no size information).

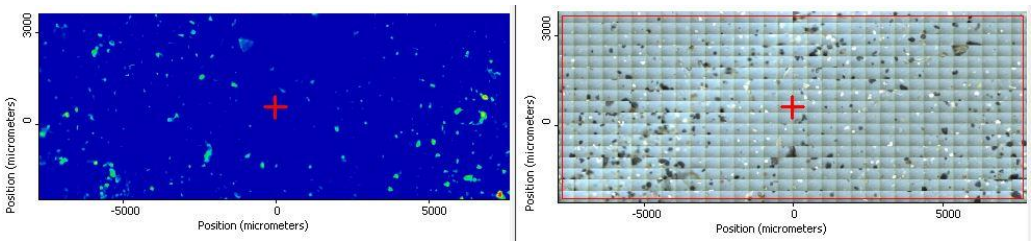


Figure 3: 2017008168

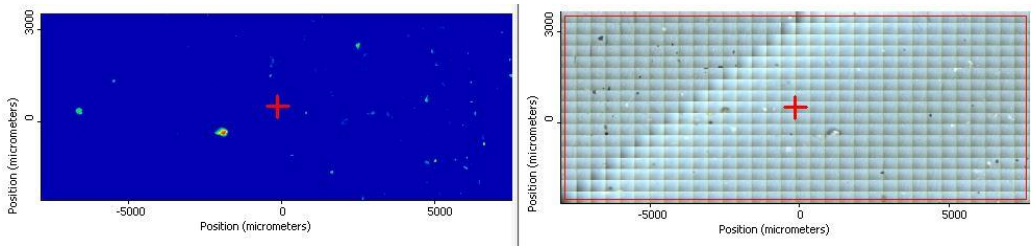
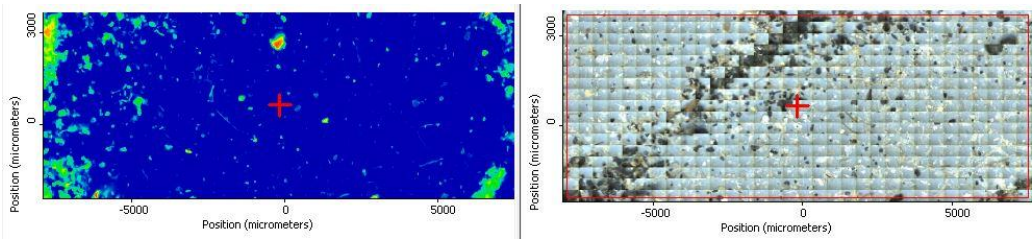


Figure 4: 2017008615



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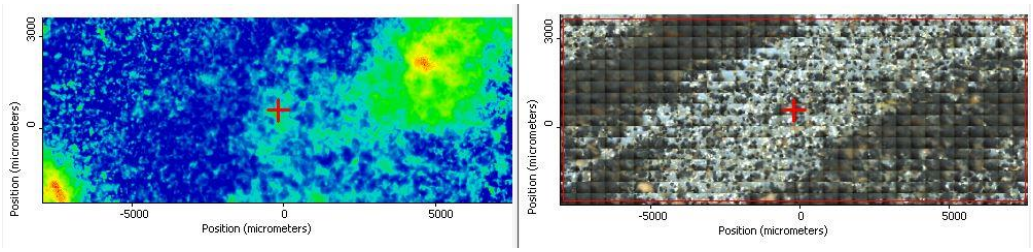

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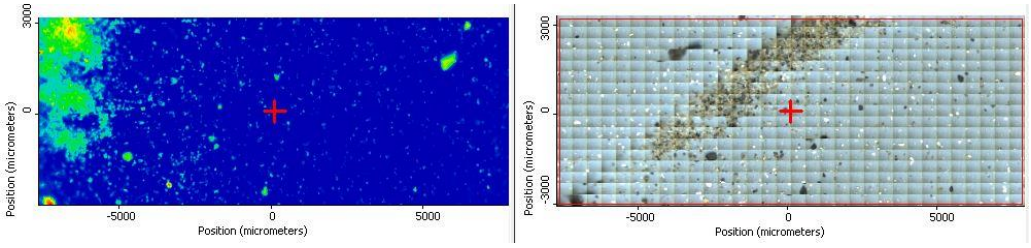

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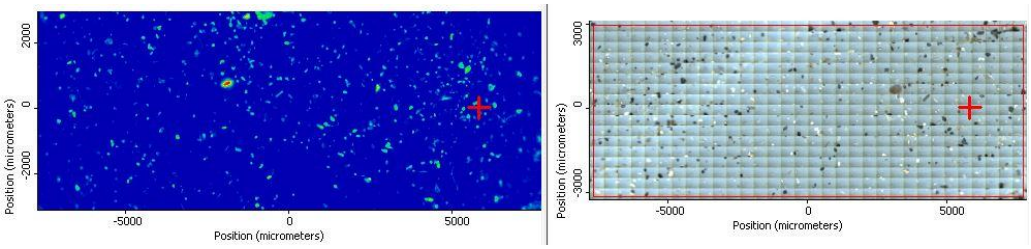

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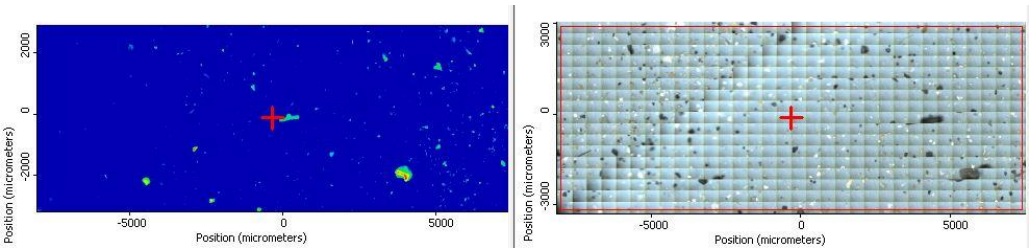

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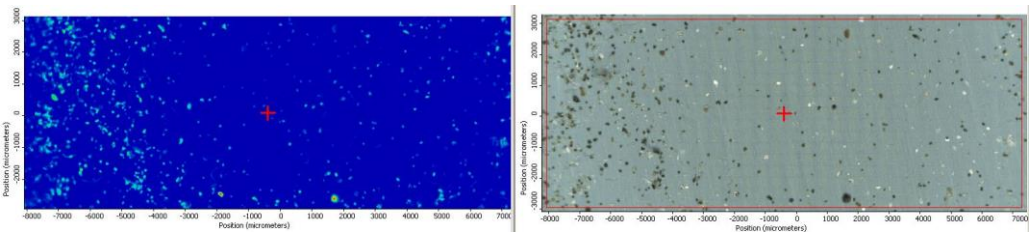

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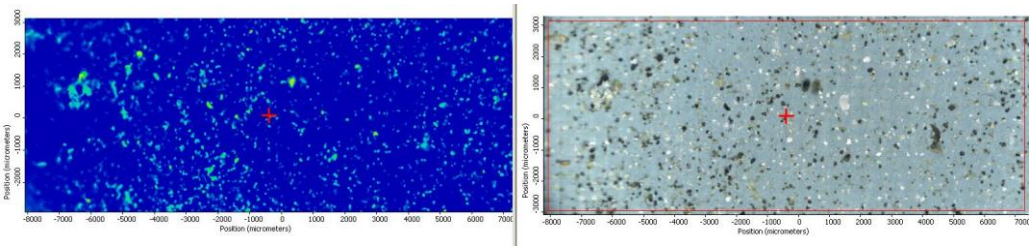
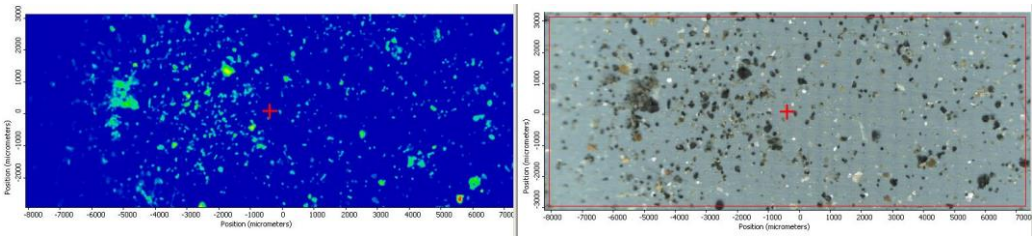


