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RESEARCH ARTICLE

Comparative Analysis of Methods for Separating Microplastics from Aquatic Environments

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ABSTRACT: The improper disposal of plastics contributes to the contamination of terrestrial and, eventually, marine ecosystems. Recently, reports of negative consequences from the ingestion of microplastics by living organisms through food and water have been increasing. Although still under investigation, evidence shows that these particles can accumulate inside organisms, obstruct circulation, and even trigger diseases. Therefore, there is an urgent need to control their intake, mainly by removing them from aquatic environments and ensuring treated water is suitable for consumption. This work compiles and compares several laboratory methods that enable the filtration of microplastics. The evaluation criteria focused on accessibility, ecological benefits, cost-effectiveness, and large-scale applicability, particularly when integrated into Water Treatment Plants (WTPs). From this comparison, an alternative approach is proposed, inspired by the principles observed in previous methodologies and the characteristics of the microplastics studied. The main hypothesis relies on the non-polar nature of plastics and their affinity with substances such as oils, which share similar polarity. By exploiting this interaction, a possible separation could occur between water, a polar resource, and the combined fraction of plastics and oils, both non-polar materials. The study aims to assess whether current methods already provide a truly effective and sustainable solution or if further innovation remains essential for future water treatment strategies.

KEYWORDS: Chemistry, Biochemistry, Microplastic, WTP, Separation Method.

Introduction

Pollution is a problem that affects flora and fauna as a whole; however, it is clear that it is particularly alarming in the aquatic environment. According to research published in the article "Plastic ingestion as an evolutionary trap: Toward a holistic understanding,"[1] man's polluting action quickly causes changes in the environment, reaching the base of the food chain, to the point that more than 1,200 marine species, including all families of seabirds and marine mammals, have been recorded ingesting plastic. The problem becomes even worse when this intake not only takes up space inside the animal, but they also have a lower capacity to assimilate nutrients, damaging their life in the long term. Likewise, there are short-term damages, such as the fact that these beings can die immediately due to the blockage of the respiratory ducts.

Therefore, as they are chemically non-polar, their toxic damage extends from the absorption of heavy metals to their fixation in structures such as lipids, blood, and other body fluids, thus leading to a series of disturbances in the essential functions of living beings. An analysis published in the specialized magazine Environmental Science and Technology reveals that the plastic particles evaluated directly affect the fish's functions, such as behavior, metabolism, and neurological functions. As a consequence, they interfere with various psychological processes such as stress neurotransmission and immunity levels.[2]

However, these effects are not restricted to animals; human beings suffer from the side effects of their own invention. Through means such as food and breathing, their progenitors also ingest plastics, and these access different parts of the human body, even combining with lipids. One of its main dangers is related to the fact that plastic can absorb heavy metals, and if it has been previously contaminated, it can cause poisoning in the person who ingests it.

Some more pessimistic studies reveal that microplastics affect male fertility in mammals.[3] According to research, the quality of mammalian sperm, when exposed to the presence of microplastics, proves to be dangerous for reproductive activities. Thinking in the long term, the main current concern is the unfeasibility of human reproduction, which could gradually lead to the extinction of the species and irreparable damage.

Origin, Definition, and General Characteristics:

Plastic is a synthetic material that, in contemporary society, is universally present in various environments. Its emergence, however, is quite recent. It was only in 1907 that Belgian chemist Leo Baekeland created the first fully synthetic resin.[4] As time went by, different types of plastics emerged, each with different characteristics and uses. With mass use came large-scale disposal, and due to its long decomposition time, it became a focus of environmental concern.

Furthermore, it is worth defining the very meaning of microplastics (MP). The term was introduced by marine biologist, director and professor at the University of Plymouth in England, Richard Thompson (2004), and refers to a microscopic particle of plastic less than 5 mm in size. This definition is globally recognized by organizations such as the European Union, the National Oceanic and Atmospheric Administration,[5] the

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International Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection,[6] among others.

Destiny of Microplastics:

Although there are systems that collect and treat waste, giving it the most appropriate destination in a way that minimizes damage to the environment, plastic waste continues to grow. The lack of awareness or care during this process ends up causing the debris to contaminate ecosystems, especially the aquatic ones. According to studies released by WWF,[7] approximately to 10 million tons end up in the oceans every year. And if it continues like this, by 2030, there will be more than 104 million tons of plastic polluting the environment.

