Science of the Total Environment 671 (2019) 411–420

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal

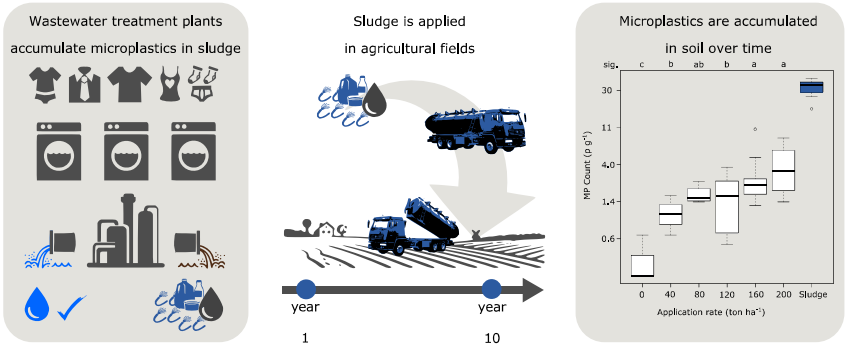
Fabio Corradini [a,b,*], Pablo Meza [a], Raúl Eguiluz [a], Francisco Casado [a], Esperanza Huerta-Lwanga [b,c], Violette Geissen [b]

[a] Instituto de Investigaciones Agropecuarias, INIA La Platina, Casilla 439, Correo 3, Santiago, Chile
[b] Soil Physics and Land Management Group, Wageningen University & Research, Droevendaalsesteeg 3, 6708PB Wageningen, the Netherlands
[c] Agroecología, El Colegio de la Frontera Sur, Unidad Campeche, Av Polígono s/n, Cd. Industrial, Lerma, Campeche, Mexico

HIGHLIGHTS

- Sludge holds microplastics that could enter soils by sludge disposal.
- 31 fields that underwent sludge applications at different rates were evaluated.
- Microplastics were found in soil samples (0.6–10.4 p g^{-1}).
- Microplastic counts increased where increased rates of sludge were applied.
- Sludge is proposed as a primal driver of soil microplastic pollution.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 January 2019
Received in revised form 18 March 2019
Accepted 23 March 2019
Available online 24 March 2019

Editor: Jay Gan

Keywords:
Microplastics
Agricultural soil
Sludge
Waste management
Diffuse pollution

ABSTRACT

Microplastics are emerging as a steadily increasing environmental threat. Wastewater treatment plants efficiently remove microplastics from sewage, trapping the particles in the sludge and preventing their entrance into aquatic environments. Treatment plants are essentially taking the microplastics out of the waste water and concentrating them in the sludge, however. It has become common practice to use this sludge on agricultural soils as a fertilizer. The aim of the current research was to evaluate the microplastic contamination of soils by this practice, assessing the implications of successive sludge applications by looking at the total count of microplastic particles in soil samples. Thirty-one agricultural fields with different sludge application records and similar edaphoclimatic conditions were evaluated. Field records of sludge application covered a ten year period. For all fields, historical disposal events used the same amount of sludge (40 ton ha^{-1} dry weight). Extraction of microplastics was done by flotation and particles were then counted and classified with the help of a microscope. Seven sludge samples were collected in the fields that underwent sludge applications during the study period. Soils where 1, 2, 3, 4, and 5 applications of sludge had been performed had a median of 1.1, 1.6, 1.7, 2.3, and 3.5 particles g^{-1} dry soil, respectively. There were statistical differences in the microplastic contents related to the number of applications that a field had undergone (1, 2, 3 < 4, 5). Microplastic content in sludge ranged from 18 to 41 particles g^{-1}, with a median of 34 particles g^{-1}. The majority of the observed microplastics were fibers (90% in sludge, and 97% in soil). Our results indicate that microplastic counts increase over time where

* Corresponding author at: Instituto de Investigaciones Agropecuarias, INIA La Platina, Casilla 439, Correo 3, Santiago, Chile.
E-mail address: fabio.corradini@inia.cl (F. Corradini).

https://doi.org/10.1016/j.scitotenv.2019.03.368

successive sludge applications are performed. Microplastics observed in soil samples stress the relevance of sludge as a driver of soil microplastic contamination.

1. Introduction

Human activities are directly responsible for aquatic and terrestrial microplastic contamination. In recent years, more research has been performed to assess the different sources of microplastics and their relative impact on the environment (Auta et al., 2017; Bläsing and Amelung, 2018; He et al., 2018; Ng et al., 2018; Pinto da Costa et al., 2018). Historically, researchers have mainly focused on examining the effects of plastic contamination arising from general littering, plastic waste dumping, and inappropriate management of landfill sites (Duis and Coors, 2016). However, over the last few years, this focus has grown to include environmental concerns arising from techniques used in the agricultural sector. The common agricultural practices of disposing of plastic mulching, water pipes, and plastic greenhouse covers have begun to raise concerns (Steinmetz et al., 2016; Brodhagen et al., 2017; Zhang and Liu, 2018). Although there is evidence supporting the fact that wastewater sludge used as a soil amendment could also be contributing to soil contamination (Zubris and Richards, 2005), a field evaluation further examining the effects of this practice has not yet been carried out.

Wastewater is a main source of microplastic contamination in freshwater environments. Wastewater is capable of transporting plastics from many different sources. Horton et al. (2017) observed that storm drains in the UK, for example, carry considerable amounts of synthetic fibers. In fact, synthetic fibers are a major source of microplastics in sewage (Ziajahromi et al., 2017; Henry et al., 2019). Fibers from textile materials originating from domestic washing machines have the potential of reaching aquatic environments even after sewage treatment (Napper and Thompson, 2016; Hernandez et al., 2017). Despite their relevance, they are not the only source of microplastics in sewage. Personal care products are also believed to contribute to microplastic pollution. Some brands of toothpaste, soaps and facial scrubs contain microplastics which could potentially reach aquatic environments through wastewater treatment plants (Napper et al., 2015). However, the contribution of these personal care has caused some scientific controversy (Duis and Coors, 2016).

The presence of microplastics in wastewater has been studied by several scientists who have come to the common agreement that overall, waste water treatment plants (WWTP) are efficiently removing microplastics from wastewater (Sun et al., 2019). This conclusion is great for aquatic environments where the wastewater eventually ends up. WWTPs effectively remove nearly 99% of microplastics from wastewater. This begs the question: Where do the microplastics go? Unfortunately, although the removal process benefits aquatic environments, the soil environment is less fortunate. Microplastics accumulate in the sludge produced in WWTPs (Li et al., 2018). This sludge is in turn used as fertilizer on agricultural fields. This practice has a positive impact on soil fertility and is still essential in many countries all over the world (Schmidt et al., 2006; Coors et al., 2016). However, this use of sludge creates a pathway for microplastics to enter agricultural soils (Zubris and Richards, 2005).

Before making conjectures, several information gaps need to be addressed. Evidence supports the finding that synthetic fibers accumulate in soils treated with sludge (Zubris and Richards, 2005). We also know that plastic debris is found in sewage sludge (Mahon et al., 2017; Li et al., 2018). To our knowledge, there are no studies that evaluate the effect of successive sludge applications on agricultural fields. Therefore, with this research, we wanted to answer the question: Are microplastics accumulating in agricultural soils as a result of sewage sludge applications? There were two aims of this study: (1) to evaluate microplastic contamination in soils from sewage sludge applications and (2) to evaluate the impact of repeated applications of sewage sludge by examining soil samples. We did this by selecting and evaluating 31 fields in the Chilean central valley with different sludge application records which covered a period of ten years of sludge application.

2. Material and methods

2.1. Study site

Chile was chosen for this case study since the country has a ten year record of sludge applications (MINSEGPRES, 2009). Sludge disposal on agricultural fields has been permitted by local authorities in Chile since 2009. Sludge producers have to report each sludge application to the Chilean Agricultural and Livestock Service, which keeps a record of each application. We looked at all of the places were sludge has been applied over the last ten years. From this data, a hot spot area was selected which we hoped would prevent soil covariates from increasing experimental noise. The area was located in Mellipilla county, in the Metropolitana region of Chile. Within this county, a 10 km^2 area near the Maipo river was selected since it included 30 fields that were successively treated with sludge over the past 10 years. In the same area, we selected one control site where no sludge had been applied.

