Soil contamination by microplastics in relation to local agricultural development as revealed by FTIR, ICP-MS and pyrolysis-GC/MS

Khawla Chouchene[1*], Tommaso Nacci [2], Francesca Modugno[2,3], Valter Castelvetro[2,3] and Mohamed Ksibi[1,4]

1) Laboratory of Environmental Engineering and Ecotechnology (LGEET-ENIS), University of Sfax, Tunisia

2) Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa (Italy)

3) CISUP—Center for the Integration of Scientific Instruments of the University of Pisa, Pisa, Italy

4) Higher Institute of Biotechnology of Sfax (ISBS), University of Sfax, Tunisia

[*] corresponding authors: chouchene.khawla23@gmail.com

Abstract

Plastic film mulching and use of wastewaters for irrigation have been common agricultural practices for over half a century in Tunisia, especially in arid regions, resulting in the undesired creation of a pathway for microplastics (MPs) to enter farmland soil. In order to assess the extent and characteristics of soil contamination by MPs in the Moknine province, an area of intensive agricultural practices, 16 farmland soil samples were collected and characterized. The total concentration of targeted MPs was 50-880 items/kg; among them, the most common MPs type being polypropylene (PP), mainly occurring as white/transparent fibers with small size (cross section <0.3 mm). SEM images of MPs surfaces revealed multiple features related to environmental exposure and degradation. ATR-FTIR spectroscopy and pyrolysis-GC/MS analyses enabled the accurate identification of MPs separated from the embedding soil micro- and macro-aggregates. Finally, contamination of the polymeric microparticles with a broad range of metals was found by ICP-MS analysis, suggesting that MPs can be vectors for transporting heavy metals in the soil and indicators of soil contamination as a result of mismanagement of industrial wastewaters.

Key words

Polymer microparticles, Intensive agriculture, Pyrolysis-Gas Chromatography-Mass Spectrometry, Infrared spectroscopy, Heavy metals, Moknine province.

1. Introduction

Recent research has been focused on assessing the main sources of MPs and their environmental distribution as a prerequisite to evaluate how they affect the contaminated biosphere (Pinto, 2018). Two broad categories can be distinguished as the sources of MPs; primary particles fabricated as such are present as micro- or nano-beads in personal care and industrial product formulations, while secondary particles result from the constant weathering and abrasion or fragmentation of plastic items occurring upon dispersion in the environmental (Chouchene et al., 2019). Waste mismanagement and uncontrolled dumping results in an increasing amount of plastics accumulating in terrestrial as well as aquatic environments (Samir et al., 2021), also due to the very slow degradation of synthetic polymers under normal photo-oxidative and hydrolytic conditions. MPs can also behave as collectors and concentrators of toxic environmental pollutants (Chouchene et al., 2020). While MPs are increasingly recognized as a serious threat for natural aquatic environments, it is inland where most of the plastic items are produced, used, and disposed of. Uncontrolled disposal and subsequent environmental (mainly photo-oxidative) polymer degradation may thus result in a significant inflow of MPs also in terrestrial environments, where they may accumulate in soils before reaching the groundwater table and eventually riverine and marine ecosystems (Afrin et al., 2020; Steinmetz et al., 2016). Studies support the alarm about using wastewater treatment plants (WWTP) sludges as fertilizers and urban or industrial wastewaters for agricultural land irrigation, both practices being important sources for soil contamination by MPs (Nizzetto et al., 2016). Indeed, WWTP and sewage sludges have been identified as the receptors of MPs including a significant fraction of synthetic fibers (Corradini et al., 2019). Additional sources of MPs in agricultural soils are plastic mulch films (mainly polyethylene, PE, and its copolymers with vinyl alcohol or butyl acrylate) and other plastic items (greenhouse covers, strings and nets supporting growing plants, etc.) widely used in intensive farming.

More than 700.000 ton of MPs are estimated to enter the soil annually in North America and Europe, a quantity larger than the 93.000-236.000 ton of MPs estimated to be floating in oceanic surface waters (Van Sebille et al., 2015). Increasing evidences indicate some MPs toxicity for organisms that are important indicators of a healty agricultural soil, as in the studies concerning the oligochaeta *Enchytraeus crypticus* and *Lumbricus terrestris* (earthworm) exposed to soils containing 10 wt% polystyrene (PS) (Zhu et al., 2018) and 28-60% PE (Lwanga et al., 2016) MPs, respectively. MPs have

the ability to convey toxic pollutants with endocrine disrupting ability within soil-plant system, whether by adsorption of toxic chemicals from their polluted environment, or from their own suite of potentially toxic additives (Zhu et al., 2018). Nevertheless, the contribution of the internal sources (i.e., plastics in agricultural practice) on the occurrence of MPs in is not well-known.

In the case of Tunisia, the limited experimental research on plastics pollution has focused until now on macroplastics. The Moknine province, located in the center-east of Tunisia, is a typical agricultural area characterized by intensive use of plastics mulches and extensive greenhouse coverage (43.5% of the total area of the greenhouses in Tunisia) for growing vegetables and fruits; thus the soil health in this area is of utmost importance. Farmers in Moknine have been using plastic film for mulching and greenhouse for quite a long time due to its convenience and cheap price. In this work, the concentration of MPs in a representative agricultural land of the Moknine province was assessed, distinguished by polymer types. This was intended as a first step to increase the knowledge on the MPs sources and accumulation in the agricultural soil, thereby providing scientific background to the setting up a management plan including reduction or redesign of the use of plastic in this ecosystem. In detail, the objectives of this study are: 1) to assess the abundance of MPs contamination in intensive agriculture soils; 2) to assess the characteristics and spatial distribution of MPs in a typical city suburban area; 3) to evaluate the efficacy and accuracy of Py-GC/MS and DSC methods as compared to the most common FTIR analysis for the identification of polymers in MPs.

2. Materials and methods

2.1. Description of the study site

Moknine city is situated in the middle of eastern Tunisia (35°37'–35°35'E and 10°55'–10°53'N), in an area separated from the Mediterranean Sea by a 4–6 km wide littoral barrier. In spite of being a small province, with an estimated population of 75.885, Moknine was selected to run this study because of the record application in agriculture of sludge, mulching and greenhouse covers for many years now. Moknine city has been connected to a wastewater treatment plant (WWTP) since 1983.

2.2. Sampling procedure

Agricultural soil samples from 16 points were collected as the top 20 cm using a soil hand auger (Fig.1). About 1 kg of soil was sampled at each point and placed into an aluminum bag, then the samples were transported to the laboratory and stored at 4 °C before treatment. Before the analyses, each soil sample was homogenized by mechanical mixing, then dried in an oven at 40 °C. Once dried the soil formed hard clods, therefore the samples were mildly hand-milled in a porcelain mortar and sieved through a cascade of 5, 2-, 1-, 0.5-, and 0.3-mm mesh size stainless-steel sieves, giving five size classes (<0.3, 0.3-0.5, 0.5-1, 1-2 and 2-5mm), prior to the extraction procedure. When present, visually detectable plastic particles were separated with tweezers and counted by hand.

2.3. Microplastic extraction and purification

MPs extraction was performed according to a previously described procedure (Chouchene et al., 2020, 2019). 200 g of dried sample for each one of the smaller sized classes (<0.3, 0.3-0.5, 0.5-1 mm) was suspended in aqueous 1.2 g/L NaCl solution in a 1 L glass beaker. The mixture was thoroughly manually stirred for 15 min, then allowed to stand for 30 min to allow density separation by flotation of the less dense MPs. The supernatant was filtered through paper filter (Prat-Dumas flat filter diam. 7-9 µm; QNDM-090-100), then the filter with the MPs debris was placed in a culture dish and digested with aqueous 10 % KOH solution to remove the organic matter soiling the MPs. Flotation and filtration steps were repeated until no more floating particles could be detected and recovered, with a minimum of 3 repetitions. The collected particles were inspected at 10×magnification using a Realux CFM-7045 B2 stereomicroscope (COFEMO, France), and sorted according to their shapes: fragment, fibers, granules, tubes, and foams, respectively. In addition, each isolated particle was classified and numbered by color and size.

2.4. Analytical techniques

Fourier-transform infrared spectroscopy (FTIR) analysis for the identification of the polymers extracted as MPs from the soil samples was performed using a Perkin Elmer Spectrum BX Fourier transformed infrared spectrometer operating in transmission mode (64 scans, 4 cm^{-1} resolution, spectral range 4000–650 cm^{-1}).

A model STA 409PC Differential Scanning Calorimeter (Netzsch, Germany) equipped with a Pt/Rh crucible with lid was used for DSC analysis. The sample was heated from

20-800 °C at 5 $K \cdot min^{-1}$ under nitrogen atmosphere (purge: 50 mL/min; protective: 20 mL/min) to suppress oxidation. An empty Pt/Rh crucible served as reference. Integration of the DSC peaks was realized using the ChemStation Software (Ver.A09.01) from Agilent Technologies.

