Olive oil-based method for the extraction, quantification and identification of microplastics in soil and compost samples

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Abstract

Microplastics (MPs) have become a pressing environmental concern over the past few years and their extraction from solid samples is a scientific challenge that needs to be faced and solved. Standardized and validated protocols for MPs extraction are lacking and the existing methodology, such as density separation, is often unable to separate high density polymers. The aim of our research was to develop a non-density based, inexpensive, simple and safe method to extract MPs from soil and compost samples. We tested an oil-based extracting technique exploiting the oleophilic properties of plastics. For validating the method, soil and compost samples were spiked with six different micro-polymers: polyethylene, polystyrene, polyvinyl chloride, polycarbonate, polyethylene terephthalate and polyurethane. The obtained results are promising, and the polymer density had only a small role in the recovery rate: low, medium and high density polymers reached a mean recovery rate of 90% ±2%, 97%±5% and 95%±4%, respectively.

Keywords: Olive oil; Compost; Recycled fertilizers; Soil; Microplastics extraction; Microplastics quantification

1 Introduction

Microplastics (MPs) pollution is an environmental issue, whose risks for human health are not yet well understood (Lee et al., 2019). What we know is that MPs are ubiquitous in marine and freshwater environments and can cause adverse health effects to the biota that mistake plastic debris for food (Andrade et al. 2019, Cincinelli et al., 2018; Dawson et al., 2018; Martellini et al., 2018; Ribeiro et al., 2018; Silva et al., 2018; Yu et al., 2018; Gewert et al., 2015; Stolte et al., 2015; Vianello et al., 2013). The vast majority of MPs studies is focused on aquatic habitats, while knowledge of microplastics pollution in terrestrial ecosystems is extremely limited. The first published research studying microplastics in soil–under environmental conditions was carried out

close to the industrial areas in Sydney, where Fuller et al. (2016) developed a method based on pressurized fluid extraction (PFE) to analyze MPs in soil samples and showed that the soil mass was composed of 0.03-6.7 w/w% MPs. In Switzerland, 90% of the studied floodplain soils were found to contain MPs with concentrations up to 55.5 mg kg^{-1} or 593 particles kg^{-1} (Scheurer and Bigalke, 2018).

One possible source of plastic in soils may be soil amendments like the compost made from biowaste and sewage sludge, particularly rich in organic carbon and plant nutrients (Bläsing and Amelung, 2018). Wastewater treatment plants remove up to 90% of MPs from the incoming water, with the plastic debris remaining in the sludge (Mason et al., 2016; Estahbanati and Fahrenfeld, 2016; Carr et al., 2016; Mintenig et al., 2017) and reaching a concentration of 1000–24,000 plastic items kg^{-1} (Mintenig et al., 2017; Mahon et al., 2017; Du et al., 2020). Taking into account the compost application rate, and the MPs load in the sludge, Nizzetto et al. (2016) estimated a yearly input of 63,000–430,000 and 44,000–300,000 tons of microplastics to European and North American agricultural soils, respectively. Considering all these alarming data and how little is known about MPs in terrestrial ecosystems, there is an urgent need to collect new information.

Lack of information of pollution levels is not the only gap in the scientific MPs literature. Most of the researchers agree that MPs are plastic particles smaller than 5 mm but for others the upper limit should be reduced (Hartmann et al., 2019). The definition of MPs is thus still unclear and the lack of specifications hampers progress in research (Hartmann et al., 2019). For instance, the US EPA defines MPs as plastic particles smaller than 5 mm (Environmental Protection Agency, 2011), while Hartmann et al. (2019) have suggested to define them as plastic debris in the size range of 1–1000 μm. Furthermore, there are no standardized MPs extracting methods commonly accepted within the scientific community (Silva et al., 2018; GESAMP 2015). The discrepancy between the adopted methodologies poses serious problems regarding the comparability of different studies and the under- or overestimation of MPs concentrations (Silva et al., 2018; Scopetani et al., 2020).

66 The most adopted MPs extracting process from solid samples is density separation followed by
67 filtration (Hanvey et al., 2017). This technique exploits the density difference between the polymers
68 and the solid sample. Different brine solutions are added to the samples and after shaking and a
69 subsequent settlement period, the supernatant is filtered with a vacuum pump and MPs are collected
70 on a filter. The most common separation solution is composed of NaCl with a density of 1.2 g mL^{-1}
71 but other salts like zinc chloride ($ZnCl_2$), calcium chloride ($CaCl_2$), sodium iodide (NaI), sodium
72 polytungstate and sodium bromide (NaBr) are also used to increase the density in order to extract
73 higher density polymers (Hanvey et al., 2017; Liu et al., 2019). Despite this, the density separation
74 techniques are often unable to separate the polymers with highest density (except when NaBr is
75 used) and are usually not suitable for solid samples that contain light organic matter, such as
76 recycled fertilizers, or for clay soils, whose fine grains remain suspended in the supernatant.

77 Besides density separation, a few other techniques have recently been presented in the scientific
78 literature. Felsing et al. (2017) developed an electrostatic metal/plastic separator to physically
79 isolate plastics from solid samples, while Grbic et al. (2019) presented a method based on magnetic
80 extraction, using hydrophobic Fe nanoparticles. Crichton et al. (2017) introduced an oil extraction
81 procedure where canola oil was used to separate MPs from aquatic sediments with an average MPs
82 recovery rate of over 96%. Mani et al. (2019) used castor oil to separate MPs from suspended
83 surface solids (marine and fluvial), soil and sediment. They spiked the samples with polypropylene
84 (PP), polystyrene (PS), polymethyl methacrylate (PMMA) and glycol modified polyethylene
85 terephthalate (PET-G) polymers obtaining a mean MPs spiked-recovery rate of 99%±4%. However,
86 when analyzing non-spiked fluvial suspended solid samples, the authors noticed that the majority
87 (76%) of PS MPs were not retained in the oil layer, but instead found in the solid phase. Lares at al.
88 (2019) tested the method presented by Crichton et al. (2017) and pointed out that ethyl alcohol was
89 not able to remove all canola oil traces, thus hindering the identification with Raman and FTIR
90 spectroscopy. Furthermore, Lares et al. (2019) observed that separatory funnels, technical solution

adopted also by Mani et al. (2019), could easily get obstructed when extracting solid samples (e.g. compost matrix) and they restrict the upper size of recovered MPs.

All the presented methods have limitations and potential biases and there is an urgent need to develop a reliable and standardized method that could be globally adopted by the scientific community.

The aim of our research is to develop an alternative extracting technique process that could overcome the criticalities of the currently adopted methodologies. Inspired by Crichton et al. (2017) and Mani et al. (2019), we decided to modify and improve their oil extraction methods to find a methodology that would be suitable for treating compost and soil samples and that would avoid using separatory funnels, thus solving the weaknesses highlighted by Lares et al. (2019).

We performed preliminary experiments with oils of different density and polarity (e.g. rapeseed, mineral, synthetic, and olive oil) to check which had the strongest affinity with polymers and found that olive oil had the best performance. We therefore selected olive oil for the extraction of MPs from soil and compost samples.

2 Materials and methods

2.1. Test matrices and MPs

The efficiency of the method was tested for both soil and compost matrix. Soil samples were collected from two oat fields in Hollola, Finland: Simola (60° 56.650'N; 25° 29.895'E) and Mäkelä (60° 57.735N; 25° 26.969'E). The samples were collected from the first soil layer (1-5 cm) using a metal spoon and kept in glass jars previously rinsed with ultrapure water (18.2 MΩ).

The compost samples were collected from Labio Ltd, a waste treatment company that collects and treats biowaste and sludge from wastewater treatment plants. Both composted biowaste and

114 composted sewage sludge products were sampled using a metal shovel and kept in metal buckets
115 previously rinsed with ultrapure water. Soil and compost samples were preserved in a cold room at
116 5 °C and analysed one week after the sampling.

117 Six different self-made micro-polymers (range of dimension 0.2–2 mm) were used as test MPs. The
118 polymers were assigned to one of three groups according to their density. Group 1 (low density):
119 polyethylene (PE, 0.91–0.94 g/cm^3) and polyurethane (PU, 0.05–0.07 g/cm^3); Group 2 (medium
120 density) polystyrene (PS, 1.05 g/cm^3) and polycarbonate (PC, 1.20-1.22 g/cm^3); Group 3 (high
121 density): polyvinyl chloride (PVC, 1.30-1.45 g/cm^3) and polyethylene terephthalate (PET, 1.39
122 g/cm^3).These polymers were selected because they are among the most common plastic polymers
123 produced worldwide (Plastic Europe 2013) and their densities differ greatly. The polymers were
124 classified into three density groups: low (density <1 g/cm^2), medium (1–1.29 g/ cm^2), and high
125 (>1.3 g/ cm^2). Five sub-samples of each soil (each 25 g) and compost (10 g) matrices (20 sub-
126 samples in total) were spiked with all of the six self-made micro-polymers. Ten pieces of each
127 polymer (60 polymer items in total) were added to each sub-sample. The pieces were analysed with
128 Fourier transform infrared (FTIR) spectroscopy (Bruker Alpha-P FTIR equipped with ATR) both
129 before and after extraction.

130 The results on MPs recovery were not hampered by the potential MPs contained originally by the
131 samples. The spiked MPs were self-made and clearly recognizable with the naked eye. Furthermore,
132 the samples were carefully checked, before spiking them with MPs, to see if there was any plastic
133 particle that could resemble the self-made ones.

134 The possibility of self-contamination was considered, and during all steps of the sampling,
135 treatment and analysis of the samples, fleece clothing and other plastic items, which could release
136 MPs, were avoided (Scopetani et al., 2020). Instead, only cotton clothes were used during sampling.
137 Furthermore, procedural blanks were performed parallel with the samples to check for potential

source of contamination coming from equipment or airborne particles and no MPs were found in any of the blanks.

2.2 Extraction method

The spiked samples were mixed with ultrapure (18.2 MΩ) water in custom-made polytetrafluoroethylene (PTFE) cylinders (25 cm length, 4 cm external diameter, 3 cm inner diameter) equipped with removable caps and a piston to push out the sample. After shaking the systems, 3 ml of oil was added. The samples were then shook again, allowing the oil to get in contact with the test matrix and the MPs. After two hours of settling, the samples were frozen at -40 °C and the ice columns, together with the oil layers, were pushed out to filtering funnels. Most of ice and soil could be removed from the samples at this point. The samples were filtered through glass microfiber filters (GF/A, 90 mm diameter, Whatman). The filters were carefully rinsed with water and finally with hexane to remove oil traces, air dried, and the polymer particles were collected with tweezers and identified using FTIR. Each sample was extracted thrice in order to maximise the recovery.

We chose to use PTFE cylinders to overcome the difficulties when using separation funnels. The separation of soil and water from oil in separation funnels was found to be impractical because of the boundary layer containing both floating polymers and plant material and possible emulsion. In addition, separation funnels become easily clogged by solid material. The process of freezing the samples facilitate the separation of the oil layer from the water and the soil or compost. In this way, we were able to recover the whole oil layer without collecting any unwanted portion of the settled solid sample. The use of a freezer that reaches – 40 °C allows a fast freezing (2h) and reduce the downtime optimizing the whole process.

