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Microplastics in the abyss: a first investigation into sediments at 2443-m depth (Toulon, France)

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Reviewers' comments:

Reviewer #1: This paper aims to present an investigation of microplastics (<5 mm) distribution performed in the bottom sediments of the abyssal plain off the coast and the canyon of Toulon (France). The methodology followed is based on chemical and physical characterization of four samples of deep-sea sediment collected at the depth of 2443 m during the sea operations carried out by the French oceanographic cruises for the KM3NeT project. Items were extracted from sediments by density separation and analyzed by optical microscope and μ Raman spectroscopy.

I consider this topic very interesting and relevant, at present, for the possibility of bringing a new contribution to overcome the lack of knowledge about microplastic pollution in deep marine environments. In fact, regarding the actual and important problem of microplastic contamination of sediments, the sea bottom, especially at great depths, is known to be the final site of arrival and deposition of microplastics, however little is known about the contamination in deep environments because of their inaccessibility. Furthermore, this type of results is very important by supplementary reasons, as makes any information that can be obtained from sediments collected in these remote environments valuable and useful for understanding deep circulation mechanisms.

In my opinion, the work is developed correctly and in a proper way, the bibliography is appropriate and satisfactory, and nothing to say about the English language. At the present form the manuscript is well written and organized, and therefore is ready to be published, considering that I believe it fits in the scope of Environmental Science and Pollution Research. Therefore, I advise the manuscript to be published in the state in which it is now.

We thank Reviewer #1 for his comments and we are pleased that both the work done and the final draft were appreciated.

Reviewer #2:

Line 18) As this study investigates MP pollution at abyssal sediments, representing just a fraction of the worldwide environments, the hypothesis of a ubiquitous pollution can't be confirmed just by the outcomes of this study. It rather confirms the presence of MP in abyssal sediments in the Mediterranean Sea.

We have modified the text, as suggested.

Line 147) The reviewer agrees with the choice to increase the % of items to analyse for a better representation of the sample. Why 40%?

We have chosen to analyse 40% of the items found because the 5-10% suggested by the references seemed too low for us and because 40% was for us a good compromise between the time and cost of the analysis and the possibility of obtaining good results in the characterisation of item composition. We have added a detail explaining this choice in the text.

155) the reviewer is wondering why the authors decide not to highlight the polymer composition of the polymers analysed via μ Raman, is there any specific reason?

We have modified the text in the Results section. The polymer composition obtained by μ Raman analysis of items was reported in the text. We did not include a figure on the polymer composition of the items analysed because the description of the μ Raman results is given in the text and a figure seemed to us redundant. Furthermore, we found only three plastic polymers, so we felt that the description in the text was sufficient to highlight our results. If the Reviewer meant something else, we are willing to modify the text further.

Reviewer #3: This is an interesting study and the authors have collected a unique dataset. The paper is generally well written and structured. "Supplementary Information" section is also very useful.

However, I suggest authors to make more in-depth analyses of the data. They should focus on key critical points. The manuscript presents only experimental results but here is non comparison with previous studies (there are few studies carried out at close depth.).

Bearing in mind that our study is a short communication because it is based on only one sample (divided into 4 sub-samples), we have focused our discussion on comparing our results with results found during other deep sediment surveys and making all the considerations that were relevant to us and that might be useful to other researchers. Thanks to the Reviewer's comment, we have realised that in the discussion we had not taken into account the type of polymers found in the samples and therefore, we have added a comment on this important aspect of the results. In general, the number of studies focused on microplastics in deep sediments available in bibliography is very limited and this consequently limits the comparison with other deep sediments.



Cover letter

On October 2019, during a sea campaign organised in the field of the French oceanographic cruises in the MEUST NUMerEnv deep sea infrastructure that hosts the project KM3NeT, bottom sediments were sampled at the depth of 2443 m on the abyssal plain off the coast of Toulon (South France) thanks to the robotic arm of the HROV Ariane. This gave us the opportunity to characterise the sediments of this deep environment from a geological and chemical point of view and to investigate the presence of microplastics, which are now present in all types of environments, in a complex hydrodynamic context such as the Northwest Mediterranean Sea.

I should point out that this Short Communication was initially submitted to another Journal for a Special Issue; the four Reviewers that reviewed the manuscript gave these opinions: two indicated “out of topics”, and two indicated “minor revision”. The Editor then determined a “major revision” of the manuscript. Then we made all the changes suggested by the Reviewers (if we felt they needed to be made) and resubmitted it as requested. The Editor's final decision was “rejected because out of topics”. We have revised the article further to improve it and have decided to submit it to your Journal for evaluation.

I have not submitted my manuscript to a preprint server before submitting it to *Environmental Science and Pollution Research*

Genoa, 13/07/2021

The corresponding author:

1 **Microplastics in the abyss: a first investigation into sediments at 2443-m depth (Toulon, France)**

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9 **Abstract:** Plastic and microplastic pollutions are known to be widespread across the planet in all types of
10 environments. However, relatively little about microplastic quantities in the deeper areas of the oceans is
11 known, due to the difficulty to reach these environments. In this work, we present an investigation of
12 microplastics (<5 mm) distribution performed in the bottom sediments of the abyssal plain off the coast and
13 the canyon of Toulon (France). Four samples of deep-sea sediment were collected at the depth of 2443 m
14 during the sea operations carried out by the French oceanographic cruises for the KM3NeT project. The
15 chemical and physical characterisation of the sediment was carried out and items were extracted from
16 sediments by density separation and analysed by optical microscope and μ Raman spectroscopy. Results
17 show microplastics in the deep-sea sediments with a concentration of about 80 particles L⁻¹, confirming the
18 hypothesis of microplastics spread **to abyssal sediments in the Mediterranean Sea.**

19
20 **Keywords:** microplastics; μ Raman; deep sea; sea currents; KM3NeT project; Toulon (France)

