Free phenolic compounds in waters of the Ross Sea.

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Abstract

The presence of free phenolic compounds (PC) in Antarctic sea water has been investigated to explain their source and particle size distribution in the atmospheric aerosols, as determined in our previous research. The sea water samples were filtered to distinguish the PC concentrations in the particulate and dissolved fractions. Two sample preparation procedures were developed to quantify nine PC in both fractions. The highest concentrations were found in the dissolved fraction of Ross Sea water, with vanillin, vanillic acid, acetovanillone and *p*-coumaric acid being the most abundant PC. Dissolved PC were mainly found in the upper part of water column. This facilitated the sea water-air exchange by bubble busting processes. In the aerosol, they were mainly found in the fine fraction, where these compounds have a higher degree of oxidation than PC detected in seawater, suggesting that they were newly emitted and they have been not yet oxidized. These results supported our previous hypothesis that PC were locally emitted into the atmosphere from the Ross Sea.

Three different possible sources of PC are hypothesized for Antarctic sea waters: 1) from the intrusion of Modified Circumpolar Deep Water that may transport oceanic lignin; 2) from phytoplankton biomass that may be a source of PC in Antarctic waters since diatoms produce exudates that contain vanillic acid, *p*-coumaric acid and syringic acid; 3) from the melting of glaciers and sea ice: glaciers contain lignin that can be degraded, while in the sea ice there are diatoms that may release PC.

Statistical analysis and the low value of vanillic acid/vanillin ratio indicated that the most plausible source for PC in the dissolved fraction was the senescence of phytoplankton. As a contrast, particulate PC with higher vanillic acid/vanillin ratios were ascribed to degraded lignin or the sorption of diagenically oxidised material on particles.

1.Introduction

Phenolic compounds (PC) are compounds of plant origin because they are the building blocks of lignin. Lignin is a biopolymer that makes up one third of dry wood biomass (Jex et al., 2014; Li et al., 2012), and it is ubiquitous in the environment. Lignin contains three main phenolic groups: the vanillyl, the syringyl and the cinnamyl moieties and their relative abundances can be used to distinguish between types of plants. While softwoods contain mainly vanillyl moieties, hardwoods prevalently include syringyl groups, and grasses are rich in cinnamyl substrates (Oros et al., 2006; Oros and Simoneit, 2001a; Oros and Simoneit, 2001b). Since lignin is relatively resistant to microbial degradation in comparison to other plant components, it is widely used as an indicator of organic matter in riverine, lacustrine and marine waters (Li et al., 2012; Opsahl and Benner, 1997), as well as an indicator for specific vascular plants (Jex et al., 2014; Li et al., 2012; Opsahl and Benner, 1997). Lignin is also an important component of organic matter in soil, peats and sediments (Jex et al., 2014). As the chemical composition of lignin is indicative of wood type, it is often used during paleo-environmental research on soils and sediments (Jex et al., 2014). Lignin can be biologically and photochemically degraded (Benner and Kaiser, 2011; Jex et al., 2014). Compounds produced from lignin degradation are found in soils (Thevenot et al., 2010), in rivers and the sea.

Free PC are molecular tracers also produced by lignin pyrolysis (Simoneit et al., 1999). When they are injected into the atmosphere during biomass burning, their proportions are indicative of the type of wood combusted (Zangrando et al., 2016a). Some free PC, such as vanillic acid, have also been proposed as additional biomass burning tracers in ice core paleorecords (Giorio et al., 2018; Grieman et al., 2015; McConnell et al., 2007; Wolff et al., 2012). However, free PC have been rarely determined in river or sea water (Edelkraut, 1996; Keil et al., 2011).

Recently, a study on Antarctic aerosols collected at a coastal site and on the plateau highlighted a different particle-size distribution and different seasonal trends between levoglucosan, an unambiguous biomass burning tracer, and free PC (Zangrando et al., 2016b; Zangrando et al., 2013) .

The aim of this paper is to identify plausible sources of PC in the Antarctic environment and to explain their particle size distribution in atmospheric aerosol and their relative abundances in comparison to levoglucosan. Due to the lack of vegetation in Antarctica, and the fact that ice-free areas account for less than 2% of the surface area, the most probably local source should be the ocean. In this work have been developed two HPLC-MS/MS analytical methods to determine vanillic acid (VA), vanillin (VAH), syringic acid (SyA), syringaldehyde (SyAH), homovanillic acid (HA), isovanillic acid (IVA), *p*-coumaric acid (PA), acetovanillone (VAC) and acetosyringone (SyAC) in the dissolved and particulate phases of sea waters. These methods were applied to Ross Sea water samples collected during the 2011-2012 expedition of the Italian National Research Programme in Antarctica. To the best of our knowledge, this is the first study on free PC concentrations and their distribution in Antarctic sea waters.

