Environmental Science & Technology Letters

pubs.acs.org/journal/estlcu

Letter

On the Relative Contribution of Iron and Organic Compounds, and Their Interaction in Cellular Oxidative Potential of Ambient $PM_{2.5}$

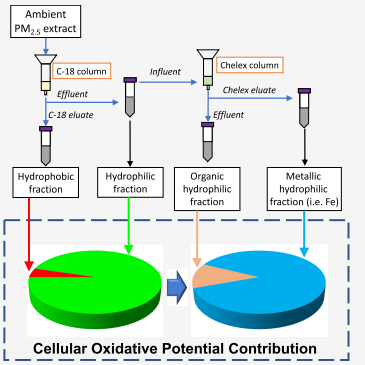
Yixiang Wang, Sudheer Salana, Haoran Yu, Joseph V. Puthussery, and Vishal Verma*

Cite This: *Environ. Sci. Technol. Lett.* 2022, 9, 680−686

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Previous studies have indicated the roles of both organic compounds and metals in driving the cellular generation of reactive oxygen species (ROS); however, their contribution has not been adequately quantified using mechanistic approaches. We developed a novel fractionation scheme for the stepwise removal of various classes of organic compounds and metals using a combination of solid phase extraction columns. We applied this fractionation scheme to 10 $PM_{2.5}$ samples collected from the midwestern United States. Because both water-soluble organic carbon (WSOC) and Fe have shown good correlations with cellular ROS, we separated them into different fractions and measured their ability to generate ROS in rat alveolar macrophages. Most of the $PM_{2.5}$ cellular ROS was attributed to the metallic fraction. To further explore the reason for the correlation of WSOC with ROS, we investigated the water solubility of Fe by measuring the total Fe in $PM_{2.5}$ samples. The water-soluble fraction of Fe was tightly correlated with WSOC ($r \geq 0.69$), indicating WSOC may have an additional role in cellular oxidative potential, probably through complexation of Fe, in enhancing its water solubility and macrophage ROS activity. This work reveals the role of both Fe and organic compounds through different mechanisms in contributing to $PM_{2.5}$-driven cellular ROS.



KEYWORDS: *cellular oxidative potential, water-soluble organic compounds, water-soluble iron, macrophage ROS, complexation, solid phase extraction, chemical fractionation*

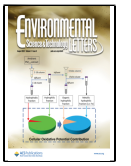
INTRODUCTION

Oxidative potential (OP) denotes the ability of fine ambient particulate matter (PM with an aerodynamic size of <2.5 μm or $PM_{2.5}$) to cause an imbalance of reactive oxygen species (ROS) in biological systems. OP could be an important factor in the $PM_{2.5}$ toxicology ladder given its reported association with several cellular end points such as mitochondrial damage,[1] inflammatory response,[2] mitophagy,[3] lipid peroxidation,[4] and autophagy.[5] Thus, understanding the contribution of $PM_{2.5}$ components to OP should be helpful in explaining their roles in $PM_{2.5}$ toxicity.

There are both chemical[6−11] and cellular[5,12,13] assays for measuring $PM_{2.5}$ OP. One of the most widely used approaches for measuring cellular OP is the macrophage ROS assay, based on a rat alveolar macrophage cell line.[14−17] Studies using the samples from various sites such as Los Angeles, CA, Riverside, CA, Seoul, Korea, Tehran, Iran, and Baghdad, Iraq, have indicated that the cellular $PM_{2.5}$ OP assessed by macrophage ROS assay is related to a few metals (i.e., Ba,[18] Cd,[15,19,20] Co,[21] Cr,[18,21,22] Cu,[15,22,23] Fe,[18,21,22,24] Mn,[20,21] Ni,[15,24] Pb,[18] V,[15,18,19,23,24] Zn,[15,22] etc.), organic carbon (OC),[24,25] and water-soluble organic carbon (WSOC).[22,25] In our previous study,[26] $PM_{2.5}$ samples collected from five midwestern U.S. sampling sites (Chicago, Champaign, and Bondville in Illinois, St. Louis in Missouri, and Indianapolis in Indiana) were analyzed to study the relationship among chemical composition, their sources, and $PM_{2.5}$ cellular OP. Interestingly, we found that among several measured organic and inorganic components, only Fe and WSOC were strongly and consistently correlated with water-soluble $PM_{2.5}$ cellular OP throughout all locations and seasons (Table S1, reproduced from ref 26). However, because both Fe and WSOC were also highly correlated to each other, it is difficult to infer if both of these species contribute to the OP and, if so, to what extent. Therefore, mechanistic techniques need to be adopted to segregate the individual role of metals and organic compounds in $PM_{2.5}$ cellular OP.

Previous mechanistic studies involving the macrophage ROS assay are limited, and they have largely relied on using a technique for removing a fraction of either component (i.e., the metallic or organic fraction). The main concern in these studies is that the authors have not adequately assessed if that technique explicitly removes the targeted fraction without affecting other components. For example, the role of metals in cellular OP has been assessed through chelation of water-

Received: May 15, 2022
Revised: July 13, 2022
Accepted: July 14, 2022
Published: July 18, 2022



https://doi.org/10.1021/acs.estlett.2c00316
Environ. Sci. Technol. Lett. 2022, 9, 680−686

soluble PM extracts using Chelex resin (to remove all metals)[21] and deferoxamine (DFOA, to specifically bind Fe).[20] Although these studies have indicated a substantial contribution of metals to cellular OP (70−97%), the effect of Chelex and DFOA treatment on the organic compounds was not reported in either of the studies. On the contrary, the role of organic compounds in cellular OP has been emphasized by Pirhadi et al.,[27] who removed both WSOC (∼80%) and macrophage ROS activity (∼72%) by heating the aerosols to 100 °C. Although the authors reported that heating did not affect the concentration of metals, its effect on other chemical properties such as oxidation states and the solubility of the metals, which are important for cellular OP, were not studied. We hypothesize that the contradicting results on the dominant role of metals[20,21] versus organic compounds,[27] as obtained from these studies, arise from the limitations of their experimental design, i.e., incomplete segregation of organic compounds and metals.

