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Evaluation of microplastic pollution in four rivers in the United Kingdom.

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Abstract:

Microplastics are plastic particles smaller than 1mm that originate from degradation of larger plastics or are discharged from primary sources such as face wash and hand cleansers. Microplastics contamination has been found across UK rivers and lakes. Human activity has resulted in microplastic contamination throughout freshwater and marine environments. As a result of widespread contamination, microplastics are ingested by many wild species including fish and shellfish, which is a great concern as plastics relates to toxic chemicals from manufacturing. The presence and the degree of microplastic pollution was investigated in sediment across four England rivers (River Nar, River Tove, Alconbury Brook and Kempston Mill). The abundance of microplastics found in the samples ranged from 0.05mm to 4.5mm in size to 168-270 microplastic particles. The findings of the investigation revealed that there was no statistical significance in the data when compared with control samples but means of river samples were higher than the control samples. It was concluded that the hypothesis needs to be tested in a larger sample from various locations of the rivers to establish a statistical significance presence of microplastics and the scale f the pollution in those rivers.

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Introduction:

The fact that rivers, lakes and oceans are polluted with a variety of substances is well known. One of the most common pollutants of waters are microplastics. The first study to confirm the presence of microplastics in river Thames in four different location was performed by Horton et al. (2017). The study linked the presence of microplastics of size 1-4mm to the manufacture process of sewage and road marking paints. According to the International Union for Conservation of Nature report from 2017, microplastics contribute to about 30% of the Great Pacific Garbage Patch (Fig 1)

Figure 1 - Location of The Great Pacific Garbage Patch (Aevia Ideas Inc, no date).

The term 'microplastic' is used to define a fragment of any type of plastic smaller than 5mm in length (Colignon et al., 2014). There are two classifications of microplastics: primary and secondary.

Primary microplastics are plastic elements that enter the environment in the size less than 5mm. There are multiple sources of primary microplastics. The most common are fibres from synthetic clothes and cosmetic products such as scrubs and toothpaste (Boucher and Friot, 2017).

Secondary microplastics come from larger pieces of plastics and separated under natural process of degradation. Most common sources of secondary microplastics are plastic bottles, fishing nets, and plastic bags (Conkle, et al., 2018).

The main focus is placed on the larger plastic, however microplastic should become a higher concern as it is found in tap water and even bottled water which is widely consumed by population worldwide (Mason et al, 2018). The main risk linked to microplastic is biological integration into organisms through consumption or inhalation. The long-term risks are not established; however, it has been shown that it may cause bleaching and stressing of coral what leads to increased mortality (Thompson et al, 2004).

In terms of human health, the most concern is associated with toxins present in the plastics. BPA commonly present in the variety of plastics may lead to cardiovascular diseases, type two diabetes and liver diseases (Thompson, et al., 2009). Additionally, microplastic can be ingested by zooplankton and other marine organisms causing negative impact on the foundation of the marine food chain. According to the …

microplastic has a negative effect on multiple factors of marine animals such as growth, development, reproduction and overall lifespan (Botterel et al., 2019). In the study performed by Duncan et al., in 2019 has shown that each of the studied wild turtles (n=102) contained some levels of microplastic in their organism. At this point there is an increased interest in studying long term risks of ingested microplastics by marine organisms, however the specific effects are not yet established.

Aims:

This study was performed to test sediment from rivers in England for the presence and degree of microplastic pollution.

Methods:

Four river sediments (table 1) were sieved to remove debris and prevent apparatuses being clogged. The sediments had been stored in plastic falcon tubes, so for the control, ulta-pure water was put into an empty tube and 10 ml was used for each control, which followed the entire method. After finding microplastics in the method control, a control with ultra-pure water was used to investigate it for being the source of contamination. Each sample and control had three repeats. A 1:1 ratio of 30% hydrogen peroxide was added to 1 g of sediment at a 1:1 radio, and left for 24 hours to decrease the organic matter and make it easy to count microplastics under the microscope.