The Pyrolysis-GC/MS instrumentation consisted of a Multi-Shot Pyrolyzer EGA/Py-3030D micro-furnace (Frontier Laboratories Ltd., Fukushima, JP) coupled to a 8890 Gas Chromatograph (GC, Agilent Technologies, Palo Alto, USA) that was equipped with a deactivated silica pre-column (2 m x 0.32 mm i.d., Agilent J&W, USA) and an HP-5MS fused silica capillary column (stationary phase 5 % diphenyl - 95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., 0.25 µm, Hewlett Packard, USA). Ten fibers, each one characterized by a different color, were selected for Py-GC-MS: four fibers (2-white and 2-blue) from sample 3, three fibers (grey, blue, and white) from sample 6, and three fibers (yellow, green and grey) from sample 11, all of which had previously been identified by FTIR analysis. About 80 µg of each one of the ten selected particles was placed into a clean stainless-steel cup, inserted in the microfurnace with an Auto-Shot sampler AS-1020E (Frontier Laboratories Ltd. Fukushima, JP) and pyrolyzed at 600 °C for 0.20 min. The detector was an Agilent 5977 Mass Selective Detector (Palo Alto, USA) single quadrupole mass spectrometer operating in electron ionization mode (EI) at 70 eV, in positive mode scanning in the range from 35-550 m/z. The MS ion source temperature was 230 °C and the MS quadrupole temperature 150 °C. The injection port was set at 280 °C, with 1:20 split ratio, and the interface temperature was 280 °C. The pyrolysis products were eluted in constant flow mode at 1.0 mL/min (carrier gas He, purity 99.995%) with the following chromatographic program: initial isothermal step at 40 °C for 6 min, heating at 20 °C/min to 310 °C, then isothermal step for 40 min. The data collected were processed by MassHunter Workstation (version10.0) and the NIST Mass Spectral Search Program 2.4.

Analysis of trace metals as contaminants of MPs was carried out by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Thermo Scientific iCAP7000series). Four representative plastic particles were selected for this purpose from the different sampling sites: a fragment from sample 7, a blue fragment from sample 1, a white fiber from samples 13, and a transparent fragment from sample 15. The fragment from the sample 7 was sonicated in an ultrasonic bath to complete the removal of residual soiling material. The plastic particles were transferred into centrifuge tubes, added with 65 %

HNO_3 and then 30 % H_2O_2, and heated for 30 min at 100 °C. Five control blank solutions containing 30 ppm, 10 ppm, 1 ppm, 0.1 ppm, 0.05 ppm, and 0.01 ppm, respectively, of combined Cr, Ag, Cu, Hg, Mn, Fe, Ni, Zn, Ti, As, Co, Al, and Cd standard solution (1000 mg/L in aqueous HNO_3) were prepared to ensure precision and accuracy.

Elemental analysis for the metal content of the soil samples was performed with a iCE3000 Series Atomic Absorption Spectrometer (Thermo Scientific, Cambridge, UK). About 1 g of soil was weighed into a PTFE vessel with 1 mL HNO_3 and 3 mL HCl for microwave digestion. All the samples were heated at 10 °C/min to 120 °C, and after 10 min digestion at 120 °C and 1000 W were allowed to cool down to room temperature. After cooling, the volume was adjusted until 25 mL with distilled water before performing the analysis.

Scanning Electron Microscopy (SEM) analysis was performed on 13 particles representative of all the different polymer types detected in the soil samples (Table S-1 in the Supplementary Data, SD). Among them, particles from sample 7 and 2 required additional cleaning before the analysis; thus, they were suspended in deionized water and sonicated in a sonication bath for 15 min at 40 °C to remove residual soiling material. Subsequently, surface topography and roughness of MPs sputter coated with platinum was observed using a scanning electron microscope (SEM Quanta FEG450) operated at 15 kV.

2.5. Quality Assurance and Quality Control

To prevent contamination by airborne MPs, strict quality control was carried out during the experiment. The metal auger was rinsed before and after taking the soil samples. All sieves, tweezers, containers, beakers and equipment were rinsed carefully with distilled water. All culture dishes and suction filters used during the experiment were washed and covered with aluminum foil for each step. Cotton laboratory coats was always worn during the sampling process and lab work. During laboratory work, procedural blanks were run by placing 250 mL of distilled water in the same glass containers used for samples extraction and leaving them uncovered on lab benches during the extraction process. Blank experiments performed in triplicate and run in parallel with the samples in the laboratory did not show any evidence of MPs contamination from the lab environment.

3. Results and discussions

3.1. Abundance and spatial distributions of MPs

In the investigated area of the Moknine province, MPs particles were detected in all samples of agricultural soil collected across the 16 sampling points, at concentrations ranging between 50-880 $p \cdot kg^{-1}$ with average abundance of 161 $p \cdot kg^{-1}$. The maximum concentration of MPs was 880 $p \cdot kg^{-1}$, that is higher than the 593 $p \cdot kg^{-1}$ reported for samples soils of Swiss floodplains (Scheurer and Bigalke, 2018), and much higher than the 4.5 $p \cdot kg^{-1}$ found in a Shanghai aquaculture farmland (Lv et al., 2019) and the 78-62 $p \cdot kg^{-1}$ in a farmland in the suburbs of Shanghai (Liu et al., 2018). On the other hand, MPs concentrations found in the present study are lower than those reported for Shaanxi agricultural soils, Hangzhou Bay soils (Ding et al., 2020) and the coastal soil of Shandong province (Zhou et al., 2018). Two kinds of shapes were detected in this study as shown in Fig. 2. The sampling area of the present study is not located in the immediate vicinity of the industrial zone or of densely populated areas, therefore the detected soil pollution by MPs cannot be ascribed to a direct input from wastewaters or from other types of anthropogenic wastes. Results of this study showed that the soil around the olive trees in Moknine province was also polluted with MPs (55-105 $p \cdot kg^{-1}$), suggesting that these inputs were randomly dumped scraps. Additional sources of pollution by MPs could be fragmentation of weathered plastic items entering the land through littering. Moreover, the comparatively low concentration of 50 $p \cdot kg^{-1}$ found in the arid land of the sampling site 16 suggests that organic/inorganic matter could facilitate the settlement and the aggregation of MPs (Chairi et al., 2010). The maximum concentration of MPs was found in the soils sampled in areas where greenhouses are more common, suggesting a correlation between such agricultural practices and the accumulation of MPs in the Moknine province.

3.2. Shapes, colors and sizes distribution

The diversity of MPs characteristics such as size, colors, and shapes were analyzed as they are thought to play a critical role in the translocation within the soil food web. Irregularly shaped fragments and curly fibrous MPs were the most abundant types, accounting for 41 % and 59 % of the total, respectively (Fig.S-1.a), while pellets were the less common particles. A predominant concentration of film fragments and fibers has been reported in several other studies (Ding et al., 2020; Zhou et al., 2018, 2019;

Ding et al., 2020; Scheurer and Bigalke, 2018), in which it has also been correlated with intensive use of plastic film, of wastewater for irrigation, and of sewage sludge for agricultural soil amendment. Among the sampling sites explored in this study, common relative number of fibers and fragments have been discovered.

The detected MPs were classified into five size ranges, namely <0.3, 0.3-0.5, 0.5-1, 1-2 and 2-5 mm (Fig.S-1.b). In all the soil samples, the size <1mm was the most abundant with 74% of the total number of particles. The <0.3 and 0.3-0.5 mm fractions counted for over 20% of the total (Fig.S-1). The higher fraction of smaller sized MPs was again in good agreement with previous results (Chen et al., 2019; Scheurer and Bigalke, 2018), and is clearly the result of oxidative degradation caused by the intensive exposure to UV radiation and high temperature (Chai et al., 2020). The number concentration of small sized MPs in the ranges <0.3 and 0.3-0.5 mm reported above, is likely due to the fragmentation of macroscopic plastic items into large MPs and then, with an obvious multiplying factor, into progressively smaller ones, posing specific threats to the terrestrial and nearby aquatic organisms.

As shown in Fig.S-1.c, MPs of ten colors were found. Colorless particles were the most abundant (49.1 %), followed by blue (20.3 %), white (18.6 %), green (3.1 %), black (3 %), grey and yellow (2.7 %), red and maroon (1.1 %) and less defined colors (1.0 %). Of these, colorless and blue/green were the dominant MPs in the smaller <0.3 and 0.3-0.5 mm classes. Overall, different size classes of MPs tended to have different color distribution. Fragments were predominantly white/transparent and blue/green in color, while fibers were more diverse in color. Soils of greenhouse sample contained mostly white/transparent fragments and blue fibers, probably associated with the improper disposal of plastic sheets and missed collection of mulching plastics, suggesting a close correlation between MPs pollution and degradation of this specific kind of plastic waste that will keep accumulating unless more appropriate agricultural plastic waste management is adopted. However, the light-colored MPs found in this study could also be the result of weathering causing discoloration and fading as mentioned by (Galafassi et al., 2019).

3.3. Identification of microplastics extracted from soil samples

FTIR results

Six different polymer kinds were identified as the constituents of the two types of MPs (fragments and fibers), based on their FTIR spectra: branched, low density polyethylene (LDPE) and linear, high-density polyethylene (HDPE, collectively PE), aliphatic polyamide (nylon, Ny), polypropylene (PP), polyacrylonitrile (PAN), polyester (polyethylene terephthalate, PET), and cellulose (natural or regenerated). Representative transmittance spectra recorded from individual MPs are shown in Fig.S-2.

