



Pollution sources affecting the oxidative potential of fine aerosols in a Portuguese urban-industrial area - an exploratory study

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Abstract

This study targets to determine the oxidative potential (OP) of fine aerosols in an urban-industrial area of the Lisbon Metropolitan Area (Portugal) and, in addition, to identify which pollution sources may have an impact on the OP levels of fine aerosols. For this purpose, thirty samples were selected from a set of 128 samples collected over one year (Dec 2019–Nov 2020), based on the highest load for each source (both mass and %) previously assessed by source apportionment studies (using Positive Matrix Factorisation, a total of 7 different sources were identified: soil, secondary sulphate, fuel-oil combustion, sea, vehicle non-exhaust, vehicle exhaust and industry). The OP associated with the water-soluble components of PM_{2.5} was assessed using the dithiothreitol (DTT) method. The samples had a mean DTT activity (normalised to the mass) of 12.9 ± 6.6 pmol min⁻¹ μg⁻¹, ranging from 3.5 to 31.8 pmol min⁻¹ μg⁻¹. The DTT activity (normalised to the volume, OP_V^{DTT}) showed to have a significant positive association with PM_{2.5} levels (R²=0.714). Considering that the mass contributions of the different sources to the PM_{2.5} levels were known, Spearman correlations were assessed and significant correlations were found between OP_V^{DTT} and three different sources: vehicle exhaust (ρ=0.647, *p*-value=0.001), fuel-oil combustion (ρ=0.523, *p*-value=0.012) and industry (ρ=0.463, *p*-value=0.018). Using a multiple linear regression analysis, these three sources were found to explain 82% of the variability in OP_V^{DTT}, with vehicle exhaust being the most influential source.

Keywords PM_{2.5} · Oxidative potential · Multiple linear regression analysis · Dithiothreitol method · Urban-industrial areas

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Introduction

The negative impact that aerosols have on human health (Liu et al. 2019), climate and the environment (Ramanathan et al. 2001) is now well established. Aerosols consist of a complex mixture of chemical components that vary depending on the sources and their consequent atmospheric processes (Perrone et al. 2013). Several studies have identified particulate matter (PM), especially its fine fraction (PM_{2.5}), as a critical parameter for adverse health effects (Delfino et al. 2013; Canha et al. 2016). However, many questions remain about the underlying drivers of PM toxicity.

Oxidative stress induced by PM is proposed as the primary mechanism responsible for the adverse health effects associated with PM inhalation and its toxicity (Valavanidis et al. 2008). Oxidative stress can occur when the body's antioxidant capacity is unable to counteract or detoxify the harmful effects of reactive oxygen species (ROS) due to an

excess presence of ROS introduced into the body by PM inhalation (Bates et al. 2019). Recently, several studies have linked measurements of oxidative potential (OP) of aerosols to adverse health effects (Fang et al. 2016; Abrams et al. 2017).

There are several cellular and acellular methods for studying the OP of ambient particles, each with its own characteristics. To fully represent the toxicity of ambient PM, a combination of chemical and cellular assays is required to account for all aerosol components and mechanisms that may contribute to its toxicity. These methods include ascorbic acid (AA) (Fang et al. 2016), antioxidant-reduced glutathione (GSH) (Weichenthal et al. 2016), electron spin resonance (ESR) (Yang et al. 2015) and dithiothreitol (DTT) (Wang et al. 2019). The DTT method (OP^{DTT}) is widely used and has been associated with airway inflammation markers (Delfino et al. 2013), cellular oxidative stress markers (Li et al. 2003), cellular cytotoxicity (Steenhof et al. 2011) and cardiorespiratory health endpoints in epidemiological studies (Bates et al. 2015; Yang et al. 2016). These findings support OP as a highly health-relevant air quality parameter.

However, the specific chemical species, aerosol sources and atmospheric processes that influence the OP of PM are not yet fully established (Paraskevopoulou et al. 2019). In Europe, most of the published studies on OP include a limited sampling for periods not exceeding a few months (Weber et al. 2018; Paraskevopoulou et al. 2019), and for Portugal, there are no studies on OP assessment of aerosols in the country. Therefore, the establishment of a relationship between PM toxicity (derived from OP evaluation), aerosol composition and emission sources remains to be defined in many European countries and, in particular, in Portugal.

The present study aimed to contribute to fulfil this lack of knowledge, being an exploratory study to characterize OP levels of $PM_{2.5}$ sampled in an urban-industrial area of the Lisbon Metropolitan Area (Seixal, Portugal). For this purpose, fine aerosols were sampled for one year (December 2019 - November 2020, with a total of 128 sampling days) and their chemical composition was assessed to perform a source apportionment study using Positive Matrix Factorisation (Gamelas et al. 2023). A total of 7 different sources were identified, namely: soil, secondary sulphate, fuel-oil combustion, sea, vehicle non-exhaust, vehicle exhaust and industry (Gamelas et al. 2023).

From the total dataset, a subset of 30 samples was selected (considering the samples with the highest load for each source, both in mass and in %) and their OP was determined for their water-soluble fraction by the dithiothreitol method. The OP levels were then evaluated taking into account the contribution of the different pollution sources, assessed by Positive Matrix Factorisation (PMF), to the $PM_{2.5}$ levels in order to understand their potential influence on PM toxicity.

Materials and methods

Study site

Fine aerosols were sampled at Aldeia de Paio Pires (38.617885°, -9.080055°), located in the municipality of Seixal, a suburban and industrial area in the Lisbon Metropolitan Area (Portugal) which is one of the most densely populated municipalities in Portugal (with 167,294 inhabitants in 95.5 km²). The sampling site was located in the vicinity of busy motorways, several small and medium sized industries (including steelworks, lime factory, and metal waste management and treatment factory), a shipyard and the ferry terminal that connects the two river banks for commuting (Seixal – Lisbon). A detailed description of the sampling site and the study area is available elsewhere (Abecasis et al. 2022; Gamelas et al. 2023).