21
22 **1. Introduction**

23 It is known that plastic litter, and among these the microplastics (items with size between 1 μ m and 5 mm),
24 impact any environment, terrestrial (Dioses-Salinas et al. 2020; Hoffman and Hittinger 2017), aerial (Dris et
25 al. 2016; Zhang et al. 2020) and marine (Alomar et al. 2016; Fossi et al. 2012; Ruiz-Orejon 2018). Since the
26 2000s, attention on microplastics has grown exponentially (Schmid et al. 2018) and there are many
27 researchers and teams conducting studies on microplastic sampling and characterisation in environment
28 (Gago et al. 2018; Yu et al. 2019), their effects on animals (Lusher et al. 2017) and humans (Campanale et al.
29 2020), and investigating their relationship with pollutants, such as metals, hydrocarbons etc. (Constant et al.
30 2019; Kutralam-Muniasamy et al. 2021; Perea et al. 2020; Tesán Onrubia et al. 2021). In marine
31 environment, microplastics are sought in the beach areas (Antunes et al. 2018; Bosker et al. 2017; Browne et
32 al. 2011; Constant et al. 2019; Pieper et al. 2019) and at different depth from the sea surface along the water
33 column (Andrady 2011; Bagaev et al. 2018; Bains et al. 2018; Pan et al. 2019; Zheng et al. 2019) and down
34 to sediments (Anderson et al. 2016; Andrady 2011; Bergmann et al. 2017; Zheng et al. 2017); their transport

35 and behaviour related to dynamics is investigated as well as their degradation process due to seawater and
36 microorganism action (Browne et al. 2011; Cutroneo et al. 2020a).
37 Concerning sediments, studies applied to their content in microplastics are mainly carried out in bottom
38 sediments of coastal areas (Cutroneo et al. 2020a; Ruiz-Compean et al. 2017; Zobkov and Esiukova 2017) or
39 on the continental shelf (Mu et al. 2019), while it is very difficult to have information on sediments at great
40 depths due to the poor accessibility of deep environments and, consequently, the very high costs of such
41 investigations. Despite difficulties, some recent studies have focused their attention on abyssal sediments,
42 verifying the presence of macrolitter by video analysis (Bergmann and Klages 2012; Chiba et al. 2020) and
43 testifying the presence of microplastics by sediment sampling and analysis even at high depths (Courtene-
44 Jones et al. 2020; Gerigny et al. 2019; Van Cauwenberghe et al. 2013; Zhang et al. 2020).
45 In this context, the testimony of the presence of microplastics in deep sediments off the coast of Toulon
46 ($42^{\circ}48.352' \text{ N} - 006^{\circ}01.613' \text{ E}$; France; **Fig. 1**) is presented here. Sediment sampling was carried out on 21
47 October 2019 during a sea campaign organised in the field of the French oceanographic cruises in the
48 MEUST NUMerEnv deep sea infrastructure that hosts the project KM3NeT (Adrián-Martínez et al. 2016), a
49 research infrastructure consisting of new generation neutrino telescopes in the deepest areas of the
50 Mediterranean. In addition, the chemical and physical characterisation of sediments was carried out by
51 analysing grain size, chemistry, mineralogy, and organic and inorganic matter content to frame the deep
52 environment and gather as much information as possible on it.

53 **2. KM3NeT infrastructure and study area**

54 KM3NeT is a new Research Infrastructure consisting of a network of deep-sea neutrino telescopes in the
55 Mediterranean Sea (KM3Net 2021). Its main objectives are (1) the discovery and subsequent observation of
56 high-energy neutrino sources in the Universe and (2) the determination of the neutrino mass ordering and
57 other fundamental physics searches, such as sterile neutrinos, neutrino non-standard interactions, dark
58 matter, and quantum gravity effects. The KM3NeT infrastructure allows performing also deep-water
59 biological studies, such as bioluminescence monitoring and acoustic detection of marine mammals.
60 Two deep-sea sites for the telescope deployment have been selected, namely Toulon (France) and Capo
61 Passero (Italy). The selection was based on the optical properties of the water, distance to shore and local
62 infrastructure. The samples analysed and presented in this paper come from the Toulon site (Fig. 1), at a
63 depth of about 2443 m.

64 The study area is located in the abyssal plain 10 km off the coast of Toulon (south-eastern France) and at the
65 base of the canyon that characterised the continental slope in front of Toulon. The abyssal plain is mainly
66 composed of fine-grained sediments and characterised by scarcity in benthic macrofauna and flora (Cartes et
67 al. 2004). From an oceanographic point of view, the study area lies on the border between the Ligurian-
68 Provençal basin and the Gulf of Lion that is characterised by the presence of a surface cyclonic circulation
69 50 km wide, the Northern Current also known as the Liguro-Provençal-Catalan Current, which flows mainly
70 counter-clockwise along the coast (Millot et al. 2005) (Fig. 1). The abyssal plain is an area of formation of
71 the Western Mediterranean Deep Waters that flow towards Catalonia and Balearic Islands Cisneros et al.

72 2019). Due to the cyclonic gyre trapping water in the middle of the basin and to the significant winter heat
73 losses due to the strong northern winds, the Gulf of Lion is affected by periodically massive events of dense
74 shelf water cascading that impact on sediment dynamics and suspended particle transport on the slope and
75 abyssal plain (Durrieu de Madron et al. 2017). These dense events erode the sediments along the slope and
76 transport a large quantity of suspended particles into the lower layer; they are associated with strong mixing
77 of the water down to the sea bottom and strong currents near the bottom in the horizontal diffusion phase of
78 the newly formed deep waters (Durrieu de Madron et al. 2017).

79 The marine area hosts military activities, Toulon being a major French military harbour, and the site is
80 subject to maritime traffic with ferry lines to Corsica. The fishing activity is very limited in the area.