Even though pollution is a problem that affects the world's flora and fauna, it is undeniable that a large part of plastic waste ends up in the oceans. The way in which this happens is diverse and more routine than we are aware of, and it often happens due to human error. Primary microplastics, such as microfibers from synthetic clothing, come loose during washing and end up in rivers, where they go to the sea. Or plastic microbeads from cosmetics and beauty products travel through effluents to water treatment plants.

Furthermore, sewage treatment plants can retain part of the microplastics, which are mixed in sludge, and later used in agriculture. Just as rain carries garbage left on the streets, in landfills, and into rivers, micro particles released by objects such as car tires can be carried away by the wind. Not to mention that there are exclusively marine circumstances, such as fishing nets, containers, and materials that are lost at sea and waste discarded by oil platforms, for example.[8]

Microplastic Control Methods:

Filtration due to size difference: Given the average thickness of microplastics, which varies from 1 millimeter to 5 millimeters, a mesh or filter is used in which the opening thickness of the holes is smaller than the average thickness of the microplastics. This blocks the majority of microplastics, which remain trapped in the mesh while the water passes through. The material that makes up the network is also relevant, since properties such as elasticity, electronegativity, and friction, among others, contribute to a greater or lesser degree of retention of microplastics.

Application of hybrid silica gel: Silica gel, when hybridized, acquires the property of agglomerating microplastics that contaminate water due to its molecular structure and electronegativity. In other words, with the addition of hybrid silica gel to water contaminated with microplastics, an aggregate of microplastics is formed large enough to be removed from the environment through scavenging.[9, 10]

Electrocoagulation: Consists of the use of an electrochemical reactor to verify the effect of electric current on the removal of particles through the formation of clots.[11]

Biodegradation by the fungus *Zalerion maritimum*: When placed in a food-scarce situation, it can use the breakdown of plastic molecules as a source of energy. As it is an aquatic fungus, it can degrade microplastics present in running water, thus contributing to the decontamination of the environment through food scarcity.[12, 13]

Sphagnum SP mosses. (Must Go) *Sphagnum* moss found in the Andes of Colombia is capable of holding up to 40 times its weight in water and releasing it during the dry season. This property of moss makes it a natural filter that retains microplastics that are floating in the water with an effectiveness of 94%, serving as a natural control of microplastics.[14]

In the meantime, it is observed that among the various ways in which microplastics end up in water, many are not the result of human error in the collection and disposal process. Therefore, it is not a problem that can be solved solely by population awareness. It is necessary to develop or apply a method that is capable of filtering microplastics from water and thus, at least, minimizing damage.

To achieve this, we seek to develop a method based on the principle of polarity, a characteristic of different materials. Since plastic is a non-polar material and water is polar, they do not mix. Therefore, if it is possible to create a non-polar structure through which the contaminated sample passes, there is a tendency for it to attract microplastics. This allows the separation of substances.

Methods

The experiment was conducted entirely in a laboratory with a fixed temperature of 22°C. Firstly, it was necessary to determine the amount of microplastics that would be inserted into each glass tube. Using as a reference point the proportion typically used by scientists researching microplastics, a proportion was calculated for the desired quantity. With this, we obtained that for every 12ml of mixture, 0.003g of microplastics would be needed, which were subsequently weighed on a scientific scale, ensuring the proportional accuracy of the research. Thus, with the polymers separated according to their tubes, it was possible to proceed to the next step.

Secondly, with the aid of an automatic pipette, exactly 6 ml of filtered distilled water was inserted into each tube to ensure a fair comparison. With all the tubes containing water, each tube continued to be labeled with the name of the substance that would be inserted into it, to avoid future identification problems. With this, 6 ml of vegetable oil, mineral oil, hexane, and filtered distilled water were sequentially added, the latter being made to be the Control, to guarantee the integrity and validation of the research.

Each substance is based on a concept. Oil, for example, because it is non-polar - just like plastic - does not mix with water, ensuring that the mixture is two-phase. Considering the polarity of the plastic, it is expected that it will tend to remain close to the oil due to the similarity of polarity and density, separating the particulate material from the water. Hexane, in turn, is already known to react strongly to plastic in high concentrations, and as it is a petroleum derivative, just like plastic, it is expected that some reaction will occur that groups them together.