PM characterization, source apportionment and selection of samples

A full description of the PM characterisation strategy and methodology can be found elsewhere (Gamelas et al. 2023). Sampling was conducted from December 2019 to November 2020, and a total of 128 samples were obtained. Briefly, mass concentration of $PM_{2.5}$ sampled over 24 h (using 47 mm diameter quartz filters) was determined by gravimetry and its chemical characterisation for 24 chemical elements was performed by Particle Induced X-Ray Emission (PIXE), together with the determination of three water-soluble inorganic ions by ion chromatography and the black carbon content by light absorption at 639 nm.

To perform the source apportionment, the receptor model PMF was used to identify the main emission sources and to estimate their contribution to the $PM_{2.5}$ mass. The original data matrix was composed by a total of 128 samples and 24 PM species (only those variables with more than 70% of the data above the detection limit were considered). A total of 7 different pollution sources were identified as contributing to the $PM_{2.5}$ mass, namely, soil (3.6%), secondary sulphate (18.4%), fuel-oil combustion (11.5%), sea (11.0%), vehicle non-exhaust (2.3%), vehicle exhaust (40.8%) and industry (12.6%). A description of the methodology and a detailed description of the results can be found elsewhere (Gamelas et al. 2023).

For the present study, thirty samples were selected from the full dataset and their selection was based on the highest load for each source (both in mass and %). Table 1 shows the selected samples, including the mass contribution of the different seven sources assessed to their $PM_{2.5}$ levels.

Table 1 Selected PM_{2.5} samples with identification of mass contribution from 7 different pollution sources

Sampling Days	Filter ID	PM _{2.5} (µg m ⁻³)	Source mass contribution to PM _{2.5} concentration (µg m ⁻³)						
			Soil	Secondary sulphate	Fuel-oil combustion	Sea	Vehicle non-exhaust	Vehicle exhaust	Industry
12/19/2019	Q3	13.9	-	1.0	0.9	11.1	-	-	0.9
12/22/2019	Q4	15.4	-	1.1	2.0	10.0	-	1.6	0.8
12/30/2019	Q8	56.3	0.4	0.1	9.9	2.3	2.1	36.8	4.7
1/6/2020	Q11	57.6	-	1.0	8.4	0.3	0.5	43.7	3.8
1/7/2020	Q12	67.8	0.1	-	9.3	0.8	0.7	55.1	1.9
1/8/2020	Q13	25.8	0.6	1.4	-	0.1	0.4	23.2	0.1
1/19/2020	Q15	7.3	0.2	0.6	-	3.1	3.5	-	-
1/20/2020	Q16	12.3	0.0	1.0	5.4	1.6	0.8	0.9	2.5
1/26/2020	Q20	24.1	0.4	1.9	1.9	-	0.2	19.7	-
1/27/2020	Q21	5.1	1.4	1.3	-	1.7	0.0	0.8	-
2/17/2020	Q36	4.5	0.0	-	-	3.0	0.5	0.4	0.6
2/27/2020	Q41	15.6	3.3	2.0	1.1	1.2	0.4	5.6	2.0
3/1/2020	Q42	9.1	2.5	1.0	0.0	5.0	0.4	0.3	-
3/2/2020	Q43	11.4	-	-	0.8	9.1	0.0	0.4	1.0
3/4/2020	Q45	4.6	1.7	0.8	-	1.7	-	0.1	0.2
3/5/2020	Q46	6.6	-	-	0.2	5.7	0.0	0.0	0.5
3/17/2020	Q54	12.8	1.1	-	2.8	1.2	0.0	-	7.7
7/8/2020	Q76	10	0.2	8.6	-	0.5	-	0.0	0.7
7/12/2020	Q78	12.5	0.6	8.3	0.1	0.3	0.1	2.0	1.0
7/15/2020	Q81	18.1	2.6	4.6	1.6	0.0	0.1	2.2	7.0
7/16/2020	Q83	23.1	4.6	0.7	1.1	0.7	0.5	-	15.4
7/19/2020	Q84	11.1	0.2	9.4	0.4	0.1	0.1	0.2	0.7
8/9/2020	Q97	5.8	0.2	4.6	-	0.2	0.0	0.5	0.3
10/6/2020	Q108	5.9	0.3	1.1	0.6	0.3	0.7	0.3	2.6
10/11/2020	Q111	14.3	1.2	1.1	1.4	1.3	1.4	-	7.9
10/12/2020	Q112	15.6	1.2	-	0.5	0.3	0.5	3.3	9.7
11/9/2020	Q133	15.1	0.0	-	7.1	3.2	1.5	1.7	1.7
11/10/2020	Q134	26.6	-	-	8.5	1.2	2.0	13.3	1.6
11/11/2020	Q135	28.1	-	-	10.0	2.0	0.4	12.8	3.0
11/12/2020	Q136	26.8	0.7	-	12.6	-	1.2	6.2	6.1
	Mean	18.8	1.0	2.6	3.8	2.4	0.7	9.2	3.2
	SD	15.9	1.2	2.9	4.1	3.1	0.8	15.2	3.7
	Min	4.5	0.0	0.1	0.0	0.0	0.0	0.0	0.1
	Max	67.8	4.6	9.4	12.6	11.1	3.5	55.1	15.4