Figure 11: 2017008167



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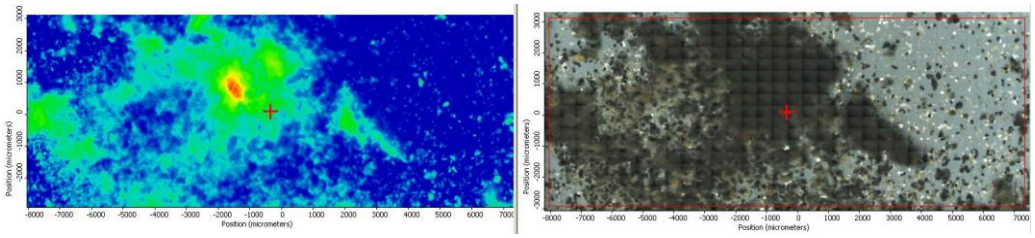

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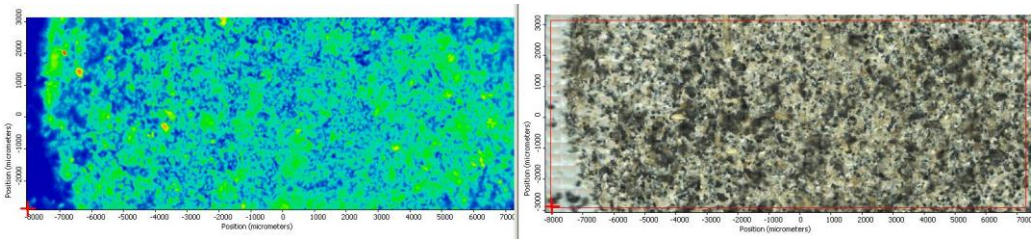

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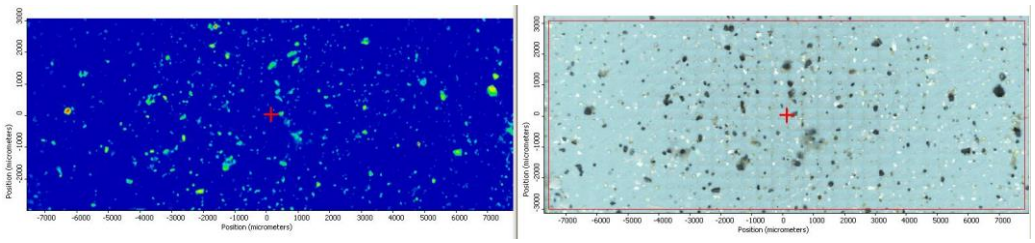

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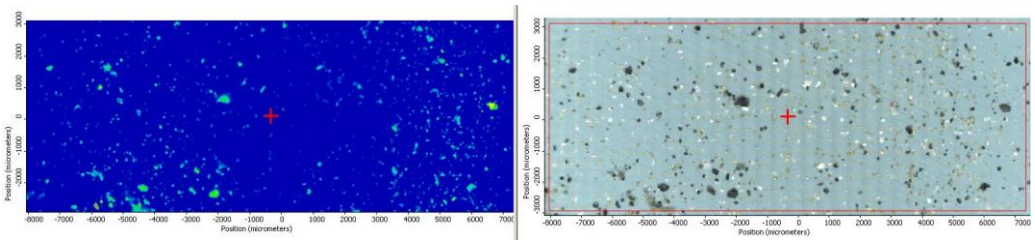

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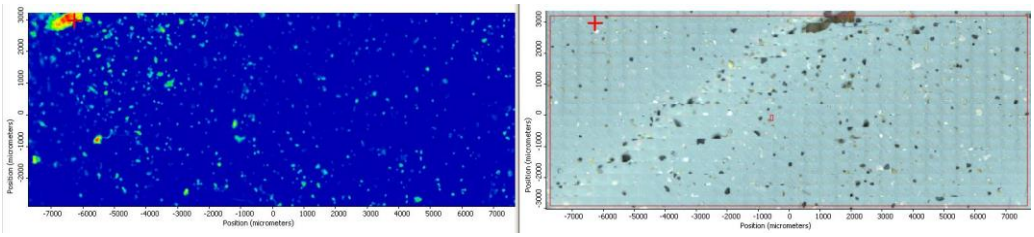

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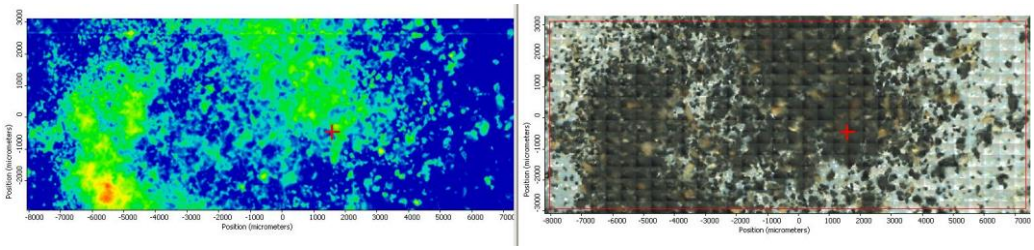

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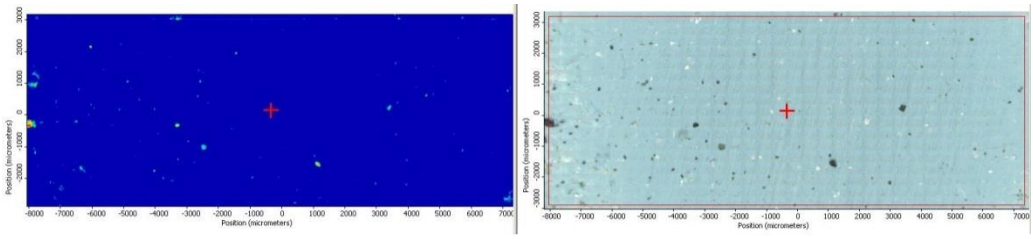

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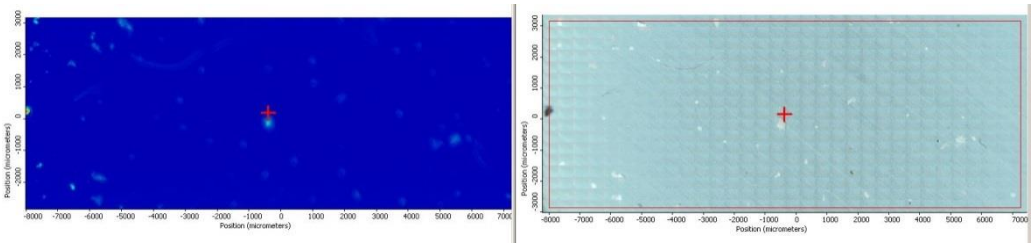

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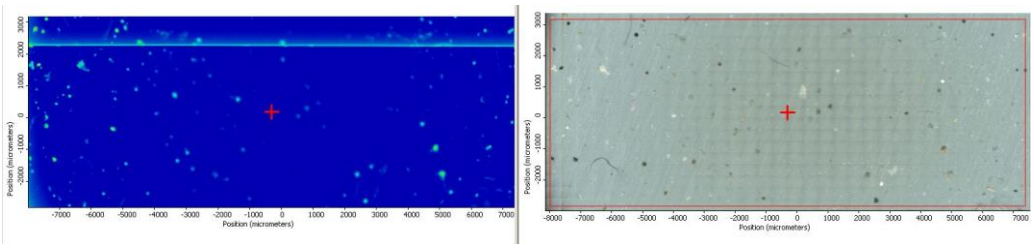

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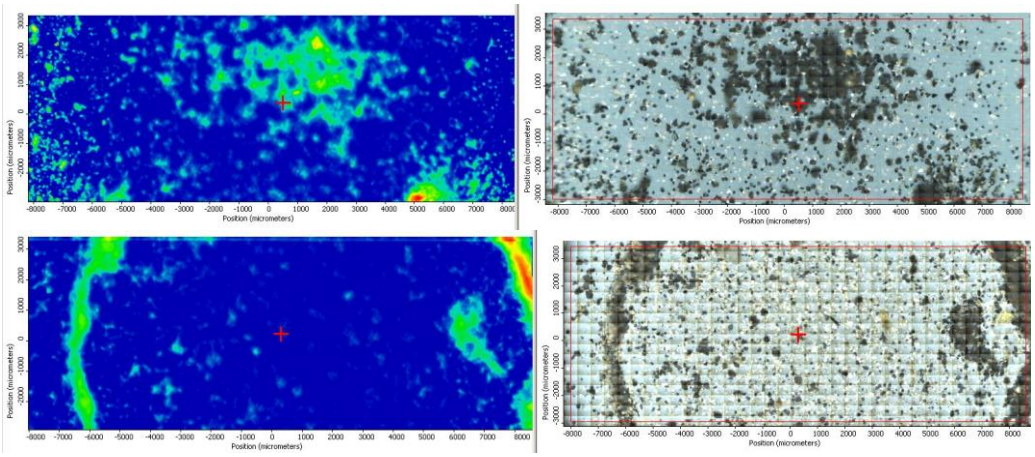


