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Visual Detection of Microplastics Derived from Plastic Mulch in Soil

Toprakta Plastik Malçtan Kaynaklanan Mikroplastiklerin Görsel Olarak Saptanması

ABSTRACT

This preliminary study aimed to visually investigate the presence and disturbance of microplastic particles among soil aggregate fractions (1000- 2000 µm - <0.053 µm) in a mulch applied soil. In the extraction of microplastics from the soil samples, two separate treatments were applied, by changing the order of applied solutions (NaCl-H_2O_2 and H_2O_2-NaCl), through density separation. In the first treatment, the soil sample was shaken first with a saturated NaCl solution, then filtered through the filter paper and the microplastics remaining on the filter paper were determined. Then, 30% H_2O_2 was added to the remaining soil sample. In the second treatment, H_2O_2 (30%) solution was applied first, and then saturated NaCl solution was added. Microplastics were counted and defined by stereo binocular microscope. In the first treatment 29 particles of 20g $soil^{-1}$ and in the second treatment 16 particles of 20g $soil^{-1}$ microplastics were found. Microplastics were found in all fractions and dominated form was "fiber" and in the form of 'round bead' were also found in the fraction smaller than 0.053 mm. As a result, in the extraction of microplastics, changing the order of application of solutions did not have a different effect on the extraction, showed that both treatments can be used for visual identification.

Keywords: Microplastics, Soil, Separation, Stereo binocular microscope

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ÖZET

Bu ön çalışmada, malç uygulanmış bir toprakta toprağın farklı agregat fraksiyonları (1000- 2000 µm - <0.053 µm) arasında mikroplastik partiküllerin varlığının ve dağılımının görsel olarak belirlenmesi amaçlanmıştır. Toprak örneklerinden mikroplastiklerin ekstraksiyonunda, uygulanan çözeltilerin sırası (NaCl-H_2O_2 ve H_2O_2-NaCl) değiştirilmiş ve yoğunluk ayrımı yapılarak iki ayrı işlem uygulanmıştır. İlk işlemde toprak örneği önce doymuş NaCl çözeltisi ile muamele edilmiş, ardından filtre kağıdından süzülerek filtre kağıdı üzerinde kalan mikroplastikler görüntülenmiştir. Daha sonra kalan toprak örneğine %30 H_2O_2 ilave edilmiştir. İkinci işlemde ise önce H_2O_2 (%30) çözeltisi uygulanmış ve ardından doymuş NaCl çözeltisi ilave edilmiştir. Mikroplastikler, binoküler bir mikroskopla görüntülenmiş ve görsel tanımlamaları yapılmıştır. İlk işlemde 20 g toprakta 29 parçacık, ikinci işlemde ise 16 mikroplastik parçacık bulunmuştur. Mikroplastiklerin tüm fraksiyonlarda bulunduğu ve baskın formun "lif" olduğu, ancak 0.053 mm'den küçük fraksiyonda "yuvarlak boncuk" şeklinde de olduğu belirlenmiştir. Sonuç olarak, topraktan mikroplastiklerin ekstraksiyonunda, çözeltilerin uygulama sırasının değiştirilmesinin ekstraksiyon üzerinde farklı bir etkisinin olmadığı, her iki işlemin de görsel tanımlama için kullanılabileceği ortaya konulmuştur.

Anahtar kelimeler: Mikroplastikler, Toprak, Ayırma, Binoküler mikroskop

INTRODUCTION

Microplastics are one of the new generation pollutants that accumulate in increasing amounts in ecosystems and are durable in nature. The extent of microplastic pollution in the world has become a growing concern for soils after oceans, freshwater sources and sediments (Zhang and Liu, 2018; Helmberger et al., 2020; Hale et al., 2020; Chen et al., 2020; Ahmad et al., 2020).

Plastic materials are widely used in many areas due to their properties such as durability, workability, lightness and low cost. On the other hand, the fact that plastics are extremely difficult to decompose in nature causes plastics to accumulate in the ecosystem and have negative effects. (Hale et al., 2020). Most microplastics can only be broken down into smaller particles rather than biodegraded (under the influence of ultraviolet, radiation, wind or water erosion, etc.) and remain in the environment for years (Wright and Kelly, 2017). Microplastics arrive at soils from agricultural activities, wastewater, irrigation pipes, spills in microplastic production sites, plastic microbeads in daily use products (Rocha-Santos and Duarte, 2015) and vehicle tires. In fact, the main source of microplastics contaminating other ecosystems is terrestrial ecosystems and soils are heavily exposed to plastic pollution. Microplastics are also transported from land to water resources such as streams and rivers and can move long distances by erosion (Andrady, 2015; Jambeck et al., 2015). It is mentioned that the use of mulch in soils is one of the most important sources of microplastic pollution (Zhang et al., 2016). Mulching is one of the cultural practices applied to increase production and quality in vegetable growing.