Therefore, the already sealed tubes were left on a rack while the tube shaker was prepared. With the machine already pre-

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pared, the tubes were inserted side by side, and the agitator was turned on for 1 hour.

The testing is complete, and a final step must be carried out to guarantee results. Therefore, after the stirring process for 1 hour, the tubes were left to rest for around 14 hours, in order to allow decantation, completely separating the substances.

Thus, with the solutions already decanted, a step was taken in which the aim was to verify the presence of microplastics. To do this, with the help of an automatic pipette, samples were collected from different parts of each tube, always changing points, in order to avoid cross-contamination and affect the reliability of the experiment. Therefore, the samples were placed in culture plates, always identifying them to avoid any future perception problems. With this, the plate was taken to a microscope, where the samples were carefully analyzed to verify the event that occurred and how the plastic reacted to the substances.

Results and Discussion

Control Tube:

For research to be validated in the scientific field, the existence of a Control Group is always necessary to establish a parameter relating to what would normally happen to the substance. Following this scientific rigor, a Control Group was adopted. After resting for 14 hours after shaking, it was possible to verify that in this group, the microplastics were well diffused throughout the water; however, due to their density being lower than that of water, there was a slightly higher concentration in the upper part, which was then the part chosen to analyze under the microscope.

With the choice of analyzing the upper part of the water and microplastic mixture, with the help of an automatic pipette, it was possible to separate 1 ml and insert it into a bacteria culture plate, to be able to analyze it later under a microscope. By analyzing the control substance under the microscope, it was possible to verify the shape of the microplastic in the aquatic environment (Figure 1), which was found to be stretched, possibly due to the time that the tube spent in the shaker. With this in mind, it is now possible to identify the plastic material in the other tubes when later analyzing them under a microscope, since there is knowledge of its shape, condition, and color, making it easier to identify and making it difficult for it to be confused or not found.

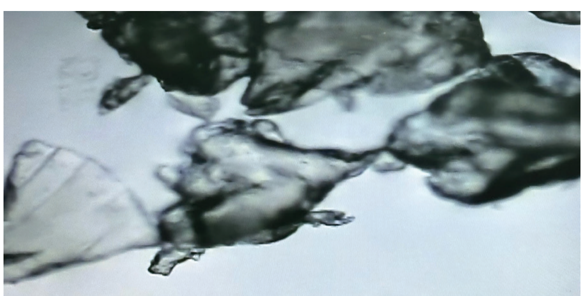

Figure 1: Photograph of a microplastic observed under 40x magnification in the control sample through a microscope, facilitating future identifications. Source: Author

Tube with Mineral Oil:

The tube mixture that contained mineral oil was divided into three well-defined phases. However, water is denser than microplastics and mineral oil; the surface phase of the mixture is made up of water, the intermediate phase is characterized as an "inverted obelisk" (Figure 2), containing the highest concentration of microplastics, and the deepest phase is mineral oil. This result is in contrast to previous research, since mineral oil has a lower density than water. Thus, the main hypothesis is that when stirred, the microplastics and mineral oil reacted with each other and their densities added up, acquiring a higher value than that of water and, thus, decanting. Thus, this result also proves the initial theory on which the method is based, since the microplastic, a non-polar compound with a lower density than water (polar), reacted and joined the mineral oil, also non-polar, remaining together even after the density of the resulting mixture was higher than that of water, thus showing that the reaction between non-polar compounds was stronger than the density force. Furthermore, there was an attempt to observe the layer of microplastics through a microscope; however, due to its opacity, the analysis was hampered, and it was not possible to identify any relevant information.

Figure 2: Tube with mineral oil, allowing the naked eye to see the "inverted obelisk", the phase in which microplastics were concentrated. Source: Author

Tube with Vegetable Oil:

The tube containing the vegetable oil, in turn, reacted as expected. This, after decantation, was divided into two phases: the surface phase, formed by vegetable oil with the microplastics dispersed in it, and the lower phase with distilled water.