The polymer type for each MP found in the soil samples has been identified based on polymer-specific absorption bands (Table S-2 in the SD). The two different PE types could be distinguished by inspection of the spectral regions between 2950 cm^{-1} and 2850 cm^{-1} (C-H stretching bands) and between 1475 cm^{-1} and 1350 cm^{-1} (methylene and methyl deformations): the presence of a shoulder at 2950 cm^{-1} (methyl asymmetric C-H stretching) and the higher relative intensity of the shoulder in the 1400-1375 cm^{-1} range (methyl symmetric bending deformation) compared to the absorption band at about 1460 cm^{-1} (methylene scissoring deformation) are indicative of branched polyolefins such as LDPE. DSC analysis of the two fragments identified by FTIR as PE gave relatively sharp melting transitions peaking at 124 °C (Fig.S-3), which is typical of a highly crystalline LLDPE or of heavily oxidized HDPE (Li et al., 2019) the latter being more likely for highly oxidized particles as detected by FTIR.

PP was the dominant polymer in the soil samples, accounting for 50 % of the total number of particles, followed by PE (24 %), and smaller amounts of other polymers such as PAN (9 %), Ny and PET (8 %). The MPs composition was variable with the sampling site, but PE and PP were a common and dominant presence. Fibers typically consisted of PE, PP, PAN, PET, and cellulose. Overall, PP was the dominant fiber type, likely as a result of its use (bags, nets) in the agricultural practice. PAN, PET and Ny fibers, along with cellulosic ones, were mainly attributed to release and dispersion of staple fibers from textiles into laundry wastewaters used for irrigation or contained in wastewater treatment plant sludge used as agricultural soil amendment. Small fragments consisted almost exclusively of PP along with some PE, most likely resulting from the mechanical fragmentation of weathered plastic sheets used for greenhouse, as protecting covers against adverse environmental factors for grapes in vineyards, and as mulching films (LDPE/HDPE) not removed from the farmed land at the end of the useful life cycle. Additional MPs from the above polyolefins may result from the environmental degradation of improperly disposed waste from e.g. pesticides and fertilizers packaging

(mainly LDPE and PP), and, to a lesser extent, from water irrigation pipes (HDPE). Indeed, a common feature of all the recorded FTIR spectra, and particularly so for those of fully hydrocarbon backbone polymers (PE, PP, PAN), was the presence of hydroxyl (3200-3500 cm^{-1}), carbonyl (1700-1800 cm^{-1}), and various C-O-C and C-O-O (1000-1200 cm^{-1} from ether, ester, alcohol, peroxide groups) absorptions, indicating extensive photo-oxidative degradation due to natural weathering; the resulting polymer embrittlement promotes then their fragmentation into secondary MPs.

The presence of all the above polymers as MPs pollutants in agricultural soils, with a significant direct contribution from agricultural activities, has been reported before (Liu et al., 2018; Lv et al., 2019). A peculiarity of the sampled sites in Moknine province is the presence of nearby sabkhas, areas in North Africa coastal flats rich in salts as they are subject to periodic flooding and evaporation. In the specific case of Moknine catchment, the sabkhas suffer from contamination by heavy metals, as a result of industrial activities disposing there about a half of their wastewaters (Wali et al., 2015), and by plastic waste originating from urban settlements. While such polluted sites in the Moknine province have not undergone any environmental remediation treatment, making their soils as likely ‘‘hotspots” for pollution by both heavy metals and MPs, they have been, and are still used today, to grow various crops.

Pyrolysis-GC/MS results

Py-GC/MS has been used to identify MPs from different matrices (Primpke et al., 2020). The GC/MS profiles recorded upon pyrolysis at 600 °C of individual sampled particles were compared with data bases from the literature (Orsini et al., 2017); specific polymer markers identified among the pyrolysis products are listed in Table S-3 in the Supplementary Data, while a more exhaustive list of the main pyrolysis products is reported in Table S-4 in the SD. Fibers of animal origin were identified by the presence of 2,5-diketopiperazines (DKPs) such as cyclo(Pro-Val), cyclo(Leu-Pro), cyclo(Pro-Pro) and cyclo(Pro-pyroGlu), as pyrolytic markers, although assignment to a given protein fiber, e.g. wool or silk, was impossible because no specific pyrolytic markers have been identified so far (Orsini et al., 2017).

The pyrogram of a typical PP particle as identified by FTIR, namely the blue fiber S3.2, is shown in Figure 3.a, while the corresponding main detected pyrolysis products are reported in Table S-4 in the SD. The intense peak from 2,4-dimethyl-1-heptene is a

321 characteristic marker of PP (Dierkes et al., 2019); such assignment is confirmed by the
322 presence of the typical clusters of peaks consisting of three signals from 1,n-diene, 1-
323 alkene, and n-methyl-1,n-diene of a given PP oligomer (Castelvetro et al., 2021).

324 The pyrolytic profile of the yellow fiber S11.8 (Table S-4 in the SD), shown in Figure
325 3.b, features many signals from unsaturated nitriles such as 2,4-dicyano-1-butene (2-
326 methylenepentadinitrile) along with one from 2-methylene-pentadinitrile, both markers
327 for PAN acrylic fibers (Castelvetro et al., 2021). A very similar pyrogram was recorded
328 for the green fiber S11.9 (Figure 3.b).

329 Pyrolysis of the MPs identified by FTIR as PET led to a broad range of fragments
330 (Fig.3.c) dominated by aromatic species, such as phenylacetaldeide, 4-methyl-benzoic
331 acid, 4-ethylbenzoic acid, 4-vinyloxycarbonyl benzoic acid, divinyl terephthalate and, in
332 particular, divinyl benzoate, in agreement with the literature (Wu et al., 2014; Dayana et
333 al., 2016). Phenyl radicals are also generated, leading to the formation of diphenyl and
334 its derivatives (Table S-4).

335 *SEM analysis*

336 Most of the ten sampled microplastic fibers and fragments selected for Scanning
337 Electron Microscopy (SEM) observation of their surface morphology were analyzed
338 without additional purification after the procedural flotation and mild oxidation
339 treatment described in the Section 2.3. Additional treatment to remove residual soiling
340 matter was only performed for the fragments 2 and 7; for this purpose they were placed
341 in a test tube with 3 mL deionized water, and mildly sonicated in a water bath for 15
342 min at 40 °C. The SEM images revealed significant differences in surface roughness in
343 both kinds (fragments and fibers) of the ten particles, indicative of different extent of
344 degradation and/or of different weathering behavior of different polymer types (Table
345 S-1in the SD). In particular, fragments seemed to be more heavily oxidized compared to
346 fibers, showing scratches, fractures and crevices, pits and pores, protrusions and
347 grooves, in addition to some residual particles soiling their surface (Fig.4). On the other
348 hand, fibers exhibited partially damaged texture with irregular filaments and fibrillated
349 structures at the ends. In addition, some fibers also showed erosion features, with small
350 pits and cracks visible even at low magnification. Samples 2 and 7 were observed
351 before and after the mild sonication, to evaluate possible damage caused to the polymer
352 surface by the cleaning procedure. Indeed, in Fig.4 (a, b and e) the surfaces appear

visibly eroded as a result of the sonication performed on oxidized particles with an already damaged surface. By comparing these morphological features before and after sonication, SEM images revealed that the PP and PE fragments showed stronger surface degradation than the PP and PE fibers. The apparently better resistance of the PE and PP fibers as highlighted by their less degraded surface morphology is possibly the result of shorter exposure to the environment and/or of a higher crystallinity of the fibers. It is worth pointing out that a damaged surfaces with fringed and sharp edges in MPs may pose a higher risk of internal injuries for the soil fauna as they travel through the gastrointestinal tract (Touil et al., 2020). Finally, the numerous pits and pores observed by SEM on the surface of the MPs could act as shelter for colonization by microorganism communities, further impacting the MPs properties (Chai et al., 2020).

3.4. Heavy metals associated with MPs

Heavy metals were analyzed in both MPs and their corresponding soils at each sampling site; the results are reported in Fig.5. The average concentrations of the heavy metals Hg, Ti, As, Al, Fe, Zn, Mn, Cu, Cr, and Ni associated with the MPs were 40.56, 28.47, 21.17, 1.88, 1.19, 0.09, 0.01, 0.01, 0.009, and 0.004 $\mu g \cdot g^{-1}$, respectively. These concentrations are lower than those measured on the corresponding soil matrices that contained the same sequence as above at concentration 11938.75, 160.81, 31.62, 7.6, 7.41, 7.22, 5.20, and 7.03 $\mu g \cdot g^{-1}$, respectively.

The highest concentrations of heavy metals adsorbed on the MPs were those of Hg, ranging from 4.27-80.58 µg/g, followed by As, up to 44.54 µg/g, and Ti, from 0.68 to 82.65 µg/g, lower concentrations having been found for Cr, Cu, Ni, and Mn, while the concentrations of Ag, Cd and Co were below the detection threshold. The content of trace metals in the soils was greater than that in the MPs, in agreement with a previous study by (Zhou et al., 2019) in which the specific surface area of the soil particle, comparatively larger than that of the MPs, was assumed as the main reason for the former to be more effective adsorbers of pollutants (including heavy metal ions) from the environment (e.g. polluted surface waters).

