

1 Coastal waters contamination by mining tailings: what triggers the stability of iron in the
2 dissolved and soluble fractions?

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30 Running head: Mining tailings impacts on dissolved iron

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33 *Abstract*

34 The solubility of iron (Fe) in seawater is greatly enhanced by complexation with organic
35 ligands, predominantly occurring as humic substances (HS) in coastal areas. Mining
36 exploitation is believed to change the natural biogeochemical cycle of Fe in coastal waters,
37 even though its impacts on the physical and chemical speciation of the Fe fractions are not
38 known. Here we show that dissolved and soluble Fe concentrations in coastal waters affected
39 by a mining catastrophe (Fundão dam, Southeast Brazil) remain very high, even almost 3
40 years later, with concentrations of dissolved Fe up to 2.8 μM (0.45 μm filtration) or 700 nM
41 (0.22 μm filtration), and soluble Fe (0.02 μm) up to 40 nM. Levels of humic substances can
42 only explain the binding of 2 % and 10 % (median values) of dissolved Fe (0.22 μm) and
43 soluble Fe concentrations, respectively, which shows that processes other than complexation
44 with humic substances are at play to maintain such high Fe concentrations. We hypothesize
45 that the colloidal phase that dominates the dissolved Fe fraction occurs as Fe(III)
46 oxyhydroxides while the soluble fraction is comprised of Fe(III) complexes with amine
47 compounds (widely used in the ore extraction process). Mass balance of dissolved Fe in the
48 water column suggests that sediment resuspension on the continental shelf is by far the
49 dominant process delivering dissolved Fe to coastal and shelf waters. Estimates of dissolved
50 Fe yearly fluxes highlight the sheer magnitude of this catastrophe that might provide a non-
51 negligible amount of dissolved Fe to the open ocean.

52 *Introduction*

53 Iron (Fe) is a bio-essential element that is limiting primary productivity in 40 % of the
54 world oceans (Moore et al. 2013). It is entering the marine system through multiple external
55 sources. The atmospheric dust is dominant to the Fe supply at the low latitudes whereas
56 continental margin and hydrothermal inputs are more important for Fe inventory at the high
57 latitudes (Tagliabue et al. 2014). Nevertheless these natural inputs, dissolved Fe (dFe)
58 residence time in the water column is much shorter than other nutrients due to its low
59 solubility (Liu and Millero 2002) and its propensity to adsorb on any reactive surface, thus
60 removing it from the water column (Boyd and Ellwood 2010). Understanding the distribution
61 of Fe between different size fractions is a key factor to describe the chemical reactivity and
62 bioavailability of Fe in seawater and its biogeochemical behaviour (Tagliabue et al. 2017).
63 Speciation is usually done in one of the three operationally-defined size fractions: particulate
64 (pFe; > 0.45 or $0.2 \mu\text{m}$), dissolved Fe (dFe; < 0.45 or $0.22 \mu\text{m}$) and to a much lesser extent,
65 soluble Fe (sFe; $< 0.02 \mu\text{m}$).

66 In the dissolved fraction, Fe speciation has been focused on the complexation with organic
67 compounds as Fe bound to natural organic ligands represents more than 99 % of the dissolved
68 Fe concentrations in marine ecosystems (Gledhill and van den Berg 1994). Organic
69 complexes increase the dissolved Fe solubility by reducing its removal by precipitation from
70 the water column (Liu and Millero 2002; Boyd and Ellwood 2010; Gledhill and Buck 2012).
71 In the absence of organic ligands, Fe concentrations in natural oxygenated seawater with a pH
72 around 8.0 would not be more than 0.01 nM (Liu and Millero 2002), due to extensive
73 precipitation. The presence of organic ligands, their reactivity and own biogeochemical
74 cycling, thus defines the distribution patterns of dissolved Fe concentrations in the ocean
75 (Hassler et al. 2017).

76 Natural Fe-binding ligands in seawater are thought to be mostly siderophores, strong
77 chelators released by heterotrophic bacteria and cyanobacteria as a biochemical strategy to
78 assimilate dissolved Fe, exopolymeric substances from cellular exudates and refractory
79 organic matter like humic substances (Gledhill and Buck 2012). These compounds are
80 different in their origin, conditional stability and dispersal patterns from coastal to offshore
81 oceanic regions (Hassler et al. 2017). In estuarine and coastal waters, there is mounting
82 evidence that humic substances dominate the bulk of organic ligands (e.g. Laglera and van
83 den Berg 2009). These humic substances have been showed to be mostly of terrestrial origin
84 (e.g. Laglera and van den Berg 2009) and although they undergo extensive removal within
85 estuaries through coagulation and flocculation processes (Sholkovitz et al. 1978), a significant
86 proportion make it through the estuarine trapping (Muller 2018).

87 Speciation of the soluble Fe fraction in terms of organic ligands has not been largely
88 addressed but the presence of soluble chelates from microbial degradation of settling biogenic
89 particles (e.g. faecal pellets) has been suggested to explain the levels of soluble Fe in the
90 North Atlantic and North Pacific waters (Wu et al. 2001). Recent studies using both the size
91 partitioning method and ligands determination by cathodic stripping voltammetry showed the
92 presence of two soluble organic ligand classes (stronger L₁ and weaker L₂) occurring in
93 excess in surface waters of the Atlantic Ocean (Fitzsimmons et al. 2015). Although the type of
94 ligands has not been specified, the range of ligand conditional stability constants K observed
95 by Fitzsimmons et al. (2015) for L₁ fall into siderophores ($\log K_1 > 12$) (Hassler et al. 2017)
96 while $\log K_2$ values for L₂ can represent a wide class of ligands with high similarity in
97 complex stability (e.g. humic and exopolymeric substances) (Hassler et al. 2017). In addition
98 to the stabilization of soluble Fe by organic complexation, recent studies have suggested that
99 stability of soluble Fe in the vicinity of hydrothermal vents is mostly related to the presence of

100 small colloids present as inorganic nanoparticles of pyrite FeS_2 , Fe_xSi_y (Gartman et al. 2014)
101 and Fe oxy-hydroxide; $\text{FeO}_x(\text{OH})_y$ (Fitzsimmons et al. 2017).

102 Besides the natural processes controlling Fe cycle and speciation, anthropogenic inputs
103 have strongly modified metal distribution and biogeochemistry in coastal seas. Extraction and
104 processing of ores have impacted several coastal environments, modifying the dispersal of
105 metals by various physical and chemical processes that are fundamentally different than those
106 that disperse natural inputs (Koski 2012). In this context, in November 2015, the collapse of
107 the Fundão dam in South-eastern Brazil spilled more than 55 million m^3 of Fe ore tailings that
108 travelled 600 km along the Doce River before reaching the adjoining coastal waters. Even
109 though this event is considered as one of the largest dam tailings failure worldwide, its
110 impacts in the processes controlling the Fe cycle on the marine affected areas are still
111 unknown (Longhini et al. 2019).

112 The aim of this study is to evaluate the impact of this catastrophic dam failure on the
113 distribution and physicochemical forms of Fe in the adjoining coastal waters as a result of a
114 massive and sustained increase in ambient Fe concentrations. We determined the distribution
115 of three size-fraction of Fe (dissolved Fe: $< 0.45 \mu\text{m}$ and $< 0.22 \mu\text{m}$, and soluble Fe: < 0.02
116 μm) and their relation with humic substances. This study brings new insights into the
117 biogeochemical factors related to the Fe fluxes and tailings deposition in coastal areas
118 impacted by mining activities and also presents the first direct measurements of humic
119 substances in marine area directly affected by mining tailings.

120 *Materials and methods*

121 *Study area*

122 The Doce River watershed (Southeast Brazil) comprises two states (Minas Gerais and
123 Espírito Santo). In Minas Gerais, this watershed is located in the Quadrilátero Ferrífero

124 mineral province, the largest Fe ore complex in Latin America. One of the most important ore
125 deposits exploited in this region is the Itabirite, a low-grade Fe ore that requires several
126 treatment steps to reach a higher mass recovery. The flotation method is mostly used to
127 reduce the presence of quartz and other impurities in Fe ore concentrates (Filippov et al.
128 2014). Tailings sludge with high Fe content is generated as a waste product of the Fe ore
129 processing and stored in dams, structures built of earthfill to contain the tailings
130 (<https://www.samarco.com/en/barragens/> accessed 03 May 2020). The activities of
131 exploitation and processing of Fe ore in this region were known to affect the metal levels in
132 water bodies near the tailings dam including a coastal lagoon located in Espírito Santo state
133 (Pereira et al. 2008).