81 **3. Materials and Methods**

82 On 21 October 2019, superficial bottom sediments were taken at the depth of 2443 m. Sediment sampling
83 was performed during a dive of the HROV Ariane operated from the RV L'Europe of Ifremer (EMSO-
84 KM3NET-LIGURE-OUEST 2019 EU)(**Fig. 2**). Originally, it was planned to use a standard corer device to
85 perform the sampling. Unfortunately, the sediment was too smooth and did not stay in the corer after the
86 sampling. We changed the sampling method and, therefore, we took sediment samples directly with the grab
87 of the ROV and put the sediments in an open plastic container placed in the basket of the ROV. The
88 container was previously coated with an aluminium foil to avoid plastic contamination of the sample. Since it
89 was not possible to also coat the container lid with aluminium foil, knowing the composition of the lid in
90 terms of plastic polymer, any finding of this polymer would have been subtracted from the results of the
91 analysed samples. In **Online Resource 1**, it is possible to see how the robotic arm of the HROV Ariane picks
92 up and stores the sample from the bottom; for short, the video playback speed is multiplied by 4 times. The
93 robotic arm took 3 portions of surface sediment approximately 5 cm thick with the grab (Fig. 2) and
94 collected them on the container. Once the ROV was recovered on the vessel, sediment was taken from the
95 container using a metallic tool and divided in four samples stored in glass jars with aluminium-coated cap.
96 From the moment of the sample recovery on board the ship until the moment of analysis, samples were
97 stored at 4 °C.

98 A portion each of the four original sediment samples was placed in Petri dishes and dried in a thermostatic
99 oven at 60 °C. Afterwards, each portion was divided in three for the grain-size, mineralogical and chemical
100 analyses to characterise the sampled sediments. Dimensional, mineralogical, and chemical analyses were
101 conducted following methodologies described in Cutroneo et al. (2017).

102 For grain-size characterisation, samples were firstly wet-sieved to divide the fine fraction (particle diameter
103 (\emptyset) <63 μ m) from the coarse fraction (\emptyset >63 μ m), and then the coarse fraction was dried and subsequently
104 dry-sieved to determine grain-size classification considering the following size classes: \emptyset <63 μ m,
105 63< \emptyset <125 μ m, 125< \emptyset <250 μ m, 250< \emptyset <500 μ m, 500< \emptyset <1000 μ m, 1000< \emptyset <2000 μ m, \emptyset >2000 μ m
106 (Cutroneo et al. 2017).

107 For the mineralogical characterisation of sediments powder X-ray Diffraction (XRD) analysis was carried
108 out on pre-grounded sediment with an agate pestle, with Co K α radiation (current 20 mA, voltage 40 kV).

109 Quantification of minerals was performed according to the Reference Intensity Ratio (RIR) method (Zhout et
110 al. 2018). Dimensional and mineralogical results were expressed in %.

111 Chemical analysis was carried out to quantify the metal and trace elements concentration of sediments (Al,
112 Fe, Mg, As, Cd, Co, Cu, Cr, Mn, Ni, Pb, V, Zn, Ag, Hg). Inductively coupled plasma mass spectrometry
113 (ICP-MS) analysis was applied to 0.5 g of dry and ground sediment sample after modified Aqua Regia
114 digestion (ISO 15587) to determine trace elements, while major element concentrations were investigated
115 with an inductively coupled plasma-atomic emission spectroscopy (ICP-AES) on 1 g of sample digested in
116 hot aqua regia. Chemical analyses were carried out by Bureau Veritas Mineral Laboratories (Canada;
117 ISO9001 Quality Management Systems). Standard quality assurance procedures include analysis of blanks
118 within each batch and a routine testing of certified reference material standards; duplicate samples included
119 in each batch to ensure that reproducible results are being achieved (Consani et al. 2017).

120 To avoid sample contamination from external microplastics, different measures of prevention were taken. All
121 the laboratory procedures described below were performed by operators wearing a white cotton coat;
122 laboratory instruments used were only in glass or steel and thoroughly rinsed with previously micro-filtered
123 water before their use; moreover, almost all the passages took place under the laboratory hood and in the
124 presence of a control filter Cutroneo et al. (2020b).

125 Fifty millilitres of sediment were taken from each sample, placed inside a beaker, and covered with watch
126 glass dish. Two hundred millilitres of Magnesium Chloride saturated solution (prepared with approximately
127 2400 g of $MgCl_2$ per L of filtered water to reach the density of 1.31 g cm^{-3}) were added to extract microliters
128 from sediment by density separation following this process:

- 129 - the mix was stirred with a glass stick for 5 minutes and then allows settling under a flow hood for 48 h
130 (duration of the process time was chosen based on the fine nature of the sediment);
- 131 - afterwards, 10 mL of supernatant were taken and placed inside a glass jar. The separation process was
132 carried out 3 times for each sample, obtaining 30 mL of supernatant for each sediment sample which was
133 filtered on a GF/F glass fibre membrane (porosity $0.42 \mu\text{m}$);
- 134 - the obtained filter was rinsed with 1 L of micro-filtered water to eliminate the eventual residual salts that
135 could create difficulties during the following analysis;
- 136 - filters were treated with 30 mL of H_2O_2 overnight to dissolve organic matter and then subjected to
137 additional washing with 1 L of micro-filtered water;
- 138 - filters were finally placed in glass Petri dishes.

139 Particles over the filters were observed with a Leica Z16 microscope and photographed with a Leica
140 Application Suite software, then measured and classified according to shape, colour, and size. Shape
141 identification classified microparticles into filaments, spheres, granules, fragments and other types (pellets,
142 foam, film, other), while size classification divided microparticles into the following categories: $\emptyset < 63 \mu\text{m}$,
143 $63 < \emptyset < 125 \mu\text{m}$, $125 < \emptyset < 250 \mu\text{m}$, $250 < \emptyset < 500 \mu\text{m}$, $500 < \emptyset < 1000 \mu\text{m}$, $1000 < \emptyset < 2000 \mu\text{m}$, $2000 < \emptyset < 5000 \mu\text{m}$.