While all fields shared similar soil chemical and physical characteristics, they were exposed to different sludge application rates over time. All of the fields that were selected had a soil classified within the USDA Entic Haploxerolls subgroup (CIREN, 1996), a medium (loam) to moderately coarse (sandy loam) soil texture, a flat surface (0–1%), and an average depth of 75 to 100 cm. The soil organic matter (SOM) ranged from 1.3% to 4.3% (median = 2.1%). The fields, however, comprised different soil map units since they presented different degrees of stoniness. Regarding sludge applications, there were fields that were treated with sludge up to five times, while others received only one application. The number of applications, the year when the last application was performed, and the crops produced after the application are shown for each field in Fig. 1. In nearly all fields, sludge was applied for the last time in 2017 and corn -either as a monoculture or in rotation- was the main crop after this last application. Hence, the only factor that significantly varied between fields was the number of sludge applications. The sludge applied was homogeneous. It originated from the same wastewater treatment plant and was stabilized before each application by solar desiccation or centrifugation.

Current Chilean regulation allows no more than one application of 90 ton ha^{-1} of dewatered sludge per year per field (dry weight basis). This rate is almost never reached in practice since applying 90 ton ha^{-1} of dewatered sludge entails several technical problems. Therefore, on average, 40 ton ha^{-1} of dewatered sludge is applied to fields during each application (dry weight basis).

2.2. Sampling and sample preparation

Three soil samples, chosen randomly within each field boundary, were collected for each field. Each sample was taken from the topsoil (0–25 cm) using a metallic soil auger. The sampling depth corresponded with the reported depth of sludge application. Samples were transported in polypropylene (PP) plastic bags (3 mm thick) to the laboratory, where they were unpacked, spread over wood trays, and dried in an forced air oven at 40 ± 2 °C. The samples were then sieved with a < 2 mm metal mesh and stored in polyethylene terephthalate (PET) jars.

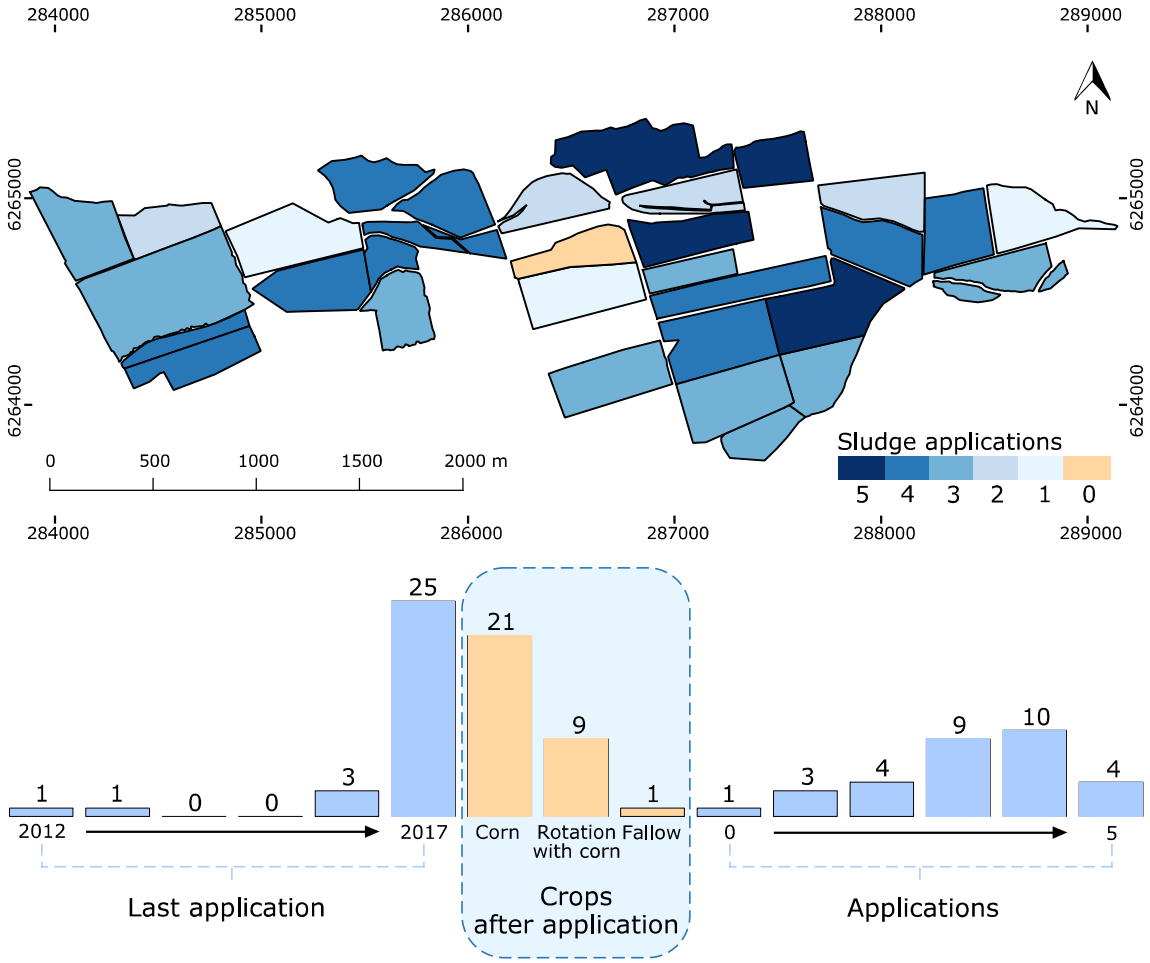


Fig. 1. Location of fields under study. The differences in the number of sludge applications carried out over time have been highlighted for each field (above). Quantity of fields (n) that: were applied until a given year (bottom-left); presented corn, a rotation with corn, or laid fallow after the last application (bottom-center), and; underwent a given number of sludge applications (bottom-right) (each application = 40 ton ha^{-1}).

When present, microplastic particles larger than 2 mm were counted by hand and noted.

A control sample without plastic was made to check whether the plastic containers polluted the samples with plastic, compromising the quality of the analysis. An agricultural soil was collected, dried, sieved, and ignited at 500 °C for 3 h. The temperature reached ensured the elimination of all plastic particles (Anuar Sharuddin et al., 2017). After ignition, three replicates (500 g) of the ignited soil were placed in PP plastic bags, and shaken at 180 strokes min^{-1} for 10 min in a platform shaker to simulate transport. Samples were then unpacked and stored in PET jars. Since it was not possible to guarantee a site with no plastic contamination, no blanks were collected in the field or used during the handling procedure.

Seven sludge samples were collected on location, as some of the fields underwent sludge applications during 2018 (not considered in the study, as soil sampling was done before). The sludge samples were transported in plastic bags and then dried in an forced air oven at 40 ± 2 °C. Since the dried sludge formed hard clods, the samples were milled with a porcelain mortar and sieved using a < 1 mm sieve, before being stored in PET containers. When present, microplastic particles larger than 2 mm were counted by hand.

2.3. Laboratory analysis

There is no standardized procedure to quantify microplastics in soil samples. Therefore, we implemented a methodology based on two recent studies (Hurley et al., 2018; Zhang et al., 2018). The method takes advantage of the fact that plastics have a lower density than soil particles. We used a wet extraction technique to float the plastic particles. A general overview of the methodology is shown in Fig. 2.