134 The object of this study is the continental shelf adjacent to the Doce River mouth (Espírito
135 Santo state) (Fig. 1) which has been impacted by the tailings sludge deposition after the
136 Fundão mining disaster. The hydrological and oceanographic conditions (winds/waves
137 regimes) are known to affect the Doce River discharge conditions, defining the flow of the
138 turbidity plume (Rudorff et al. 2018), the sedimentary processes (Quaresma et al. 2015) and
139 consequently the dispersion pattern of the tailings along the Doce River continental shelf. The
140 average Doce River flow is $541.5 \text{ m}^3 \text{ s}^{-1}$, with lower median streamflows occurring around
141 $368 \text{ m}^3 \text{ s}^{-1}$ during the dry season (from April to October), while higher median streamflows of
142 about $965 \text{ m}^3 \text{ s}^{-1}$ occurs during the wet season (between November and March) (Oliveira and
143 Quaresma 2017). Flooding events occur with highest frequencies in January and December
144 and they usually exceed $2,379 \text{ m}^3 \text{ s}^{-1}$ with a maximum flood record of approximately $9,000 \text{ m}^3$
145 s^{-1} (Oliveira and Quaresma 2017).

146 *Sampling strategy*

147 Sampling was carried out in August 2018, approximately two years and nine months after
148 the Fe tailings reached the coastal area. Three transects were established perpendicular to the
149 shoreline: North (N), Central (C) and South (S), each composed of three stations from 10 to
150 30 m isobaths. Seawater samples were also collected 6 days (the 27th of November 2015) and
151 3 months (February 2016) after the Fundão dam failure to evaluate the geochemical
152 conditions during the acute phase of the impact. Two locations were sampled in November
153 2015 and three in February 2016, as denoted by stars and crosses in Fig. 1, respectively.

154 Coastal water samples from surface (≈ 50 cm below the water surface) and bottom (≈ 2 m
155 above the bottom) waters were collected using a 5 L Niskin horizontal bottle sampler
156 (General Oceanics) ($n = 4$ in November 2015; $n = 6$ in February 2016; $n = 18$ in August 2018)
157 into 500 mL acid-cleaned LDPE bottles. Temperature and seawater pH were measured on-
158 board using a portable pH meter (Metrohm model 826). The pH was calibrated using
159 Metrohm buffer solutions of 4.01, 7.00 and 9.00 pH (NBS scale). Salinity was measured with
160 a CTD instrument (*11 plus* Sea-Bird in Nov 2015 and *9 plus* Sea-Bird in Feb 2016). In Aug
161 2018 this parameter was measured using a refractometer.

162 *Hydroclimatic conditions during the samplings*

163 The Fundão dam rupture occurred in the context of an extreme hydrological drought in the
164 region (Rudorff et al. 2018). After the dam failure on the 5th of November 2015, the Fe ore
165 tailings reached the coast on the 21st of November 2015 by low streamflow conditions (267.5
166 m^3s^{-1}) (Fig. 2). The samplings were conducted under low Doce River discharge which ranged
167 from $291.3 \text{ m}^3 \text{ s}^{-1}$ in Nov 2015, $457.8 \text{ m}^3 \text{ s}^{-1}$ in Feb 2016 and $231.8 \text{ m}^3 \text{ s}^{-1}$ in Aug 2018 (Fig. 2).
168 A flooding event occurred towards the end of January 2016 reaching a maximum of $3,667.1$
169 $\text{m}^3 \text{ s}^{-1}$ on the 22nd of January (Fig. 2).

170 The winds predominantly came from N, NE and S in Nov 2015 causing the Doce River
171 turbidity plume to disperse southward, southwest-ward and northward. In Feb 2016 the plume
172 was dispersed mainly to the southern region while in Aug 2018 it was concentrated parallel to
173 the coast and moved southwest-ward (MODIS-Aqua imagery).

174 *Processing and preservation of samples*

175 The samples were filtered once back at the laboratory, immediately after collection.
176 Unfiltered seawater was passed either through 0.45 μm or through 0.22 μm cellulose acetate
177 membrane (Millipore[®]) using a vacuum pump system to evaluate the Fe concentration in two
178 dissolved size fractions: dissolved Fe (0.45 μm) and dissolved Fe (0.22 μm), respectively. The
179 aliquots filtered through 0.22 μm membrane were also used to determine humic substances.
180 The filtrates for dissolved Fe (0.45 μm) were acidified to reach $\text{pH} < 2.0$ by adding HNO_3 65
181 % (previously distilled using sub-boiling procedure; boiling sub-distill acid mark Berghof
182 BSB-939-IR model) and stored at 4°C until analysis. Samples for dissolved Fe (0.22 μm)
183 determination were stored in a frozen state and transported in a thermic box filled with dry ice
184 from Brazil to the University of Liverpool (Liverpool, UK). The soluble fraction was obtained
185 by filtration in the laboratory after thawing of 0.22 μm filtrate samples through acid-cleaned
186 0.02 μm Anotop filter syringes (25 mm diameter). Samples were always analysed within 24 h
187 of thawing. Fe was thus determined in three different size fractions: a) dissolved Fe (0.45 μm)
188 by ICP-MS which corresponds to the total dissolved Fe; b) dissolved Fe (0.22 μm) and
189 soluble Fe (0.02 μm) by voltammetry. Humic substances were measured by voltammetry in
190 the 0.22 μm fraction.

191 Chlorophyll-*a* pigments were concentrated by filtration on board through Glass
192 Microfibre Filter (Sartorius) of 25 mm diameter. The filters were kept frozen until the
193 extraction procedure. The suspended particulate material (SPM) was determined by

194 gravimetric method after filtration through pre-weighted glass fibre membranes (porosity of
195 0.45 μm).

196 *Chemical analyses*

197 *Mass spectroscopy*

198 Dissolved Fe (0.45 μm) in seawater samples was determined by Inductively Coupled
199 Plasma – Mass Spectroscopy (ICP-MS, Agilent, 7500 cx). The pH in the pre-acidified
200 aliquots was adjusted using 1 M NaOH to a final pH around 7.0. The samples were then pre-
201 concentrated by passing through a cationic resin column (Chelex[®]). The trace metal extracts
202 were eluted from the resin column with HNO_3 2 % solution. The compounds were quantified
203 according to the EPA 6020A method for multi-element determination using ICP-MS (U.S.
204 EPA 1998). The fluctuations in the signals during the measurements were corrected by using
205 a multi-element internal standard (Internal Standard Mix - Bi, Ge, In, Li, Sc, Tb and Y,
206 Agilent Technologies). Multi-element standards (ICP multi-element standard solution XXI for
207 MS, CentiPUR[®] MERCK, Darmstadt - Germany) were used to carry the calibration curve
208 (concentration range from 0 to 300 $\mu\text{g L}^{-1}$). The dissolved Fe (0.45 μm) concentrations were
209 determined by the linear regression ($y = ax + b$) plotted as a result of the calibration curve.
210 Three analytical modes were used (no gas, Helium and Hydrogen) and the optimum one, as
211 determined by the method of recovery from spiked samples, was used. All solutions were
212 prepared with ultra-pure water processed using a PURELAB ultra (Model Ultra an MKZ, the
213 brand Elgar).

214 *Adsorptive cathodic stripping voltammetry*

215 The voltammetric system used for dissolved Fe (0.22 μm), soluble Fe and humic
216 substances determination was a $\mu\text{Autolab(III)}$ potentiostat connected to a VA663 Stand
217 (Metrohm, Switzerland) and a 663 IME interface and controlled with the GPES software. The

218 working, counter and reference electrodes were a static mercury drop electrode, a glassy
219 carbon rod and an Ag/AgCl//KCl (3 M), respectively. The voltammetric cell was an acid
220 cleaned polytetrafluoroethylene cell placed into a home-made acrylic holder to allow analysis
221 of small sample volume (5 mL). The stirrer, a specially made rotating polytetrafluoroethylene
222 rod, was set to a fixed stirring speed of 5. All the materials used during the analytical
223 procedure and for storing samples and reagents were cleaned by 1 M HCl (1 week), 0.1 M
224 HCl (1 week), 0.01 M during storage and rinsed using Milli-Q water between steps and prior
225 to usage.

226 *Dissolved Fe (0.22 μm) and soluble Fe determination:* Diluted Fe standard solution (1 μM
227 and 10 μM Fe) was prepared from a 1,000 mg L⁻¹ standard (SpectrosoL®) diluted with ultra-
228 pure water (Millipore, UK, 18 M Ω cm⁻¹ resistivity) and acidified to pH 2 using 9 M HCl (10
229 μL 9 M HCl/10 mL). HCl trace analysis grade (Fisher Scientific), and ammonia trace metal
230 grade (Fisher Scientific) were employed. The buffer was 0.2 M HEPPS (3-[4-(2-
231 hydroxyethyl)-1-piperazinyl]propanesulfonic acid; Merck, \geq 99.0 %) prepared in ultra-pure
232 water and the pH was adjusted using adequate amount of solid sodium hydroxide (Fisher,
233 99.2 % trace reagent grade) to a final pH_{NBS} of 8.15. The HEPPS buffer was purified by two
234 overnight equilibrations with 100 μM MnO₂, removed by filtration using 0.2 μm cellulose
235 acetate membrane (Whatman) (Obata and van den Berg 2001). The added ligand was 2 mM
236 DHN (2,3-dihydroxynaphthalene) which was prepared by dissolving DHN (Fluka, \geq 98.0 %)
237 in Milli-Q water.

