Research Article

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Assessment of the Effects of Physical Disturbances in Simple Micro-Universes by Measurement of DOC-levels

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Abstract
An increasing problem with today's way of living is the rising of anthropologically influenced pollutions. Quite recently has research been directed towards the effects and consequences these disturbances may cause within freshwater environments, which has inspired this study. Hoping to assess the effects of different disturbances that are likely to occur in lakes, DOC-levels (Dissolved Organic Carbon) were measured to get an idea of what might happen to the microbial ecology when these are introduced. The research was conducted by introducing a heavy reduction of bacteria, micro plastics, SDS (Sodium dodecyl sulfate) and micro plastics and SDS together to enclosed environments. The purpose of this, and also the aims of this study being to 1) find out of DOC-levels were affected, if 2) a difference could be observed between exposure to micro plastics + SDS versus micro plastics and SDS alone, and 3) to assess the recovery after each disturbance. Following exposure DOC-levels remained generally unchanged in the reduced bacteria and micro plastics mesocosms, whereas these levels were heavily reduced after exposure to SDS. It was also found that SDS + micro plastics required a shorter exposure time before mortality was observed than SDS alone, and resulted in a much higher mortality rate than micro plastics alone.

Introduction
An increasing problem in today's world is dispense of anthropogenic materials and general pollution caused by human interference, such as use of fossil fuel, dispense of chemical waste and physical disturbances such as debris. Only quite recently has any major focus been directed towards the effect these pollutants might have on aquatic environments; for example, I know plastic debris can cause heavy disturbances to aquatic animals due to ingestion and entanglement [1]. Other aspects of aquatic pollution also starting to surface, for example the effects of acidification and how it dismantles the ecology of freshwater and halocline environments [2].

More recently have studies been made regarding the fallouts of these plastics, for instance what might happen to the debris after long time exposures. Plastics has a very long decomposition time which means that items that may enter an environment will remain there for quite some time. However, it has been found that these plastics may break down into small, microscopic pieces known as microscopic plastics (microplastics, < 1 mm in diameter) [3]. These microscopic beads have later, to quite large extents, been found in soaps and personal care products. Estimates shows that 450 tones are used yearly within the EU, where most are disposed in domestic sewer systems and therefore translocated via WwTW (Wastewater Treatment Works) to aquatic environments [4]. The extents of micro plastic pollution has also been shown to be more extensive than first thought, as these beads has been found in not only halocline waters, as initially expected, but also to a large amount in freshwaters [5].

The effect of this has previously been determined as problematic as the microplastics can be ingested by several different types of planktons and zooplanktons, which in turn has a very negative effect on these species [6]. Ingestion has caused altered feeding capacity, paralysis and death [7]. Some studies also suggest that microplastics can serve as transport for various chemicals into organisms as these are absorbed into pores of the microplastics, which in turn makes organisms more vulnerable for exposure of toxic chemicals [8]. This theory also suggests that microplastics can worsen the effects of bioaccumulation as more toxins are introduced to the food web when ingested by the zooplankton [9]. However, it is also suggested that microplastics might have the opposite effect and actually decrease the negative effects of bioaccumulation as the pores in the microplastics might cleanse organisms from polluting chemicals. The reason of doubt being that previous experiments showing negative effects of ingesting microplastics has been executed in controlled environments, containing few disturbances other than those being tested. In a natural environment where other pollutants and disturbances are present the results might be different, however, few studies have been executed on this [10].

SDS (Sodium dodecyl sulfate) is a synthetic organic compound heavily used in a range of areas for its hygienic properties, and is to a large abundance found in soaps, toothpastes and other personal care products along with microplastics [11,12]. Previous studies has shown that SDS has quite noticeable toxic properties, for example it is known to cause lethal conditions in smaller organisms and cause heavy disturbances in higher trophic levels in aquatic food webs [13].

However, few studies has been done regarding the effects SDS and microplastics may cause when introduced to the same environment.

