

Nanoplastics in Aquatic Environments: Origin, Separation and Characterization (review)

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Scientists discovered plastic in the early 1900s, but didn't realize the detrimental effects its fragmentation could have on the environment 100 years later. In particular, nanoplastics (NPs) particles ranging in size from 1 to 100 nm can cause major problems in the living world due to their high specific surface area for the adsorption other polluting substances from water, and their further bioaccumulation through the food chain. There is no distinctive method to identify, characterize, and quantify nanoplastics in aquatic environments. Although many of the methods developed to study microplastics are not directly applicable to nanoplastics, conventional methods of characterizing nanoplastics are usually tedious because they study individual nanoparticles in isolation. Since nanoplastics resulting from the decomposition of microplastics have different properties than engineering plastic nanoparticles, new techniques need to be developed to help us better understand the seriousness of the nanoplastic problem. Nanoplastic can be isolated from the water environment by a combination of filters and ultracentrifugation. A recent publications states that combining microscopy and spectroscopy, supported by chemometric techniques, will allow a better understand he behavior of nanoplastic particles in the environment and organisms. High hopes are placed on microscopies combined with neural networks for the quantification and characterization of nanoplastics in complex systems. This article describes the degradation pathways of plastics and the formation of nanoplastics in aquatic environments, and possible methods for separation and characterization of nanoplastics in relation to recent publications.

Key Words: nanoplastic, fragmentation, separation, characterization, environmental impact

1. INTRODUCTION

Plastic first appeared on the market in the 1950s, and its worldwide production has increased steadily since then [1]. Production has skyrocketed, especially in recent decades, and in 2020 it is estimated at 367 million tons [1]. It is expected that this will triple by

2050. [1]. This has caused an exponential increase in plastic waste in the oceans. In fact, out of 359 million tons produced in 2018, an estimated 14.5 million tons ended up in the ocean [2].

Discarded in the aquatic environment, these plastics are slowly eroded and fragmented into small particles by various physical, chemical, and biological processes. A growing number of aqueous media and sediment samples have been found to contain plastic particles smaller than 55 mm (microplastic), which can be harmful to the general public's health [3, 4].

Particularly nanoplastics between 1 and 100 nm in size (EFSA 2016) have completely different physical

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(e.g. mobility), chemical (e.g. surface functional groups), and biological (e.g. diffusion through the cell membrane of the organism, toxicity) properties than microplastics and macroplastics, which can cause a variety of issues in the living world [3, 4]. Nanoplastics are present in both terrestrial and aquatic organisms, as well as in the air, water, and sediments. This nanoplastic can be created either directly from natural or artificial sources, or it can be produced indirectly through the breakdown of microplastics [5]. Additionally, it can get past wastewater treatment facilities that aren't made to take them out of the water [6]. The primary sources of nanoplastics are textiles used in agriculture, disposable plastics, cosmetics, paints, rubber, and urban dust [7]. Since the structure and composition of nanoplastics are related to the source material, the most common plastic polymers are polystyrene (PS), polypropylene (PP), polyethylene (PE), polyamide (PA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and cellulose acetate (CA) [7].

Nanoplastics (NPs) particles have a large specific surface area relative to volume, which makes them more reactive and potentially more toxic to humans and the ecosystem than microplastics [8]. There is still controversy over definitions related to NP size, here we have adopted the definition of size for nanoplastics ranging from 1 to <100 nm [9]. While the fragment size from 100 to <1000 nm is used the term submicron plastic. As the appearance and origin of nanoplastics largely depend on the degradation of microplastics, nanoplastics are polydisperse in size, reactivity and surface chemistry and nonuniform in composition [10]. Nanoplastics created by the degradation of microplastics in the aquatic environment are not the same as synthesized nanoplastics [8].

2. ORIGIN OF NANOPLASTICS IN AQUATIC ENVIRONMENT

Once in the aquatic environment, plastic degradation first begins with photo-oxidation, when depolymerization and polymer chain scission reactions are initiated. Physico-chemical, mechanical, and biological degradation then occur as plastic fragments are formed. Figure 1 shows the degradation pathways of plastics and the formation pathways of nanoplastics (NPs) in the aquatic environment. Studies show that nanoplastics can form in the aquatic environment through four main pathways: photodegradation, thermo-oxidative degradation, hydrolysis, and microbial biodegradation [11]. First, the photodegradation of larger plastic residues into microplastics occurs with solar radiation in the ultraviolet (290-400 nm) and visible part of light spectrum (400-700 nm) [12]. Photo-excitation triggers the production of free radicals that automatically accelerates reaction and decreases the

average chain length of the polymer structure over time. When the polymer structure is heavily degraded, it degrades to MP and then to NP, making it even more brittle. These plastic debris can accumulate in water bodies. Especially small plastic particles can be ingested by marine life and have other dangerous effects.

