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**Electronic Supplementary Information for:**

**Reactive Oxygen Species Activity and Chemical Speciation of Size-Fractionated Atmospheric Particulate Matter from Lahore Pakistan: An Important Role for Transition Metals.**

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### Summary of ROS Methodology

The sample extracts were prepared for ROS analysis by adding an aliquot of 10X concentrated solution of salts glucose medium (SGM). Cultured non-adherent macrophage cells were harvested and gently concentrated by centrifugation at 750 RPM for 5 minutes, the culture media removed, and replaced with SGM to produce a cell suspension of 1000 cells/ $\mu\text{L}$ . Macrophage cells (100  $\mu\text{L}$  =100,000 cells) were dispensed into each well of a 96 well plate and incubated for 2 hours in a 37 °C incubator under a 6%  $\text{CO}_2$  atmosphere. Approximately 15 minutes before the end of the incubation period, DCFH-DA was added to each prepared sample to achieve a final concentration of 45  $\mu\text{M}$ . After the incubation period, during which time >98% of the cells have settled and adhered to the well bottom, the SGM was pipetted off and immediately replaced with 100  $\mu\text{L}$  of the SGM-buffered sample extract or control sample. The plate was returned to the 37 °C incubator for 2.5 hours after which time the fluorescence intensity of each well was determined at 504 nm excitation and 529 nm emission (515 nm cutoff) using a m5e microplate reader (Molecular Devices). Un-opsonized zymosan, a  $\beta$ -1,3 polysaccharide of D-glucose, which is recognized by TLR-2 receptors on macrophage cells, activating a strong immuno-chemical response<sup>S1</sup> was used as a positive control for ROS induction.<sup>S2-S3</sup> Raw fluorescence data was blank-control corrected and normalized to the zymosan controls to account for minor variations in method sensitivity and to aid comparisons between studies. All samples, as well as positive (zymosan) and negative (method blanks) controls were analyzed in triplicate (3-wells). A minimum of six dilutions (each of them in triplicate) of every sample extract

were run to ensure that a linear dose-response region could be identified. Overall uncertainty in the ROS method was estimated by propagating the standard deviation of the triplicate ROS measurements with the standard deviation of the applied method blank. These values ranged from 8.3 to 50% (median = 17.7%, n=52). Our previous studies<sup>31</sup> demonstrated that sample duplicate precision (i.e. filter splits carried through the full extraction and analysis procedure) in the range of 3 to 4% RPD can be expected.

S1 A.C. Mörk, R.J. Helmke, J.R. Martinez, M.T. Michalek, M. L. Patchen and G.H. Zhang, *Immunopharmacology*, 1998, **40**, 77-89.

S2 J. Giron-Calle and H.J. Forman, *American Journal of Respiratory Cell and Molecular Biology*, 2000, **23**, 748-754.

S3 J.F. Long, P.K. Dutta and B.D. Hogg, *Environ. Health Perspect.*, 1997, **105**, 706-711.

### **Further Discussion of Chelex and DFO Methods, Controls and Validation**

Outcomes of the negative and positive controls (refer to Methods section) suggest that the Chelex and DFO treatments did not introduce significant ROS contamination or suppression of ROS activity; therefore we believe the sample outcomes to be reasonable free of biases sourced from the ligands themselves and general method handling. The Chelex separations were run under conditions that our previous work has demonstrated is essentially quantitative in removing labile metals from dilute aqueous solutions.<sup>S4</sup> In environmental samples, natural ligands will compete with the immobilized-Chelex ligands for metals, and thus metal removal will to some extent depend upon the competing kinetics of complexation, thus the term Chelex non-labile for the species not retained on the column. While this separation paradigm may at one level appear problematic, in actual biochemical terms it is actually very relevant, as metal acquisition and subsequent toxicity by organisms/cells can in many situations be viewed and modeled as a competitive ligand process.<sup>S5</sup> Thus, we consider the Chelex-labile fraction to have enhanced biological toxicity relevance. To check whether with natural samples we were saturating the Chelex column or operating in a sensitive kinetic region, two Chelex-processed samples were subsequently re-processed through new columns under the same operating conditions. While some metal retention was noted, the additional fractional removal was small (4-12%) and the impact on our estimates of Chelex-labile ROS, negligible. Additionally, we observed no statistically significant relationship between the percentage removal of ROS activity and the total ROS activity (n=24). The 30-minute DFO equilibration time applied is ample time for

equilibrium to be established between labile iron species and the DFO-ligand.<sup>S6-S7</sup>

