

Separation and Measurement of Microplastics in Paddy Soil

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Abstract

Accumulation of microplastics (MPs) in soil is a worldwide concern, but a standardized analysis method for soil MPs has not been established yet. In this study, we compared deionized water, zinc chloride and sodium iodide solution with specific gravities of 1.0, 1.4 and 1.5, respectively, for the separation of MPs in paddy soil that had a history of coated fertilizer application. Organic matter in the paddy soils was digested with Fenton's reagent (30% peroxide and 0.05 M iron sulfate) before density separation. Separation efficiency of MPs in soil was significantly high for sodium iodide, followed by zinc chloride, then water. The abundance of MPs in the soil ranged 100 - 5800 pieces/kg. The particle sizes of MPs in the soil were found to be extremely small, ranging from 1 - 111 μm . Since paddy fields are waterlogged during plantation and are drained about two weeks before harvest, large MPs might be flushed out by water. It can be concluded that MPs in paddy soil may have large range of plastic materials with different gravity and small particle size.

Keywords

Microplastics, Gravity Separation, Soil, Paddy Field

1. Introduction

Plastics have been mass-produced since the 1950s, and their production is estimated to reach around 12,000 Mt by 2050 [1]. Most of the plastics produced continue to exist in the environment because of their persistent nature. In addition, some of these plastics flow into the ocean through rivers and other sources, causing serious impacts on marine life [2]. On land, plastics derived from agricultural vinyl greenhouses and tunnels, mulch films, and coated fertilizers have mixed with the soil and have accumulated, raising concerns regarding their effect on the properties and qualities of soil and agricultural products [3]. Microplastics (MPs) with a

diameter of 5 mm or less are difficult to be removed from soil, and they may affect the physicochemical properties of soil and may cause adverse effects on soil microbes and other organisms and even affect plant growth [1] [4]. In addition, it has been pointed out that MPs contained in soil and water may enter the human body through the food chain and accumulate in organs, causing various diseases [5].

The extraction of MPs from soil samples is an essential step for monitoring MPs pollution in soil environments, and density separation is most often used. However, at present, no standardized density separation methods are available for the analysis of MPs in the soil [6]. Previous studies have used a variety of solutions for the extraction of MPs based on the type of plastic polymers studied [6]. Several experimental studies have examined the recovery rate of spiked MPs in soil samples using different solutions [7]. However, the MPs in paddy field soil might be more diverse in type, shapes and size because MPs can accumulate by several routes, such as from agricultural plastic materials, water flow, and wind flow. Soil is a heterogeneous medium, and aged MPs can be more strongly bound to soil aggregates. Thus, the performance of these methods on field soil samples needs to be examined; however, few studies have been reported [8].

In this study, we conducted comparative experiments using deionized water, zinc chloride, and sodium iodide for extracting MPs from paddy soil, with the aim to evaluate the separation efficiency of MPs in soil using different separation media.

2. Materials and Methods

Soil samples of the paddy field from Kumamoto Prefecture, Japan, were used in this study. Application of coated fertilizer has been in practice in the field since 2016. Two plots F169 (producing edible rice) and F170 (yielding feed rice) were selected from the field. Area of F169 and F170 were 2996 m² and 1735 m², respectively. Two topsoil (0 - 15 cm) from each plot were collected as composite sample in January 2020, while the rice cultivation season is from Jun to November in these fields. After air-dried, the samples were passed through a 2 mm sieve and were subsequently used for the analysis of general properties. A 1:5 soil: water solution was prepared and shaken for 1 hour, and the pH was determined by glass-electrode analysis (LAQUA F-71; HORIBA, Japan). Exchangeable cations (Ca²⁺, Mg²⁺, K⁺ and Na⁺) were extracted three times from each solution with 1 M ammonium acetate at pH 7.0. The ammonium ion absorbed in each residue was replaced by 10% sodium chloride and the cation exchange capacity (CEC) was determined using Kjeldahl distillation and titration methods. Total carbon and nitrogen contents were determined by dry combustion with a CN CORDER (JM 1000; J-Science Lab). The particle size distribution in the soil samples was determined using the pipette method (<2 µm for clay, 2 - 20 µm for silt, 20 - 2000 µm for sand fractions) [9].