In aquatic environments it has been shown that decreasing pH-values harms plankton and bacterial flora to the extent of population decline, decreasing pH being a direct effect of acidification, mainly caused by anthropological influences such as use of fossil fuel [14]. The decline of bacterial population is believed to cause disruptions in local microbial loops [15]. Aside from the primary production, bacteria forms a necessary gateway of additional nutrients to enter the food web by decomposing DOC (dissolved organic carbon) [16]. Originating from dissolved remains of dead organisms, DOC is a broad definition for all types of organic molecules in amphibious systems. As aquatic entities typically cannot utilize the DOC themselves, bacterium contribute to the recycling of these lost nutrients by ingesting it for growth and therefore making it available for higher trophic levels in the food web [17]. Together with the primary production, the microbial loop makes out the total production of any aquatic environment [18]. The decline of bacteria population is therefore very likely to disrupt the production, and in turn entire ecosystems.

In this study the endeavoured aim has been to assess the significance physical disturbances could have in a freshwater ecosystem. It was hypothesized that DOC-levels would change when a physical disturbance was introduced, these disturbances being heavy reduction of bacteria as well as introduction of SDS, microplastics and microplastics together with SDS. This study was aimed towards finding if 1) DOC-levels were altered in relation to physical disturbances, if 2) a difference could be observed after exposure to microplastics and microplastics together with SDS and to 3) assess the recovery after each disturbance. As these eruptions are both relevant and as of yet not very widely studied they were chosen for this project.

Materials & Methods

Materials

1. Glass bottles 1l,2l.
2. pH-measuring machine
3. Filters 0.2 µm, 0.45 µm
4. Plastic bottles for filtration
5. Thermometer
6. Vacuum filtration machine
7. Microscope
8. Stereoscope
9. DOC measurement machine
10. Freezer
11. Pipets
12. Scales

Water sampling

This study was executed in Lake Erken, which is a freshwater, dimictic lake located in southern Sweden, (Nortällje, 59°510N, 18°360E). The lake is meso-eutrophic, with a good chemical composition. The average depth is 9 meters, with the deepest point measuring 21 m, and a surface area covering about 24 km2. The surrounding area of the lake consists of a dominating coniferous flora, and because of the high concentration of limestone in surrounding bedrock Erken has been resilient against acidification [19].

Water samples were collected from two general areas of Lake Erken. The idea was to experiment on water that has been exposed to a lot of human activity (HA), but to assess if this really had any significance, water was also sampled from an area free of human activity (NHA). A 6.1l sample was collected from four different spots with frequent anthropological disturbance, and a 1.6 l sample was extracted the same way from an isolated area to be used as control.

Freshwater Heteroptera sampling

Heteroptera Corixidae is a small bug most commonly found in still, freshwater environments. Most species are herbivores, feeding on phytoplankton and various types of plants found in the water, although exceptions can be found within this family. Typically these bugs spend their lives close to the bottom in shallow waters. The Corixidae samples were collected from the anthropogenically disturbed area together with the water samples. The experiment required 385 insects in total.

Freshwater heteroptera

Mesocosm conditions and DOC measurements

The sampled water coming from the anthropologically active area was divided into nine 500 ml containers to serve as mesocosms during the experiment and two 800 ml containers where nothing was changed in order to be used as controls (HAC). Samples from the non-human active area was divided into two 800 ml containers to serve as additional controls (NHAC). Each sample was filtrated of plankton and larger organisms in order to 1) rid the samples from unwanted, larger organisms and 2) easily separate and add the Heteroptera, 25 insects were used in each 500 ml mesocosm and 40 in each 800 ml sample. The remaining plankton was then added into each control sample and mesocosm, except for those meant to contain the bacteria reduced environment. 3 ml from each mesocosm and control container was then filtered using vacuum filtration (MILLIPORE) with 0.2 µm filters and later frozen to be preserved for DOC-measurement. At the end of the research period the same procedure was repeated and all collected DOC samples where measured.