The main objective of this study is to segregate and quantify the role of metals (particularly Fe) and WSOC in water-soluble cellular OP measured by the macrophage ROS assay. Recognizing the limitations of the past studies, we have developed a mechanistic scheme based on a combination of solid phase extraction (SPE) columns, which can effectively segregate the water-soluble $PM_{2.5}$ into recoverable metallic and organic subfractions. Finally, through a combination of statistical and mechanistic approaches, we showed that both organic compounds and metals play an important role in determining the cellular OP of the ambient $PM_{2.5}$.

MATERIALS AND METHODS

Sampling. Integrated $PM_{2.5}$ samples (72 h continuously from Tuesday 0:00 to Friday 0:00) were collected in parallel by the high-volume samplers (Thermo Andersen, flow rate of 1.13 m^3 min^{-1}) installed at five different locations: Chicago, IL (CHI), St. Louis, MO (STL), Indianapolis, IN (IND), Champaign, IL (CMP), and Bondville, IL (BON). A detailed description of these sites is provided in our previous publications.[10,28] The sampling schedule (Table S2) is provided in the Supporting Information. A total of 241 samples were collected from May 22, 2018, to May 30, 2019. However, for the purpose of this study, we chose only a few samples for various segregation experiments, specifically, two samples from each site (one collected during the summer and another during the winter); thus, a total of 10 samples were used for complete $PM_{2.5}$ component segregation, and 51 samples collected from various sites, i.e., BON (N = 11), CHI (N = 10), CMP (N = 8), IND (N = 11), and STL (N = 11), were selected to assess the solubility of Fe and its association with WSOC (see Table S2).

Extraction of $PM_{2.5}$ Filters and Preparation of SPE Columns. The $PM_{2.5}$ water-soluble extracts were obtained by cutting a circular section [∼32 cm^2; corresponding to 1/12th of a whole high-volume filter (8 in. × 10 in. in size)] of the filter, immersing it in 20 mL of deionized water (DI), and sonicating it for 1 h. The potential impact of sonication on the alteration of the chemical composition and ROS generation is discussed in Section S1 of the Supporting Information. After sonication, the water-insoluble fractions such as fiber debris were removed by the 0.45 μm syringe filter. The C-18 column and Chelex columns were prepared separately by packing ∼2 g of C-18 and Chelex 100 resins, respectively, into polypropylene gravity flow chromatographic columns (Econo-Pac from Bio-Rad). The C-18 column was preconditioned by first rinsing the resin with 10 mL of methanol, followed by 10 mL of DI. Methanol was used to activate the column. Because Chelex 100 resin is basic, it was pretreated with DI (∼1 L) until the pH of the effluent decreased to 7−8.

$PM_{2.5}$ Fractionation Scheme. First, to assess if the commercial chelation agents affect the relevant organic compounds (i.e., compounds with known redox activity) present in the ambient $PM_{2.5}$, 9,10-phenanthrenequinone (PQN, a model compound for ROS-active hydrophobic organic substances) was used. We treated the PQN solution with both Chelex and DFOA separately to assess if these treatments may also affect the hydrophobic organic compounds present in ambient $PM_{2.5}$. Although it was not expected, both of the chelating agents removed the cellular OP of PQN (Figure S1), showing the inadequacy of the previous approaches[20,21] based on a chelation-only technique for segregating the contribution of organic compounds and metals to ROS generation and thus justifying the need for our segregation scheme as discussed below.

Water-soluble organic and metallic fractions of $PM_{2.5}$ were separated by a two-step segregation procedure. $PM_{2.5}$ water extracts of 10 samples (18 mL each) were first passed through the C-18 column. The hydrophilic part was collected as the effluent, while the hydrophobic part was retained on the C-18 resin and eluted in methanol (10 mL). The retained fraction contains mostly hydrophobic organic compounds, also called humic-like substances (HULIS), while the passed-through fraction has hydrophilic organic compounds and metals. Methanol-eluted HULIS were evaporated by N_2 gas to almost dryness (i.e., ∼20 μL), after which they were reconstituted in DI to obtain the "water-soluble hydrophobic fraction". Part of the water-soluble hydrophilic effluent (12 mL) was passed through a Chelex column. Metals in the water-soluble hydrophilic fraction are retained on Chelex resin. Twenty milliliters of DI was passed through the Chelex column to wash away any WSOC residue left in the column. After this rinsing, metals in the column were eluted by passage of 10 mL of 1 N hydrochloric acid (HCl). Then, 9.5 mL of 1 N NaOH was added to the HCl eluate to increase the pH to ≥2. This solution was called the "water-soluble metallic hydrophilic fraction", and the metal-free effluent, which directly passed through the Chelex column, was called the "water-soluble organic hydrophilic fraction". The schematic of our fractionation technique is illustrated in Figure S2. The negative control for each fraction and/or sample was collected by treating the DI in the same way as the $PM_{2.5}$ extract and analyzing it via the macrophage ROS assay. The results of all of these negative controls are shown in Figure S3.