The relatively high concentrations of some less naturally common toxic metals found in this study are closely associated with human activities (uncontrolled dumping, industrial and household wastes) suggesting that the presence of most of them be the result of

adsorption from polluted agricultural soil and/or surface waters, although contributions from bulk contaminants in the original plastic formulation could not be excluded.

The results from both laboratory experiments (Turner and Holmes, 2015) and analyses on environmental soil samples e.g. (Wang et al., 2016) actually suggest that MPs are potential carriers of metals from even a lightly polluted aqueous environment. Metals may in this case accumulate onto the MPs surface, with increasing efficiency as the particle size becomes smaller and thus the specific surface area increases. Soiling material (clay, silt, etc., as well as adhered organic matter) could also contribute to the accumulation of metals onto MPs by modifying their surface properties and effective surface area.

4. Conclusions

The results of this study, aiming at assessing the state of contamination by MPs in the farmland soil of Moknine province in Tunisia, showed significant pollution mostly related to the local intensive agricultural practices, and in particular to extensive use of plastics mulches and greenhouse coverage. The concentration of MPs in the greenhouse farms was higher than in enclosed farms away from, suggesting a possible risk of agricultural activities on the side of the greenhouse. The MPs isolated from soil samples were identified by a combination of FTIR and Py-GC/MS. Polymer identifications by Py-GC/MS and FTIR analyses on the same particle were generally in agreement confirming their reliability as complementary techniques for MPs identification in agricultural soil; better accuracy can obviously be achieved when both techniques are applied.

The presence of MPs from textiles and of heavy metals, as detected by ICP-MS, suggested a contribution to the overall contamination by wastewaters from nearby urban and industrial sites, as they are commonly used for irrigation.

Acknowledgements

K. Chouchene acknowledges the University of Sfax for providing financial support for her internship at the University of Pisa in Italy. Scanning Electron Microscopy with FEI QUANTA 450 ESEM-FEG and Py-GC/MS analyses with Frontier EGA/Py-3030D Multi-Shot Pyrolyzer plus Agilent 8890 Gas Chromatograph were performed at the Center for Instrument Sharing of the University of Pisa (CISUP).

References

Afrin, S., Uddin, K., Rahman, M.M., 2020. Heliyon Microplastics contamination in the soil from Urban Land fi ll site , 6. https://doi.org/10.1016/j.heliyon.2020.e05572

Castelvetro, V., Corti, A., Nasa, J. La, Modugno, F., Ceccarini, A., Giannarelli, S., Vinciguerra, V., Bertoldo, M., 2021. Polymer Identification and Specific Analysis (PISA) of Microplastic Total Mass in Sediments of the Protected Marine Area of the Meloria Shoals 13, 1–18. https://doi.org/https://doi.org/10.3390/polym13050796

Chai, B., Wei, Q., She, Y., Lu, G., Dang, Z., Yin, H., 2020. Soil microplastic pollution in an e-waste dismantling zone of China. Waste Manag. 118, 291–301. https://doi.org/10.1016/j.wasman.2020.08.048

Chairi, R., Derenne, S., Abdeljaoued, S., Largeau, C., 2010. Sediment cores representative of contrasting environments in salt flats of the Moknine continental sabkha (Eastern Tunisia): Sedimentology, bulk features of organic matter, alkane sources and alteration. Organic Geochemistry 41, 637–652. https://doi.org/10.1016/j.orggeochem.2010.04.006

Chen, Y., Leng, Y., Liu, X., Wang, J., 2019. Microplastic pollution in vegetable farmlands of suburb Wuhan, central China. Environ. Pollut. 113449. https://doi.org/10.1016/j.envpol.2019.113449

Chouchene, K., Pinto, J., Wali, A., Girão, A. V, Hentati, O., Duarte, A.C., Rocha-santos, T., Ksibi, M., 2019. Microplastic pollution in the sediments of Sidi Mansour Harbor in Southeast Tunisia. Mar. Pollut. Bull. 146, 92–99. https://doi.org/10.1016/j.marpolbul.2019.06.004

Chouchene, K., Rocha-santos, T., Ksibi, M., 2020. Selected case studies on the environment of the mediterranean and surrounding, occurrence , and distribution of microplastics and metals contamination in sediments from south west of Kerkennah archipelago , Tunisia. https://doi.org/https://doi.org/10.1007/s11356-020-09938-z

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-lwanga, E., Geissen, V., 2019. Science of the Total Environment Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Sci. Total Environ. 671, 411–420. https://doi.org/10.1016/j.scitotenv.2019.03.368

Dayana, S., Sharuddin, A., Abnisa, F., Mohd, W., Wan, A., 2016. A review on pyrolysis of plastic wastes. Energy Convers. Manag. 115, 308–326.

https://doi.org/10.1016/j.enconman.2016.02.037

Dierkes, G., Lauschke, T., Becher, S., Schumacher, H., Földi, C., Ternes, T., 2019. Quantification of microplastics in environmental samples via pressurized liquid extraction and pyrolysis-gas chromatography. https://doi.org/https://doi.org/10.1007/s00216-019-02066-9

Ding, L., Zhang, S., Wang, X., Yang, X., Zhang, C., Qi, Y., Guo, X., 2020. Science of the Total Environment The occurrence and distribution characteristics of microplastics in the agricultural soils of Shaanxi Province , in north-western China. Sci. Total Environ. 720, 137525. https://doi.org/10.1016/j.scitotenv.2020.137525

Galafassi, S., Nizzetto, L., Volta, P., 2019. Science of the Total Environment Plastic sources : A survey across scienti fi c and grey literature for their inventory and relative contribution to microplastics pollution in natural environments , with an emphasis on surface water. Sci. Total Environ. 693, 133499. https://doi.org/10.1016/j.scitotenv.2019.07.305

Li, D., Zhou, L., Wang, X., He, L., Yang, X., 2019. Effect of Crystallinity of Polyethylene with Different Densities on Breakdown Strength and Conductance Property. https://doi.org/https://doi.org/10.3390/ma12111746

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. https://doi.org/10.1016/j.envpol.2018.07.051

Lv, Weiwei, Zhou, W., Lu, S., Huang, W., Yuan, Q., Tian, M., Lv, Weiguang, He, D., 2019. Science of the Total Environment Microplastic pollution in rice- fi sh co-culture system : A report of three farmland stations in Shanghai , China. Sci. Total Environ. 652, 1209–1218. https://doi.org/10.1016/j.scitotenv.2018.10.321

Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Ploeg, M. Van Der, Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the Terrestrial Ecosystem : Implications for Lumbricus. https://doi.org/10.1021/acs.est.5b05478

Majewsky, M., Bitter, H., Eiche, E., Horn, H., 2016. Science of the Total Environment Determination of microplastic polyethylene (PE) and polypropylene (PP) in environmental samples using thermal analysis (TGA-DSC). Sci. Total Environ. 568, 507–511. https://doi.org/10.1016/j.scitotenv.2016.06.017

Nizzetto, L., Langaas, S., Futter, M., 2016. Pollution: Do microplastics spill on to farm soils? Nature 537, 488. https://doi.org/10.1038/537488b

Orsini, S., Parlanti, F., Bonaduce, I., 2017. Journal of Analytical and Applied Pyrolysis Analytical pyrolysis of proteins in samples from artistic and archaeological objects. J. Anal. Appl. Pyrolysis 124, 643–657. https://doi.org/10.1016/j.jaap.2016.12.017

Pinto, J., 2018. ScienceDirect Micro- and nanoplastics in the environment : Research and policymaking. Curr. Opin. Environ. Sci. Heal. 1, 12–16. https://doi.org/10.1016/j.coesh.2017.11.002

Primpke, S., Fischer, M., Lorenz, C., Gerdts, G., Scholz-Böttcher, B., 2020. Comparison of pyrolysis gas chromatography / mass spectrometry and hyperspectral FTIR imaging spectroscopy for the analysis of microplastics. https://doi.org/https://doi.org/10.1007/s00216-020-02979-w

Samir, S., Elsamahy, T., Koutra, E., Kornaros, M., El-sheekh, M., Abdelkarim, E.A., Zhu, D., Sun, J., 2021. Science of the Total Environment Degradation of conventional plastic wastes in the environment : A review on current status of knowledge and future perspectives of disposal. Sci. Total Environ. 771, 144719. https://doi.org/10.1016/j.scitotenv.2020.144719

Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss Floodplain Soils. https://doi.org/10.1021/acs.est.7b06003

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., Ellen, G., 2016. Science of the Total Environment Plastic mulching in agriculture . Trading short-term agronomic bene fi ts for long-term soil degradation ? Sci. Total Environ. 550, 690–705. https://doi.org/10.1016/j.scitotenv.2016.01.153