River Name	Date Collected	Notes
1 - River Nar	10/05/2018	TF69845
2 - Alconbury Brook	09/05/2018	TF1880475595
3 - River Tove	09/05/2018	SP8015642150
4 - Kempston Mill	21/05/2018	TL0238347665

Table 1 - Four river sediments used in this study and date collected

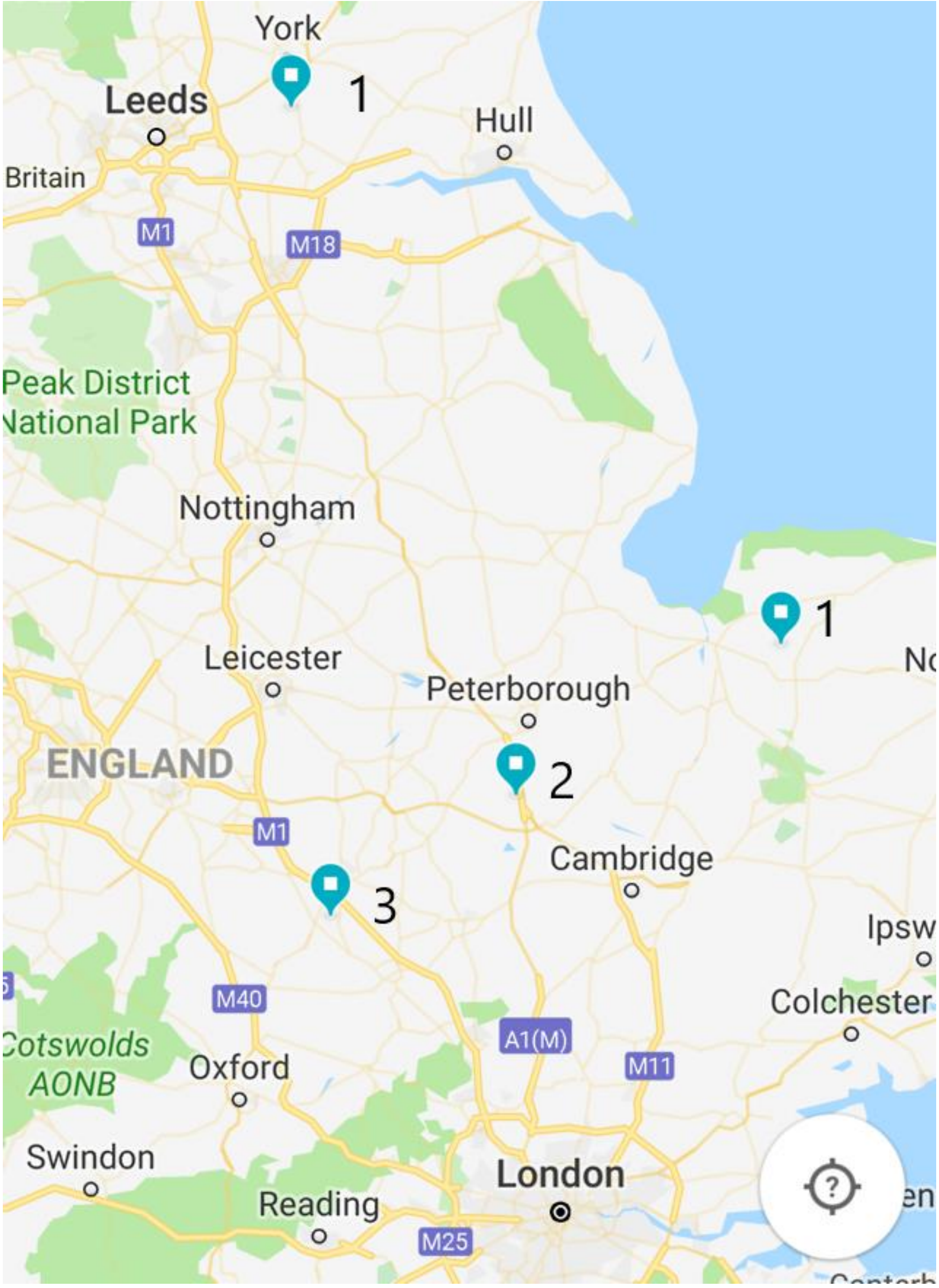


Figure 2 - Rivers plotted on a map of the UK using google maps (table 1 shows River names)

Plastics have the density of 0.8-1.4 g/cm^3 and sand/sediment is 2.65 g/cm^3 (Hidalgo-Rus et al., 2012), and these can be separated by density separation using zinc chloride ($ZnCl_2$) at 1.3 g/dm^3. $ZnCl_2$ was made with ratios of salt to ultra-pure water from Coppock et al., 2017 were used. Ultra-pure water being used to reduce background contamination of microplastics as distilled water was suspected to have more. $ZnCl_2$ was poured into glass separation funnels and then the treated sediment were added and these were left for 24 hours to density separate. Then the separation funnel tap was opened to remove the dense sediment layer and leave the floating microplastic layer.