Oxidative potential analysis using the DTT assay

The oxidative potential of the water-soluble fraction of the sampled PM_{2.5} was assessed following the DTT assay, as fully described elsewhere (Chirizzi et al. 2017). The water-soluble fraction was extracted from 1/4 of each filter in 15 mL deionized water (DI, Milli-Q; > 18 MΩ) by sonication in a water bath for 30 min. The extracts were then filtered through 0.45 µm pore PTFE (polytetrafluoroethylene) syringe filters to remove insoluble materials and residual fibres. Sample extracts were then incubated at 37 °C with DTT (100 µM) in 0.1 M potassium phosphate buffer at pH 7.4 (5 mL total volume) for a period of 90 min. At specified times (namely 5, 10, 15, 20, 30, 45, 60, and 90 min), a 0.5 mL aliquot of the incubation mixture was picked up and 0.5 mL of 10% trichloroacetic acid was added to stop

the reaction. This reaction mixture was then mixed with 2 mL of 0.4 M Tris-HCl, pH 8.9 containing 20 mM EDTA and 25 µL of 10 mM DTNB. The concentration of 5-mercapto-2-nitrobenzoic acid formed was measured by its optical density absorption at 412 nm, using a Cary 50 UV-Vis spectrophotometer (Varian Inc.). The rate of DTT consumption (δDTT, pmol/min) was determined from the slope and intercept of linear regression of measured absorbance versus time, where N₀ is the initial moles of DTT used (Cho et al. 2005). Two replicates were made for 7 random samples and the variability (standard deviation) between replicates was taken as the uncertainty of the measured DTT activity.

The average DTT consumption on the blanks was 0.947 ± 0.119 nmol/min (total of 4 different filter blanks, with 2 replicates each). The final DTT activity for a sample was calculated by subtracting the average value of the blanks

from the value of each sample. The final DTT activity was normalised with respect to (1) the sampled air volume (V), which provides DDT_V and depends on the emission rates and dilutions and characterises the human exposure to particles at a given location, and it indicates the oxidative potential per unit volume of air of the aerosol component; and (2) the collected aerosol mass (M), which provides DDT_M and represents an intrinsic property of particles linked to sources (Chirizzi et al. 2017), which reflects the oxidative potential of PM per unit mass (Farahani et al. 2022).

Statistical analysis

Data handling and statistical analyses were performed using Excel and XLSTAT 2023.1.6 software programmes. The data were analysed using non-parametric statistics with a significance level of 0.050. Associations between parameters were obtained using Spearman correlations. Origin version 7.5 (OriginLab Corporation) was used to plot the results.

A multiple linear regression (MLR) analysis was performed between the contribution of the sources that showed a significant influence on OP_V^{DTT} (from the analysis of the Spearman correlations) and the OP_V^{DTT} values (as the dependent variable), in order to have an independent estimate of the source contributions to OP. For this, OP was assumed to be linearly linked to $PM_{2.5}$ due to the different sources, according to the formula (Weber et al. 2018):

$$OP_V^{DTT} = m_{PM} \times \beta + \epsilon$$

Where OP_V^{DTT} is the dependent variable, i.e., a matrix ($n \times 1$) in $\text{nmol min}^{-1} \text{m}^{-3}$, m_{PM} is the ($n \times p$) matrix with

the PM contributions attributed to each source by the PMF study in $\mu\text{g m}^{-3}$, ϵ is ($n \times 1$) uncertainty matrix in $\text{nmol min}^{-1} \text{m}^{-3}$, n is the number of samples and p is the number of sources. The estimator β matrix ($p \times 1$) represents the intrinsic OP of the sources (i.e., the OP per mass unit of PM attributed to a given source, expressed in $\text{nmol min}^{-1} \mu\text{g}^{-1}$) and the intercept is expressed in $\text{nmol min}^{-1} \text{m}^{-3}$.

The MLR was performed using the XLSTAT tool, taking into account the replacement of the source's contributions by the overall mean when no value was available and imposing the intercept equal to zero.

Results

Oxidative potential of $PM_{2.5}$

Figure 1 presents the oxidative potential (normalised to the mass) together with the $PM_{2.5}$ levels of the studied fine aerosol filters. The samples had a mean DTT activity (normalised to the mass, OP_M^{DTT}) of $12.9 \pm 6.6 \text{ pmol min}^{-1} \mu\text{g}^{-1}$, ranging from 3.5 to $31.8 \text{ pmol min}^{-1} \mu\text{g}^{-1}$. The mean $PM_{2.5}$ levels of the selected samples was $18.8 \pm 15.9 \mu\text{g m}^{-3}$, ranging from $4.5 \mu\text{g m}^{-3}$ to $67.8 \mu\text{g m}^{-3}$. Figure 1 also highlights the great variability of OP_M^{DTT} levels between the analysed samples, and also that they clearly do not follow the trend of $PM_{2.5}$ concentrations. It is noteworthy to highlight that the selection of samples was based on the most representative samples for each of the sources identified by the PMF study (due to their higher contribution in mass and % to the $PM_{2.5}$ levels). In addition, Fig. 1 shows that samples having comparable $PM_{2.5}$ concentrations may have significantly different OP_M^{DTT} and OP_V^{DTT} according to the sources influencing