The main goal of this paper was to compare PC concentrations found in sea water samples with those previously reported in atmospheric aerosols samples (Zangrando et al., 2016b).

2.Experimental

2.1.Materials

The list of materials used is reported in the Supporting material section: Materials

2.2.Water sampling in the Ross Sea

The twenty-seven water samples were collected in the Ross Sea during the R/V Italica cruise from January 26 to February 8, 2012. The sampling area was divided as described in the sampling plan

(Figure 1 and sampling details in Table 1) into five transects near Cape Adare (CA)(transect A), Coulman Island (CL) (B), Cape Washington (CW) (C), and in three polynya areas: Terra Nova Bay (TNB) (D), Mc Murdo Sound (MMS) (E) and the Ross sea (F).

Sea water samples were collected at the fluorescence profile maximum obtained from CTD fluorescence measurements in the µg L^{-1} range (Chelsea Technologies Group Aqua 3 Chlorophyll a sensor) to collect specific samples at the primary biomass production maximum.

Sampling was performed using a *rosette* of 24 12 L Niskin bottles with a companion SBE9/11 plus CTD probe (Sea Bird Scientific) with sensors for dissolved oxygen, temperature, fluorescence, salinity and conductivity. The sea water samples were immediately filtered onboard using a glass microfiber filter GF/F (porosity 0.7 µm, diameter 47 mm, Whatman, Maidstone, UK), previously cleaned at 400°C for 4 h, to separate the dissolved and particulate fractions.

At 4 sampling sites (E1, E3, D3 and D7, Figure 1) three samples that bracketed the fluorescence maximum were collected (above, below and at fluorescence maximum) to define the vertical distribution. The wet filters were enveloped in a double layer of aluminum foil, whilst the water samples were transferred to polyethylene bottles. Both samples were stored at -20°C until analysis.

An aliquot of the water samples collected at the fluorescence maxima were buffered with a 4 % (v/v) formalin solution for phytoplankton counting.

2.3.Sample processing

Free phenolic compounds in the dissolved fraction.

A 500 mL sample of filtered sea water acidified with formic acid (2 % v/v, pH=5) in a volumetric flask was spiked with 250 ng (absolute amount) of $^{13}C_6$ labeled VAH (VAH*) and $^{13}C_1$ labeled VA (VA*). Samples clean-up and pre-concentration was performed using OASIS HLB SPE cartridges (6cc, 200 mg sorbent per cartridge, Waters). The cartridges were conditioned under vacuum with methanol (5

mL), and equilibrated with formic acid (2% v/v) in water (5 mL). The sample was then loaded onto the cartridge. The sea salt matrix was eliminated by washing the SPE cartridge with 5 mL of ultrapure water before elution. PC were eluted from the cartridge into a 7 mL vial at atmospheric pressure with 5 mL of methanol, however, this solvent strength would cause the immediate elution of the compounds during injection causing peak broadening. To prevent this a 250 µL aliquot of the sample was diluted to a final volume of 1 mL with water to reduce the eluent strength of the solvent in which the samples are dissolved, as reported by Kromidas (2000).

2.4.Free phenolic compounds in particulate fraction.

The determination of free PC in the particulate fraction was performed after breaking the filter into small pieces and transferring them into a 1.5 mL Eppendorf tube that was previously washed with methanol, then extracting them with 1.5 mL of a 50:50 water-methanol, solution for 30 min. To the samples, 38 ng (absolute amount) of VAH* and VA* were added and the extract was then filtered using a PTFE syringe filter (4mm, 0.2 µm, Phenomenex, Torrence, CA, USA). A 500 µL of sample in methanol was then diluted with 500 µL of water before analysis, to improve peak shape as described above. Field blanks were obtained analyzing GF/F previously cleaned by heating them at 400°C for 4h using the same extraction procedure.

2.5.Instrumental methods

The HPLC/(-)ESI-MS/MS instrumental method used in the present paper was the same reported by Zangrando et al. (2013) with the introduction of the mass spectrometer parameters to simultaneously determine acetovanillone and acetosyringone. Briefly: the chromatographic separation was obtained using a Zorbax Extend C18 (150 mm × 4.6 mm, 3.5µm, Agilent) column fitted to an Agilent 1100 Series HPLC system (Agilent, Waldbronn, Germany), with a gradient elution using a binary mobile

