

# SURVEY OF MICROSCOPIC ANTHROPOGENIC PARTICLES IN SKAGERRAK.

Pilot study October- November 2010



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## SUMMARY

During two pelagic surveys between Arendal and Hirtshals, the concentration of potential anthropogenic particles was measured using a new sampling method for monitoring purposes. The sampling method worked well and potential anthropogenic particles in size range of 10 – 500 µm were quantified with the following main results:

1. The concentration of textile fibers and microscale plastic particles could not be qualitatively distinguished to the control samples that were analyzed or be excluded as contamination.
2. A large number of black particles was found (median 41\*lit<sup>-1</sup>, max: 779\*lit<sup>-1</sup>,min: 8\*lit<sup>-1</sup>). Of these a large, but not yet quantified, number is minerals and other natural particles but a measurable amount could be anthropogenic particles such as rubber particles, road wear etc. One suspicious anthropogenic particle type that was found was the round black particles that partially were dissolved in an organic solvent.

The method can easily be modified for sampling of plastic particles larger than 0,3mm by using a larger pump in the sampler and a wider mesh (333/450 µm). Such samples could then be compared to enumerations of plastic micro litter from other areas.

## BACKGROUND

Marine macroscopic litter is of well known concern and has been studied for several decades. Today both national and intergovernmental organizations work to minimize the littering, such as IMO regulations and UN programs.

The littering by microscopic plastic particles is of more novel interest (even if there were some research on the topic in the 1970's such as Carpenter (Carpenter, Miklas et al. 1972) and (Colton, Knapp et al. 1974)). Articles by e.g Thompson (Thompson, Olsen et al. 2004), (Lattin, Moore et al. 2004) and More (Moore, Moore et al. 2001) where they found concentrations of plastic particles in the range of  $\sim 0,01 - 1 - (20)$  plastic particle per  $m^3$  received a strong response from both governments, media and researchers, see Table 1 for a brief summary. A notable fact from those studies is that the researchers used zooplankton net in their studies. The mesh openings in the nets are most often  $333\mu m$  or  $450\mu m$  which excludes particles smaller than that specific size.

**Table 1 Summary of some research results on marine microscopic litter.**

Reference	Particle conc. (per $m^3$ )	Sea	Sample type
(Moore, Lattin et al. 2005)	0.43 – 2.23	North Pacific	Offshore, surface
-II-	5.0 – 7.25	North Pacific	Inshore, surface
-II-	0.017	North Pacific	Offshore, subsurface
(Thompson, Olsen et al. 2004)	0.01 – 0.06	Scotland-Shetland	
(Lattin, Moore et al. 2004)	0.5 – 18	Kalifornien	Inshore, surface
(Carpenter, Miklas et al. 1972)	0.01 – 2.6	USA north east	Coastal waters

One well known problem with plastic pollution in the sea is that animals such as filter-feeders, fish and sea birds ingest the plastic which causes them harm, sometime even lethal. Another problem is the transport of organic pollutants such as PAH, PCB etc. on the surface of the particles which also is well known (Mato, Isobe et al. 2001), but the very uptake into the animals has not been quantified.

During 2007 KIMO Sweden (Kommunenenes Internasjonale Miljøorganisasjon) performed a study to see if the same amount of particles that Thompson found around Great Britain was found in Swedish waters. They assigned N-research which performed the study using a phytoplankton net as sampler in parallel with a WP-2 zooplankton net ( $450\mu m$ ). This led to a hitherto unstudied fraction was revealed, with a size distribution of anthropogenic particles from  $10\mu m$  to  $500\mu m$ . The particles consisted of polyethen pellets, textile fibers etc, and was in the order of thousand times higher than previously reported (Table 1 values). This led to further studies in Swedish waters on the behalf of Swedish Environmental Protection Agency published 2009 (Norén, Ekendahl et al. 2009). The first study used the same phytoplankton net as sampler and the results was consistent with the 2007 KIMO report (i.e. concentrations of anthropogenic particles (fibers included) in the range  $1000-100000$  per  $m^3$ ). In the first study the concentration of black particles was counted for the first time (they were only noted in the first study) and their hypothetical origin was suggested as road and rubber wear. This was based on the fact that  $\sim 100.000$  tons of asphalt roads wears every year in Sweden alone and  $\sim 10.000$  tons of rubber