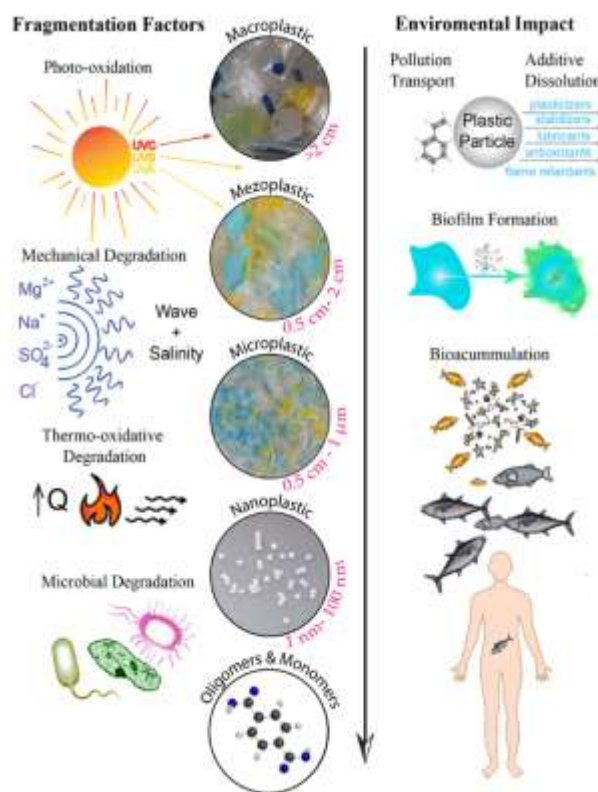


Figure 1 - Degradation of plastics in aquatic environments, fragmentation factors, and the emergence of nanoplastics. Potential harmful effects of nanoplastics on the environment.

Nanoplastics, like macroplastics, contain a large number of toxic compounds, such as antioxidants, plasticizers, pigments, heat stabilizers, flame retardants, acid scavengers, light stabilizers, antistatic agents, and lubricants [13]. Apart to these additives, plastic nanoparticles may also adsorb hydrophobic or hydrophilic organic pollutants from the aquatic environment, thanks to their enormous surface area and affinity for these contaminants [14]. In NPs collected from the environment, persistent organic pollutants such as polychlorinated biphenyls, aromatic hydrocarbons, and phthalates have been detected [14]. Indeed, NPs are often associated with toxic chemicals and act as vectors of their transport through the environment. In recent years, the adsorption and transport of various pharmaceuticals have been discussed [15]. Additionally, NPs represent a mixture of harmful compounds that are added as additives to polymers during production to improve their quality and extend their lifespan.

NPs are also suitable for biofilm formation [16]. Scientific studies have shown that MP and NP have minimal impact on various species such as yeast, bacteria, and protozoa [17, 18]. Various toxicological effects on organisms such as invertebrates, vertebrates, seabirds, and mammals were also discussed [19-21].

3. SEPARATION OF NANOPLASTICS FROM AQUATIC ENVIRONMENT

It is vital to monitor NPs in diverse biotic and abiotic environmental matrices to establish contamination status, flow, and risk of biotic exposure. Follow-up studies require methodologies that are trustworthy and comparable. Nevertheless, detecting NPs of varying composition, shape, and size using a single approach is a tough objective.

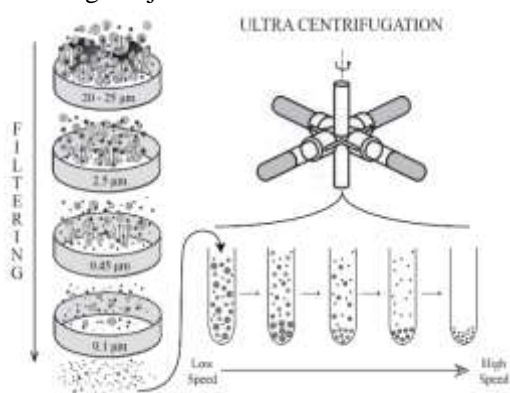


Figure 2 - Separation of nanoplastic by filtration and ultracentrifugation

However, as there are still no particular techniques for its detection and quantification, it is very challenging to distinguish and define nanoplastics from microplastics. The same methods that are used for other engineered nanoparticles can be applied here. Figure 2 shows a size-based separation of microplastic and nanoplastic using filters with various pore sizes. Microplastics with diameters ranging up to 2.5 µm are typically separated either separately or in sequence using mesh sieves [22]. Small mesh filters (e.g. 2.5 µm to 0.1 µm) are also employed to separate tiny MPs or NPs. Only the nanoplastic fraction can be isolated at the end of a filter with pores smaller than 0.1 µm. The nanoplastics can be entirely separated from one another and concentrated by ultracentrifugation [23]. Ultracentrifugation can be used for complete separation and concentration of the nanoplastics [23]. Depending on the number of revolutions of the centrifuge, but also the diameter of the cuvette, nanoplastics can be classified by size. The use of a centrifugal field is such a powerful method that it is possible to separate plastic nanoparticles by other properties, such as, for example, density, optical properties, color, and others [24, 25].