Touil, S., Nacer, M., Omira, C., 2020. Impact assessment of long treated wastewater irrigation on soil and crops in Algeria. Euro-Mediterranean J. Environ. Integr. 7, 1–10. https://doi.org/10.1007/s41207-020-00187-7

Turner, A., Holmes, L.A., 2015. Adsorption of trace metals by microplastic pellets in fresh water. https://doi.org/https://doi.org/10.1071/EN14143

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F., Law, K.L., 2015. A global inventory of small floating plastic debris. Environ. Res. Lett. 10, 124006. https://doi.org/10.1088/1748-9326/10/12/124006

Wali, A., Kawachi, A., Seddik, M., Bougi, M., 2015. Effects of Metal Pollution on Sediments in a Highly Saline Aquatic Ecosystem : Case of the Moknine Continental Sebkha (Eastern Tunisia). https://doi.org/10.1007/s00128-015-1469-9

Wang, Xiaoning, Bi, W., Zhai, P., Wang, Xiaobing, Li, H., Mailhot, G., Dong, W., 2016. Applied Surface Science Adsorption and photocatalytic degradation of pharmaceuticals by BiOCl x I y nanospheres in aqueous solution. Appl. Surf. Sci. 360, 240–251. https://doi.org/10.1016/j.apsusc.2015.10.229

Wu, T., Hu, H.L., Du, Y.P., Jiang, D., Yu, B.H., 2014. International Journal of Polymer Analysis and Characterization Discrimination of Thermoplastic Polyesters by MALDI-TOF MS and Py-GC / Discrimination of Thermoplastic Polyesters by MALDI-TOF MS and Py-GC = MS 37–41. https://doi.org/10.1080/1023666X.2014.920126

Zhou, Q., Zhang, H., Fu, C., Zhou, Y., Dai, Z., Li, Y., 2018. Geoderma The distribution and morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea. Geoderma 0–1. https://doi.org/10.1016/j.geoderma.2018.02.015

Zhou, Y., Liu, X., Wang, J., 2019. Science of the Total Environment Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. Sci. Total Environ. 694, 133798. https://doi.org/10.1016/j.scitotenv.2019.133798

Zhu, B., Fang, Y., Zhu, D., Christie, P., Ke, X., Zhu, Y., 2018. Exposure to nanoplastics disturbs the gut microbiome in the soil oligochaete Enchytraeus crypticus *. Environ. Pollut. 239, 408–415. https://doi.org/10.1016/j.envpol.2018.04.017

541

542

Figure captions

Figure.1: Sampling location in Moknine area, and the sixteen sampling points of agriculture soils.

Figure.2: Microscopic images of observed fibers detected in the agricultural soils by A, B, C, D: Dark field and A1, B1, C1, D1 UV light of PP and PAN.

Figure.3: Chromatogram Pyrolysis-GC/MS of (A): PP (Blue, Grey and White fiber), (B): PAN (Green fiber) and (C): PET (Grey fiber).

Figure.4: SEM images of the surface morphology of some microplastic particles; A (S2), B (S1) and E(S7) (Sonicated): surface of PE fragment., C (S12) and D (S4): PP fibers surface and F(S13): the surface of Nylon fiber.

Figure.5: Heavy metal contents in MPs and in the corresponding soils ($\mu g \cdot g^{-1}$) of each sampling site.

Supplementary material

Figure.S-1: The polymer: (A): types, (B): size and (C): colors distribution of MPs within sixteen samples from the region of Moknine province.

Figure.S-2: Chemical identification of MPs polymers via FTIR; *A: HDPE: high-density Polyethylene; B: LDPE: low-density Polyethylene or LLDPE: Linear low-density Polyethylene; C: PP: Polypropylene; D: PAN: Polyacrylonitrile E: Nylon; F: PET: Polyester; G: cellulose.*

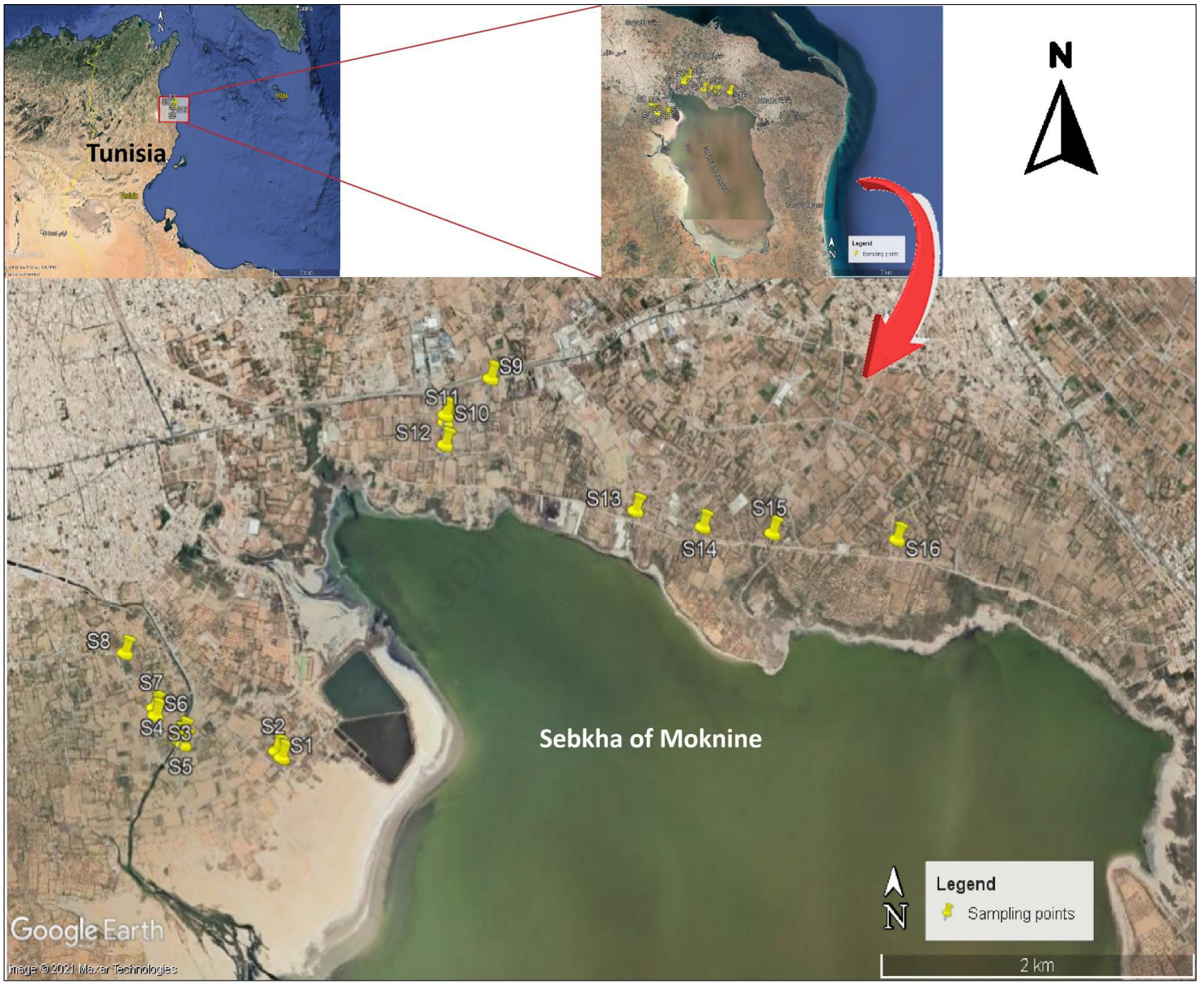
Figure.S-3: Differential scanning calorimetry thermal of two polyethylene fragments.

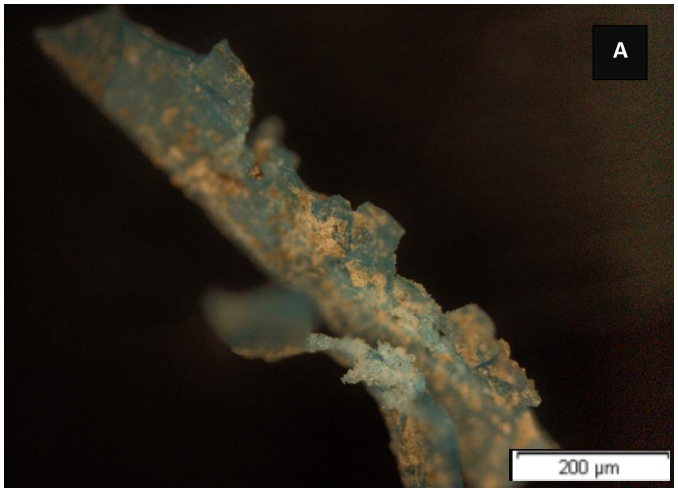
Table.S-1: Observation of the morphology of microplastics by SEM: interpretation of the sign degradation referred to figure legend**.**

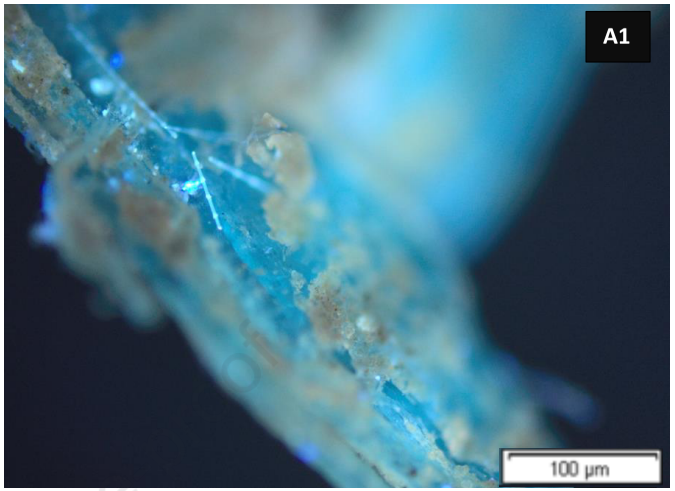
Table.S-2: Important absorption bands and assignment for -FTIR spectra of MPs detected in the agriculture soils.