Because during the elution from the Chelex column, Fe(II) is oxidized to Fe(III) (see Figure S4), which has very low activity in the macrophage ROS assay,[29] ascorbate was used to recover Fe(II) in the metallic hydrophilic fraction. Then, 188 μL of ascorbate [concentration varying on the basis of the sample (see Figure S5 for more details about choosing the concentration of ascorbate)] mixed with 642 μL of 3 mg/L dithiothreitol (DTT, to prevent the consumption of ascorbate by Cu) was added to 1100 μL of the metallic hydrophilic fraction, and the mixture was diluted by DI to 5000 μL. The mixture was left at room temperature for 30 min to convert Fe(III) to Fe(II). The concentrations of ascorbate and DTT in the metallic hydrophilic fractions measured after 30 min were <50 and <90 μg/L, respectively, in the PM samples. The

https://doi.org/10.1021/acs.estlett.2c00316
Environ. Sci. Technol. Lett. 2022, 9, 680−686

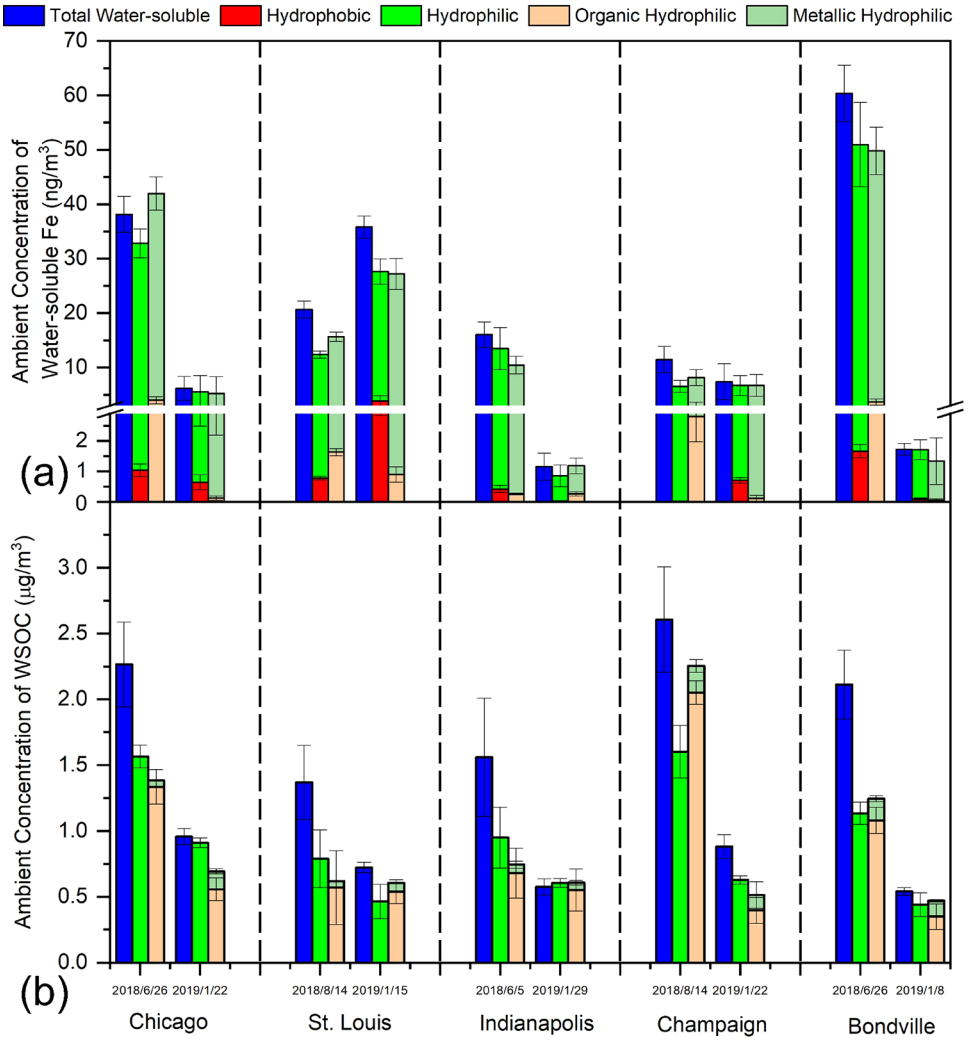


Figure 1. Distribution of (a) Fe and (b) WSOC in different water-soluble $PM_{2.5}$ fractions, i.e., hydrophobic (retained on the C-18 column), hydrophilic (passed-through C-18 column), metallic hydrophilic (part of the hydrophilic fraction retained on the Chelex column), and organic hydrophilic (part of the hydrophilic fraction passed through the Chelex column). Due to the presence of a trace of methanol, the hydrophobic fraction was not analyzed for WSOC content.

metallic hydrophilic fraction was then neutralized by NaOH to pH 7.4. This neutralization step increased the salinity of this fraction; therefore, it was again diluted with DI to keep the sodium chloride (NaCl) concentration below 9 g/L. Experiments were conducted to ensure that the residual concentrations of DTT, ascorbate, and NaCl did not have any significant impact or interaction with PM components for affecting the cellular OP. Details are presented in Section S2 of the Supporting Information (Figure S6).

Macrophage ROS Assay. The cellular OP of different $PM_{2.5}$ fractions was measured by the macrophage ROS assay, which is conducted on rat macrophage cell line NR 8383. A detailed description of this assay is provided in our previous publication[26] and also in Section S3 of the Supporting Information. Briefly, cells, DCFH-DA, and the sample were incubated at 37 °C and 5% CO_2 for 2.5 h, after which a small aliquot of the mixture was withdrawn and measured for its fluorescence at 488 nm excitation and 530 nm emission wavelengths. Cellular OPs of the samples were expressed as the percentage of the cellular ROS increase normalized by their respective negative controls, as discussed above. Experiments were conducted to confirm that the conversion of DCFH to DCF was mostly by the cellular ROS, and not from the direct oxidation by metals (Section S4 of the Supporting Information and Figure S7).