238 The voltammetric procedure used to determine Fe levels in dissolved Fe (0.22 μm) and in
239 soluble Fe fraction was the method of Caprara et al. (2015), based on the reduction of the
240 electroactive complex Fe-DHN by adsorptive cathodic stripping voltammetry, but slightly
241 modified to suit the high Fe concentrations present in our samples. Standard additions were

242 used to determine Fe in each sample, with a minimum of 2 additions and a minimum of 3
243 voltammetric scans per addition. Prior to the analysis, the samples were acidified to $\text{pH} < 2.0$
244 by addition of HCl (Fisher, Trace Analysis) ($3.6 \mu\text{L}$ of 30 % HCl/3 mL sample) and UV-
245 digested for 2 hours using a home-built system with a 100-W, high-pressure, mercury vapour
246 lamp in 30 mL quartz tubes. Then 1 M NaOH ($21 \mu\text{L}$ of 1 M NaOH/3 mL sample) was added
247 to adjust the pH to around 8.0 before measurements. For dissolved Fe ($0.22 \mu\text{m}$)
248 determination, the samples were diluted using oceanic seawater (dissolved Fe concentration
249 was $1 \pm 0.1 \text{ nM}$; $n = 6$) which was UV-irradiated (1 hour) freshly prior to use. This oceanic
250 seawater is referred below as the background seawater.

251 The deposition time and the dilution factor were defined by a series of calibration curves
252 using 30 s, 15 s, 10 s and 5 s deposition time and a range of Fe concentrations from 5 nM to
253 40 nM. The best linear range was achieved using 5 s deposition time until 14 nM Fe
254 concentration. Subsequently the dilution factor for dissolved Fe ($0.22 \mu\text{m}$) samples was
255 adjusted so that a maximum of 14 nM was present in the cell at the end of the standard
256 addition (minimum of 2 additions). soluble Fe determinations were mostly made in undiluted
257 samples.

258 Regarding dissolved Fe ($0.22 \mu\text{m}$) analysis, 5 mL of the background seawater was
259 pipetted into the polytetrafluoroethylene cell then DHN (final concentration $10 \mu\text{M}$) and
260 HEPPS buffer (final concentration 10 mM) were added. The solution was air-purged using
261 cylinder air (1 bar pressure) for 90 s and five voltammetric scans were made to determine the
262 background peak height. Then the sample was added to the background seawater with a
263 dilution factor of at least 30 to ensure an increase of the signal of between 2 and 3 times the
264 background seawater signal. The solution was air-purged again for 90 s and six more
265 voltammetric scans were made. The first Fe standard addition was made sufficient to

266 approximately double the original peak and a minimum of three voltammetric scans were
267 carried out. A second Fe standard addition was made to confirm the sensitivity. The
268 sensitivity (nA/nM) was calculated from the slope of the increase in peak height by the Fe
269 standard additions and used to calculate the Fe concentration in the sample. The Fe
270 concentration in the background seawater was calculated in the same way and subtracted from
271 those of the samples. A number of samples were analysed from repeated measurements to
272 determine the precision (standard deviation) of the method. For samples of soluble Fe no
273 dilution was required because the Fe concentrations were sufficiently low. The sample (5 mL)
274 was pipetted directly into the polytetrafluoroethylene cell and the same procedure as that
275 described for dissolved Fe (0.22 μM) analyses was performed. The method used for soluble
276 Fe determination was validated by using the sample C-10 Bottom (Aug 2018) as control
277 which was previously analysed in triplicate to confirm its concentration. This control sample
278 was measured several times along the days of analysis. Only the certified results for soluble
279 Fe are given here (n = 14, from a total of n = 28) because of a contamination issue resulting in
280 higher soluble Fe values and non-validation from the control sample. Measurement errors
281 ranged around 5.1 % when dilution was required (dissolved Fe (0.22 μM) analyses) and
282 around 4.4 % for non-diluted samples (soluble Fe determination).

283 The polytetrafluoroethylene cell was rinsed five times using Milli-Q water and then with
284 Milli-Q water plus 10 μM DHN between the analyses to avoid adsorption on the cell wall.
285 Measurements were made using square wave cathodic stripping voltammetry with a
286 deposition time of 5 s at -0.1 V, a 5 s equilibration time (no stirrer) and stripping from -0.35 to
287 -0.75 V (5 mV step, 50 mV amplitude and 10 Hz frequency).

288 *Humic substances determination:* The borate buffer (boric acid, BDH, UK; 99.8 %) and
289 the oxidant (potassium bromate, Fisher) were prepared in a mixed solution of 0.2 M borate

290 and 0.4 M potassium bromate. The pH of this mixture was adjusted with ~0.08 M ammonia to
291 a final pH_{NBS} of 8.30. The boric acid solution was UV-irradiated for 1 h to ensure that the
292 solution was free of organic contamination. The borate/bromate mixture was purified twice by
293 overnight equilibration with 100 μ M MnO₂, as previously described (Obata and van den Berg
294 2001). Fulvic acid stock solutions of Suwannee River Fulvic Acid (SRFA; 0.2 g SRFA L⁻¹)
295 were prepared in ultra-pure water from standard reagents (Suwannee River, IHSS reference
296 material, 2S101F) and stored on dark and refrigerated conditions. The SRFA standard
297 solution was saturated with Fe prior to use to ensure the voltammetric signal reflected the
298 contribution of all Fe-binding groups (Sukekava et al. 2018). Previous work (Laglera and van
299 den Berg 2009) has shown that the binding capacity of SRFA is 16.7 nM Fe /mg FA. We used
300 this value as a guide to saturate the SRFA standard.

301 Concentrations of humic substances were determined on dissolved samples (< 0.22 μ m)
302 using the method described by Laglera et al. (2007) which includes a step of saturation of the
303 sample with Fe in high concentration, equilibration time for 2 to 16 h followed by catalytic
304 analysis and internal calibration with Suwannee River Fulvic Acid (SRFA) standard. To
305 confirm that the concentration of added Fe was sufficient to saturate all the humic binding
306 groups, experiments were performed to evaluate the reaction kinetic in two samples (higher
307 and lower Fe concentration) after two Fe spikes of 100 nM (Sukekava et al. 2018).
308 Immediately after the first Fe standard addition, a rapid increase followed by a slower
309 decrease of the Fe signal was observed (Fig 3). This decrease corresponds to the precipitation
310 of excess, unbound Fe(III) as Fe(III) oxyhydroxides while the signal at equilibrium is due to
311 Fe-humic complexes. After the second Fe addition of 100 nM, the catalytic signal decreased
312 back to its original value after 2 h 30 min confirming that the first addition of 100 nM was
313 enough to saturate all humic binding groups (see Fig. 3). These results led us to adopt the

314 following protocol. An aliquot of 10 mL of each sample was pipetted into sterilin tubes
315 (Sterilin, polyethylene, Fisher Scientific), saturated with 100 nM Fe and 500 μ L of 0.2 M
316 borate/0.4 M bromate mixed solution were added (final concentration of 10 mM borate
317 buffer/20 mM bromate). The solution was left to equilibrate for 2 h 30 min to allow Fe in
318 excess to compete with other cations and then saturate all the humic binding groups. The
319 sample was transferred to the polytetrafluoroethylene cell for measurements and the oxygen
320 was removed by purging the solution with nitrogen for 300 s. The catalytic analysis consisted
321 in five voltammetric scans followed by internal calibration with two additions of Fe-saturated
322 SRFA standard solution (with a minimum of 3 voltammetric scans per addition) to determine
323 the original concentration of humic substances in μ g SRFA L⁻¹ units. The instrumental
324 parameters were: sampled direct current mode at 50 mV s⁻¹, deposition potential -0.1 V, 60 s
325 deposition time, 10 s equilibration time (no stirrer), scan from 0 to -1.0 V.

326 *Biological parameters - chlorophyll-a and phaeophytin*

327 Filters were left overnight into centrifuge tubes filled with 10 mL of 90 % acetone
328 (HPLC grade acetone) on dark and refrigerated conditions to allow the extraction of the
329 pigments (APHA 2012). Extracts were centrifuged at 5,000 rpm for 10 minutes. The
330 absorbance in the extract were determined spectrophotometrically (BEL Photonics UV-M51-
331 Vis spectrophotometer) at 665 nm, 647 nm, 630 nm and 750 nm wavelength before and after
332 acidification with 0.1 mL of 1 N HCl. Chlorophyll-*a* and phaeophytin (phytoplanktonic
333 detrital material) concentrations were calculated by the equations from the monochromatic
334 method (Lorenzen 1967). The detection limit (DL) was 0.01 μ g L⁻¹ for both analytes.