The dried soil samples were weighed and 5 ± 0.01 g was placed in 50 ml glass centrifuge tubes. 20 ml of deionized water was added to each tube and the samples were then stirred at ~21,000 rpm for 30 s. A Dremel® 3000 (Robert Bosch Tool Corporation, IL, USA) with a custom-made rod and mixer palette was made to stir the samples (rod ∅ = 3.2 mm, palette width = 5 mm). The high speed used allowed the soil to be completely suspended in our experimental set up. Samples were centrifuged at 2000 rpm (2240*g*) for 15 min and the supernatant was filtered using a Whatman No. 42 filter paper (retention >8 μm). 20 ml of sodium chloride (NaCl) 5 M (ρ = 1.20 g cm^{-3}) was added to the precipitate, which was then stirred and centrifuged a second time. The supernatant was once again filtered through the same Whatman No. 42 filter paper or through a new one if the first one became clogged. If the filter was replaced, the first filter was saved in a Petri dish for optical inspection. 20 ml of a concentrated zinc chloride solution ($ZnCl_2$ 5 M, ρ = 1.55 g cm^{-3}) was added to the centrifuge tubes with the precipitate for one final extraction. Since the $ZnCl_2$ solution had a higher viscosity than the previous solutions, the samples were stirred at 32,000 rpm for 30s. Centrifugation was carried out at 2000 rpm (2240*g*) for 15 min and the supernatant was filtered through Whatman No. 42 filter paper, while taking into account the replace-if-clogged indication. Filter papers were then saved in Petri dishes for optical inspection.

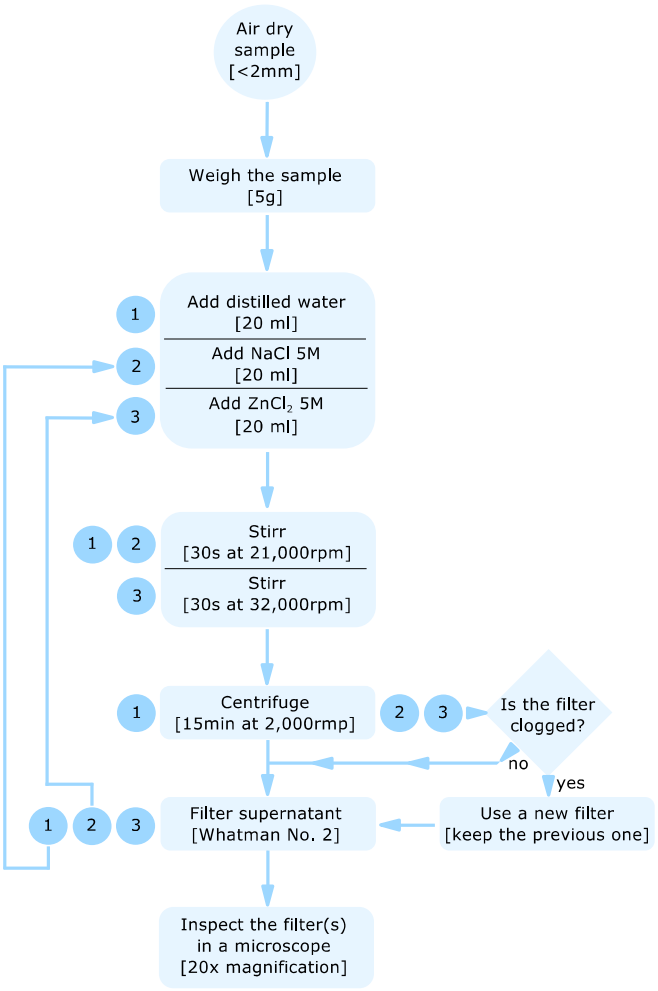


Fig. 2. Diagram of the method used. Samples were dried, sieved and weighted. Different solutions were added in sequential time steps to extract the plastics by flotation. First water was used ① then the sample was stirred, centrifuged, and filtered. Next, a solution of NaCl was used ② and the sample is again stirred, centrifuged, and filtered. The first filter was replaced only if it was clogged. Thirdly, a solution of $ZnCl_2$ was used ③ then the sample was stirred, centrifuged, and filtered for the last time. The previous filter was replaced only if it was clogged. The filters were then inspected in a microscope to identify plastic particles.

After microplastic extraction, the filters were inspected using a stereo microscope (model SMZ 745 T coupled with a NI-150 high intensity illuminator, Nikon, Tokyo, Japan) at 20×. The microplastic particles collected on each filter were counted twice. Microplastic particles were considered to have shiny surfaces, strong colors, and sharp geometrical shapes. Synthetic fibers were considered to have smooth sides and strong colors, as laid out by Horton et al. (2017). Particles were classified according to their shapes: fibers, fragments (angular and solid), films (flexible and thin), or pellets (rounded and solid). Together, fragments, films, and pellets are referred to as non-fiber particles. A random sample of the examined microplastics were photographed (Micrometrics® camera model 519CU CMOS 5.0 Megapixel, ACCU-SCOPE Inc., NY, USA) to measure the length and width if fibers and the surface area for non-fiber particles. ImageJ 1.5 software was used for this purpose (Schneider et al., 2012). Results were reported as number of microplastic particles per 5 g of dry soil (p 5 g^{-1}).

An estimation of the weight of the microplastics was performed using the measured area (Simon et al., 2018). The fiber weight was calculated using the width as the fiber diameter while considering a 40% of void fraction. The area of pellets was measured within a circle to estimate the volume of a perfect sphere. Fragment volumes were approximated using ellipsoids. A thickness of 13 μm was used to calculate film volumes. The weight was estimated using a density of 1.38 g cm^{-3} for fibers (polyester) and 1.35 g cm^{-3} for non-fibers (polyvinyl chloride).

Sludge samples were analyzed in a similar way but microplastic counts turned out to be too high in sludge samples so only 1 g of sludge was per tube was measured.

As a quality control measure, each set of samples (n = 20) included one reagent blank. The filter from this blank was saved in a Petri dish and inspected at the end of the analysis. The measurement should have accounted not only for the quality of the reagents used but also for any contamination inside the lab (Mahon et al., 2017; Scheurer and Bigalke, 2018). All materials used in the sample analysis were made of glass (funnels, Petri dishes, centrifuge tubes) and the stirrer rod was made of stainless steel. White cotton lab coats were used by the analysts during analysis and sample manipulation.

2.4. Method validation

In order to test our method, 10 soil samples from the region were selected for the validation experiements. Microplastics were then added to the soil samples which were then put through the same treatment as the rest of our soil samples. This allowed us to check the recovery rates of the added microplastics. The selected soils had between 1.0 and 4.0% SOM and were 12 to 44% clay, with textural classes ranging from clay loam to sandy loam.

Acrylic, polyester, and nylon fibers, as well as low density polyethylene and polyvinyl chloride particles were used to pollute the samples. Each of these polymers were sourced using a different method. A ball of acrylic wool was cut into pieces and the acrylic threads were cut with an electric hair cutter. Ready-made polyester fibers normally used as cushion stuffing were purchased and cut with scissors into shorter lengths. A pair of pantyhose (98% nylon - 2% elastane) was processed as an acrylic sample since the elastane compound was present only in the waist support, which was discarded before preparing the fabric for the samples. Low density polyethylene (LDPE) particles were obtained by freezing and milling LDPE pellets (SABIC® LDPE). Lastly, a polyvinyl chloride (PVC) water poncho was cut and rasped with a rectangular rasp. After size reduction, all polymer particles were sieved using a > 2 mm mesh. A sample of each polymer was photographed under the microscope and their dimensions were measured using ImageJ 1.5 (Schneider et al., 2012). The size of the polymers used in the recovery test are shown in Table 1.

3 fibers or particles of each polymer were included in the microplastic sample that was used in the validation of the method. 5 g of the soil samples were put into centrifuge tubes along with 3 fibers/particles of each polymer. 10 ml of distilled water was added to the prepared samples, which were then mixed with a glass stirring rod and allowed to dry. Samples were wetted and air dried twice more in order to emulate natural wetting and drying cycles, as suggested by Hurley et al. (2018). Three replicates per soil sample were analyzed. Recovery was expressed as a proportion of observed polymers after the extraction process. The recovery rate from the first two solutions (H_2O and NaCl 5 M) was registered separately from the third ($ZnCl_2$ 5 M) and two filter papers were used per extraction.

Along with this recovery assessment, the method repeatability was evaluated. Five random samples were analyzed a total of five times.

Table 1
Average particle size by polymer type used in the validation set and their standard deviation.