tires together with the striking morphological similarity with particles from road side snow-melt (Fig. 1). This lead to a focus on the black particles due to the fact that the oil constituent in asphalt roads, bitumen, is rich in toxic hydrocarbons such as PAH:s and rubber particles has been shown to be toxic to aquatic animals (Wik and Dave 2006). So one on the ongoing projects with high priority is to discriminate between sources of marine black particles: Naturally degraded organic particles, peat, volcanic ash, black mineral particles such as biotit and amphibolit together with anthropogenic sources as asphalt/bitumen particles, rubber tire wear, oil-spill droplets and fly ash. It is of uttermost importance that we analyze the particle origin before we can assess the environmental risk of the anthropogenic particles. To our knowledge there is no such studies conducted before, but several studies has studied the concentrations of PAH:s in marine environment (e.g. Broman 1988) where the PAH concentration in marine waters is coupled to urban areas.

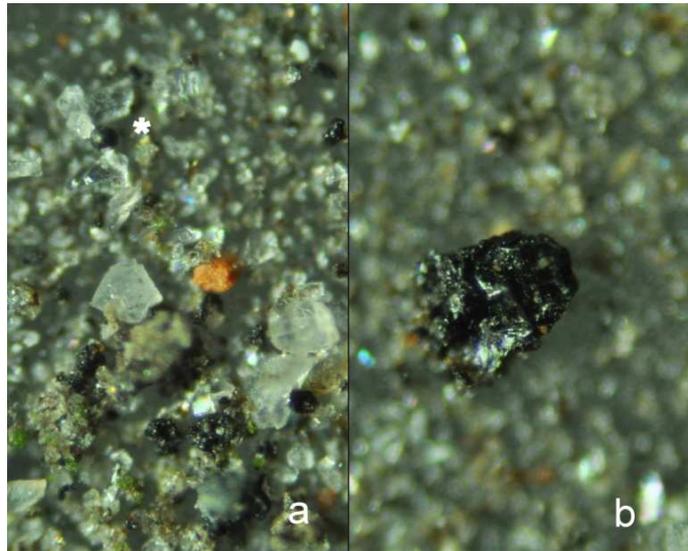


Figure 1 Black particles from road dust. a. 10x, note the black round particle (\*) which is found in all marine samples b. 20x.

During the following period Swedish EPA has granted more research funding for improvement of the sampling methodology and analysis of both environmental concentrations and material composition. During the second survey we have concluded that contamination of the samples is a serious threat for overestimation of particle concentrations and that previous reported concentrations are not reliable. Most contamination originated mostly from textile fibers.

So the need for a contamination free sampler was urgent. Because of the impracticality of using a clean-room technique in routine monitoring we have weighed the need for low contamination with an easy to use method and the method today lets us take control samples on board. Those controls can be seen as blanks. During the development of the sampler used in this actual KLIF cruise, at least seven different samplers has been tested and rejected due to impracticability or high contamination. We see the sampler used today as still under development, even if we are currently satisfied with the main principle: placing the sampling filter directly in contact with sea water and the use of controls.

## MATERIAL & METHODS

### GENERAL

During two journeys with the research vessel G. M. Dannevig, the concentration of microscopic anthropogenic particles was assessed. The first journey was 2010-10-XX and the second was 2011-11-12. See figure 1 for route and location of sampling stations.

All procedures were made with the aim to minimize contamination from both equipment and handling. This aim was weighed with the need of having a method useful in routine monitoring, i.e. some contamination cannot be excluded due to the practicality of sampling onboard under time restraints. This does not exclude further improvements of the methodology.