335 *Statistical analyses*

336 Shapiro-Wilk normality test was used to test the normality of the data distribution, and
337 indicated a departure from normality ($p < 0.05$). The non-parametric Kruskal-Wallis test was

338 used to identify significant differences between the sampling periods (Nov 2015; Feb 2016;
339 Aug 2018), the isobaths (10, 20 and 30 m) or the inshore-offshore coastal transects (North,
340 Central and South sectors). When the result of the Kruskal-Wallis test was $p < 0.05$, the
341 Mann-Whitney U test was then used to reveal which pairs of samplings/isobaths/transects
342 differed from each other, and how. The Mann-Whitney U test was also applied to reveal the
343 depth stratification for the physico-chemical, biological parameters, dissolved and soluble Fe
344 fractions and humic substances concentrations. Spearman rank order correlation test was used
345 to test the correlation of Fe size-fractions with humic substances, suspended particulate
346 material and biological parameters. All statistical analyses were based on $\alpha = 0.05$. Statistics
347 analyses were performed using PAST software (version 3.26).

348 *Results*

349 *Physico-chemical and biological parameters*

350 Over the course of the 3 sampling campaigns, there were no significant differences with
351 depth, neither for the physico-chemical nor the biological parameters (Mann-Whitney, $p >$
352 0.05). Significant differences in the temporal distribution were only observed for suspended
353 particulate material (Kruskal-Wallis, $p < 0.05$), which reached the highest values in Nov 2015
354 (from 30 to 300 mg L⁻¹) (Table 1). Because of the low quantity of sampling stations in Nov
355 2015 and Feb 2016 we were not able to test statistically spatial changes between the isobaths
356 and inshore-offshore coastal transects separately for these samplings. In Aug 2018, the levels
357 of suspended particulate material decreased with distance from the Doce River mouth
358 (Kruskal-Wallis, $p < 0.05$), ranging from 16.5 to 25.3 mg L⁻¹ at the 10 m isobaths (i.e. near the
359 coast) and from 14.1 to 17.3 mg L⁻¹ at the 30 m isobaths (i.e. far from the coast) (Table 1).

360 The temperature varied between 20.9 to 26.7°C with generally, lower values in the bottom
361 waters when compared to surface (Table 1). There was no trend of depth stratification for the

362 salinity (Mann-Whitney, $p > 0.05$) which ranged from 36.0 to 36.6 at bottom and from 34.5 to
363 36.8 at surface (Table 1). The pH was measured between 8.00 and 8.15 in the last sampling
364 campaign; it was not recorded in Nov 2015, nor in Feb 2016.

365 Chlorophyll-*a* levels were comparable to phaeophytin levels in Nov 2015 and Feb 2016
366 but markedly lower in Aug 2018 (Table 1). Most of the Chlorophyll-*a* concentrations in
367 surface waters were below detection limit. Near the bottom, concentrations averaged 1.5 ± 1.6
368 $\mu\text{g L}^{-1}$ for Chlorophyll-*a* ($n = 9$) and $1.3 \pm 1.1 \mu\text{g L}^{-1}$ for phaeophytin ($n = 12$) (Table 1).

369 *Distribution of dissolved Fe (0.45 μm), dissolved Fe (0.22 μm), soluble Fe and humic*
370 *substances*

371 Over the course of the 3 sampling campaigns, the parameters dissolved Fe (0.45 μm),
372 dissolved Fe (0.22 μm), soluble Fe and humic substances presented homogeneous
373 distributions along the water column depth (Mann-Whitney, $p > 0.05$) and distance from the
374 Doce River mouth (Kruskal-Wallis, $p > 0.05$).

375 The dissolved Fe (0.45 μm) concentrations in surface and bottom waters were similar,
376 ranging from 784 to 2,784 nM at the surface, and between 739 and 2,570 nM for the bottom
377 waters (Table 2). During the acute phase of the impact (Nov 2015), the highest dissolved Fe
378 (0.45 μm) values in both the surface and bottom waters were found at station C-10, located in
379 front of the Doce River mouth (Fig. 4). For the Central and North sections, dissolved Fe (0.45
380 μm) concentrations decreased with time at similar rates, while the South section showed a
381 different behaviour, with concentrations increasing from Feb 2016 to Aug 2018 (Fig. 4a). In
382 Aug 2018, it is remarkable that the Central and North measurements were within a narrow
383 concentration range (863 ± 90 nM on North stations; $1,166 \pm 166$ nM on Central stations)
384 while much higher concentrations were observed for the South stations ($2,010 \pm 248$ nM)
385 (Kruskal-Wallis, $p < 0.05$) (Fig. 4a and Table 2). The same pattern was observed for

386 sedimentary Fe distribution (Supplementary, Table S1; Fig. S2) indicating a trend of material
387 accumulation on the south sector over time.

388 The concentrations of dissolved Fe ($0.22 \mu\text{m}$) were higher during the acute phase of the
389 disaster (Nov 2015; Kruskal-Wallis, $p < 0.05$) and increased again in Aug 2018 (Kruskal-
390 Wallis, $p < 0.05$) (Fig. 4b). The levels ranged from 30 to 245 nM in surface waters, whereas
391 the values in bottom waters were found to vary between 30 and 698 nM. In Aug 2018 the
392 highest values occurred at station N-10 for the surface waters and N-20 at bottom. The
393 sediment collected at station N-20 in Aug 2018 presented a marked reddish colour
394 characteristic of the presence of Fe oxide. Processes such as resuspension, remobilisation and
395 diffusion may thus explain the elevated value of dissolved Fe ($0.22 \mu\text{m}$) concentration
396 measured in the water column just above the sediment. The lowest dissolved Fe ($0.22 \mu\text{m}$)
397 concentrations were observed in Feb 2016 at the isobaths of 30 meters (station C-30 for the
398 surface samples and N-30 for those at bottom) (Fig. 4b; Table 2).

399 The concentrations of soluble Fe ranged from 10 to 25 nM (stations S-30 and N-20,
400 respectively) in the surface waters and varied between 10 and 37 nM at bottom, with the
401 lowest concentrations at station C-30 in Aug 2018 and the highest occurring at station C-30 in
402 Feb 2016 (Fig. 4c; Table 2).

403 Similar distribution patterns were observed for humic substances and dissolved Fe (0.22
404 μm) in surface waters. In general, the concentrations of dissolved Fe ($0.22 \mu\text{m}$) and humic
405 substances showed a trend of decrease with the distance from the Doce River mouth in the
406 surface samples and increased again in the 30 m isobaths (Fig. 4d; Table 3). The highest value
407 of humic substances was found at station N-10 in Aug 2018 ($350 \mu\text{g SRFA L}^{-1}$) and the
408 lowest occurred at station C-30 in Feb 2016 ($15 \mu\text{g SRFA L}^{-1}$) (Fig. 4d; Table 3). In bottom
409 waters, the highest concentration of humic substances occurred at station C-30 in Feb 2016

410 (241 $\mu\text{g SRFA L}^{-1}$) while the lowest value occurred at station N-20 in Nov 2015 (44 $\mu\text{g SRFA}$
411 L^{-1}). The dissolved Fe (0.22 μm) concentrations increased from North to the South sectors
412 whereas the humic substances decreased until the station C-20 and increased again from the
413 station S-20 (Fig. 4d; Table 3).

414 *Size-fractionated Fe proportion and Fe-binding by humic substances*

415 The concentrations of dissolved Fe (0.22 μm) were up to 98 % smaller than those of
416 dissolved Fe (0.45 μm) (Table 2; Fig. 5) suggesting that most Fe is present in a large (> 0.22
417 μm) colloidal form. Similarly, ultrafiltration (0.02 μm) of some pre-filtered samples (0.22
418 μm) caused significant removal, from 39 to 95 % of the Fe from dissolved Fe (0.22 μm) to the
419 soluble fraction (Table 2; Fig. 5), also suggesting that a significant proportion of dissolved Fe
420 (0.22 μm) fraction was present in a colloidal form higher than 0.02 μm . The high presence of
421 orange particulate matter retained on 0.22 μm membrane is an additional evidence of colloidal
422 dominance in our samples (Supplementary, Fig. S1).

423 Assuming a ratio of 16.7 nmol Fe (mg FA) $^{-1}$ for the binding capacity of humic substances
424 (Laglera and van den Berg 2009), the maximum concentration of Fe-binding by humic
425 substances would have been 0.2 to 5.8 nmol L^{-1} (Table 3). Based on previous studies, most of
426 dissolved Fe would have been anticipated to occur as organic complexes, mainly with humic
427 substances (Laglera and van den Berg 2009). In reality, the binding capacity of humic
428 substances could only explain a maximum of between 0.2 and 13 % (median is 2 %) of the
429 dissolved Fe (0.22 μm) (Table 3). We did not measure levels of humic substances in the
430 soluble fraction but assuming that those are the same as in the $< 0.22 \mu\text{m}$ fraction, they could
431 only bind from 0.2 to 43 % (median is 10 %) of the soluble Fe levels (Table 3). These
432 percentages correspond to the maximum binding that would occur if there was no competition
433 from other metals. However, when carrying out our measurements of humic substances (Fig.