4. IDENTIFICATION, CHARACTERISATION AND QUANTIFICATION OF NANOPLASTICS

To be able to predict the behavior of nanoplastic particles in the environment, various physical and chemical properties such as transport, stability, aggregation, absorption, and sorption should be characterized [26]. A basic classification of micro- and nanoplastic characterization methods can be divided into visualization techniques, physico-chemical and chemical characterization [3].

NPs particles are commonly characterized using classical techniques that are applied to characterize nanoparticles, however, it is a time consuming job. Polymer identification is carried out by methods such as Fourier-transform infrared spectroscopy (FTIR), Raman and micro-Raman spectroscopy (RS and MRS), energy dispersive x-ray spectroscopy (EDX), x-ray photoelectron spectroscopy (XPS), infrared nuclear magnetic resonance (IR-NMR), and mass spectrometry (MS) [27, 28]. The advantages of these methods are that they are simple, non-destructive and do not require sample preparation. The disadvantage is that they are time consuming. In addition to them, both thermal gravimetry (TG-DSC) and high-performance liquid chromatography (HPLC) are also used to identify polymers, as well as additives. Different microscopic techniques are used to characterize the size, color, and morphology, such as optical microscopy (OM), atomic force microscopy (AFM), confocal scanning laser microscopy (CSLM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) [27, 28]. All of them require sample preparation before imaging. Various physicochemical properties, such as surface charge, hydrodynamic diameter, dispersion, and distribution, can be characterized by dynamic light scattering (DLS) [5, 29, 30].

However, all of these methods individually have the limitation of providing incomplete results when analyzing nanometric particles. Currently, a combination of microscopic and spectroscopic techniques (chemical analysis) overcomes these limits [31]. Statistical methods are powerful tools that can correlate data obtained by microscopy and spectroscopy techniques [31]. Microscopy and spectroscopy techniques, together with recently introduced statistical chemometric data processing approaches, can lead to a more accurate understanding of the problem of nanoplastics.

Based on their inherent fluorescence [27, 32], fluorescence microscopy is a good tool for analyzing nanoplastics, notably white and transparent plastics. Fluorescence microscopy records the fluorescence emission from a material that has been stimulated by a certain wavelength [12]. In traditional fluorescence

methods, lasers generally excite in the visible (blue) and ultraviolet (UV) areas, and light flows through a tiny aperture and is focused on the area of interest on the sample. As the incoming photons must release more energy, the wavelength of the excitation laser should be roughly 50-200 nm shorter than the light generated by the fluorophore. In confocal laser scanning microscopy, the light beam illuminates just a tiny section (spot) of the specimen [33].

As a result, the detector sees just the intensity of light emanating from the excitation point and not the entire picture. By moving points along a plane or within a volume, cross-sectional or three-dimensional representations of the sample may be recreated. In addition, the acquired data may be processed statistically to describe and quantify the nanoparticles in the sample. The results of the PET nanoparticles autofluorescence investigation give a novel method for characterizing nanoplastics [34].

In recent years, holographic microscopy combined with neural networks has received much attention in identifying transparent nanoplastics in aquatic environments [27]. Figure 3 shows a scheme for nanoplastic

identification, characterization, and quantification using digital microscopy supported by neural networks. Recently, a new technique called digital holography (DH) has been identified that can distinguish plastic debris from marine microalgae or microplankton in water samples [35]. The proposed method uses artificial intelligence and holographic sensors to obtain information from the analyzed elements through holographic microscopy. Additionally, in order to obtain as many images as possible with a higher spatial resolution during 3D imaging, three laser light sources in the visible part of the spectrum are used [27]. The information thus obtained allows artificial intelligence systems to be „trained“ to distinguish nanoplastics from other natural substances that are very similar in size and shape. DH represents optical technology for qualitative and quantitative 3D imaging of objects. Digital cameras can be used to detect transparent objects at various depths and search for the presence of large quantities of underwater plastic waste. Thanks to this technology, it is possible to detect, count, and quantitatively measure nanoplastics throughout the volume of thousands of objects of various classes [27].