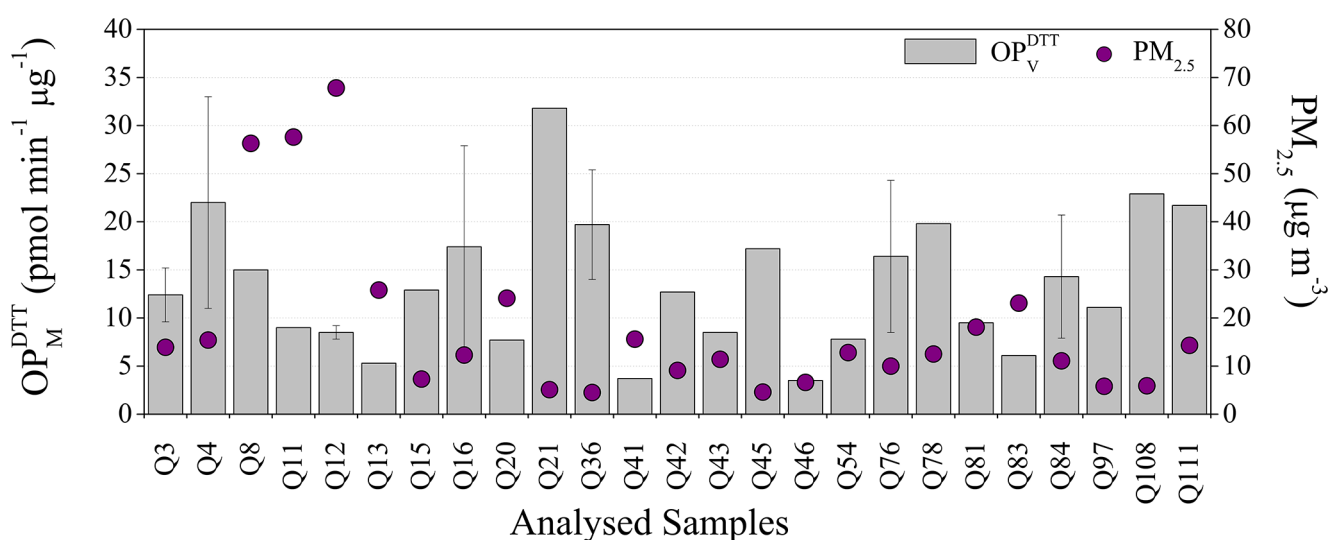


Fig. 1 Oxidative potential, normalized to the mass (OP_M^{DTT}) – mean and standard deviation when two replicates were done, and $PM_{2.5}$ levels of the selected samples

those samples. For example, sample Q97 influenced mainly by sulphate has significantly lower OP compared to sample Q108 mainly influenced by industry source. This is in agreement with previous works (Molina et al. 2020; Daellenbach et al. 2020; Clemente et al. 2023) that suggested that a linear relationship between OP and PM concentrations is not always observed. This is the reason why OP could be a metric for toxicity complementary to mass concentration of PM.

The DTT activity (normalised to the sampled volume, OP_V^{DTT}) of the selected samples showed mean levels of $0.21 \pm 0.17 \text{ nmol min}^{-1} \text{ m}^{-3}$, ranging from 0.02 to $0.85 \text{ nmol min}^{-1} \text{ m}^{-3}$. As seen for OP_M^{DTT} , the analysed samples also show a high variability for the OP_V^{DTT} levels, taking into account the selection of samples done considering the highest load (in mass and %) for each identified source. Table 2 provides an overview of the OP_V^{DTT} levels of $PM_{2.5}$ from different types of environments and countries. The results of the present study are similar to others studies carried out in other countries, such as in an urban-industrial area in France (Dunkerque $-0.36 \pm 0.24 \text{ nmol min}^{-1} \text{ m}^{-3}$ (Moufarrej et al.

2020), urban backgrounds in Italy (Sarno $-0.19 \pm 0.10 \text{ nmol min}^{-1} \text{ m}^{-3}$ (Cesari et al. 2019), Lecce $-0.40 \pm 0.26 \text{ nmol min}^{-1} \text{ m}^{-3}$ (Chirizzi et al. 2017) and $0.29 \pm 0.19 \text{ nmol min}^{-1} \text{ m}^{-3}$ (Giannossa et al. 2022), urban environments in Greece (Athens $-0.33 \pm 0.20 \text{ nmol min}^{-1} \text{ m}^{-3}$ (Paraskevopoulou et al. 2019) and in India (Mumbai - median of $0.12 \text{ nmol min}^{-1} \text{ m}^{-3}$ in a low-traffic slum and median of $0.20 \text{ nmol min}^{-1} \text{ m}^{-3}$ in a high-traffic slum (Anand et al. 2022); and in different types of environments (urban, roadside, near-road and rural) especially during summer and fall in Atlanta, USA (Fang et al. 2015). Our results were found to be lower than those obtained in different types of urban environments in China (Liu et al. 2018; Chen et al. 2022; Wu et al. 2022), India (Patel and Rastogi 2018), Iran (Khoshnamvand et al. 2023), Brazil (Serafeim et al. 2023), Spain (in 't Veld et al. 2023) and different types of environments in Switzerland (Grange et al. 2022).

However, it is important to highlight that some of the above-mentioned studies had applied a slightly different extraction method of the $PM_{2.5}$ filters, which can be

Table 2 Comparison of OP_V^{DTT} levels of $PM_{2.5}$ from different types of environments and countries

