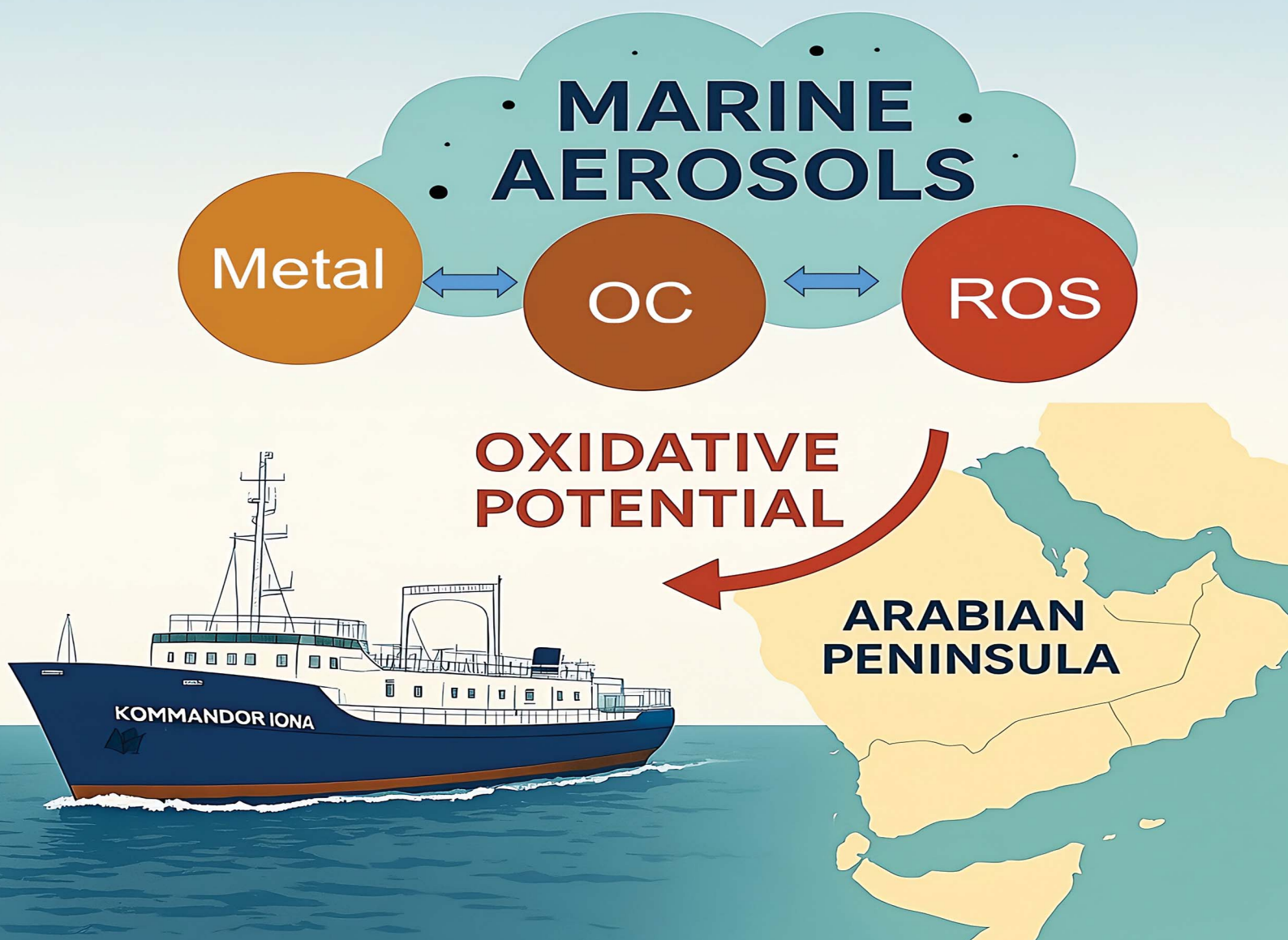


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Influence of redox-active components and particle size on reactive oxygen species production and oxidative potential of marine aerosols around the Arabian Peninsula