434 3), it was clear that humic substances present in the sample were not saturated with Fe since a
435 significant increase (52 % for the sample shown in Fig. 3) of the signal of Fe-humic
436 complexes was observed upon additions of Fe (light grey and black markers in Fig. 3). This
437 increase can be explained by the presence of competing metals for complexation with humic
438 substances (Sukekava et al. 2018). It is thus clear that levels of humic substances measured in
439 this study cannot explain the Fe dissolved and soluble concentrations, indicating that there are
440 other processes and/or other compounds that maintain the Fe in the dissolved/soluble fraction.

441 *Correlation analyses*

442 No significant correlation was observed between dissolved Fe ($0.45 \mu\text{m}$) and suspended
443 particulate material, neither with Chl-*a* nor Phaeophytin (Supplementary, Table S2). For the
444 dissolved Fe ($0.22 \mu\text{m}$), on one hand, a positive correlation with humic substances was found
445 in surface waters (Supplementary, Table S2; Fig. 6a; $R = 0.62$; $p = 0.02$; $n = 12$) suggesting
446 that these variables originate from the same source. The coefficient of correlation in these
447 samples was relatively low due to three outliers (open circles in Fig. 6a). Exclusion of these
448 points resulted in a strong linear fit ($R = 0.98$; $r^2 = 0.97$; $p = 0.00001$; $n = 9$), as shown in the
449 inserted diagram (Fig. 6a). On the other hand, no significant correlation was found between
450 humic substances and dissolved Fe ($0.22 \mu\text{m}$) concentrations in bottom waters ($R = -0.44$; $p =$
451 0.15 ; $n = 11$). The dissolved Fe ($0.22 \mu\text{m}$) concentrations increased with the levels of
452 suspended particulate material in surface waters (Supplementary, Table S2; $R = 0.60$; $p =$
453 0.02 ; $n = 14$). A weak positive correlation was also found between phaeophytin and dissolved
454 Fe ($0.22 \mu\text{m}$) (Supplementary, Table S2; Fig. 6b; $R = 0.40$, $p = 0.04$; $n = 25$) which may be
455 due to the export of phytoplanktonic debris to the marine environment.

456 *Discussion*

457 *Dissolved Fe concentrations and sources*

458 The results of this study demonstrate that the concentrations of dissolved Fe in coastal
459 waters impacted by the Fundão dam collapse are very high across all size fractions, even
460 almost three years after the disaster, without showing any obvious signs of decrease. The
461 dissolved Fe ($0.45 \mu\text{m}$) levels found here are much higher than those reported in other marine
462 environments affected by mining tailings (Supplementary, Table S3). Only the lowest value
463 recorded in this study is comparable to the mean concentration of dissolved Fe ($0.22 \mu\text{m}$)
464 observed in coastal systems impacted by abandoned lead mining (Tovar-Sánchez et al. 2016),
465 while the range of dissolved Fe ($0.45 \mu\text{m}$) concentrations found here is similar to the values
466 recorded in coastline impacted by acidic metal-rich fluids from abandoned mines (Koski et al.
467 2008) (Supplementary, Table S3).

468 The fluxes of dissolved Fe to the coastal areas vulnerable to mining impacts are highly
469 intensified by the Fe inputs from mining tailings. These loads can reach the coastal zone by
470 riverine inputs, pore-water exchange and submarine groundwater discharge (Longhini et al.
471 2019). In our case, the spatial distribution pattern of dissolved Fe ($0.45 \mu\text{m}$) and dissolved Fe
472 ($0.22 \mu\text{m}$) concentrations indicates that there are two main sources of dissolved Fe to
473 consider: the fluvial input of suspended particulate material from the Doce River and the
474 resuspension of sediment/tailings particles deposited on the continental shelf.

475 The positive correlation between dissolved Fe ($0.22 \mu\text{m}$) and suspended particulate
476 material (Supplementary, Table S2) in surface waters is in agreement with the substantial
477 increase in the Doce River transport of Fe rich suspended particulate material observed by
478 Hatje et al. (2017) after the Fundão dam disaster (maximum values of suspended particulate
479 material was 33 g L^{-1} and particulate Fe reached up to 48 mg Fe g^{-1}). Iron in Fundão dam

480 tailings is mainly present as crystalline oxyhydroxides ($89 \pm 2.3 \%$) in forms of stable goethite
481 (Almeida et al. 2018; Queiroz et al. 2018), which possibly reduce the estuarine removal of
482 dissolved Fe. This suggests a limited estuarine trapping with particulate and colloidal organic
483 matter (Muller 2018) and then a high stability of the dissolved Fe forms that reached the
484 coastal area after the dam failure.

485 The transport of tailings mud from the river to the coastal sea in the acute stage of the
486 disaster occurred as a massive loading of sediment/tailings highly enriched with Fe (44.3%;
487 Pereira et al. 2008) of finer grain size compared to pre-disaster conditions (Gomes et al.
488 2017). This modified sediment deposited on the continental shelf remains fluid and subject to
489 resuspension by high winds/waves dynamics (Rudorff et al. 2018). The tailings mud transport
490 at the bottom then explains the increase of sedimentary Fe and dissolved Fe ($0.45 \mu\text{m}$)
491 concentrations on South stations over time and higher values of bottom dissolved Fe (0.22
492 μm) at those stations. These increasing concentrations are in agreement with the bottom
493 sediment transport (Quaresma et al. 2015) and with the southward trajectory of the Doce
494 River plume when trade winds are prevalent (Marta-Almeida et al. 2016). Mobility tests using
495 the Fundão dam tailings confirm this sedimentary/tailings source as they have shown that Fe
496 (and other metals like Ba, Sr, Mn and Al) can enter the dissolved phase from mud particles,
497 especially in environments with high calcium content, like seawater (Segura et al. 2016). This
498 phase transfer leads to an increased Fe bioavailability from the sediment during the transport
499 from the riverine portion to the estuarine mouth, which has been quantified as a 16 % increase
500 by Hatje et al. (2017).

501 *Concentrations and sources of humic substances*

502 Our study is the first one to inform about the role of humic substances on Fe transport in
503 waters impacted by Fe ore tailings. Even if humic substances show the same distribution

504 pattern as dissolved Fe ($0.22 \mu\text{m}$) in surface waters and show higher concentration near the
505 coast, attesting of their continental input from the Doce River, dissolved Fe-humic complexes
506 can explain only from 0.2 to 13 % of the dissolved Fe ($0.22 \mu\text{m}$) levels. To compare with
507 studies not subject to ore tailing, humic substances represented from 23 to 58 % of the ligand
508 bulk in North Atlantic (Whitby et al. 2020b), while they could account for almost the all Fe-
509 binding ligands in Liverpool Bay (Laglera and van den Berg 2009) and in the Arctic
510 (Sukekava et al. 2018). Furthermore, humic-binding capacity of Fe usually shows a good
511 agreement with dissolved Fe ($0.22 \mu\text{m}$) concentrations (Sukekava et al. 2018) and dissolved
512 Fe-humic complexes accounts for 80 % of the dissolved Fe speciation (Laglera et al. 2019).

513 The imbalance that we observe between humic substances and the dissolved Fe
514 concentrations is likely caused by the abnormal processes that mobilize the dissolved Fe from
515 the tailings material, whereas humic substances are delivered to the area from natural sources.
516 Likewise, the lack of significant correlation between humic substances and dissolved Fe (0.22
517 μm) concentrations in the bottom waters might be because different processes are decoupling
518 those components, as dissolved Fe shows substantial inputs from the sediment/tailings
519 resuspension. In natural systems, humic substances and Fe are known to be subject to co-
520 precipitation in the estuarine mixing zone reaching a removal rate ranges from 90 % to 95 %
521 (Boyle et al. 1977; Sholkovitz et al. 1978). At our location, dissolved Fe ($0.22 \mu\text{m}$)
522 concentrations reached $21.8 \pm 46.1 \mu\text{M}$ in continental water bodies affected by the tailings
523 (Segura et al. 2016) and part of this material was deposited during the transport, probably
524 carrying downward the humic substances and other compounds.

525 Our results also suggest some influence of humic substances from marine sources as their
526 concentrations increased at the 30 m isobaths (i.e. far from the coast). The high concentrations
527 of phaeophytin against the very low *Clh-a* levels are proxies of the presence of

528 phytoplanktonic debris/senescence which can be transformed by microorganisms to marine
529 humic-like compounds and possibly contribute to the bulk of humic substances in our system.
530 Indeed, marine-derived humic substances produced by microbial transformation of biogenic
531 particles has been recently described as the most important Fe-binding ligand in surface
532 waters of the Mediterranean Sea and Southern Ocean (Whitby et al. 2020a). In coastal waters
533 of Western Antarctic Peninsula and Drake Passage, humic-like compounds was attributed to
534 the mucus production (as exopolymeric substances) by phytoplanktonic assemblages
535 (Trimborn et al. 2015) confirming the marine origin of these compounds. Senescence of
536 biological material is also a source of exopolymeric substances released to the water column
537 as cellular exudates, which is believed to be included in the pool of humic-like compounds
538 (Norman et al. 2015).