Continent	Country	City	n	Type of environment	OP_V^{DTT} (nmol min ⁻¹ m ⁻³)	Reference	
Asia	China	Shanghai	38	Urban	7.21 ± 1.69	(Wu et al. 2022)	
		Ningbo	55	Urban	3.65 ± 1.71	(Chen et al. 2022)	
		Jinzhou	100	Urban	4.4 ± 2.6	(Liu et al. 2018)	
		Tianjin	100	Urban	6.8 ± 3.4		
		Yantai	100	Urban	4.2 ± 2.7		
	India	Mumbai	44	Urban - low traffic	0.12 (median) (0.04–0.51)	(Anand et al. 2022)	
			40	Urban - high traffic	0.20 (median) (0.03–1.06)		
		Patiala ¹	48	Semi-urban	3.8 ± 1.4 (1.3–7.2)	(Patel and Rastogi 2018)	
	Iran	Tehran	30	Urban background	0.87 ± 0.14 (spring), 0.70 ± 0.28 (summer), 0.94 ± 0.38 (autumn)	(Khoshnamvand et al. 2023)	
	America	USA	Atlanta	28	Urban	0.29 ± 0.02 (summer), 0.43 ± 0.03 (winter)	(Fang et al. 2015)
80				Roadside	0.27 ± 0.00 (fall), 0.36 ± 0.02 (winter)		
60			Near-Road	0.20 ± 0.00 (fall), 0.25 ± 0.02 (winter)			
55			Rural	0.28 ± 0.01 (summer and winter)			
Brazil		São Paulo	58	Urban	$1.22 + 0.55$ (0.06–2.22)	(Serafeim et al. 2023)	
Europe		Italy	Sarno	48	Urban background	0.19 ± 0.10	(Cesari et al. 2019)
			Milan	72	Urban	0.85 ± 0.10 (summer), 3.38 ± 0.46 (winter)	(Hakimzadeh et al. 2020)
			Lecce	30	Urban background	0.40 ± 0.26	(Chirizzi et al. 2017)
				124	Urban background	0.29 ± 0.19	(Giannossa et al. 2022)
			Bologna	10	Urban	0.3–1.7	(Visentin et al. 2016)
	Greece	Athens	361	Urban	0.33 ± 0.20 (0.02–1.16)	(Paraskevopoulou et al. 2019)	
	France	Dunkerque	57	Urban-Industrial	0.36 ± 0.24	(Moufarrej et al. 2020)	
	Spain	Barcelona ²	102	Urban	1.2	(in 't Veld et al. 2023)	
		Montseny ²	106	Rural	0.4		
	Switzerland	Bern ²		-	Urban-Traffic	1.1	(Grange et al. 2022)
Zürich ²			-	Urban	0.8		
Cadenazzo ²			-	Rural	0.7		
Payerne ²			-	Rural	0.6		
Basel ²			-	Suburban	0.6		
Portugal	Seixal	30	Urban-industrial	0.21 ± 0.17 (0.02–0.85)	This study		

¹ Extraction done with mixture 1:1 of methanol and Milli Q water; ² Extraction done with Gamble and dipalmitoyl phosphatidylcholine solution

translated into different levels of OP, such as the use of a 1:1 mixture of methanol and Milli Q water (Patel and Rastogi 2018) and the use of a simulated lung fluid (SLF) solution consisting of a mixture of Gamble's solution and dipalmitoylphosphatidylcholine (DPPC) (Grange et al. 2022; in 't Veld et al. 2023). However, OP_V^{DTT} measured in Milli-Q water, Gamble and Gamble+DPPC solution have been shown to have no statistically significant differences (Calas et al. 2017). This highlights the need for a standardised OP procedure to allow direct comparisons of OP metrics between studies in different countries and performed by different laboratories.

Even limiting to cases with similar protocols, there is still a large spatial and seasonal variability, likely associated not only to $PM_{2.5}$ mass concentrations but to the differences in chemical composition due to the sources acting on the different sites. It is therefore useful to mention that the values found here are comparable with average values observed in Europe and USA but significantly lower than those observed in China and India, likely as a consequence of both a higher $PM_{2.5}$ concentration in these countries and to the difference on sources contributing to the observed concentrations.

OP_V^{DTT} levels were found to increase linearly with $PM_{2.5}$ concentrations, with a R^2 of 0.714 (p -value < 0.0001), as shown in Fig. 2. A similar trend in the correlation between $PM_{2.5}$ and OP_V^{DTT} has already been reported in other studies.

In Shanghai (China), a study in urban environments found a high correlation between $PM_{2.5}$ and OP_V^{DTT} ($\rho=0.69$) (Wu et al. 2022). In the USA, OP_V^{DTT} was also found to be fairly well correlated with $PM_{2.5}$ (with Pearson's correlation coefficient, r , ranging from 0.49 to 0.86) sampled at seven sites in the southeastern USA (Fang et al. 2016). Similar results were also observed in Lecce (Italy), where a significant correlation ($r=0.57$) was found between OP_V^{DTT} and $PM_{2.5}$ levels (Chirizzi et al. 2017); in Patiala (India), OP_V^{DTT} levels were also found to increase linearly with $PM_{2.5}$ concentrations ($R^2=0.44$, $p<0.001$) (Patel and Rastogi 2018), as well as in Tehran (Iran) for the summer dataset ($R^2=0.63$) (Khoshnamvand et al. 2023). However, in the particular case of the present study, the high linear correlation found is highly dependent of the 3 samples with higher $PM_{2.5}$ levels. Without those samples, the R^2 decreases to a value of 0.24.

Influence of pollution sources on oxidative potential of $PM_{2.5}$

Considering that the mass contribution of the different sources to the $PM_{2.5}$ levels was known from the previous source apportionment study by PMF, Spearman correlations between OP_V^{DTT} and the contribution of each source to the $PM_{2.5}$ levels were assessed and are described in Table 3. Three different sources showed a significant

Fig. 2 Correlation between $PM_{2.5}$ levels and their associated oxidative potential, normalized to the volume (OP_V^{DTT})