### SAMPLING

The sampling equipment used was principally a submersible water pump (12V) inside a water proof case (Pelicase) with the sampling filter located directly to the sea. See Figure 2 for a schematic drawing of the sampling equipment and Figure 3 for a photo.. The Pelicase was of orange color (Polypropylen) and the filter holder was a modified stainless pressure filter holder from Sterlitech (their # XXX, but similar filter holders are available from common filter vendors). The modification consisted in constructing a new outlet fitting (1/2") and a new larger inlet was lathed with a smooth surface and a semi-enclosed volume before the filter to avoid resuspension on the filter from wave turbulence. From the submersed pump a water hose (enhanced polyester hose, ordinary used for compressed air, i.d. 10mm). On the end of the hose, which was located on the ship deck, a electronic flow and volume meter was placed (Great Plains Industries). The sampler was hold 2 meter outside the ship hull by a metal pole where on the rope (white polyester) that hold the sampler was fastened. The sampler was hold in position in the surface water at 0,5 meters depth, but this depth ranged from 0,1 to 1,5 meter due to large waves.

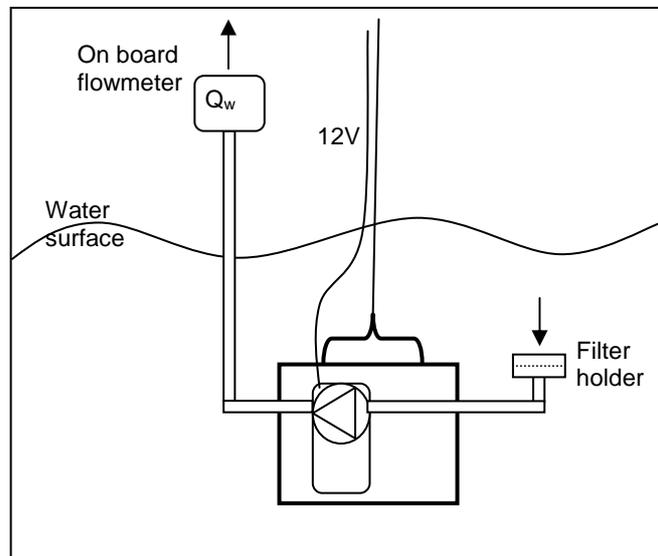


Figure 2 Schematic drawing of the submersible sampling equipment.

### FILTER HANDLING

The filters used was polycarbonate *hydrophobic* pore filters with a defined pore size of 10 $\mu$ m (Sterlitech #XX, but similar filters are available from different sources). A 30  $\mu$  mesh nylon filter was used for as a supporting filter (this enhanced the filtration capacity of the 10 $\mu$ m filter). Before and after filtration the filters were placed in protective filter holders (Millipore #XX) which reduced the risk of contamination from airborne dust since the filter could be microscopically analyzed inside the filterholder. The pump was started before the pump was submersed and shut off after the filter had been removed from the filter holder. This latter procedure was done because to keep the filter dry and onto the filter holder.

The procedure of filter handling during the sampling is crucial for avoiding airborne contamination, see Results & discussion on this topic. As contamination control we took two kinds of controls during the sampling; The first control was to try to evaluate contamination from the filterholder and filter handling itself, and was done by filtering 25 liters of MilliQ water on deck and using the same filter handling techniques as done during sampling. This control was performed in duplicate the second journey. The second control was performed to find out if there was airborne contamination from the sampling procedure due to the time after sampling when pumping air through the filter, this was done by placing a wet filter in the filter holder and let the pump work for 3-5 minutes. Except from not submersing the sampler into the water, the filter was handled the same way as an ordinary sample.

All the material that was removeable from the filter holder was cleaned before sampling in a heated ultrawave cleaner (60°, manufacturers strong detergent] and stored in clean petridishes before use.

During the sampling, purple nitrile gloves was used and fibrous textiles was avoided by the sampler (i.e no Helly Hansen-, fleece- or wool clothes was used).

#### *ANALYSIS*

The filters was analyzed at a laboratory under a metallurgical Olympus BHM microscope (reflected light) at 100x to 200x. To get a illumination that showed the natural colours and surface morphology of the particles, extra white light was used (cold light, fiber optics). In our survey samples was taken in duplicate and the variation in concentration could be estimated as the mean value and the variation thereof. For the second survey only one of the two samples are analyzed yet due to the short time between survey and report.