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Oxidative potential (OP) is a metric for assessing the potential toxicity of ambient particulate matter (PM). However, it is unclear how the OP of PM relates to the presence of redox-active components, the particle size, or their ability to produce reactive oxygen species (ROS) in aqueous solution. For size-segregated marine PM samples collected during a ship cruise around the Arabian Peninsula, we determined the OP of PM using acellular assays, *i.e.*, dithiothreitol (DTT) depletion and H₂O₂ formation assays. The content of environmentally persistent free radicals (EPFRs) as well as the production of radicals upon dissolution of the PM in water were determined by electron paramagnetic resonance spectroscopy. Generally, sub-micrometer particles contributed more strongly to the particle mass-normalized OP (DTT_m, in units of pmol min⁻¹ μg⁻¹), whereas the production of radicals upon dissolution in water was higher in coarse particles. PM_{0.49} (*i.e.*, PM with diameter < 0.49 μm) sampled in the Northern Red Sea showed the highest OP out of all samples. The range of DTT_m is lower than previously observed on both land and sea. The OP assays (DTT, H₂O₂) showed positive correlation with concentrations of both water-soluble transition metals (WSTMs) and water-soluble organic carbon (WSOC), while EPFR content and radical production upon dissolution were significantly correlated with WSTMs only. Overall, the OPs of the marine PM samples investigated in this study were substantial, but below levels reported previously from continental or urban sites.

Environmental significance

Oxidative potential (OP) is an important metric for assessing air pollution toxicity. However, the relationship of particle size, redox-active components, and reactive oxygen species yields with the OP of marine aerosols is not clear. Based on dithiothreitol depletion and H₂O₂ formation assays, we determined the OP of size-segregated marine aerosols around the Arabian Peninsula. We found stronger contribution of submicron particles to particle mass-normalized OP, higher radical yields of coarse particles in water, and positive correlation of OP with water-soluble transition metals and water-soluble organic carbon. These findings are of critical importance for unraveling aerosol health effects at molecular level, understanding source-specific aerosols toxicity and their contribution to overall health risks, and developing tailored mitigation approaches.

1 Introduction

Airborne particulate matter (PM) plays an important role in climate change and human health. The environmental health effects of PM have been linked to its oxidative potential (OP), *i.e.*, the ability to produce reactive oxygen species (ROS) such as

H₂O₂, ·OH, and O₂^{·-}, as well as oxygen- and carbon-centered organic radicals.¹ Quantitative understanding of OP, ROS yield, and redox-active components of PM, as well as their interconnections, is of critical importance for unraveling the atmospheric and health effects of PM.

To evaluate the OP and adverse health effect of PM, various cellular and acellular assays have been developed.²⁻⁴ As a cellular assay, macrophage assay has been used to assess the ROS activity and toxicity of atmospheric PM_{2.5} from different urban areas.^{5,6} Dithiothreitol (DTT) depletion assay has been used as an acellular assay to evaluate the OP of both ambient PM^{7,8} and laboratory-generated secondary organic aerosols (SOA).⁹⁻¹¹ Moreover, surrogate lung fluid assays and antioxidant depletion assays with ascorbic acid (AA) or glutathione (GSH) have been used to characterize the OP of PM with different sources,¹² composition,¹³ or sizes.¹⁴ Electron paramagnetic resonance (EPR) measurements have been conducted to quantify atmospheric environmentally persistent free radicals (EPFRs) as well as the radical yields of laboratory-generated SOA and ambient PM in liquid water.¹⁵⁻¹⁷ EPFRs are a class of pollutants composed of a series of long-lived radicals such as carbon-centered radicals and semiquinone radicals, which can be formed *via* combustion or pyrolysis of organic matter,¹⁸⁻²⁰ heterogeneous reactions of ozone with polycyclic aromatic hydrocarbons,²¹ and aging of plastics²² or ambient PM.^{23,24}

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EPFRs can undergo redox-active reactions to generate ROS, which may contribute to cellular oxidative stress, cytotoxicity, and hospitalization.^{25–27}

The numerous recent reports on acellular assays reflect their promise in PM health studies. However, the relationship between *in vitro* assay-based OP and the toxicity of atmospheric PM — characterized by diverse size distributions, complex compositions, and various emission sources — remains inadequately understood. The diverse sensitivity, selectivity, and reactivity of different assays result in substantial inconsistencies and various correlations with health outcomes,² which need further composition-resolved analysis at a molecular level.