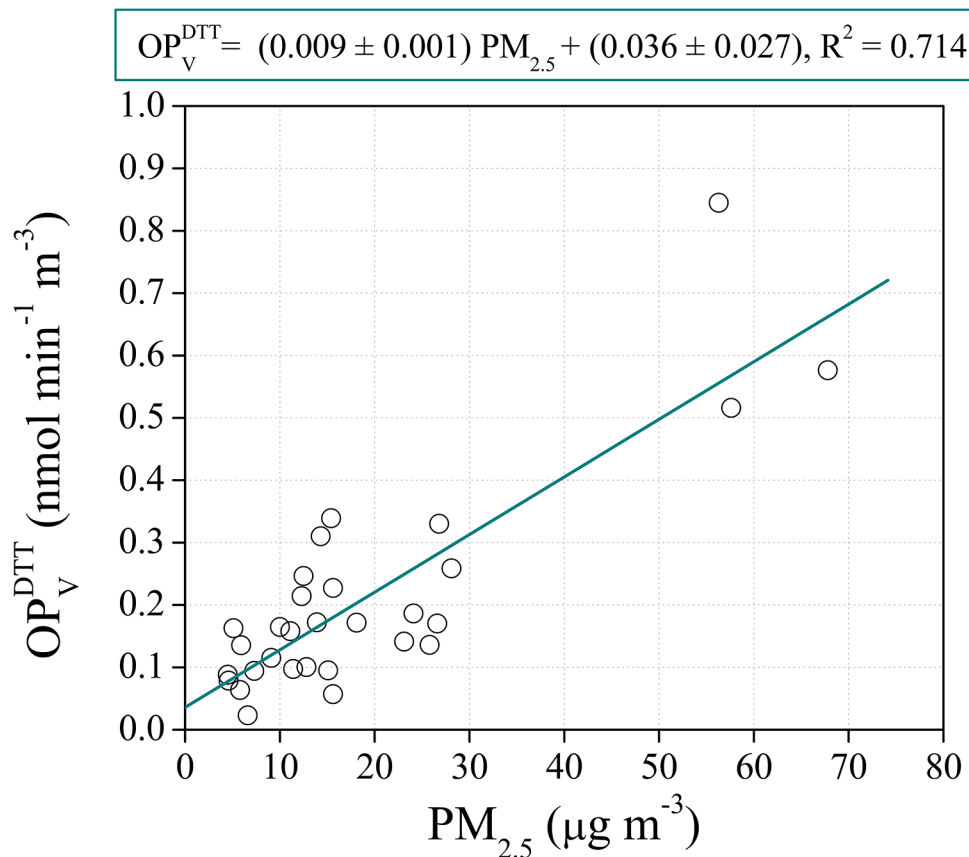


Table 3 Spearman correlations between OP_V^{DTT} and the contribution of the different assessed pollution sources for $PM_{2.5}$ levels

Source	Spearman's rank correlation coefficient (ρ)	<i>p</i> -value
Soil	-0.056	0.799
Secondary sulphate	-0.108	0.649
Fuel-oil combustion	0.523	0.012
Sea	-0.136	0.489
Vehicle non-exhaust	0.369	0.064
Vehicle exhaust	0.647	0.001
Industry	0.463	0.018

Table 4 Multiple linear regression (MLR) analysis results and parameters regarding the goodness of the fit produced by the model for each source

Source	β Coefficients ($\text{nmol min}^{-1} \text{m}^{-3}$)	Standard Error ($\text{nmol min}^{-1} \text{m}^{-3}$)	<i>p</i> -value	Lower 95%	Upper 95%
Fuel-oil combustion	0.019	0.006	0.005	0.006	0.032
Vehicle exhaust	0.008	0.002	0.000	0.004	0.012
Industry	0.093	0.005	0.025	0.002	0.024

positive correlation with OP_V^{DTT} , namely fuel-oil combustion ($\rho=0.523$, p -value=0.012), vehicle exhaust ($\rho=0.647$, p -value=0.001) and industry ($\rho=0.463$, p -value=0.018), indicating their influence on OP levels and, consequently, their potential impact on the health of the citizens of the study area.

To estimate the contributions of the significant PM sources to OP_V^{DTT} , a multiple linear regression (MLR) was applied considering OP_V^{DTT} as the dependent variable and the contributions of fuel-oil combustion, vehicle exhaust and industry as the independent variables, following the methodology described elsewhere (Weber et al. 2018; Cesari et al. 2019; Giannossa et al. 2022). The results of the MLR methodology are characterised by a $R^2=0.824$ (R^2 correct=0.804) and a RMSE (root mean squared error) of 120. In addition, the F-test results (at a 95% confidence level) indicated that the model provided a good fit of the three variables considered. Considering the R^2 , 82% of the variability of the dependent variable OP_V^{DTT} is explained by the contribution of the three sources and, based on the Type III sum of squares, the variable vehicle exhaust was the most influential independent variable.

Table 4 presents the results of the MLR analysis in terms of coefficients of β , standard errors, p -values and 95% confidence intervals. The model provided a good fit for all the sources considered (p -values below 0.050). Results showed that the different sources contributed differently to OP_V^{DTT} of $PM_{2.5}$ levels in the study area. The source with the highest contribution for the intrinsic OP_V^{DTT} of the studied aerosols was vehicle exhaust (standardized β coefficient=0.471), followed by fuel-oil combustion (standardized β coefficient=0.366) and, finally, by industry (standardized β coefficient=0.222).

Figure 3 presents the correlation between the measured and the reconstructed OP_V^{DTT} using the MLR, which shows a good correlation ($R^2=0.60$), but with larger scatter on the comparable data, as also observed in other studies (Cesari et al. 2019; Giannossa et al. 2022).

Figure 4 presents the relative contributions of the different sources to the $PM_{2.5}$ and OP_V^{DTT} levels. For the OP_V^{DTT} levels, only the three identified sources used for the MRL methodology were considered. A high standard deviation in both cases is found since there is a high variability on the type of selected samples (which had influence of different sources). In average, fuel-oil combustion contributed 15.6% to the $PM_{2.5}$ levels and 41.9% to the OP_V^{DTT} levels, while vehicle exhaust showed a relative contribution of 27.0% to $PM_{2.5}$ levels and 30.4% to the OP_V^{DTT} levels. The industry source had a relative contribution of 19.3% to $PM_{2.5}$ levels and 25.7% to the OP_V^{DTT} levels. Overall, the contribution of sources to the $PM_{2.5}$ levels were, in decreasing order, vehicle exhaust (27.0%), secondary sulphate (24.2%), sea (23.1%), industry (19.3%), fuel-oil combustion (15.6%), soil (8.7%) and vehicle non-exhaust (5.1%). Regarding the OP_V^{DTT} levels of the $PM_{2.5}$ sampled in the studied urban-industrial area, the highest contribution was from fuel-oil combustion ($41.9 \pm 37.3\%$), followed by vehicle exhaust ($30.4 \pm 34.7\%$) and, then, by industry ($25.7 \pm 30.0\%$).