125 phase of a 0.01% formic acid water-based solution (solvent A) and a solution of methanol/acetonitrile
126 80/20 (solvent B), with an elution flow of 500 µL min^{-1}.
127 The PC were detected by mass spectrometric analysis with an API 4000 triple quadrupole mass
128 spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Ontario, Canada), equipped with a Turbo V
129 source operating in negative polarity with source parameters as reported in Zangrando et al. (2013).
130 Data acquisition was in multiple reaction monitoring mode with a 50 ms dwell time/transition.
131 Precursors and fragment ions, declustering potential, entrance potential, collision energy and collision
132 cell exit potential monitored are reported in Table S1. Due to the low PC concentrations in polar waters,
133 300 µL of sample were injected without any observed fronting or tailing effects on the
134 chromatographic peaks.
135
136 *2.6.Quality control*
137 The analytes were quantified using VA* and VAH* as internal standards. The native compounds peak
138 areas were compared with those of the labeled compounds. The results were corrected for the
139 instrumental response factors. During method validation, the instrumental linear response for PC was in
140 the concentration range of 1 to $3 \cdot 10^3$ ng L^{-1} using VA*, and 1 to $2.7 \cdot 10^5$ ng L^{-1} with VAH*. Good
141 linearity was obtained for all compounds and the R^2 values were always above 0.99.
142 Due to the lack of reference standard materials and the limited availability of Antarctic waters, the
143 analytical method was validated using waters collected from the Venice lagoon.
144 For the validation of the dissolved fraction method, 250 ng (absolute amount, abs) of native standard of
145 PC, and 250 ng abs of VA* and VAH* were added to 500 mL of filtered Venice lagoon water. The
146 unspiked lagoon water was considered as the blank. The validation for the particulate fraction was
147 performed adding spikes of the native target (38 ng abs) and VA* and VAH* as internal standards (38

ng abs) onto pre-combusted GF/Fs. Procedural blanks were prepared by only adding the internal standards to pre-combusted filters.

The reproducibility (expressed as CV%), trueness (as percent error), and the efficiency of the sample preparation procedure (yield %) were evaluated by preparing and analysing 5 replicate samples. The trueness was always < 10%, the CV%, were generally < 10%, and the yield% was generally > 80% for PC in the dissolved phase and > 67 % for each compound in the particulate fraction. The validation data and the internal standard compounds used for the quantification of each analyte are reported in Table S2 for the dissolved fraction, and Table S3 for the particulate fraction.

Sample contamination introduced during sample preparation was estimated employing ultrapure water purified using an OASIS HLB SPE cartridge, so that every trace of phenolic compounds in the water was eliminated. Procedural blanks were prepared using this treated ultrapure water and the blank values were subtracted from the PC concentrations found in the seawater samples. The values of procedural blanks, Method Detection Limit (MDL) and Method Quantification Limit (MQL) are reported in Table S4.

Matrix effects (ME) were evaluated at 50, 250 and 500 ng L^{-1} in the particulate fraction and in dissolved fraction considering ME (%) (Matuszewski et al., 2003) with and without the use of the internal standard (Table S5). The use of internal standard helped to reduce the matrix effects.

2.7.Chlorophyll a and phaeophytin a determination in Antarctic sea water

Chlorophyll *a*, found in every phytoplanktonic cell, is one of the most widely used proxies for determining the phytoplankton biomass. Assays of Chl *a* and phaeophytin *a* provide a useful information on the spatial and temporal variability of phytoplankton biomass and allows us to

171 determine the composition and ecological status of the phytoplankton community as well as estimate
172 the health water body.
173 Chl *a* and Pheo *a* in Antarctic seawater samples were assayed using an LS55 Fluorescence
174 Spectrophotometer (Perkin Elmer, Waltham, MA - USA). To assay Chl *a* and Pheo *a,* the seawater
175 samples (1 to 2 L) were filtered using Millipore filters (MCE, mixed cellulose esters, 0.45 µm) . The
176 filters were then stored in centrifuge vials at -20 °C in the dark until extraction. The pigment extraction
177 was carried out by harmonizing the methods of Lorenzen & Jeffrey (1980), Arar & Collins (1997) and
178 Smith et al. (1981). The reagents prepared in advance were a 90% acetone solution and 1 mol L^{-1}
179 hydrochloric acid solution (HCl). To extract the pigments, 10 mL of 90% acetone was added to each
180 filter in the centrifuge vial, this was then shaken well to dissolve the filter. The centrifuge vials with the
181 dissolved filters were then kept in the dark at 4 °C for 16 hours. After this time, the centrifuge vials
182 were centrifuged for 5 to 10 minutes at 4000 rpm and then the supernatant were collected in clean vials
183 for fluorescence measurement.
184 Standard solutions of pure Chl *a* at different concentrations (0.01-835 µg L^{-1} range) were prepared to
185 calibrate instrument sensitivity to this pigment. The supernatant of a blank filter dissolved in 10 ml of
186 90% acetone was used as the blank. Standards solutions and sample supernatants were analyzed in a 1
187 ml quartz cuvette, excitation/emission wavelengths were 435 nm and 667 nm. The calibration for Pheo
188 *a* was carried out 2-3 minutes after the blank and standard solutions (0.01-835 µg L^{-1}) were acidified
189 with a few drops of 1 mol L^{-1} HCl. The supernatants of seawater samples were acidified as well and
190 their fluorescence (excitation/emission wavelengths 390 nm and 667 nm) was recorded.
191 The formulas reported in Arar & Collins (1997) and Lorenzen & Jeffrey (1980) were used to calculate
192 the concentrations of Chl *a* and Pheo *a*.
193