Previous OP studies mainly focused on anthropogenic source PM in urban or semiurban areas,^{20,28–32} and less in remote environments.^{33–35} OP is often found highest in fine PM, especially the sub-micrometer fractions, due to the prevalence of water-soluble transition metals (WSTMs) and water-soluble organic carbon (WSOC) in particles of these smaller sizes.^{36–38} Previously, in the central Arabian Sea and Indian Ocean, DTT depletion rate-based OPs had been found to be associated with WSTMs, WSOC and organic nitrogen with OPs ranging from 6–28 pmol min⁻¹ μg⁻¹ (300–2200 pmol min⁻¹ m⁻³).^{33–35} As mass concentrations and redox activities of marine PM are typically low, the particle size-dependence of their OP has been rarely addressed before.³⁵

The aim of this study was to obtain data from remote marine environments and address the particle size dependence of OP in these regions. We collected PM in marine air around the Arabian Peninsula and the Mediterranean Sea and investigated ROS (OH radical and H₂O₂) yields and DTT depletion of PM size fractions, besides quantifying relevant PM components like WSOC and WSTMs.

2 Chemicals and methodology

2.1 Chemicals

The following chemicals and equipment were used: dithiothreitol (DTT, > 98%, Sigma-Aldrich, Germany), 5-*tert*-butoxycarbonyl-5-methyl-1-pyrroline-*N*-oxide (BMPO, high-purity, Enzo Life Sciences, Lörrach, Germany), H₂O₂ assay kit (MAK165, Sigma-Aldrich), ultrapure water (14211-1L-F, Sigma-Aldrich), H₂O₂ (30% w/w, Sigma-Aldrich), 47 mm diameter Teflon filters (JVWP04700, Omnipore membrane filter, Merck, Darmstadt, Germany), KH₂PO₄ (≥99%, Fisher Scientific GmbH, Schwerte, Germany), and Na₂HPO₄ (≥99.999%, Fluka, Buchs, Switzerland). High quality EPR quartz tubes (ER 221 TUB/3, Bruker, Karlsruhe, Germany) and micropipettes (50 L, Brand, Wertheim, Germany) were used for particle phase and liquid phase samples analysis.

2.2 PM sampling

Marine PM was collected during the Air Quality and Climate in the Arabian Basin (AQABA) campaign from 24 July to 27 August 2017.³⁹ Size-segregated PM was collected using a high-volume sampler (Baghirra HV 100-P, Prague, Czech Republic) equipped with a cascade impactor inlet (TE-235, Tisch Environmental, Cleves,

USA). PM with six size fractions (five stages + backup filter) within PM₁₀ was collected. The sampling duration of each filter sample was 24 h. PM_{0.49} ($D_p < 0.49 \mu\text{m}$), PM_{0.49–0.95} ($0.49 \mu\text{m} < D_p < 0.95 \mu\text{m}$), and PM_{3–7} ($3 \mu\text{m} < D_p < 7 \mu\text{m}$) were studied. The sampling regions and cruises are shown in Fig. 1. Sampling was performed in the Mediterranean Sea (MS), Northern Red Sea (NRS), Southern Red Sea (SRS), Arabian Sea (AS), Gulf of Oman (OG), Aden Gulf (AG), and Persian Gulf (PG). More details about the sailing route can be found in other reports from this cruise.^{39,40}

2.3 DTT depletion assay

The assay uses an aqueous (pH 7.4) PM extract; more details on the method applied can be found in previous studies.^{10,41} Briefly, a buffer solution composed of 0.01 mol KH₂PO₄ and 0.04 mol Na₂HPO₄ was used to keep the solution sample pH at 7.4. Additionally, 1 mM ethylenediaminetetraacetic acid (EDTA) solution was used as a chelator, whereas 0.5 mM DTT and 1 mM dithiobisnitrobenzoic acid (DTNB) were used as probes to determine the depletion rate of DTT. A mixture of 1.5 mL working buffer, 75 μL of 0.5 mM DTT and 300 μL of ambient PM extract was incubated at 37 °C and measured for 30 min. At 15 min intervals, 300 μL of the solution was mixed with 16 μL of 1 mM DTNB solution and the mixture's UV absorption at 412 nm was measured. The decay rate of DTT in 30 min was used as an OP indicator for PM from marine air around the Arabian Peninsula. A plate reader (Synergy NEO, BioTek Instruments, Waldbronn, Germany) was used for the absorption quantification. The DTT depletion rates are shown in Fig. 2 and 3, and Tables S1 and S2.