Removal of soluble organic material (WSOC) on Chelex is expected to be low at the circumneutral pH of the extracts ( $6.4 \pm 0.4$ ,  $n=30$ ) (WSOC at these pHs is typically negatively charged and interaction with the Chelex functional groups and support resin is minimal).

S4 M.M. Shafer, S.R. Hoffmann, J.T. Overdier and D.E. Armstrong, *Environ. Sci. Technol.*, 2004, **38**, 3810-3819.

S5 V. Slaveykova and K.J. Wilkinson, *Environmental Chemistry*, 2005, **2**, 9-24.

S6 S-F. Cheah, S.M. Kraemer, J. Cervini-Silva and G. Sposito (2003). *Chem. Geol.* 2003, **198**, 63-75.

S7 B. Faller and H. Nick, *J. Am. Chem. Soc.*, 1994, **116**, 3860-3865.

### Statistical Summary

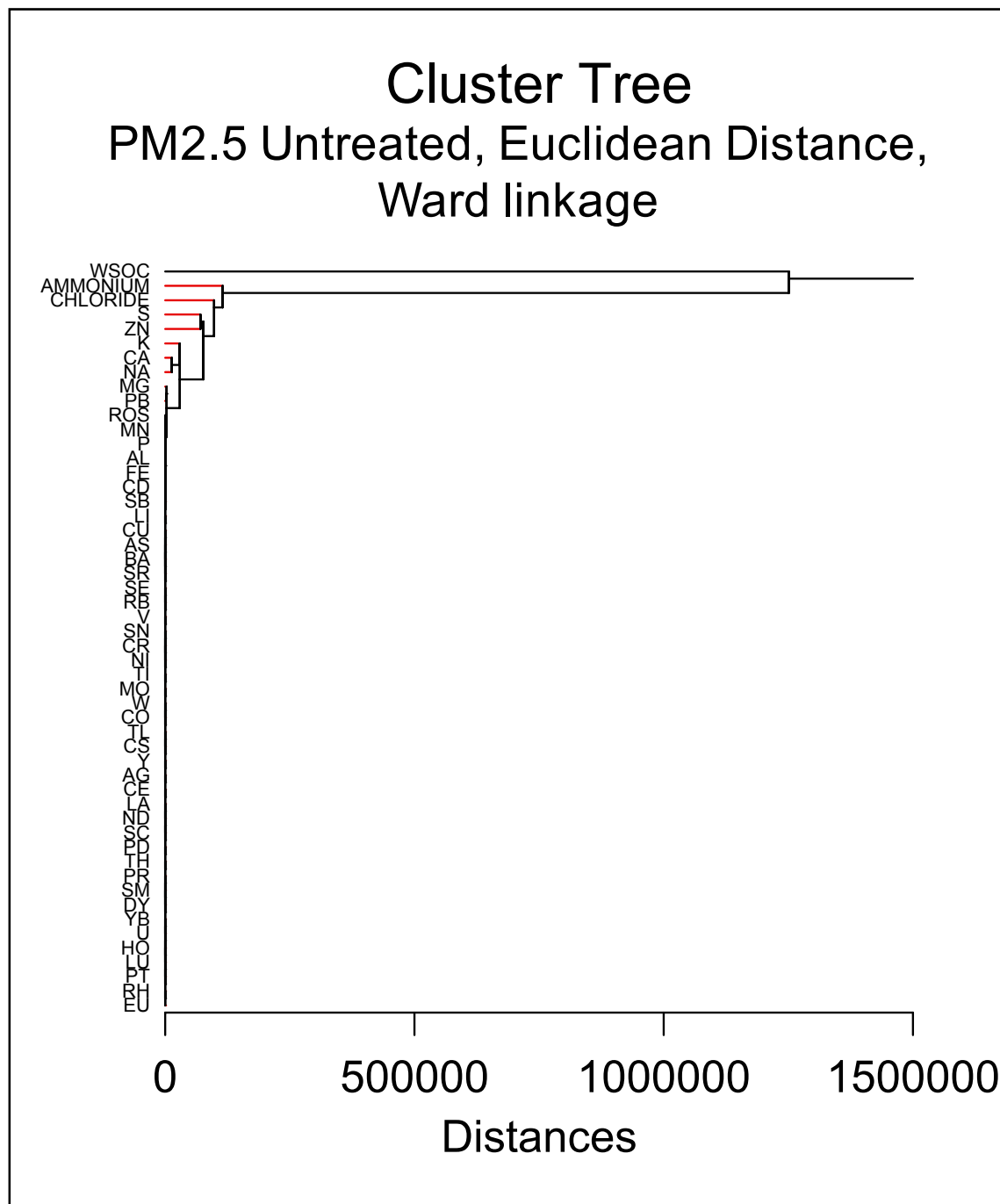
**Table S1: Pearson Correlation Coefficients, Ranked, Pooled PM10 + PM2.5.**