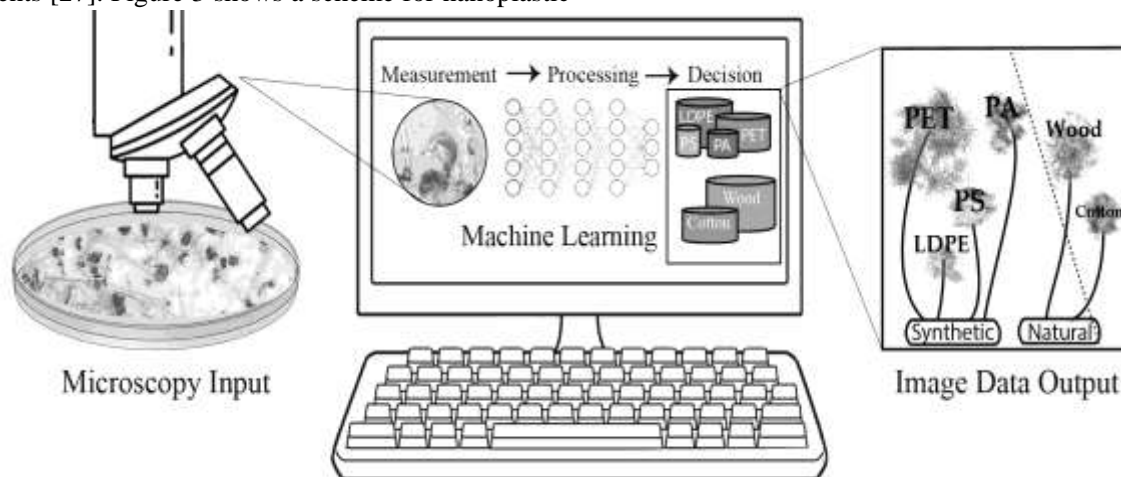


Figure 3 - Identification, characterisation and quantification of nanoplastic using digital microscopy

5. CONCLUSION

The fragmentation of plastic waste in the aquatic environments, which leads to the formation of microplastics and nanoplastics, has been recognized as a major pollution problem in the last few decades. Currently, there is no uniform method for the reliable and rapid identification and characterization of nanoplastics in the aquatic environment.

The separation of nanoplastics can be achieved by using a system of filters with different porosities, as well as ultracentrifugation for complete separation at the nano level. Combining microscopy techniques with spectroscopy followed by statistical methods can overcome some prominent problems of nanoscale characterization.

Holographic microscopy supported by artificial intelligence can also identify and quantify transparent nanoplastics in complex systems. Standardized methods for the detection and characterization of nanoplastics must be developed that are effective for establishing and maintaining long-term monitoring programs for nanoplastics in aquatic environments. This is the only way to assess its effects on the environment and the living world.

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REZIME

NANOPLASTIKA U VODENOJ SREDINI: POREKLO, SEPARACIJA I KARAKTERIZACIJA (PREGLED)

Naučnici su otkrili plastiku ranih 1900-ih, ali nisu shvatili kakve štetne efekte njena fragmentacija može imati na životnu sredinu 100 godina kasnije. Konkretno, čestice nanoplastike (NP) veličine od 1 do 100 nm mogu izazvati velike probleme u živom svetu zbog svoje velike specifične površine za adsorpciju drugih zagađujućih materija iz vode i njihove dalje bioakumulacije kroz lanac ishrane. Ne postoji poseban metod za identifikaciju, karakterizaciju i kvantifikaciju nanoplastike u vodenoj sredini. Iako mnoge metode razvijene za proučavanje mikroplastike nisu direktno primenljive na nanoplastiku, konvencionalne metode karakterizacije nanoplastike su obično zamorne jer proučavaju pojedinačne nanočestice u izolaciji. Pošto nanoplastika koja je rezultat razgradnje mikroplastike ima drugačija svojstva od sintetisanih plastičnih nanočestica, potrebno je razviti nove tehnike koje će nam pomoći da bolje razumemo ozbiljnost problema nanoplastike. Nanoplastika se može izolovati iz vodenog okruženja kombinacijom filtera i ultracentrifugiranja. U novijim publikacijama se navodi da će kombinovanje mikroskopije i spektroskopije, uz podršku hemometrijskih tehnika, omogućiti bolje razumevanje ponašanja čestica nanoplastike u životnoj sredini i organizmima. Velike nade se polažu u mikroskopiju u kombinaciji sa neuronskim mrežama za kvantifikaciju i karakterizaciju nanoplastike u složenim sistemima. Ovaj rad opisuje puteve degradacije plastike i formiranje nanoplastike u vodenim sredinama, kao i moguće metode za odvajanje i karakterizaciju nanoplastike u odnosu na nedavne publikacije.

Ključne reči: nanoplastika, fragmentacija, separacija, karakterizacija, uticaj na životnu sredinu