When observing the microplastics dispersed in the vegetable oil through the microscope, it was possible to notice that a well-defined round bubble encompassed them, differing strongly from the microplastics analyzed in the control. Thus, it can be seen that the vegetable oil acted in a similar way to a cell in the process of phagocytosis, "swallowing"/engulfing microplastics (Figure 3). Thus, it proves both the effectiveness of the oil in separating microplastics from the aquatic environment and that, during this process, the oil remains around the microplastics, precisely due to its polarity affinity.

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Figure 3: Photograph of the microscopic analysis of a vegetable oil sample, observed under 40x magnification, allowing the identification of microplastics "engulfed" by the oil. Source: Author

Tube with Hexane:

After resting for 14 hours, during which the substances had time to react and settle, it was possible to observe that the tube containing hexane reacted with the microplastic during agitation, forming sizable agglomerates (Figure 4). The initial hypothesis is that, because both hexane and plastic are hydrocarbons and are also derived from petroleum, this common origin contributed to a reaction between the two substances, causing the microplastics to be forced together, forming these clusters. Due to the size of the agglomerates, it was not possible to remove the microplastics using the pipette so that they could be observed under the microscope.

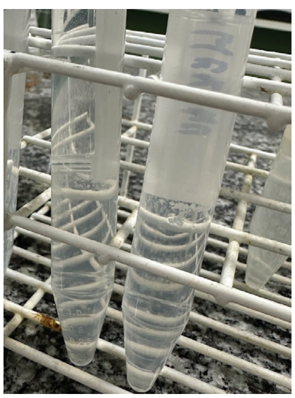

Figure 4: Comparison of the control sample and the hexane mixture, showing, in the latter, the formation of microplastic agglomerates. Source: Author

Discussion:

Primarily, the previously collected methodologies must be compared. It should be noted that all methods have already been proven effective, and, for the group, one that pollutes less and is accessible is better than one that presents better results. This is explained by the fact that a reduced cost proposal has a greater chance of being accepted by WTPs and the Brazilian government. Therefore, considering the alarming situation the world finds itself in, any way of minimizing damage is already a huge step forward for public health.

Starting with some of which were given greater investigative emphasis, there is the Portuguese sea fungus, *Zalerion maritimum*. Due to its nature and the fact that it can develop freely in an aquatic environment, many expectations were placed on this method, especially in comparison to the others, which were in conditions restricted to laboratories. With this in mind, work developed by Ana Beatriz Silva and Ana Sofia Bastos, from the University of Aveiro, was investigated, and this continues with that of Ana Paço, who was a finalist in Biotechnology.

Although recent research has shown good results with different types of plastics, furthermore, those responsible for this discovery are interested in expanding their studies because they see their ability to be adapted to larger-scale systems. However, some problems arise.

Firstly, both works took around a month to complete, which, if applied in one station, is practically unfeasible due to the enormous flow and speed with which the water must be treated. Another is that the culture medium in which the fungus is grown has some restrictions regarding temperature, agitation, and, mainly, the quantity of nutrients, which makes its creation difficult. The fungus must necessarily be deprived of food, but not completely, and a restricted proportional amount must be supplied. This ends up preventing its use in different climates, such as Brazil, or else the costs originally saved with the simplicity of the organism would be used to maintain the environment.

Continuing with biologicals, *sphagnum* moss is used in the system developed by the company MustGo. The big problem with this is that, because of the physical limitations of moss, it cannot be used in structures much larger than those already being put into practice. Furthermore, expanding its structure and also having to change it every two months would result in immense exploitation and deforestation of this plant.

Methods for separating microplastics that use the physical properties of materials and media have continually been found. One of them, and probably the most obvious, is a filter with pores so small that they prevent microplastics from passing through. However, the filter would quickly accumulate microplastics and consequently, the holes would gradually be plugged. As a result, if the difficulty of water passing through the filter were not enough, as it is used, an increasingly greater force would have to be applied for the liquid to continue passing in the same quantity and at the same speed. Thus, they need a source of energy and a resistant material. The same logic applies to others that start from the principle of molecular size of microplastics and nanoplastics, such as disc filtration. The others, although commented on in the introduction, ended up not being given much depth due to the lack of availability of information. Or because they are very similar to what is being incorporated by WTPs and are not completely efficient.