Introducing the disturbances

After temporarily removing the insects, three of the 500 ml samples from the anthropologic active environment were filtered, using the vacuum filtration process to free them from bacteria and other microorganisms. Of course, as the insects were added back into the samples after doing this a bacterial population was once again introduced into the mesocosms along with them. However, the reinstated bacteria population was considered to be small in comparison to the original flora and diversity, thus creating an environment experiencing a heavy reduction of its bacterial populations (BF). In three of the mesocosms 40 µml of microplastics (0.1 µm in diameter) was added, creating a concentration of about

4000 pieces of microplastics ml-1 in each solution (MP). 19 µg of SDS was introduced to three separate mesocosms. The same amount of microplastics and SDS was added to additionally three mesocosms in order to compare the effects these two disturbances have together versus the effects they have alone. As SDS and microplastics are used together (i.e in personal care products) they are also released into the same environment when discarded. This makes it worthwhile to study how they might influence each other when introduced to the same environment, and what the effects might be.

External variables and conditions

The mesocosms were stored in the lake with bare openings in order to keep the natural conditions as unvaried as possible, such as rain entering and temperature variations, as these would normally occur in the lake. One of my aims being to assess any recovery of DOC-levels, this was important in order to possibly answer and predict what might happen when these disturbances are introduced to a real environment. Each day around 13.10 all samples would be gathered for measurement of pH-levels and examined for changes (i.e insect mortality) and later put back into the lake, the point of this being to monitor the developments on a daily basis. On the third day of the experiment, live insects from the MP and MP+SDS were examined under a fluorescent microscope in order to ascertain if microplastics has been consumed or not.

Results

Environmental conditions

Throughout the experiment water temperatures were measured with 24 hour intervals in order to identify and detect external variables that might be correlated to insect mortality or other changes. Weather condition data was also collected for the same purpose, as factors like these presumably has an impact on other data, such as pH and DOC-levels. The lake temperatures varied between 17.0 and 21.3 (Figure 1), which is deemed as rather stable conditions. The weather has been predominantly sunny, with a slight increase of clouds towards the final half of the research period, whereas rain occurred on the 24th and 26th of June (Figure 2).

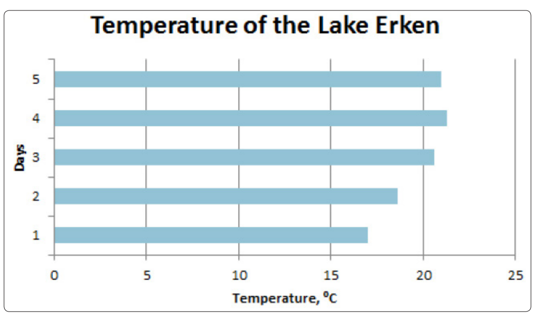


Figure 1: The temperature of Lake Erken in the shore, where the samples were kept

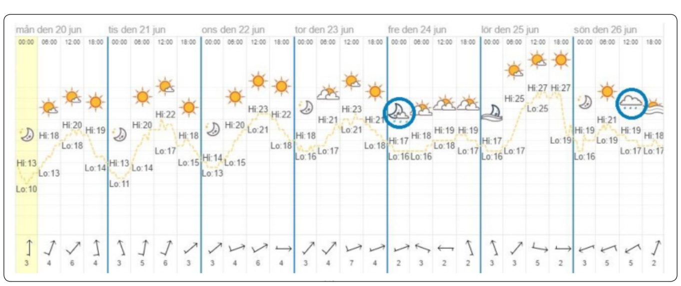


Figure 2: Weather. June 20th - June 26th (2016) [20].

pH levels and mortality

At the start of the experiment, pH levels in all samples were located at 8.10 ± 0.1, form which point each sample-group changed with similar patterns. Most of the samples alternated towards higher values for the majority of the research period, whereas MP and MP + SDS first experienced a steady decline, followed by a rising trend towards the end of the study. During this time pH-levels in the MP+SDS samples had decreased from 8.13 to 7.74 (figure 3), also worth noting is the slight increase in pH found after the 7 day exposure time, whereas the pH rose from 7.66 to 7.78. The MP samples alone varied from 8.6 to 7.66 (figure 3), whereas a similar incline could be observed towards the final days of the study, this time rising from 7.9 to 8.44. The BF samples remained fairly unchanged during the course of the research period, inclining from 8.11 to 8.37. Amongst the HAC samples the pH increased from 7.91 to 8.46 whereas the NHAC samples varied from 8.02 to 8.42, measured at the end of the research period (Figure 3).