144 Once the observation under microscope was completed, 40% of microparticles for each sample were
145 analysed with Raman spectroscopy (Galgany et al. 2013). Despite Galgani et al. (2013) indicate a percentage

146 equal to 5-10% of the items observed under the optical microscope, we opted for analysing 40% of the items
147 by μ Raman spectroscopy to have a better detail of the plastic component observed and as compromise
148 between time and cost of the analysis and the intended results. In our study, only μ Raman analysis can assess
149 with certainty that the particles identified in the samples are composed of plastic polymers, and therefore
150 identified as microplastics. Only the resulting spectra with a correspondence of more than 70% to the
151 reference spectra were considered acceptable and entered in the results.

152 Throughout the period in which the samples were exposed to air in laboratory, a control filter was placed on
153 the work surface and then analysed by microscope and μ Raman to remove particles from the results due to
154 contamination of the sample by the laboratory environment (laboratory contamination was quantified as an
155 average of 6% of the particles analysed, and consist of cellulose fibres, mineral particles and microplastics).

156 **4. Results**

157 The results of the sediment grain-size analysis (**Table 1**) show the general predominance of fine sediment
158 ($\emptyset < 63 \mu\text{m}$), while the most representative classes of the coarse fraction ($\emptyset > 63 \mu\text{m}$) were very fine sand and
159 fine sand.

160 Mean mineralogical results are reported in Table 1. The main minerals found in all the samples were quartz,
161 calcite, plagioclase and pyroxenes, with muscovite and chlorite in minor percentage, and zircon and rutile in
162 trace.

163 Metal concentrations are reported in **Table 2**.

164 From the optical results obtained from the four starting 50-mL samples, a total of 446 items was classified,
165 with an average of 111 particles per sample. Among them, the main categories represented were fragments,
166 filaments, granules, while only a few particles ($< 10\%$) were classified as 'other' (film, foam, other types)
167 and spheres (**Fig. 3**). In addition, most of the items fell within the size class 63-125 μm (Fig. 3), followed by
168 microparticles smaller than 63 μm and the size range 125-250 μm in accordance with sediment size results
169 (Table 1). The remaining size classes were poorly represented ($< 7\%$) and the size class over 2000 μm was
170 represented by only 3 fibres found in only one sample. Considering the colour (Fig. 3), most of the particles
171 were white, grey, and black.

172 From the total of 446 items, 40% was analysed by μ Raman spectroscopy and only 4% of the items analysed
173 have been recognised as microplastics (polymers, additives, and industrial dyes; **Fig. 4**). Two per cent of the
174 analysed particles has been identified as organic material (such as cellulose; Fig. 4A, C), whereas the
175 majority of particles (94%) has mineral origin (such as carbon and quartz; Fig. 4D,E) and therefore are
176 classified as inorganic material. Regarding these, the main minerals found are quartz (mean of 38.8%),
177 muscovite (mean of 21.0%; Fig. 4G), and plagioclase (mean of 8.4%) which match the mineralogical
178 composition of the sediments found through mineralogical analysis (Table 1). Plastic polymers found consist
179 of polyurethane (PU) foam in the form of 2 grey particles of 50 μm of dimension (Fig. 4B), polyvinyl
180 chloride (PVC) as 1 transparent particle of 183 μm (Fig. 4F), and copper-phthalocyanine ($\text{C}_{32}\text{H}_{16}\text{CuN}_8$) as 3
181 blue particles of 10-20 μm . Copper-phthalocyanine is a blue synthetic pigment with numerous applications
182 in industrial coatings, textile and paper manufacturing, fine art pigments, organic and photovoltaic cells

183 (Ahmed Basha et al. 2013; Matoko-Ngouma et al. 2020). Although not a recognised microplastic, as we
184 cannot determine the composition of the material making up the particle because the dye prevents it from
185 being analysed with the μ Raman, we have considered the blue particles to be part of the microplastic
186 category as it is an artificial dye.

187 Starting from particles found in the samples, considering 446 microparticles found in 200 ml of total
188 sediment analysed, a concentration of about 80 microplastics L^{-1} has been found in this study.

189 **5. Discussion**

190 Size characteristics of sediments were in accordance with the general low dynamism of abyssal environment
191 (Cisneros et al. 2019) and to results found by Durrieu de Madron et al. (2017) in the abyssal plain of the Gulf
192 of Lion where particles distribution is dominated by a principal mode around 8 μm and a secondary mode
193 around 30 μm . Metal concentrations show generally low values like those found in lightly contaminated
194 areas of the bay of Toulon by Tessier et al. (2011).

195 Regarding microplastic contamination of sediments, the sea bottom, especially at great depths, is the final
196 site of arrival and deposition of MPs, as reported by da Courtenes-Jones et al. (2020), but little is actually
197 known about the deep environments because of their inaccessibility. This makes any information that can be
198 gleaned from sediments taken in these remote environments valuable and useful for understanding deep
199 circulation mechanisms. As regards the Mediterranean Sea, several studies have been carried out on surface
200 waters (mainly by manta trawl sampling) (Suaria et al. 2014; Ruiz-Orrejon et al. 2016; Cincinelli et al. 2019;
201 Akarsu et al. 2020; De Haan et al. 2019; Zayen et al. 2020) and on coastal sediments (Alomar et al. 2016;
202 Cutroneo et al. 2020a; Missawi et al. 2020; Tata et al. 2020), but only one research article focused on deep
203 sediments (Woodall et al. 2014). Thus, our results bring a new contribution to the lack of knowledge of
204 microplastic pollution in deep marine environments.

205 Considering other studies at similar depths, Woodall et al. (2014) searched only microfibers in deep-sea
206 sediments in different part of the world by analysing 50 mL of sample. They found 10 and 15 fibres in open
207 slope in the North East Atlantic and in the subpolar region of the North Atlantic (2000 m depth),
208 respectively, while in canyon in the western part of the Gulf of Lion they found 35 and 10 items at 300 and
209 1300 m of depth, respectively. Contrary to the findings of Woodall et al. (2014), no plastic fibres were found
210 in our samples as all microfibers consisting of cellulose. In this case, our results seem to support the fact that
211 most of the fibres are concentrated near the coast and especially near population centres, as indicated by
212 Alomar et al. (2016), and in areas of accumulation. Our study area is far from the coast and from the city of
213 Toulon, and the high depth may be a determining factor for the absence of this type of microplastics. Strong
214 currents and winds, such as those affecting the Gulf of Lion, may transport microlitter, especially the most
215 buoyant, far away from its source (van Sebille et al. 2020) and so fibres are perhaps not facilitated to deposit
216 at the study site, but more to be transported along the water column by general circulation.