Preliminary experiments were performed to test a larger set of polymers including polytetrafluoroethylene (PTFE) (Scopetani et al., 2019b). The obtained recovery was promising for all the polymers extracted from soil samples except for PTFE (Scopetani et al., 2019b). PTFE MPs

did get up to the oil layer when the matrix was composed of oil and water only, but when soil or compost was added, the MPs stayed on the bottom. Therefore, as the method presented here is not suitable to extract PTFE MPs from solid samples, the use of PTFE tubes does not pose contamination issues.

2.3 Oxidation of the organic matter

When complex and organic-rich solid substrates like soil and compost are analyzed for MPs, it is often necessary to remove the organic content of the samples that hampers the identification of MPs. In this study, we applied a Fenton system protocol described by Qin et al. (2015), slightly modified, and checked if it could be combined with the oil extraction method. Hurley et al. (2018) identified the Fenton's reagent as the most effective method for removing organic material without causing degradative changes to plastic particles.

Briefly, four sub-samples of Mäkelä soil and biowaste compost (8 sub-samples in total) were spiked with the same MPs (PE, PU, PC, PET, PVC, PS) used in the previous test. In addition, here we also used micro-particles from car tires, known to be one of the major sources of microplastics in the environment (Sundt et al., 2014; Essel et al., 2015; Lassen et al., 2015). Knowing that car tire rubbers are a mixture of natural rubber, styrene butadiene rubber and butadiene rubber, we wanted to see how the method works with such different material.

The spiked samples were put in glass bottles and the following reagents were added for each gram of sample: 10 mL 30% H_2O_2, 1 mL of 2 mmol/L $FeSO_4*7H_2O$, 1 mL of 2mmol/L protocatechuic acid and 5 mL of H_2O (Qin et al., 2015). The bottles were first kept in the fume hood for one hour and then overnight in the oven at 40 °C. We checked that the oxidation reaction did not create heat that could affect the polymers. The oxidized samples were transferred to the PTFE tubes and the oil extraction method was applied as described earlier for non-oxidized samples. The recovered

polymers were analyzed using an Alpha-P FTIR-ATR spectrometer as described later in section 2.4 to check for potential changes in the polymer structures due to the oxidation process.

Loss on ignition (550 °C, overnight) of compost samples showed that their average organic content was 38%±2%. The average mass loss of compost samples in oxidation was 36%±5%, which shows that the selected oxidation method was able to oxidize on average 95% of the total organic content. These results are in compliance with an earlier study, which found 80–87% removal of organics in sludge and 96–108% removal of organics in soil after applying an oxidation protocol using Fenton reagent (Hurley et al., 2018).

2.4 FTIR-ATR analysis

The PE, PU, PC, PET, PVC, PS and car tire MPs were analyzed before and after extraction using an Agilent Cary 630 FTIR Spectrometer equipped with a diamond crystal ATR (Attenuated Total Reflection) unit. Black samples (PE, rubber) were also analyzed with a germanium crystal ATR. The analyses were performed in an absorbance mode and using a resolution of 4 cm^{-1} with 32 scans for each spectrum in a spectral range from 4000 to 650 cm^{-1}. The MP particles were picked by hand using forceps and placed onto the ATR crystal. The particle sizes ranged from 0.2 to 2 mm.

2.5 Microscope FTIR analysis

To further test the method and to investigate if smaller MPs originally occurred in the collected soil and compost samples, three replicates of each matrix were prepared following the procedure described above, but without spiking MPs. The dried filters were analyzed with a Cary 620-670 Fourier transform infrared spectroscopy (FTIR) microscope (Agilent Technologies) equipped with an FPA (Focal Plane Array) 128 × 128 detector (Agilent Technologies), which allows performing 2D imaging-FTIR analysis on MPs directly on the filters. This analytical approach for the identification of MPs has been validated in several studies by different research groups (Harrison et

al., 2012; Tagg et al., 2015; Mintening et al., 2017; Andrades et al., 2018; Simon et al., 2018; Scopetani et al., 2019a.). The analyses were performed in a reflectance mode using open aperture and a spatial resolution of 4 cm^{-1} acquiring 128 scans for each spectrum. Each analysis consisted of a map of 700 µm x 700 µm (128 x 128 pixels) and each pixel had a dimension of 5.5 µm x 5.5 µm (Imaging map spatial resolution of 5.5 µm). MPs were detected and identified in five randomly chosen squares (2 cm × 2 cm) on each filter, comprising 31.4% of the filter area.

2.6 Statistical analysis

The main effects of polymer density group (low, medium and high density) and polymer identity (two polymers in each density group) and the interaction effects of polymer density group × matrix class (compost and soil) and polymer density group × oxidation on MPs extraction efficiency were tested using data of numbers of missed MPs items for each polymer in each test cylinder (N = 168) and a generalized linear model with negative binomial distribution and log link function. The main effects of oxidation, matrix class and matrix identity (two different compost and soil materials) and the interaction effect of matrix class × oxidation were tested using data of total number of missed MPs items in each test cylinder (N = 28) and a generalized linear model with normal distribution and log link function. In the models, matrix identity was nested within matrix class and polymer identity was nested with polymer density group. Statistically significant differences among polymer density groups were tested using Bonferroni corrected pair-wise comparisons of density group means.

3 Results and discussion

3.1 Recovery of MPs

When polymers were collected and analyzed using an Alpha-P FTIR equipped with ATR, no oil traces were detected that could affect the identification. The FTIR spectra recorded before and after

extraction revealed that the use of hexane and the oxidation process did not modify the FTIR spectra of MPs and no changes in the morphology or size of the MPs was observed after a visual examination.

. Both the reference and the oxidized samples shared the majority of peaks (Fig. 1 and Supplementary Info) and in case of Fig. 1 they are identifiable as polystyrene. The intense absorption at 1742 cm^{-1} observed in the oxidized samples might indicate the development of carbonyl groups due to oxidation (Verleye et al., 2001).

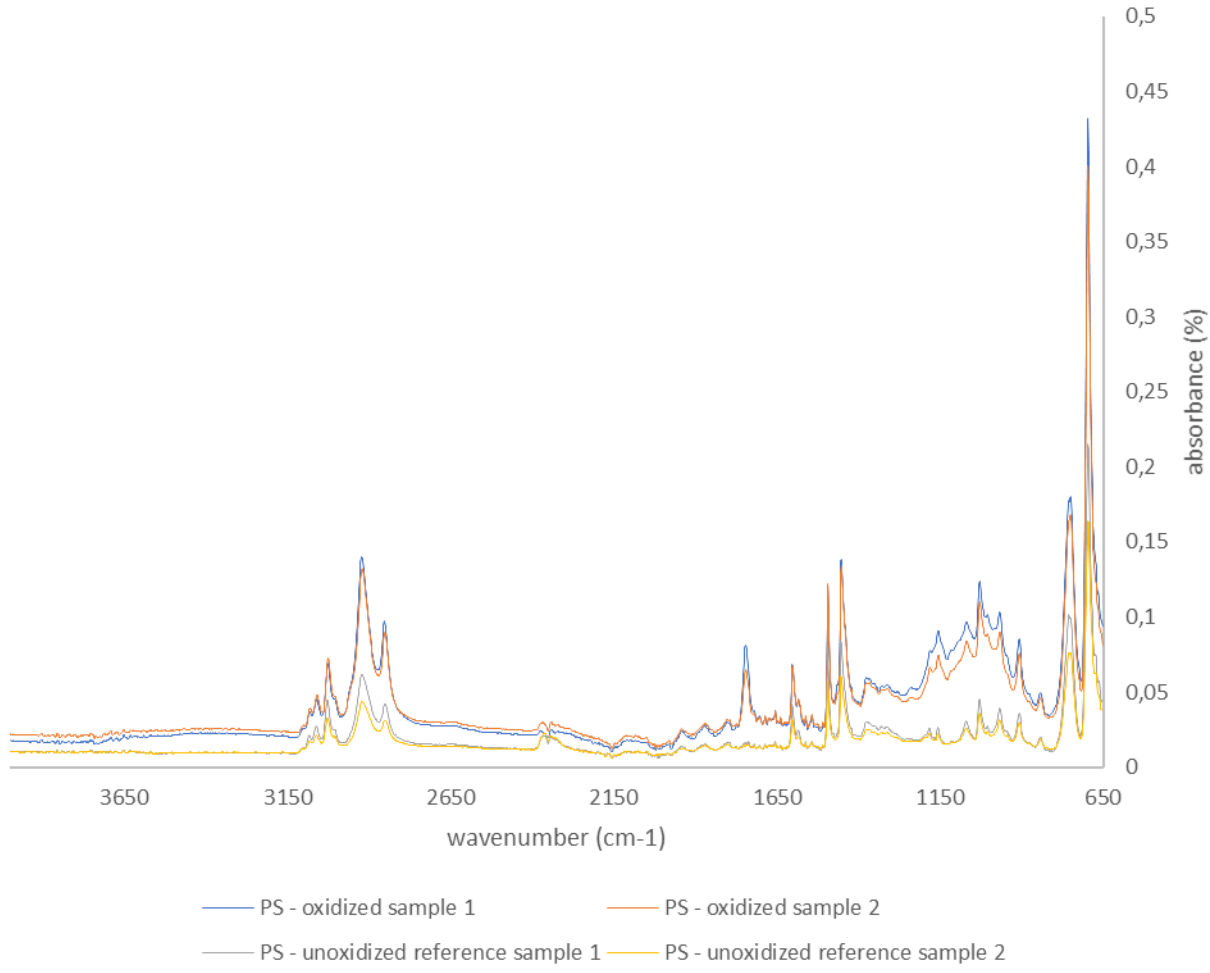


Figure 1. FTIR spectra of two polystyrene MPs before and after the oxidation and extraction procedure.

The number of missed MPs during the extraction was affected by polymer density group (df = 2, Wald Chi^2 = 12.3, P = 0.002), polymer identity, which represents polymer structure (df = 3, Wald Chi^2 = 10.4, P = 0.015) and matrix class, i.e. whether soil or compost was used as a test material (df

250 = 1, Wald Chi^2 = 6.85, P = 0.009), whereas matrix identity, i.e. which compost or which soil was
251 used (df = 2, Wald Chi^2 = 4.15, P = 0.125) and the oxidation treatment (df = 1, Wald Chi^2 = 0.60, P
252 = 0.439) had no effect. Matrix class × polymer density group interaction effect was not significant
253 (df = 2, Wald Chi^2 = 0.93, P = 0.628), which indicates that differences among polymer density
254 groups did not depend on whether they were tested in compost or soil matrix.

When the results are illustrated using MPs recovery rate (% of added items extracted), it appears that the recovery was on average lower in compost (mean recovery rate 90.5% of added items) than soil (96.5%) and on average lower for the low density polymer group (89.1%) than for the medium density (95.9%) and high density (95.5%) polymer groups (Fig. 2). Within polymer density groups, the recovery rate of polymers differed on average by 4.9% (Fig. 3).