Finally, among the physicochemical methods, the so-called electrocoagulation stands out. This appeared to have no problems; however, when testing it, they found that the clots formed very close to the electrode, quickly preventing the formation of other clots. Furthermore, another promising one was found: hybrid silica gel. By using only hybrid silica, it appeared to satisfy the idea of evaluating its large-scale application in Water Treatment Plants (WTP). The recovery rate of microplastics was enormous, in addition to forming a large plastic clot that was easy to remove. Unlike others, hybrid silica gel did not depend on a specific pH or a certain temperature for its operation, further opening up the range of possibilities of circumstances in which it could be used. However, upon further research, it was found that the hybridization process took months, with a high process cost. Furthermore, it was discovered that silica can cause serious damage to health, making the

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method even more unfeasible when it comes to treating water for subsequent human use.

The new methodology developed by the group is still in a similar stage to the other processes evaluated. At this first moment, the large amount of oil and hexane that is used in proportion to the water acts as a major obstacle. Just as the fact that the contaminated substances were not separated prevents the full assessment of the possibility of risk that the experiment presents when placed in a WTP, all this, however, does not negate the accessibility and sustainability it provides. Unlike the others that were discussed, even if this one does not yet meet the previously established parameters, it is possible to continue through a continuous experimentation process that improves its effectiveness.

Conclusion

In view of the above, it is concluded that no existing method to date is capable of being applied in a WTP, or if it is applied, it is not fully functional. It takes many years of testing and exploring the most diverse resources to explore the functionalities of different methodologies, adapting them to different scenarios or using substitute resources. Everyone has chances; it is up to researchers to invest in their potential.

Among those that stood out the most, and there are hopes for uses in the future, there is the use of fungi, especially Zalerion maritimum. Currently, it is unfeasible, but if research continues with the organism, it is one of the most likely to succeed. There is speculation that it can ingest other types of plastic and also make the conditions for its growth more flexible. In any case, the use of these beings can be present even though they are not capable of carrying out the filtration themselves. The fungus acts as an ecological final destination for microplastics, definitively removing them from ecosystems.

Regarding those that use physical properties, such as filters, which are still in use, they are very limited to the form of the MP. Even so, it represents an important start in dealing with this problem; it is up to governments to decide whether it is worth dealing with the costs of cleaning, materials, and energy to apply the methods.

On the other hand, physical chemicals, such as hybrid silica gel, have the advantage of acting on all particles that share properties characteristic of plastic molecules. In the meantime, what is really making its use impossible is the fact that the reacting substances are often difficult or impossible to separate from water and are toxic for human consumption.

Regarding the method developed by the group itself, its effectiveness can be seen from the results, since in all the compounds used, microplastics were removed from the distilled water after shaking the mixtures. Furthermore, as it is a simple and inexpensive process, it can continue to be tested and evaluated in various situations until it is suitable for a treatment plant.

However, regardless of the results, the absolute need for mass awareness is evident to avoid a catastrophe in the near future. Taking into account the various harms that have already been proven or are being studied, the obstacles in containing the dispersion of plastic, and mainly, the prolonged decomposition time of this material, the best way to minimize this current situation is a drastic change in habits. Therefore, the spread of the problem is avoided by replacing plastic with biodegradable alternatives. Another measure is to follow the correct disposal of waste because, as seen previously, objects that do not follow the appropriate destination are more likely to end up interfering with terrestrial and aquatic environments.

Therefore, the relevance of water treatment processes and the importance of guaranteeing them for the world population are highlighted. Through WTPs, contamination and disease transmission are avoided, making it an efficient way to prevent plastic contamination.

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My name is Felipe Leirião Riva, and I'm a student deeply interested in research and innovation, planning to study abroad and pursue engineering. This work has been invited to and presented at national and international fairs, such as FEBRACE and LIYSF, and now I'm looking to publish it.

Camila Castellani Souza, Brazilian student, graduated from high school in 2024, currently (2025) in the first year of graduation at the medical college of "University of São Paulo" in Ribeirão Preto. Planning to use the project to support studies on the impacts of microplastics on personal and public health issues.

Pedro Duarte da Costa, born in Santos, Brazil, and graduated from high school in "Colégio Jean Piaget" in 2024, plans to pursue a career in the engineering area, using the research knowledge of this project to ground the skills of academic life.