217 Bergmann et al. (2017), analysing sediments at 2340–5570 m depth in the Fram Strait in the western part of
218 Svalbard (Norway), found concentrations (mean of 2264 microplastics L^{-1}) much higher than those found in
219 Toulon deep sediments. Showing relatively low microplastics concentrations, our results are like what found

220 by Courtene-Jones et al. (2020) in the deep-sediments of the Rockall Trough (North Atlantic Ocean), and by
221 Van Cauwenberghe et al. (2013) in different part of the world (Polar front in the Southern Ocean, Porcupine
222 Abyssal Plain in the North Atlantic Ocean, distal lobe of Congo Canyon in Gulf of Guinea, and the Nile
223 Deep Sea Fan in the Mediterranean Sea).

224 Regarding the polymeric composition of the plastics found in deep sediments, due to the low variety of
225 plastic polymers found (i.e. 3), only the following evidence can be highlighted. Polyvinyl chloride is a high-
226 density polymer that is extensively present in sediments worldwide and was also found in deep sediments by
227 Courtene-Jones et al. (2020). Copper-phthalocyanine, widely used pigment in multiple industrial processing,
228 was also extracted from deep sediments by Van Cauwenberghe et al. (2013). Finally, polyurethane is a dense
229 polymer that tends to settle quickly and becomes incorporated into sediment (Uddin et al. 2021).

230 Considerations about the origin of the microplastics found cannot be made due to the great depth of the
231 sampling site and the great distance between the sampling site and the coast or the main anthropogenic
232 activities in the area. In fact, Kane et al (2020), studying the complexity of mechanisms of the microplastic
233 transport and diffusion on the sea bottom, highlighted that microplastics are not simply affected by vertical
234 setting, but are subject to wind mixing, current transport along the water column, dense down-canyon flows
235 and strongly near-bed thermohaline currents, as well as and biological interactions (biofouling, inclusion in
236 the trophic chain, and inclusion in marine snow). Notwithstanding, microplastics found in our samples are all
237 definable as secondary microplastics, i.e. resulting from the degradation and fragmentation of larger plastics.
238 This, together with their small size ($\varnothing < 200 \mu\text{m}$), may be a clue as to the type of microplastics that, in
239 relation to their site and time of origin, manage to reach great depths. Kooi et al. (2020) found that small
240 fragments are more susceptible to wind mixing, because of their low buoyant terminal rise velocity, which
241 strengthens the hypothesis that wind mixing causes a size selective loss of plastics from the surface and
242 facilitate small particles transport along the water column. In addition, the study site is subject to the
243 formation of very extensive nepheloid bottom layers events (Tessier et al. 2011) that can lead to the
244 resuspension of the bottom sediment along the water column and thus to the dispersion and subsequent re-
245 deposition of the particles even at great distances from the origin site. In a recent study conducted by
246 Gerigny et al. (2019) on the presence of macro-debris on the sea bottom by ROV, the Toulon Canyon,
247 located between the continental shelf and the abyssal plain off Toulon, does not appear to be strongly
248 contaminated by plastic debris (1 item km^{-1}), but rather by metallic materials (about 3 item km^{-1}) due to the
249 presence of the large naval base of Toulon and the metal materials that have been dumped regularly in the
250 past. This may support the relatively low concentrations of microplastics found in the abyssal sediments.
251 Further considerations on the origin of the fragments found in the deep sediments in front of Toulon can be
252 made based on previous findings in the Gulf of Lion by Lefebvre et al. (2019) and Schmidt et al. (2018).
253 Lefebvre et al. (2019), analysing water column in the Gulf of Lion found microplastics in 93% of water
254 column samples consisting only of fibres composed by polyethylene terephthalate (PET; 61%). This could
255 support the hypothesis that the fibres are diffuse more along the water column and are unlikely to reach great
256 depths. Schmidt et al. (2018) found that particles $< 1 \text{ mm}^2$ clearly dominated water sampling stations in the

257 Northern Current, the Rhône River and its plume, suggesting a long exposure time in the environment, while
258 items between 1 mm² and 5 mm² in size were the most abundant microplastics near the coast in the Marseille
259 Bay, suggesting coastal pollution sources or the removal of smaller particles from surface waters by
260 ballasting owing due to the presence of epibionts. This may support the hypothesis that the small particles
261 found in the deep sediment have come a long way over a long period of time before depositing on the deep
262 sediments.

263 Settled particles are likely to be affected by patterns of deep circulation, such as deep-sea currents acting as
264 conveyor belts which can transport small plastic items across the seafloor, possibly creating microplastics
265 hotspots on the sea bottom. Poor information still exists about the conditions of deep ocean across the world
266 and small-scale deep circulation, in addition to the abundance of factors affecting microplastics settling, such
267 as density, polymeric compositions, shape and interaction with organisms, making it difficult to predict
268 microplastics transport from coastal environment to the sea bottom (Kane et al. 2020; Abel et al. 2021).
269 However, considering the described morphology and characteristics of the study area and the local
270 circulation in relation to the relative low number of microplastics found and the lack of fibres, it can be
271 assumed that the seabed in front of the Toulon canyon is not to be considered as a microplastic hotspot due
272 to the periodic strong resuspension phenomena present, which generate shear stress on the seabed, and
273 resuspension and dispersion of microplastics.