The analysis of MPs in soil was performed with slight modification of the method used by Vermeiren *et al.* [10]. First, about 30% hydrogen peroxide and 0.05 M ferrous sulfate were added to 50 g of the air-dried soils, and the organic matter was decomposed on a hot plate at 50°C for several days until no bubbles

were observed. After filtering the supernatant, the residue was transferred to a gravity separator. Three kinds of separation media, namely deionized water, zinc chloride with the gravity of 1.4 and sodium iodide with the gravity of 1.5 were used for separating MPs from the soils. Around 1L of deionized water was added into the separator, the mixture was stirred and left to stand overnight (**Figure 1**). Subsequently, 200, 150, and 100 ml of the supernatant from the gravity separator were overflowed into beakers at 5-minute intervals, then filtered through pre-combusted Whatman GF/C glass fiber filters (pore size 1.7 μm) to retain the MPs on the filter. Same procedure was followed for zinc chloride and sodium iodide separation. The filter papers were then dried at 60°C for 24 hours. Nile red staining was applied to the MPs retained on the filter, by dropping 1 $\mu\text{g}/\text{ml}$ Nile red solution in methanol and the filters were then oven-dried at 60°C for 1 hour and then photographed under green light using a stereomicroscope equipped with an orange filter. The images were subsequently analyzed with ImageJ to measure the abundance of MPs and their size [11].

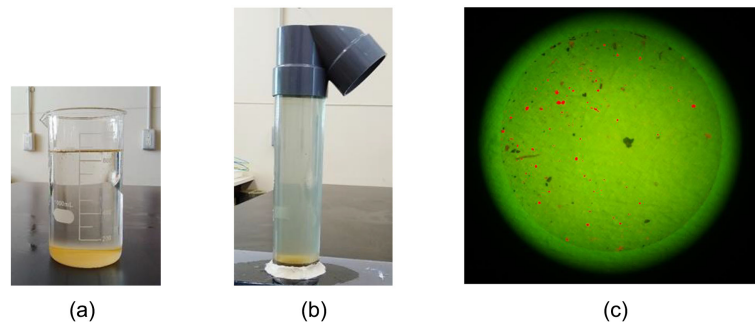


Figure 1. Soil microplastics (MPs) analysis. (a) Soil after decomposition; (b) Specific gravity separator; (c) The filter that collected the MPs, red spots indicate MPs stained by Nile red.

Statistical analysis: Tukey's multiple comparison tests were used to identify significant differences ($p \leq 0.05$) in the abundance of MPs in soil between the three-separation media used.

3. Results and Discussion

Table 1. Characteristics of the paddy soils.

Soil parameters	169 SW	169 SE	170 N	170 SE
pH	5.10	4.77	5.16	5.26
CEC (cmol./kg)	19.7	21.0	18.8	18.8
Total carbon (g/kg)	21.7	19.8	15.1	15.8
Total nitrogen (g/kg)	1.83	1.71	1.35	1.44
Sand (%)	40.4	39.6	36.8	39.2
Silt (%)	30.2	27.1	29.1	31.6
Clay (%)	29.4	33.2	31.7	31.5

The pH was found to be acidic at around 5 in all soil samples (**Table 1**), because no neutralization with lime has been carried out in these fields. CEC, total carbon and nitrogen contents tended to be higher in F169 than in F170. But there were no significant differences in particle size distribution of the soils.

MPs were more abundant in samples separated using sodium iodide (1700 to 5800 pieces/kg) compared to zinc chloride and deionized water (40 to 1940 pieces/kg and 100 to 380 pieces/kg, respectively) (**Figure 2**). Content of MPs in sodium iodide was significantly higher than that of deionized water ($p < 0.01$) and zinc chloride ($p < 0.05$), although there was no significant difference between the contents in zinc chloride and deionized water. This is consistent with the results of Li *et al.* [8]. They compared three different salts (NaCl, ZnCl₂ and NaI) for removing MPs from upland soil and sewage sludge, and their results indicated that floatation solutions with high densities were effective in removing higher proportions of MPs.