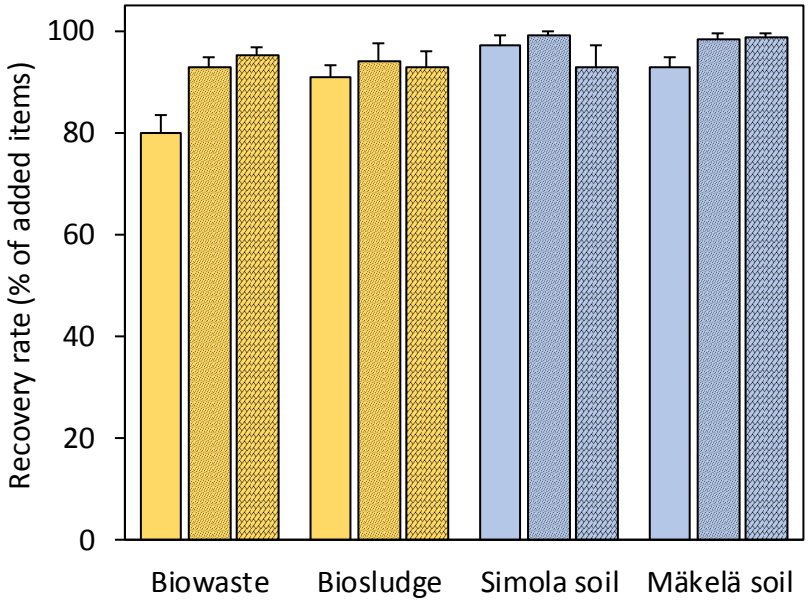


Figure 2 MPs recovery rate (mean + SE; n = 10–18) for low density (plain), medium density (dotted) and high density (tiled) polymer groups in compost (yellow) and soil (blue) matrixes as averaged across the oxidation treatment.

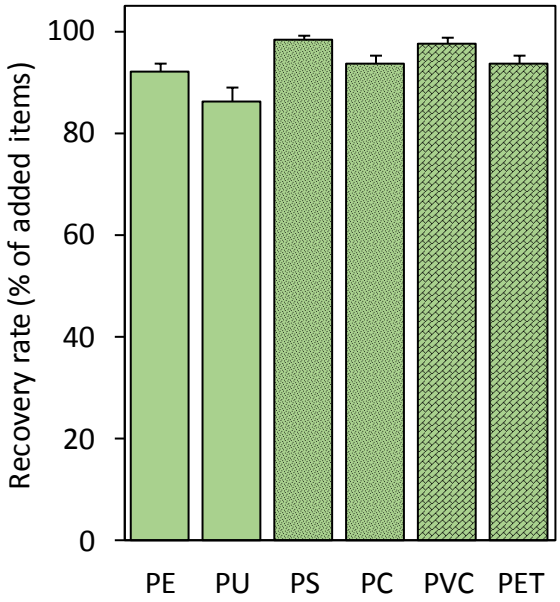


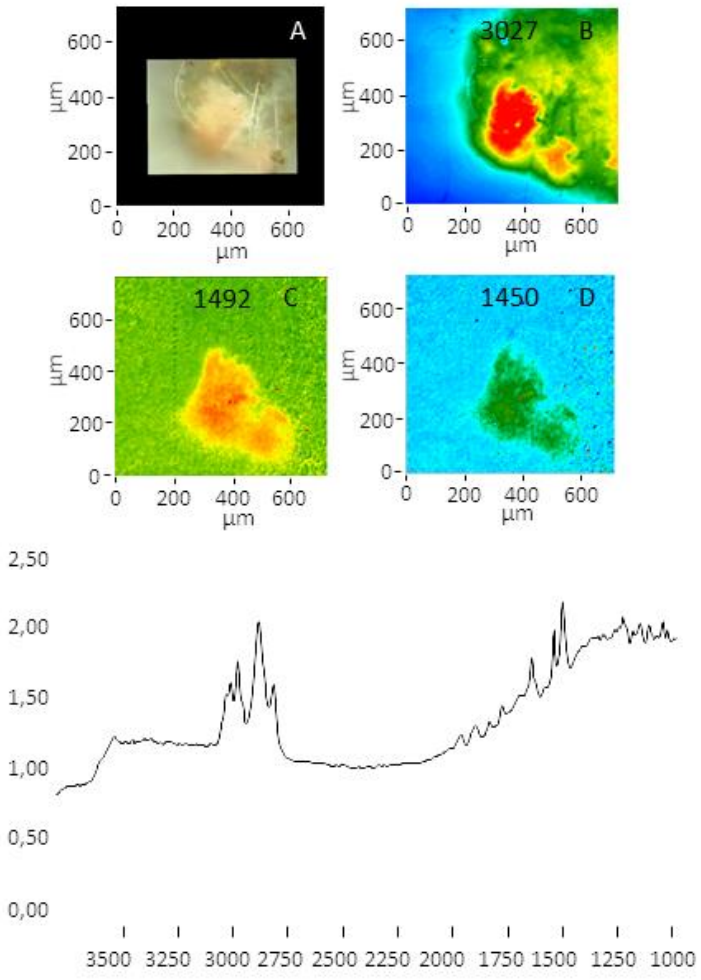
Figure 3 MPs recovery rate (mean + SE; n = 28) for each polymer in low density (plain), medium density (dotted) and high density (tiled) polymer groups as averaged across all matrices and the oxidation treatment.

These results suggest that the presented method has a high recovery rate of MPs. Compost materials seem to have a slightly lower recovery rate than agricultural field soils and the density and identity of polymers also play a role. Our results suggest that low density MPs are somewhat harder to extract than higher density MPs. However, this result needs to be treated cautiously as differences between polymers within polymer density groups were of similar magnitude as differences among the density groups. Significant differences within density groups suggest that other characteristics of polymers, on top of density, are also important for recovery, and therefore, the lower recovery rate of the low density group might simply arise of the characteristics of the two polymers other than density.

The mechanism of the extraction was called oleophilic interaction by Crichton et al. (2017), meaning attraction between the long chain fatty acids of the oil and the polymer back-bone. Our experiments suggest that this interaction is strong enough to bring many dense polymers to the oil layer, but not strong enough to extract fluorinated polymers, like polytetrafluoroethene from solid

282 samples. The oil separation method can be used for the extraction of many soil types without the
283 oxidation step. Sandy sediments, and in particular sea sand, may be an exception, however, as we
284 have earlier shown that especially the densest polymers (e.g. poly methyl methacrylate, PC, PVC,
285 PET) are attached to the sand (Scopetani et al., 2019b).
286
287 3.2 Recovery of car tire MPs
288 Car tire MPs was the only MPs type that had a low recovery rate and thus seemed to behave
289 differently from others. The recovery rate also differed between the soil (mean recovery rate
290 73%±5% of added items) and compost (30%±18%). These results suggest that the oleophilic
291 interaction between car tire MPs and olive oil is weaker than for the other polymers, or then the
292 interaction between the matrix and tire rubber is stronger than the interaction between oil and the
293 rubber. Further studies are clearly needed to clarify these findings.
294
295 3.3 Microscope FTIR analysis
296 To demonstrate the usefulness of the method for particles smaller than 300 μm, we checked the
297 occurrence of MPs in our matrix materials using the olive oil extraction. The dried filters were
298 analyzed using a Cary 620-670 Fourier transform infrared spectroscopy (FTIR) microscope
299 (Agilent Technologies). In the sample collected from the Mäkelä field, two different polymers were
300 found: one acrylonitrile (butadiene styrene) (ABS) fiber and one PS fragment. Instead, no polymers
301 were identified in the soil collected from the Simola field. This organic field was the most isolated
302 sampling area in this study, far away from the highway and other possible sources of MPs. Two
303 ABS fragments and a PE fiber were detected in the sewage sludge compost, while in the biowaste
304 compost, four polymers were recorded: one PE fragment, one PET fiber, one acrylate fragment and
305 a blend acrylate/PET fiber. These findings show that the method also works with smaller particles
306 (from 5 μm to 300 μm).

307 The FTIR spectra of the fragment found in the Mäkelä soil (Fig. 4) present intense absorptions at
308 3027 cm^{-1} (aromatic C–H stretching vibrations), 2908 and 2850 cm^{-1} (CH_2 stretching), 1944 and
309 1871 cm^{-1} (combination bands), 1743 cm^{-1} (C=O stretching), 1601 and 1492 cm^{-1} (aromatic ring
310 stretching) and 1450 cm^{-1} (CH_2 bending), which are characteristic of polystyrene (Verleye et al.,
311 2001; Noda et al., 2007; Asensio et al., 2009; Biazar et al., 2010; Olmos et al., 2014; Jung et al.,
312 2018).



313

314 **Figure 4. FTIR spectra, visible and IR imaging of a plastic fragment found in the Mäkelä field**
315 Visible light map of a plastic fragment (A). 2D FTIR Imaging maps, where the intensity of the following bands was mapped: 3027
316 (aromatic C–H stretching vibrations) (B), 1492 and 1450 cm^{-1} (carbon–carbon stretching vibrations in the aromatic ring) (C and D).
317 The chromatic scale of each map qualitatively shows the increasing absorbance intensity as follows: blue, green, yellow, red. Maps
318 have dimensions of 700 x 700 μm^2. The bottom panel shows the FTIR Reflectance spectrum of the plastic fragment, which relates to
319 a single pixel (5.5 x 5.5 μm^2) of the 2D Imaging maps.
320
321

322 The plastic fibre found in the same soil (Fig. 5) has intense absorptions at 2927 cm^{-1} (CH
323 stretching), 2240 cm^{-1} (C≡N stretching), 1450 and 1369 cm^{-1} (CH_2 and CH bending), which are

characteristics of polyacrylonitrile (Coates, 2000; Verleys et al., 2001), as well as bands at 1735 cm^{-1} (CO stretching) and 1246 cm^{-1} (C-O stretching), typical for acrylates (Balamurugan et al., 2004; Duan et al., 2008; Ramesh et al., 2017; Jung et al., 2018). This makes us hypothesize that the fibre is acrylonitrile-acrylate copolymer.

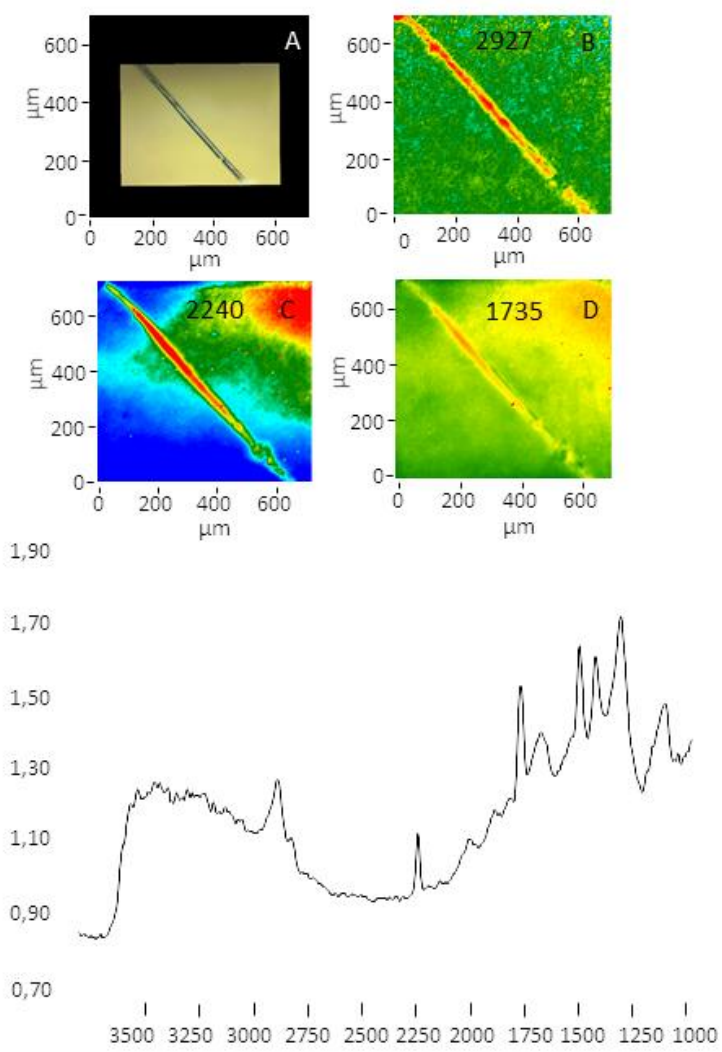


Figure 5 FTIR spectra, visible and IR imaging of a plastic fibre found in the Mäkelä field
Visible light map of a plastic fibre (A). 2D FTIR Imaging maps, where the intensity of the following bands was mapped: 2927 (CH stretching) (B), 2240 (C-N stretching) (C), and 1735 cm^{-1} (C=O stretching). The chromatic scale of each map qualitatively shows the increasing absorbance intensity as follows: blue, green, yellow, red. Maps have dimensions of 700 x 700 μm^2. The bottom panel shows the FTIR Reflectance spectra of the plastic fibre and of the neighbouring filter. Each spectrum relates to a single pixel (5.5 x 5.5 μm^2) of the 2D Imaging maps.