274 **5. Conclusions**

275 Our study, although preliminary due to the small number of samples considered, confirms the ubiquity of
276 microplastics in environment, finding them also in the deep-sea environment of the abyssal plain. Thanks to
277 the KM3NeT infrastructure and the periodical project campaigns, further sampling and in-depth study of the
278 number and morphology of microplastics present in this kind of environment are underway. This will allow
279 us to broaden knowledge of the origins and diffusion of abyssal microplastics and study possible variations
280 over the years, as well as have indirect information on sea-bottom circulation and the shear stress on the
281 seabed.

282

283 **Supplementary Information:** Online Resource 1: HROV Ariane picks up and stores the sample from the
284 bottom; for short, the video playback speed is multiplied by 4 times. In the video, it is possible to see the
285 robotic metallic arm of the HROV Ariane that opens the lid of the sample storage container that was
286 previously coated with an aluminium foil to avoid plastic contamination of the sample, picks up the sediment
287 samples from the sea bottom, stores them in the container, and, finally, closes the lid of the container. The
288 robotic arm takes 3 portions of surface sediment approximately 5 cm thick with the grab and collected them
289 on the container.

290

291 **Declarations**

- 292 • Ethics approval and consent to participate: Not applicable
- 293 • Consent for publication: Not applicable

- 294 • Availability of data and materials: The datasets used and/or analysed during the current study are
295 available from the corresponding author on reasonable request
- 296 • Competing interests: The authors declare that they have no competing interests
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- 298 • Authors' contributions: Conceptualization, L.C., M.C., A.D., P.L., M.A.; Methodology, L.C., A.R.;
- 299 Investigation, V.B., I.G.; Data curation, P.C., S.C., D.D., A.R.; Writing—original draft preparation,
300 L.C., M.C., A.D., P.L., P.C., V.B., D.D., A.R., I.G.; Project administration, M.C., P.L., M.A.;
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306

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Figure captions

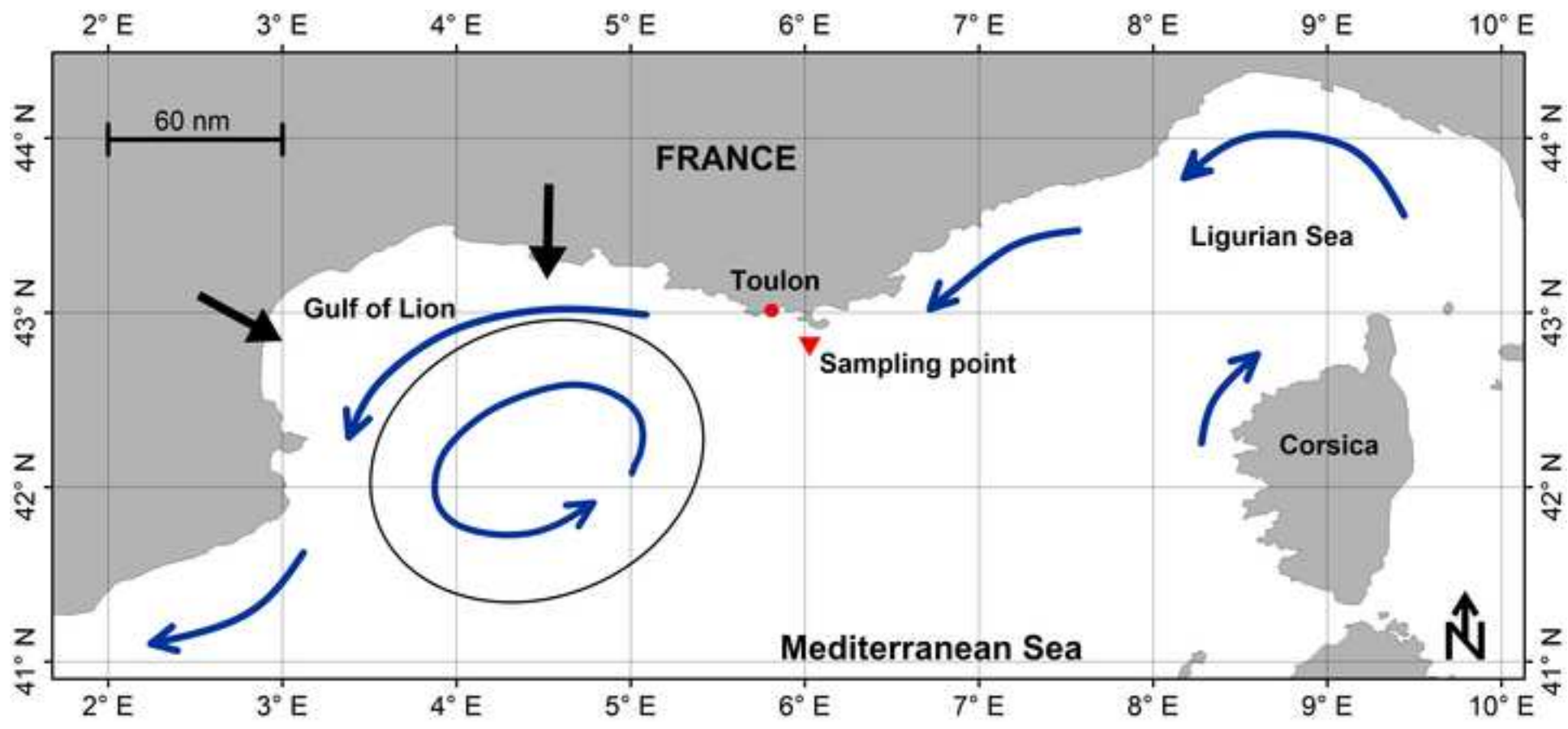
Fig. 1 Localisation of the sampling point off the coast of Toulon (France) in the north-western Mediterranean. Blue arrows outline the cyclonic general circulation, black arrows the strong winds affecting the Gulf of Lion, and black circle the area of trapping water by the cyclonic gyre. Red point indicates the city of Toulon and the red triangle the sampling point

Fig. 2 HROV Ariane (left) and sediment sampling with the grab (right)