Untreated		Untreated-Chelexed		Chelexed	
r	Element	r	Element	r	Element
<b>0.827</b>	<b>Mn</b>	<b>0.812</b>	<b>Co</b>	<b>0.913</b>	<b>W</b>
<b>0.816</b>	<b>Co</b>	<b>0.802</b>	<b>Mn</b>	<b>0.893</b>	<b>Fe</b>
<b>0.765</b>	<b>Ni</b>	<b>0.741</b>	<b>Ni</b>	<b>0.869</b>	<b>Al</b>
<b>0.746</b>	<b>Mg</b>	<b>0.731</b>	<b>Mg</b>	<b>0.864</b>	<b>Sn</b>
<b>0.741</b>	<b>Ba</b>	<b>0.728</b>	<b>Ce</b>	<b>0.862</b>	<b>As</b>
<b>0.735</b>	<b>Na</b>	<b>0.724</b>	<b>Ba</b>	<b>0.860</b>	<b>Ti</b>
<b>0.724</b>	<b>Mass</b>	<b>0.699</b>	<b>Pd</b>	<b>0.847</b>	<b>Cr</b>
<b>0.720</b>	<b>Pd</b>	<b>0.680</b>	<b>Ca</b>	<b>0.816</b>	<b>Sc</b>
<b>0.719</b>	<b>K</b>	<b>0.675</b>	<b>K</b>	<b>0.793</b>	<b>Th</b>
<b>0.715</b>	<b>Ce</b>	<b>0.672</b>	<b>Sr</b>	<b>0.778</b>	<b>V</b>
<b>0.706</b>	<b>Ca</b>	<b>0.627</b>	<b>Li</b>	<b>0.776</b>	<b>P</b>
<b>0.705</b>	<b>S</b>	<b>0.617</b>	<b>La</b>	<b>0.771</b>	<b>Yb</b>
<b>0.699</b>	<b>Sulfate</b>	<b>0.617</b>	<b>Eu</b>	<b>0.768</b>	<b>Ho</b>
<b>0.698</b>	<b>Sm</b>	<b>0.600</b>	<b>Pt</b>	<b>0.765</b>	<b>Lu</b>
<b>0.695</b>	<b>U</b>	<b>0.589</b>	<b>Cd</b>	<b>0.761</b>	<b>Pr</b>
<b>0.689</b>	<b>Sr</b>	<b>0.584</b>	<b>Rb</b>	<b>0.760</b>	<b>Sm</b>
<b>0.684</b>	<b>Y</b>	<b>0.581</b>	<b>Cs</b>	<b>0.754</b>	<b>Nd</b>
<b>0.683</b>	<b>REE</b>	<b>0.573</b>	<b>U</b>	<b>0.754</b>	<b>Dy</b>
<b>0.675</b>	<b>La</b>	<b>0.552</b>	<b>Rh</b>	<b>0.750</b>	<b>Eu</b>
<b>0.673</b>	<b>Nd</b>	<b>0.540</b>	<b>Y</b>	<b>0.748</b>	<b>REE</b>
<b>0.670</b>	<b>Yb</b>	<b>0.527</b>	<b>Sm</b>	<b>0.747</b>	<b>Mn</b>
<b>0.667</b>	<b>Eu</b>	<b>0.522</b>	<b>Yb</b>	<b>0.747</b>	<b>Sr</b>
<b>0.666</b>	<b>Li</b>	<b>0.519</b>	<b>REE</b>	<b>0.746</b>	<b>La</b>
<b>0.665</b>	<b>Dy</b>	<i>0.500</i>	<b>Fe</b>	<b>0.739</b>	<b>Y</b>
<b>0.649</b>	<b>Ho</b>	<i>0.498</i>	<b>Se</b>	<b>0.738</b>	<b>Cd</b>