Polymer	Shape	Length (mm)	Width (mm)	Area (mm^2)
Acrylic	Fiber	2.7 ± 1.4	0.04 ± 0.01	0.12 ± 0.06
Polyester	Fiber	1.60 ± 1.1	0.04 ± 0.01	0.07 ± 0.06
Nylon	Fiber	2.30 ± 0.8	0.05 ± 0.01	0.98 ± 0.37
LDPE	Fragment	–	–	0.16 ± 0.10
PVC	Fragment	–	–	0.10 ± 0.08

2.5. Statistical analysis

2.5.1. Method validation

The data from the validation procedure was analyzed using a split-plot design where the whole plots corresponded to the SOM and the split-plots to the plastic polymer. Total recovery was analyzed. In order to look for significant differences, a logistic regression model was fitted to data and an ANOVA test was performed using a significance >95%. A logistic regression was used since the recovery rate corresponded directly to the added polymers recovered. The overall effect of SOM and the hypothesis which focused on finding differences between each plastic polymer were tested using the Wald chi-squared test (R Package **A**nalysis of **O**verdispersed **D**ata 'aod', Lesnoff and Lancelot, 2012).

The microplastic counts from the repeatability trial were analyzed after grouping the data by sample. The average, standard deviation, and coefficient of variation were calculated and the repeatability reported as the overall mean of the standard deviation and the coefficient of variation.

2.5.2. Soil microplastic contamination

The results from the 3 samples taken from each field were averaged. The difference in soil microplastic counts was evaluated as a function of the quantity of sludge applied. The microplastic count was the dependent variable, while the number of sludge applications was the independent variable. In this way, the number of sludge applications was set as the treatment with five levels (1, 2, 3, 4, and 5 sludge applications). An ANOVA test was performed to look for statistical differences. Counts were transformed using the natural logarithm to satisfy ANOVA assumptions. Significant differences were considered to occur when a significance >95% was observed. Fisher's last significant different test (LSD) was used to compare results between treatments (number of sludge applications).

Since there was only one control site (no replicates), zero sludge applications was not considered as a level of the independent variable. Instead, the Wilcoxon signed rank test was used to check whether or not the mean of the control site was different from 0. The microplastic counts of each treatment (number of sludge applications) were compared with the mean of the control site by a one-sample Student's *t*-test after the data were transformed using the natural logarithm.

The fiber to microplastic ratio was calculated as the number of fibers divided by the total microplastic count. The ratio was defined as the dependent variable with the sample matrix (sludge or soil) as the independent variable. An ANOVA model was fitted to the data to look for significant differences in the proportion of fibers with respect to the total count of plastics between sludge and soil samples. Differences were evaluated for a 95% confidence interval (CI).

Descriptive statistics were used to characterize sludge and soil microplastic content, fiber length and width and non-fiber-particle surface area.

All statistical analysis were performed in the R environment (R Core Team, 2018).

2.6. Method validation

Three sequential extractions were enough to get high recovery rates. All plastic polymers used presented statistically different recovery rates, which were influenced by SOM (Fig. 3). The recoveries observed were LDPE > Polyester > PVC > Nylon > Acrylic. Only acrylic fibers presented a poor recovery (mean = 49%) while other polymers showed a relatively high recovery rate (>77%). The recovery rate of LDPE particles stood out with 98% of the particles being recovered. In all cases, recovery was increased as a result of a third extraction carried out with $ZnCl_2$. This was especially true for PVC. Although soil organic matter did affect the recovery rate of each polymer, this effect was considered negligible for the study area.

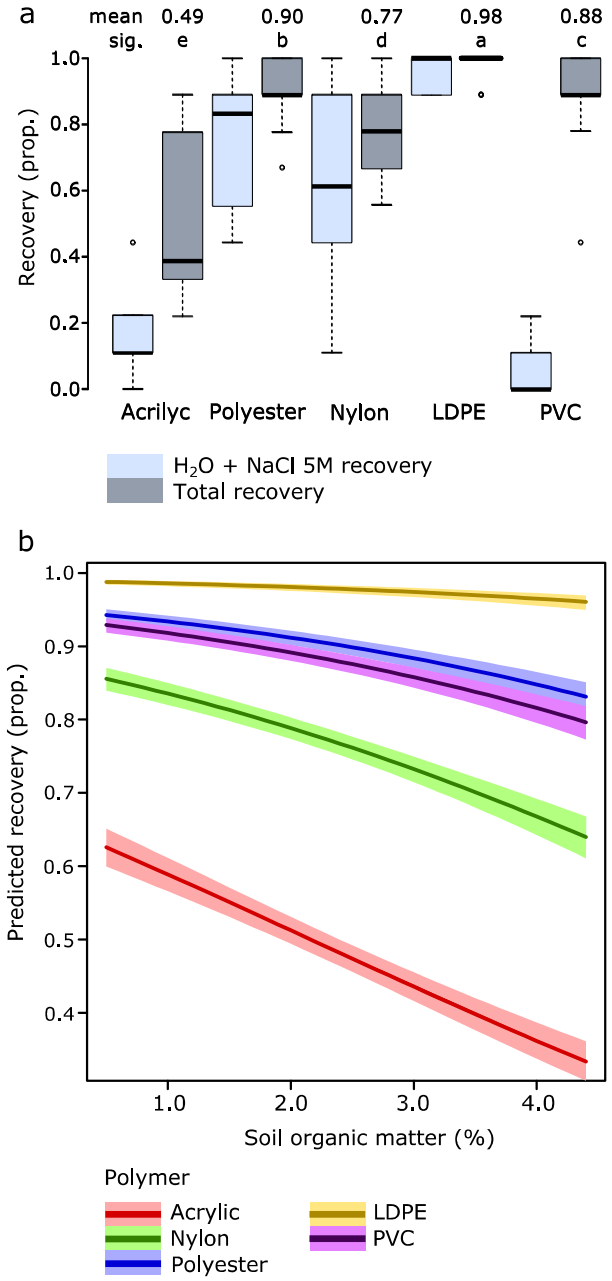


Fig. 3. Results of the validation test: (a) Recovery rates by polymer, splitting the recovery as total (grey) and by using only two steps (H_2O and NaCl 5 M) (blue); (b) Predicted recovery rates by content of soil organic matter (CI = 95%).

The repeatability was acceptable, with a standard deviation of 1.5 p 5 g^{-1} and a coefficient of variation of 12% in samples that ranged between 8 and 20 p 5 g^{-1}. With regard to packaging/transport contamination, only 1 fiber was found in one of the three ignited soil samples. It was a blue fiber, which did not match the colour of the PP plastic bags or the PET jars. Although the effects of the packaging was negligible, the use of plastic bags should be avoided whenever possible. Around 33% of reagent blanks were polluted with 1 fiber, while the remaining 66% had a null count. The analysis of a set of 20 samples -including a reagent blank- took a day (~7.5 h).

3. Results

3.1. Microplastics in soil samples

Overall, with each successive sludge application, there was an increase in the median (Fig. 4). The control site had the lowest microplastic count. Two of the three samples from the control site scored 1 p 5 g^{-1}, while the other had 3 p 5 g^{-1}. The mean of the control was different from zero (p-value = 0.08). The means of the treatments were different from the control mean (p-value < 0.01 for 5, 4, 3, and 2 applications, and p-value = 0.05 for 1 application). One, two, and three sludge applications had a similar effect with regards to microplastic accumulation in the topsoil. There were no differences between the means of 2, 4, and 5 sludge applications. The data dispersion was the highest for 3 sludge applications, where data ranged from 2.3 to 19 p 5 g^{-1}. Sludge presented a high microplastic content (median = 170 p 5 g^{-1}), which stood out from the soil observations. The weight estimates are presented in Table 2.

3.2. Microplastic characterization

The majority of microplastics observed in both soils and sludge samples were fibers. While the mean fiber to microplastic ratio for sludge was 0.90 ± 0.05, the proportion of fibers for soil samples was 0.97 ± 0.03. The ANOVA result indicates that the fiber to microplastic ratio was statistically different between soils and sludge samples. Most of the fibers observed were small, with only a few fibers having a width > 50 μm (Fig. 5a). The median fiber width was 20 μm, while the interquartile range (IQR) was 10 μm (p.25 = 17 μm and p.75 = 27 μm). The fiber length showed a similar distribution, having a median of 0.97 mm and a IQR of 1.05 mm (p.25 = 0.62 mm and p.75 = 1.67 mm) (Fig. 5b). Twenty percent of the fibers observed had a length > 2 mm, and only 5% had a length > 4 mm. The shortest fiber observed was 0.16 mm, and the narrowest was 8 μm.