Chemical Analysis. We measured the concentrations of WSOC and water-soluble Fe in different fractions of the PM samples. In addition to water-soluble Fe, water-soluble Fe(II) was measured in the hydrophilic and metallic hydrophilic fractions to determine the recovery Fe(II) from the Chelex column. Both water-soluble Fe(II) and water-soluble Fe [i.e., Fe(II) and Fe(III)] were measured using the ferrozine method, as described in our previous study.[30,31] A brief description of the Fe and WSOC measurement protocol, as adapted from our previous publication,[32] is provided in section S5 of the Supporting Information.

In addition to the analysis of segregated PM fractions, 51 ambient samples (see Table S2) were analyzed to assess the water solubility of Fe (expressed as water-soluble Fe divided by total Fe) and its relationship with WSOC content. Water-soluble Fe on these samples was measured using a NexION 300X inductively coupled plasma mass spectrometer (ICP-MS; PerkinElmer, Waltham, MA).[26] The total Fe was measured with a Shimadzu EDX-7000 energy-dispersive X-ray fluo-

https://doi.org/10.1021/acs.estlett.2c00316
Environ. Sci. Technol. Lett. 2022, 9, 680−686

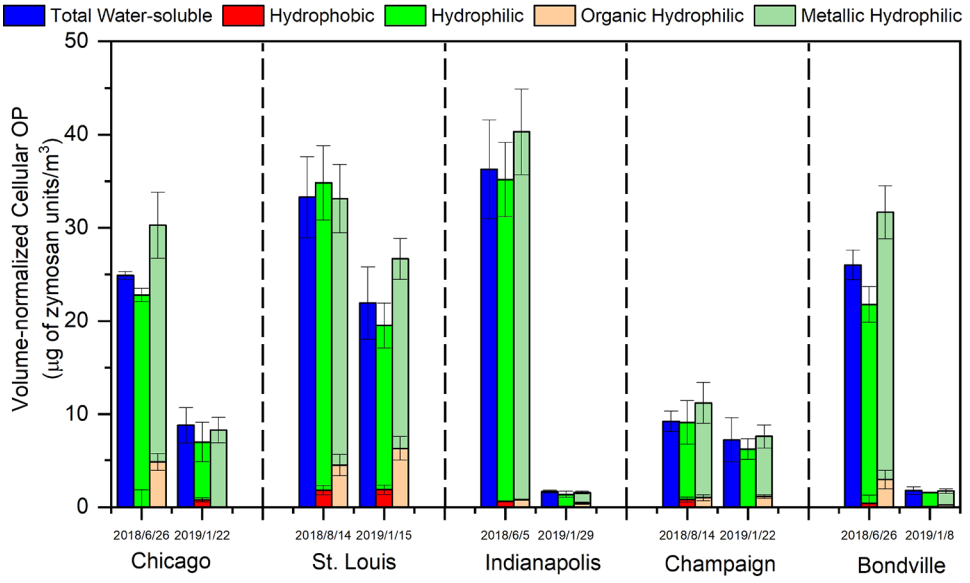


Figure 2. Distribution of cellular OP in different water-soluble $PM_{2.5}$ fractions, i.e., hydrophobic (retained on the C-18 column), hydrophilic (passed through the C-18 column), metallic hydrophilic (part of the hydrophilic fraction retained on the Chelex column), and organic hydrophilic (part of the hydrophilic fraction passed through the Chelex column).

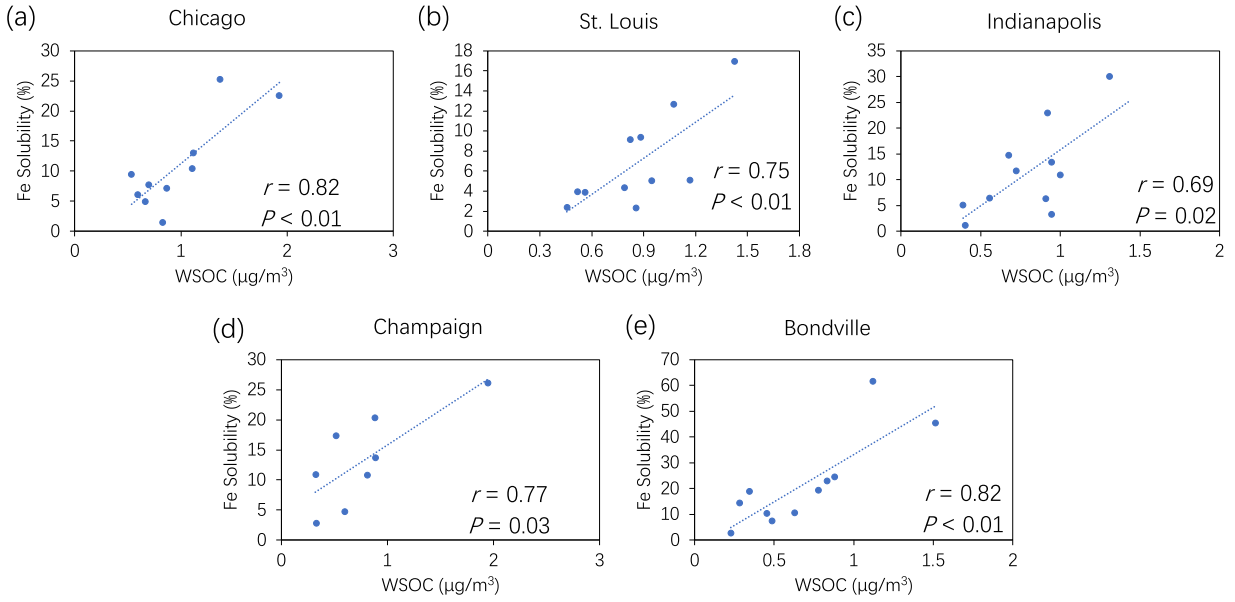


Figure 3. Correlations of WSOC with the water-soluble fraction of Fe in the $PM_{2.5}$ samples collected from various sites, i.e., (a) Chicago, (b) St. Louis, (c) Indianapolis, (d) Champaign, and (e) Bondville.

rescence spectrometer (Shimadzu Co.). A circular section with a diameter of 16 mm was punched from the filter and loaded into a small polyethylene cup, which used an ultralean film at the bottom. The instrument yielded the results in units of Fe mass per unit area (micrograms per square centimeter).