Figure 22: 2017010217_v1 and v1 (Two filters for this sample) *

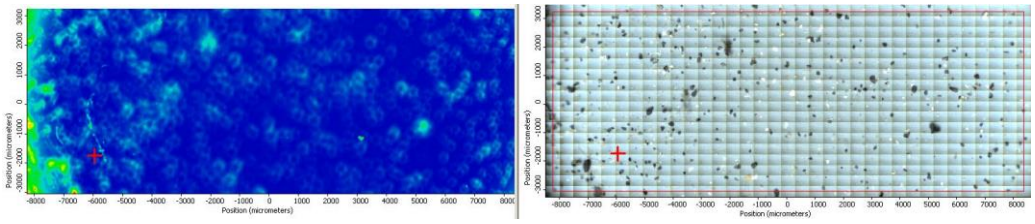


Figure 23: 2017008178

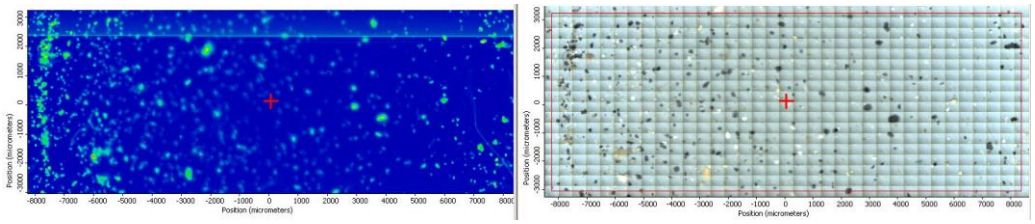


Figure 24: 2017008177

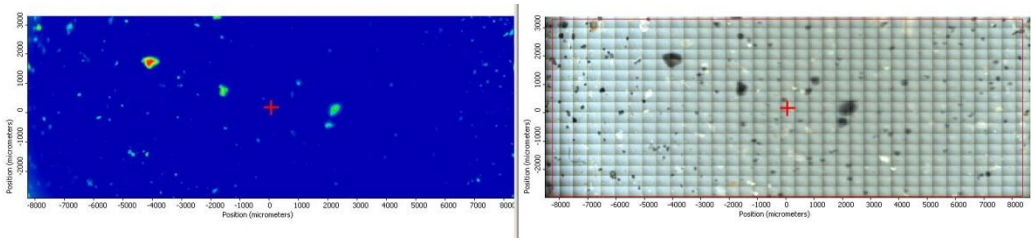


Figure 25: 2017008179