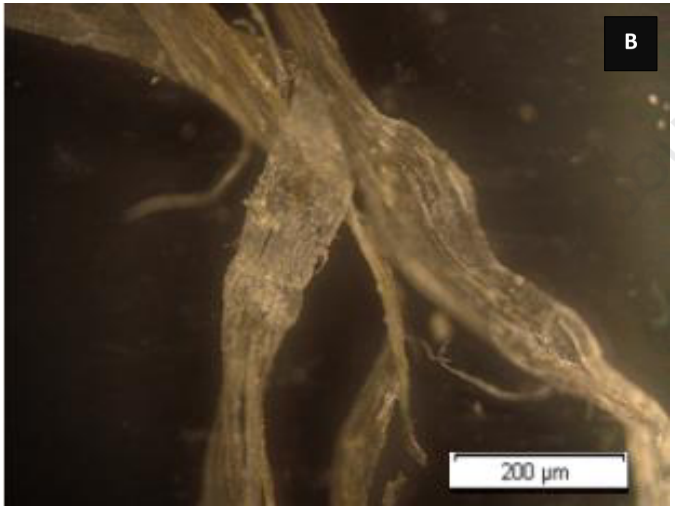
Table.S-3: Main Pyrolysis product of different fiber microplastics.

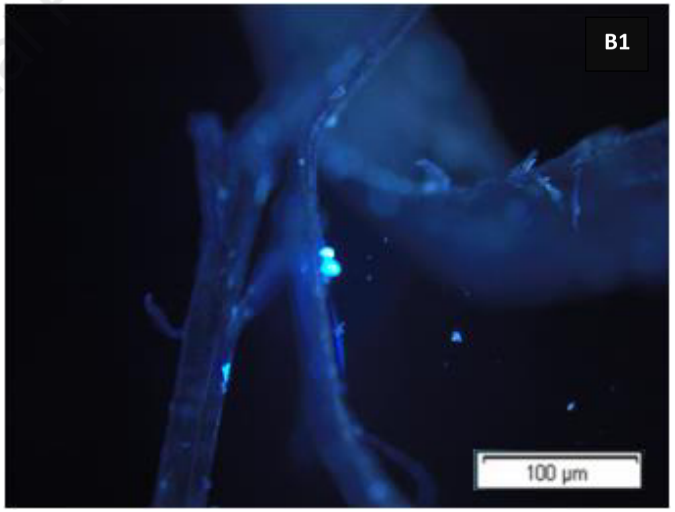
Table.S-4: Application of pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC/MS) to the characterization of fibers in environmental samples, in the context of the study of MPs contamination in agricultural soil in Moknine, Tunisia.


Tunisia
N
S9
S11
S10
S12
S13
S15
S14
S16
S8
S7
S6
S4
S3
S2
S1
S5
Sebkha of Moknine
Legend
Sampling points
N
2 km
Google Earth
Image © 2021 Maxar Technologies


A
200 µm


A1
100 µm


B
200 µm


B1
100 µm

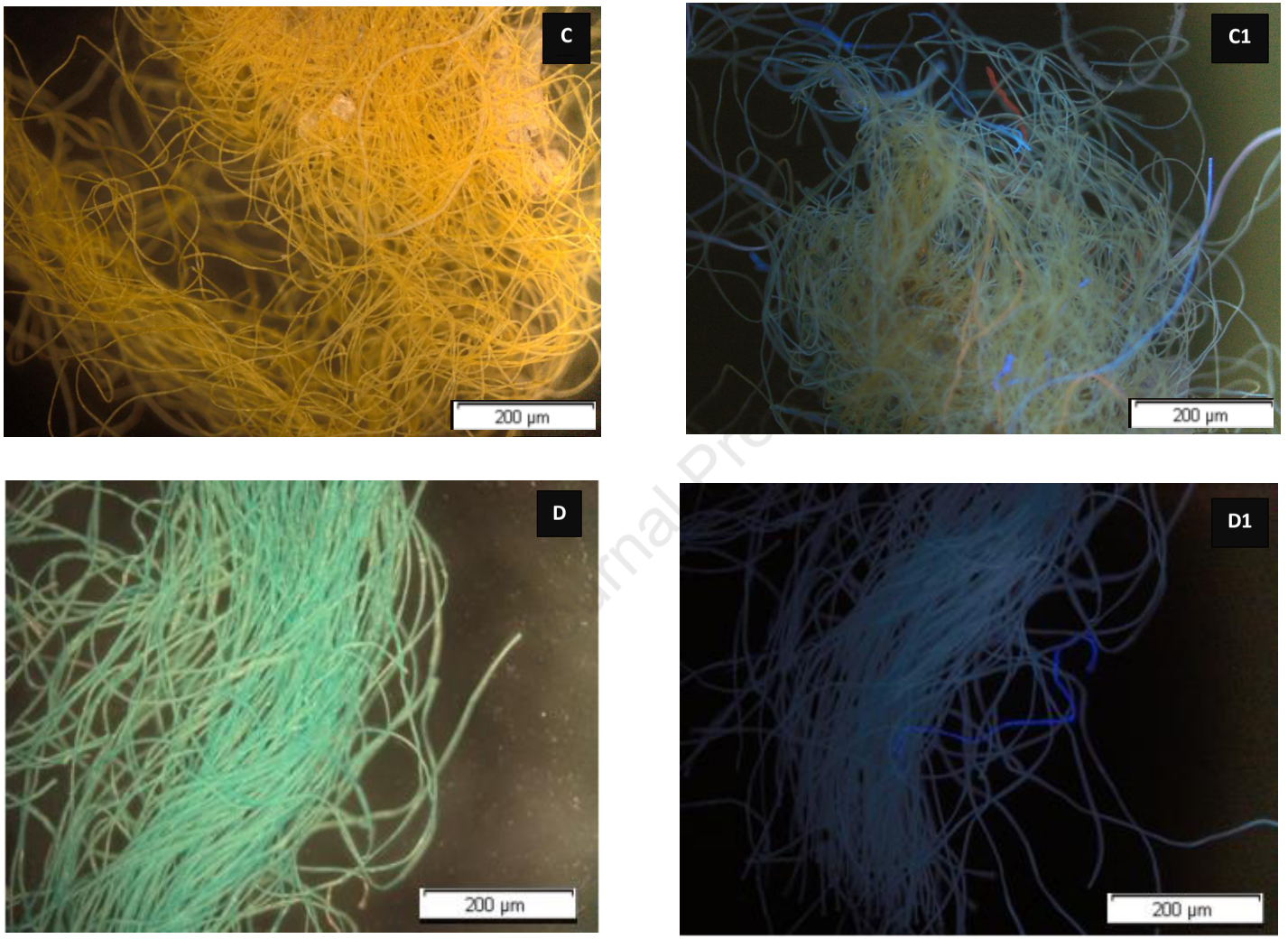
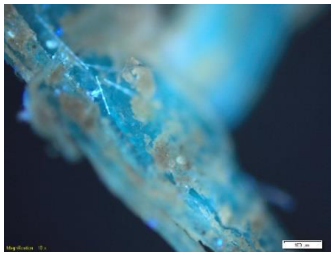
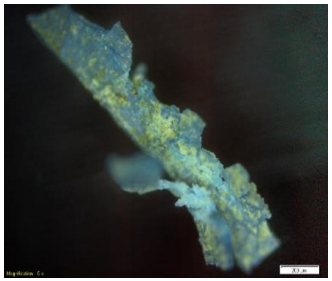
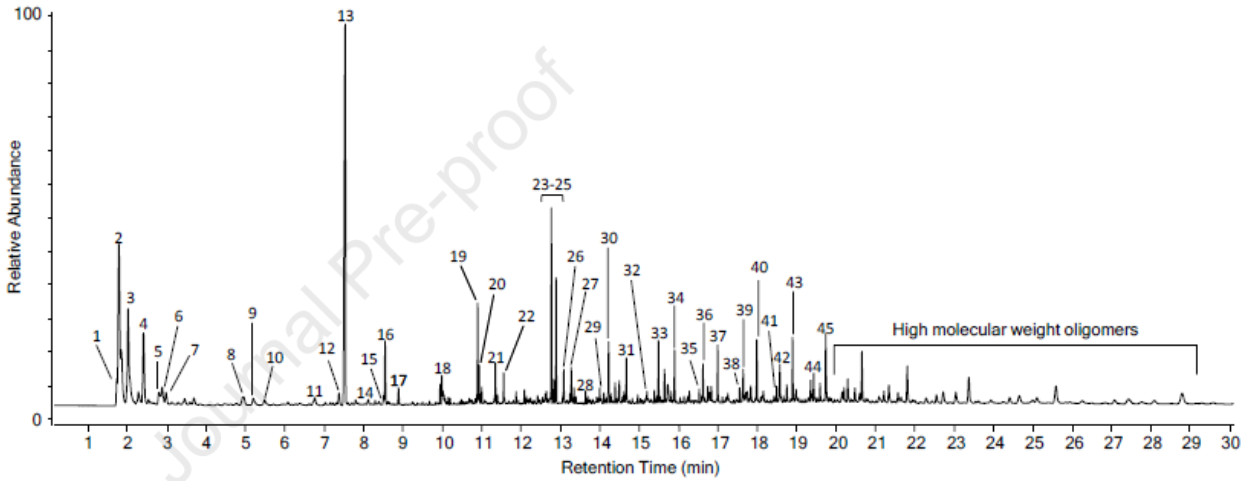