The influence of these three specific sources on the OP_V^{DTT} of $PM_{2.5}$ levels obtained in the present study is consistent with several studies around the world that have also identified their contribution to the OP levels of fine aerosols. A study in the urban environments of Shanghai (China) found that the sources contributing to the OP_V^{DTT} of $PM_{2.5}$ levels (using PMF) were mainly biomass burning (42%), followed by vehicle emissions (28.9%), road dust (17.4%) and incomplete combustion (11.8%) (Wu et al. 2022). In the coastal city of Ningbo (China), a PMF study identified five sources (out of six contributing to $PM_{2.5}$ levels) that contributed to OP_V^{DTT} , namely, road dust (71%), marine vessels (9%), industry emissions (8%), vehicle emissions (7%) and secondary inorganic aerosol (5%) (Chen et al. 2022). In Italy, several works have investigated the main sources contributing to the oxidative potential of fine aerosols. A study in Sarno (Italy) identified three major sources contributing to the OP_V^{DTT} of $PM_{2.5}$ levels (using PMF), namely, vehicle

Fig. 3 MLR reconstructed vs. measured OP_V^{DTT} of fine aerosol

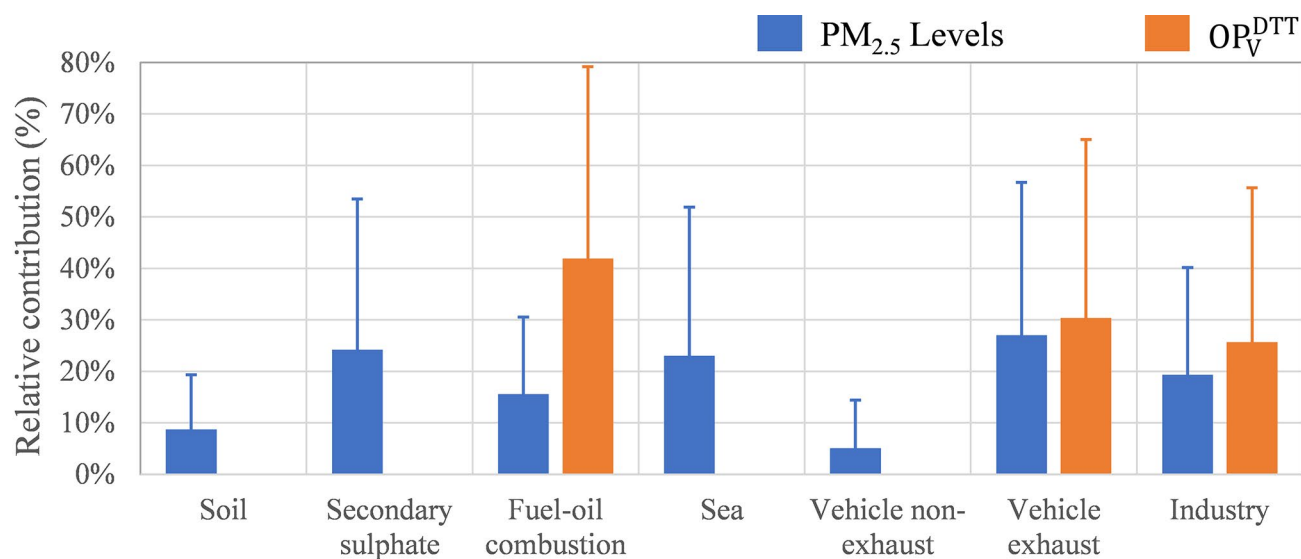
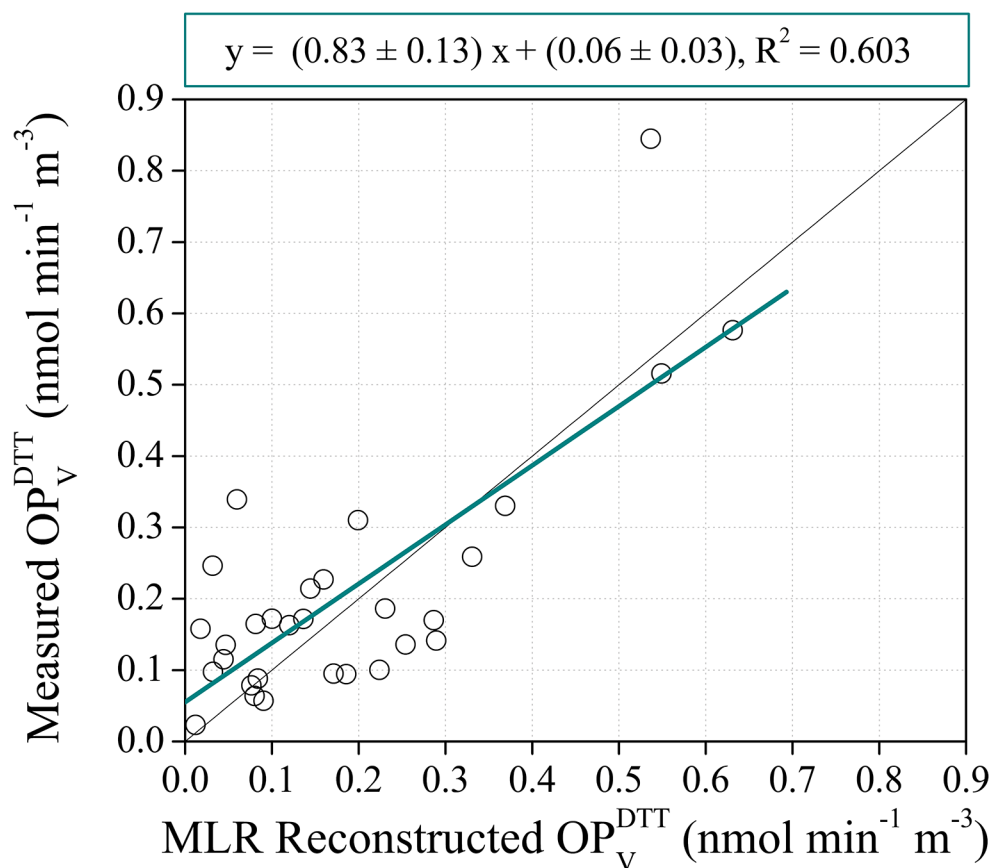