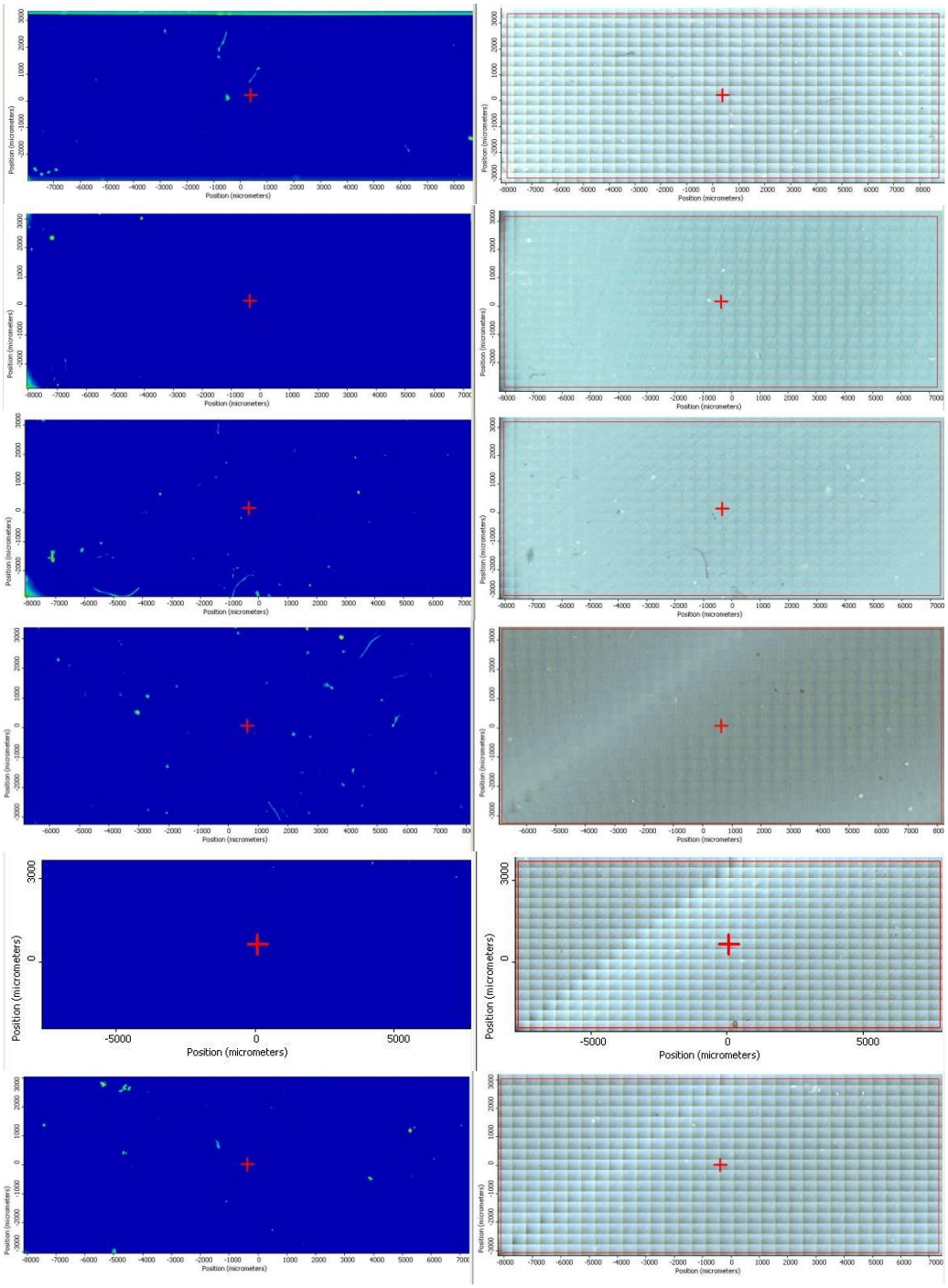
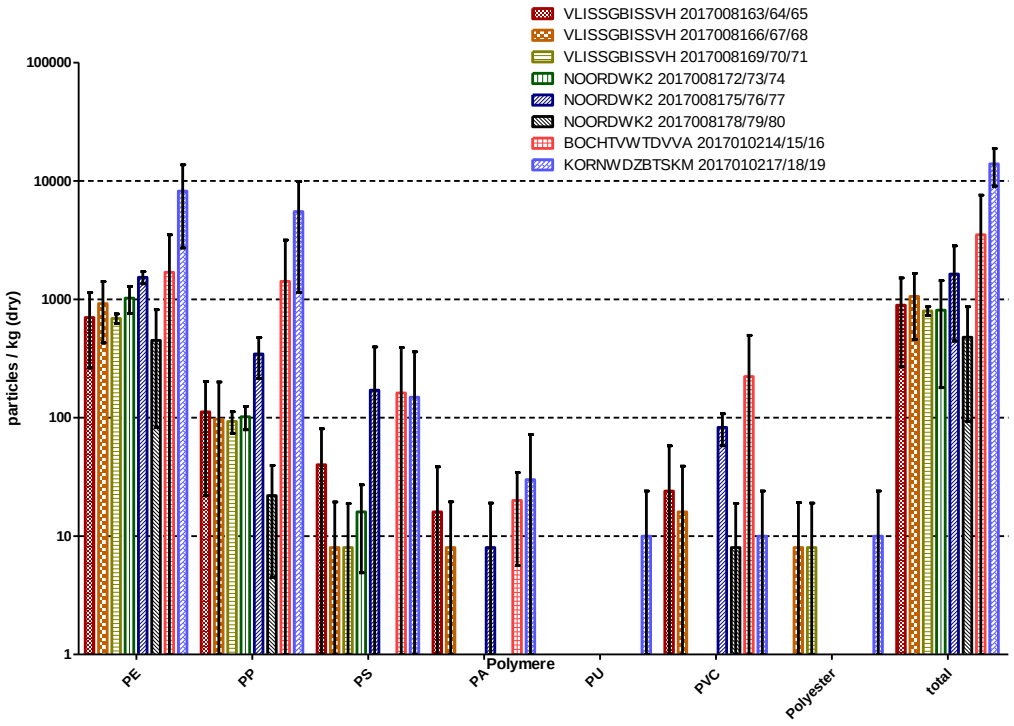
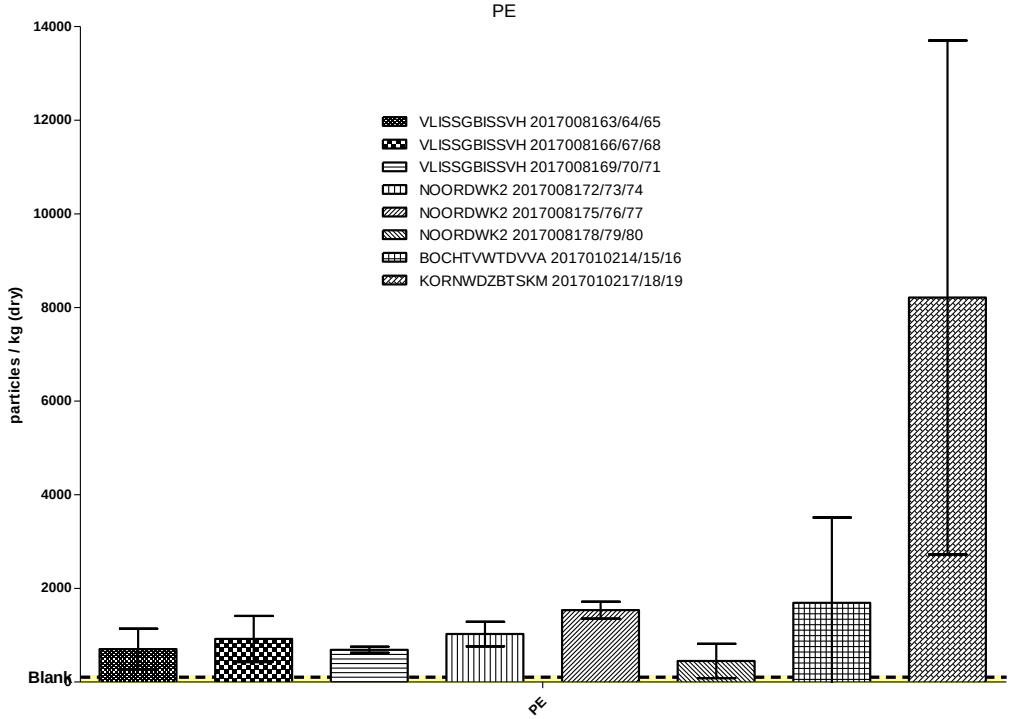
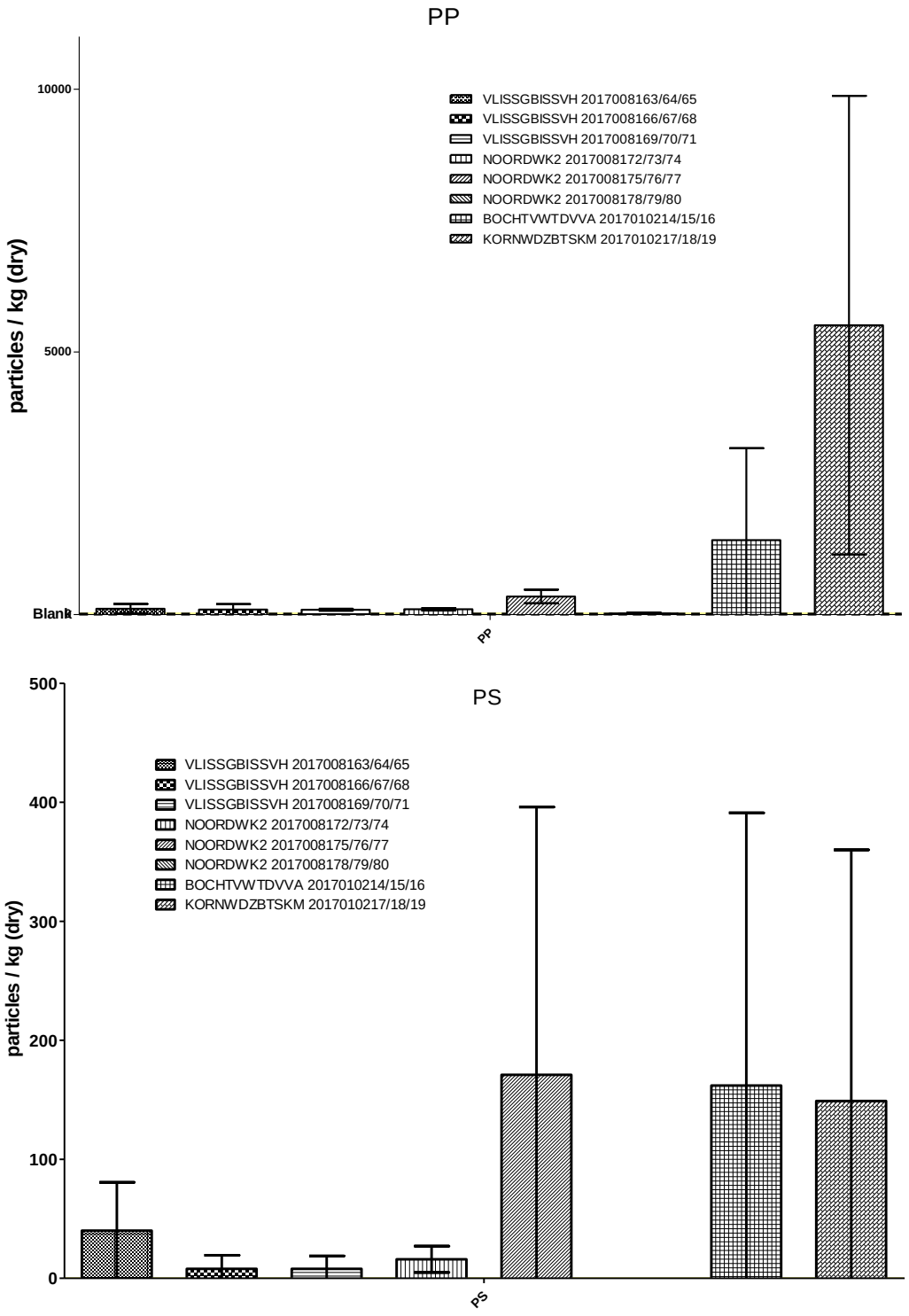


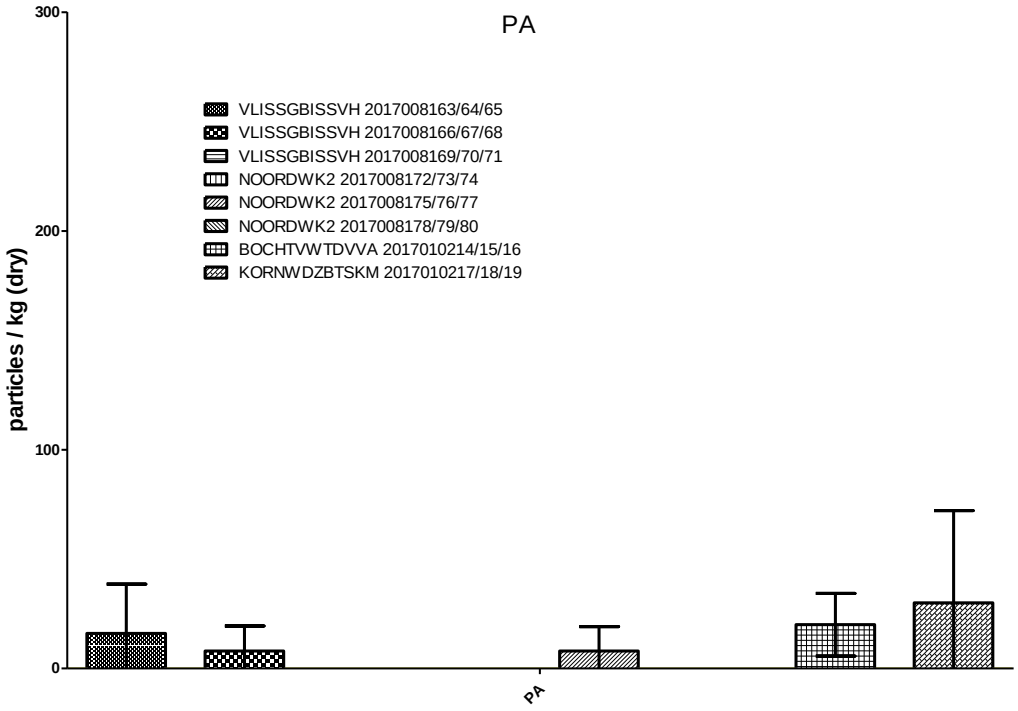
Figure 26: Blanks 1 to 6 from top to bottom.