RESULTS AND DISCUSSION

Assessing the Effectiveness of Our Fractionation Scheme. Panels a and b of Figure 1 show the concentration of Fe and WSOC in various fractions obtained by our $PM_{2.5}$ segregation scheme. Consistent with previous studies,[31,33,34] the C-18 column is capable of separating the metallic fraction from HULIS. Fe in the hydrophilic fraction was almost the same as in the original $PM_{2.5}$ extract. Moreover, the Chelex column does not retain any organic compound present in the hydrophilic fraction. This difference in the results compared to our previous results showing the retention of quinones on the Chelex column (Figure S1) is due to the first step of our segregation scheme, i.e., removal of hydrophobic organic compounds from the $PM_{2.5}$ extract using the C-18 column. Finally, the Chelex column is highly efficient in retaining Fe (almost negligible Fe in the passed-through fraction of the Chelex column), which is effectively recovered in the eluent (i.e., HCl). Thus, by using this scheme, we can achieve a nearly complete segregation of organic compounds and metals from the water-soluble $PM_{2.5}$ matrix.

Macrophage ROS Activity of Fractionated PM Components. The results of the macrophage ROS assay conducted on different PM fractions obtained by our segregation scheme are shown in Figure 2. The hydrophobic

https://doi.org/10.1021/acs.estlett.2c00316
Environ. Sci. Technol. Lett. 2022, 9, 680−686

fraction of water-soluble $PM_{2.5}$ shows very little activity in the macrophage ROS assay (average ± 1σ = 4.2 ± 3.3% of the water-soluble $PM_{2.5}$ cellular OP). In contrast, the hydrophilic fraction was strongly active in the macrophage assay, explaining on average 85.8 ± 8.2% of the water-soluble cellular OP. Note that the hydrophilic fraction contains not only the metals but also the hydrophilic organic compounds. Interestingly, the chelated hydrophilic fraction (i.e., hydrophilic organic compounds) accounted for only 16.3 ± 9.9% of the OP of the hydrophilic fraction, while the rest of it was attributed to the metallic hydrophilic fraction, which was eluted in HCl.

Collectively, these results, i.e., low activity of the hydrophobic and organic hydrophilic fraction and substantial activity of the metallic hydrophilic fraction, show that it is mostly the metals (particularly Fe in our case given the strong correlation of Fe with OP), which drives the macrophage ROS response.

Effect Modification of WSOC on Fe. Although our experimental design described above could show a dominant effect of metals on the water-soluble $PM_{2.5}$ macrophage ROS activity, it could not explain the strong correlation of WSOC with OP (Table S1). There could be two possible explanations. (1) WSOC could synergistically interact with Fe to enhance its macrophage ROS activity, and (2) WSOC enhances the solubility of Fe through complexation, making it available for macrophage ROS generation. Although there are studies showing the interaction of Fe with HULIS in the acellular[34−36] and cellular assays,[37,38] the results shown in Figure 2, i.e., the close agreement of the sum of the OPs from hydrophobic and hydrophilic fractions with the total activity of the original $PM_{2.5}$ water-soluble extract, do not support that.

Several studies have shown the effect of complexation of organic compounds such as humic acid, tartrate, malonate, and oxalate on the solubility of Fe.[39−44] Therefore, finally, we tested the effect of WSOC on Fe solubility, by measuring both water-soluble and total Fe in 51 ambient $PM_{2.5}$ samples collected from all five sites. Figure 3 shows the plot of Fe solubility versus WSOC concentration at different sites. Interestingly, there was a significant ($P < 0.05$) and tight correlation between WSOC and the soluble fraction of Fe at all five sites, with r varying from 0.69 to 0.82. These results are consistent with previous atmospheric studies generally showing a strong correlation of Fe solubility with WSOC.[43−46] It suggests that the correlation of WSOC with water-soluble $PM_{2.5}$ macrophage ROS activity is not coincidental but an effect modification. WSOC through complexation makes Fe more water-soluble, which plays an active role in the water-soluble $PM_{2.5}$ cellular OP.

The novel fractionation scheme based on a combination of C-18 and Chelex techniques developed in our study not only segregated the water-soluble $PM_{2.5}$ into metallic and organic subfractions but also recovered the metallic fraction without affecting its activity. For the first time, we were able to effectively decouple the effect of organic and metallic fractions of ambient $PM_{2.5}$ in the cellular OP. Our study reveals different roles of two important aerosol components and their interaction through effect modification on altering the cellular OP of ambient $PM_{2.5}$. However, we note that our results were obtained from a single cellular OP assay, and the relative contributions of metallic and organic fractions could vary substantially based on different OP metrics. Moreover, only water-soluble components were targeted in our study, although water-insoluble components could also play an important role in $PM_{2.5}$ OP and toxicity. Nevertheless, the segregation technique developed in our study could be used in future toxicological studies to comprehensively understand the contribution of water-soluble metallic and organic fractions in the cytotoxicity of $PM_{2.5}$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.2c00316.