Figure.2

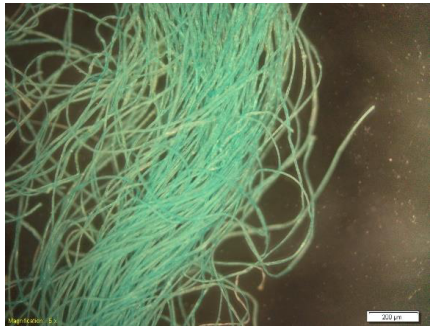
A: Py-GC-MS pyrogram of PP fibers (Blue, Grey and White fiber)

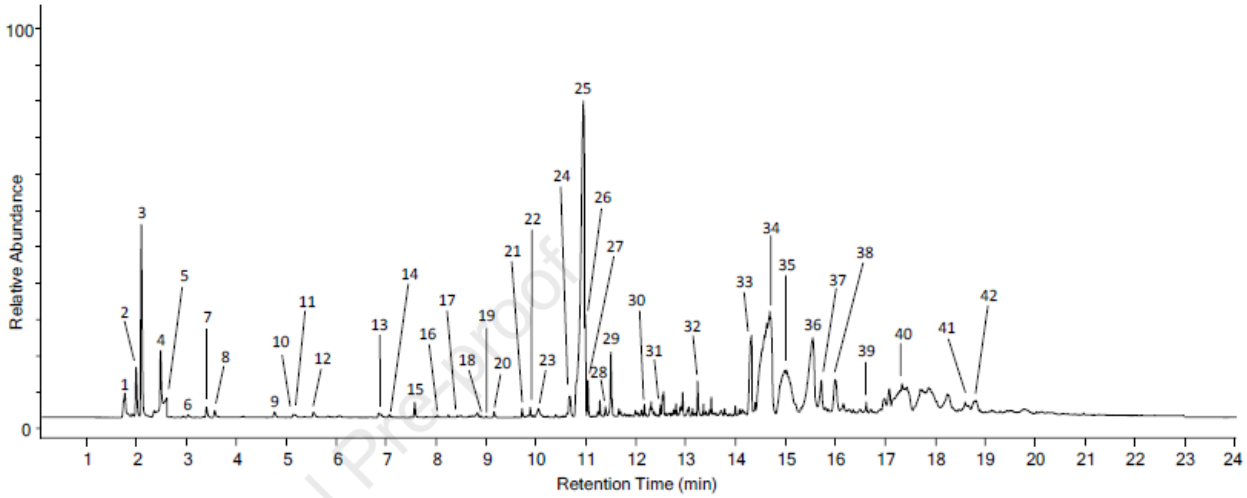




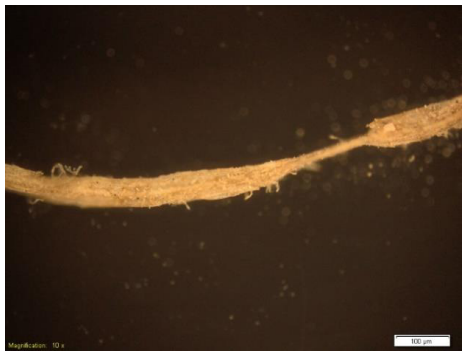


B: Pyrolysis-GC-MS of PAN (Green fiber)





C: Pyrolysis-GC-MS of PET (Grey fiber)



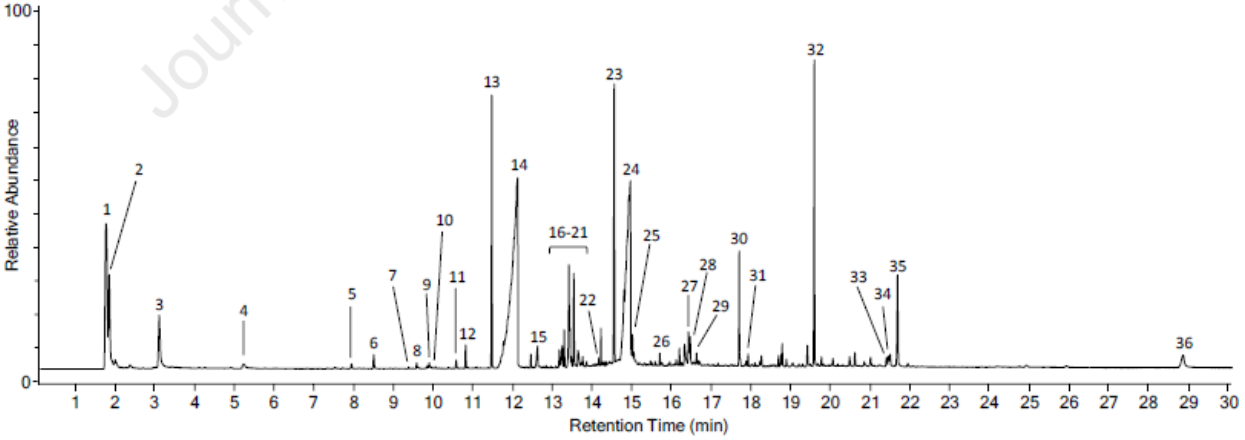
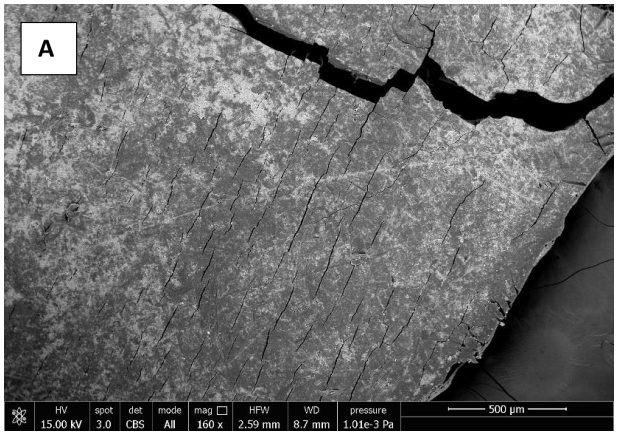
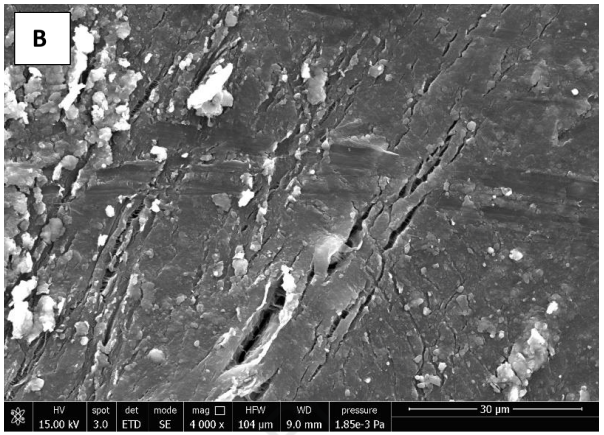
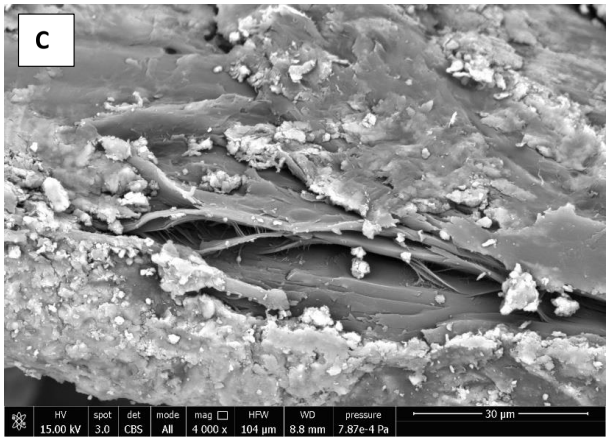
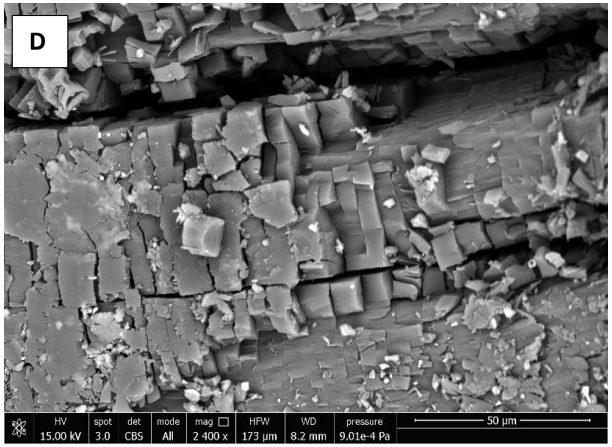