539 The kinetic experiments revealed the presence of humic under-saturated by Fe as the
540 signal of humic substances in the sample N-20 bottom (Aug 2018) shown in Fig. 3 was
541 increased by 52 % after the saturation with 100 nM Fe(III). Similarly, Sukekava et al. (2018)
542 observed an increase of about 67 % on signal of humic substances after the saturation with 60
543 nM Fe(III) in seawater samples from Arctic Ocean. This result could indicate that the
544 behaviour of humic substances to outcompete Fe and other metals may be similar, irrespective
545 of the origin of the seawater, as our samples are fundamentally different than those analysed
546 by Sukekava et al. (2018) in terms of metal concentrations, physico-chemical conditions and
547 possibly organic ligands contents. So, the increase of the initial signal after Fe saturation can
548 be explained by two hypotheses: 1) the dissolved Fe is already complexed by other
549 compounds or stabilized as colloidal forms (see next item); and/or 2) humic substances are
550 bound by other metals.

551 The co-existence of multiple metals should change the Fe organic speciation due to the
552 differences on binding capacity of specific organic compounds with different metals (Hassler
553 et al. 2017). Humic substances are also important ligands for Cu, Zn, Co and Al in seawater
554 (Yang and van den Berg 2009). Considering the levels of dCu ($0.45 \mu\text{m}$) were lower than
555 dissolved Fe ($0.45 \mu\text{m}$) (Supplementary, Table S4), competition with Cu does not seem to be
556 a plausible explanation. On the other hand, dAl ($0.45 \mu\text{m}$) reached levels as high as 9.9 to
557 $12.5 \mu\text{M}$ in Nov 2015, from 0.39 to $9.2 \mu\text{M}$ in Feb 2016 and from 3.4 to $14.8 \mu\text{M}$ in Aug
558 2018 (Supplementary, Table S4) suggesting that Al could potentially outcompete Fe for
559 binding to humic substances in this coastal area. Similarly, dZn ($0.45 \mu\text{m}$) was a possible
560 candidate for humic competition in the acute phase of the impact as its concentrations
561 occurred between 0.7 and $1.1 \mu\text{M}$ in Nov 2015 (Supplementary, Table S4).

562 *Forms of dissolved Fe and soluble Fe*

563 The rapid change in Fe concentrations across the size spectrum indicates that a very
564 significant portion of Fe occurs in colloidal forms. Indeed, Fe in seawater was shown to be
565 primarily in the colloidal size fraction, considering the size range from 0.02 to $0.4 \mu\text{m}$ (Wu et
566 al. 2001) and from 10 kDa to $0.2 \mu\text{m}$ (Fitzsimmons et al. 2015). In our case, these colloids,
567 highly modified by unnatural chemical and physical processes (mineral treatment for Fe ore
568 pre-concentration), may be the main reason for the high dissolved Fe levels in the $< 0.45 \mu\text{m}$
569 and $< 0.22 \mu\text{m}$ size fractions.

570 This is also in agreement with changes to flocculation processes that were demonstrated
571 by settling experiments performed with mud sediment fraction ($< 63 \mu\text{m}$) from the continental
572 shelf before and after the disaster. The post-disaster decrease in the settling velocity of fine
573 flocs was suggested to be a result of changes of the sediment composition, like the presence of
574 porous Fe oxide goethite (Grilo et al. 2018). In addition, adsorptive characterisation of tailings

575 from the Fundão dam was consistent with a monolayer model with low adsorption capacity
576 and low interaction among adjacent molecules (Almeida et al. 2018) which means that this
577 material was probably less prone to processes of flocculation.

578 Humic substances played a minor role in stabilizing the soluble Fe fraction. Therefore,
579 soluble Fe speciation can be assumed to be mostly comprised of various small organic
580 complexes, nanoparticles (essentially Fe oxy-hydroxide) and a minute amount of free metal
581 ions (Tagliabue et al. 2017). Considering that humic substances are generally the most
582 important organic ligands in coastal waters affected by riverine inputs (Laglera and van den
583 Berg 2009), it is therefore likely that in our case, processes other than complexation by humic
584 substances are at play to stabilise soluble Fe in the water column. Even though other natural
585 organic substances, such as siderophores, are important ligands to soluble Fe in marine
586 systems (Gledhill and Buck 2012), these compounds are unlikely to be the predominant
587 ligand in view of the low levels of Chl-*a* that were consistently measured in our study.

588 The complexation of soluble Fe with unnatural organic ligands is one hypothesis to be
589 considered. Organic compounds are frequently used during the flotation stage employed to
590 concentrate the mineral product as a richer Fe ore pulp (Filippov et al. 2014). Reverse cationic
591 flotation using amine-based collectors is the most common method employed by the Fe ore
592 processing industry (Filippov et al. 2014). Amine adsorb on silicates (mainly quartz) surface
593 and also on metal oxides causing the flotation of quartz and other associated mineral (Filippov
594 et al. 2014), such as goethite. Querol et al. (1999) and Alzaga et al. (1999) were the first
595 authors to reference aromatic amine compounds as a geochemical tracer of mine spillage after
596 the Aznalcóllar dam disaster (Los Frailes, SW Spain). Similarly to the Fundão, ore processing
597 in Aznalcóllar generated waste products with very high Fe content and other chemical
598 contaminants, like amine compounds from flotation reagents, were deposited as tailings

599 (Querol et al. 1999). Aromatic amine was widespread recorded either in atmospheric
600 particulate matter (Querol et al. 1999) or in water bodies affected by that mining disaster
601 (Alzaga et al. 1999). Likewise, infrared spectroscopy analyses confirmed the presence of
602 primary and secondary amines and amides in the Fundão tailing composition (Almeida et al.
603 2018) whereas ether amine was quantified in high concentrations (up to 57.8 mg kg⁻¹) when
604 comparing sediments contaminated by those tailings against preserved areas (Santos et al.
605 2019). According to these authors both amine and Na are the main toxic compounds found in
606 the Fundão tailings and both are related to reverse flotation procedure (Santos et al. 2019).
607 These compounds would appear to be highly stable as aromatic amine have been frequently
608 identified in seawater samples collected on the continental shelf adjacent to the Doce River
609 mouth since the disaster (R.R. Neto unpubl.). Stability of the complex soluble Fe-amine and
610 concentrations of that amine ligand could be high enough to compete with the binding from
611 other natural ligands, such as humic substances, but this is presently unknown.

612 The presence of colloidal Fe species smaller than 0.02 μm is also a likely hypothesis to
613 support the levels of soluble Fe in our study area. Analyses of sedimentary metal partitioning
614 in areas affected by the Fundão dam tailing showed that pyrite accounts for only 1 % of all
615 metals whereas crystalline Fe oxyhydroxides are the most important solid phases for Fe and
616 other metals (Queiroz et al. 2018). Fitzsimmons et al. (2015) reported that inorganic Fe
617 occurring as nanoparticles was probably ubiquitous in the colloidal phase (10 kDa < cFe < 0.2
618 μm) of samples collected in surface oceanic waters. Inorganic colloids as nanoparticulate
619 pyrite (FeS₂) (Gartman et al. 2014), Fe-containing silicate particles (Gartman et al. 2014) and
620 nanoparticulate Fe(III) oxyhydroxides (Fitzsimmons et al. 2017) have been identified to
621 encompass the bulk of dissolved Fe in fluids leaking from hydrothermal vents. These
622 inorganic entities fall into the colloidal and soluble size classes as their aggregates reach from

623 3 nm to 200 nm in size (Gartman et al. 2014; Fitzsimmons et al. 2017). They are characterized
624 by a relatively long half-life, slow settling rates and thus a potential to travel long distances
625 (Gartman et al. 2014). Another process controlling the stability of nanoparticles is the
626 reversible scavenging mediated by desorption/sorption ligand exchanges from particulate Fe
627 (pFe) to the colloidal phase which protect it from precipitation and settling (Fitzsimmons et
628 al. 2017). These processes are expected to occur in ocean regions with high particulate Fe
629 inputs, including continental margins (Fitzsimmons et al. 2017).

630 Taking all the above factors into consideration, the different dissolved Fe size fractions
631 occur as a continuum of inorganic and organic components regulating the dissolved Fe
632 speciation (Tagliabue et al. 2017). The major dissolved Fe form in the study area is colloidal
633 Fe, mostly as nanoparticulate Fe(III) oxyhydroxides which may be stabilised by amine or
634 humic compounds. Amine compounds in particular, are thought to strongly bind Fe, thus
635 controlling its solubility in the coastal waters off the Doce River (Fig. 7).