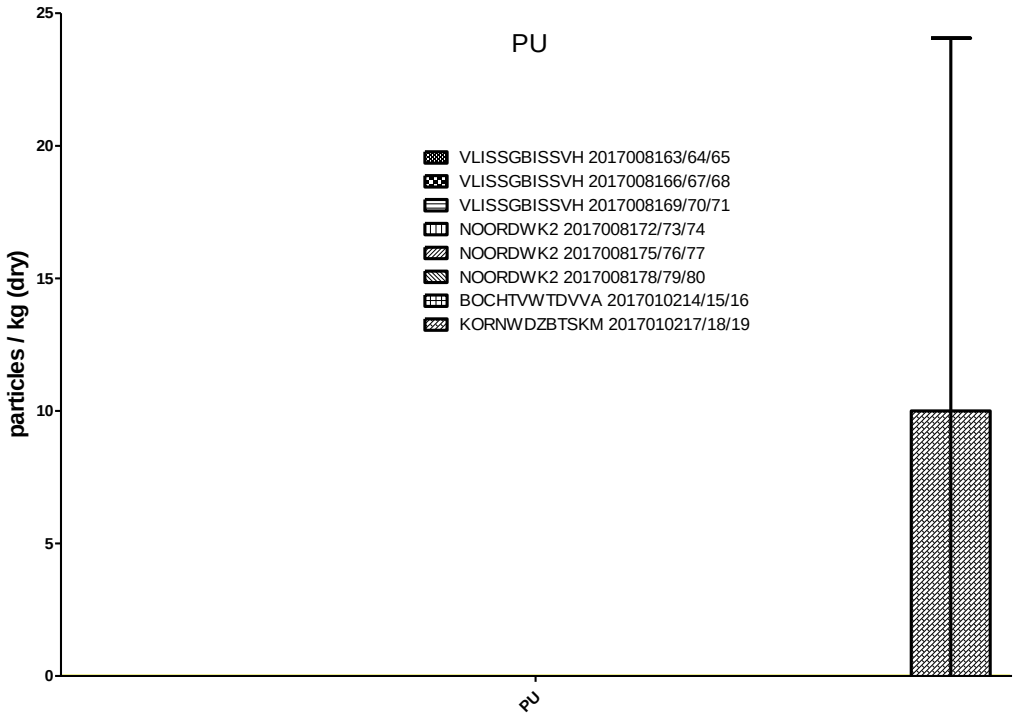
Appendix II - Size fraction 100-300 µm

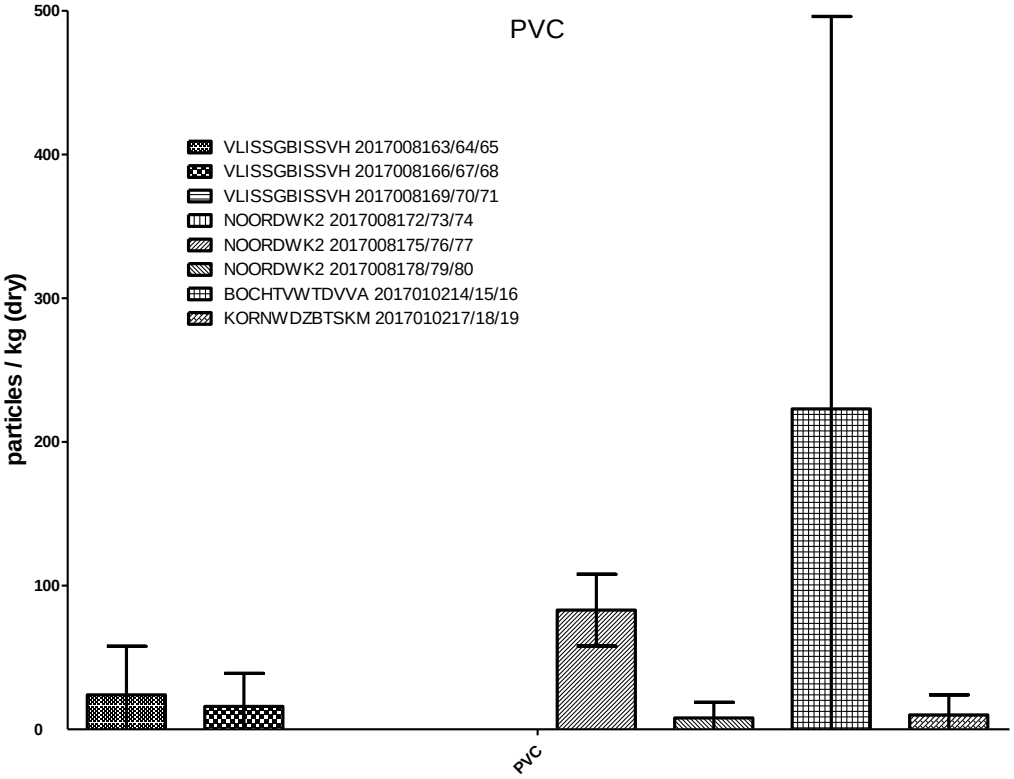


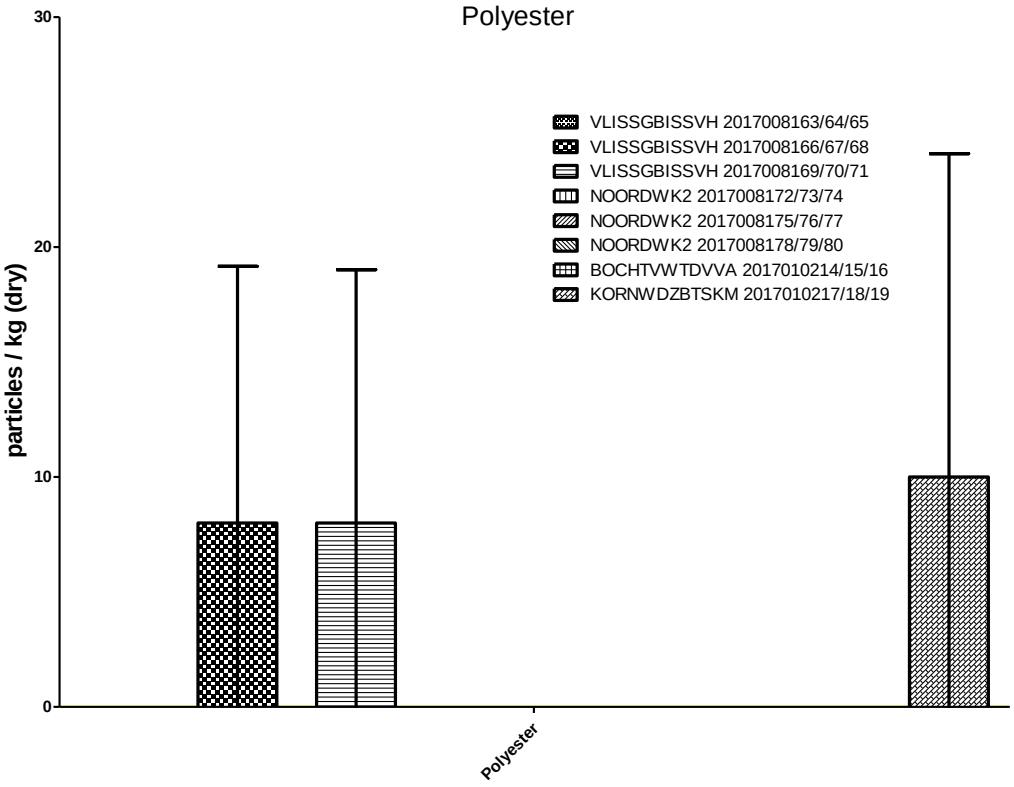


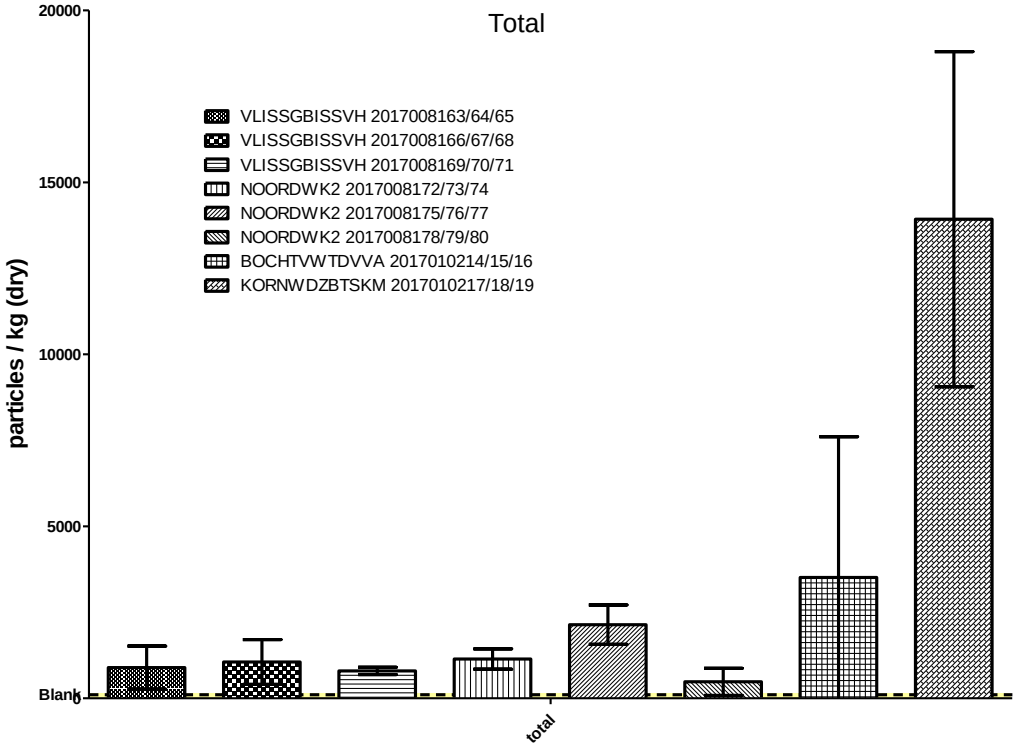

PP
particles / kg (dry)
10000
5000
Blank
PP
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
PS
particles / kg (dry)
500
400
300
200
100
0
PS
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19


PA
particles / kg (dry)
300
200
100
0