4 Conclusions

A clear understanding of the risks associated with MPs pollution requires reliable and accurate data on MPs occurrence in environmental samples.

339 The method presented here for the extraction of MPs is easy and inexpensive and can be applied to
340 isolate various polymers from solid samples like soil and compost.

341 The method was validated using six different micro-polymers: PE, PS, PVC, PC, PET and PU and
342 low, medium and high density polymers reached a mean recovery rate of 90% ±2%, 97%±5% and
343 95%±4%, respectively. The density of the polymer seems to have only a small role in the recovery.
344 The method is a good alternative to density separation methods as it provides as good recovery rates
345 but no hazardous salt solutions are needed. Furthermore, density separation-based methods are often
346 unable to extract high density polymers like PET or PVC, unlike the method presented here. The
347 olive oil methodology can be applied to MPs of size 0.2-5 mm if a bench-top FTIR is used for the
348 identification and down to 5 µm with a microscope-FTIR instrument.

349 Lares at al. (2019) showed that the weakness in the method presented by Crichton et al. (2017) is
350 that ethyl alcohol is not able to remove all canola oil traces, thus hampering the identification with
351 Raman and FTIR spectroscopy. Furthermore, Lares et al. (2019) pointed out that using separatory
352 funnels, a technical solution also adopted by Mani et al. (2019), limits the upper size of recovered
353 MPs and may not be suitable for samples that contain larger particles (e.g. compost matrix) able to
354 obstruct the funnel. We showed that after oxidation and flushing the samples with hexane, neither
355 oil traces nor spectral modifications were detected that could affect polymer identification. By
356 freezing the samples, we could also avoid separation funnels and could collect the oil layer without
357 a risk of mixing the phases.

358 When analyzing non-spiked fluvial suspended solid samples, Mani et al. (2019) noticed that a
359 majority (76%) of PS MPs was not retained in the oil layer, but found in the solid phase. That we
360 reached higher PS MPs recovery rate with our method and found PS MPs in non-spiked soil and
361 compost samples suggest that oil olive may have better affinity for PS than the castor oil. In fact,
362 our results suggest that olive oil may have high affinity for a wide set of polymers. Clearly though,

363 further studies are needed to test the method with different sample matrices and to improve the
364 efficiency in the extraction of car tire rubber and fluoropolymers.
365

Authors contributions section

Costanza Scopetani: Conceptualization; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing - original draft; Writing - review & editing. **Jukka Pellinen**: Conceptualization; Methodology; Project administration; Supervision; Validation; Writing - original draft; Writing - review & editing. **David Chelazzi**: Investigation; Writing - original draft; Writing - review & editing. **Juha Mikola**: Formal analysis; Writing - original draft; Writing - review & editing. **Alessandra Cincinelli**: Writing - original draft; Writing - review & editing. **Ville Leiniö**: Investigation; Writing - original draft. **Reijo Heikkinen**: Conceptualization; Methodology.

Declaration of competing interest

None.

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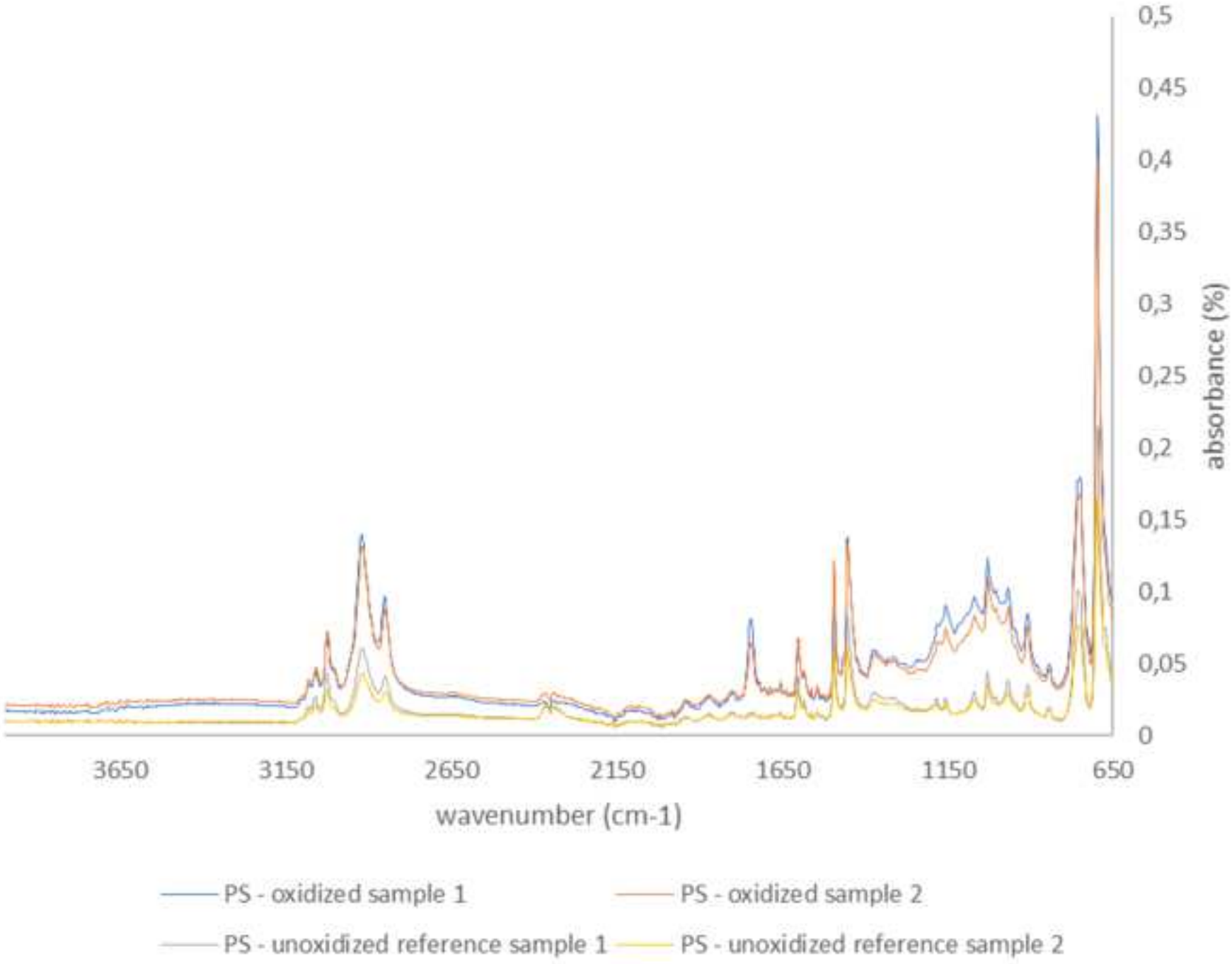

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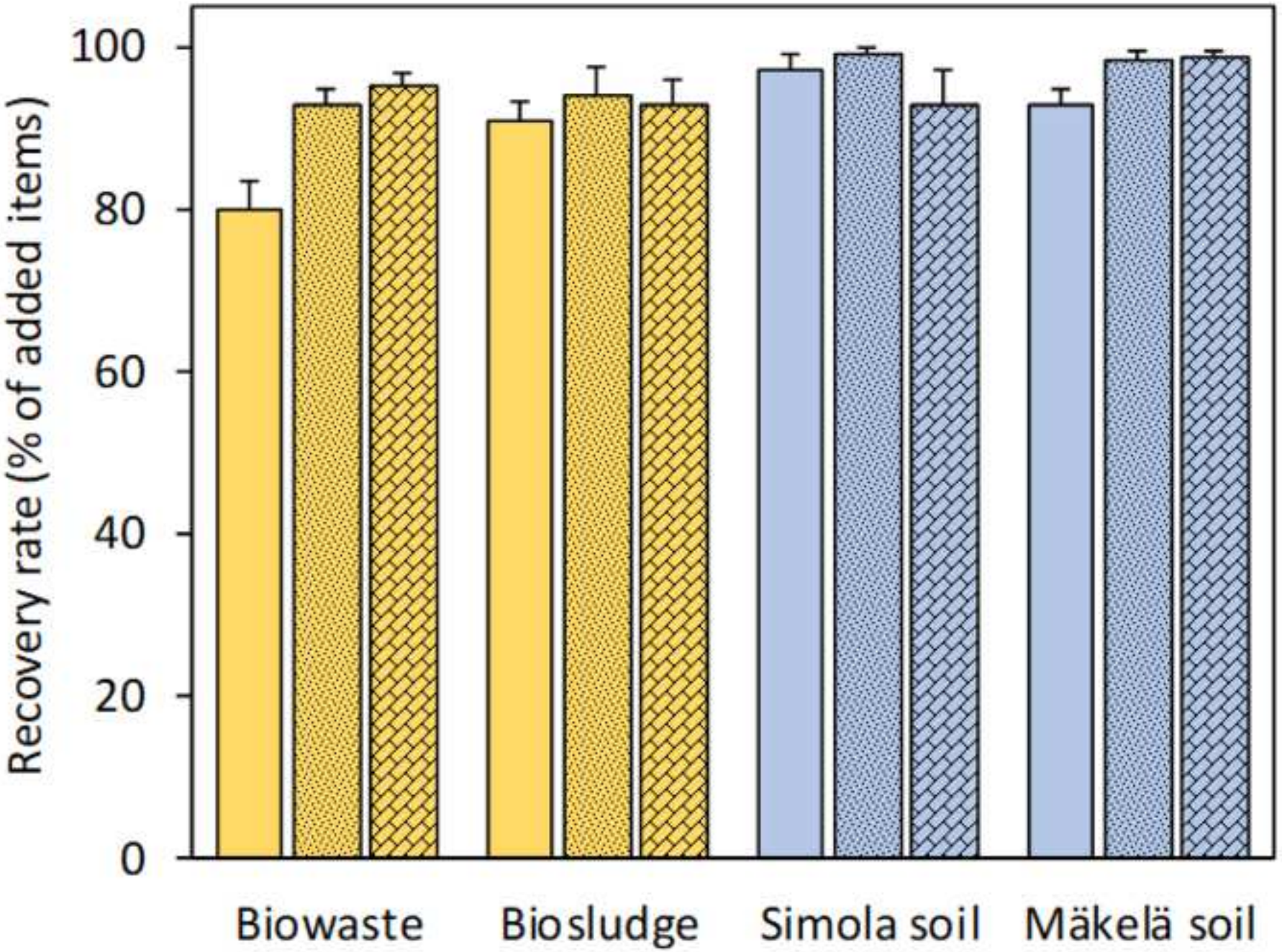