194 ## 3.Results and Discussion

195 *3.1.Phenolic compounds in Ross Sea water.*

196 Analysis of the twenty-seven sea water samples highlighted that mainly VAH, VA, VAC and PA acid
197 were present in the dissolved and particulate fractions (Table 2-3). SyA, SyAH and HA were observed
198 only at residual concentrations while IVA and SyAC were below the detection limit (BDL) in all
199 samples.

200

201 *3.2.Free phenolic compounds in the dissolved and in the particulate fraction.*

202 In the dissolved fraction of our samples, VAH had the highest concentration, ranging from 52 to 859 ng
203 L^{-1} (Table 2), with a mean concentration of 191 ng L^{-1}, which accounted for 92.8% of the total PC
204 present. There are few literature reports of VAH concentrations in marine waters: a mean concentration
205 of 100 ng L^{-1} was found in samples collected in March 2010 in Puget Sound (Washington State, USA)
206 while a mean value of 10 ng L^{-1} was determined in Barkley Sound (British Columbia, Canada) (Keil et
207 al., 2011). In the estuarine waters of the Elbe river (Germany) (Edelkraut, 1996) VAH had a mean
208 concentration of 8000 ng L^{-1}.

209 In Antarctic sea waters, VA and VAC were the next most concentrated compounds with mean
210 concentrations for VA of 7 ng L^{-1} (range 2 to 47 ng L^{-1}, 3.3% of the total PC), while VAC had a mean
211 concentration of 10 ng L^{-1} (range BDL-70 ng L^{-1} and 3.0% of the total PC). PA concentrations ranged
212 from 0.1 to 1.0 ng L^{-1}, with a mean concentration of 0.4 ng L^{-1}. This value was obtained after excluding
213 sample F7_108m where the PA concentration was 32.6 ng L^{-1}, two orders of magnitude higher than the
214 other samples. SyA, SyAH and HA were present only sporadically (Table 2) representing collectively
215 only 0.2% of the total PC concentrations.

216

217 In the particulate fraction, mean concentrations of PC were lower than those in the dissolved fraction
218 (Table 3). As before, the most concentrated compound was VAH at a mean concentration of 1.0 ng L^{-1}

(range 0.1-3.4 ng L^{-1}, 85.7% of total PC concentrations), while VAC and VA accounted for 8.8% and 4.3% respectively of the PC total, with mean concentrations of 0.10 and 0.05 ng L^{-1}. PA was only present occasionally in the particulate fraction. Interestingly, in this phase, a higher number of samples contained SyAH and HA were at detectable levels.

3.3.Spatial distribution of tracers in the Ross Sea water.

To explain the spatial distributions, we have reported the PC concentrations on maps (Ocean Data View, Weighted-average gridding, Schlitzer, R., Ocean Data View, odv.awi.de, 2017). In doing so we have obtained a spatial distribution of the analytes along Victoria Land coast. The site F7_108m (F7 indicates the sampling site, 108m the depth) was excluded because the high concentration of PA observed at this site caused a flattening of the map colors.

In both the dissolved (D) and particulate (P) fractions, VA, VAH and VAC were the most significant PC in Ross Sea water and vanillin was the most concentrated compound (Table 2 and 3). The reason for the prevalence of vanillyl phenols in the environment lies in the relatively high reactivity of syringyl phenol in comparison to the vanillyl moiety. The higher number of methoxy groups in the aryl ring make syringyl molecules more prone to oxidation by UV-radiation (Benner and Kaiser, 2011).

For VAH in the dissolved fraction (VAH D, Figure 2A), the highest concentrations were observed in the Terra Nova Bay and Mc Murdo Sound areas (D and E transects). Similar distributions were observed for VA D and VAC D (Figure 2C and 2E).

VAH in the particulate fraction (VAH P, Figure 2B) was located mainly in the Southern coastal areas of Victoria Land, while VA P showed higher concentrations in the Northern coastal areas of the Ross

Sea in transect A (Figure 2D). The VAC P distribution (Figure 2F) showed high concentrations of this compound near Coulman Island (sample Cl3), and in transect D.

SyA, SyAH and HA were only sporadically found in the dissolved fraction. Whilst SyAH P (Figure 2H) and HA P were quantified in some samples with the highest concentrations in A and B transects (Table 3).

PA in the dissolved fraction was observed in each sample, and was predominantly in the Southern section of Ross Sea in McMurdo Sound (Figure 2G). On the contrary it was found in only some of the particulate fraction samples at very low concentrations. The most interesting sample was F7-108m collected in front of the Ross Ice Shelf which showed the highest concentration of PA D as well as significant concentrations of VA D, VAH D, SyA D and SyAH D (Table 2).