2.4 H₂O₂ formation assay

The fluorimetric hydrogen peroxide assay kit (MAK165, Sigma) was used to determine the concentrations of H₂O₂ in aqueous

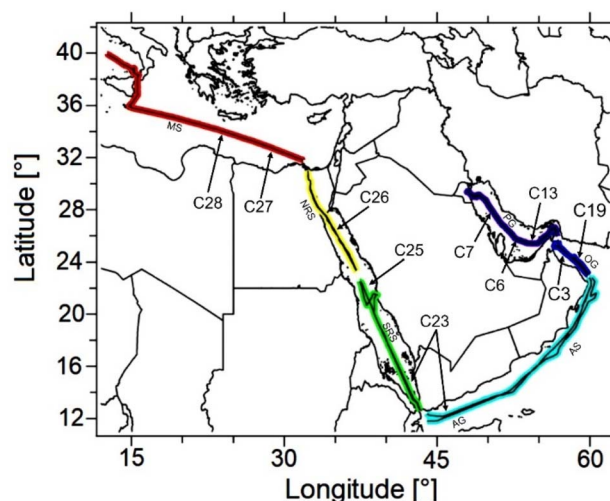


Fig. 1 Overview of the AQABA campaign's ship cruise route and sampling regions (rearranged from Tadic *et al.*, 2020).³⁹ Red: Mediterranean Sea (MS). Yellow: Northern Red Sea (NRS). Green: Southern Red Sea (SRS). Turquoise blue: Aden Gulf (AG) and Arabian Sea (AS). Blue: Oman Gulf (OG). Purple: Persian Gulf (PG). C3–28: cascade impactor sample sets.



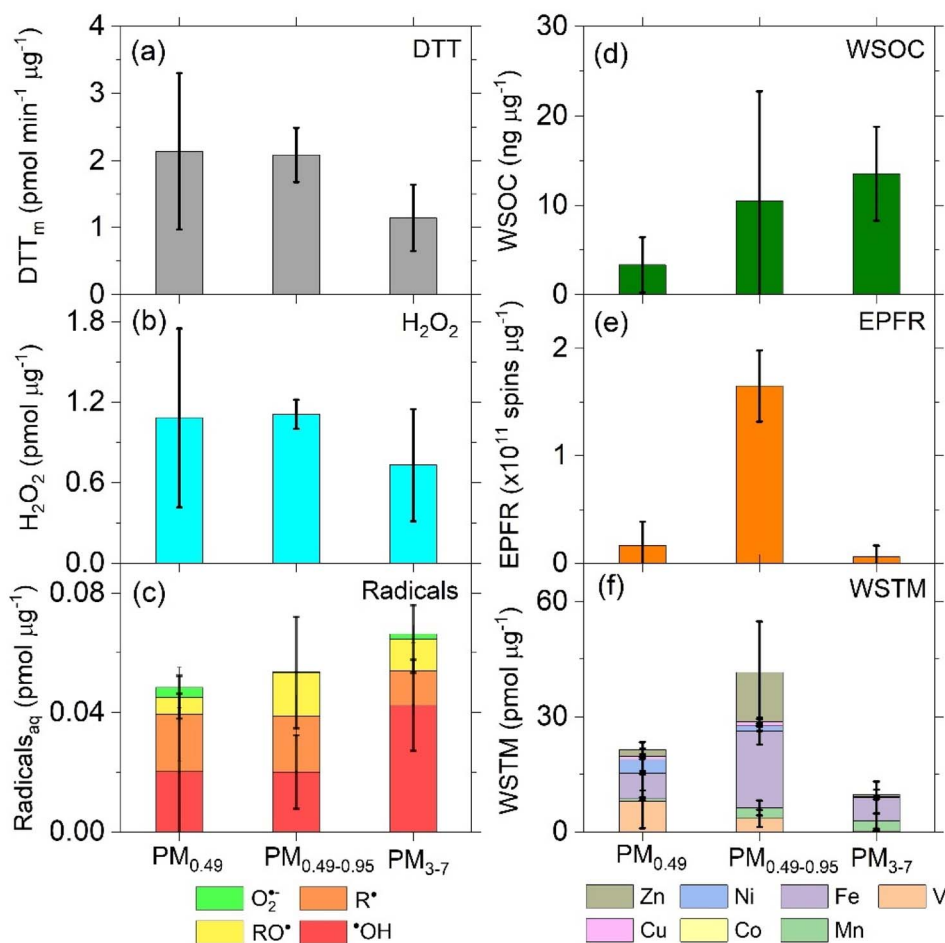


Fig. 2 Particle mass-normalized oxidative potential (OP), reactive oxygen species yields, and concentration of redox-active components of different PM size fractions, displayed as means over the measurement campaign. (a) DTT depletion rates in aqueous extracts of PM. (b) H₂O₂ yields of PM in water. (c) Radicals yield in aqueous PM extracts (Radicals_{aq}). Concentrations of (d) PM-bound water-soluble organic carbon (WSOC), (e) environmentally persistent free radicals (EPFRs), and (f) seven water-soluble transition metals (WSTMs). The error bars represent standard deviations ($n = 2-9$).