Synthetic polyethylene plastics (PE) are widely used (Iqbal et al., 2020). Black polyethylene plastics are the most preferred materials due to their lightweight, mechanical properties and cheapness (Ngouajio and McGiffen, 2004). Since these petroleum-derived materials are not degradable, they remain undecomposed in the soil and cause a number of agronomic and environmental problems (Briassoulis et al., 2015; Makhijani et al., 2015). On the other hand, the extremely thin (approximately 8-50 mm thick) films make it difficult to physically remove them from the soil after harvest. With tillage, UV rays and biodegradation, this residual mulch is slowly broken down into parts that form the macro, micro and nano plastic in the soil (Qi et al., 2020).

Agricultural soils potentially have more microplastic storage potential than ocean floors (Nizzetto et al., 2016). Zhang et al., (2018) reported that the number of microplastic particles in soils is over 40000 kg^{-1}, and approximately 92 (%) of the dominant microplastics are composed of microplastic fibers (%) and fragments (particles) constitute 4.1 (%). Fibers and fragments are secondary plastic products formed by the breakdown or degradation of larger plastics.

Although microplastics have been extensively detected in aquatic environments, studies on the detection of microplastics in soils have been much more limited than in other receptors. Both the variety of microplastics and the more complex structure of soils compared to water or sediments make it difficult to detect microplastics in soil (Hidalgo-Ruz et al., 2012; Zhang et al., 2016). On the other hand, despite the development and implementation of new methods, inconsistencies in methods limit the determination and fate of microplastics in soil (Thomas et al., 2020).

Visual identification is one of the most widely used methods for the identification of microplastics. This technique provides simple visual identification of macro and microplastics. The structure of the identified and collected plastics can then be determined with modern equipment (Zhang et al., 2018).

In the extraction of microplastics from water and sediments, floating method is used with saturated solutions of NaCl, NaI, $ZnCl_2$, or sodium polytungstate (SPT) by density separation (Hidalgo Ruz et al., 2012; Imhof et al., 2012; Nuelle et al., 2014b). Screening through sieves of different sizes is also used to separate microplastics into several size categories. After the sieving process, particles of different sizes are kept in different sieves (Schwinghammer et al., 2021).

Microplastics detected in studies are expressed in units such as g g^{-1} or particle g^{-1}. They were defined using various advanced technology devices such as FTIR, ATR, Raman and GC (Silva et al., 2018). However, due to the differences in the properties of microplastics and receiving environments, standardization has not yet been achieved in the determination of microplastics. Therefore, more studies are needed on the determination of microplastics in soil (Bläsing and Amelung, 2018).

In this study, it was aimed to extract and determine in a simple and fast way the presence of microplastics in the fractions of different aggregate sizes of the mulch applied to soil with NaCl-H_2O_2 and H_2O_2-NaCl treatments and to define them under the microscope in order to contribute to the limited number of microplastic studies in soils and to prepare for more detailed studies.

MATERIALS AND METHODS

Soil Sampling

Soil samples were taken from the surface (0-10 cm depth) of a field where mulching material used intensively. A partial sample from each square meter from a matrix of 10mx10m was sampled at the site to prepare representative composite samples. Soil samples were air-dried and sieved at 2 mm. For wet-sieving, 20 g soil samples were taken and sieved into 6 soil aggregate fractions (1000-2000 µm, 1000-500 µm, 500-250 µm, 250-125 µm, 125-0.053 µm, and <0.053 µm) for the treatments.