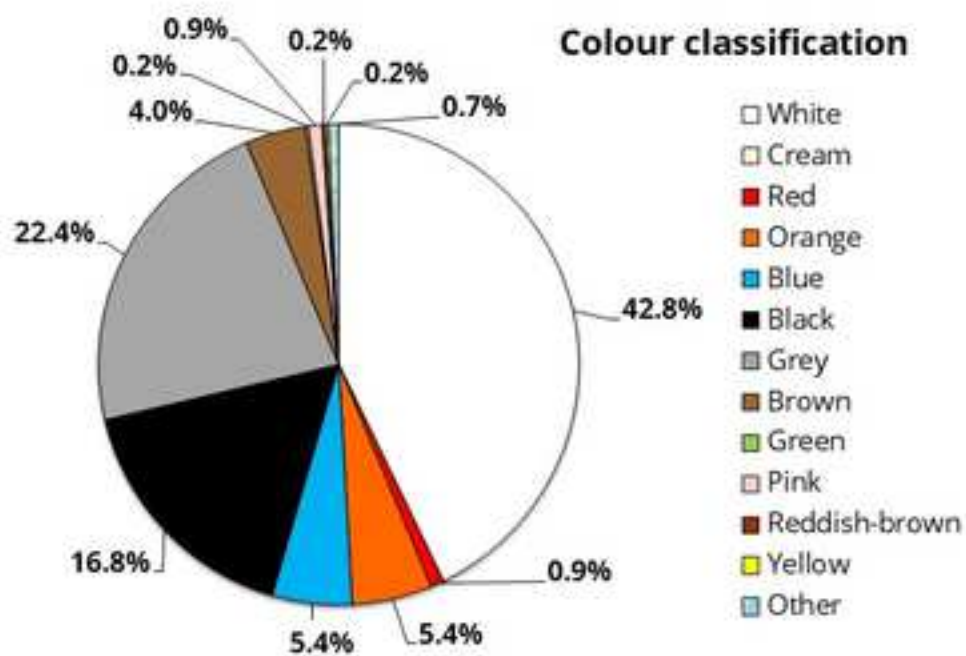
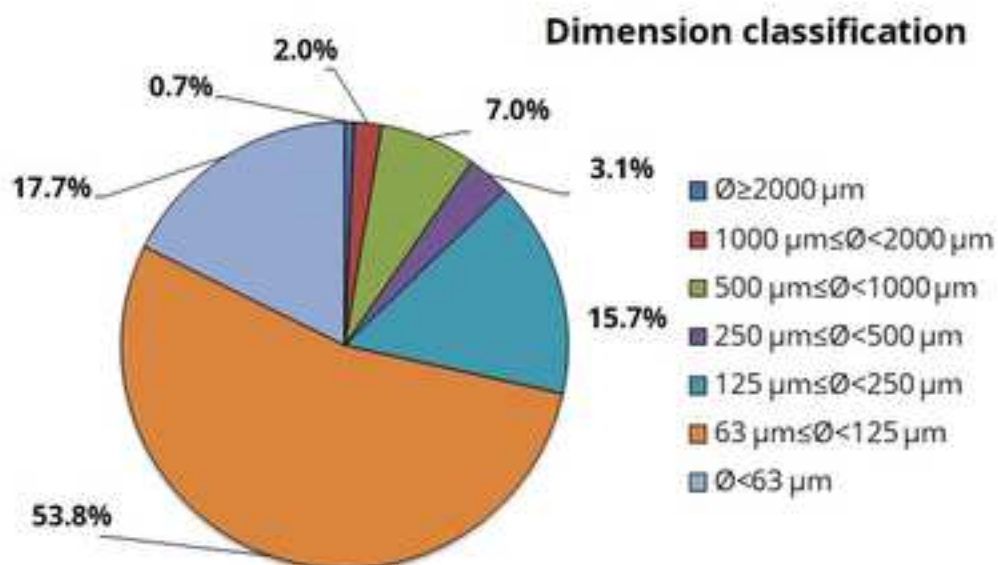
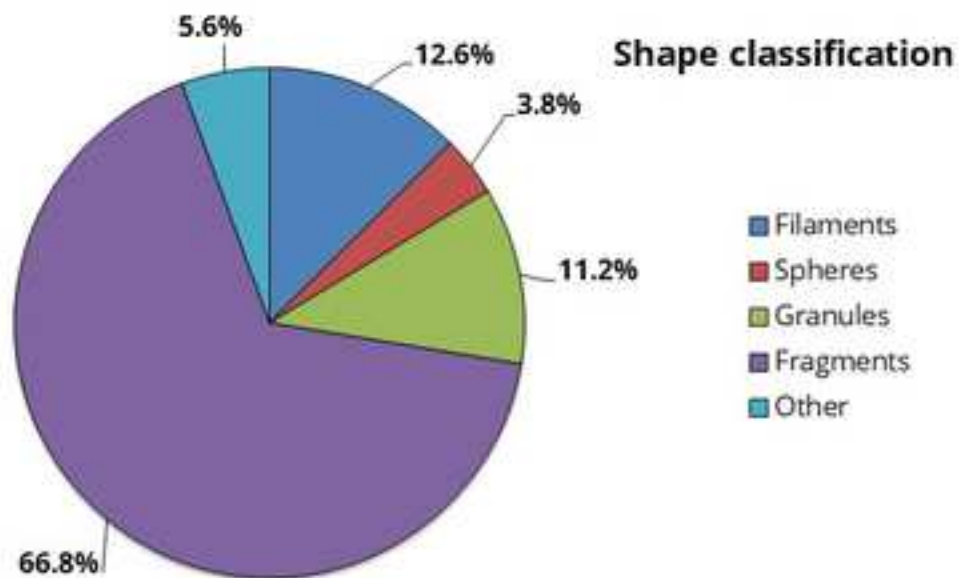
Fig. 3 Particle classification according to shape, size, and colour