During the analysis particles that had certain properties was counted. The properties aimed to exclude natural particles such as unicellular protists and multicellular zooplankton as well as natural debris, both organic (e.g detrital particles) and inorganic (e.g. various minerals ). The focus was to count anthropogenic particles, which in the microscope was distinguished by the combination of colour and texture (morphology). Particles that had unnatural colours was counted, e.g. blue, red, yellow etc. and particles that had a “mad made” structure such as plastic or textile fibres.

Hydrographical data was obtained from the ordinary monitoring program for the surface water.



Figure 3 Control sampling, 25 liter MilliQ

## RESULTS & DISCUSSION

The sampling method worked well. The sampler could be located approx. 2 meters away from the ship hull to avoid potential contamination. Between the first and the second cruise some improvements in the methodology was conducted: 1) the volume was measured with the electronic flow meter with good result. 2) the filters was immediately placed in the Millipore filter holders 3) the use of ultrasonic cleaning of the filter apparatus.



This survey used a 10 $\mu$ m polycarbonate filter as sampling filter and the method was designed to study the abundance of particles in the size range of 10-500 $\mu$ m, with > 95% of the particles in the range of 10-100 $\mu$ m. The drawback of this method is that we cannot compare the results with previous results on microscopic plastic litter from other seas. But we argue that this fraction in one way is more important to study based on 1) the size of those particles are in the same dimension as phytoplankton and hence can be ingested by filter feeding animals and hence can be incorporated into the food-chain 2) All persistent organic pollutants (POP) is absorbed on particle surfaces, and the surface per volume quota of an particle increases with smaller size leading to smaller particles transports much more POPs than the larger particles previous studied 3) Plastic in itself is not very toxic (a simplification – eg. except for plasticizers and other additives) but oil particles and rubber particles is toxic in themselves due to the cancerogenicity of e.g the high PAH constituent.

We do not exclude the need for monitoring larger particles. This can be done by using the same kind of zooplankton net as other studies use (WP-2 or manta trawl) or use a modification of the sampler used in this study with a larger pump and wider mesh openings (333 $\mu$ m). By using the latter method the constant risk of contamination could be minimized and measured using controls. The use of controls has not been practiced by other studies due to the impracticability when using a large zooplankton net.

### *CONTROLS*

The number of particles in controls taken on board before sampling is summarized in Table 2 and the fact that there was lower number of particles in the control the second survey could be explained by overall improvement of methodology mentioned above. Notable is that there is fibers in the control. This implies that there could be a constant airborne contamination from clothes or other sources that is hard to avoid during ordinary sampling, and we gets an actual baseline (zero value) for the method. By constant improvement of the technique this could be lowered. The necessity for this is that the concentration of textile fibers in the water samples is slightly higher than the control concentration – so we cannot say if this slightly higher concentration reflects a real abundance in the water or if all fibers come from contamination.

For coming surveys we will recommend that at least three controls are made in the beginning and in the end of each survey. Another explanation for the particles/fibers in the control water could be impure control water or impure storage cans – it is very easy that just some fibers are contaminating the process.

**Table 2 Summary of control samples**

	Black particles * lit <sup>-1</sup>	Blue particles * lit <sup>-1</sup>	Fibers (all colours)* lit <sup>-1</sup>
Control survey #1	7,0	0	1,0
Control survey #2	0,4	0	0,2
Control for air <sup>a</sup>	9 pieces	0 pieces	0 fibers

<sup>a</sup> Only air was filtered in this control and hence no concentrations are given. The pump was filtering air for 3-5 minutes at the same place as ordinary sampling.