Separation of Microplastics

Treatment 1: The method of density separation with saturated NaCl (1.202 g cm^{-3}) was used with the somewhat modifications to extract microplastics from the soil sample a quite simple way was followed (Nuelle et al., 2014; Zhang et al., 2018). In this simplified method instead of NaI, NaCl solution was applied and ultra sonification was not used. Fractionated soil samples were transferred directly to the Erlenmeyer flask for density separation, then saturated 100 ml NaCl solution was added to samples. Soil samples were stirred at high intensity for 30 min then allowed to settle for 24 hours. After soil samples settled, supernatants including plastic were collected by moving a 50 ml pipette across the solution surface in a conical flask. The steps above were repeated 3 times to fully extract microplastic from the soil. The supernatants were passed through Whatman filter paper (No: 42). After the density separation, in order to remove organic matter and dispersion of aggregates (Bläsing and Amelung, 2018), 50 ml of 30% H_2O_2 was slowly added under constant stirring. The degradation of the organic matter process continued for 24 h at 50 °C under a water bath in order to fully digest organic matter in the extracts until the foaming stopped. This procedure of digestion was applied to all 6 size fractions. After that, digested solutions were passed through again Whatman filter paper (No: 42). Then filter papers were transferred into the glass petri dishes and dried at room temperature.

Treatment 2: In this treatment, the order of solutions for the procedure mentioned above was changed and soil samples were treated with 10-50 ml 30% H_2O_2 solution first. Microplastics that enter the soil can adhere to the organic matter of the soil. For this reason, H_2O_2 oxidizing treatments were also used for plastic analyses in sediments (Imhof et al., 2012). The aim here is to remove the organic matter, disperse the particles and separate the plastics from the soil particles before density separation. Then, microplastic density separation was made by adding saturated NaCl. After that same procedure steps were applied as mentioned in treatment 1. This procedure was applied to all 6 size fractions. Then filter papers were transferred into the glass petri dishes and dried at room temperature.

Apart from the above-mentioned treatments, the soil sample was examined under the microscope to see if the sample was seen after drying the soil directly after treatment with H_2O_2 and the following images were recorded (Figure 1). Microplastic images from this study were similar in line with the literature (Choi et al., 2021; Liu et al., 2022).

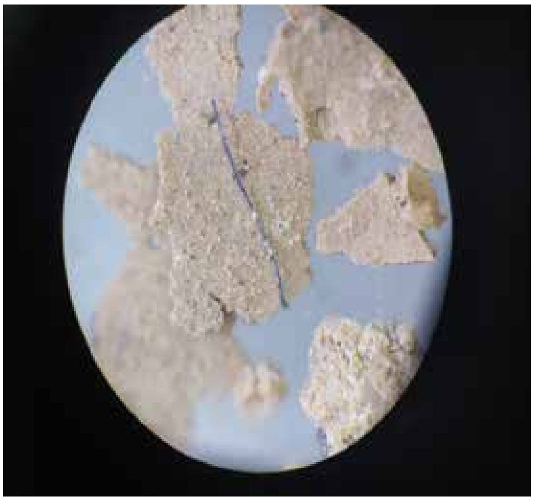

Figure 1. Microplastic appearance of soil with mineral part after H_2O_2 application

Microplastics Observation

Microplastics on filter papers were examined under a binocular stereomicroscope (Leica S8AP0, simple light) (1.0-8.0 X Zoom). Suspected microplastics were preliminarily identified and recorded based on their shape, size, color, and identified according to Zhang et al. (2018). Their images were captured with a camera.

Microplastic Collection

Tweezers and needles were used to collect microplastics under a microscope view. A needle threader, which is touched with glue, was found to be more functional than tweezers in collecting microplastics from filter paper and treated soil. Because it is easier to use to pick up small microplastics with its thinner tip than tweezers. The point to be considered here is that the needle threader or tweezers, which glue is applied to the tip during the removal of the microplastic seen, sticks to the soil particle very rarely. To eliminate this, the collected microplastics were placed in Eppendorf tubes containing pure water. The microplastics were collected by immersing them in distilled water in Eppendorf tubes.

RESULTS AND DISCUSSION

As a result of examination with a binocular stereomicroscope in soil fractions of different aggregate sizes, microplastics were found in all 6 fractions of both treatments. (Table 1 and Table 2). Microplastics were evaluated qualitatively by examining their properties such as color, shape, number and length (short or long). In the first treatment, a total of 29 particles $20g^{-1}$ soil microplastic was counted.