Fig. 4 Combined results obtained from microscope and Raman analysis. Starting from the top, examples of fibres in cellulose, polyurethane foam, and particles of carbon, quartz, polyvinyl chloride, and muscovite. In the Raman spectrum box on the right, the reference molecule spectrum is shown in red, while the spectrum resulting from the analysis of the sample item is shown in black

Figure 1







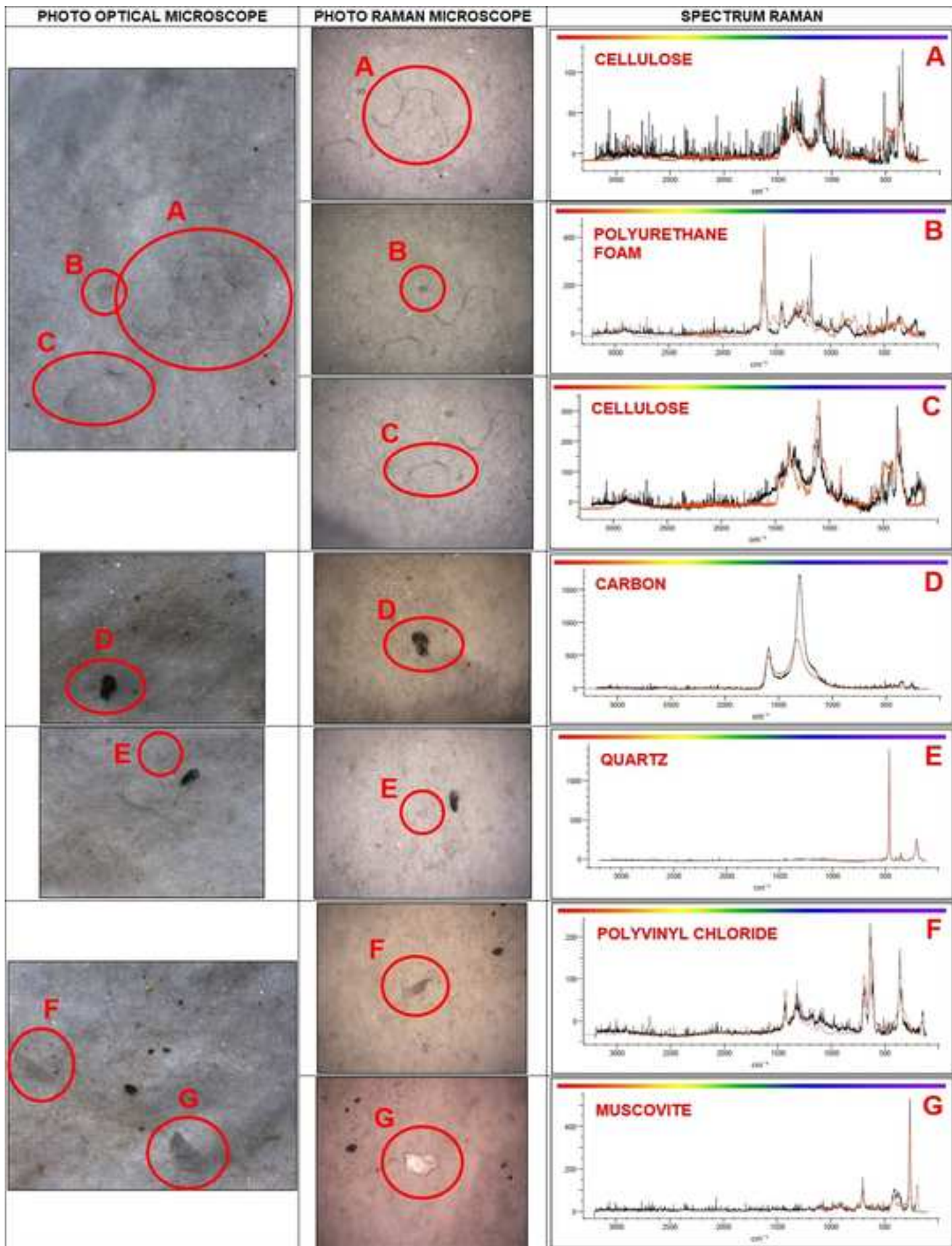


Table 1 Mean grain-size and mineralogical composition (in %) of sediments

Grain size	Mean (%)	Standard Deviation (%)
Fine material ($\phi < 63 \mu\text{m}$)	53.7	3.2
Coarse material ($\phi > 63 \mu\text{m}$)	46.3	3.2
Very fine sand ($63 < \phi < 125 \mu\text{m}$)	31.3	1.9
Fine sand ($125 < \phi < 250 \mu\text{m}$)	13.9	2.7
Mean sand ($250 < \phi < 500 \mu\text{m}$)	0.9	0.1
Coarse sand ($500 < \phi < 1000 \mu\text{m}$)	0.1	0.0
Minerals	Mean value (%)	Standard Deviation (%)
Zircon	0.8	1.6
Rutile	0.8	1.5
Muscovite	9.0	2.7
Plagioclase	14.8	3.1
Calcite	25.6	0.6
Quartz	29.0	2.3
Pyroxenes	12.8	2.6
Chlorite	7.2	2.7

Table 2 Detection limit, mean concentration, and standard deviation for selected metals to characterise the study area

Metal	Detection limit	Mean concentration	Standard deviation
Al (%)	0.01	0.89	0.13
Fe (%)	0.01	1.52	0.18
Mg (%)	0.01	0.95	0.06
As (ppm)	0.10	8.35	1.20
Cd (ppm)	0.01	0.08	0.01
Co (ppm)	0.10	7.75	0.90
Cu (ppm)	0.01	18.60	3.26
Cr (ppm)	0.50	31.08	4.30
Mn (ppm)	1.00	612.50	62.88
Ni (ppm)	0.10	31.33	4.29
Pb (ppm)	0.01	18.10	2.93
V (ppm)	1.00	23.25	3.10
Zn (ppm)	0.10	40.95	5.57
Ag (ppb)	2.00	46.75	21.62
Hg (ppb)	5.00	136.50	15.02