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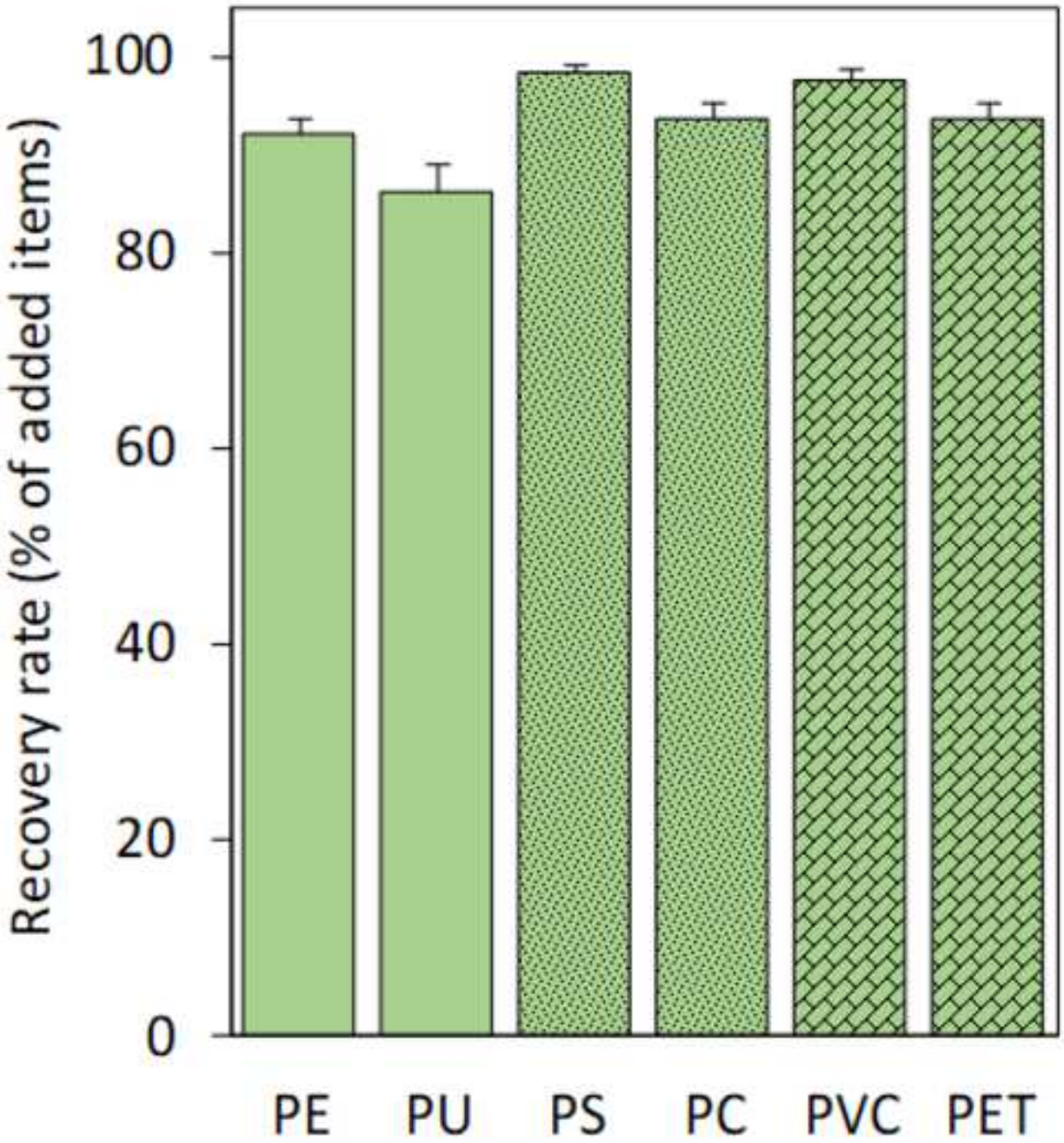

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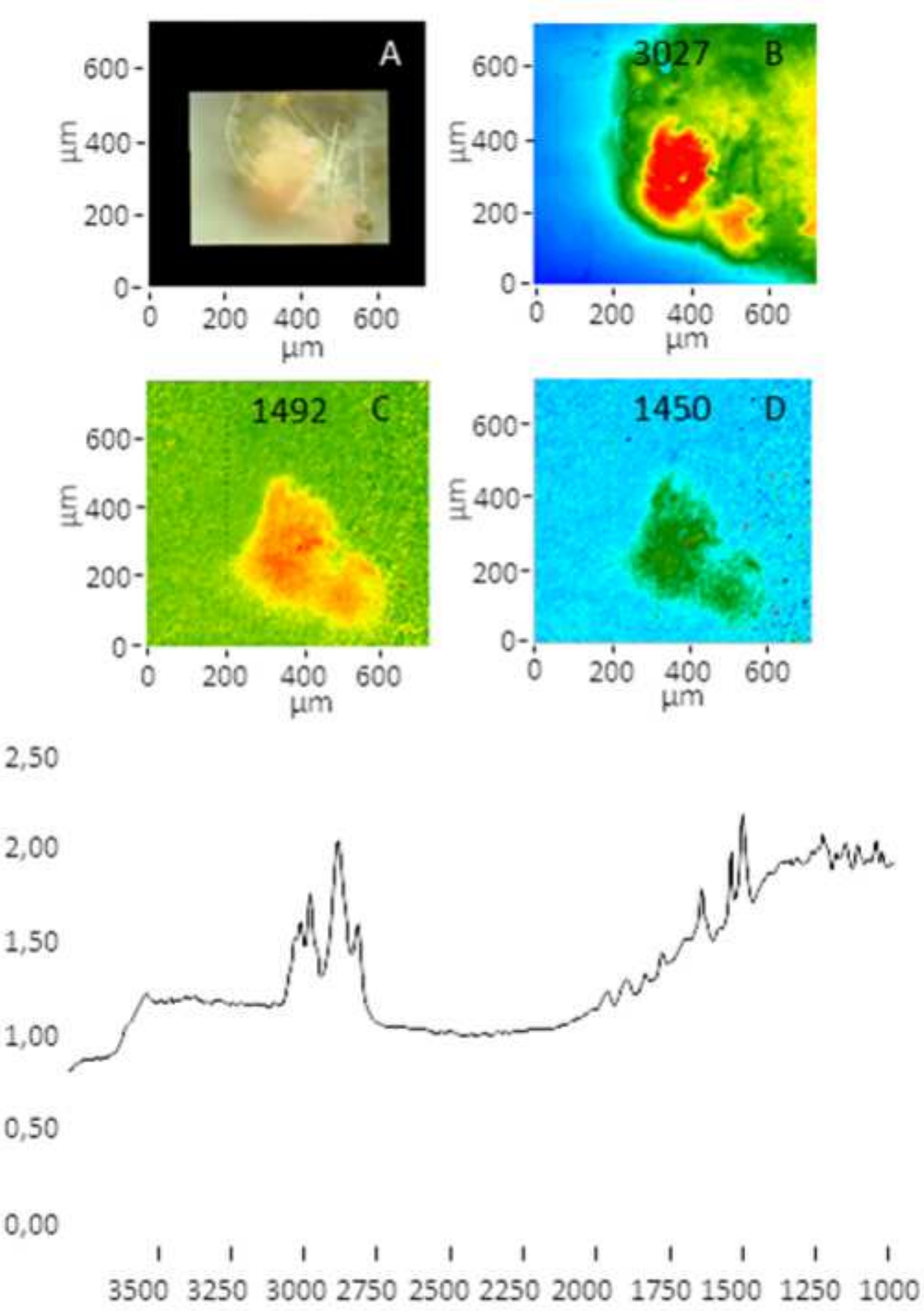

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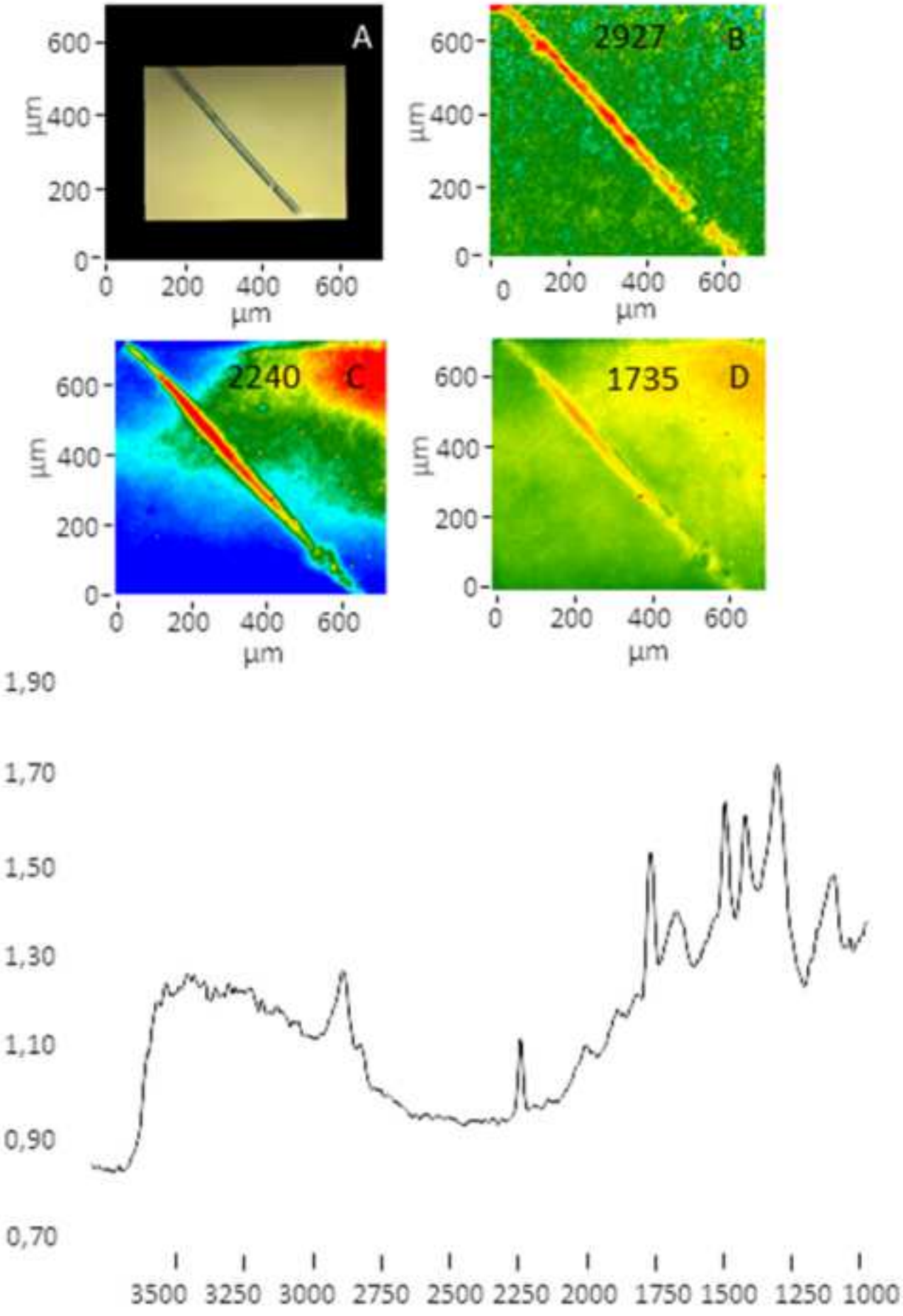