3.4.Vertical distribution of tracers in the Ross Sea water.

At the four sampling sites D3, D7, E1 and E3, samples were collected above, below and at the fluorescence maximum to investigate the vertical distribution of PC (Table 4). Using potential temperature and salinity data Table S6 (Orsi and Wiederwohl, 2009; Turetta et al., 2010; Turetta et al., 2017) for each sampling site at different depths, it was possible to assign the samples to different water masses.

The shallower samples collected at the fluorescence maximum can be said to be Antarctic Surface Water (AASW) produced by ice melting during the summer. At the TNB polynya (transect D) the samples at greater depth (D3_112m and D7_60m) were ascribed to High Salinity Shelf Water (HSSW). This region is where HSSW is generated by the continuous formation of sea ice, which increases sea water salinity (Budillon et al., 2003; Budillon and Spezie, 2000). In transect D the highest dissolved fraction concentrations of VA, VAH and VAC were observed in the shallower sample (D7_2m). While the highest concentrations of VAH P were in the samples (D7_10m).

At site E3 in the MMS polynya (transect E), close to the coast, higher concentrations of dissolved and particulate PC were observed in the intermediate sample (E3_29m). The vertical distribution for samples collected at E1 (transect E) is more complex, since PC D showed the same concentrations in the shallower (1.5 m) and in the deeper sample (70 m). At this sampling site the water at a depth of 1.5 m is AASW, while that at 70 m is Low Salinity Shelf Water (LSSW). LSSW is a large volume of water present at intermediate depths in the central eastern Ross Sea. The formation of LSSW is attributed to the interaction of AASW and the colder waters in the subsurface layer (Budillon et al., 2011). The different origins of these water masses could explain the higher concentrations observed in the deeper sample (E1_70m).

3.5.Comparison between phenolic compound concentrations in Antarctic aerosols and in seawater.

The determination of biomass burning compounds (levoglucosan and PC) in Antarctic aerosol in our previous work (Zangrando et al., 2016b) suggested possible other sources of PC in the Antarctic area based on the following observations. In coastal Antarctic atmospheric aerosols, PC were found in the fine fraction while levoglucosan, an unequivocal tracer of biomass burning, was mainly distributed in the coarse particles. If levoglucosan and PC were produced from the same emission source, they should have been present in the same atmospheric particles. The vanillic acid/vanillin ratio is linked to the oxidation of PC, these ratios were lower in coastal atmospheric aerosols than in those collected on the Antarctic plateau, indicating a lower degree of transformation at the coastal site. Finally, the ratios of levoglucosan/PC were very different when compared with ratios observed in aerosols affected by biomass burning from Zangrando et al. (2016b) and references therein. Furthermore, these indications were consistent with the literature that includes results from the Arctic (Fu et al., 2009; Zangrando et al., 2013). These evidence suggest another possible source that contributes to the presence of PC.

291 The prevalent PC abundances in Antarctic aerosols normalized to the total concentration were VAH,
292 (74%), VA (10%) and PA (6%). Similarly, in Antarctic sea waters, in both the dissolved and
293 particulate fraction, VAH and VA were the dominant compounds present.
294 In the dissolved fraction VAH was 93% of the total, while VAC, VA accounted for around 3% each
295 and PA for 1%. The spatial distribution study found the highest concentrations in the dissolved fraction
296 in the polynya area (D and E transects), while the vertical PC distribution highlighted that the highest
297 concentrations were generally in the shallower samples of Ross Sea water. The ocean through bubble
298 bursting can eject fine particulates (< 0.1 μm) containing organic compounds into the atmosphere,
299 principally during periods of high biological activity (Ault et al., 2013; O'Dowd and De Leeuw, 2007).
300 In accordance with the literature we mainly found PC in the < 0.49 μm particle fraction of Antarctic
301 aerosols, while levoglucosan was relatively enriched in the coarse particles.
302 The VA/VAH ratio is indicative of the oxidation state (Net et al., 2011) of PC, high values indicate a
303 high degree of oxidation of PC in aerosol. In the atmospheric aerosols, the ratio values were
304 significantly lower at coastal sites (0.13) than on the plateau (0.4-0.5), suggesting a local source of
305 young aerosol at the coast (Zangrando et al., 2016b). The mean VA/VAH ratio in the dissolved fraction
306 of our sea water samples was 0.04±0.02 whilst in the Antarctic coastal aerosol it was 0.13±0.06
307 (Student t-test p=000002). This increase in the VA/VAH ratio between the sea surface and the
308 atmosphere suggests a transformation process during emission..
309
310 *3.6. Possible sources of PC in Antarctic sea waters*
311 Different hypotheses were tested to try explain the presence of PC in Ross Sea water..
312
313 *3.6.1. Could free PC be from lignin contained in the Modified Circumpolar Deep Water?*