Table 1. Qualitative evaluation of microplastic microscope images in samples obtained by first treatment

Soil aggregate fractions (µm)	Microplastic properties		
	Shape	Number	Color
1000-2000	fibrous	6	red, blue, black, white
1000-500	fibrous	8	black, blue, white
500-250	fibrous	3	white, black
250-125	fibrous	3	white, blue
125-0.053	fibrous	3	white, black
<0.053	spherical, fibrous	4	red, white,

Table 2. Qualitative evaluation of microplastic microscope images in samples obtained by second treatment

Soil aggregate fractions (µm)	Microplastic properties		
	Shape	Number	Color
1000-2000	fibrous	2	white, black
1000-500	fibrous	3	white, black
500-250	fibrous	3	white, black
250-125	fibrous	3	white
125-0.053	fibrous	1	black
<0.053	spherical, fibrous	4	white, yellow, blue, fuchsia

Short or long filamentous images in different colors (such as red, blue, black, white) were recorded for each of the fractions, (Figure 2). In addition to filament, microplastic in spherical form was also observed in the <0.053 µm fraction. More microplastics were determined in the 1000-500 µm fraction than in the other fractions (8 fibrous, black, blue and white). In the smallest fraction (<0.053 µm), 4 very short red and white fibrous microplastics were detected. The dominant microplastic structure was fiber, which is the most common form as noted by Zhang et al. (2018).

Figure 2. Microplastic image in the first treatment in the fraction of 1000-2000 µm

In the second treatment, a total of 16 particles 20 g^{-1} soil was found in all fractions. Microplastics in white, black, blue, fuchsia and yellow colors were detected. In addition to the filamentous form, the spherical microplastic form (microbeads) was also encountered (Figure 3). Microbeads are defined as spherically manufactured plastics, no greater than 1 mm in size (Wardrop et al., 2016). Microbeads have become an increasing concern in recent years, due to their persistence in the environment. Most likely, this form is thought to be a secondary microplastic released as a result of the decomposition of a larger macroplastic.

Figure 3. Microplastic image of the second treatment in the fraction of 0.053 µm

Results show that both treatments are successful in extracting microplastic from soil. Microplastics were detected in all the differences and determined as 45 particles /40 g^{-1} dry soil.

China is the country with the highest number of studies based on the pollution of agricultural microplastics. Huang et al. (2020) determined 0.1-324.5 kg ha^{-1}, an average of 83.6 kg ha^{-1} mulch plastic particles in 384 soil samples collected from 19 agricultural areas in China.

In the studies carried out, microplastics may be found due to the use of mulch in the soil, as well as microplastic structures that may come from plastic materials used during harvest such as sacks and ropes. The red filamentous structure found in this study is thought to be a piece of string or red colored sack. In the second treatment, less material was displayed compared to the first treatment. No doubt, it is not expected that the homogenized distribution of microplastics in the field during the soil sampling. Therefore, reproducibility of the method was not considered here. Lv et al. (2021) also stated that microplastics can be easily determined by visual identification, however, new methods are needed for nanoplastic determination. The presence of nanoplastics in soils can be checked with more advanced microscopic methods such as scanning transmission X-ray microscopy (STXM).

Considering the amount used for analysis here, it becomes clear that the sample should be taken much more than the amount required for ordinary soil analysis. The amount of microplastic obtained by extraction should be sufficient for further descriptive analysis. When sufficient samples cannot be extracted, it is not possible to determine the type of microplastics in FTIR or ATR.

This study is a preliminary study, the amount of microplastic obtained from the screened soil was not sufficient to run FTIR or Raman to determine the microplastic type. Therefore, it is necessary to enrich the sample amount by working with more soil samples.

Schwinghammer et al. (2020) used wet-sieving, 500 g and 1 kg material for microplastic determination in dewatered digested sludge, co-substrates, and compost samples. In fact, it can be predicted that the presence of microplastics in the research soil may be higher from the presence of so many microplastics in a soil sample as low as 20 g. During this study, a soil sample taken from a corn field was also scanned with a stereo microscope for simple comparison purposes, but no microplastic was found. Nuelle et al. (2014) suggested the use of more aggressive methods if a specific type of plastic is sought, and less aggressive methods if the screen is to be made.

CONCLUSION

This preliminary study shows that in soils where microplastic pollution will be investigated, first of all, it is necessary to investigate what the microplastic input might be. It is suggested that the use of the land should be included in the studies of microplastics. If it is not known, a qualitative preliminary screening should be performed as stated above and then the microplastic type should be determined with more detailed instruments. Scanning and collecting microplastics in the soil with a microscope is time-consuming, but it is thought to be a good preliminary step that guides the researcher. If the land use is known, it should be visualized first, choosing the methods that can be applied for the separation of different microplastic types (low/high density, PE, PVC, etc.) for later definitions according to the type, density, size and shape of the microplastics will provide simplicity and speed in the studies.

Conflict of Interest

The authors declared that there is no conflict of interest.

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