<b>0.635</b>	<b>Pr</b>	<i>0.495</i>	<b>Dy</b>	<b>0.710</b>	<b>Mg</b>
<b>0.621</b>	<b>Cd</b>	<i>0.467</i>	<b>Nd</b>	<b>0.705</b>	<b>Cu</b>
<b>0.610</b>	<b>Fe</b>	<i>0.466</i>	<b>Cu</b>	<b>0.698</b>	<b>Pb</b>
<b>0.608</b>	<b>Rb</b>	<i>0.463</i>	<b>Ho</b>	<b>0.691</b>	<b>Ce</b>
<b>0.596</b>	<b>Lu</b>	<i>0.449</i>	<b>Pr</b>	<b>0.684</b>	<b>U</b>
<b>0.591</b>	<b>Mo</b>	<i>0.437</i>	<b>Ag</b>	<b>0.667</b>	<b>Zn</b>
<b>0.586</b>	<b>W</b>	<i>0.397</i>	<b>Tl</b>	<b>0.635</b>	<b>S</b>
<b>0.583</b>	<b>Se</b>	<i>0.376</i>	<b>Cr</b>	<b>0.597</b>	<b>Ba</b>
<b>0.583</b>	<b>Cs</b>	<i>0.352</i>	<b>Zn</b>	<b>0.582</b>	<b>Ni</b>
<b>0.583</b>	<b>Nitrate</b>	<i>0.306</i>	<b>W</b>	<b>0.556</b>	<b>Mo</b>
<b>0.562</b>	<b>Pt</b>	<i>0.284</i>	<b>As</b>	<i>0.488</i>	<b>Se</b>
<b>0.559</b>	<b>WSOC</b>	<i>0.271</i>	<b>Sc</b>	<i>0.488</i>	<b>Ca</b>
<b>0.555</b>	<b>Cr</b>	<i>0.263</i>	<b>Sn</b>	<i>0.453</i>	<b>K</b>
<b>0.553</b>	<b>Sc</b>	<i>0.250</i>	<b>Al</b>	<i>0.450</i>	<b>Tl</b>
<b>0.549</b>	<b>Rh</b>	<i>0.244</i>	<b>Lu</b>	<i>0.366</i>	<b>Co</b>
<b>0.542</b>	<b>Ti</b>	<i>0.224</i>	<b>Th</b>	<i>0.358</i>	<b>Li</b>
<b>0.536</b>	<b>Cu</b>	<i>0.222</i>	<b>Ti</b>	<i>0.345</i>	<b>Rb</b>
<b>0.502</b>	<b>Ag</b>	<i>0.210</i>	<b>P</b>	<i>0.246</i>	<b>Cs</b>
<i>0.494</i>	<b>As</b>	<i>0.204</i>	<b>Mo</b>	<i>0.214</i>	<b>Pd</b>
<i>0.471</i>	<b>Sn</b>	<i>0.188</i>	<b>Pb</b>	<i>0.090</i>	<b>Sb</b>
<i>0.461</i>	<b>Th</b>	<i>0.143</i>	<b>Sb</b>	<i>0.043</i>	<b>Rh</b>
<i>0.433</i>	<b>EC</b>	<i>0.105</i>	<b>S</b>	<i>-0.058</i>	<b>Ag</b>
<i>0.410</i>	<b>Al</b>	<i>0.079</i>	<b>V</b>	<i>-0.204</i>	<b>Pt</b>
<i>0.407</i>	<b>OC</b>				
<i>0.399</i>	<b>P</b>				
<i>0.388</i>	<b>Tl</b>				
<i>0.343</i>	<b>Zn</b>				
<i>0.334</i>	<b>Chloride</b>				
<i>0.219</i>	<b>Pb</b>				
<i>0.213</i>	<b>Sb</b>				
<i>0.199</i>	<b>V</b>				
<i>0.161</i>	<b>Ammonium</b>				

