KING'S College LONDON

University of London

Manor Road
PM_{10} Toxicity Study

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Environmental Research Group
King's College London
Franklin-Wilkins Building
150 Stamford St
London SE1 9NN
Tel 020 7848 4044
Fax 020 7848 4045

	Name	**Contact**	
Authors	Timothy Baker	tim.baker@erg.kcl.ac.uk	
	Gary Fuller	gary.fuller@erg.kcl.ac.uk	
	Prof. Frank Kelly	frank.kelly@erg.kcl.ac.uk	
	Dr. Ian Mudway	Ian.mudway@kcl.ac.uk	

	Name	**Signed**	**Date**
Reviewed by	Gary Fuller		
Approved by	Frank Kelly		

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Summary

Elevated concentrations of PM_{10} at the Manor Road site in Bexley have been noted on weekdays and Saturday mornings. The issue is of concern since part of Manor Road is residential and was declared an air quality management area in 2001.

In 2001, ERG undertook a study that showed that local tail pipe sources of PM_{10} were relatively minor in Manor Road. The vast majority of local PM_{10} was found to originate from non tail pipe sources and was closely linked to vehicle activity on Manor Road. The diurnal pattern of local non tail pipe PM_{10} suggested that the elevated PM_{10} arises from a combination of re-suspension from the road and from dust being lifted directly from dirty vehicles.

Given the proposed nature of this re-suspended material it was hypothesised that although the mass concentration of PM in Manor Road was high, its toxicity per unit mass may be lower than other neighbourhood sites.

To examine this possibility ERG undertook a sampling campaign between June and August 2002 to collect PM_{10} from Manor Road and for comparison, the nearby Greenwich 4 site.

Toxicity was determined by accessing the 'oxidative activity' of PM_{10} samples. The oxidative activity of PM is currently believed to be the best explanation for its impact on human health Specifically, we examined the ability of PM_{10} samples to consume small molecular-weight antioxidants that are present in respiratory tract lining fluid – a technique which has been pioneered by the Lung Biology group at King's College London (KCL) with Medical Research Council (MRC) support.

Contrary to expectation, the data obtained indicate that particles collected from the Manor Road site have a greater oxidative activity (on an equal mass basis) than particles collected from the Greenwich site during the working week. Interestingly, the weekend collections tended to indicated that particles collected from the Greenwich site were more reactive suggesting that Greenwich 4 may not be representative of background conditions at Manor Road. If Manor Road is considered in isolation a significant pattern can be seen in the oxidant activity with activity being greatest on weekdays, lower on Saturday and lowest on Sunday suggesting that PM_{10} at Manor Road is more oxidatively active when local sources are making a contribution.

The mechanisms driving the oxidant activity at each site appear to be different although oxidant reactions at both sites are dependent on transition metals in PM_{10}.

Total metal content of PM_{10} at both sites were analysed by ICP/MS following acid digestion. Metal concentrations were similar at both sites. Oxidant activity was found to be linked with iron, lead and total transition metals at Manor Road but not at Greenwich 4, further indicating different mechanisms for the oxidant activity at each site.

At Manor Road the concentrations of iron, lead and total transition metals suggest that they may originate from the same source(s) and show a similar daily pattern to the local PM_{10} at the site.

The water leachable iron, perhaps a better indicator of biological available iron is different at the two sites suggesting that the metal PM_{10} at Manor Road may be in a different chemical form to that at Greenwich 4.

An overall health effects index has been created using the oxidant activity and the mass of PM_{10} at each site. This health effects index suggests that the PM_{10} burden at Manor Road has a greater health effect than the PM_{10} burden at Greenwich 4. It is likely that this increased health effect is due to the local sources of PM_{10}.

1 Expertise

1.1 King's College London

King's is one of the two oldest and largest colleges of the University of London with some 12,200 undergraduate students and over 4,500 postgraduates in ten schools of study. It is in the top group of five UK universities for research earnings.