### *PARTICLE CONCENTRATIONS*

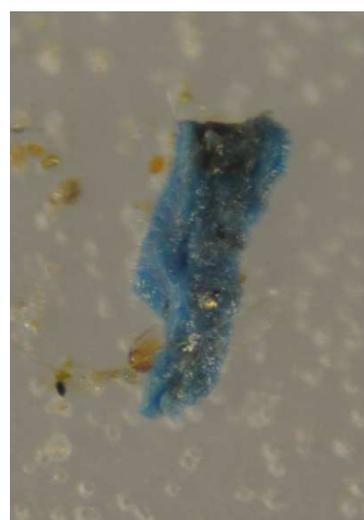
Several kinds of particles were found in the samples that had anthropogenical origin. We found textile fibers (both polyester like and wool/cotton like) that has been found in all samples

### **Fibers**

Since we suspect that fibers easily contaminate the samples we have a restrictive way to interpret the concentrations found. By comparing only the number of fibers on each sample filter we really cannot distinguish between samples and the two controls. The number of controls is too few for making any statistical test between samples and controls, so we have to look at the figures qualitatively. For the control in survey #2 there was 4 fibers on the filter after filtering 25 liters MilliQ. For all other stations the number of fibers on the filter ranged between 0 and 7 independent on the volume filtered. By including the fact that there were 20 fibers on the control filter in the first survey we conclude that we can't separate the control concentrations from the sample concentrations.

### **Blue particles**

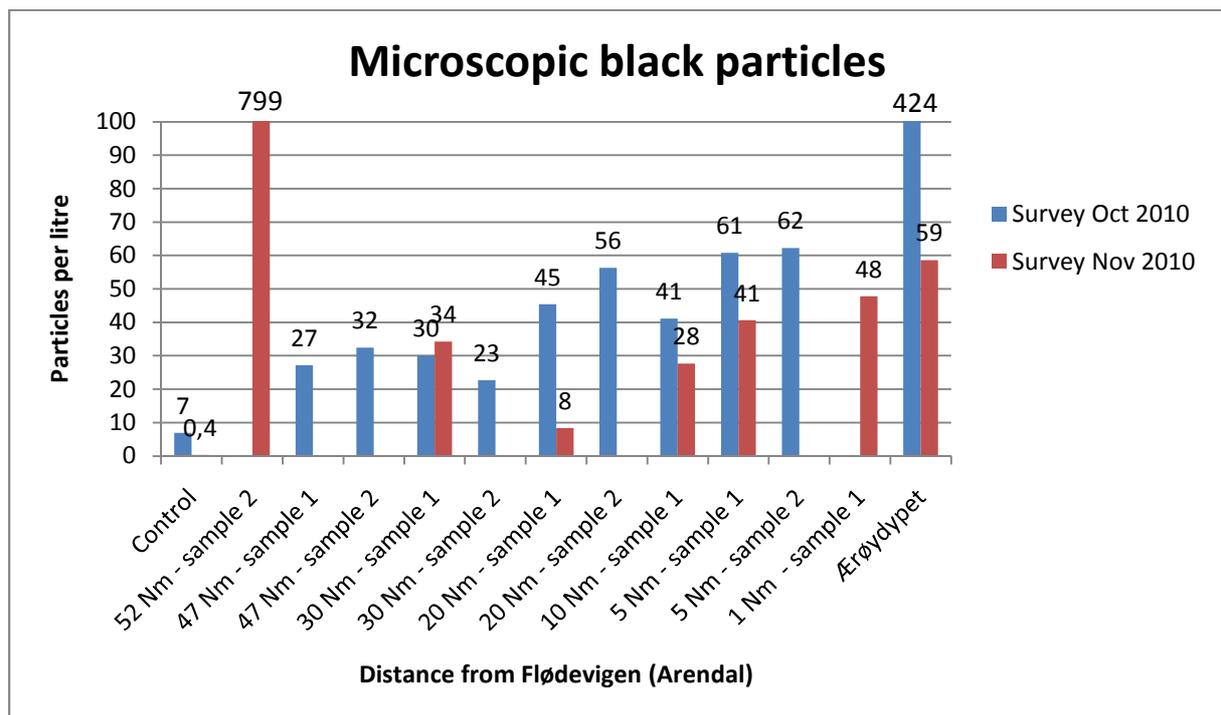
Blue particles with dimensions from 10µm to 300µm was found in 15 out of 17 samples. The same kind of particles has been recorded from earlier investigations from Swedish waters. During this survey the particles were compared to small samples of paint from G.M. Dannevig. The particles found in the samples were identical with the paint samples. So it was concluded that the particles were contamination and hence excluded from further analysis. In next step a FTIR spectra will be made for the paint and the samples for a chemical identification as well. Earlier FTIR analyses on blue particles from surveys in Swedish waters did show that the blue particles were epoxybased blue paint. Interestingly we found blue particles in samples where no blue-painted ship was used, such as samples from the harbor of Lysekil and the sampling with the Swedish research vessel Argos (a white painted ship). So even if we exclude the blue particles in this study, it is too early to exclude them as microscopic marine litter.