extracts of ambient PM. The method details can be found in our previous studies.^{10,42} Briefly, aliquots of assay buffer, horseradish peroxidase, infrared peroxidase substrate stock, and PM extracts were incubated for 15–30 min at 37 °C. Then, the fluorescence of this aqueous mixture was measured with the microplate reader (same as the DTT depletion measurement, excitation: 540 nm; emission: 590 nm). Standard H₂O₂ calibrating solutions of five different concentrations, ranging from 0 to 100 μM, were also measured.

2.5 EPR measurements

A continuous wave electron paramagnetic resonance (CW-EPR) spectrometer (EMXplus10/12, Bruker) was applied for detecting PM-bound environmentally persistent free radicals (EPFRs) in untreated dry filter samples (see the spectra in Fig. S1). The parameter sets for EPR measurements can be found in our previous study.²⁹ Briefly, a modulation frequency of 100 kHz, a microwave frequency of 9.84 GHz, a microwave power of 2.15 mW, a modulation amplitude of 1.0 G, a sweep

width of 60.0 G, a sweep time of 10.49 s, a receiver gain of 40 dB, a time constant of 0.32 ms, a conversion time of 10.24 ms, and a scan number of 10 have been used. To measure the concentrations of different types of aqueous radicals, *i.e.*, the radical yield in aqueous PM extracts (Radicals_{aq}), a spin trapping technique with BMPO as probe was applied (10 mM). To quantify spin numbers for each type of radical, deconvolution of the EPR spectrum and spin counting were conducted with the Bruker software Xenon. A calibration curve based on stable radical TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl) was tested and used for radical concentration correction.⁴³

2.6 Quantification of water-soluble transition metals

An inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7900) was used to quantify the concentrations of seven water-soluble transition metal (WSTM) species (Zn, Cu, Ni, Co, Fe, Mn, and V) from the supernatants of ultrapure water-based aqueous extracts (pH 4–7) of PM_{0.49}, PM_{0.49-0.95}, and PM₃₋₇,