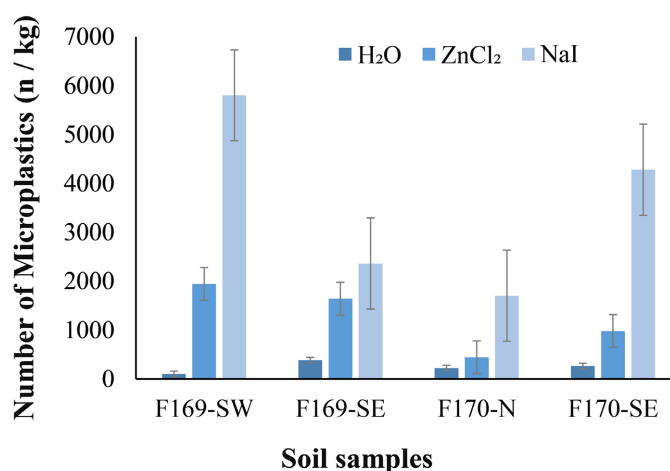


Figure 2. Microplastics abundance in the paddy soil (Deionized water, zinc chloride and sodium iodide were used as separation media. $n = 4$).

The specific gravity of plastics varies greatly depending on the material, most of them ranging from 0.84 to 2.2 [12]. The resin components of the coated fertilizer used in the paddy field of this study are polypropylene glycol (PPG) and polyester (PES), whose specific gravities are 1.128 and 1.38, respectively. It is presumed that plastics can also be mixed in by water and wind flow in the paddy fields, so there is a possibility that plastics other than PPG and PES were also present. Deionized water can extract low density MPs such as polyethylene (PE) and polypropylene (PP), which have a specific gravity of less than 1. The zinc chloride solution used in this study had a specific gravity of about 1.4, so it could extract a wide variety of MPs with a specific gravity less than 1.4, for example polystyrene (PS) and polyethylene terephthalate (PET). The sodium iodide solution used in this study had a specific gravity of about 1.5, so in addition to the MPs extracted by zinc chloride solution, it could also extract MPs with a specific gravity of 1.4 to 1.5.

The particle size of MPs in this study was extremely small, ranging from 1 - 111 μm . Extracted MPs with deionized water, zinc chloride and sodium iodide ranged in size from 1 - 46 μm , 1 - 111 μm , and 1 - 52 μm , respectively, with no distinct difference between three media (**Table 2**). This is contrast to the particle sizes of MPs in upland soil and sewage sludge ranged from 20 - 5000 μm as shown in Li *et al.* [8]. Because paddy fields are waterlogged during plantation and are drained before harvest, bigger-size MPs might be flushed out by the water. Katsumi *et al.* [13] measured microcapsules of 0.9 - 2.0 mm size derived from coated fertilizer in paddy soil by wet sieving method and found that the contents ranging from 2 - 123 pieces/kg. Our results suggest that MPs may exist in smaller size in paddy soil. It can be concluded that MPs in soil may have large range of plastic materials with different gravity and particle size. MPs below 2 μm may behave similarly to clay minerals in soils, therefore, analysis of small size MPs is important for evaluating the impact of MPs on the material dynamics in soil.

Table 2. Size distribution of microplastics in the paddy soils (μm) (Mean values with number of MPs and ranges of size).

Samples	NaI	ZnCl ₂	H ₂ O
169 SE	10 (n = 118) (1 - 52)	22 (n = 73) (1 - 111)	2 (n = 19) (1 - 6)
169 SW	10 (n = 290) (1 - 38)	154 (n = 97) (1 - 34)	9 (n = 5) (1 - 22)
170 SE	9 (n = 241) (1 - 34)	14 (n = 49) (1 - 45)	18 (n = 13) (1 - 46)
170 N	12 (n = 85) (1 - 37)	12 (n = 22) (1 - 23)	23 (n = 11) (1 - 9)

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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