**Figure 4** A blue particle (possibly blue paint). Size approx. 30\*70µm

## Black particles

The number of black particles ranged from 799 per litre to 8 per litre with the median value of 41 per litre. There was a difference in concentrations between the two surveys as shown in Figure 5. At both occasions there was high numbers of black particles closest to land. After survey one this fact led us to take samples in a small rivulet unaffected by traffic – could the black particles stem from rivers and natural particles therein? In samples from this water there was a high concentration of black particles (170 per liter). This result led us further to compare the black particles found with photos of minerals of different kind, such as biotit, amphibolit and volcanic ash and the similarity was striking. As well were photos for natural degraded organic matter (such as peat and similar material). This finding can explain the high concentration close to land.



Figur 5 Summary of concentrations of black particles.

But we still can't determine the origin of the black particles and whether they are of natural or anthropogenic source or the quota thereof.

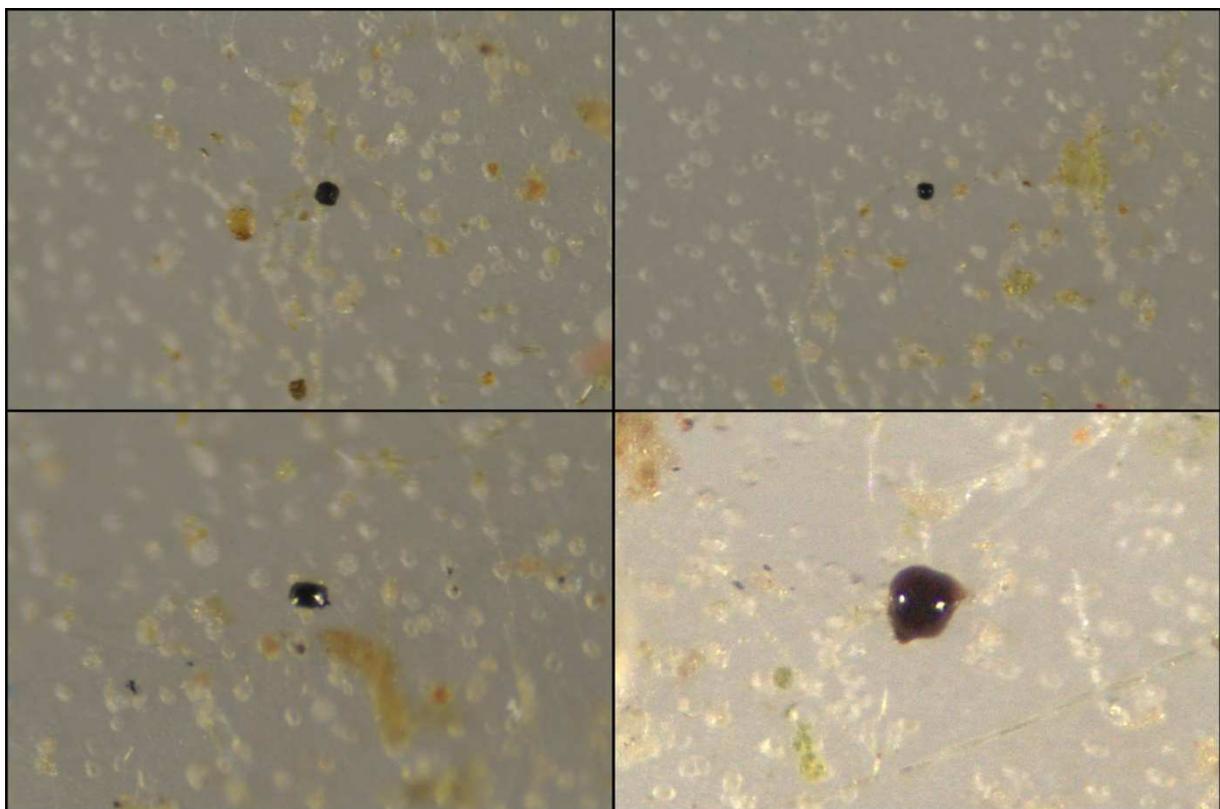
To do this we need to do further analysis on the black particles by methods such as FTIR (Fourier Transformation Infra Red spectroscopy) or SEM-EDX (Scanning electron microscope with energy dispersive X-ray spectroscopy). Unfortunately the FTIR microscopy which is very powerful to identify material composition of any material has one drawback – it can't study black particles. This is due to the fact that infra red energy is more or less completely absorbed by black particles. There are ways to overcome this, by e.g. pyrolysis of organic particles before FTIR analysis, but then the problem is that the black particles are way too small for ordinary analytical methods, which often requires samples of one gram or more. So we need develop special methods for this kind of studies. The SEM-EDX analytical procedure could be used to distinguish between organic and mineral black particles, and if possible tell us what kind of mineral the particle consist of. The SEM-EDX has been used in studies of road wear particles with some success and methodology are used from that field of knowledge. But they got, and