1.2 The Environmental Research Group

The Environmental Research Group at King's College London (KCL) was formally part of the South East Institute of Public Health (SEIPH). SEIPH was formed in 1991, as part of the National Health Service and became part of the University of London in 1993. The Environmental Research Group (ERG) is now part of the School of Health and Life Sciences within King's.

The ERG is not a profit orientated organisation. Being part of a university we are motivated by the desire to produce high quality research. We are therefore always keen to work with our clients on a partnership basis, implementing innovative solutions, taking a pro-active role in fulfilling our contractual obligations.

ERG and other researchers within KCL have a long history of particulate measurement modelling and analysis. Particulate research programmes have included early measurement of PM_{10}, comparison of measurement techniques, chemical speciation and regional modelling of current and future PM_{10}. ERG also has extensive experience of successfully operating air pollution measurement programmes for (Department for the Environment, Food and Rural Affairs) DEFRA, local authorities and private industry.

The ERG has been working closely in conjunction with Bexley Council on air pollution issues since 1993. Data from the Council's continuous monitoring sites are collected by the ERG as part of the London Air Quality Network (LAQN). This allows pollution in Bexley to be compared to that in neighbouring London Boroughs and elsewhere, contributing to a wider understanding of air pollution in Bexley, and throughout London and the South East. This perspective is unique to ERG and is essential to the proposed programme.

The ERG also has considerable expertise in air pollution modelling having undertaken strategic studies for the Greater London Authority, London Transport and numerous local councils. The ERG has undertaken air pollution modelling to supporting Bexley Council's air quality management programme.

1.3 The Lung Biology Group

The Lung Biology Group researches the health impact of air pollution. Many air pollutants are powerful oxidants and can cause damage to the structure and function of the lung. Investigations have demonstrated that air pollutants such as O_3, NO_2 and PM_{10} all interact with, and deplete, lung lining fluid antioxidant defences.

The group is investigating why around 10-15% of the population are particularly sensitive to air pollution and how their susceptibility can be reduced. They are also examining whether particles from different locations have equivalent toxicity, or whether there are there some areas where particles are more dangerous.

The Lung Biology Group has developed a screening model used to examine the oxidative capacity of PM_{10}. The Group has since won competitive funding from the Medical Research Council in the UK and the EU to use this technique. No other group is undertaking such work.

2 Background

2.1 PM_{10} at Manor Road

The Manor Road monitoring site is located on the south side of Manor Road, Erith. The site began operating in spring 1999 and measures PM_{10} using a Tapered Element Oscillating Microbalance (TEOM). Measurements of PM_{10} at the site are amongst the highest on the London Air Quality Network (LAQN). During 2000 the site measured 78 exceedence days of the EU Limit Value, compared to a limit of 35 days to be achieved by the end of 2004. Elevated concentrations of PM_{10} at the site have been noted on weekdays and on Saturday mornings. The issue is of concern since part of Manor Road is residential. An Air Quality Management Area was declared on 22nd August 2001 for the residential section of Manor Road.

During 2001 Bexley Council commissioned ERG to investigate the sources of PM_{10} at Manor Road. The study method focused on the enhanced PM_{10} and NO_X measured at Manor Road compared to background sites in the area. For the study period (6thJuly – 18th October 2001) additional NO_X monitoring equipment was installed at the Manor Road site to allow analysis to differentiate between tail pipe and non tail pipe sources of PM_{10}. Diurnal and wind direction analysis was undertaken.

The study (Baker and Fuller 2001) showed that local tail pipe sources of PM_{10} were relatively minor. The vast majority of local PM_{10} was found to originate from non tail pipe sources but was closely linked to vehicle activity on Manor Road. The diurnal pattern of local non tail pipe PM_{10} suggested that the elevated PM_{10} arises from a combination of re-suspension from the road and dust being lifted directly from dirty vehicles.