Non-fiber particle shapes were predominantly films (58%) and a particle's surface area was generally <0.5 mm^2 (Fig. 6). The median for the particle surface area was 0.03 mm^2 and the IQR was 0.12 mm^2 (p.25 = 0.01 mm^2 and p.75 = 0.13 mm^2). The smallest particle observed was 0.0023 mm^2 (2254 μm^2). Example images of the observed microplastics are provided in Fig. 7.

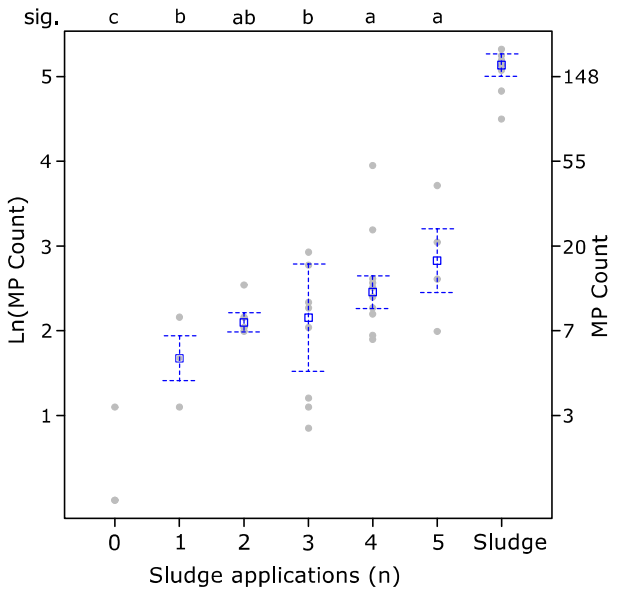


Fig. 4. Microplastic (MP) counts by number of sludge applications. Average of counts per field (grey dots), treatment medians (blue squares), and inter quantile range (blue dotted arrows). Differences at α < 0.05 are shown in the top axis with lower case letters.

Table 2
Microplastic weight in soil by number of sludge application events and in sludge (mg kg^{-1}).

Applications (n)	Min	1st Qu.	Median	3rd Qu.	Max
1	0.73	1.05	1.37	1.78	2.18
2	1.79	1.90	2.03	2.38	3.16
3	0.57	0.79	2.22	2.97	4.56
4	1.76	2.25	2.88	3.38	12.9
5	1.79	3.03	4.38	6.56	10.3
Sludge	22.0	37.3	45.5	50.2	53.0

4. Discussion

4.1. Accumulation of microplastics in soils

Our evidence suggests that microplastics accumulate in soils with successive sludge applications. While the presence of synthetic fibers in sewage sludge has been known since the end of the 90's (Habib et al., 1998), their accumulation in soil by sludge disposal was first acknowledge almost ten years later (Zubris and Richards, 2005). These authors reported a mean count of 1.21 ± 0.25 synthetic fibers per g^{-1} of soil five years after dewatered sludge was applied to soil columns for the last time. While the amount of sludge applied in the Zubris and Richards study was high (215 dry ton ha^{-1} to simulate 30 years of agronomic applications), the amount of sludge that is usually applied on Chilean fields are also exceptionally high (200 dry tons ha^{-1} can be reached within five years). In our study, fields where sludge had been deposited 5 times (200 dry tons ha^{-1}) had an median of 3.5 p g^{-1} soil one year after application, which is almost three times the amount reported by Zubris and Richards (2005). To complement the experiment of the soil column, Zubris & Richards evaluated a soil amended with 300 ton ha^{-1} of alkaline-stabilized sludge two years after application, reporting ~2.5 synthetic fibers g^{-1} soil (topsoil). As the alkaline-stabilization process abrades synthetic fibers, this result can be considered similar to ours.

Despite the evidence of synthetic fibers accumulating in soils after sludge application and the growing concern surrounding plastic contamination of soils, there are only a few recent studies evaluating plastic accumulation in agricultural soils (Pinto da Costa et al., 2018). Liu et al. (2018) reported that farmland soils near Shanghai in China had a maximum microplastic content of 0.28 p g^{-1} soil, with an average of 0.078 ± 0.013 p g^{-1} in the topsoil. Although these authors pointed to sludge as a possible source of the observed microplastics, they were unable to report on application rates. A different study in China evaluated the microplastic content in soils under intensive agriculture (Zhang and Liu, 2018). The authors reported that soils where approximately 23 tons ha^{-1} $year^{-1}$ of sludge were applied had between 7 and 43 p g^{-1} of microplastics at 0 to 10 cm depth. The reported range is by far greater than ours, but the authors had additional sources of microplastics polluting the soil such as plastic mulch and other plastic covers, which were not included in our study area. Nonetheless, the authors found that the majority of microplastics were fibers (92%), which match our findings.

Other studies that reported expected microplastic concentrations in soils did not evaluate agricultural soils with sludge applications. While they are not relevant for comparison, they emphasize the relevance of the sludge contribution to microplastic soil contamination. Home gardens in rural Mexico, where plastic household waste accumulates, averaged 0.9 ± 1.9 p g^{-1} soil (Huerta Lwanga et al., 2017). An agricultural field in China, where plastic mulch is used to reduce soil water evaporation, averaged 0.10 ± 0.14 p g^{-1} soil (Zhang et al., 2018). As previously mentioned, data concerning plastic soil contamination is scarce, but if the average reported by Zhang et al. (2018) is taken as a reference and

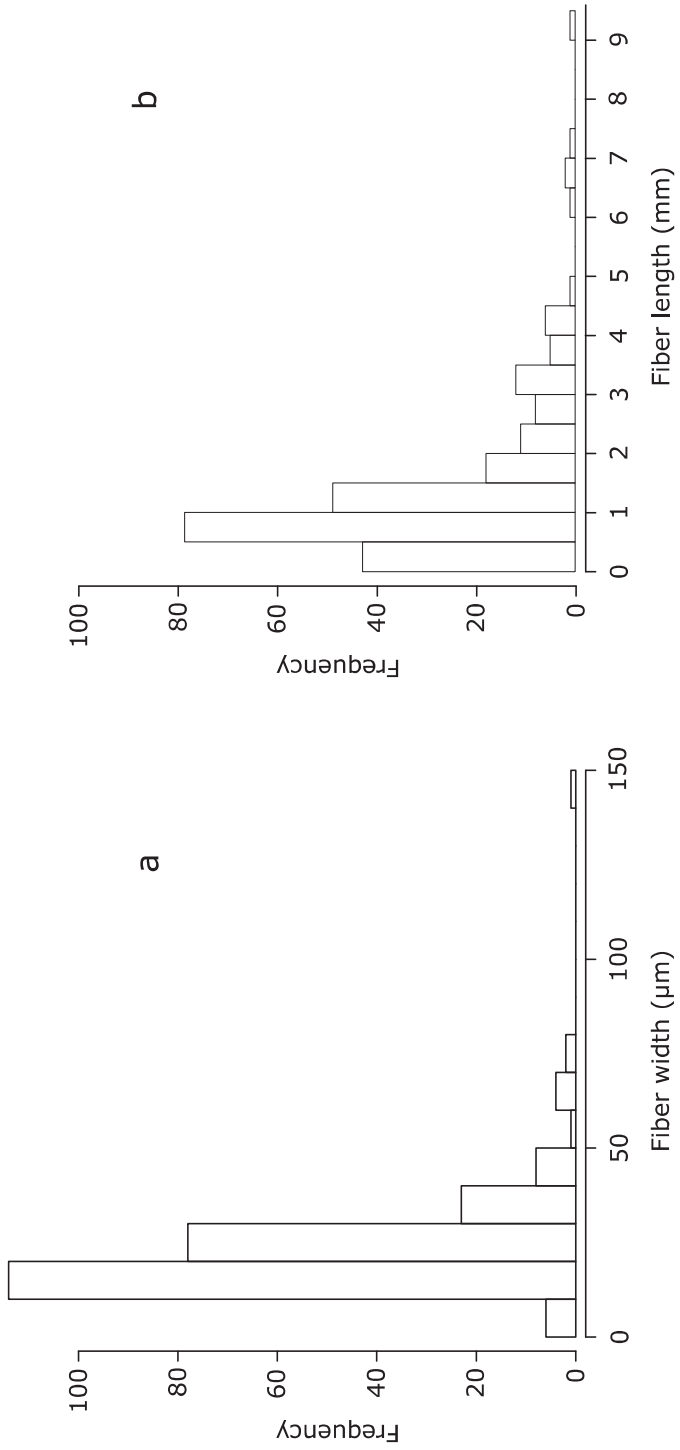


Fig. 5. Histograms for (a) fiber width, and (b) fiber length.