Correlation of $PM_{2.5}$ components with cellular OP, sampling information, segregation scheme, cellular OP of various negative controls, possible effect of sonication, recovery of Fe(II) by ascorbate, impact of added impurities on $PM_{2.5}$ cellular OP, experimental protocols of the macrophage ROS assay, effect of direct oxidation of DCFH from metals, and water-soluble Fe(II), Fe(III), and WSOC measurements (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Vishal Verma − *Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States;* orcid.org/0000-0001-8415-9454; Email: vverma@illinois.edu

Authors

Yixiang Wang − *School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, China;* orcid.org/0000-0003-0530-8664

Sudheer Salana − *Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States*

Haoran Yu − *Air Quality Research Center, University of California, Davis, Davis, California 95616, United States*

Joseph V. Puthussery − *Department of Energy, Environmental, and Chemical Engineering, Washington University in St. Louis, St. Louis, Missouri 63130-4899, United States;* orcid.org/0000-0002-0185-1187

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.estlett.2c00316

Author Contributions

Y.W.: conceptualization, methodology, validation, formal analysis, investigation, resources, writing of the original draft, and visualization. S.S.: methodology, validation, investigation, and reviewing. H.Y.: investigation and reviewing. J.V.P.: investigation and reviewing. V.V.: conceptualization, resources, review and editing, supervision, project administration, and funding acquisition.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Science Foundation under Grant CBET-1847237. The total Fe measurement by XRF was carried out in the Illinois Materials Research Laboratory at the University of Illinois Urbana-Champaign. The authors appreciate Dr. Gregory Macdougall for training us to use XRF. The authors also thank Dr. Martin Shafer and Dr. Dagmara Antkiewicz from the University of Wisconsin−

Madison (Madison, WI) for providing us the training on the macrophage ROS assay.

REFERENCES

(1) Jin, X. T.; Xue, B.; Zhou, Q. F.; Su, R. J.; Li, Z. Y. Mitochondrial damage mediated by ROS incurs bronchial epithelial cell apoptosis upon ambient PM2.5 exposure. *J. Toxicol Sci.* **2018**, *43* (1−3), 101−111.

(2) Das, A.; Habib, G.; Vivekanandan, P.; Kumar, A. Reactive oxygen species production and inflammatory effects of ambient PM2.5-associated metals on human lung epithelial A549 cells "one year-long study": The Delhi chapter. *Chemosphere* **2021**, *262*, 128305.

(3) Qiu, Y. N.; Wang, G. H.; Zhou, F.; Hao, J. J.; Tian, L.; Guan, L. F.; Geng, X. K.; Ding, Y. C.; Wu, H. W.; Zhang, K. Z. PM2.5 induces liver fibrosis via triggering ROS-mediated mitophagy. *Ecotox Environ. Safe* **2019**, *167*, 178−187.

(4) Wang, Y.; Tang, M. PM2.5 induces ferroptosis in human endothelial cells through iron overload and redox imbalance. *Environ. Pollut.* **2019**, *254*, 112937.

(5) Deng, X. B.; Zhang, F.; Rui, W.; Long, F.; Wang, L. J.; Feng, Z. H.; Chen, D. L.; Ding, W. J. PM2.5-induced oxidative stress triggers autophagy in human lung epithelial A549 cells. *Toxicol in Vitro* **2013**, *27* (6), 1762−1770.

(6) Kumagai, Y.; Koide, S.; Taguchi, K.; Endo, A.; Nakai, Y.; Yoshikawa, T.; Shimojo, N. Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles. *Chem. Res. Toxicol.* **2002**, *15* (4), 483−489.

(7) Vidrio, E.; Phuah, C. H.; Dillner, A. M.; Anastasio, C. Generation of Hydroxyl Radicals from Ambient Fine Particles in a Surrogate Lung Fluid Solution. *Environ. Sci. Technol.* **2009**, *43* (3), 922−927.

(8) Charrier, J. G.; McFall, A. S.; Richards-Henderson, N. K.; Anastasio, C. Hydrogen Peroxide Formation in a Surrogate Lung Fluid by Transition Metals and Quinones Present in Particulate Matter. *Environ. Sci. Technol.* **2014**, *48* (12), 7010−7017.

(9) Mudway, I. S.; Duggan, S. T.; Venkataraman, C.; Habib, G.; Kelly, F. J.; Grigg, J. Combustion of dried animal dung as biofuel results in the generation of highly redox active fine particulates. *Part. Fibre Toxicol.* **2005**, *2* (1), 6.

(10) Yu, H. R.; Puthussery, J. V.; Verma, V. A semi-automated multi-endpoint reactive oxygen species activity analyzer (SAMERA) for measuring the oxidative potential of ambient PM2.5 aqueous extracts. *Aerosol Sci. Technol.* **2020**, *54* (3), 304−320.

(11) Puthussery, J. V.; Singh, A.; Rai, P.; Bhattu, D.; Kumar, V.; Vats, P.; Furger, M.; Rastogi, N.; Slowik, J. G.; Ganguly, D.; Prevot, A. S. H.; Tripathi, S. N.; Verma, V. Real-Time Measurements of PM2.5 Oxidative Potential Using a Dithiothreitol Assay in Delhi, India. *Environ. Sci. Tech Let* **2020**, *7* (7), 504−510.

(12) Landreman, A. P.; Shafer, M. M.; Hemming, J. C.; Hannigan, M. P.; Schauer, J. J. A macrophage-based method for the assessment of the reactive oxygen species (ROS) activity of atmospheric particulate matter (PM) and application to routine (daily-24 h) aerosol monitoring studies. *Aerosol Sci. Technol.* **2008**, *42* (11), 946−957.