Nile Red dye stains microplastics and causes fluorescence under a fluorescent microscope, so they are easier to count compared to using a light microscope (Maes et al., 2017) (Emi-Cassol et al., 2017). Two drops of 10 μg mL^{-1} dye made up in acetone were added to the density separators and then left for 30 minutes to stain plastics. Pure water was added to dilute the acetone to prevent damage when filtering on to 2.0 μm TTTP filter papers. The filter papers were then placed into petri dishes. Next the Zeiss Axioskop 2 fluorescence microscope with coloured filters was used at x10 magnification to count fluorescing microplastic particles with use of a click counted to avoid losing count. Images of some of the particles were also captured (see results).

The Ethics A form was submitted to the University and no ethical issues were raised by this study. There were potential safety risks, so a COSHH form was filled out and stored in the project notebook in the laboratory. The COSSH form was followed for handling of chemicals and equipment For example, wearing gloves and a lab coat while in the laboratory and wearing goggles when handling hydrogen peroxide.

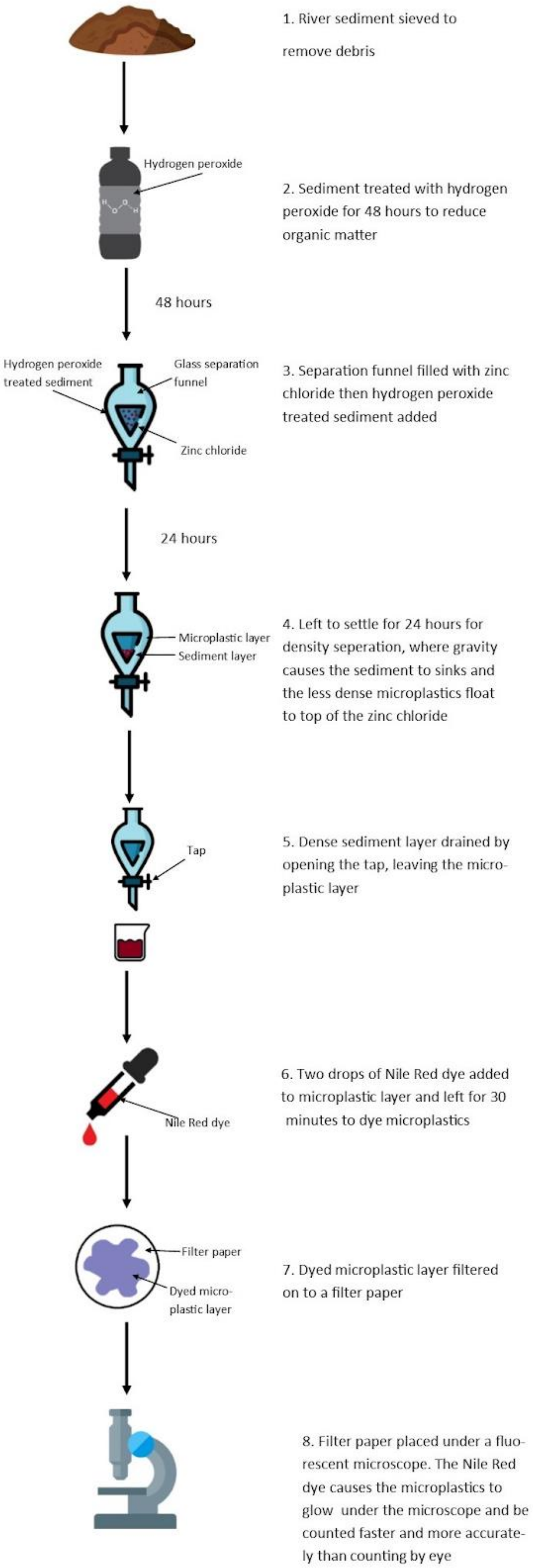


Figure 3 - Flow chart of the method

Results

Site	Repeats	Microplastic particles counted	Mean number of microplastic (standard deviation)	Mean number of microplastics with background contamination (pure water) corrected
River Nar	a b c	242 362 354	319 (±67)	182
Alconbury Brook	a b c	518 181 -	350 (±238)	213
River Tove	a b c	394 156 364	305 (±130)	168
Kempston Mill	a b c	390 486 336	407 (±81)	270
Method control	a b c	315 183 309	269 (±75)	132
Pure Water	a b c	130 157 124	137 (±18)	-

Table 2 - Raw Data and averages of microplastics counted in river sediment samples, control of method and pure water controls.