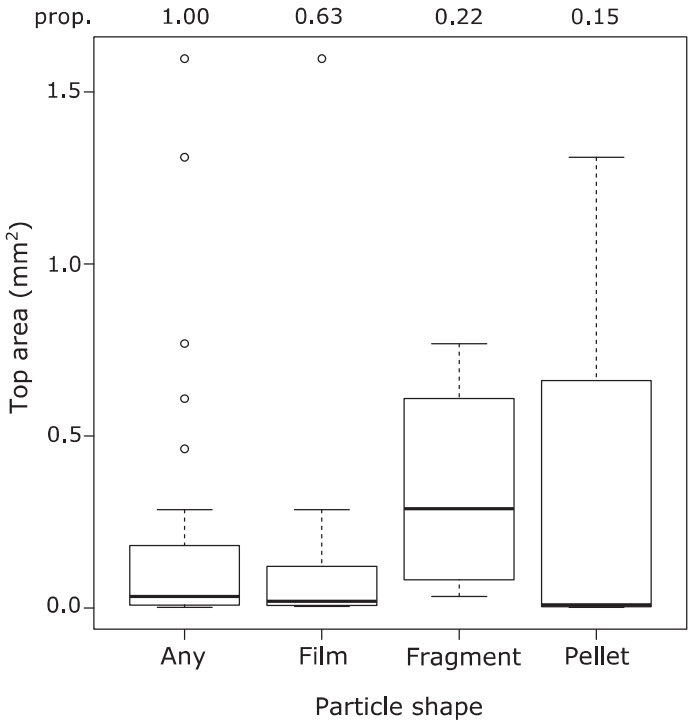


Fig. 6. Non-fiber particles surface area by shape.

compared to the data in Table 2, sludge can contribute 101 times more plastic particles in a year than plastic mulching.

Other studies reporting microplastic content in sewage sludge present slightly lower results. An extensive survey of microplastic contamination of sewage sludge in China reported an average of 22.7 ± 12.1 p g^{-1}, from which 63% were fibers (Liu et al., 2018). In Ireland, where sewage sludge undergoes different stabilization treatments, researchers found microplastic concentrations that ranged from 4.1 to 15.4 p g^{-1} (Mahon et al., 2017). When they evaluated a composted sludge, they found that 91% of the particles were fibers, which match our findings. At the study site, sludge underwent a simple process before disposal (centrifugation or solar desiccation), which could explain the higher microplastic counts observed.

An explanation for the fact that the control site had between 1 and 3 p g^{-1} soil can be difficult to confirm. On the one hand, it has been reported that microplastics can reach remote places by aeolian transport processes that are only partially understood (Prata, 2018). In Switzerland, a country with a successful plastic waste management strategy, researchers found evidence of aeolian and fluvial deposition of plastic in rural areas far from any polluting source (Scheurer and Bigalke, 2018). Generally speaking, the ubiquity of microplastics in agricultural environments makes it difficult to find a control site where absolutely no plastic is present. This was the case for studies performed in China (Zhang and Liu, 2018) and in the USA (Zubris and Richards, 2005). On the other hand, contamination could occur during the sample collection and handling (Section 4.2).

Considering the fact that the ratio of fibers to total microplastic particles was different between sludge and soil samples, the question could be raised: Do non-fiber microplastic particles have a greater mobility in soil and thus, are they being washed out of fields while fibers remain?

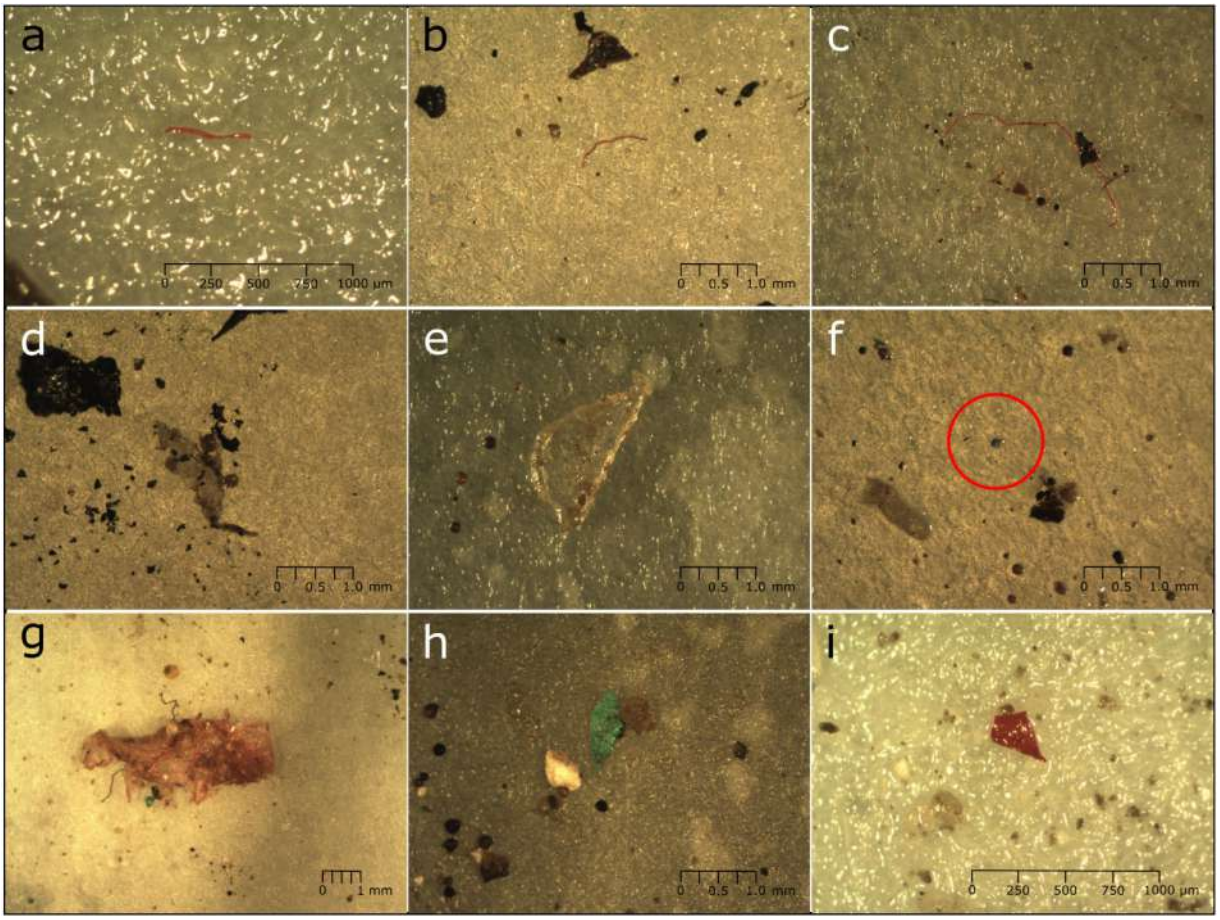


Fig. 7. Example images of the microplastics observed. Fibers (a, b, c), films (d, e), pellets (f), and fragments (g, h, i).