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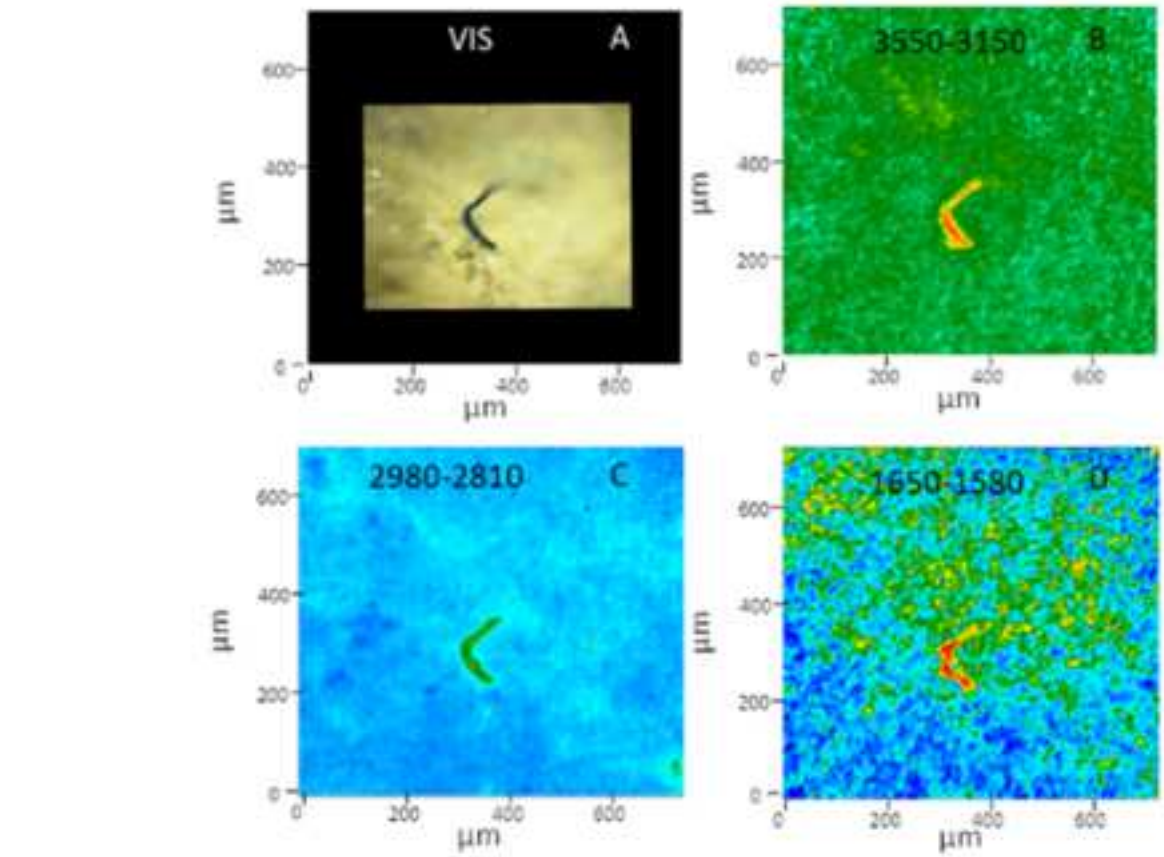

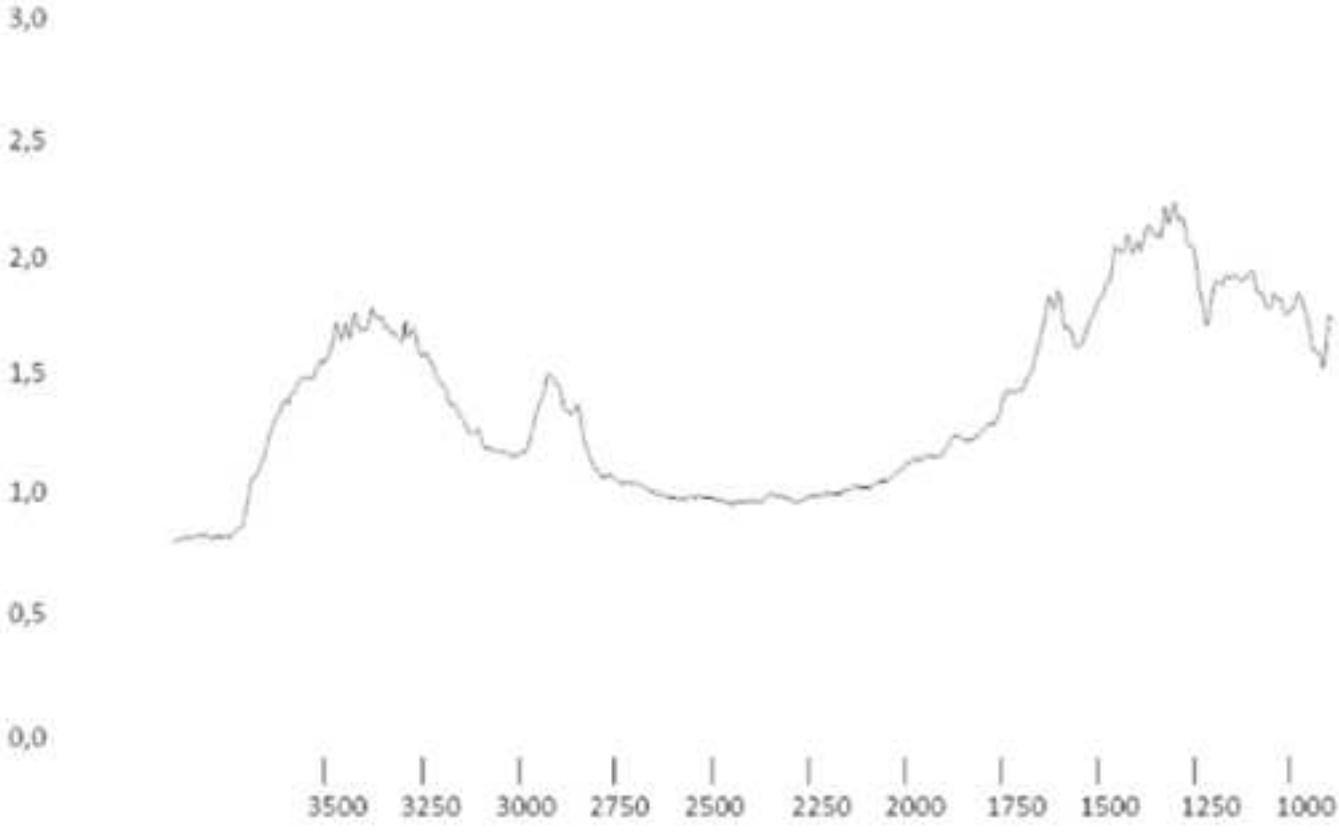

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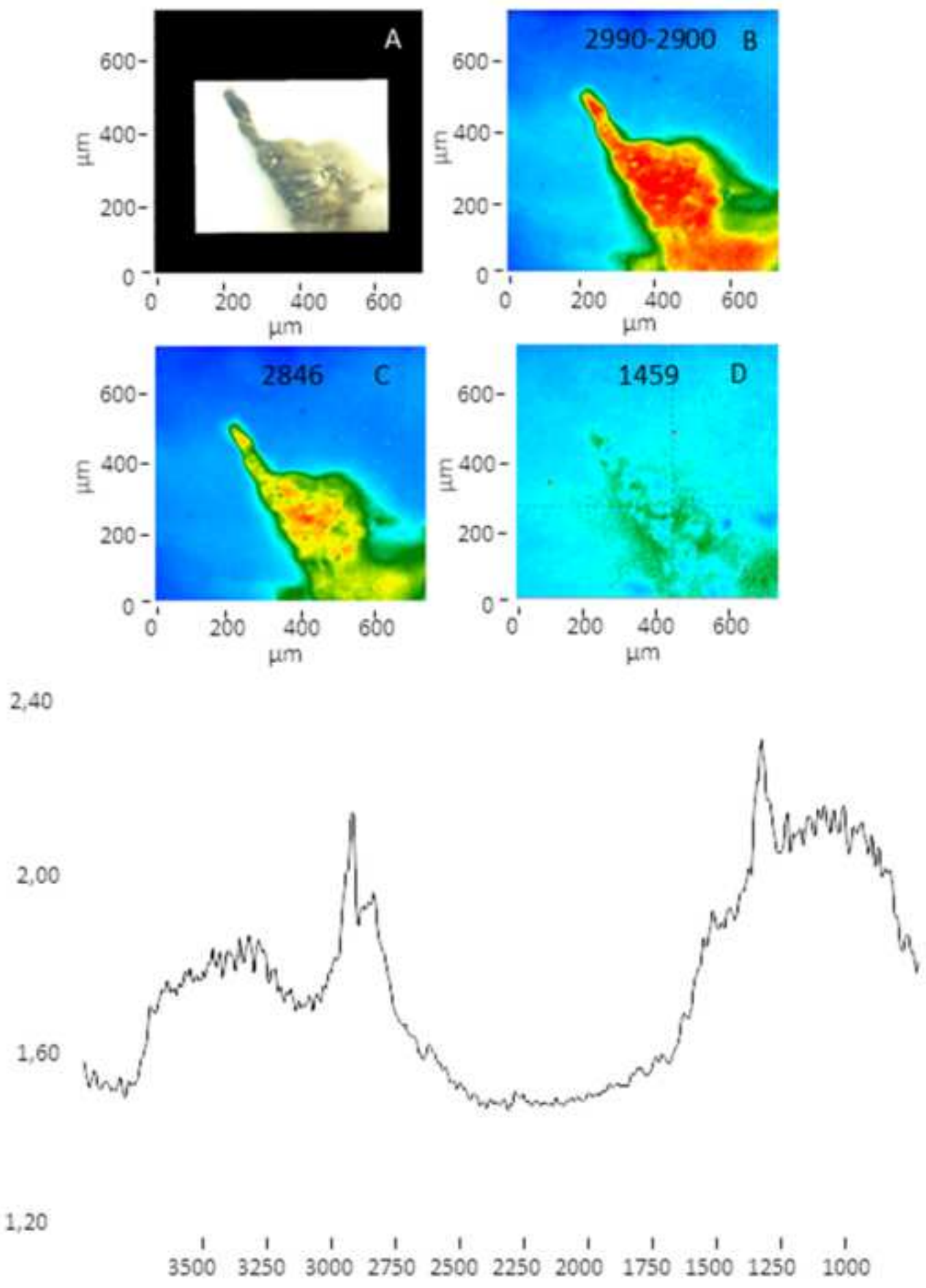