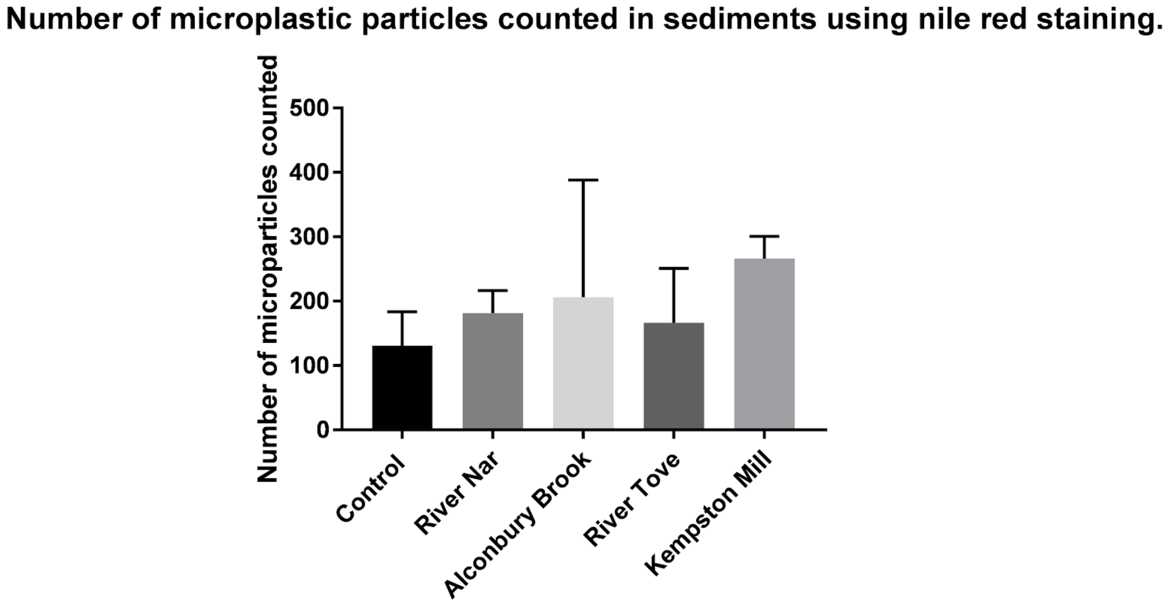


Figure 4 - Average number of microplastic particles counted for each site.

Microplastics were found in the four river sediments used in this study so the method was successful at retrieving microplastics. Some of these microplastics have been captured in the images below (figures 5-9) The results were not significantly significant by ANOVA. Although, the means of all four rivers range from 168-270 microplastic particles which are all higher than the control which has a mean of 132 (table 2). Kempston Mill has the highest mean number of microplastics with an average of 270 particles which is 138 particles higher than the mean, suggesting heavy microplastic contamination at this river.

The pure-water control had an average of 137 microplastic particles, which was subtracted from means of each river and the control to remove background contamination. The control sample had 132 particles after accounting for plastics introduced by using ultra-pure water, suggesting another source of microplastic contamination. This may be from the plastic falcon tubes the control was taken from. The sediments were also stored in these plastic tubes, thus microplastic contamination may have been introduced by this. Although, the means from the river samples are higher than the control, which shows this may be environmental microplastic pollution and not background contamination.