By the final day of this project, the average freshwater heteroptera mortality in all controls had reached 25%, equivalent of ten dead insects. In HAC the mean mortality at this time was 17.5%, and in NHAC 32.5% resulting in 7 respectively 13 dead insects. The MP samples had during this period experienced a mean lethality rate of 40%, respectively 10 dead individuals. The highest mortality percentage was observed in the SDS+microplastics mesocosm, where a 100% mortality rate was reached on the third day. In comparison, the SDS only contained a mortality rate of 100% as well, however, no deaths were observed until the third day. This would indicate that microplastics+SDS does more damage to Freshwater heteroptera than SDS and microplastics alone. As for the BF samples, the mean lethality rates had reached 36% after the 7 day exposure, resulting in 9 dead insects (Figure 4).

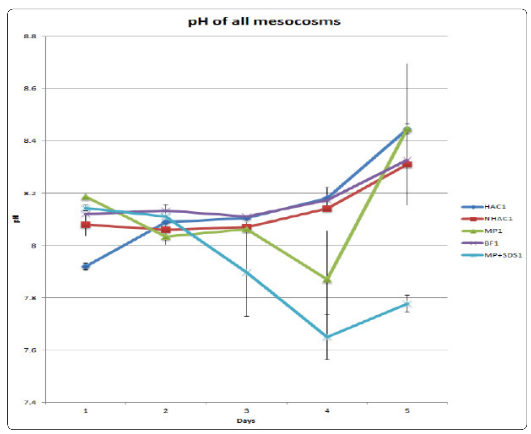
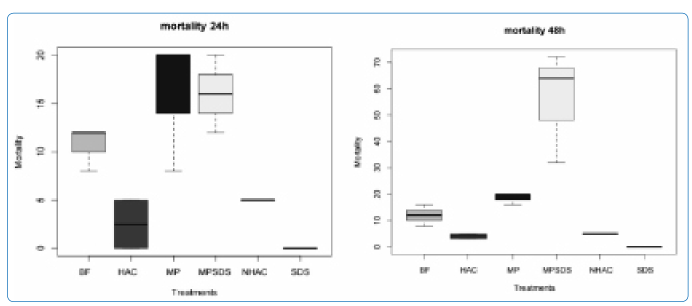


Figure 3: pH-levels of all the mesocosms



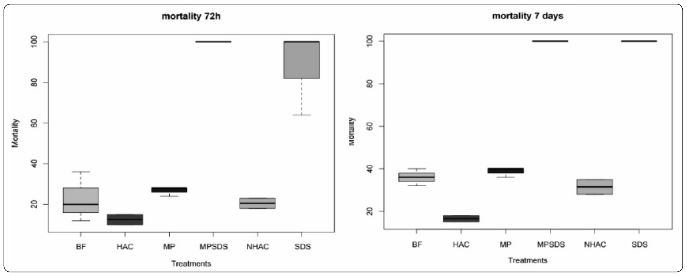


Figure 4: The changes in mortality rates

DOC-levels

Within the HAC samples DOC measures inclined from 12.21 mg/l to 14.17 mg/l (116.05%). In the NHAC treatments these levels grew quite dramatically, from 12.02 mg/l to 26.0 mg/l (213.11%) during the seven-day exposure time. As for the MP samples, DOC grew gently from 13.18 mg/l to 13.24 mg/l (100.46%), resulting in the most unchanged DOC levels obtained in this study. Similarly, the BF samples did not vary to any greater extents either, from 12.84 mg/l to 12.41 mg/l (96.65%). Within the samples containing the MP+SDS treatments, DOC dramatically decreased from 24.38 mg/l to 14.53 mg/l (59.60%), whereas the SDS treatments followed a similar pattern, dropping from 22.70 mg/l to 15.49 mg/l (Figure 6).