which we lack, a possibility to create samples of known material such as different asphalt or rubber materials. Even so a review paper by Thorpe (2008) states “It is concluded that ... unequivocal identification of particles from other sources [than brake dust particles] is likely to prove extremely difficult”. We have the same problem, in that the particles change chemical profiles during transport in marine environment and the samples are minute small.

Using polarized light microscopy and fluorescence microscopy could be a way forward in parallel with the more analytical methods.

### **Black round particles**

One kind of black particle that always has been found in all samples is the conspicuous round black particles, see Figure 6. The same kind of particles has also been found in most of the previous samples from Swedish surveys. They are even found in sediment samples (Stefan Agrenius pers. com) but then in larger dimensions (~0,5 mm).



**Figure 6 Round black particles (diameters from 10 to 50  $\mu\text{m}$ )**

During survey two (November) the concentration of those particles was ranging from 2 to 22 particles per litre, see Figure 7. Which, based on subjective memory, is the highest records of those particles found yet. The material composition of the particles will be further elucidated at SP (Swedish Technical Research Institute, Borås) where the particles can be tested with SEM-EDX and FTIR. As a first microscopical analysis THF (tetrahydrofuran) was added. THF is known to dissolve bitumen and other organic substances. In 6 out of 7 analyzed particles the black colour was dissolved by THF after 10-30 second, se Figure 8. The interpretation of this will be discussed with material experts at SP.