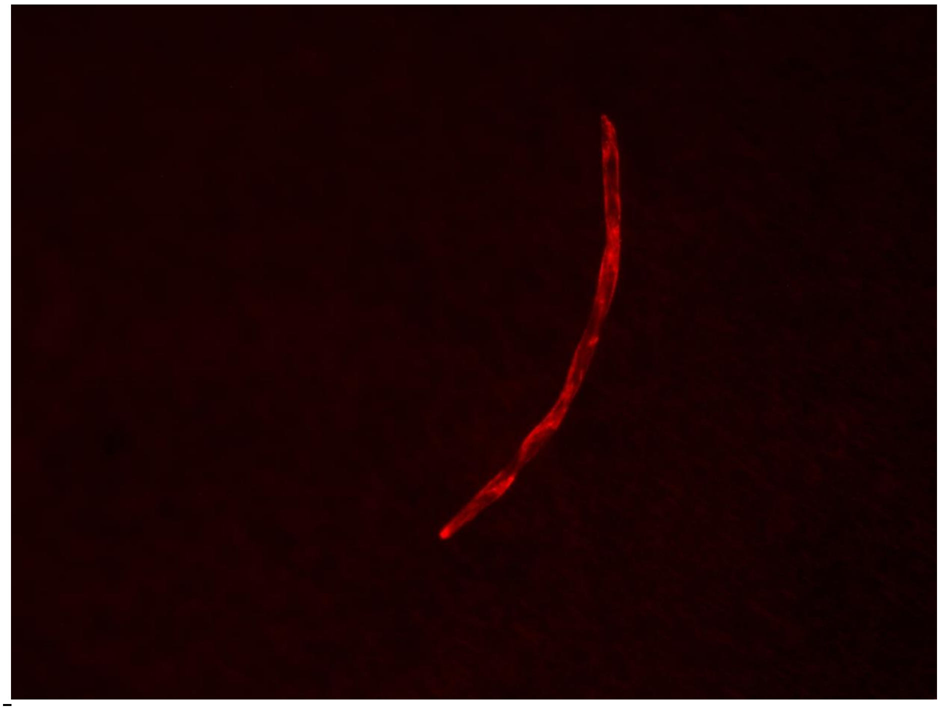


Figure 5 - Fluorescent microplastic fibre taken with a Zeiss Axioskop 2 fluorescent microscope at 10 x magnification. Suggested to be a textile fibre introduced by for example, washing clothes made of polyester. Approximately 4.5 mm x 0.12 mm.

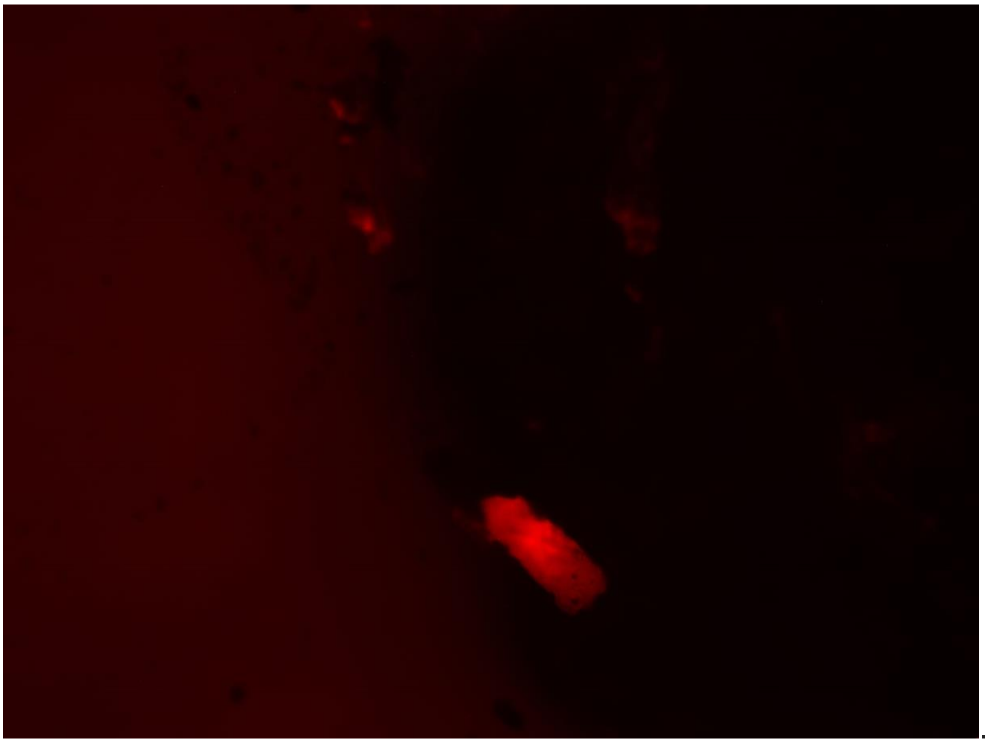


Figure 6 - Fluorescent microplastic fragment taken with a Zeiss Axioskop 2 fluorescent microscope at 10 x magnification. Appears textured and weathered, so may have been eroded off of a large piece of plastic debris. Approximately 1.4 mm x 0.5 mm.