Generally PC in oceanic waters (Opsahl and Benner, 1997) are related to the photochemical and microbiological (Benner and Kaiser, 2011; Hernes and Benner, 2003; Opsahl and Benner, 1998) degradation of lignin (Opsahl and Benner, 1995) contained in dissolved organic material. A possible source of lignin of terrigenous origin could be from the intrusion of the Modified Circumpolar Deep Water (MCDW) derived from the mixing of Circumpolar Deep Water (CWD) which is a very old water and newly formed shelf waters (Rivaro et al., 2015). A strong incursion of MCDW into the Ross Sea has been observed each Austral summer in December and around the beginning of January (Castagno et al., 2017) and the inflow of MCDW onto the Ross Sea continental shelf also influences the TNB polynya (Rusciano et al., 2013). The MCDW could be a possible origin of the PC found in the particulate fraction, especially in the Northern sector (transects A and B).

3.6.2. Could free PC have a phytoplankton origin?

The Ross Sea is reportedly one of the most productive regions in the Antarctic, and it sustains an annual primary production of ca. 23.4 ± 9.98 Tg C yr^{-1}, accounting for more than one third of total shelf production in the Southern Ocean (Arrigo et al., 2008). The Ross Sea polynya is further known as a hyperproductivity area (Smith and Gordon, 1997). For this reason, we note the possibility that phytoplankton could be the source of PC. Although a limited number of studies report the profile of PC in phytoplankton, the literature supports this hypothesis because numerous PC (among them VA, PA, SyA) have been observed in extracts and exudates of diatoms (Rico et al., 2013), which were observed in the phytoplanktonic community assemblages studied in our samples. Diatoms regulate speciation, bioavailability and toxicity of trace metals such as iron and copper by producing and excreting PC, so the environmental availability of these metals influences the phenolic profile of the diatoms (Rico et al., 2013). PA has also been identified as a breakdown compound of algal lignin that is symptomatic of aging of *Emiliania huxley* (Seyedsayamdost et al., 2011). Further investigation and characterization of

338 molecules released from phytoplankton would be useful to better understand the chemical composition
339 of seawater. To investigate possible links with phytoplankton, sampling was carried out at the
340 maximum of fluorescence. In these samples, Chl *a* and Pheo *a,* as proxies of phytoplankton biomass
341 were determined together with PC.
342
343 The concentrations of Chl *a* and Pheo *a* in all the Antarctic seawater samples collected are reported in
344 Table 5. The concentrations of Chl *a* range from 0.01 μg L^{-1} to 0.72 μg L^{-1}, while the concentrations of
345 Pheo *a* range from 0.002 μg L^{-1} to 0.110 μg L^{-1}.
346 The Pheo *a*/Chl *a* ratios, can be used to explain changes in the residual Pheo *a* and changes in the
347 growth stages of the phytoplanktonic community assemblage, are reported in Table 5. The higher is the
348 ratio, the higher the number of phytoplanktonic cells which are degraded, damaged or in a senescence
349 state (Reynolds, 2006).
350 Further information on the concentrations of Chl *a* and Pheo *a*, microscopic counts of the
351 phytoplankton community assemblages and discussion on Pheo *a*/Chl *a* ratios the Antarctic seawater
352 are in Supplementary material.
353
354 If the chlorophyll concentrations (Chlorophyll concentration, NASA) and sea ice extent (Sea Ice
355 Concentration and Snow Extent Global, NASA) maps are shown side by side, Figures 3A, 3B and 3C
356 and 3D are obtained. Comparison of these with the maps of the spatial distribution of PC Figure 2, we
357 find that that the highest target compound concentrations were observed in transects D and E in areas
358 of the polynyas of Terra Nova Bay and Mc Murdo Sound and near the marginal ice zones (Figure 1).
359 Remote sensing data of chlorophyll *a* concentrations agreed with on-site CTD multiparameter
360 measurements of fluorescence (including that of chlorophyll a) (Figure 4A). This coincided well with
361 the distribution of dissolved oxygen (originating from the atmosphere and from photosynthetic activity)