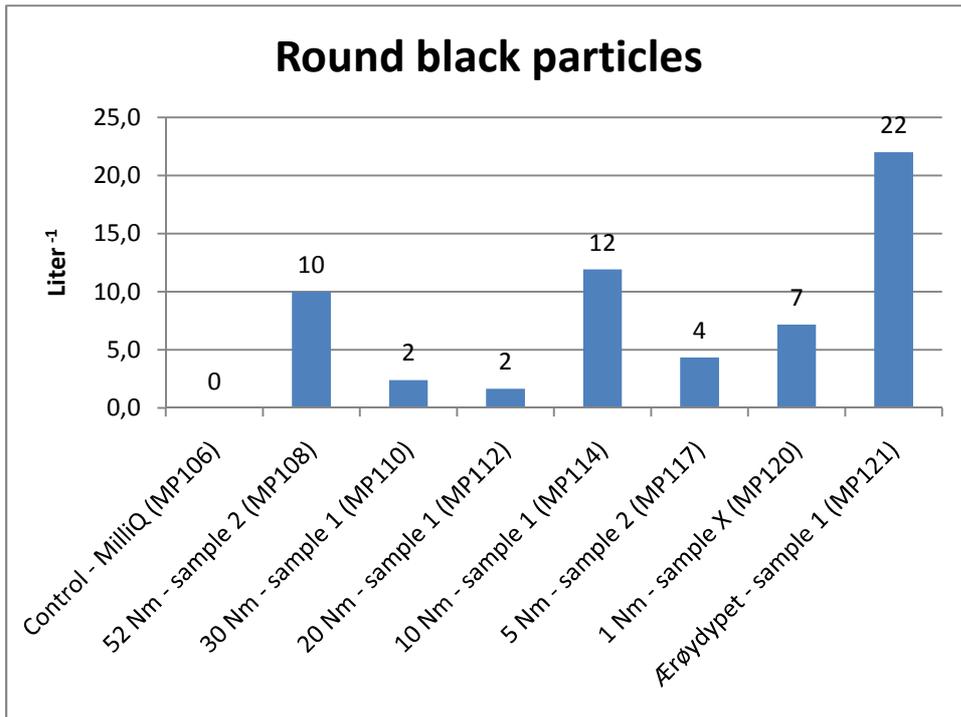


Figure 7 Summary of black round particles found in survey two.

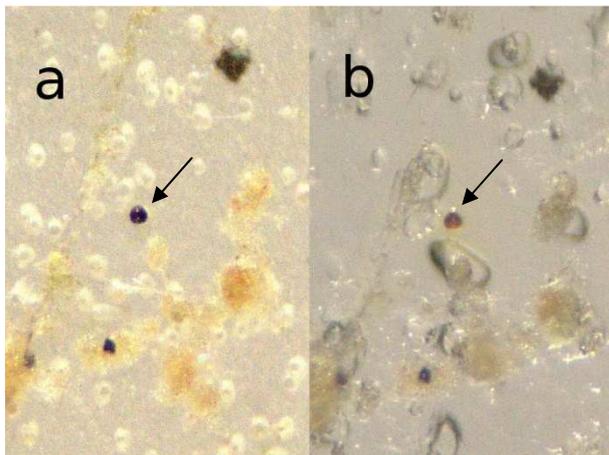


Figure 8 Black round particle,  $\phi$  10 $\mu$ m. a. Original particle on sampling filter b. After THF addition.

## References

- Broman, D. (1988). "MULTI-SEDIMENT-TRAP STUDY ON THE TEMPORAL AND SPATIAL VARIABILITY OF POLYCYCLIC AROMATIC-HYDROCARBONS AND LEAD IN AN ANTHROPOGENIC INFLUENCED ARCHIPELAGO." *Environmental Science & Technology* **22**(10): 1219-1228.
- Carpenter, E. J., H. P. Miklas, et al. (1972). "Polystyrene spherules in coastal waters." *Science (Washington)* **178**(4062): 749-750.
- Colton, J. B., F. D. Knapp, et al. (1974). "Plastic Particles in Surface Waters of the Northwestern Atlantic." *Science* **185**: 491-497.
- Lattin, G. L., C. J. Moore, et al. (2004). "A comparison of neustonic plastic and zooplankton at different depths near the southern California shore." *Marine Pollution Bulletin* **49**(4): 291-294.
- Mato, Y., T. Isobe, et al. (2001). "Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment." *Environmental Science & Technology* **35**(2): 318-324.
- Moore, C. J., G. L. Lattin, et al. (2005). Density of Plastic Particles found in zooplankton trawls from Coastal Waters of California to the North Pacific Central Gyre. The Plastic Debris Rivers to Sea Conference, Redondo Beach, California, USA.
- Moore, C. J., S. L. Moore, et al. (2001). "A Comparison of Plastic and Plankton in the North Pacific Central Gyre." *Marine Pollution Bulletin* **42**(12): 1297-1300.
- Norén, F., S. Ekendahl, et al. (2009). Mikroskopiska antropogena partiklar i Svenska hav. N-research.
- Thompson, R. C., Y. Olsen, et al. (2004). "Lost at sea: Where is all the plastic?" *Science* **304**(5672): 838.
- Thorpe, A. (2008). "Sources and properties of non-exhaust particulate matter from road traffic." *A review* **400**(1-3): 270-282.
- Wik, A. and G. Dave (2006). "Acute toxicity of leachates of tire wear material to *Daphnia magna* - Variability and toxic components." *Chemosphere* **64**(10): 1777-1784.