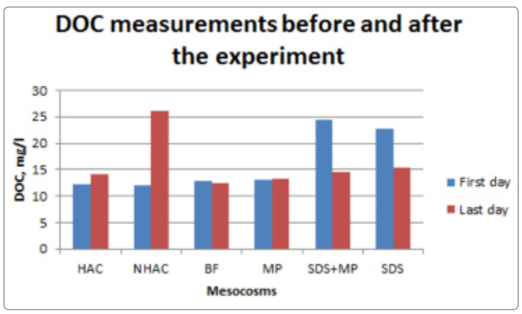


Figure 6: DOC measurements before and after the experiment. Changes in levels in Human-Active Control, Non-Human Active Control, Bacteria-Free, Microplastics, SDS+Microplastics and SDS mesocosms.

Ingestion of microplastics

As shown in figures 7 and 8, it was clear after examination under fluorescent microscope that the heteroptera had consumed microplastics in both MP and MP+SDS samples. This also revealed that quite a large abundance of plastics had been absorbed, and that these appears to 12 have spread to other tissues within the insect. Also another hypothesis is that microplastics weaken the skeleton and they stuck on the insect (shiny places in the pictures below).

Figure 7: Microplastics shining in the Freshwater heteroptera body taken from MP treatment

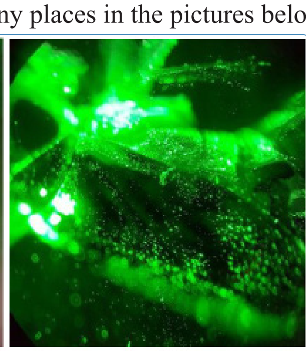


Figure 8: Microplastics shining in the Freshwater heteroptera body taken from MP+SDS treatment

Discussion

pH values and Heteroptera mortality

After 168 hours exposure time mortality in the control samples had reached 25%, the mean mortality from the HAC samples at this time being about 17.5% whereas the NHAC mortality had risen to 32.5%, heavily influencing the total average. The reason of this major difference in mortality most likely originates from the fact that the NHAC water was not taken from the heteroptera natural habitat. The environmental change that the heteroptera may have experienced i.e. differences in biodiversity or chemical compositions could have resulted worsened living conditions, since it may have lead to less food or other uncomfortable circumstances. These possible factors are probable explanations for higher mortality rates in the NHAC-samples. The 17.5% mortality found in the HAC samples could be deemed as effects of age or other health deficiencies. As these insects were collected directly from Lake Erken there is no way of knowing what possible sources of errors were brought into the samples. It is also possible that other factors, i.e. damage inflicted on the insects during transfers lead to increased lethality in these samples.

The highest and fastest rising mortality rates were observed in the SDS and MP + SDS samples, where 100% of the heteroptera population was dead within 72 hours. As no mesocosm other than these reached a mortality rate of 100% it would be safe to assume that the SDS had toxic effects on the insects, agreeing with previous studies [21]. However, heteroptera mortality was observed earlier in the MP+SDS samples, where mortality rates had surpassed 50% after 48 hours, compare this to the 0% mortality found in the SDS samples at this time. This major difference in lethality progression suggests that microplastics does worsen the effect of bioaccumulation as it appears the insects were exposed to lethal levels of SDS after shorter exposures, disagreeing with the speculations that negative effects might be reduced when microplastics are present in natural environments [22]. It appears instead that the effects were worsened, supporting the idea that larger amounts of toxins are ingested and introduced to the food web when microplastics are present [23]. The samples containing microplastics alone experienced a quite rapid progression regarding lethality rates, reaching nearly 20% within the first 24 hours, and had by the end of the research period progressed to nearly 40%. It was clear from the results that the insects ingested these microplastics, suggesting that this may cause heightened lethality. However, obtained data cannot specify if this result was due to microplastics themselves damaging the heteroptera (i.e by harming intestines and entering other tissues), or if ingestion of the plastics lead to higher exposures of toxic chemicals present in the lake water.