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
PA

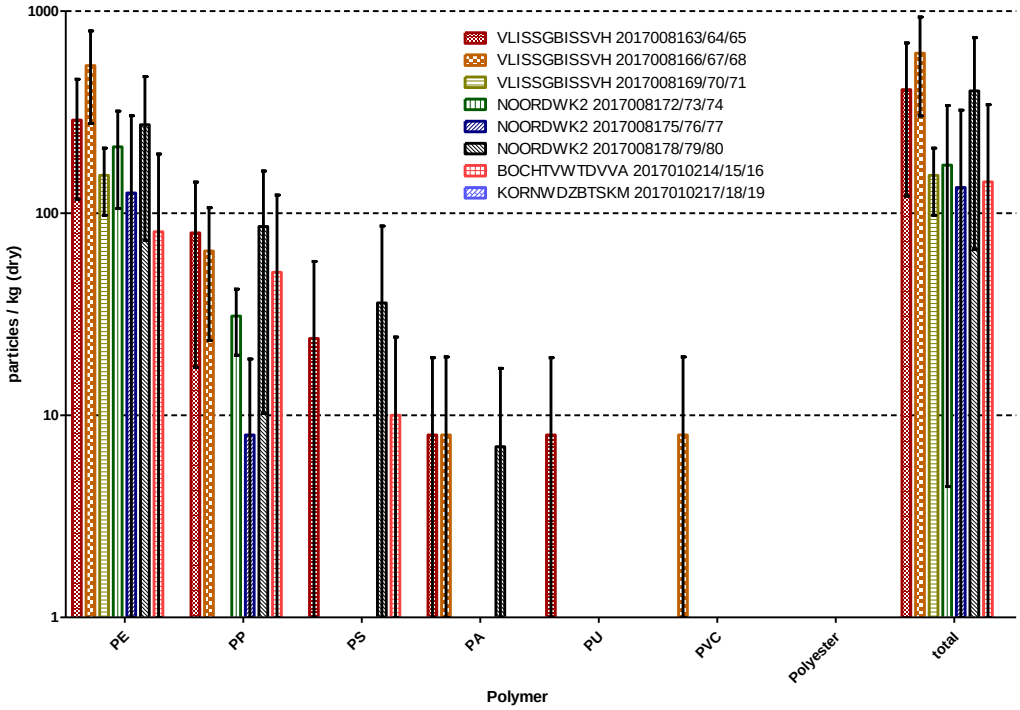

PU
particles / kg (dry)
25
20
15
10
5
0
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
PU

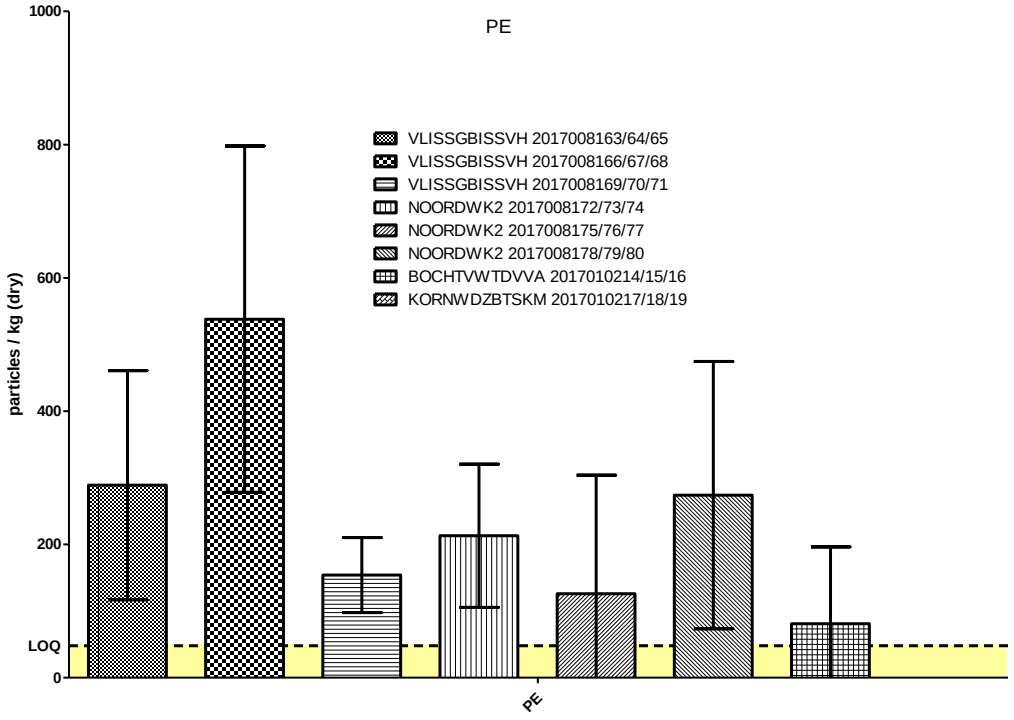

PVC
particles / kg (dry)
500
400
300
200
100
0
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
PVC

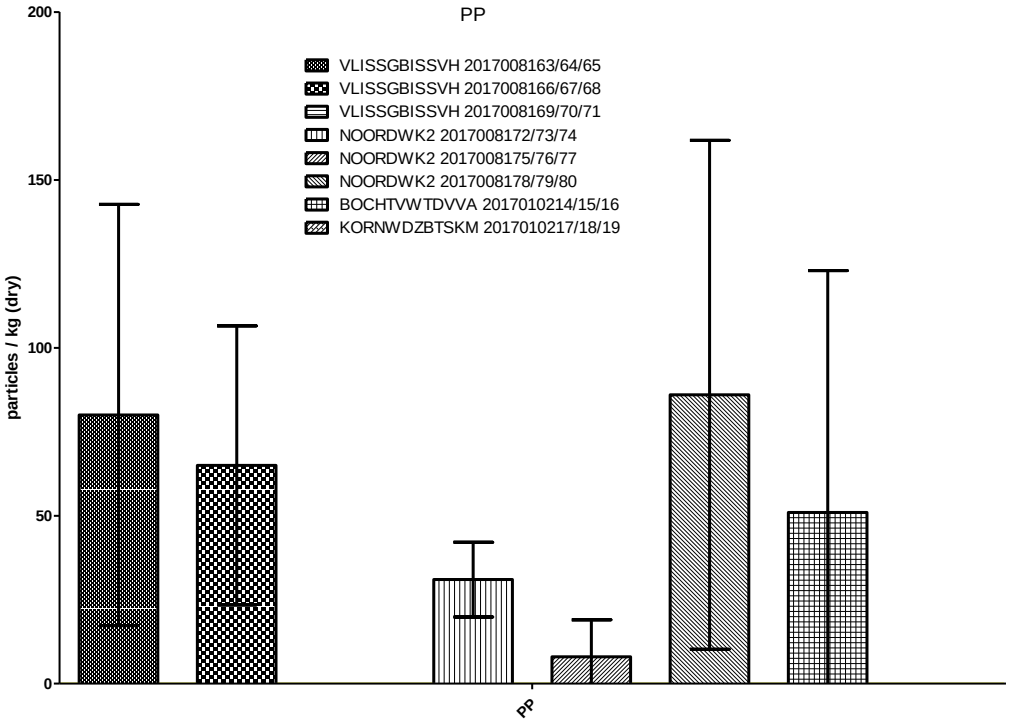

Polyester
particles / kg (dry)
30
20
10
0
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
Polyester