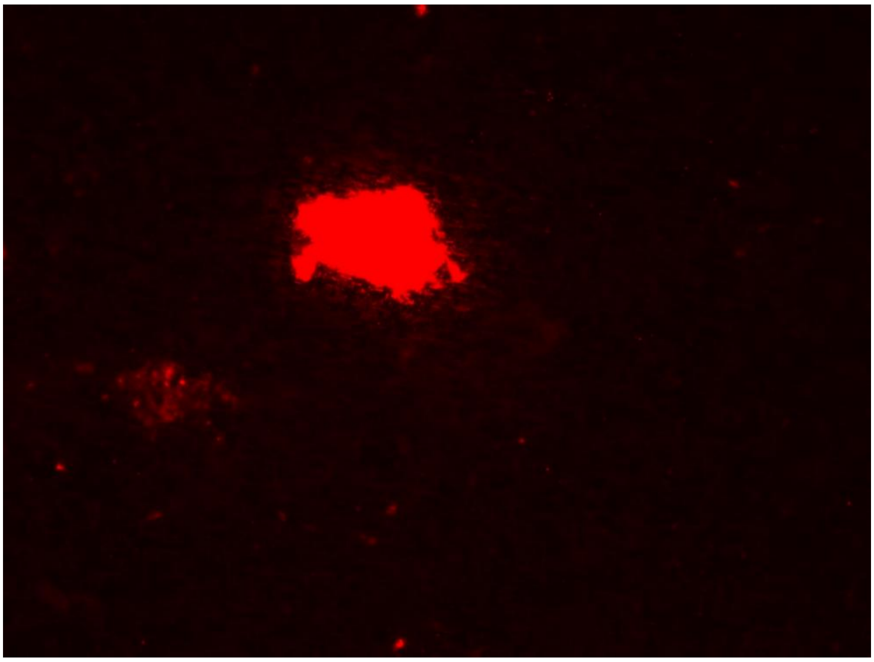


Figure 7 - Fluorescent microplastic fragments and small spherical fragments taken with a Zeiss Axioskop 2 fluorescent microscope at 10 x magnification. Large fragment may have been eroded off of a large piece of plastic debris due to the irregular shape. Approximately 1.6 mm x 1.3 mm. Small glowing fragment at bottom of image is approximately 0.1 mm and may be a spherical microplastic bead from a facewash before the ban on microplastics on products was introduced in several countries (Beat the Microbead, 2019).