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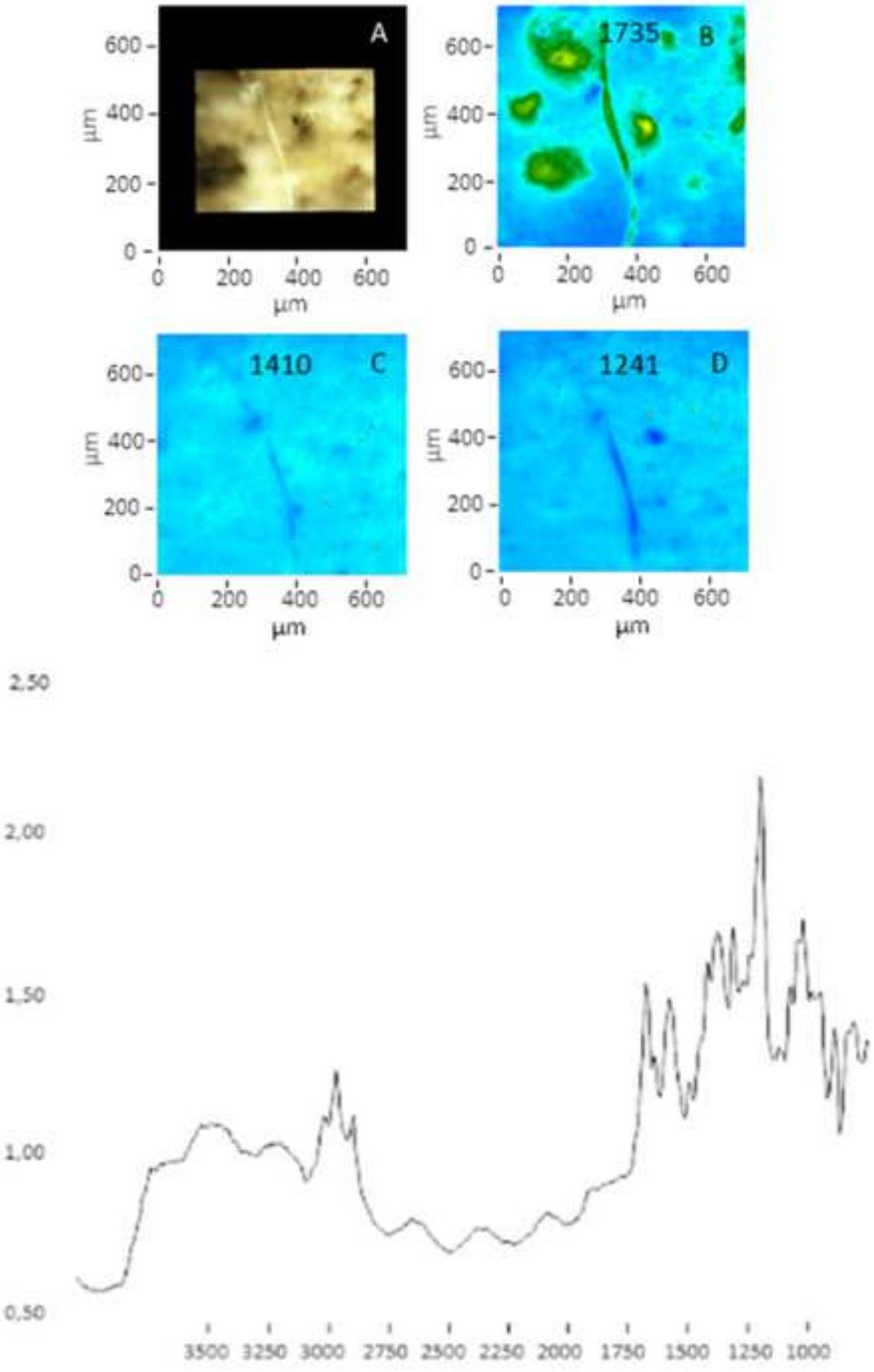

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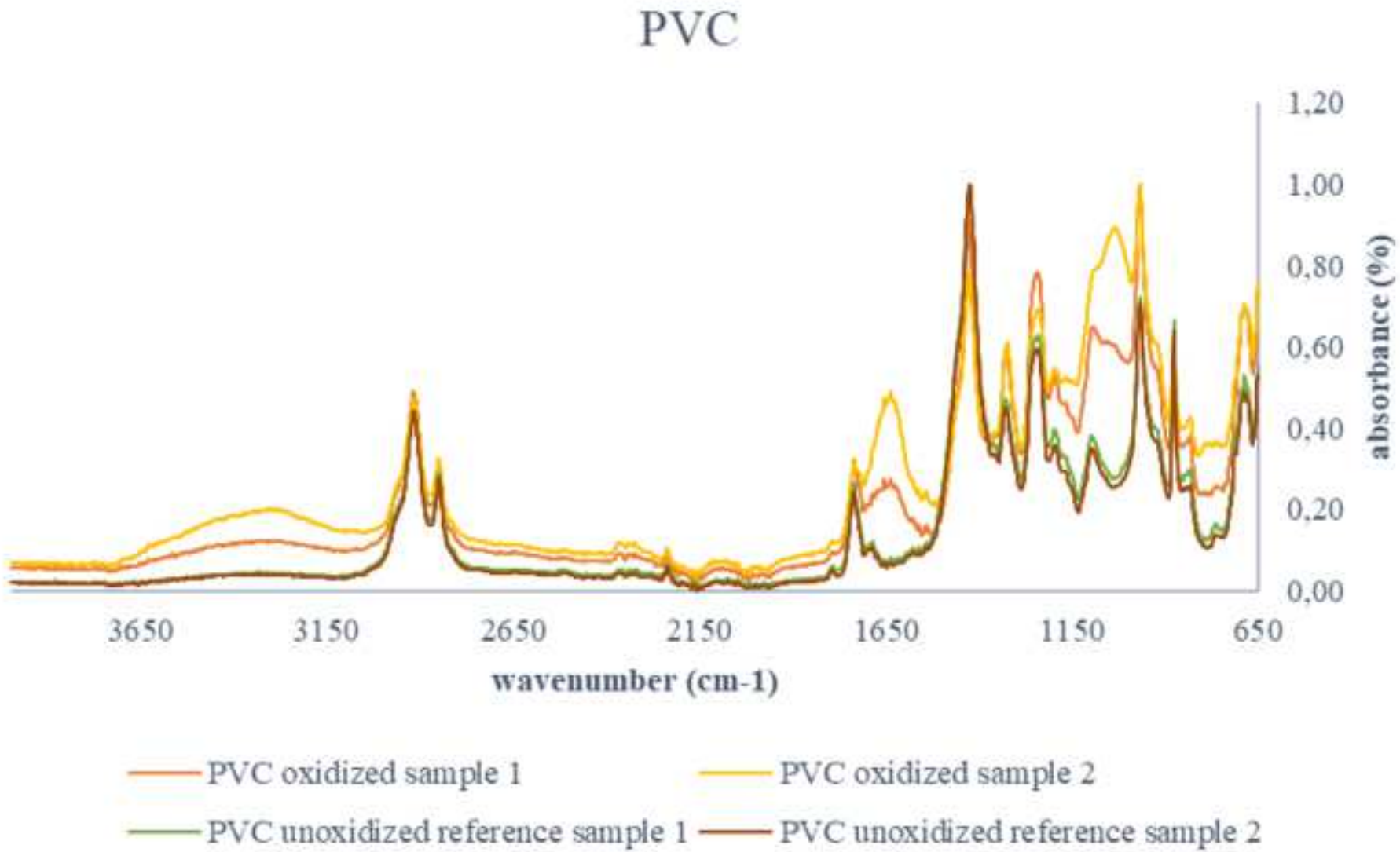

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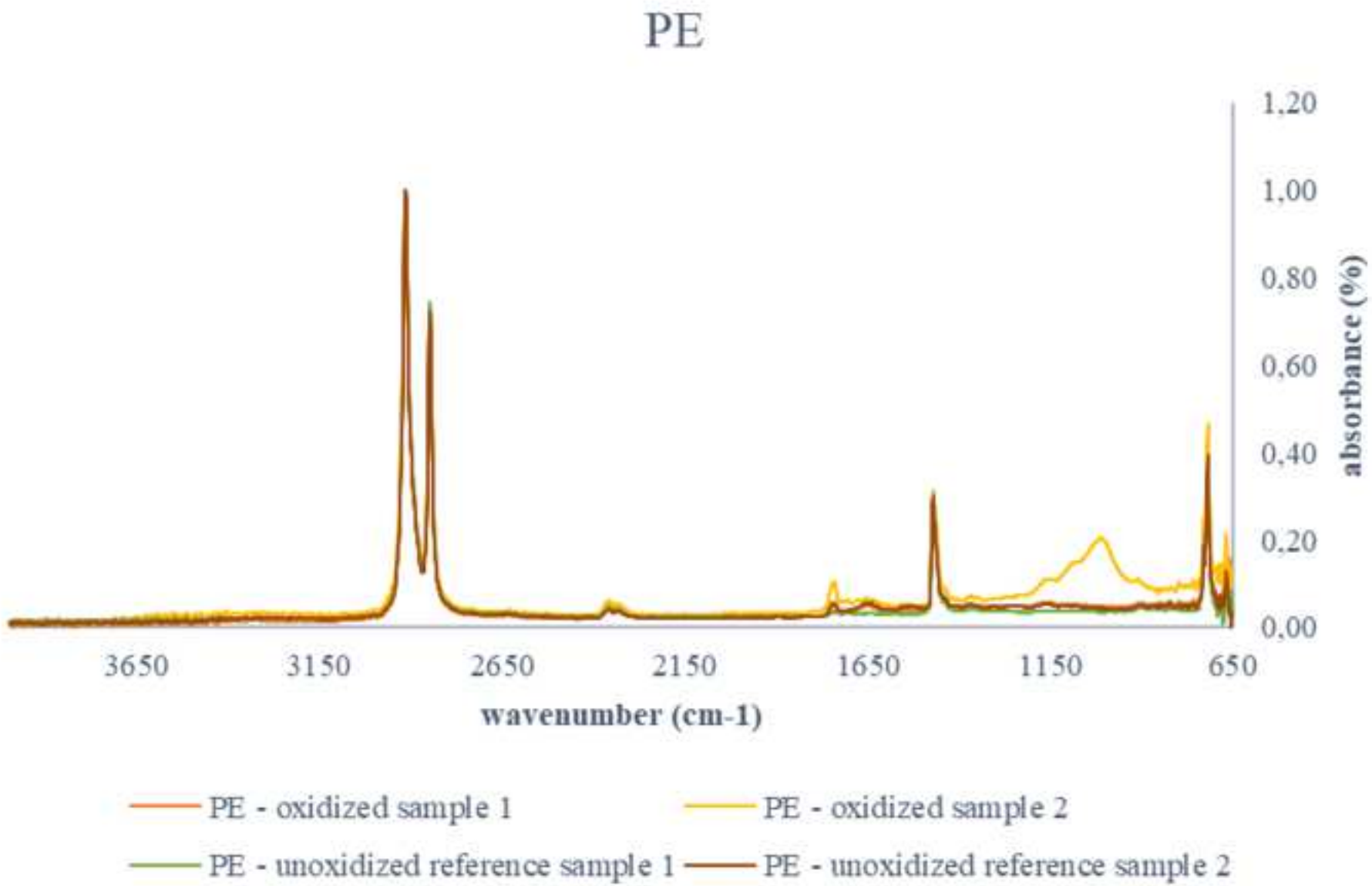