2.2 PM_{10} Toxicity

Numerous epidemiological studies have shown associations between ambient particulate matter (PM) concentrations and various health outcomes. The vast majority of epidemiological studies have used ambient mass concentration as a surrogate of PM exposure. This measure is neither sensitive to the varying composition and physico-chemical properties of PM, over space and time, nor to the sources that contribute to the total mass concentration. Thus, current studies give only limited guidance to understanding the underlying biologic pathways that lead to the associated effects and to designing optimal abatement strategies. To better understand how different species of PM may cause long-term health effects in humans, we need biologically relevant PM characteristics, applied to epidemiological studies.

A unifying biologic model to explain the health effects of air pollution considers oxidative damage and antioxidative repair capacities in the lung as key pathophysiologic pathways. The model integrates results from observational and experimental studies, showing oxidative stress (resulting from either increased exposure to oxidants or reduced antioxidative capacities) being a fundamental mode of injury. Respiratory tract lining fluid is a layer of fluid that covers the surface of the lung and it has a rich complement of antioxidant defences, e.g. vitamin C or ascorbate; reduced glutathione and urate. Figure 1 illustrates how PM, which enters the lung, has to first cross the respiratory tract lining fluid before it interacts with the delicate lung cells which lie underneath. In this manner, the respiratory tract lining fluid antioxidant defences (particularly ascorbate and reduced glutathione) protect the lung from injury. Accordingly, oxidative stress in the respiratory lining fluid, epithelial cells and macrophages are coupled to persistent inflammatory responses which lead to tissue damage, decreased ventilatory capacity and increased bronchial reactivity. Clinically, these processes lead to acute effects such as asthma exacerbations or increases in infections and symptoms, and chronic injury affecting lung function growth and decline. In line with this model we have shown that asthmatics have decreased concentrations of protective antioxidants in their lung lining fluids (Kelly et al, 1999) revealing one reason why these patients may have increased sensitivity to air pollution. Trials have also shown that antioxidant intake can protect from air pollution related respiratory symptoms and changes in lung function.

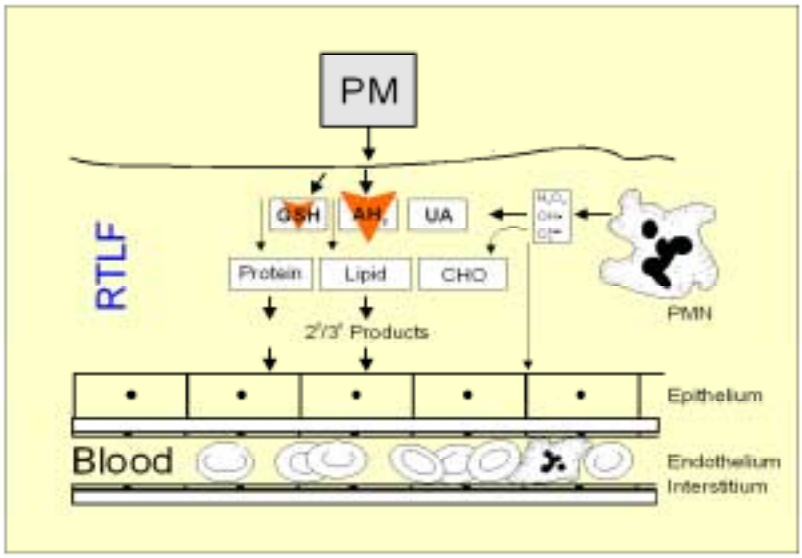


Figure 1. The surface of the lung is covered by a layer of fluid rich in antioxidant defences – the respiratory tract lining fluid (RTLF); see Kelly et al, 1999a for further detail.