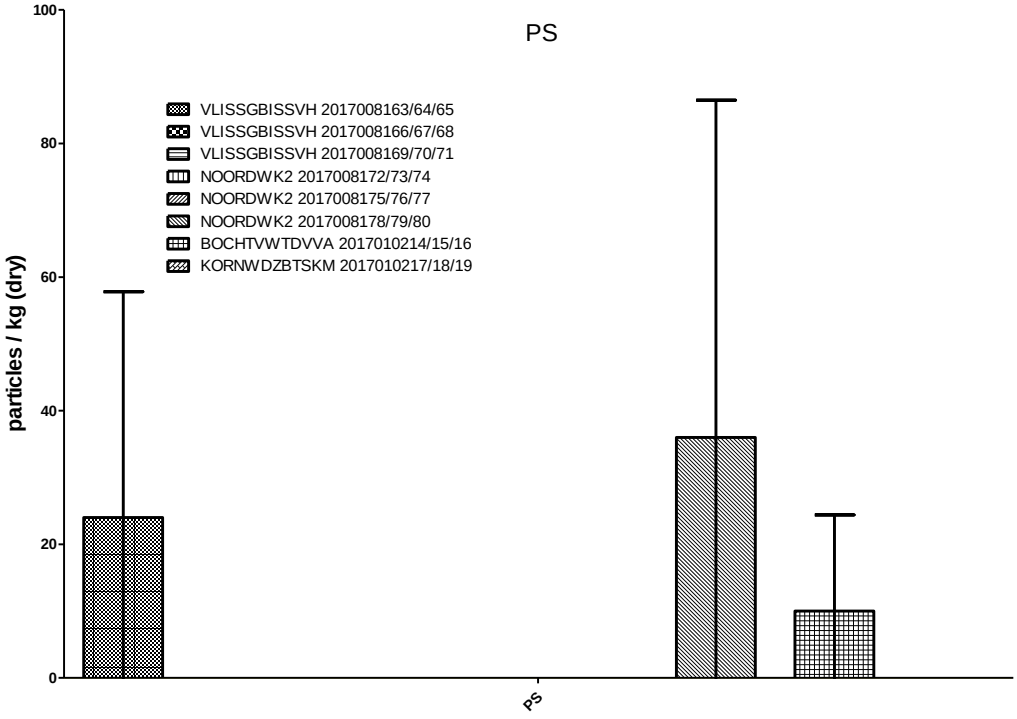

Total
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
particles / kg (dry)
20000
15000
10000
5000
0
Blank
total

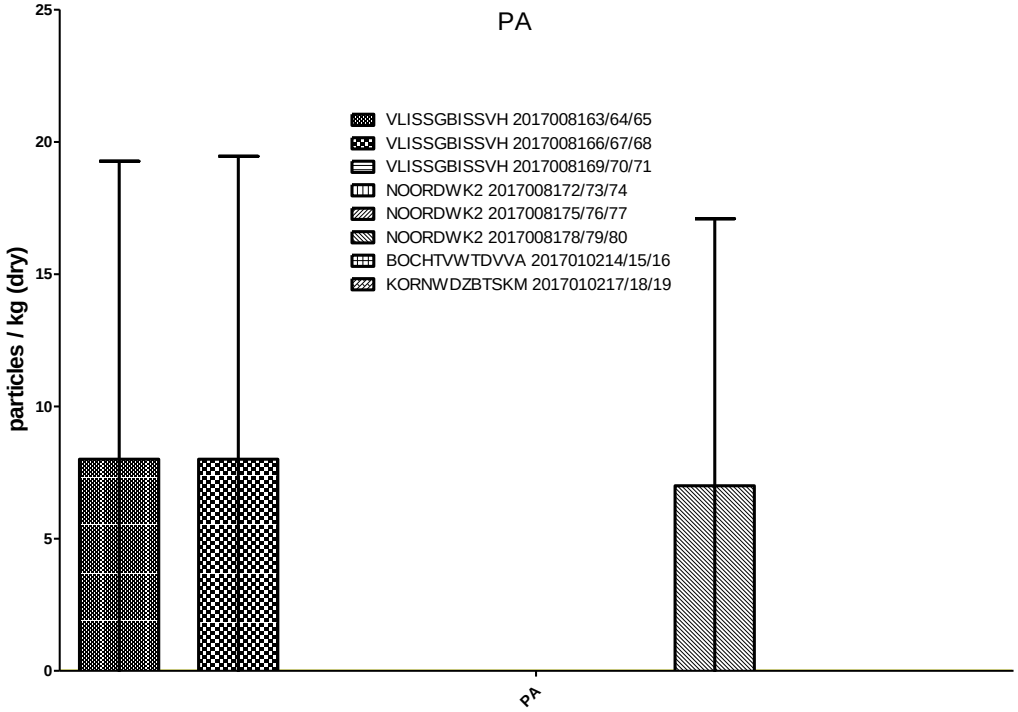
Appendix III - Size fraction 300–1000 µm