362 (Queste et al., 2015) (Figure 4B), as well as with the spatial distributions of Chl *a*, Pheo *a* (proxies
363 related to phytoplankton biomass, Figure 4C-D), and PC (Figure 2) which were measured in the
364 Antarctic sea water samples. These spatial distributions seem to suggest a relationship between
365 phytoplanktonic biomass and PC.
366
367 *3.6.3. Could free PC be related to lignin compounds and/or biota in melted ice?*
368 The sampling plan also considered possible differences in PC concentrations at different depths. The
369 vertical distributions showed that the highest dissolved concentrations of PC were determined in the
370 superficial samples, while in the particulate fraction higher concentrations generally coincided with
371 higher fluorescence, Chl *a* and Pheo *a* values.
372 In the Southern sector of the Ross Sea (transects D and E), during the austral summer the sea ice is
373 melting, at the ice shelf front as well as basal melt at the base of the ice shelf (Rignot et al., 2013;
374 Schodlok et al., 2016). This results in a large freshwater release into the mixed layer together with
375 organisms, nutrients and micro elements of atmospheric origin (Budillon et al., 2003) giving origin to
376 AASW. In the sea ice, the organisms responsible for photosynthetic production are almost always
377 diatoms representing up to almost 90% of the photosynthetic community (Arrigo, 2017). Considering
378 the PC determined in diatoms by Rico et al. (Rico et al., 2013), this could explain for the high
379 concentration of PC in the shallower layer.
380 An additional source of free PC in this area could be from melting glaciers. In snows from the South
381 Pole and East Antarctica a significant presence of lignin was observed (Antony et al., 2014; Grannas et
382 al., 2004). The presence of free PC in seawater influenced by glacial melt water suggests a possible
383 photochemical degradation of lignin in the snow. Other authors have reported the degradation of lignin
384 due to photooxidation in water (Benner and Kaiser, 2011; Hernes and Benner, 2003; Opsahl and
385 Benner, 1998), that could be facilitated in shallow waters since the penetration of light is easier. In

386 water, biological degradation can also contribute (Benner and Kaiser, 2011; Hernes and Benner, 2003)
387 because bacterioplanckton could use lignin as a carbon source (Sala et al., 2005).
388
389 *3.6.4. Indication on the origin from statistical analysis and diagenic degradation.*
390 A similar spatial distribution of PC and phytoplankton proxies (Chlo *a* and Pheo *a*) was found at the
391 fluorescence maximum. Analysis of their vertical distribution showed a different behavior between the
392 dissolved (PC D) and particulate (PC P) fractions of PCs. The PC D seemed to be linked to the
393 superficial layer of water, whereas the higher concentration of PC P coincides with the proxies of
394 phytoplanktonic biomass (Chl *a* and Pheo *a*) which were found at different depths (see Table 4). To
395 identify the different sources that contribute to the composition of the water samples, two statistical
396 approaches were used: Hierarchical Cluster Analysis and Factorial Analysis. The compounds which
397 were mainly below the detection limit (BDL) were excluded from the statistical analysis (eg VA P).
398 When a variable was BLD in less than 50% of samples, the result is reported as half the limit of
399 detection for the samples BDL.
400 Hierarchical Cluster Analysis (CA) was performed using Statistica 8.0 (StatSoft, Inc., 2007) (Ward's
401 method, Squared Euclidean Distance). CA produced a tree diagram whose variables were divided into
402 two macro-clusters A and B. The macro-cluster A (Figure 5) collects phenolic compounds in the
403 dissolved fraction and the Pheo *a*/Chl *a* ratio, indicating a connection between phenolic compounds and
404 algal senescence. The macro-cluster (B), shows two sub-clusters: cluster (a) contains salinity, pH and
405 VAC and VAH in the particulate fraction and the second cluster (b) collected Pheo *a*, Chl *a*, potential
406 temperature, fluorescence and PA, dissolved oxygen and depth. The concomitant presence of VAC P,
407 VAH P, PA D and phytoplanktonic proxies (Chl *a* and Pheo *a*) in the same macrocluster suggests a
408 relationship between these PC and phytoplankton.

409 Factorial Analysis (FA) was also performed using Statistica 8.0 (StatSoft, Inc., 2007) and the results
410 confirmed the observation of CA. Varimax rotation was applied and the four factors explained 77.6%
411 of the variance. High values of factor loadings (Table 6) were shown in factor 1 for fluorescence, Chl *a*,
412 Pheo *a* and PA in the dissolved fraction, in agreement with CA. The difference with CA was that VAH
413 P and VAC P had high factor loadings in factor 4, suggesting that for these PC P there is a different
414 source. In factor 2, factor loadings were high for PC in the dissolved fraction and for the ratio Pheo
415 *a*/Chl *a*. Factor 2 suggests that these compounds could be released into the water during the senescence
416 of algae. Factor 3 shows high factor loadings for depth and dissolved oxygen, while very low loadings
417 were found for salinity that resulted anti-correlated.
418 Both statistical approaches suggest a link between PC in the dissolved fraction and the Pheo *a*/Chl *a*
419 ratio, which indicates a connection between PC and algal senescence. Factorial analysis confirmed this
420 result and the high factor loading in factor 4 of the PC in the particulate fraction indicate they may have
421 different sources.
422 The discussion on the possible sources of PC in the Ross Sea above (section 3.6.1-3.6.3), highlights the
423 complexity of this environment, where many sources that can be linked to lignin can also contribute to
424 the presence of PC.
425
426 Studies on the diagenic degradation of lignin using the ratio between VA and VAH demonstrated that
427 diagenetic degradation increases the oxidation state of PC. Literature reports that the ratio of VA/VAH
428 for lignin in fresh wood ranges between 0.1-0.2, and 0.1-0.5 in fresh herbaceous tissues (Opsahl and
429 Benner, 1995). Lignin in river DOM has VA/VAH 0.89±0.22 in Amazon river water and 0.93±0.10 in
430 the Mississippi river (Hernes et al., 2007). In older oceanic DOM, VA/VAH ratios ranged from 0.9-1.7
431 (Opsahl and Benner, 1997). A study on the fractionation of lignin during leaching and sorption (Hernes