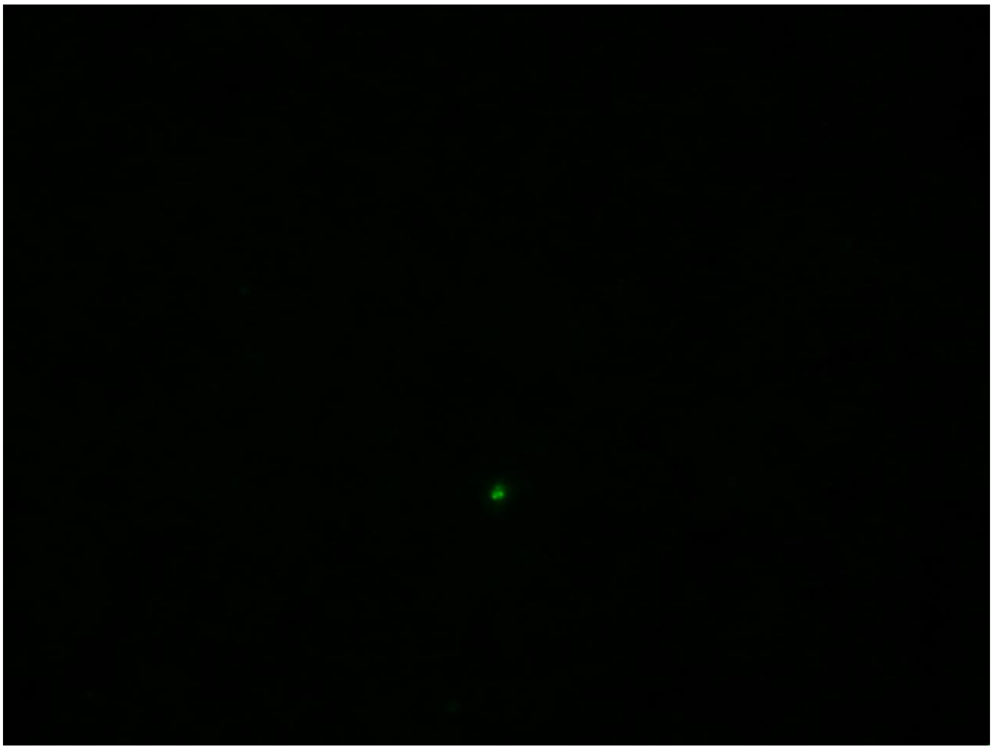
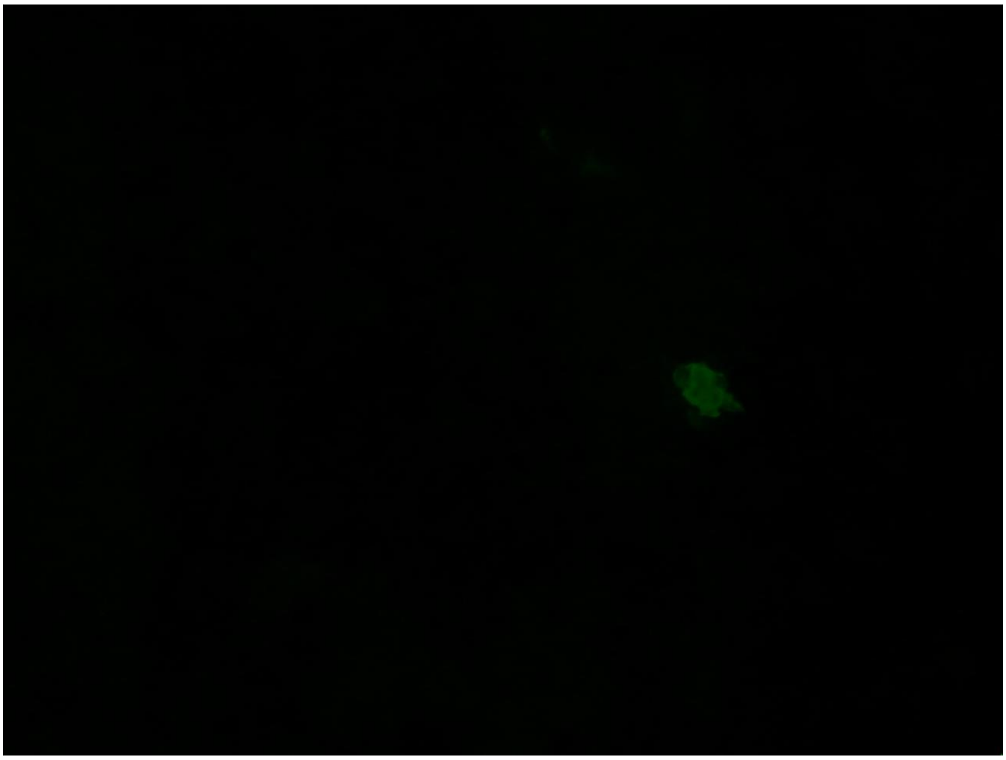


Figure 8 - Small fluorescent spherical microplastic fragment taken with a Zeiss Axioskop 2 fluorescent microscope at 10 x magnification. Spherical shape suggests this microplastic may have been discharged into the river sediment from a face wash containing microplastic beads. Approximately 0.1 mm x 0.05 mm.



<u>Figure 9 -</u> Textured fluorescing irregular shaped microplastic fragment taken with a Zeiss Axioskop 2 fluorescent microscope at 10 x magnification. May have been eroded off a larger plastic due to the weathered irregular shape. Approximately 0.6 mm x 0.35 mm.

Discussion:

The aim of this study was to test sediment from rivers in England for the presence and degree of microplastic pollution. As shown in our methods we investigated sediment samples from four rivers in England and found microplastics in all river sediments and repeats. Our results have shown no significance using ANOVA carried out in SPSS, however because all rivers had higher means than our control, it shows environment microplastic contamination in the rivers. The most polluted river is Kempston Mill and least polluted is River Tove, however as the standard deviation for River Tove was (±130) more repeats for this river is needed for accuracy. Potentially, microplastics are increasing in the rivers because there is an increasing amount of plastic entering them, but further study is required to test for this. Previous studies have found microplastic contamination in rivers, supporting our findings. For example, a microplastics found in the River Thames was identified as coming from road marking paint. Other studies across the UK have been carried out and found macro and micro plastics in rivers including the Thames, Mersey, Trent, Tamar, Usk, Taff and Wye (Horton *et al*. 2017; Tibbets *et al*. 2018; Hurley *et al*. 2018; Morrit *et al*. 2014; Kay *et al*. 2018; Sadri & Thompson 2014; Gallagher *et al*. 2015). There is little public knowledge about microplastic pollution, as the main focus is placed on the larger plastic that have been found to be harmful for marine animals. Sharing awareness about the risks of microplastic on environment and human health is the key. Daily habits and choice of materials of clothes may have a significant impact on the level of pollution in water. In order to reduce primary microplastic from entering environment there is a need to reduce washing synthetic fabrics, wear fabrics made of natural fabrics such as cotton and most importantly buy cosmetics and cleaning products that do not contain microplastics (Boucher and Friot, 2017). In terms of secondary microplastic, more complex actions must be undertaken to prevent their entry into freshwater and marine environments. Plastic pollution is one of the most serious problems which will require involvement of multiple organisations.