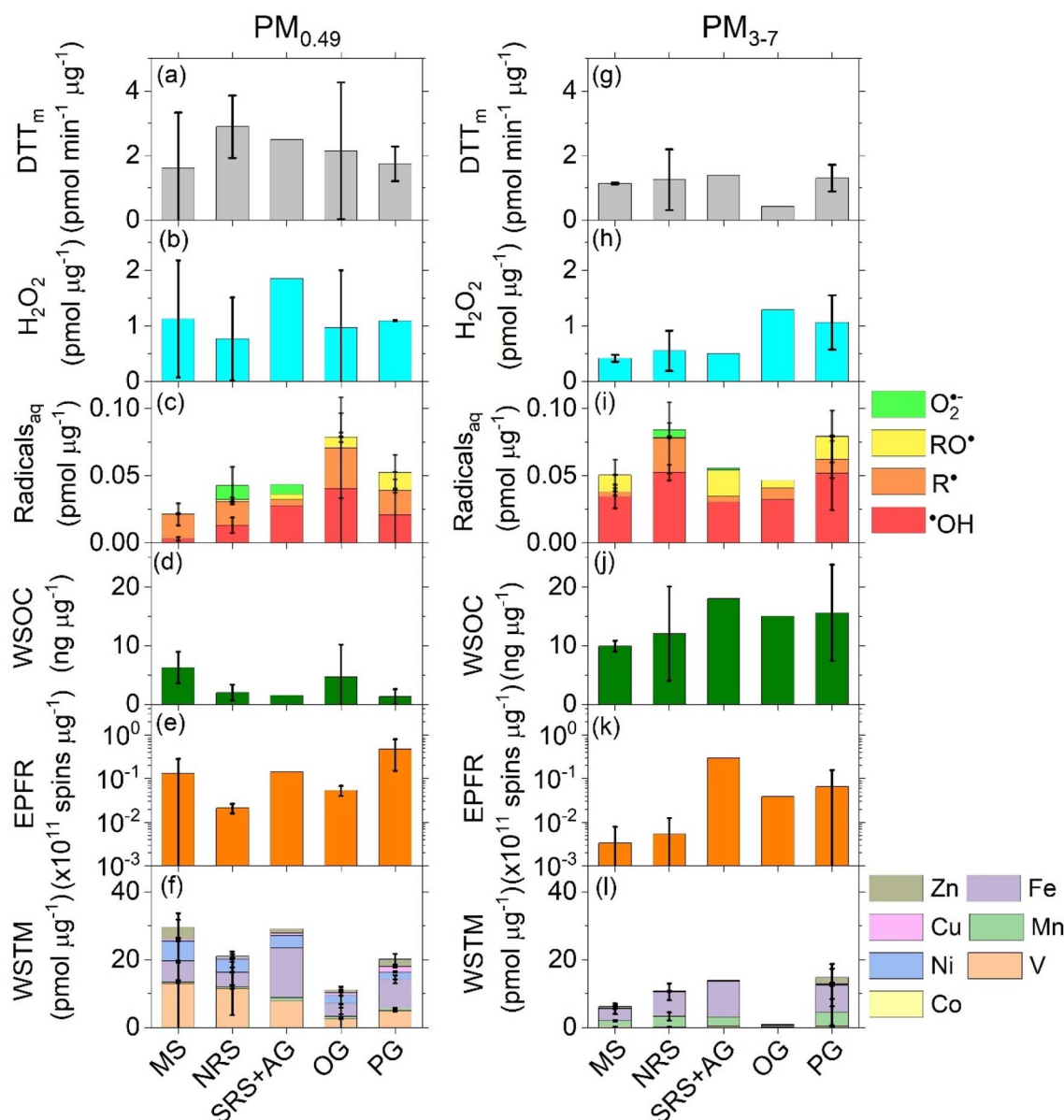


Fig. 3 Particle mass-normalized oxidative potential (OP), reactive oxygen species yields, and concentrations of target redox-active components of $\text{PM}_{0.49}$ (a–f) and PM_{3-7} (g–l), displayed as means from different locations during the measurement campaign. (a and g) DTT depletion rates in aqueous extracts of PM. (b and h) H_2O_2 yields of PM in water. (c and i) $\text{Radicals}_{\text{aq}}$ of PM in water. Concentrations of (d and j) seven WSTMs, (e and k) EPFRs, and (f and l) WSOC in PM. Seas: Mediterranean Sea (MS), Northern Red Sea (NRS), Southern Red Sea (SRS), Arabian Sea (AS), Gulf of Oman (OG), Aden Gulf (AG), and Persian Gulf (PG). The error bars indicate standard deviations.

respectively (see Table S3). These seven WSTMs were chosen for analysis, due to their high concentrations (Fe, Zn) and OP (Cu, Mn, Ni, Co, V).⁷ Moreover, Mn, Cu and Zn were also found in marine aerosols before.^{33,35} The method details for ICP-MS measurement can be found in previous studies.^{42,44} Briefly, an aliquot of the supernatants of PM extracts was diluted ($\sim 1:3$ v/v) and acidified using a mixture of HNO_3 solution (5%) and HF solution (1%) before analysis. The measured WSTM concentrations were blank corrected. Calibration ($R^2 > 0.99$) of ICP/MS was performed using standard multielement solutions of predefined concentrations (custom grade, Inorganic Ventures, Virginia, USA).

3 Results and discussion

3.1 Size distribution of oxidative potential, redox activity and composition of PM

Fig. 2a and b illustrate a trend indicating that sub-micrometer particles exhibit higher particle mass-normalized DTT depletion (DTT_m) and increased H_2O_2 formation compared to coarse particles. This size dependency had been reported earlier, related to combustion particles from traffic, shipping, industrial activities, cooking, and biomass burning being prominent in PM_1 .^{45–51} Indeed, previous work on the AQABA campaign cruise showed that the sub-micrometer marine particles'



composition was most influenced by biomass burning and fossil fuel combustion, with ship exhaust playing a significant role.^{34,52,53} Based on the significant quantities of previously identified ship emission plumes⁵² and that OP of PM from ship exhaust is generally high,⁵⁴ we presume that shipping emissions may have driven the OP of ambient marine PM around the Arabian Peninsula.