636 *How much Fe can be transported to the open ocean?*

637 In view of the continuing input and high stability of dissolved Fe and soluble Fe in those
638 waters, it is worth considering the possibility of Fe transport from the continental shelf to the
639 open ocean. Modelling study evaluating the dispersion of contaminated Doce River plume
640 two months after the disaster showed a net flow mainly southward along the outer shelf, shelf
641 break and reaching the open ocean about 200 km from the coast (Marta-Almeida et al. 2016).
642 The riverine water residence time on the shelf is a key factor to determine the transport of
643 inorganic N and P to the oceans and similar behaviour is expected for trace metals (Sharples
644 et al. 2017). Shelves with short residence time of riverine plume (essentially located at low
645 latitudes) deliver higher loads of nutrients to the open ocean (Sharples et al. 2017). In this
646 context, the mean shelf residence time at Abrolhos (17.5 S, 37.5 W; 250 km off the Doce

647 River mouth) was estimated to be 7 days, with the result that 84 % and 97 % of the dissolved
648 inorganic nitrogen and phosphorus (DIN and DIP, respectively) reached the open ocean,
649 which is also believed to occur for metals (Sharpes et al. 2017). Considering that Abrolhos is
650 a wider shelf (250 km) than that at our study site (50 km), the residence time on the
651 continental shelf adjacent to the Doce River mouth is expected to be lower.

652 Using a conservative residence time of 7 days, a 12 x 15 km impacted area (area
653 monitored in this study; Fig. 1), an average depth of 20 m and an average dissolved Fe (0.45
654 μm) of $1.44 \mu\text{M}$ (average concentration in Feb 2016 and Aug 2018; Table 2), the total Fe flux
655 to the open ocean is estimated at c.a. $260 \text{ Mmoles y}^{-1}$, which represents approximately
656 between 1 and 5% of the net Fe fluvial worldwide flux in the oceans ($5.4 - 23 \text{ Gmoles y}^{-1}$ –
657 Chester 2003), highlighting the sheer magnitude of this event. Furthermore, this 260 Mmoles
658 y^{-1} Fe flux is a lower approximation since: 1 - the impacted area might be significantly larger
659 than the area considered here (the Doce River turbidity plume extended more than 75 km into
660 the continental shelf during the acute phase of the impact according to Rudorff et al. 2018);
661 and 2 - the sampling campaigns were conducted during conditions where remobilisation
662 processes were probably at their lowest (low river discharge; Fig. 2; and no cold front
663 passage). In addition, using an average Doce River flow of $540 \text{ m}^3 \text{ s}^{-1}$ (Oliveira and Quaresma
664 2017) and an average dissolved Fe ($0.45 \mu\text{m}$) concentration of c.a. $1.6 \mu\text{M}$ (mean
665 concentration found in Feb 2016 in comparable river discharge conditions; Table 2) a net
666 dissolved Fe flux from the Doce River is estimated at c.a. 27 Mmoles y^{-1} suggesting that in
667 post-disaster conditions almost c.a. 90 % of dissolved Fe present in the water column is
668 coming from continuous remobilisation of the sediment deposited on the continental shelf
669 (Fig. 7). This contribution of the Doce River for the dissolved Fe fluxes to the continental
670 shelf is also in agreement with dissolved Fe fluxes calculated by Hatje et al. (2017) which

671 reached 58.8 mg dFe s⁻¹ (c.a. 35 Mmoles y⁻¹) in river discharge conditions of about 1,000 m³
672 s⁻¹.

673 *Issues to be further discussed: the fate of submarine tailings disposal*

674 Considering the huge fluxes of dissolved Fe estimated here and the main role of
675 sediment/tailings resuspension on the continental shelf, it is also worth to consider the role of
676 submarine tailings disposal and deep-sea tailings placement for dissolved metal release and
677 consequent ocean enrichment (Ramirez-Llodra et al. 2015). Tailings disposal in marine shore
678 was used in the 70s and has been recently discussed as an alternative strategy to deal with
679 land tailing disposal problems, including catastrophic rupture (Kwong et al. 2019). Currently,
680 there are fifteen mines in activity using submarine tailings disposal or deep-sea tailings
681 placement whereas most of operations started in the past were closed and generated
682 abandoned mine wastes along the coast (Ramirez-Llodra et al. 2015). The accumulation of
683 tailings in marine systems are object of concern since most of the impacts, including chemical
684 processes of dissolved and particulate metals released from the submarine tailings, are still
685 unknown (Ramirez-Llodra et al. 2015). Our study suggests that mining tailings deposited on
686 marine bottom might be non-negligible sources of stable forms of dissolved Fe and soluble Fe
687 to the oceans and may affect the global Fe ocean budget considering all the coastal areas
688 affected by mining activities worldwide (Longhini et al. 2019).

689 *Conclusion*

690 The Fe ore tailings spillage after the Fundão dam disaster lead to huge and stable
691 concentrations of dissolved and soluble Fe in the adjoining coastal areas even three years
692 later. This dissolved and soluble Fe stability over time suggests that processes are at play to
693 help Fe remobilization, e.g. complexation or stabilisation of Fe rich colloids through
694 adsorption of organic molecules. While humic substances have been previously identified as

695 the main Fe ligands in coastal waters, the Fe-humic concentrations observed in our study
696 indicate that other processes are able to stabilise Fe in the water column. Colloidal particles
697 are the main forms encompassing the bulk of dissolved Fe fractions, while other organic
698 substances (here supposed to be amine compounds) are probably acting to stabilise small
699 colloids as nanoparticulate Fe(III) oxyhydroxides. Further studies should evaluate the relation
700 between dissolved Fe and synthetic organic ligands used during Fe ore processing
701 (specifically amine groups). Microscopy studies to identify the presence of nanoparticulate
702 colloidal species could also shed light on the processes at play that maintain Fe in the soluble
703 fraction.

704 At present, the main source of dissolved Fe to the water column is the resuspension of fine
705 mud on the continental shelf which has a high content of goethite, whereas the offshore
706 transport of aquatic colloids by the Doce River and the remobilisation of accumulated
707 material from the river bed seem to be less representative. The Fe fluxes estimated here are
708 comparable to 1 – 5 % of the net Fe fluvial worldwide inputs to the oceans (as calculated by
709 Chester 2003) highlighting the sheer magnitude of this catastrophe. The role of mining
710 tailings as sources of stable forms of dissolved and soluble Fe in a global context is currently
711 unknown but this study suggests that it should be investigated further.

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886 *Figure legends*

887 **Fig. 1.** Distribution of sampling stations on the continental shelf adjacent to the Doce River
888 mouth (Southeast, Brazil) after the Fundão dam rupture. N, C and S correspond to the sectors
889 North, Central and South, respectively; 10, 20 and 30 indicate the isobaths 10, 20 and 30
890 meters.

891 **Fig. 2.** Daily average of the Doce River discharge ($\text{m}^3 \text{s}^{-1}$) for the months November 2015,
892 December 2015, January 2016, February 2016 and August 2018. Black dashed arrow
893 represents the day that the iron ore tailings reached the coastal waters after the Fundão dam
894 rupture; Light grey arrows indicate the sampling days. Data from Agência Nacional de Água
895 – ANA (Colatina station): <https://www.snirh.gov.br/>.

896 **Fig. 3.** Catalytic adsorptive cathodic stripping voltammetry used for determination of humic

897 substances. Peak heights were obtained from the sample N-20 bottom (Aug 2018) before and
898 after two iron additions of 100 nM and kinetic stabilization for 2 h 30 min. Light grey markers
899 define the original Fe-humic complexes in the sample; black markers are Fe-humic complexes
900 after saturation of all humic substances binding with iron; triangle markers: internal
901 calibration with iron-saturated SRFA standard to determine the humic substances
902 concentration. Dissolved Fe ($0.22 \mu\text{m}$) concentration in the sample was $698 \pm 44 \text{ nM}$.
903 Procedure as described by Sukekava et al. (2018). SRFA: Suwannee River Fulvic Acid.

904 **Fig. 4.** Spatial and temporal distribution of dissolved Fe ($0.45 \mu\text{m}$), dissolved Fe ($0.22 \mu\text{m}$),
905 soluble Fe ($0.02 \mu\text{m}$) and humic substances concentrations (average between the surface and
906 bottom data) of the continental shelf adjacent to the Doce River mouth. dFe: dissolved Fe;
907 sFe: soluble Fe; HS: humic substances.

908 **Fig. 5.** Spatial distribution of dissolved Fe ($0.45 \mu\text{m}$) (dark grey boxplots), dissolved Fe (0.22
909 μm) (light grey boxplots) and soluble Fe ($0.02 \mu\text{m}$) (white boxplots) to highlight the
910 difference in concentrations between the size-factions (see scale breaks) in each transect over
911 the course of the three samplings (Nov 2015, Feb 2016 and Aug 2018). dFe: dissolved Fe;
912 sFe: soluble Fe.