Fig. 4 Relative contributions of the different sources to PM_{2.5} levels and to OP_V^{DTT} levels

traffic with secondary nitrate ($\cong 50\%$), biomass burning ($\cong 35\%$), and industrial emissions ($\cong 12\%$) (Cesari et al. 2019). In Milan, a study using an MLR analysis identified four main sources contributing to the measured DTT activity in PM_{2.5} levels, namely, biomass burning (41%), secondary organic aerosols (20%), resuspended road dust (18%),

and vehicular emissions (16%) (Hakimzadeh et al. 2020). In Lecce, the majority of OP_V^{DTT} (about 50.6%) in PM_{2.5} was due to combustion sources (biomass burning and traffic), while natural and soil sources (sea spray, crustal and carbonates) contributed only 13.6% (Giannossa et al. 2022).

In Switzerland, road traffic (especially non-exhaust emissions) and wood burning were identified as the most important drivers of OP_V^{DTT} of fine aerosols (Grange et al. 2022). In the USA, the main sources contributing to OP_V^{DTT} of $PM_{2.5}$ levels (using PMF) were biomass burning (35%), secondary water-soluble organic carbon (WSOC) (19%), vehicle emissions (both from mechanically generated – brake/tire wear – and from combustion – tail pipe emissions) (16%), secondary sulfate (12%), road dust (9%) and residual (9%) (Fang et al. 2016). A study in São Paulo (Brazil) identified four major sources contributing to OP_V^{DTT} by using a PMF-MLR approach, namely biomass burning ($40.7 \pm 26.3\%$), followed by industrial emissions ($24.3 \pm 22.5\%$), vehicle emissions ($20.5 \pm 15.2\%$), and secondary aerosol ($14.5 \pm 15.0\%$) (Serafeim et al. 2023).

Considerations

The present study provides the first insights into the OP of fine aerosols in Portugal, using a limited number of samples (30) and focusing only on samples with the higher contributions from the different assessed pollution sources (by higher mass and %). Despite the lower number of samples, it was still possible to identify three pollution sources that significantly influence the OP levels of $PM_{2.5}$. Future work will aim to assess the OP of the remaining samples (total dataset of 128 $PM_{2.5}$ filters, corresponding to a one year of sampling done between December 2019 and November 2020) to confirm the influence of the sources identified in this study on OP levels of the fine aerosols in the study area. Moreover, the evaluation of the complete dataset (corresponding to one year of sampling) will also allow to assess the seasonal variability of the OP and to provide a more complete characterisation of the OP of the fine aerosols of this Portuguese urban-industrial area.

It is important to highlight the relevant role of wood burning as one of the main sources contributing to the oxidative potential of aerosols, as shown by the various studies cited above and others (Borlaza et al. 2021). Naturally, the nature of the sources affecting a particular region may have different origins, from local to regional sources. The previous source apportionment study (Gamelas et al. 2023) carried out on the $PM_{2.5}$ samples analysed in this study did not identify wood burning as a source in the study area. However, specific tracers of this source were not evaluated during the PM characterisation (as levoglucosan) (Calvo et al. 2013), and this fact may have contributed to the non-identification of this source for the PM levels in the study area. Considering the known impact of wood burning to the OP levels of aerosols, future studies in the study area should include the evaluation of tracers of this source to assess whether its contribution to the OP levels is significant or not.

Conclusions

To the best of our knowledge, this is the first study providing information on the OP levels of fine aerosols in Portugal. The values of OP_V^{DTT} in the studied urban-industrial area (with a mean of 209 ± 174 pmol min⁻¹ m⁻³) were in agreement with others evaluated in European countries, India and the USA, and lower than those registered in some Asian countries, Brazil and some European countries (namely, Spain and Switzerland, where the extraction method of $PM_{2.5}$ used was slightly different).

OP_V^{DTT} was found to be significantly correlated with $PM_{2.5}$ levels. Furthermore, based on the information on the pollution sources contributing to the $PM_{2.5}$ levels, it was possible to identify three pollution sources that had a significant influence on the OP of these fine aerosols, namely, fuel-oil combustion, vehicle exhaust and industry. Using an MLR approach, it was found that these three sources contributed to 82% of the OP_V^{DTT} of the studied fine aerosols. This information is useful to define targeted mitigation measures to tackle these sources of pollution, in order to minimise the health impacts due to their exposure.

Future work should aim at evaluating of the seasonal OP variability in the studied area and in other types of environments in Portugal, in order to strengthen the information on this indicator of the health potential of aerosols. In addition, special efforts should be made to understand which are the drivers of OP and the influence of the different pollution sources.

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Author contributions Nuno Canha – conceptualization, funding, methodology, OP analysis, data curation, formal analysis, writing – original draft, review and editing. Sara Gonçalves – methodology, OP analysis, formal analysis. Diogo Sousa – literature review. Carla Gamelas – PM sampling, PMF for source apportionment, data curation, review and editing. Sergio Mendez - literature review, methodology. Sandra Cabo Verde – conceptualization, methodology, OP analysis. Susana Marta Almeida – conceptualization, PMF for source apportionment, data curation. Anna Rita de Bartolomeo - methodology, OP analysis. Maria Rachele Guascito - methodology, OP analysis, data curation, review and editing. Eva Merico – methodology, data curation. Daniele Contini - methodology, OP analysis, data curation, review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets used or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval and consent to participate Not applicable.

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