The yields of radicals from dissolution of PM in water (Radicals_{aq}) are one order of magnitude lower than H₂O₂ yields, with the largest contribution coming from OH radicals. Radicals_{aq} is higher for coarse particles (Fig. 2c), which is the opposite trend compared to DTT_m. The particle size trend of Radicals_{aq} might be related to OC, especially WSOC, which showed the same trend as particle size (Fig. 2d).⁴⁰ WSOC is generally associated with radical formation in water.^{26,43,55–57} Petrochemical industries and open flares, which dominated emissions especially in the PG,⁵⁸ have been reported to not be connected with high OP.⁵⁹ Note that in the few PM samples published previously from the coastal and open Arabian Sea and Indian Ocean,^{33–35} the DTT_m samples were higher than in this study and ranged from 6.00–28.02 pmol min⁻¹ μg⁻¹. Unlike Radicals_{aq} (Fig. 2c), WSTMs of PM_{0.49–0.95} exhibit higher solubility than PM_{3–7} and PM_{0.49} (Fig. 2f), agreeing with previous reports for soluble metal from anthropogenic sources.^{60–62} Fig. S2 and S3 also show consistent natural logarithmic size distributions of OP and H₂O₂ yields, which can be explained by the similar size distribution patterns of EPFRs and WSTMs. EPFR concentrations around the Arabian Peninsula (10⁷–0.7 × 10¹¹ spins μg⁻¹) are found to be overall lower than previously reported at urban continental sites, where the EPFR concentrations are typically in the range (0.1–220) × 10¹² spins μg⁻¹.^{20,26,29–31,63–65} Such a finding can be explained by the usually higher humidity of marine air⁶⁶ and higher hygroscopicity of marine aerosols,⁶⁷ which may promote radical degradation.⁴⁰ Additionally, the lack of major combustion sources in the region, other than occasional emissions from ship traffic, might account for the relatively weak EPFR signal.^{33,34,57}

3.2 Spatial distribution of oxidative potential and redox activity of PM

DTT_m, reactive oxygen species (ROS) yields, and redox-active components' concentrations in sub-micrometer (PM_{0.49}) and coarse (PM_{3–7}) PM across five sea regions are compared in Fig. 3. Both DTT depletion rates and targeted components' concentrations for PM_{0.49} and PM_{3–7} exhibit significant statistical differences, reflecting the distinct spatial variations of particle mass-based PM composition and toxicity.^{40,58}

PM_{0.49} in marine air west of the Arabian Peninsula (NRS) exhibits higher DTT depletion rates than in air east of the Arabian Peninsula (OG and PG), which is not supported by association with Radicals_{aq}, WSOC or EPFRs (Fig. 3a). Possibly, this could indicate a significant contribution of oxidized aromatics to PM's OP, as higher concentrations of ∑₂₆PAHs (polycyclic aromatic hydrocarbons) and oxidized derivatives thereof were reported in samples collected from west of the Arabian Peninsula during this campaign.⁴⁰

DTT_m of PM_{3–7} across different sea regions increased in the order of OG < MS < NRS < PG < SRS + AG (Fig. 3g). The overall higher particle mass-based DTT_m of PM_{3,7} and PM_{0.49} (Fig. 3a and g) at SRS + AG indicates locally emission-enhanced toxicity of aerosol in the continental outflow from East Africa.^{68,69} During the AQABA campaign, the aerosol was dominated by mineral dust only in this sea region. The dust substantially originates from local emissions and was mixed with black and brown carbon in both the PM₁ and PM₁₀ size fractions.⁵⁸ Correspondingly, we find WSTMs elevated in SRS + AG as compared to other sea regions, with Fe contributing most to both sub-micrometer and coarse particles (Fig. 3f and l). Mineral dust contains WSTMs, most prominently Fe, and increasingly so if aged and mixed with organic and acidifying pollutants.^{70–72} Compared to typical urban and continental PM (DTT_m ranges from 1–100 pmol min⁻¹ μg⁻¹),^{73–78} less OP is found in sub-micrometer particles (PM_{0.49}) and coarse marine PM in this study, with DTT_m ranging from 0.4–3.6 pmol min⁻¹ μg⁻¹ (see Tables S1 and S2). The particle mass-normalized OP (DTT_m) detected in this study is lower than earlier measurements in both terrestrial and marine air. Because of limited coverage of particle size fractions, comparison of volume-normalized concentrations (*e.g.*, DTT_v) is not possible.