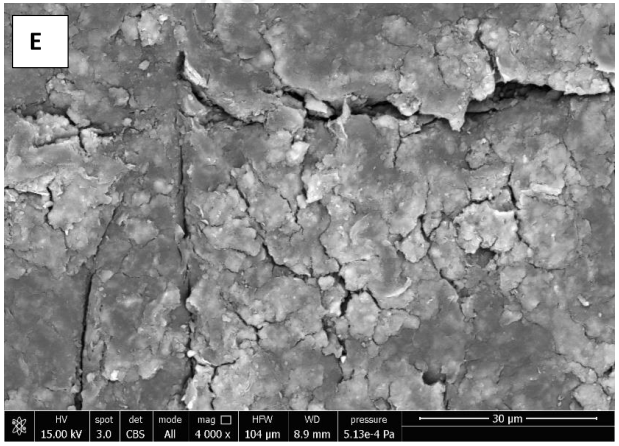
Figure.3


A
HV 15.00 kV
spot 3.0
det CBS
mode All
mag 160 x
HFW 2.59 mm
WD 8.7 mm
pressure 1.01e-3 Pa
500 µm


B
HV 15.00 kV
spot 3.0
det ETD
mode SE
mag 4 000 x
HFW 104 µm
WD 9.0 mm
pressure 1.85e-3 Pa
30 µm


C
HV 15.00 kV
spot 3.0
det CBS
mode All
mag 4 000 x
HFW 104 µm
WD 8.8 mm
pressure 7.87e-4 Pa
30 µm


D
HV 15.00 kV
spot 3.0
det CBS
mode All
mag 2 400 x
HFW 173 µm
WD 8.2 mm
pressure 9.01e-4 Pa
50 µm


E
HV 15.00 kV
spot 3.0
det CBS
mode All
mag 4 000 x
HFW 104 µm
WD 8.9 mm
pressure 5.13e-4 Pa
30 µm

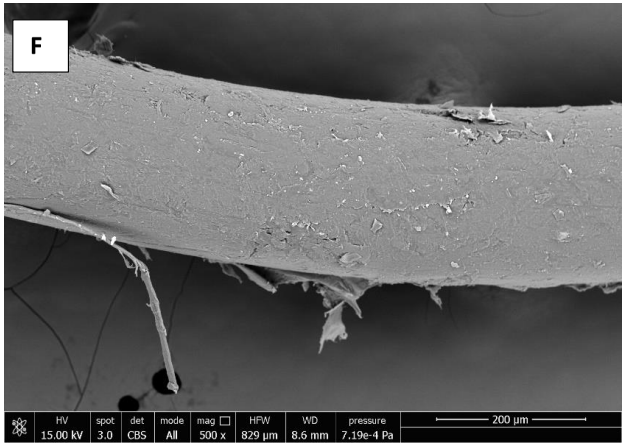
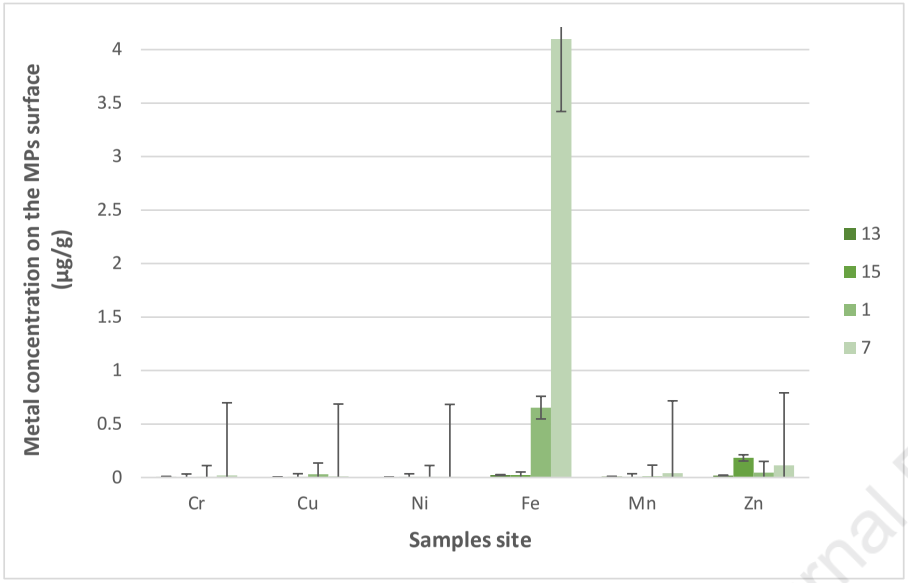
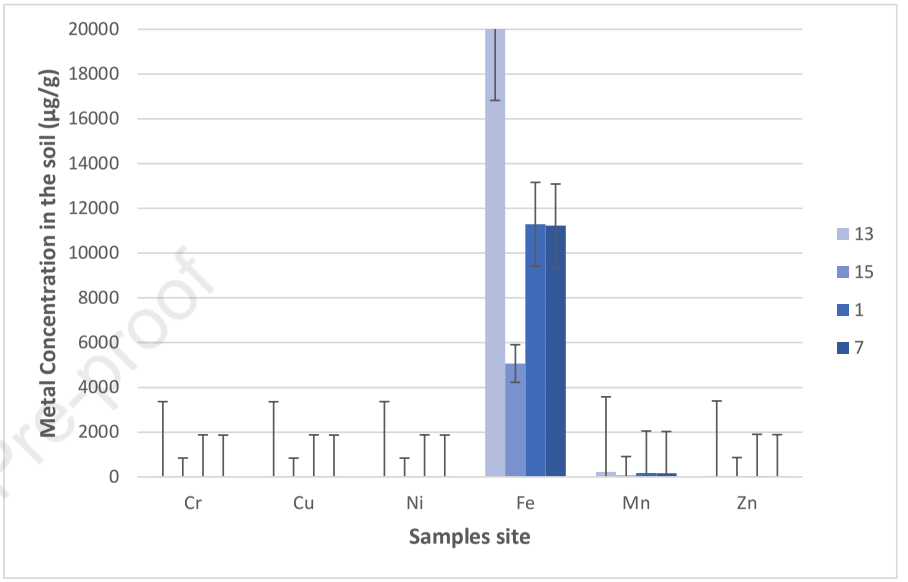

F
HV 15.00 kV
spot 3.0
det CBS
mode All
mag 500 x
HFW 829 µm
WD 8.6 mm
pressure 7.19e-4 Pa
200 µm

Figure.4: SEM images of the surface morphology of some microplastic particles; A (S2), B (S1) and E(S7) (Sonicated): surface of PE fragment., C (S12) and D (S4): PP fibers surface and F(S13): the surface of Nylon fiber.





A **B**

Figure.5: Heavy metal contents in MPs and in the corresponding soils ($\mu g \cdot g^{-1}$) of each sampling site.

- Microplastics (MPs) were isolated from intensive farming soils of Tunisia
- The concentration of MPs in the soils investigated ranged from 50 to 880 items/kg
- Synthetic textile fibers shorter than 1 mm were the dominant MPs types
- The greenhouse and plastic mulch have been identified as possible MPs point source.
- DSC and Py-GC/MS technique offer a promising option in the field of MPs analysis

AUTHORSHIP STATEMENT

Manuscript title: Soil contamination by microplastics in relation to local agricultural development as revealed by FTIR, ICP-MS and pyrolysis-GC/MS

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in *the journal of Environmental Pollution*.

Authorship contributions

Please indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Cheung). The name of each author must appear at least once in each of the three categories below.

Category 1

Conception and design of study: k. Chouchene, M. Ksibi , , ;
acquisition of data: K. Chouchene , T. Nacci , , ;
analysis and/or interpretation of data: k. Chouchene, M. Ksibi , , , .

Category 2

Drafting the manuscript: K. Chouchene , M. Ksibi , , ;
revising the manuscript critically for important intellectual content: V. Castelvetro , F. Modugno ,
T. Nacci , .

Category 3

Approval of the version of the manuscript to be published (the names of all authors must be listed):
K. Chouchene , T. Nacci , F. Modugno , V. Castelvetro , M. Ksibi ,
, ,

Acknowledgements

All persons who have made substantial contributions to the work reported in the manuscript (e.g., technical help, writing and editing assistance, general support), but who do not meet the criteria for authorship, are named in the Acknowledgements and have given us their written permission to be named. If we have not included an Acknowledgements, then that indicates that we have not received substantial contributions from non-authors.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: