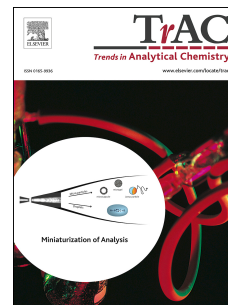# Journal Pre-proof

Assessing reactive oxygen and nitrogen species in atmospheric and aquatic environments: analytical challenges and opportunities

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1 **Assessing reactive oxygen and nitrogen species in atmospheric and aquatic**

2 **environments: analytical challenges and opportunities**

3

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12

13 **Abstract**

14 Reactive oxygen and nitrogen species (ROS and RNS) play a crucial role in biotic and

15 abiotic processes. In the atmosphere, ROS/RNS are usually associated with air

16 pollution. The ability of certain air particulate matter constituents to influence the

17 formation and cycling of ROS/RNS at the atmosphere-biosphere-hydrosphere interfaces

18 is important for the observed linkages between atmospheric aerosols and adverse health

19 and climate effects. Atmosphere-hydrosphere ROS/RNS exchange fluxes affect the

20 chemical composition of the atmosphere and surface waters compartments, acting both

21 as a source and sink for ROS/RNS. Therefore, detecting and measuring ROS/RNS in

22 this interface is of utmost importance. This article presents a critical review on the

23 analytical challenges and limitations of the existing methodologies to measure

24 ROS/RNS in air particles and surface waters. It also addresses the suitability of novel

25 methodologies based on carbon nanoparticles as potential tools for the detection of

26 ROS/RNS in atmospheric aerosols and aquatic compartments.

27

28

29 **Keywords:** Reactive Oxygen Species, Reactive Nitrogen Species, Surface Waters, Air

30 Particulate Matter, Anthropogenic Stressors, Oxidative Potential, Carbon Nanoparticles

31 Sensors, Online Methods, Offline Methods

32

33

1. Introduction

35 Reactive oxygen and nitrogen species (ROS and RNS, respectively) are of common

36 occurrence in both biotic and abiotic compartments. At the atmosphere-biosphere-

37 hydrosphere interfaces, ROS/RNS are an important group of short-lived health- and

38 climate-relevant air pollutants [1]. In the atmosphere, ROS/RNS are present in both

39 gaseous and particulate phases [2,3], with atmospheric lifetimes spanning from less than

40 a second to more than a day [1]. The growth of population and the consequent increase

41 of industrial activity and intensive agriculture has led to an increased emission of

42 pollutants to the atmosphere. Under these conditions, the occurrence of photochemical

43 and gas-phase, heterogeneous and multiphase reactions involving atmospheric oxidants

44 and aerosol particles can enhance the generation of ROS/RNS in both gaseous and

45 particulate phases [4] (Figure 1). The atmospheric ROS/RNS are also implicated in the

46 aging of biogenic and carbonaceous aerosols, thus contributing to the formation and

47 growth of secondary organic aerosols (SOA). Furthermore, a multiplicity of reactions

48 involving atmospheric ROS/RNS exchange can also occur in various biosurfaces (e.g.,

49 skin, respiratory tract, and plant leaves), inducing oxidative stress and damaging cells

50   and tissues, thus triggering a diverse suite of respiratory-related diseases from the

51   clinical side or it may diminish ecosystem diversity from an environmental point of

52   view (e.g., see references [1,5,6] and references therein). For example, reactions of

53   ROS/RNS with volatile organic compounds (VOCs) in the atmosphere may lead to

54   modifications both in the leaf surface and inside the leaves [6]. The activity of oxidative

55   pathways in the plant leaves can be strongly enhanced under oxidative stress conditions

56   induced by exposure to pollutants, such as those of ROS/RNS. On the other hand,

57   several stress pathways associated to ROS formation can also lead to emission of VOCs

58   [6].

59   <FIGURE 1 here>

60   Atmospheric ROS/RNS can also interact with abiotic surfaces (e.g., lakes, rivers, ocean

61   surfaces, and soil surfaces), upon being removed by dry and/or wet deposition, leading

62   to modifications within these environmental compartments. The sea-surface microlayer

63   is a large sink of atmospheric ROS/RNS, which interacts with the surface constituents,

64   leading to the formation and emission into the atmosphere of VOCs [1] (Figure 1).

65   Notwithstanding the huge differences in the composition, physical properties, and

66   multiphase chemical processes between the atmosphere and those abiotic surfaces, the

67   underlying chemistry involving atmospheric ROS/RNS interaction has many

68   similarities. In aquatic environments, the main source of ROS/RNS has usually been

69   assumed to be abiotic photochemical processes, where the absorption of solar radiation

70   by dissolved organic matter (DOM) in sea-surface microlayer can lead to the

71   photochemical production of diverse reactive transients, including ROS/RNS [7]. These

72   ROS/RNS may have damaging effects on bacteria and phytoplankton, by affecting cell

73   membranes or inhibiting photosynthesis. Since the sea-surface microlayer is also a

74   source of both marine organic aerosols and VOCs, the air-sea exchange of ROS/RNS is

75   likely to exert a significant influence on the global climate and, ultimately, it could

76   determine the global distribution and fate of those reactive chemical trace species.

77   Despite extensive knowledge implicating ROS/RNS as health- and climate-relevant

78   players, there are still methodological challenges related to the accurate measurement of

79   ROS/RNS in complex environmental matrices. Although a myriad of analytical

80   techniques and assays have been developed to measure the oxidative potential of

81   ROS/RNS from a human health perspective [8], the prospect of applying these

82   techniques to measure ROS/RNS in complex atmospheric and aquatic matrices is a truly

83   exciting challenge. Here, we review a number of such analytical techniques with

84   potential to meet this challenge. This review article begins by setting the scene on ROS

85   and RNS identity that are climate-relevant air pollutants interacting at the

86   atmosphere−hydrosphere interface, followed by a critical overview of the relative merits

87   and weaknesses of the existing analytical tools for ROS/RNS analysis in atmospheric

88   and aquatic matrices. A distinction is made between offline and real-time methods, with

89   the latter stemming from the need to analyze samples on short timescales. A complete

90   survey on the molecular structures and mechanisms of action of these chemical assays is

91   well beyond the scope of this review. Readers are encouraged to consult references [9–

92   11] and references therein to obtain a more complete understanding of the mechanisms

93   of action of the described molecular assays. Finally, this review highlights the potential

94   and suitability of novel methodologies based on carbon nanoparticles (CNPs) for direct

95   measurement of ROS/RNS in air particles, freshwater, and seawater. Overall, this

96   review article aims to provide the atmospheric chemistry research community with a

97   new perspective on the benefits of using NPs-based sensors for gaining new insights

98   into those reactive gaseous pollutants at the atmosphere−hydrosphere interface.

99

2. Setting the scene on ROS and RNS identity

Although referring to different chemical species, RNS and ROS usually appear in the literature under the same umbrella of "short-lived health- and climate-relevant air contaminants", since they are tightly connected through multiphase reactions in both the atmosphere and biosphere [1]. The most common ROS include superoxide ($O_2^{\bullet -}$), peroxide hydrogen (H_2O_2), hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^1O_2$), and ozone (O_3) [1,12]. Some of these ROS are not of concern to wide regions of the planet, although they might be highly toxic at very high, environmentally unrealistic levels. Other species, such as O_3, constitute the primary threat to terrestrial ecosystems and biodiversity at their current ambient levels [5]. Additional ROS include organic peroxy radicals ($ROO^{\bullet}$), alkoxy and phenoxy radicals ($RO^{\bullet}$), ozonides, organic hydroperoxides ($ROOH$), organic peroxides ($ROOR$), and chlorite ions (OCl^-) [1]. In natural waters, including seawater, $HO^{\bullet}$ radicals can be formed by the photolysis of DOM, nitrate, and nitrite [13]. In the atmosphere, the primary source of $HO^{\bullet}$ comes from the photolysis of O_3 [14], even though it has been reported that the global $HO^{\bullet}$ evolution is highly dependent on anthropogenic NO_x emissions [15], with the $HO^{\bullet}$ radicals playing an essential role in the oxidizing capacity of the atmosphere. The $HO^{\bullet}$ radicals are involved in the oxidation of volatile and semi-volatile organic compounds [16], resulting in water-soluble species that are easily removed by wet deposition into earth's surface [17]. H_2O_2 is the most stable ROS, being ubiquitous in natural waters [18] and in the atmosphere (e.g., reference [1] and references therein). In the aquatic compartments, the dominant pathway for H_2O_2 formation entails the photochemical oxidation of chromophoric DOM by solar irradiance [18]. Atmospheric wet deposition has been also identified as an important source of H_2O_2 into surface waters, which could influence the redox chemistry of the receiving watersheds [19]. In the atmosphere, the most common

125 RNS includes nitric oxide (NO) and peroxynitrite (ONOO$^-$). NO can be converted into

126 peroxynitrous acid (ONOOH), and ultimately into HO$^\bullet$ radical and nitrite anion (NO$_2^-$).

127 As reviewed by Pöschl and Shiraiwa [1], anthropogenic NO emissions are a major

128 source of RNS in the atmosphere, with NO being a key specie in catalytic radical

129 reaction cycles leading to photochemical production or destruction of O$_3$. In this regard,

130 the importance of anthropogenic NO emissions has been recently addressed during the

131 lockdown due to coronavirus disease (COVID-19) pandemic. The lockdown caused a

132 substantial reduction in NO in four Southern European cities, whereas the O$_3$ production

133 increased during this period [20]. This study further highlights the challenge of reducing

134 the formation of O$_3$ in the atmosphere despite the strict measures to control primary

135 pollutant emissions [20]. NO$_2^-$, nitrous acid (HNO$_2$), nitric acid (HNO$_3$), and NO$_3$

136 radicals are also key species in atmospheric cycling of RNS, playing also an important

137 role in the interaction of RNS with ROS [1]. Additionally well-known forms of RNS in

138 the atmosphere include NOx, which are dominated by emissions of NO and NO$_2$ that

139 react relatively rapidly (hours to days) to form HNO$_3$ [21]. NOx is produced by the

140 reaction of nitrogen and oxygen gases in the air during combustion at high

141 temperatures. In urban locations, NOx is typically produced from fossil fuel combustion

142 processes, although it can also originate from natural (e.g., forest fires and

143 thunderstorms) and biogenic (e.g., fertilization in agricultural activities, or the use of

144 nitrogen fixing plants) sources [22].

145 Due to the transient nature of ROS/RNS, encompassing high reactivity, short half-life,

146 low ambient concentrations, rapid diffusion, and potential interferences, their detection

147 and/or quantification at the air-water interface is not a straightforward process.

148 Therefore, fast, and more efficient analytical methods are preferred over more

149 traditional ones, since usually the later ones entail a lengthy time gap between sample

150   collection and ROS/RNS analysis. Nonetheless, accurate measurement of ROS/RNS is

151   a very challenging task, due to the instability of sensor probes and the potential

152   interferences from other gaseous species. Moreover, the preparation and manipulation

153   of standard solutions and environmental samples is not straightforward, due to the

154   reactivity and low ambient concentrations of ROS/RNS in those matrices. Hence, at this

155   point, one question arises: ''Among the currently available analytical methods, which

156   one appears to hold the greatest potential for investigating ROS/RNS at the atmosphere-

157   hydrosphere interface?''. The following sections will be devoted to the answer to this

158   question considering the level of information desired.

159

160   **3. Analytical tools for assessing ROS/RNS in atmospheric and aquatic matrices**

161   *3.1. Analytical methods for quantifying ROS/RNS in aquatic matrices*

162   The formation of ROS has been recognized as an important process in aquatic

163   environments (i.e., in surface waters and seawater) since these reactive species are

164   important to balance the chemical redox state of aquatic systems, where they can have

165   variable impacts on aquatic organisms and ecosystem (e.g., by affect DOM cycling,

166   trace metal speciation, and biological processes) [23]. In aquatic environments, abiotic

167   photochemical processes induced by sunlight irradiation are considered to be the main

168   source of ROS [23]. The H_2O_2 is one of the most common reactive species in aquatic

169   environments, playing a key role in contaminant decomposition in both fresh waters

170   (rivers [24], and lakes [25–27]) and seawater [28]. In fact, most of the existing studies

171   have been focused mainly on the assessment of H_2O_2 in aqueous samples, mostly

172   because of its stability and higher concentrations when compared to other ROS/RNS,

173   such as $HO^{\bullet}$ radical, NO and $O_2^{\bullet-}$. H_2O_2 are intermediate molecules generated during the

174   one-electron reduction of oxygen to water through photochemical oxidation [28,29] or

175 through "dark" (biological or chemical) processes [30]. In surface waters, the H_2O_2 is

176 mainly produced by means of the interaction of ultraviolet radiation with natural

177 dissolved organic carbon [27]. Nonetheless, atmospheric wet deposition has been also

178 identified as a source of H_2O_2 to surface waters [31]. The concentration of H_2O_2 in rain

179 is higher than those measured at fresh water or seawater surfaces, which means that

180 upon deposition, the rain will contribute to an increase of the concentration of H_2O_2 in

181 the water column [32]. Recently, it has been also demonstrated that H_2O_2 is not only

182 widespread in oceanic and atmospheric systems, but also in the groundwater domain.

183 Yuan and co-workers [33] provided evidence for the light-independent generation of

184 H_2O_2 in groundwater. These authors further suggested that the dark formation of H_2O_2 is

185 likely to occur in transitional redox environments where reduced elements (e.g., reduced

186 metals and natural organic matter) meet oxygen, such as oxic–anoxic interfaces [33].

187 Most of the analytical methodologies for the detection of H_2O_2 in aquatic matrices,

188 including seawater and lake waters, are based in fluorescence and chemiluminescence

189 methodologies. In this regard, Table 1 summarizes the most important features and

190 drawbacks of the methodologies available for ROS analysis in both atmospheric and

191 aquatic matrices. The fluorescence methods typically apply a peroxidase-mediated

192 oxidation procedure of a reagent molecule, such as the 7-hydroxy-6-methoxychromen-

193 2-one (scopoletin) and the p-hydroxyphenyl acetic acid (POHPAA), by H_2O_2 or organic

194 peroxides [27,34–37]. However, the application of fluorescent methods in many coastal

195 waters show that these methodologies are prone to interferences from the absorbance

196 and/or fluorescence of DOM at high concentrations. Chemiluminescence methods for

197 H_2O_2 determination are usually based on metal-catalysed oxidation of luminol [38]. For

198 example, a flow injection-chemiluminescence method coupled with Co(II)-catalysed

199 oxidation of luminol was successfully applied for H_2O_2 detection in the harsh conditions

200 of seawater samples, showing excellent repeatability (<5%) and reproducibility (1.8–

201 2.5%) [39]. On the other hand, an alternative chemiluminescent method involving the

202 reaction of H_2O_2 with acridinium ester 10-methyl-9-(p-formylphenyl)-acridinium

203 carboxylate trifluoromethanesulfonate was used to determine H_2O_2 in natural waters

204 [40]. This method did not require a catalyst or metal ion complexes, and an analytical

205 precision of 4% (relative standard deviation) has been reported at typical natural water

206 concentrations. The method provided a linear response over the H_2O_2 concentration

207 range of 5×10^{-9} to 60×10^{-6} M, with a detection limit of 5×10^{-9} M. Nevertheless, it has

208 been also reported that this chemiluminescence method is prone to interference in high

209 ferrous ion (Fe^{2+}) containing samples due to the formation of H_2O_2 via reduction of O_2

210 by Fe^{2+}, especially at pH 9 and above [40]. An intercomparison study has been also

211 performed between the fluorescent scopoletin method and the chemiluminescent

212 method involving reaction with acridinium ester 10-methyl-9-(p-formylphenyl)-

213 acridinium carboxylate trifluoromethanesulfonate for the determination of H_2O_2 in

214 oligotrophic seawater samples [41]. It has been concluded that the disparity between

215 results obtained by the two methods is well within analytical uncertainty with no

216 statistical difference between analytical results, suggesting that neither analytical

217 method is superior in the determination of H_2O_2 in complex aquatic samples [41].

218 Non-enzymatic fluorescent methods have been also developed for the determination of

219 H_2O_2 in aquatic samples. These methods are based on the oxidation of a probe

220 compound by $HO^{\bullet}$ radical formed in the reaction of H_2O_2 with Fe^{2+} (Fenton reaction).

221 One of these methods was applied for the determination of H_2O_2 in coastal seawater

222 samples and it involves the hydroxylation reaction of terephthalate (TP) by the $HO^{\bullet}$

223 radical, resulting in the formation of a strongly fluorescent 2-hydroxyterephthalate

224 (HTP) [42]. The authors reported a detection limit of 3 nM and 1.0% precision at 200

225   nM, with the fluorescent intensity being unaffected by coexisting sea salts. However,

226   the presence of NO_2^- at concentrations higher than 10 μM may interfere with the

227   formation of HTP. The Fenton reaction is also at the base of a method involving the

228   reaction of the $HO^{\bullet}$ radical with benzene to produce phenol for the nanomolar

229   determination of H_2O_2 in seawater [43]. The phenol was separated from the reaction

230   mixture by reversed phase high-performance liquid chromatography and detected with a

231   fluorescence detector. The authors reported a detection limit for H_2O_2 in the seawater

232   samples of 4 nM, whereas the presence of NO_2^- at a concentration of 50 μM can also

233   interfere by promoting a decrease of the fluorescence intensity signals of phenol by

234   almost 40% [43]. An intercomparison assessment of this method with the enzymatic

235   fluorescent POHPAA method also showed excellent agreement between the two

236   methods [43].

237

238   *3.2. Offline analytical methods for quantifying ROS/RNS in atmospheric matrices*

239   The first available methodologies for assessing ROS/RNS in atmospheric matrices were

240   offline. In the case of air particles, the offline quantification of ROS/RNS relies on

241   laborious intensive procedures involving sample collection on filters. In this regard,

242   Yang et al. [44] assessed how the extraction solvent and filter type might affect the

243   measurement of the oxidative potential (OP) of air particles, where OP is considered to

244   be a measure of the presence and formation of ROS in air particles. Although quartz

245   fiber filters are traditionally employed for assessing air particles composition, Teflon

246   filters are the substrate of choice to measure ROS in air particles [44]. Aerosol samples

247   collection using filter media is an offline sampling methodology with exceptional

248   collection efficiency, practicality, and low cost. However, this sampling methodology

249   also exhibits some disadvantages, including the poor recovery of particles from the

250   filters substrates, long sample collection times, and chemical aging of particles

251   deposited onto the filter surfaces, all contributing to an underestimation of ROS/RNS in

252   air particles [3]. Previous studies using filter collection media have also reported a high

253   and variable blank concentrations for ROS/RNS [2]. Yang et al. [44] also concluded

254   that the extraction solvents dichloromethane and methanol had a higher effect on the OP

255   assessed by means of dithiothreitol (DTT) than that of ascorbic acid (AA). Recently, the

256   presence of ROS/RNS in air particles has been assessed in the corresponding aqueous

257   aerosol extracts [45–47]. Although water is a natural solvent with relevance in a series

258   of atmospheric processes, the role of the water-soluble organic fraction from air

259   particles in the production of ROS/RNS is only now beginning to be studied [45–47].