3.3 Correlation of oxidative potential with redox activity of PM

The correlations (Spearman *r*) between DTT_m as well as reactive oxygen species (ROS) yields and redox-active components are shown in Fig. 4.

DTT_m of PM_{0.49} exhibits significant correlations (*P* < 0.05; Student's *t*-test) with Radicals_{aq} (*r* = 0.77), R' (*r* = 0.70), and Ni (*r* = 0.67) (Fig. 4a), which may be associated with radical chemistry in the presence of WSTMs (Fig. 3f) and WSOC (Fig. 3d).^{79,80} For instance, metal–organic peroxide interactions and metal–ligand complexation have been found to be able to increase the dissolution of particulate metals and ROS formation by atmospheric PM.^{15,81,82} This finding is different from a report of PM₁₀ compositional analysis over the Arabian Sea, which indicated that WS-Mn, WS-Cu, and WS-Zn correlated significantly with DTT_m.³³ The correlations of Radicals_{aq} with different WSTM species for PM_{0.49} and PM_{3–7} may indicate the influence of WSTM-initiated pseudo-Fenton chemistry in PM. The Radicals_{aq} in PM_{0.49} are found to be significantly correlated with 'OH and R', indicating that these two radical species contribute most to the radical sum. Conversely, in the PM_{3–7} size fraction (Fig. 4b), Radicals_{aq} are significantly correlated with Co and Mn, yet no significant correlation is observed with individual radical species. While the H₂O₂ yield of PM_{0.49} is significantly correlated with WSTMs (*P* < 0.01; *r* = 0.85) and with Fe, Co, Ni, Mn, and Zn (*P* < 0.03; *r* = 0.73–0.85), the H₂O₂ yield of PM_{3–7} (Fig. 4b) is correlated with WSOC (*P* < 0.02; *r* = 0.79) but much less with the metals. This suggests a different radical and H₂O₂ chemistry of submicrometer PM and coarse PM in liquid water, as suggested and observed previously.^{83–85}

DTT_m of PM_{3–7} is found to be significantly correlated (*P* < 0.01; Student's *t*-test) with WSTMs, especially Fe (*r* = 0.76,



Fig. 4b). This observation is in line with previous findings in polluted continental air. For instance, Fang *et al.* found that water-soluble Cu contributed significantly to OP of PM₁₀ in an urban roadside environment.³⁶ Charrier and Anastasio also found that Cu, Mn, and Fe contribute ~80% of DTT_m of the hypothetical typical PM_{2.5} sample and ambient PM_{2.5} collected in the San Joaquin Valley of California.⁷ The possible significance of WSOC and WSTMs for DTT_m (Fig. 4b) had been observed for marine aerosol before, *i.e.*, for PM_{2.5} in the northern Indian Ocean³⁴ and for PM₁₀ over the Arabian Sea.³³

4 Conclusions

Similar to continental and urban environments, we find that the sub-micrometer ambient marine PM size fraction (PM_{0.49}) exhibits a higher particle mass-normalized DTT consumption rate (DTT_m, in units of pmol min⁻¹ μg⁻¹) than coarse mode particles (PM₃₋₇) in the marine environment of marginal seas in an arid region. The range of DTT_m values is found to be lowest in the range of previous findings on both land and sea. DTT_m appears to be most associated with radicals formed upon dissolution in water, water-soluble organic compounds (WSOC), and water-soluble transition metals (WSTMs). The formation of H₂O₂ is found to be most influenced by WSTMs in sub-micrometer particles and associated with WSOC in the coarse mode particles. The new data confirms the significance of particle size-resolved redox-active components for OP of marine aerosols. The findings provide new insights into the association of redox-active PM components and OP in the marine environment. More data from marine aerosols, with various continental influences, including mineral dust, is needed for better understanding of PM's OP in marine environments. Distinguishing the contributions of surface-mediated reactions and aqueous-phase Fenton-like processes to ROS formation is warranted in follow up studies.

Author contributions

HT and GL designed the research. SL conducted experiments on H₂O₂, WSTMs, and DTT. AF conducted the EPR measurements. HT performed the data analysis and wrote the manuscript. All authors discussed the results and edited the manuscript.

Conflicts of interest

The authors declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this published article, supplied as supplementary information (SI) or are available from the corresponding author on reasonable request. Supplementary information is available. See DOI: <https://doi.org/10.1039/d5ea00093a>.

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