913 **Fig. 6.** Scatterplot diagrams between a) humic substances and dissolved Fe ($0.22 \mu\text{m}$)
914 concentrations in surface seawater, $R = 0.62$, $p = 0.04$, $n = 12$ (excluding the points out of the
915 linear fit as showed in the inserted diagram: $R = 0.98$, $p = 0.00001$, $n = 9$); b) Phaeophytin and
916 dissolved Fe ($0.22 \mu\text{m}$) concentrations including surface and bottom results, $R = 0.40$, $p =$
917 0.04 , $n = 25$. Dashed lines represent the upper and lower 95% confidence limit ($\alpha = 0.05$).
918 dFe: dissolved Fe; HS: humic substances.

919 **Fig. 7.** Schematic presentation of the dissolved and soluble Fe speciation in the water column
920 of continental shelf in the vicinity of the Doce River. ^a dissolved Fe fluxes and the relative
921 importance of Doce River flow *vs* sediment resuspension for dissolved Fe inputs were
922 estimated as described in the text. dFe: dissolved Fe.

923 **Table 1.** Physico-chemical (temperature, salinity, pH and suspended particulate matter -
 924 SPM) and biological parameters (chlorophyll-*a* and phaeophytin) recorded in the continental
 925 shelf adjacent to the Doce River mouth at two depths (surface and bottom) and at three
 926 different times after the environmental disaster (November 2015, February 2016; August
 927 2018). ND: not determined; BDL: below detection limit.

SAMPLE	T (°C)		Salinity		pH		SPM (mg L ⁻¹)		Chl- <i>a</i> (µg L ⁻¹)		Phaeophytin (µg L ⁻¹)	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
<i>Nov 2015</i>												
N-20	26.5	21.8	36.5	36.6	ND	ND	30.0	42.0	0.62	5.35	0.68	BDL
C-10	24.8	24.4	36.4	36.5	ND	ND	85.0	300.0	BDL	BDL	1.87	3.03
<i>Feb 2016</i>												
N-25	26.7	20.9	36.7	36.5	ND	ND	2.93	1.20	1.23	BDL	BDL	0.50
C-30	26.1	21.1	36.8	36.5	ND	ND	1.60	0.27	0.45	BDL	0.18	0.67
S-20	24.0	21.9	34.5	36.5	ND	ND	1.07	62.0	1.78	1.78	0.30	3.21
<i>Aug 2018</i>												
N-10	24.8	25.1	36.0	36.5	8.07	8.09	16.5	25.3	BDL	1.91	2.51	BDL
C-10	24.5	25.3	36.0	36.5	8.11	8.11	23.3	24.7	BDL	1.49	2.86	0.59
S-10	25.0	24.6	36.0	36.5	8.09	8.07	18.3	21.4	BDL	BDL	2.27	3.70
N-20	23.6	26.5	35.0	36.0	8.13	8.07	16.6	16.9	BDL	0.41	1.70	1.01
C-20	25.4	25.0	36.5	36.5	8.09	8.07	14.1	15.8	BDL	0.41	2.96	0.73

S-20	25.7	24.5	36.0	36.0	8.14	8.12	16.9	16.2	2.03	0.89	BDL	0.98
N-30	24.0	23.6	36.0	36.5	8.15	8.12	17.3	14.1	BDL	BDL	1.42	0.85
C-30	25.0	25.3	36.5	36.5	8.06	8.04	14.3	15.7	BDL	0.45	2.67	0.49
S-30	25.1	24.7	36.5	36.5	8.00	8.02	14.4	15.5	BDL	0.42	1.42	1.06
Min	23.6	20.9	34.5	36.0	8.00	8.02	1.07	0.27	0.45	0.41	0.18	0.49
Max	26.7	26.5	36.8	36.6	8.15	8.12	85.00	62.00	2.03	5.35	2.96	3.70
Average	25.1	23.9	36.1	36.4	8.09	8.08	19.44	20.86	1.22	1.45	1.74	1.25
SD	0.9	1.8	0.6	0.2	0.05	0.03	20.50	16.20	0.69	1.58	0.97	1.11

928 **Table 2.** Iron concentrations for dissolved dFe (< 0.45 μm), dissolved Fe (< 0.22 μm) and
 929 soluble Fe (< 0.02 μm) fractions in the continental shelf adjacent to the Doce River mouth.
 930 The dissolved Fe (0.45 μm) concentrations were determined by ICP-MS; dissolved Fe (0.22
 931 μm) and soluble Fe were analysed by cathodic stripping voltammetry. The standard deviation
 932 of the dissolved Fe (0.22 μm) indicated for some samples was obtained from replicate
 933 measurements. The percentages shown in parentheses indicate the proportion of dissolved Fe
 934 (0.45 μm) found in the < 0.2 μm or < 0.02 μm fraction. dFe: dissolved Fe; sFe: soluble Fe;
 935 ND: not determined.

Sample	dFe (0.45 μm) nM		dFe (0.22 μm) nM		sFe (0.02 μm) nM	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
<i>Nov 2015</i>						
N-20	1,136	1,177	204 \pm 11 ($n = 3$) (17%)	109 (10%)	10 (1%)	ND
C-10	2,607	2,570	182 (6%)	ND	15 \pm 1 (1%)	ND
<i>Feb 2016</i>						
N-25	1,756	2,184	35 \pm 2 ($n = 2$) (2%)	30 (2%)	ND	ND
C-30	1,666	1,550	30 (3%)	61 (5%)	ND	38 \pm 2 (3%)
S-20	1,032	1,337	97 (6%)	71 (5%)	11 \pm 0.4 (1%)	ND
<i>Aug 2018</i>						

N-10	964	739	245 (26%)	75 (10%)	ND	11 ± 0.4 (1%)
C-10	990	964	137 (14%)	91 (9%)	16 ± 1 (2%)	29 ± 1 (3%)
S-10	1,641	1,776	148 (9%)	206 (12%)	9 ± 1 (1%)	ND
N-20	784	925	117 (15%)	698 ± 44 (<i>n</i> = 2) (76%)	25 ± 1 (3%)	ND
C-20	1,238	1,281	64 (5%)	51 (4%)	ND	17 ± 1 (1%)
S-20	2,170	1,975	102 (5%)	194 ± 13 (<i>n</i> = 2) (10%)	ND	ND
N-30	837	927	125 (15%)	176 ± 5 (<i>n</i> = 2) (19%)	18 ± 1 (2%)	28 ± 1 (3%)
C-30	1,140	1,383	155 (14%)	185 (13%)	ND	10 ± 1 (1%)
S-30	2,784	1,716	186 ± 7 (<i>n</i> = 3) (7%)	95 (6%)	10 ± 0.4 (0.4%)	ND
Min	784	739	30	30	9	10
Max	2,784	2,570	245	698	25	38

936 **Table 3.** Humic substances concentrations in 0.22- μm filtered samples, and corresponding Fe
 937 binding capacity of humic calculated according to Laglera and van den Berg (2009). % dFe (0.22
 938 μm)-HS and % sFe-HS indicate the maximum contribution that Fe-humic complexes can make to
 939 the dissolved Fe and soluble Fe pool, respectively. dFe: dissolved Fe; sFe: soluble Fe; HS: Humic
 940 substances; SRFA: Suwannee River Fulvic Acid; ND: not determined.

Sample	HS ($\mu\text{g SRFA L}^{-1}$) ($< 0.22 \mu\text{m}$)		^(a) Fe bound by HS (nmol L^{-1})		%dFe (0.22 μm)-HS		%sFe-HS ^(b)	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
	<i>Nov 2015</i>							
C-10	219	ND	4	ND	2	ND	24	ND
N-20	247	44	4	1	2	1	43	2
<i>Feb 2016</i>								
S-20	ND	129	ND	2		3	ND	ND
C-30	15	241	0.2	4	1	7	0.2	11
N-25	ND	229	ND	4		13	ND	8
<i>Aug 2018</i>								
N-10	350	196	6	3	2	4	15	31
C-10	328	91	6	2	4	2	34	5
S-10	ND	79	ND	1	ND	1	ND	2
N-20	127	65	2	1	2	0.2	8	3
C-20	56	68	1	1	2	2	1	7

S-20	257	181	4	3	4	2	6	13
N-30	103	141	2	2	1	1	10	8
C-30	189	99	3	2	2	1	4	16
S-30	47	ND	1	ND	0.4	ND	8	ND
Min	15	44	0.2	1	0.4	0.2	0.2	2
Max	350	241	6	4	4	13	43	31

941 ^(a)Normalized with $16.7 \text{ nmol Fe (mg FA)}^{-1}$ according Laglera and van den Berg (2009).

942 ^(b)Assuming levels of humic substances measured in dissolved Fe ($< 0.22 \mu\text{m}$).