(13) Longhin, E.; Holme, J. A.; Gutzkow, K. B.; Arlt, V. M.; Kucab, J. E.; Camatini, M.; Gualtieri, M. Cell cycle alterations induced by urban PM2.5 in bronchial epithelial cells: characterization of the process and possible mechanisms involved. *Part. Fibre Toxicol.* **2013**, *10*, 63.

(14) Park, J.; Park, E. H.; Schauer, J. J.; Yi, S. M.; Heo, J. Reactive oxygen species (ROS) activity of ambient fine particles (PM2.5) measured in Seoul. *Korea. Environ. Int.* **2018**, *117*, 276−283.

(15) Al Hanai, A. H.; Antkiewicz, D. S.; Hemming, J. D. C.; Shafer, M. M.; Lai, A. M.; Arhami, M.; Hosseini, V.; Schauer, J. J. Seasonal variations in the oxidative stress and inflammatory potential of PM2.5 in Tehran using an alveolar macrophage model; The role of chemical composition and sources. *Environ. Int.* **2019**, *123*, 417−427.

(16) Taghvaee, S.; Sowlat, M. H.; Diapouli, E.; Manousakas, M. I.; Vasilatou, V.; Eleftheriadis, K.; Sioutas, C. Source apportionment of the oxidative potential of fine ambient particulate matter (PM2.5) in Athens, Greece. *Sci. Total Environ.* **2019**, *653*, 1407−1416.

(17) Wang, D. B.; Pakbin, P.; Shafer, M. M.; Antkiewicz, D.; Schauer, J. J.; Sioutas, C. Macrophage reactive oxygen species activity of water-soluble and water-insoluble fractions of ambient coarse, PM2.5 and ultrafine particulate matter (PM) in Los Angeles. *Atmos. Environ.* **2013**, *77*, 301−310.

(18) Verma, V.; Polidori, A.; Schauer, J. J.; Shafer, M. M.; Cassee, F. R.; Sioutas, C. Physicochemical and Toxicological Profiles of Particulate Matter in Los Angeles during the October 2007 Southern California Wildfires. *Environ. Sci. Technol.* **2009**, *43* (3), 954−960.

(19) Verma, V.; Ning, Z.; Cho, A. K.; Schauer, J. J.; Shafer, M. M.; Sioutas, C. Redox activity of urban quasi-ultrafine particles from primary and secondary sources. *Atmos. Environ.* **2009**, *43* (40), 6360−6368.

(20) Shafer, M. M.; Perkins, D. A.; Antkiewicz, D. S.; Stone, E. A.; Quraishi, T. A.; Schauer, J. J. Reactive oxygen species activity and chemical speciation of size-fractionated atmospheric particulate matter from Lahore, Pakistan: an important role for transition metals. *J. Environ. Monitor* **2010**, *12* (3), 704−715.

(21) Verma, V.; Shafer, M. M.; Schauer, J. J.; Sioutas, C. Contribution of transition metals in the reactive oxygen species activity of PM emissions from retrofitted heavy-duty vehicles. *Atmos. Environ.* **2010**, *44* (39), 5165−5173.

(22) Daher, N.; Ruprecht, A.; Invernizzi, G.; De Marco, C.; Miller-Schulze, J.; Heo, J. B.; Shafer, M. M.; Shelton, B. R.; Schauer, J. J.; Sioutas, C. Characterization, sources and redox activity of fine and coarse particulate matter in Milan, Italy. *Atmos. Environ.* **2012**, *49*, 130−141.

(23) Cheung, K.; Shafer, M. M.; Schauer, J. J.; Sioutas, C. Diurnal Trends in Oxidative Potential of Coarse Particulate Matter in the Los Angeles Basin and Their Relation to Sources and Chemical Composition. *Environ. Sci. Technol.* **2012**, *46* (7), 3779−3787.

(24) Hamad, S. H.; Schauer, J. J.; Antkiewicz, D. S.; Shafer, M. M.; Kadhim, A. K. H. ROS production and gene expression in alveolar macrophages exposed to PM2.5 from Baghdad, Iraq: Seasonal trends and impact of chemical composition. *Sci. Total Environ.* **2016**, *543*, 739−745.

(25) Delfino, R. J.; Staimer, N.; Tjoa, T.; Gillen, D. L.; Schauer, J. J.; Shafer, M. M. Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel. *J. Expo Sci. Env Epid* **2013**, *23* (5), 466−473.

(26) Wang, Y.; Puthussery, J. V.; Yu, H.; Liu, Y.; Salana, S.; Verma, V. Sources of Cellular Oxidative Potential of Water-soluble Fine Ambient Particulate Matter in the Midwestern United States. *J. Hazard Mater.* **2021**, *425*, 127777.

(27) Pirhadi, M.; Mousavi, A.; Taghvaee, S.; Shafer, M. M.; Sioutas, C. Semi-volatile components of PM2.5 in an urban environment: Volatility profiles and associated oxidative potential. *Atmos. Environ.* **2020**, *223*, 117197.

(28) Yu, H.; Puthussery, J. V.; Wang, Y.; Verma, V. Spatiotemporal Variability in the Oxidative Potential of Ambient Fine Particulate Matter in Midwestern United States. *Atmos. Chem. Phys.* **2021**, *21*, 16363−16386.

(29) Salana, S.; Wang, Y.; Puthussery, J.; Verma, V. A Semi-automated Instrument for Cellular Oxidative Potential Evaluation (SCOPE) of Water-soluble Extracts of Ambient Particulate Matter. *Atmos. Meas. Tech.* **2021**, *14*, 7579−7593.

(30) Majestic, B. J.; Schauer, J. J.; Shafer, M. M.; Turner, J. R.; Fine, P. M.; Singh, M.; Sioutas, C. Development of a wet-chemical method for the speciation of iron in atmospheric aerosols. *Environ. Sci. Technol.* **2006**, *40* (7), 2346−2351.