The movement of fiber-like particles through porous media involves complexities that differ greatly from other geometrical forms, as pointed out by Engdahl (2018). The author reported one of the first efforts in modeling microplastic movement in porous media. Although it was a simulated experiment, his findings indicate that sometimes counter-intuitive transport processes take place. The concept of microplastic mobility through the soil profile was reviewed in Rilling et al. (2018) and in Steinmetz et al. (2016). Since our study presented limited data and had methodological restrictions, the microplastic content of the sludge samples was not directly comparable with the soil data. Hence, our initial question remains unanswered and is set as a challenge for future investigations. After all, if plastics are remobilized from soil to water bodies, the main aim of waste water treatment plants to prevent water contamination could be compromised by current sludge disposal methods.

4.2. Method limitations

We were able to quantify microplastic particles in sludge and soil samples as units per unit of weight with the method we used. Reporting results as units per weight has the advantage that simpler and cheaper methods can be used for soil evaluation. One benefit is that the method offers alternatives for microplastic detection for developing economies. For now, the method may be used in semiarid environments, with low organic matter and calcareous soils. The method was not fast so extensive assessment campaigns using this method could prove to be time consuming if used in other contexts. There are faster methods currently being developed but they have higher analytical costs (e.g. Paul et al., 2018; Corradini et al., 2019). Reporting values as units per given weight is a known limitation since it could be difficult to compare results between studies (Bläsing and Amelung, 2018; Scheurer and Bigalke, 2018). So far, most studies assessing microplastics in soils present their data in this form (e.g. Zubris and Richards, 2005; Huerta Lwanga et al., 2017; Liu et al., 2018; Scheurer and Bigalke, 2018; Zhang and Liu, 2018; Zhang et al., 2018). In some of these studies, researchers used diameter and average density to try and estimate the plastic content on a weight to weight (w/w) basis. The disadvantage of this is that uncertainty is increased because the converted data is only an approximation. For example, fibers had a void fraction which may vary and thus assumptions had to be made in order to perform conversions to w/w (Simon et al., 2018). Despite the uncertainty, new evidence shows that simultaneous quality-quantitative assessments are important for inference of effects (de Souza Machado et al., 2018).

Visual sorting depends on the operator's criteria. Therefore, additional identification techniques are often used to avoid false positives. These additional techniques were not included in our approach. Although it is advised to couple visual techniques with spectroscopic approaches, the use of such techniques considerably increases the cost and complexities of the analysis. The additional gains in accuracy may not be relevant depending on the study purpose. Horton et al. (2017) reported a 7% rate of misclassification (particles that were natural, as opposed to anthropogenic) when they validated the method (using soils with ~6% organic matter).

Organic matter removal steps were not included in the method used. These steps are used to avoid false positives during optical inspection. Commonly used reducing agents degrade microplastics. Hurley et al. (2018) reported that Fenton's reagent could be an alternative. However, when we tried the Fenton's reagent protocol with fibers, the recovery of nylon and acrylic fibers decreased considerably (data not shown). Organic matter removal is not always mandatory and may reduce extraction efficiencies (Wang et al., 2018). The removal of organic matter is recommended when organically rich samples with a high potential interference are analyzed (Fuller and Gautam, 2016; Mahon et al., 2017). The soils surveyed had between 1.3% and 4.3% organic matter. This did not represent a problem during analysis.

Additionally, when the methodology was tuned up, we noticed that carbonates hindered organic matter removal (data not shown). For example, Fenton's reaction is partially impeded when carbonates are present (Liu et al., 2016). Therefore, if organic matter removal is needed, carbonates should be eliminated first. If this is the case, one should note that carbonate elimination is performed by acids (Soil Survey Staff, 2014), thus unnecessary particle abrasion could take place. The study sites were located in a semiarid region where carbonates range from 1.0% to 2.5% ($CaCO_3$). Consequently, organic matter removal steps were avoided, on the one hand due to the low organic matter, and on the other, due to the presence of $CaCO_3$.

Observed recovery rates were similar to other findings. Li et al. (2018) evaluated the recovery rate of polyethylene (LDPE) from sludge samples using NaCl extraction. These researchers reported a success rate of 86%, which is similar to the recovery observed in our experiements after the first two extractions. Similarly, using a higher density solution (NaI, $\rho = 1.8$ g cm^{-3}), Hurley et al. (2018) recovered 92–98% of small LDPE beads, almost 100% of large LDPE beads, and 79–86% of polyester fibers. To our knowledge, there are no studies evaluating the recovery rate of nylon or acrylic fibers or PVC. High recovery rates may be due to the high density of 5 M $ZnCl_2$ ($\rho = 1.55$ g cm^{-3}), compared to the densities of the polymers ($\rho = 1.38$, 1.17, and 1.14 g cm^{-3} for polyester, acrylic, and nylon (Qin, 2016), $\rho = 1.35$ g cm^{-3} for flexible PVC (Titow, 2012), and $\rho = 0.92$ g cm^{-3} for LDPE (Zhang et al., 2018)).

There was evidence of a laboratory sample contamination. It could be considered negligible when compared to the observed microplastic counts. Zhang and Liu (2018) reported an average of ~4 fibers in control samples, which is comparable to the 2 fibers per filter paper reported by Horton et al. (2017). Exploring microplastic content in beach sand, Lots et al. (2017) reported an in lab contamination in 3 out of 5 control replicates (60%). Scheurer and Bigalke (2018) reported that 3 out of 9 blank filters were contaminated with fibers when evaluating Swiss floodplain soils, which is similar to the proportion observed in our work (30%). Sample contamination is a recurrent issue across current studies. To keep contamination at negligible levels, sample weight should be defined carefully. For the purposes of this study, using only 5 g of a sample was sufficient to reduce the noise caused by sample contamination.

The repeatability reached by the method was good, being within the ranges expected for soil tests (McLain et al., 2018; Vaughan, 2018). The coefficient of variation (12%) implies that the probability of two replicate measurements differing by a factor of 1.5 or more is <0.025 (Reed et al., 2002). Repeatability has not been addressed directly in any of the studies that propose new methodologies for soil microplastic assessment. Repeatability reflects how much of the variation between samples is due to the analytical method and how much is due to the treatments, thus it is an important indicator of quality control.

5. Conclusion

Sludge applications on soils resulted in increased microplastic counts in soil samples. By evaluating agricultural fields with different sludge application records, we provided evidence of microplastic accumulation over time. The data revealed a high concentration of microplastics in the soils, supporting the fact that sludge is a driver of soil microplastic contamination. Also, evidence showed that there could be plastic remobilization away from fields, revealing challenging new research questions.

The method can be used as a guide to examine microplastic contamination in semiarid regions but further methods need to be adapted for other environments. There are yet only a few studies addressing soil microplastic contamination, thus the true scale of the problem has yet to be assessed. Research on plastic weathering and transport processes within the soil profile are still pending and are greatly needed to understand the fate of the pollutants in the overall environment.

Funding

This work was supported by Comisión Nacional de Investigación Científica y Tecnológica, CONICYT [grant 72170044].

Acknowledgement

We thank Servicio Agrícola y Ganadero and Aguas Andinas for providing access to the sludge application reports.

References

Anuar Sharuddin, S.D., Abnisa, F., Wan Daud, W.M.A., Aroua, M.K., 2017. Energy recovery from pyrolysis of plastic waste: study on non-recycled plastics (NRP) data as the real measure of plastic waste. Energy Convers. Manag. 148, 925–934.

Auta, H., Emenike, C., Fauziah, S., 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176.

Bläsing, M., Amelung, W., 2018. Plastics in soil: analytical methods and possible sources. Sci. Total Environ. 612, 422–435.

Brodhagen, M., Goldberger, J.R., Hayes, D.G., Inglis, D.A., Marsh, T.L., Miles, C., 2017. Policy considerations for limiting unintended residual plastic in agricultural soils. Environ. Sci. Pol. 69, 81–84.

CIREN. 1996. Estudio agrológico Región Metropolitana. Descripciones de suelos. Publicación No. 115. Centro de Información de Recursos Naturales. Santiago, Chile.

Coors, A., Edwards, M., Lorenz, P., Römbke, J., Schmelz, R.M., Topp, E., Waszak, K., Wilkes, G., Lapen, D.R., 2016. Biosolids applied to agricultural land: influence on structural and functional endpoints of soil fauna on a short- and long-term scale. Sci. Total Environ. 562, 312–326.