et al., 2007) reported the VA/ VAH ratio for litters was of 0.27±0.01, for leachates 0.50±0.15 whilst for the sorption of supernatants to soil particles a value of 0.81±0.35 was found.

VA/VAH mean ratio for free PC dissolved in the Ross Sea water was 0.04±0.02 indicating a very fresh material. While the mean ratio for PC P was 0.94±1.07 (ranging between 0.10 - 4.05, median 0.73), this ratio was closer to the values observed for DOM in rivers, the ocean, or after sorption on particles. Therefore, these outcomes agreed with those from the statistical analysis suggesting two different sources for dissolved and particulate fractions. The first probably linked to phytoplankton and the second to lignin and the possibly sorption of material onto particles in the water.

4.Conclusions

In this work for the first time the quantification of free phenolic compounds in Antarctic Ross Sea water has been performed. We have developed two analytical methods that were used to quantify vanillic acid, vanillin, syringic acid, syringaldehyde, homovanillic acid, isovanillic acid, *p*-coumaric acid, acetovanillone and acetosyringone in the particulate and dissolved fractions of these waters.

The PC found were mainly VA, VAH, VAC and PA. Highest concentrations were observed for VAH in the dissolved fraction at a mean concentration of 191.4 ng L^{-1} which represented 92.8 % of total PC concentration. In the particulate fraction the concentrations of VA, VAH, VAC and PA were much lower, eg the vanilllin mean concentration was 1.01 ng L^{-1}.

We believe that the results of this work demonstrate that the Ross Sea is a local source of PC based of the following observations: i) PC in the dissolved fraction were mainly from the superficial layers of the Ross Sea which helped the water-air transfer of these compounds. ii) These seawater data help us to explain previous atmospheric results, where PC were observed mainly in the fine fraction of coastal Antarctic aerosols. iii) The VA/VAH ratio indicating the oxidation of vanillin to vanillic acid, was lower in sea water (0.04±0.02) than in the atmospheric aerosols at the Antarctic coastal site (0.13±0.06)

suggesting that the PC in seawater have been newly emitted so the airborne oxidation reactions have not yet started.

We have also examined the possible sources of PC in the Antarctic environment. Firstly, the intrusion of MCDW carrying oceanic lignin, Secondly, the possible release of PC from phytoplankton. As higher concentrations were concomitant with higher concentration of algal proxies (fluorescence, Chl *a* and Pheo *a*). Finally, the major presence of PC in surface layer of the Ross Sea is explained by the melting of glaciers containing lignin and sea ice where diatoms are present. Statistical analysis and the evaluation of VA/VAH indicated the senescence of phytoplankton as a possible source for PC in the dissolved fraction due to the very low degree of PC oxidation. A possible origin for PC in the particle fraction linked to degradation lignin or the sorption of diagenically evolved material onto particles, was explained by the high VA/VAH ratio. Further future investigation, will hopefully improve our knowledge of the origin of PCs in Ross Sea waters.

However, this work proves that PC can have a marine origin and these results highlight that PC should be treated very carefully when considering them as biomass burning tracers in a remote area such as Antarctica, since, if confirmed, they may also be used as phytoplankton markers. Since in the environment PC can be produced by many sources such as: biomass burning, lignin degradation, phytoplankton exudates and senescence, they are non specific biomarkers. Emission sources should be assigned using unambiguous tracers, specific to a particular environmental process like levoglucosan, which is a specific marker for biomass burning. The quantification of PC in a sample is additional valuable information that aids in the interpretation of the results.

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Supplementary material available

Materials, description of the instrumental MS/MS set up (Table S1), summary of validation data for the determination of PCs in dissolved and particulate fraction (Table S2-S3). In Table S4 procedural blanks, MDL and MQL for Antarctic water determination. In Table S5 evaluation of matrix effect and in Table S6 the summary of the chemical and physical parameter for each sea water sample. And further details regarding The concentrations of Chl *a* and Pheo *a* and Microscopic counts of the phytoplankton community assemblages all the Antarctic seawater.

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