260   Nevertheless, the use of ultrapure water as extraction solvent is favored over the use of

261   organic solvents, as some of the offline methods for quantifying ROS/RNS require the

262   elimination of the organic solvents prior to analysis [46].

263   The most common offline measurement assays for assessing radical generation capacity

264   consists either in mimicking the consumption of antioxidants (e.g., AA [45] and reduced

265   glutathione, GSH) or using a surrogate for biological reducing agents (e.g., DTT) [48–

266   50]. The DTT assay has been widely used to determine the OP of atmospheric aerosols

267   [48–51]. The DTT activity have been shown to exhibit a positive correlation with H_2O_2

268   formation, but not with OH formation [52]. DTT assay measures the presence of $O_2^{\bullet-}$

269   anion radicals via formation of the DTT-disulfide due to the transfer of electrons from

270   DTT to oxygen by oxidized species, such as quinones [51]. Cho et al. were the first to

271   present the DTT assay to quantitatively measure the OP of atmospheric particulate

272   matter (PM) [51]. After that, the DTT assay has been used to assess the OP of different

273   aerosol samples, including primary particles and secondary aerosols [53]. It has been

274   reported that the DTT activity is dependent on particle size [51,54] and particle

chemical composition [46]. For example, biomass burning aerosols, vehicle-generated emissions, and soil dusts showed high DTT activity [45]. Besides reacting with highly oxidized species (e.g. quinones), transition metals can also oxidize DTT [55,56]. Lin and Yu found DTT loss in solutions of Cu(II) and Zn(II), but not with Fe [55]. A study measured DTT losses mediated by metals, quinones, and polycyclic aromatic hydrocarbons (PAHs) to identify which species contribute most to DTT loss induced by ambient PM, concluding that metals play a major role in OP [57]. Another study conducted by Li et al. [58] showed that aged-diesel exhaust PM showed higher DTT activity than the fresh diesel exhaust PM. Nevertheless, DTT method poses some drawbacks, of which the long period of incubation (up to 90 min), and its reactivity towards a limited number of species are the most important shortcomings of the method. However, a more efficient ROS semi-automated system using the DTT assay was developed for quantifying the ability of aerosol aqueous extracts to generate ROS. The instrument was further validated for accuracy by comparing with the manual procedure using ambient PM samples, being capable of one DTT activity measurement per hour [49].

The AA assay has been also used to determine the OP of transition metals present in atmospheric PM [54]. Similar to the DTT assay, the AA assay involves the incubation of the antioxidant (AA) with the PM aqueous extracts at a controlled temperature and pH, followed by the measurement of the rate of depletion of the antioxidant over time (typically detected as a decrease in light absorption at the 265 nm) [54]. The antioxidant loss rate is interpreted as a measure of the ability of aerosol redox active species to catalytically transfer electrons from AA to O_2.

Due to a high sensitivity and fast response, fluorescent-based probes have been also used to quantify atmospheric PM-related ROS, usually $HO^{\bullet}$ and H_2O_2. This fluorescent

300   method is based on the principle that a fluorescent product is generated when the

301   nonfluorescent probe molecule reacts with ROS. The most used probe for sensing PM-

302   related ROS is 2,7-dichlorofluorescein (DCFH) [2,3,59,60]. In this assay, the non-

303   fluorescent reagent DCFH is oxidized to dichlorofluorescein (DCF) by ROS in the

304   presence of horseradish peroxidase (HRP), with the ROS concentration calculated in

305   terms of H_2O_2 equivalent. The 2',7'-dichlorofluorescin diacetate (DCFH-DA) is

306   hydrolyzed to form the non-fluorescent DCFH, which in the presence of ROS is rapidly

307   oxidized to DCF. A major drawback of the DCFH probe is that it is unselective towards

308   a specific reactive species because it reacts with multiple ROS, including $HO^{\bullet}$, H_2O_2,

309    $ROO^{\bullet}$ radicals, and $ONOO^{-}$. This is largely due to the easy removal of the hydrogen

310   atom located at the 9′ position of the DCFH molecule [61]. The DCFH-DA probe has

311   been also integrated into several online instruments [61–67] for the analysis of ROS in

312   ambient air particles. These online methodologies will be addressed in detail in section

313   3. 2.

314   Dihydrorhodamine 6G (DHR-6G), a reduced form of rhodamine 6G, is an alternative

315   ROS indicator that can be oxidized to cationic, highly fluorescent rhodamine upon

316   reaction with carbon-centered $ROO^{\bullet}$ and $RO^{\bullet}$ as well as $HO^{\bullet}$ radicals [68] .

317   Quantification is based on the concentration of rhodamine formed during the reaction of

318   the free radicals with DHR-6G. This ROS indicator is air- and photo-sensitive, yielding

319   substantial background fluorescence, which is the major weakness of this approach.

320   To quantify aerosol-borne ROS in urban air, an aerosol-phase ROOH sampling

321   collection method has been also employed [69]. The HRP enzyme was used to catalyze

322   the reaction between ROOH with para-hydroxyphenyl acetic acid (POHPAA) to

323   produce a dimer that fluoresces strongly, under alkaline conditions at

324   excitation/emission wavelengths of 320/400 nm.

325   Profluorescent nitroxides (PFN)-based fluorescence methods have been also used to

326   quantify ROS in air particles, namely in combustion-generated particles such as

327   cigarette smoke [70], diesel and biodiesel exhausts [71]. For additional details on the

328   reaction between ROS and PFN, readers should consult the work of Fairfull-Smith et al.

329   [72]. Different PFN-based methods have been developed at the Queensland University

330   of Technology, one of which is the 9,10-bis-(phenylethynyl) anthracene-nitroxide

331   (BPEAnit) fluorophore [70]. The excitation/emission wavelength (430/~500 nm) of the

332   BPEAnit are long enough to avoid overlapping with the background fluorescence

333   coming from other optically active compounds present in atmospheric PM (e.g. PAH

334   and their derivatives).

335   The offline methods currently in use for quantifying ROS/RNS in air particles typically

336   involves the implementation of an aerosol sampling procedure (in sampling intervals of

337   hours to days). Under this scenario, the assessment of ROS/RNS in the atmospheric PM

338   samples becomes prone to significant sampling artifacts that contribute to an

339   underestimation of the real particle-bound ROS/RNS concentrations due to the

340   reactivity of these species, where some ROS/RNS constituents might rapidly

341   decompose during PM sampling and samples processing. The short ROS lifetime is one

342   of the main limitations of those offline particle-bound ROS measurements, thus

343   suggesting the need to develop and implement online and field-deployable approaches

344   for ROS/RNS analysis. These automated methodologies, addressed in section 3.2, are

345   considered better options than the traditional methods of PM collection in filter

346   substrates for assessing ROS/RNS in atmospheric samples.

347   <TABLE 1 here>

348

349   *3.3. Online analytical methods for quantifying ROS/RNS* **in atmospheric matrices**

350   The development of an automated system involves the selection of the best available

351   analytical approach to quantify ROS/RNS, and its subsequent integration in a suitable

352   sampling system. The choice of a suitable sampling approach that allows real-time

353   measurements of atmospheric ROS/RNS must avoid both the potential sampling

354   artifacts and the long turn-around time for ROS/RNS analysis when using the PM filter

355   sampling methodology. One of the online sampling methodologies that have been

356   applied for atmospheric ROS/RNS analysis is a steam jet aerosol collector (SJAC) [73],

357   where air particles are mixed with water steam for condensational growth, after which

358   the grown droplets are collected by cyclones for subsequent ROS/RNS analysis in real-

359   time. Besides SJAC, particle-into-liquid sampler (PILS) has been also used for real-time

360   atmospheric ROS/RNS analysis [61,67]. In PILS, the particles are grown in

361   supersaturated water vapor, thereby creating droplets sufficiently large to be collected

362   by a single-nozzle impactor. The liquid sample collected on the impactor surface is then

363   removed by a small, constant flow of purified water [66,74,75] for subsequent real-time

364   chemical analysis (e.g., water-soluble organic carbon, and water-soluble inorganic ions

365   [75]), including also ROS.

366   Recently, a particle sampler for aerosol suspensions (PSAS) has been also developed to

367   collect fine ambient air particles ($PM_{2.5}$) directly as liquid suspensions. The collection

368   impactor employs the use of several configurations compared to conventional inertial

369   impactors, including a Teflon gasket for restraining impacted droplets from bouncing

370   back to the air stream, and a mesh surrounding the impaction surface to wick the

371   collected droplets into sample outlet [76].

372   When it comes to ROS analysis in these real-time PM sampling approaches, several

373   probes used in offline methodologies have been adapted into these online strategies. For

374   example, the BPEAnit assay has been combined with PILS to measure ambient fine

15

375 particulate ROS [77]. The BPEAnit assay has been further modified to allow real time

376 detection of ROS. The air particles are bubbled through an impinger with fritted nozzle

377 tip containing a fluorescent BPEAnit solution [70]. To skip the extraction procedure, the

378 air particles were collected directly into a liquid medium (e.g., water-DCFH or dimethyl

379 sulfoxide (DMSO) – BPEAnit). The use of a continuous, automated particle-bound

380 ROS system, combining PILS with chemical assays, has been described in several

381 works [61,66,67,78,79]. Online systems for OP evaluation based on PILS and DTT

382 assay were also developed to provide real time measurements (3 min to 3 hours) of

383 oxidative capacity [78,79]. One of these online methodologies combines a PILS with

384 microfluidic-electrochemical detection of reduced DTT using a cobalt(II)

385 phthalocyanine electrode [79], while another uses a liquid spot sampler [78].

386 Venkatachari and Hopke [61] developed an automated PILS DCHF-based systems for

387 the continuous sampling of ambient aerosols and measurement of ROS concentrations

388 on the collected samples. This automated DCFH method was found to be the best non-

389 specific method, being a general indicator of total particle-bound oxidants in real time

390 [61]. Wang et al. [67] and King and Weber [66] employed the PILS approach to collect

391 $PM_{2.5}$ into a aqueous slurry that contained a DCFH/HRP solution at room temperature

392 [66,67]. Figure 2 shows a schematic representation of this continuous automated flow

393 system [66], which includes a mist chamber collection module coupled to a fluorescent

394 system, employing DCFH/HPR as a probe. The $PM_{2.5}$ samples are collected 5 min

395 before ROS analysis, which is a drawback of this method since it could lead to losses of

396 reactive components [66]. One additional disadvantage of the PILS system relates to the

397 high temperature steam used to promote particle growth. These high temperatures can

398 affect the analysis of ROS in the air particles samples due to the unstable nature of some

399 of the ROS constituents.

400   <FIGURE 2 here>

401 Another methodology, known as Particle Into Nitroxide Quencher (PINQ), has been

402 suggested by Brown et al. [80] for measuring PM-bound ROS using the BPEAnit

403 chemical probe. In this online method, an insoluble aerosol collector (IAC)

404 continuously collects air particles, regardless of their size or composition, directly into a

405 liquid medium with a collection efficiency of > 97% and a cut-off size of < 20 nm. The

406 PM-bound ROS quantification is performed by measuring the fluorescence increase

407 using a flow-through fluorimeter aimed to obtain fast and accurate measurements.

408 Figure 3 shows a schematic representation of this continuous automated flow system

409 [80]. When using DMSO as liquid medium, BPEAnit showed to be sensitive to HO$^{\bullet}$

410 radical and other ROS, such as ROO$^{\bullet}$ [81].

411   <FIGURE 3 here>

412 Other online techniques aiming to quantify the air particle-bound ROS with DCFH/HRP

413 have been also developed with the purpose of reducing time between air samples

414 collection and ROS analysis [61,66,67]. These online techniques employed distinct

415 particle collection procedures. One of the procedures consists in a gentle particle

416 extraction that collects PM in an aqueous HRP solution on a paper filter that then flows

417 through Teflon tubing immersed in a water bath for 15 min [64]. This particle extraction

418 is crucial considering the reactive and short-lived nature of ROS [64]. This instrument

419 was further developed to a portable field deployable apparatus (Online Particle-bound

420 ROS Instrument, OPROSI) for automated continuous measurements, covering hours to

421 days [63].

422

423 *3.4. CNPs sensors as potential tools to detect ROS/RNS in environmental matrices*

424 Sensors based on nanoparticles (NPs) have attracted much attention due their own

425 specific characteristics. Particularly, CNPs display features such as tunable

426 fluorescence, water solubility, photo- and physic-chemical stability, biocompatibility,

427 and low toxicity. CNPs can also be functionalized and conjugated with various

428 compounds and, therefore, could be used in several analytical and bioanalytical

429 applications [82]. This makes CNPs also very attractive materials for the construction of

430 fluorescent sensors [83]. The CNPs sensors have already been successfully used for the

431 sensitive and selective detection of ROS/RNS in biological samples [84]. In the

432 presence of the very oxidant ROS/RNS constituents, the chemical structures at the

433 surface of the CNPs are oxidized. The outcome is a decrease in the fluorescence

434 intensity of the sensors, whose magnitude is proportional to the amount of ROS/RNS

435 present in the samples. A few examples of those biological applications include the

436 quantification of NO at pH 4 and $ONOO^-$ at pH 7 and 10 using CNPs doped with

437 ethylenediamine in standard and in fortified serum solutions [85]. The CNPs sensors

438 have showed also sensibility towards the detection of ClO^- at pH 4 and $ONOO^-$ at pH 9

439 in serum samples, with minimum detection limits of 0.5 and 1.5 μM for ClO^- and

440 $ONOO^-$, respectively [86]. CNPs doped with tryptophan were also designed for the

441 detection of $ONOO^-$, showing a linear response between 5 to 25 μM, and limits of

442 detection and quantification of 1.5 μM and 4.9 μM, respectively [87]. CNPs synthetized

443 with glutathione also showed sensibility for H_2O_2 detection, exhibiting a linear response

444 in the range of 20 to 200 μM [88]. CNPs doped with boron tribromide (BBr_3) were also

445 developed for the detection of H_2O_2. In this case, the developed sensor exhibited a linear

446 response in the range of 0.1 to 1.0 mM [89], which is a less sensitive system than those

447 of functionalized CNPs (e.g., reference [87]).

448 In environmental matrices, however, CNPs have yet to be applied into ROS/RNS

449 detection; instead, CNPs have been employed in the detection of Hg^{2+} in mineral water

18

450 [90], tap and drinking water [91], and river and seawater [92] samples. Additionally, the

451 CNPs have been used to detect sulfide in tap and drinking water samples [91], Cu^{2+} in

452 seawater [93], Cr^{6+} in river water samples [94], Fe^{3+} in river [94], lake [95] and tap

453 water [95] samples, as well as an herbicide (pretilachlor) in soil samples [96].

454 Nevertheless, due to their own characteristics, namely selectivity and sensitivity

455 towards ROS/RNS, CNPs sensors are a simple, efficient, fast, and low-cost alternative

456 to be implemented in the environmental field. Furthermore, CNPs exhibit low toxicity,

457 which make them exceptional candidates for environmental applications. Moreover,

458 some of these CNPs sensors allow the simultaneous detection of at least two different

459 ROS/RNS present in the same media (e.g., the quantification of NO at pH 4 and

460 $ONOO^-$ at pH 7 and 10 [85]), which is an advantage for the selective analysis of

461 different reactive species in the same run and batch of samples. Indeed, CNPs sensors

462 could be applied for the determination of ROS/RNS in atmospheric air particles but also

463 for the determination of ROS/RNS in aquatic samples (e.g., ocean surface), as well as

464 other environmental matrices. In order to assess real-time variations in ROS/RNS

465 emissions and fate at the air-sea interface, the development of a CNP-based field-

466 deployable tool becomes also of utmost importance.

467

468 **4. Conclusions**

469 Anthropogenic activities have disturbed the cycles of several important atmospheric

470 constituents, including ROS/RNS. Since the chemical interactions at the interface of

471 ocean surface and lower atmosphere are a two-way process, ocean emissions of

472 ROS/RNS and marine aerosols can also impact atmospheric chemistry and air quality.

473 Finding the most adequate methodology for an accurate measurement of ROS/RNS at

474 the air-sea interface is an important requirement to better understand the environmental

475   impact of the OP and consequent generation of ROS/RNS. There are a multiplicity of

476   methodologies and there is no consensus on which assay is the most appropriate for

477   measuring OP related to ROS/RNS; even within each assay, protocols can vary, making

478   results difficult to compare. In offline techniques, involving the collection of air

479   particles in filters, the chemical aging of air particles deposited into the filter surface can

480   cause underestimation (due to the evaporation of some organic species) or

481   overestimation (if the particles become oxidized) of ROS/RNS concentrations.

482   Additional shortcomings of offline methods for ROS/RNS analysis include, the type of

483   filters used for PM collection (e.g. quartz filters or Teflon filters), PM extraction

484   solvents (e.g., methanol extract both hydrophilic and hydrophobic organic species,

485   resulting in higher OP than those of water-soluble extracts), incubation times, and metal

486   chelators. Online aerosol sampling techniques (e.g., PILS, SJAC), on the other hand,

487   simplify the aerosol sampling procedure and they can be fully automated by coupling to

488   a specific chemical assay, resulting in a more efficient and reliable approach for

489   ROS/RNS analysis (e.g., reducing time of analysis, experimental errors, and labor

490   costs). Nonetheless, to ensure the acquisition of reliable data on ROS/RNS, these online

491   methods should follow optimization procedures to achieve a high analytical sensitivity,

492   repeatability, and reproducibility, while simultaneously reducing the cost of a single

493   analysis. CNPs sensors are capable of meeting these criteria in the detection of

494   ROS/RNS at the air-water interface. These sensors allow selective detection of

495   ROS/RNS, they can be easily synthetized using low cost methods, and their application

496   is based in a simple fluorescent method. Moreover, the CNPs-based sensors can be

497   implemented in online flow systems, which makes them suitable for in-situ real-time

498   measurements.

499

500 **Acknowledgments**

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870 **FIGURES CAPTIONS**

871 **Figure 1.** Sources, effects, and multiphase exchanges of ROS/RNS at the air-water

872   interface. Adapted with permission from [1]. Abbreviations: DOM -

873   Dissolved Organic Matter, VOC - Volatile Organic Compounds, SOA -

874   Secondary Organic Aerosols, OCl^- - Hypochlorite, $O_2^{\bullet-}$ - Superoxide, O_3 -

875   Ozone, $^1O_2$ - Singlet Oxygen, H_2O_2 - Hydrogen Peroxide, $HO^\bullet$ - Hydroxyl

876   Radical, $ONOO^-$ - Peroxynitrite, NO - Nitric Oxide, NO_2^- - Nitrite, NO_3^- -

877   Nitrate [1].

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879 **Figure 2.** Online ROS methodology combining particle-into-liquid sampler (PILS)

880   and the fluorometric probe 2,7-dichlorofluorescein/horseradish peroxidase

881   (DCFH/HRP). Reprinted with permission from [66].

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883 **Figure 3.** Online ROS methodology combining Particle Into Nitroxide Quencher

884   (PINQ) based on 9,10-bis-(phenylethynyl) anthracene-nitroxide (BPEAnit)

885   probe. Reprinted with permission from [80].

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891 **FIGURE 1**

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FIGURE 2

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899   **FIGURE**   **3**

Table 1. Summary of analytical methodologies available for ROS analysis in aquatic and atmospheric samples.

Methodology	Observations	Drawbacks	References
Aquatic matrices			
Scopoletin	• Allow quantification of H_2O_2 by the decrease in scopoletin fluorescence	• Prone to interferences due to the absorbance and/or fluorescence of aquatic DOM at high concentrations • Requires an enzyme, so it is unsuitable for on-site analysis due to enzyme's instability	[27,35,36,41]
POHPAA	• Applied into H_2O_2 analysis in both seawater and air particles samples, based on the formation of a fluorescent dimer whose signal intensity is proportional to H_2O_2 concentration • POHPAA does not undergo autooxidation on exposure to light or air during the analysis of atmospheric aerosol samples	• Cannot differentiate between organic peroxides and H_2O_2 • Requires an enzyme, so it is unsuitable for on-site analysis due to enzyme's instability • Possible underestimation of total air particle-bound ROS concentrations	[34,37,69]
Luminol	• Reaction with H_2O_2 originating a luminol radical in the presence of carbon dioxide and metal ions or heme-containing enzymes • Not prone to interferences from the absorbance and/or fluorescence of aquatic DOM at high concentrations	• Some trace metal species (e.g., Fe(II), Fe(III), V(IV)) originate an overestimation in the determination of H_2O_2 in seawater samples using the luminol-chemiluminescent method	[38]
Acridinium ester	• Provide good sensitivity and precision for determination of H_2O_2 in natural waters • Significantly less prone to interference from naturally occurring chromophores, fluorophores, and organic peroxides	• Prone to interference in high Fe^{2+} containing samples (especially at pH $\geq$ 9)	[36,40,41]

Table 1. *Cont.*

Methodology	Observations	Drawbacks	References
Terephthalate (TP)	• Based on hydroxylation reaction of TP by $HO^\bullet$ radical, originating a strong fluorescent 2-hydroxyterephthalate (HTP) specie • Fluorescence intensity is unaffected by coexisting sea salts	• NO_2^- concentrations higher than 10 μM may interfere with the formation of HTP	[42]
Fenton reaction	• Based on the reaction of $HO^\bullet$ radical with benzene to produce fluorescent phenol	• NO_2^- at concentrations of 50 μM promotes a decrease in the fluorescence of phenol by almost 40%, thus interfering with H_2O_2 analysis	[40]
Atmospheric matrices			
DTT-based systems	• Commonly used chemical assay in atmospheric samples analysis • Low-cost, easy-to-operate, with high repeatability • Strong reducing agent that measures the formation of ROS by quinones • Remaining DTT reacts with Ellman Reagent • Detection (at UV = 412 nm) based on TNB production	• Laborious and time-consuming protocol • Weak correlations between DTT consumption and ROS generation • Reactive toward limited number of species • Incubation time up to 90 min • No standardized protocols	[48–53]
AA- based systems	• Sensitive to transition metals	• Less effective indicator of OP than DTT	[45,54]

Table 1. *Cont.*

Methodology	Observations	Drawbacks	References
DCFH-DA based systems	• Simple responsive fluorescent method • Becomes fluorescent after being oxidized by H_2O_2 • Needs a catalyst, usually the HRP • Sensitive towards ROOH, organic peroxides, alcohols, aldehydes and ClO^-	• Prone to autooxidation upon exposure to air and sunlight • HRP promote an increase in the fluorescence intensity, lack of sensitivity, and relatively complex chemistry set-up in terms of implementation	[61,62,64,65]
DHR-6G	• DHR-6G can be oxidized by $ROO^{\bullet}$ radical originating rhodamine 6G.	• Air- and photo-sensitive, yielding significant background fluorescence • Does not directly react with diluted H_2O_2 or lipid peroxides, which at low concentrations are not as reactive as the other radical species	[68]
PFN based systems	• Suppresses fluorescence emission in the presence of nitroxide moieties • React with radicals, leading either to reduction of the nitroxides to hydroxylamines or oxidation to oxoammonium cation	• Contains relatively labile linkages, which are prone to hydrolysis, leading to the separation of the nitroxide from the fluorophore	[72]
BPEAnit based systems	• Stable for long periods of time • Detect carbon and sulfur-centered free radicals as well as $ROO^{\bullet}$ and $HO^{\bullet}$ radicals	• Laborious protocol • High reagent consumption	[70,80,81]