**Bold: P < 0.01**   Normal: P < 0.05   *Italic: P > 0.05*



**Figure S2.** Cluster analysis summary. Cluster tree, Euclidean distance, Ward linkage.  
Panel B = PM2.5.





**Table S2: PCA Covariance Matrix, PM2.5 (Untreated – Chelexed)**

<u>PM2.5</u>				
Component loadings				
	1	2	3	4
ZN	79291.996	-12958.824	1365.873	-202.316
K	21019.689	34558.211	-8489.649	1571.107
S	6034.731	27679.033	9679.636	2502.171
CA	5712.736	22439.442	1874.286	-5579.924
MG	669.133	1979.115	267.174	-565.733
ROS	388.084	376.794	-224.949	121.729
FE	33.338	360.573	101.419	56.031
AL	98.296	215.233	140.442	48.175
PB	2667.380	1479.918	328.124	45.211
P	84.771	561.477	133.455	30.832
SR	4.178	32.179	-0.073	-14.696
LI	37.961	42.801	-9.024	-11.324
CU	2.720	33.905	6.763	10.879
MN	503.644	613.972	46.657	-7.589
AS	5.435	23.778	8.750	4.542
SE	28.585	2.443	-4.399	4.125
V	-5.569	8.367	6.420	3.386
RB	20.582	4.266	-4.216	-3.094
BA	1.115	24.427	-0.517	2.769
SN	2.546	14.221	2.593	2.530
SB	28.843	20.620	13.518	-2.424
NI	4.643	11.341	2.892	1.990
CD	332.734	274.202	21.354	1.110
CR	2.873	6.241	2.889	0.941
MO	0.230	1.376	0.086	0.490
TI	0.928	5.462	1.202	0.295
W	0.368	1.889	0.434	0.127
TL	1.184	0.109	0.021	-0.113
Y	0.033	0.279	0.072	0.059
CS	0.855	0.010	0.243	-0.039
CO	0.439	1.638	0.120	-0.026
CE	0.033	0.091	-0.010	0.020
LA	0.023	0.086	0.025	0.020
ND	0.015	0.113	0.035	0.019
AG	0.069	0.185	0.003	0.015
SC	0.016	0.082	0.016	0.011
TH	0.005	0.036	0.008	0.006
EU	0.000	0.008	-0.001	0.005
PR	0.004	0.027	0.007	0.004
SM	0.002	0.027	0.005	0.004
DY	0.002	0.025	0.007	0.004
PD	0.007	0.048	-0.002	-0.003
RH	0.007	0.004	-0.006	-0.002
PT	0.006	0.001	-0.002	0.001
U	0.002	0.011	0.002	0.001
YB	0.002	0.014	0.003	0.001
HO	0.001	0.005	0.002	0.001
LU	0.000	0.002	0.001	0.000