(31) Wei, J. L.; Yu, H. R.; Wang, Y. X.; Verma, V. Complexation of Iron and Copper in Ambient Particulate Matter and Its Effect on the Oxidative Potential Measured in a Surrogate Lung Fluid. *Environ. Sci. Technol.* **2019**, *53* (3), 1661−1671.

(32) Wang, Y.; Plewa, M. J.; Mukherjee, U. K.; Verma, V. Assessing the cytotoxicity of ambient particulate matter (PM) using Chinese hamster ovary (CHO) cells and its relationship with the PM chemical

https://doi.org/10.1021/acs.estlett.2c00316
Environ. Sci. Technol. Lett. 2022, 9, 680−686

composition and oxidative potential. *Atmos. Environ.* **2018**, *179*, 132−141.
(33) Verma, V.; Rico-Martinez, R.; Kotra, N.; King, L.; Liu, J. M.; Snell, T. W.; Weber, R. J. Contribution of Water-Soluble and Insoluble Components and Their Hydrophobic/Hydrophilic Subfractions to the Reactive Oxygen Species-Generating Potential of Fine Ambient Aerosols. *Environ. Sci. Technol.* **2012**, *46* (20), 11384−11392.
(34) Yu, H. R.; Wei, J. L.; Cheng, Y. L.; Subedi, K.; Verma, V. Synergistic and Antagonistic Interactions among the Particulate Matter Components in Generating Reactive Oxygen Species Based on the Dithiothreitol Assay. *Environ. Sci. Technol.* **2018**, *52* (4), 2261−2270.
(35) Gonzalez, D. H.; Cala, C. K.; Peng, Q. Y.; Paulson, S. E. HULIS Enhancement of Hydroxyl Radical Formation from Fe(II): Kinetics of Fulvic Acid-Fe(II) Complexes in the Presence of Lung Antioxidants. *Environ. Sci. Technol.* **2017**, *51* (13), 7676−7685.
(36) Lin, M.; Yu, J. Z. Assessment of interactions between transition metals and atmospheric organics: Ascorbic Acid Depletion and Hydroxyl Radical Formation in Organic-metal Mixtures. *Environ. Sci. Technol.* **2020**, *54* (3), 1431−1442.
(37) Wang, Y.; Puthussery, J. V.; Yu, H.; Verma, V. Synergistic and antagonistic interactions among organic and metallic components of the ambient particulate matter (PM) for the cytotoxicity measured by Chinese hamster ovary cells. *Sci. Total Environ.* **2020**, *736*, 139511.
(38) Lu, S. L.; Win, M. S.; Zeng, J. Y.; Yao, C. H.; Zhao, M. F.; Xiu, G. L.; Lin, Y. C.; Xie, T. T.; Dai, Y. F.; Rao, L. F.; Zhang, L. Y.; Yonemochi, S.; Wang, Q. Y. A characterization of HULIS-C and the oxidative potential of HULIS and HULIS-Fe(II) mixture in PM2.5 during hazy and non-hazy days in Shanghai. *Atmos. Environ.* **2019**, *219*, 117058.
(39) Ito, A.; Shi, Z. Delivery of anthropogenic bioavailable iron from mineral dust and combustion aerosols to the ocean. *Atmos Chem. Phys.* **2016**, *16* (1), 85−99.
(40) Li, K.; Fang, X.; Wang, T.; Gong, K.; Ali, T. M.; Wang, W.; Han, J.; Cheng, H.; Xu, G.; Zhang, L. Atmospheric Organic Complexations Enhanced Sulfate Formation and Iron Dissolution on Nano α-Fe2O3. *Environ. Sci.: Nano* **2021**, *8*, 698−710.
(41) Tao, Y.; Murphy, J. G. The Mechanisms Responsible for the Interactions among Oxalate, pH, and Fe Dissolution in PM2.5. *Acs Earth Space Chem.* **2019**, *3* (10), 2259−2265.
(42) Tapparo, A.; Di Marco, V.; Badocco, D.; D'Aronco, S.; Solda, L.; Pastore, P.; Mahon, B. M.; Kalberer, M.; Giorio, C. Formation of metal-organic ligand complexes affects solubility of metals in airborne particles at an urban site in the Po valley. *Chemosphere* **2020**, *241*, 125025.
(43) Paris, R.; Desboeufs, K. V. Effect of atmospheric organic complexation on iron-bearing dust solubility. *Atmos Chem. Phys.* **2013**, *13* (9), 4895−4905.
(44) Paris, R.; Desboeufs, K. V.; Journet, E. Variability of dust iron solubility in atmospheric waters: Investigation of the role of oxalate organic complexation. *Atmos. Environ.* **2011**, *45* (36), 6510−6517.
(45) Wozniak, A. S.; Shelley, R. U.; McElhenie, S. D.; Landing, W. M.; Hatcher, P. G. Aerosol water soluble organic matter characteristics over the North Atlantic Ocean: Implications for iron-binding ligands and iron solubility. *Mar Chem.* **2015**, *173*, 162−172.
(46) Wozniak, A. S.; Shelley, R. U.; Sleighter, R. L.; Abdulla, H. A.; Morton, P. L.; Landing, W. M.; Hatcher, P. G. Relationships among aerosol water soluble organic matter, iron and aluminum in European, North African, and Marine air masses from the 2010 US GEOTRACES cruise. *Mar Chem.* **2013**, *154*, 24−33.

NOTE ADDED AFTER ASAP PUBLICATION

Originally published ASAP on July 18, 2022; Manuscript title updated July 19, 2022.

https://doi.org/10.1021/acs.estlett.2c00316
Environ. Sci. Technol. Lett. 2022, 9, 680−686