We had a few limitations from sample repeats, sample size to the collection of the sediment's location. From each river we were provided with only one sediment sample, instantly decreasing our sample size. The more samples collected from the specified site, the more accurate the averages will be and representative. In addition, we decided to have only three repeats per sample however this was a limitation because the more repeats, the more reliable the average microplastics observed will be. Furthermore, an increase in repeats could have led to a statistical significance in ANOVA. In addition, the sediments were collected by the Environmental Agency not specifying which part of the river sediments were collected, for example, the river bank, the river bed, the river sides or even 2 meters away from the river. The specificity of the location of the sediments collection is importance as we do not know whether the collection of all sediments were the same or random parts of different rivers. This could have been easily controlled by placing a note explaining which part of the rivers the sediments should be taken from. Another limitation we came across was the removal of background contamination. As we are investigating microplastics, we avoided using plastic laboratory equipment, instead using glass, to avoid contamination of the sediment leading to false positive microplastics. We were able to switch some equipment, such as plastic beakers to glass beakers however some equipment proved harder to replace, for example, pipettes. The plastic falcon tubes may have been a source of background contamination, we could not control this because the sediment was sent in these tubes. We did not use these tubes in the water control and the average microplastic number is lower that the method controls, suggesting some background microplastic contamination came from the plastic tubes. In a future study, storing sediment in glass containers would reduce this. Even though there may have been background contamination, the same method was used for all the controls and all the samples, thus the use of the same method controlled the background contamination.

Conclusion

The aims of the study were achieved as ethics approval was obtained, the study was conducted, and data was collected and analysed. Various shapes and sizes of plastics were found in the samples from River Nar, River Tove, Alconbury Brook and Kempston Mill sediment. The hypotheses were put to the test, however there was no significance in the data as the sample size of the study was very small. As discussed, the presence of microplastics in the sediments were detected and identified however, it did not provide any statistical significance. In the future, research on a larger number of repeats than three from various locations of the rivers would be needed in order to establish the degree and presence of microplastics. It is also suggested that other rivers in England should be tested for microplastic pollution to ensure accuracy and reliability of the experimental results.

Lessons Learned

We learnt that there was microplastic contamination in the river sediments we looked at. After searching the scientific literature, we believe this is the first study looking for microplastics in these rivers. There is a need to study and test for microplastic contamination in water bodies because it can have adverse effects on organisms that ingest microplastic or get entangled in plastic debris. A large part of our project was education to the public by raising awareness about microplastic pollution as general knowledge of the public about sources, risks and long term effects of microplastics in fresh waters is poor. We had a stall on April 24th 2019 at the 16th Annual Schools Science Conference, where we could present our main aims of the study was a good opportunity to have a conversation with members of academic staff and other students about microplastics. There was great interest of the public in the presented topic, as we were asked multiple direct questions about the project. This event was an occasion where we could observe the interest in improving situation about enormous plastic pollution. Large number of people did not know how or where to introduce changes to their daily actions to reduce plastic production. We also raised awareness by sharing instagram and twitter posts about our project. The general population’s knowledge on this topic is important because the public can make changes in their daily lifestyle such as purchasing clothes made of natural fabrics, reducing one-use plastic consumption and recycling to avoid plastic contamination of rivers and oceans.

Group Reflection

This project was a great opportunity to develop a wide range of skills. For example, communication within the team and time management to organise meetings and laboratory work. We also practiced written communication skills via this report and keeping a lab notebook, as well as verbal communication with the general public, when we had a stand at the 16th Annual Schools Science Conference on April 24th, 2019 where we educated secondary school students on plastic pollution in the marine environment. We used a wide range of laboratory techniques such as staining, microscopy and pipetting and we sometimes had to come up with solutions to problems, thus developing our problem-solving skills. Being a student led independent team was an interesting experience, we found it very skill building as it was different to group projects for university coursework, we learned a lot from it as we had to arrange our project, plan, do our own research, be responsible and reliable. We are immensely thankful to our supervisor Dr Caroline Smith as she was a great support to use and gave us advice when we required it.

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