Variance Explained by Components				
	1	2	3	4
	6.80620E+09	2.63906E+09	1.71429E+08	4.02497E+07

Percent of Total Variance Explained				
	1	2	3	4
	70.467	27.323	1.775	0.417

**Table S3: PCA Covariance Matrix, PM10 (Untreated – Chelexed)**

<u>PM10</u>				
Component loadings				
	1	2	3	4
ZN	86207.860	-13168.843	-2320.460	-2512.325
CA	23572.308	30038.421	15610.194	-3662.729
K	18336.816	27872.616	-14096.480	8706.140
S	8720.012	-10081.444	10519.906	16530.586
PB	2366.573	-385.880	-304.502	-565.978
MG	1739.557	2465.070	-236.478	269.849
AL	117.219	173.289	238.303	187.532
FE	111.956	331.748	64.130	150.035
ROS	441.778	693.353	-153.237	-35.643
CD	341.883	233.922	-76.640	-27.498
MN	473.851	384.621	36.177	-26.491
P	-29.329	115.888	88.598	25.393
CU	11.021	23.841	19.633	20.895
SN	5.644	0.547	3.746	13.799
SR	27.021	44.259	19.080	-13.408
LI	56.888	16.692	-8.701	10.576
AS	12.047	4.047	5.576	7.580
SE	30.012	-0.689	-5.605	5.289
BA	16.406	22.702	-1.815	-5.256
RB	19.598	5.171	-7.250	3.711
V	-7.119	7.702	11.210	3.148
SB	88.818	-26.475	7.303	2.873
TI	-1.204	0.621	2.247	1.224
MO	0.748	0.629	0.396	0.967
CR	6.390	2.382	2.325	0.277
NI	10.129	13.261	8.254	0.276
AG	0.074	0.487	0.012	0.176
TL	1.468	0.060	-0.094	0.166
CS	1.057	0.317	-0.247	-0.145
CO	1.208	1.910	0.562	0.106
Y	0.064	0.148	0.016	0.053
LA	0.063	0.119	0.008	0.042
W	0.123	0.509	0.090	0.036
ND	0.035	0.043	0.010	0.032
PD	0.033	0.107	0.006	0.032
SC	-0.007	0.013	0.029	0.016
CE	0.154	0.235	0.005	-0.016
PR	0.007	0.010	0.003	0.007
SM	0.005	0.010	0.002	0.005
DY	0.003	0.012	0.003	0.004
U	-0.000	0.005	-0.001	0.003
EU	0.014	0.005	0.005	-0.003
YB	0.002	0.006	0.001	0.002
TH	-0.006	0.005	0.020	-0.002
RH	0.022	0.005	-0.001	-0.001
HO	0.000	0.002	0.000	0.001
LU	-0.000	0.001	0.001	0.000
PT	0.008	0.002	-0.001	0.000
Variance Explained by Components				
	1	2	3	4
	8.40893E+09	1.96131E+09	5.58691E+08	3.69240E+08
Percent of Total Variance Explained				
	1	2	3	4
	74.396	17.352	4.943	3.267

There are important similarities between the PCA and Cluster analyses, which provides added confidence in the overall statistical inferences. Focussing on the trace elements and ROS: in the PM<sub>10</sub> PCA model, factor 2 is defined by a dominant ROS loading, high loadings from Mn, Fe, Cd, Al and a negative loading from Zn. The major elements (Ca, Mg, K, S, P) all tend to group/load together. These results are nearly identical to those presented from the Cluster analysis. Quite similar outcomes were observed in factor 2 in the PM<sub>2.5</sub> PCA model, with the exception that Pb is more prominent (similar contrasts in Pb behaviour were also observed in the Cluster analysis between the two size-fractions). Factor 1 is dominated by Zn, but again ROS, Mn, Cd, and to a lesser extent than in Factor 2, Al, and Fe are significant in defining the factor. The behaviour of Zn in Factor 1 and 2 may be reflected in its position as the most distant trace element cluster in the Cluster analysis.