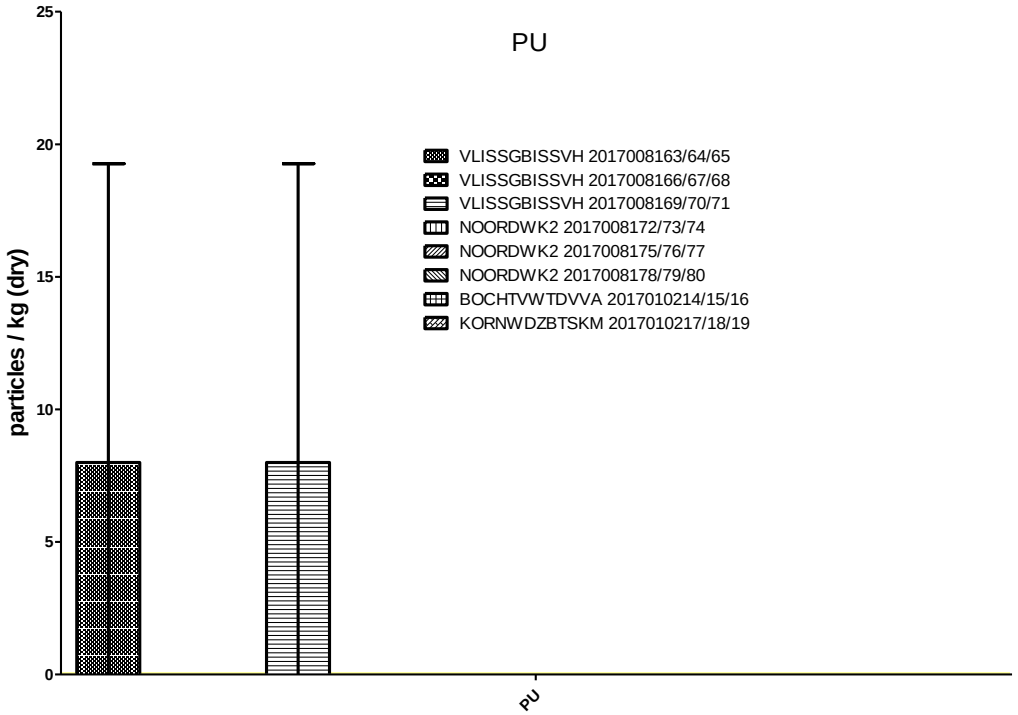


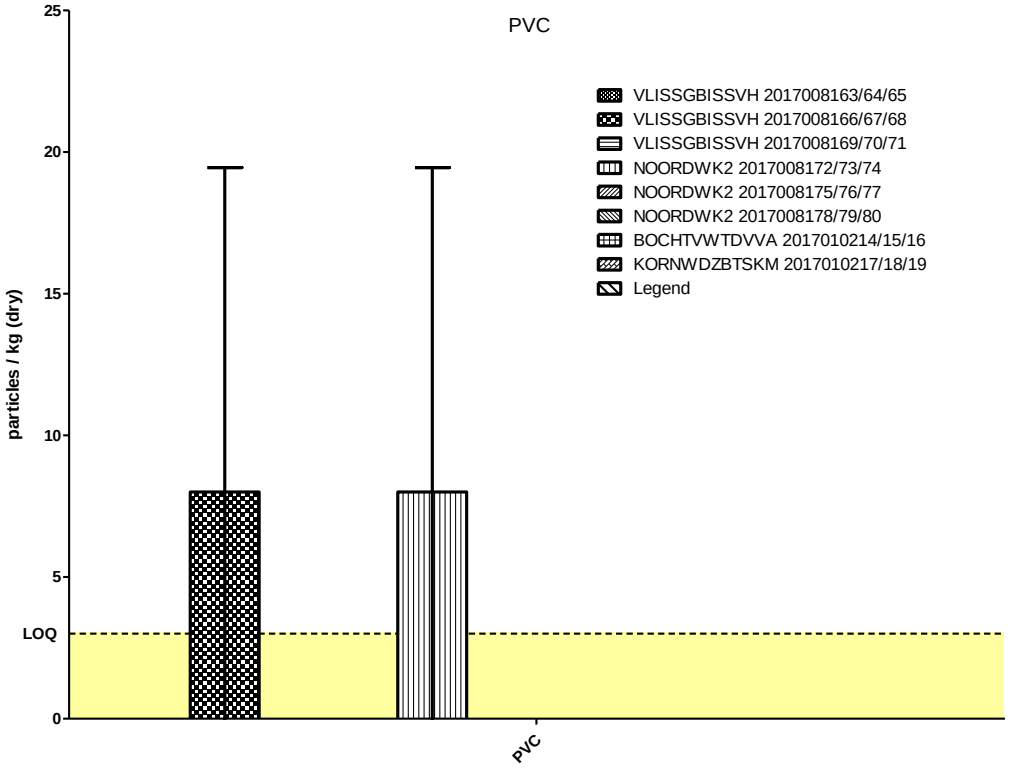


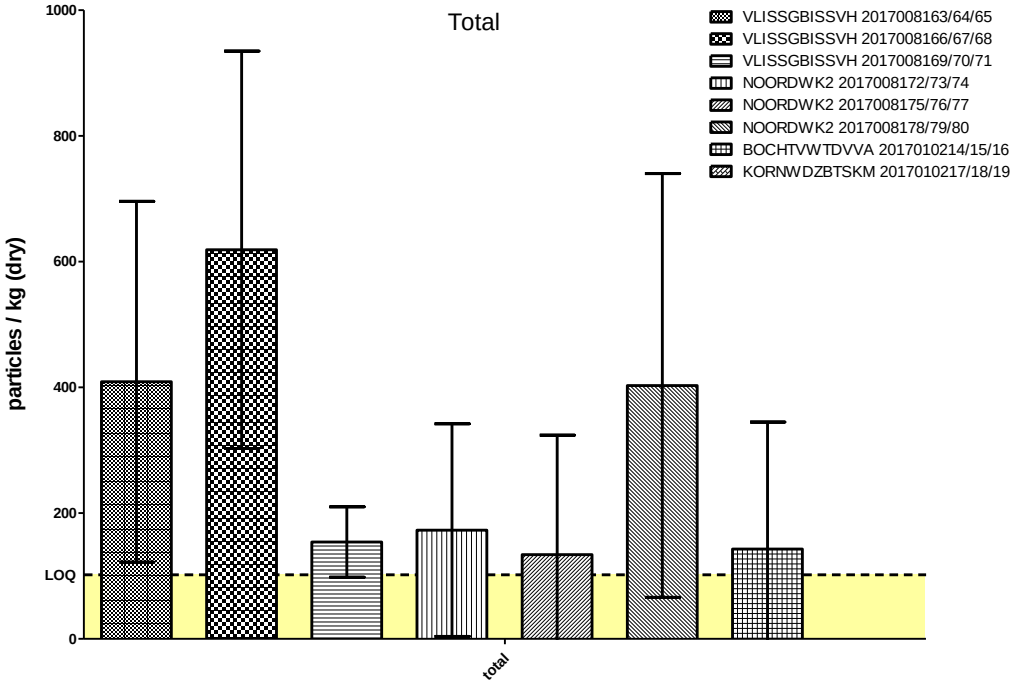

PP
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
particles / kg (dry)
200
150
100
50
0
PP


PS
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
particles / kg (dry)
100
80
60
40
20
0
PS


PA
particles / kg (dry)
25
20
15
10
5
0
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
PA


PU
particles / kg (dry)
25
20
15
10
5
0
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
PU


PVC
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
Legend
particles / kg (dry)
25
20
15
10
5
LOQ
0
PVC


Total
VLISSGBISSVH 2017008163/64/65
VLISSGBISSVH 2017008166/67/68
VLISSGBISSVH 2017008169/70/71
NOORDWK2 2017008172/73/74
NOORDWK2 2017008175/76/77
NOORDWK2 2017008178/79/80
BOCHTVWTDVVA 2017010214/15/16
KORNWDZBTSKM 2017010217/18/19
particles / kg (dry)
1000
800
600
400
200
LOQ
0
total

Appendix IV - Example data

Sample No.: VLISSGBISSVH [2017008163-01/VBC]

MP identifier	Coordinates [pixels]		Coordinates [µm]		Polymer group	Area on map [µm²]	Major dimension [µm]	Minor dimension [µm]	Volume [µm³]	Mass [ng]
MP_1	[3	149]	[90	4470]	pe	35100	281,9	158,6	2226186	2114,877
MP_2	[0	170]	[0	5100]	pe	6300	124,9	64,2	161882	153,788
MP_3	[3	37]	[90	1110]	pe	15300	180,0	108,2	662339	629,222
MP_4	[20	137]	[600	4110]	pe	45900	333,7	175,1	3215252	3054,489
MP_5	[31	168]	[930	5040]	pe	14400	212,5	86,3	497017	472,166
MP_6	[59	148]	[1770	4440]	pe	37800	525,7	91,6	1384338	1315,121
MP_7	[61	69]	[1830	2070]	pe	24300	216,1	143,2	1391580	1322,001
MP_8	[90	121]	[2700	3630]	pe	28800	253,4	144,7	1666798	1583,458
MP_9	[104	196]	[3120	5880]	pe	4500	90,0	63,7	114592	108,862
MP_10	[135	98]	[4050	2940]	pe	34200	247,4	176,0	2408122	2287,716
MP_11	[150	162]	[4500	4860]	pe	22500	191,6	149,6	1345990	1278,690
MP_12	[209	29]	[6270	870]	pe	26100	222,1	149,6	1562122	1484,015
MP_13	[235	198]	[7050	5940]	pe	36000	270,9	169,2	2436194	2314,384
MP_14	[311	84]	[9330	2520]	pe	26100	211,2	157,3	1642374	1560,255
MP_15	[314	0]	[9420	0]	pe	6300	124,9	64,2	161882	153,788
MP_16	[329	164]	[9870	4920]	pe	23400	191,6	155,5	1455823	1383,032
MP_17	[350	43]	[10500	1290]	pe	24300	202,3	152,9	1486449	1412,127
MP_18	[374	39]	[11220	1170]	pe	125100	507,8	313,7	15696646	14911,814
MP_19	[408	13]	[12240	390]	pe	21600	183,0	150,3	1298663	1233,730
MP_20	[426	156]	[12780	4680]	pe	26100	222,1	149,6	1562122	1484,015
MP_21	[436	5]	[13080	150]	pe	22500	204,9	139,8	1258151	1195,243
MP_22	[469	1]	[14070	30]	pe	29700	330,7	114,3	1358269	1290,355
MP_23	[476	137]	[14280	4110]	pe	33300	245,3	172,9	2302599	2187,469
MP_24	[478	112]	[14340	3360]	pe	46800	327,7	181,8	3403626	3233,445
MP_25	[497	18]	[14910	540]	pe	30600	245,3	158,9	1944342	1847,125
MP_26	[507	184]	[15210	5520]	pe	22500	191,6	149,6	1345990	1278,690
MP_27	[56	148]	[1680	4440]	pp	67500	478,0	179,8	4854650	4611,918
MP_28	[237	122]	[7110	3660]	pp	29700	222,1	170,3	2022771	1921,632
MP_29	[455	29]	[13650	870]	pp	14400	164,2	111,7	643305	611,140