Other than this, mortality rates and their growths has not differed to any extremes. However, every mesocosms containing a disturbance had by the end of the experiment reached higher mortality rates than the HAC samples, which could indicate that the disturbances introduced had negative effects on the insect's health. As for the BF samples, mortality quickly increased to about 20% within the first 72 hours, which could possibly be explained by disturbances caused to the gut bacteria. Reducing bacterium and other planktons in the water, giving the heteroptera heavily altered food and nutrient resources, could quite possibly affect the microbial life in heteroptera intestines. It has previously been presented that gut microbiota affects and communicates with the central nervous system [24], influencing behaviour, brain functions and general health [25], possibly leading to lethal conditions for the heteroptera. Another possibility could be that the observed mortality was caused due to starvation as a result of heavily limited food resources. Both situations could be supported by the decreased growth of the mortality rates, where the majority of deceased individuals died within the first 72 hours. At this point it is likely that the bacterium and plankton populations had recovered enough to provide a sustainable source of food and nutrients for the insects, hence slowing the mortality growth.

In all control groups pH-levels experienced a slight increase during the course of the research. It is likely that these elevated pH levels originated with the production of DOC, a relationship which has previously been found to be positively correlated [26]. This pattern could also be observed in the BF samples, whereas the MP and MP + SDS, as previously stated, experienced a decline in pH values during the majority of the research period.

Correlation between DOC and physical disturbances

The DOC-levels did not differentiate to any larger extent in the HAC, BF and MP samples, contradicting the original hypothesis that these physical disturbances would cause DOC levels to alter. However, this may not be safe to assume as it is possible that these levels fluctuated during the experiment as insect mortality increased and then re-stabilized due to bacteria population growth [27]. Alternatively, the levels did not vary at all. This could be due to the fact that the disturbances did not affect relevant factors even though insect mortality rates increased. Possibly, the decomposition of the dead heteroptera was not fast enough to result in any major amounts of DOC being created in the samples.

As expected, higher levels of DOC was measured in the samples containing SDS as this substance itself falls into the definition of organic carbon, hence adding SDS would naturally mean adding DOC into the samples. However, at the end of the research period the DOC levels had decreased quite dramatically, falling back to about the same concentration that could be 14 found in the other samples. The most probable explanation for this decline would be consumption by bacteria in the samples [28], and probably a major incline of microbial populations.

NHAC were the only samples where a dramatic incline of DOC-levels was observed at the end of the research period. This water coming from a different area of the lake, might have such a different bacterial flora compared to the water from the anthropogenically disturbed area that the DOC created from deceased insects was not consumed. Considering that these samples were collected from surface water at larger depths this outcome would be likely, as decomposing bacteria should be concentrated where DOC-levels are most prominent. Since dead organisms are to be found in the lake sediment, this is where the decomposing microbial flora should be found [29].

External variables

The temperature and weather conditions of the lake may have influenced the mortality of insects even if they were living in their natural conditions. These factors may also have affected the data collected from pH and DOC measurement. As warm temperatures and sunny weather conditions likely decreased the water volume in the samples other chemical components such as DOC and various substances that may affect pH was left with a higher concentration. Naturally, rain would in turn increase the volume of water and perhaps add other substances to the samples, altering the chemical composition and its concentrations.

Conclusion

To address the questions this study was aimed towards answering, differences in DOC-levels could not be correlated to the introduced disturbances and would require further studies in order to fully determine this. Suggested methodologies would be more frequent DOC-level measurement as well as sequencing and abundance measurements of microbial life to better determine effects of these disturbances. However, this project gave clear results regarding the combined effects of SDS and microplastics versus the effects of these alone, as they heavily differentiated. SDS proved to be a lethal toxin to the heteroptera, where microplastics also seemed to increase insect mortality, although to much smaller extents. SDS and microplastics together proved to have the same lethal effects as SDS alone, but were observed after shorter exposure times due to microplastic agglomerans and absorption of these by heteroptera. As for the recovery after these disturbances, the results obtained in this project together with previously made studies suggests that microbial life did recover from introduced disturbances. This likely occurred in the BF-samples, as shown from decreased growth of heteroptera mortality. However, this correlation cannot be made with absolute certainty, and would too require additional studies in order to be determined. More focus should be directed towards the changes that may occur within microbial populations, as these would be necessary to observe in order to draw conclusions about recovery within disturbed ecologies.

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