Corradini, F., Bartholomeus, H., Lwanga, E.H., Gertsen, H., Geissen, V., 2019. Predicting soil microplastic concentration using Vis-NIR spectroscopy. Sci. Total Environ. 650, 922–932.

de Souza Machado, A.A., Lau, C.W., Till, J., Werner, K., Lehmann, A., Becker, R., Rillig, M.C., 2018. Impacts of microplastics on the soil biophysical environment. Environ. Sci. Technol. 52 (17), 9656–9665.

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 2.

Engdahl, N.B., 2018. Simulating the mobility of micro-plastics and other fiber-like objects in saturated porous media using constrained random walks. Adv. Water Resour. 121, 277–284.

Fuller, S., Gautam, A., 2016. A procedure for measuring microplastics using pressurized fluid extraction. Environ. Sci. Technol. 50, 5774–5780.

Habib, D., Locke, D.C., Cannone, L.J., 1998. Synthetic fibers as indicators of municipal sewage sludge, sludge products, and sewage treatment plant effluents. Water Air Soil Pollut. 103, 1–8.

He, D., Luo, Y., Lu, S., Liu, M., Song, Y., Lei, L., 2018. Microplastics in soils: analytical methods, pollution characteristics and ecological risks. TrAC Trends Anal. Chem. 109, 163–172.

Henry, B., Laitala, K., Klepp, I.G., 2019. Microfibres from apparel and home textiles: prospects for including microplastics in environmental sustainability assessment. Sci. Total Environ. 652, 483–494.

Hernandez, E., Nowack, B., Mitrano, D., 2017. Polyester textiles as a source of microplastics from households: a mechanistic study to understand microfiber release during washing. Environ. Sci. Technol. 51, 7036–7046.

Horton, A.A., Svendsen, C., Williams, R.J., Spurgeon, D.J., Lahive, E., 2017. Large microplastic particles in sediments of tributaries of the river Thames, UK – abundance, sources and methods for effective quantification. Mar. Pollut. Bull. 114, 218–226.

Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., Chi, J. d. l. A., Sanchez del Cid, L., Chi, C., Escalona Segura, G., Gertsen, H., Salánki, T., van der Ploeg, M., Koelmans, A. A. and Geissen, V. 2017. Field evidence for transfer of plastic debris along a terrestrial food chain. Scientific Reports 7: 14071-.

Hurley, R.R., Lusher, A.L., Olsen, M., Nizzetto, L., 2018. Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environ. Sci. Technol. 52, 7409–7417.

Lesnoff, M., Lancelot, R., 2012. Aod: analysis of Overdispersed data. R package version 1.3. URL. http://cran.r-project.org/package=aod>.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y., 2018. Microplastics in sewage sludge from the wastewater treatment plants in China. Water Res. 142, 75–85.

Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., Zhou, W., Cao, C., Shi, H., Yang, X., He, D., 2018. Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. Environ. Pollut. 242, 855–862.

Liu, N., Ding, F., Weng, C.-H., Hwang, C.-C., Lin, Y.-T., 2016. Minimizing the interference of carbonate ions on degradation of SRF3B dye by Fe0-aggregate-activated persulfate process. Sep. Purif. Technol. 169, 230–240.

Lots, F.A., Behrens, P., Vijver, M.G., Horton, A.A., Bosker, T., 2017. A large-scale investigation of microplastic contamination: abundance and characteristics of microplastics in European beach sediment. Mar. Pollut. Bull. 123, 219–226.

Mahon, A.M. adn, O'Connell, B., Healy, M., O'Connor, I., Officer, R., Nash, R., M., L., 2017. Microplastics in sewage sludge: effects of treatment. Environ. Sci. Technol. 51, 810–818.

McLain, K.W., Bumblauskas, D.P., White, D.J., Gransberg, D.D., 2018. Comparative analysis of repeatability and reproducibility of compaction testing. J. Struct. Integ. Maint. 3, 106–113.

MINSEGPRES, 2009. Decreto 4. Reglamento para el manejo de lodos generados en plantas de tratamiento de aguas servidas. Chile. Available at, Ministerio Secretaría General de la Presidencia http://bcn.cl/1uzdu, Accessed date: 3 January 2019.

Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Mar. Pollut. Bull. 112, 39–45.

Napper, I.E., Bakir, A., Rowland, S.J., Thompson, R.C., 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. Mar. Pollut. Bull. 99, 178–185.

Ng, E.-L., Lwanga, E.H., Eldridge, S.M., Johnston, P., Hu, H.-W., Geissen, V., Chen, D., 2018. An overview of microplastic and nanoplastic pollution in agroecosystems. Sci. Total Environ. 627, 1377–1388.

Paul, A., Wander, L., Becker, R., Goedecke, C., Braun, U., 2018. High-throughput NIR spectroscopic (NIRS) detection of microplastics in soil. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-018-2180-2 [in press].

Pinto da Costa, J., Paço, A., Santos, P.S.M., Duarte, A.C., Rocha-Santos, T., 2018. Microplastics in soils: assessment, analytics and risks. Environ. Chem. 16 (1), 18–30.

Prata, J.C., 2018. Airborne microplastics: consequences to human health? Environ. Pollut. 234, 115–126.

Qin, Y., 2016. A brief description of textile fibers. In: Qin, Y. (Ed.), Medical Textile Materials. Woodhead Publishing.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/>.

Reed, G.F., Lynn, F., Meade, B.D., 2002. Use of coefficient of variation in assessing variability of quantitative assays. Clin. Diagn. Lab. Immunol. 9, 1235–1239.

Rilling, M.C., Ingraffia, R., de Souza Machado, A.A., 2018. Microplastic incorporation into soil in agroecosystems. Front. Plant Sci. 8, 1805.

Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss floodplain soils. Environ. Sci. Technol. 52, 3591–3598.

Schmidt, J., Christensen, N., Batstone, D., Trably, E., Lyberatos, G., Stamatelatou, K., Kornaros, M., Metzger, L., Amellal, N., Watson, J., García, K., Ayuso, S., Patureau, D., 2006. Safe recycling of sewage sludge on agricultural land-biowaste. Process Saf. Environ. Prot. 84, 253–257.

Schneider, C., Rasband, W.S., Eliceiri, K., 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.

Simon, M., van Alst, N., Vollertsen, J., 2018. Quantification of microplastic mass and removal rates at wastewater treatment plants applying focal plane Array (FPA)-based Fourier transform infrared (FT-IR) imaging. Water Res. 142, 1–9.

Soil Survey Staff. 2014. Kellogg soil survey laboratory methods manual. Soil Survey Investigations Report No. 42, Version 5.0. R. Burt and Soil Survey Staff (Eds.). U.S. Department of Agriculture, Natural Resources Conservation Service.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., Schaumann, G.E., 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci. Total Environ. 550, 690–705.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M. C. and Ni, B.-J. 2019. Microplastics in wastewater treatment plants: Detection, occurrence and removal Water Research 152: 21–37.

Titow, M., 2012. PVC Technology. Springer, Netherlands.

Vaughan, B., 2018. How to determine an accurate soil testing laboratory. Site-specific management guidelines 4 (SSMG-4). International Plant Nutrition Institute (IPNI). Lincoln, NE, USA. Available at. http://www.ipni.net/ssmg, Accessed date: 4 January 2019.

Wang, Z., Taylor, S.E., Sharma, P., Flury, M., 2018. Poor extraction efficiencies of polystyrene nano- and microplastics from biosolids and soil. PLoS One 13, 1–13.

Zhang, G., Liu, Y., 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. Sci. Total Environ. 642, 12–20.

Zhang, S., Yang, X., Gertsen, H., Peters, P., Salánki, T., Geissen, V., 2018. A simple method for the extraction and identification of light density microplastics from soil. Sci. Total Environ. 616–617, 1056–1065.

Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D., 2017. Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. Water Res. 112, 93–99.

Zubris, K.A.V., Richards, B.K., 2005. Synthetic fibers as an indicator of land application of sludge. Environ. Pollut. 138, 201–211.