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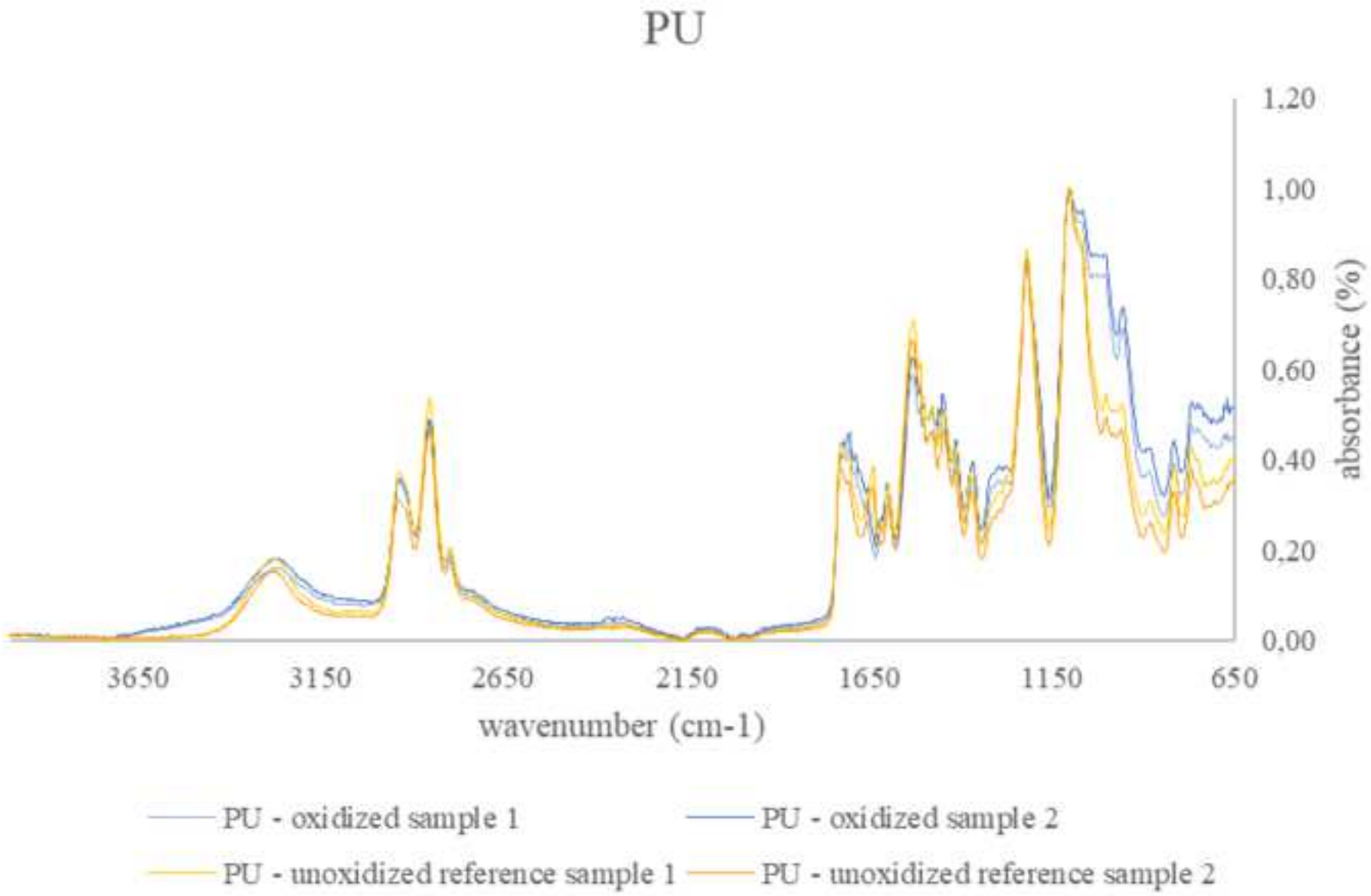

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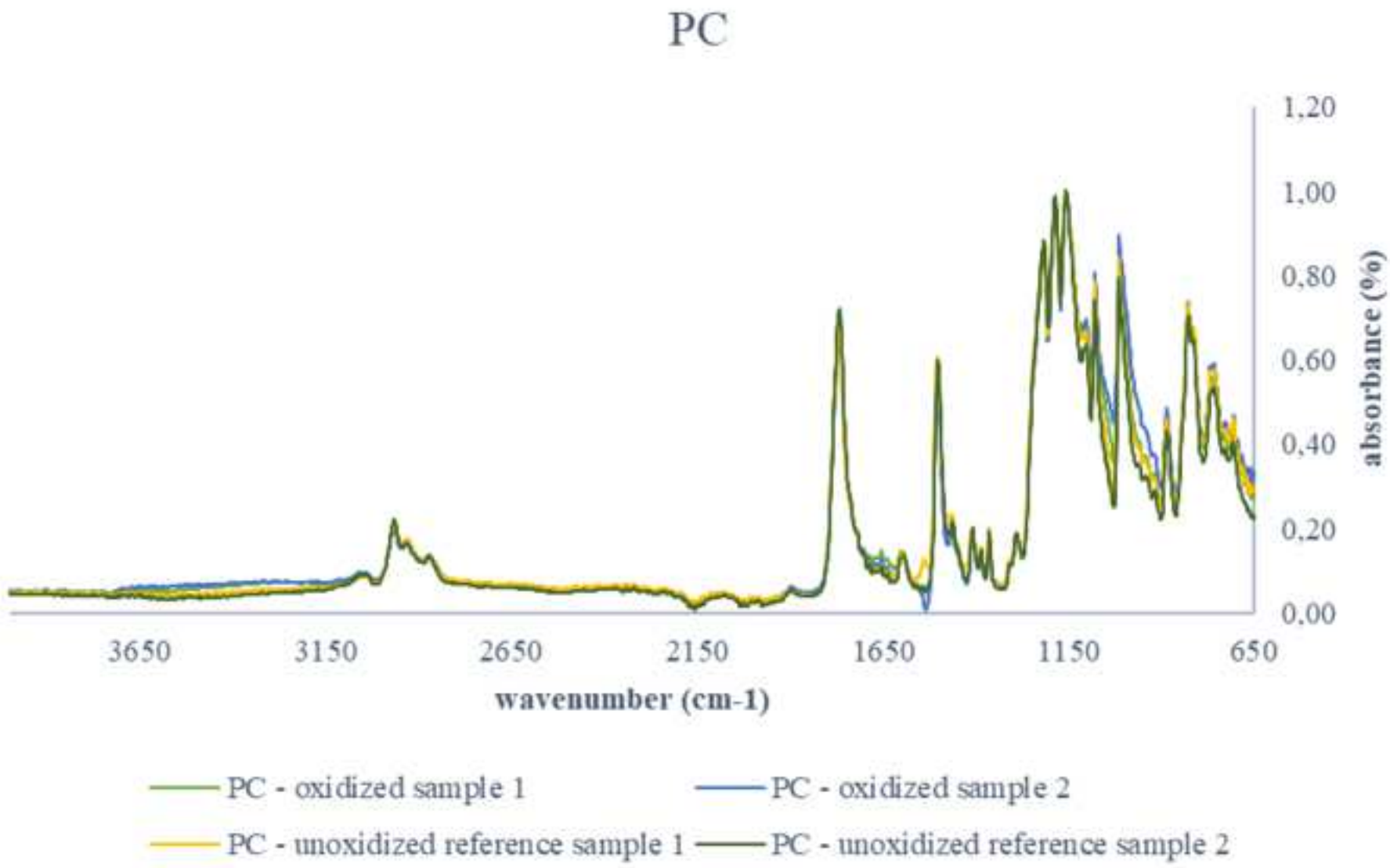

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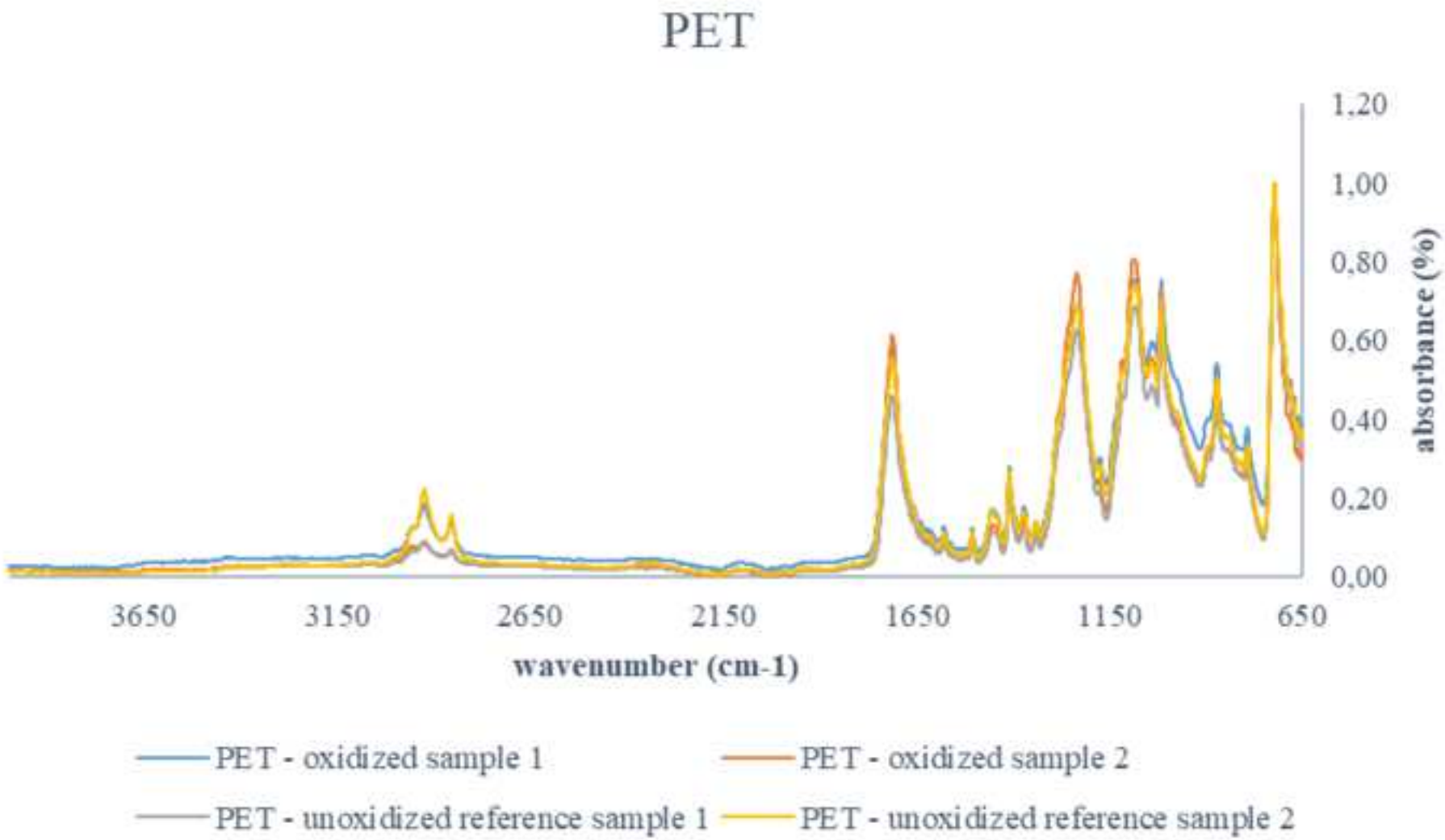

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