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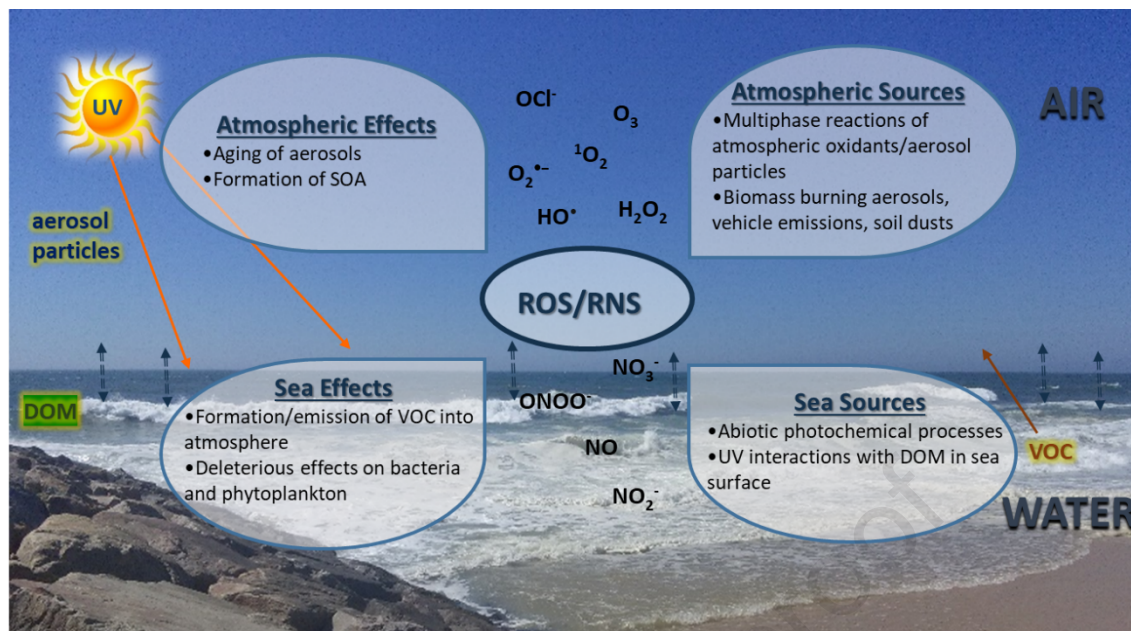**Table 1.** Summary of analytical methodologies available for ROS analysis in aquatic and atmospheric samples.

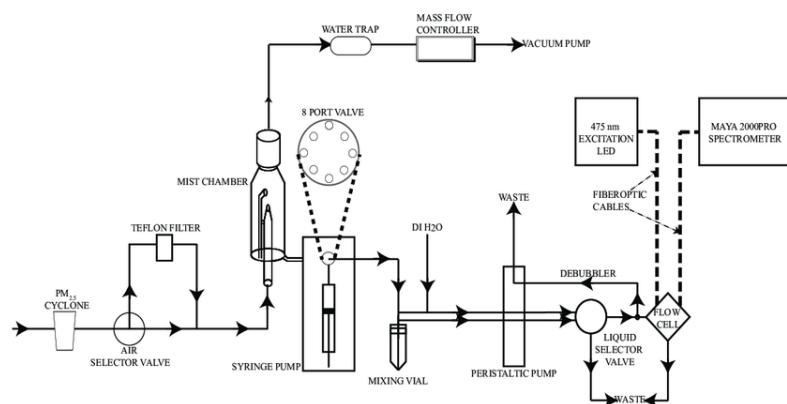| Methodology	Observations	Drawbacks	References
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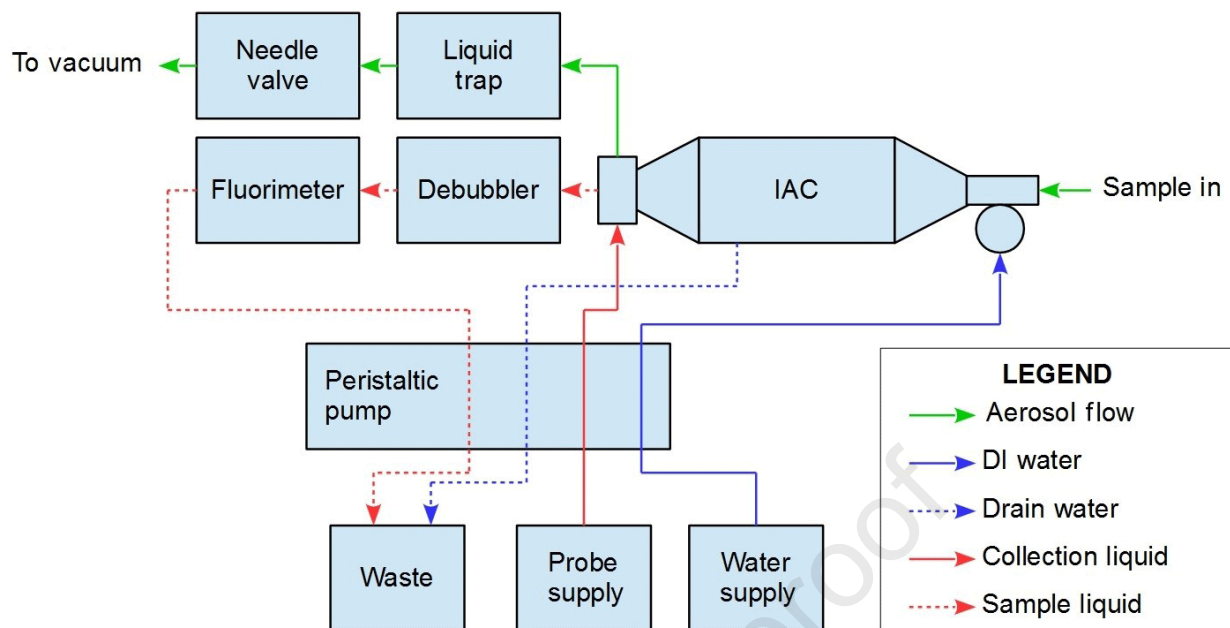| Methodology	Observations	Drawbacks	References
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To vacuum ← Needle valve ← Liquid trap ←

Fluorimeter ← Debubbler ← □ IAC ← Sample in

Peristaltic pump

Waste   Probe supply   Water supply

LEGEND
→ Aerosol flow
→ DI water
⤑ Drain water
→ Collection liquid
⤑ Sample liquid

TrAC – Trends in Analytical Chemistry

Manuscript: *"Assessing reactive oxygen and nitrogen species at the atmosphere-hydrosphere interface: analytical challenges and opportunities"* (Eliana F. C. Simões *, Antoine S. Almeida, Armando C. Duarte, Regina M. B. O. Duarte)

Highlights

> Progress in the most prominent offline and online methodologies for ROS/RNS analysis

> Limitations, challenges, and opportunities for ROS/RNS sensing at air-water interface

> Potential of CNPs as sensors for ROS/RNS analysis in environmental matrices

Declaration of interests

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

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