Currently there is an intensive research effort to further investigate the "oxidative stress model" and to examine the oxidative properties of ambient PM. In a recent study in Germany, the oxidative activity of $PM_{2.5}$ samples from urban Hettstedt versus rural Zerbst (Germany) showed remarkable differences between the two sites at similar mass concentration. The higher activity in Hettstedt was mainly related to the high Cu content of the particles. A second study in Germany showed that there is temporal variation in OH-generation in coarse samples during different seasons in Düsseldorf, although total metal content was not different. These findings suggest that the speciation of metals is important and that other radical generating components such as quinones might play a role. In the lung, it is unlikely that all Fe (or indeed other metals) will be available to drive oxidative activity. A more important measure may therefore be the water leachable (freely accessible and soluble) iron pool.

Recent, but as yet unpublished work by Kelly's group has shown that environmental PM collected in Germany, Holland and the UK has a high level of endogenous oxidant activity which appears to be related to its size and, in part, to its transition metal content. Activities of PM collected at London kerbsides were particularly strong (more so than residual oil fly ash), indicating that oxidative properties may be used as traffic specific indicators of PM toxicity. Moreover, the variation in oxidant activity apparently is greater than the variation in mass, suggesting that this metric is a useful new indicator of particulate biological activity. In conclusion, measures of the *oxidative properties* of PM offer a promising new approach to investigate health effects of PM both in epidemiological as well as experimental studies.

2.3 The Manor Road PM_{10} Toxicity Study

Given that a large proportion of the elevated PM_{10} concentration at Manor Road was due to non tailpipe sources it is possible that the biological activity, on a mass basis, of this PM is different to that from adjacent areas where traffic is the primary source of ambient PM_{10}. The study therefore sought to determine the toxicity of the PM_{10} at Manor Road relative to that found at a background location, allowing the Manor Road PM_{10} issue to be viewed within a health context. Central to this, is the hypothesis that not all particles are equally as damaging to health, and consequently that biological toxicity, and information on particle composition are essential denominators in relating mass exceedences to public health risk.

Toxicity was determined by accessing the oxidative activity of PM_{10} samples. The 'oxidative activity' of PM is currently believed to be the best explanation for its impact on human health Specifically, we examined the ability of PM_{10} samples to consume small molecular-weight antioxidants that are present in respiratory tract lining fluid – a technique which has been pioneered by the Lung Biology group at KCL.

3 Method

3.1 Sampling

Samples of PM_{10} were collected from the Manor Road site and, for comparison, the nearby background site Greenwich 4. Samples were collected on Teflon filters using the Automated Cartridge Collection Unit (ACCU) systems installed as part of the TEOM PM_{10} analysers at each site. The ACCU uses the by-pass flow from the TEOM sampling system.

Between 0.5 and 1.0 mg of PM_{10} are required on each filter for the measurement of oxidative activity. A sample regime was devised to collect sufficient material on each filter, and to simplify interpretation of results, by building upon the established diurnal weekly pattern of PM_{10} concentrations at Manor Road. From analysis of the PM_{10} measured at both sites during 2001 it was estimated that each filter would have to be exposed for between 3 and 4 days.

To highlight any daily variation in PM it was planned to expose each filter for the same day on 4 successive weeks. This exposure regime was repeated three times including blanks where possible.

Filters were loaded into each ACCU system by ERG local site operators. The exposure times were configured and monitored remotely.

Samples, reflecting pooled daily collections and blanks were archived at -20^{o}C in air-tight desiccation chambers until required for toxicity screening or metals analysis.

3.2 Selecting Filters for Analysis

Seven contemporaneous filter pairs and one pair of blank filters were selected for oxidant activity and metal analysis. Filters were selected to provide one pair for each weekday and to represent a range of elevated PM_{10} at Manor Road. Each filter was cut into two and each half weighted. One half filter was used for the oxidant activity analysis while metals analysis was performed on the other.

3.3 Oxidant Activity Analysis

These analyses aimed to determine whether PM_{10} sampled from Manor Road had more oxidative activity than PM_{10} from Greenwich 4, where composition is more indicative of background conditions. The capacity of PM_{10} obtained from both sites to elicit the oxidation of a range of protective antioxidant molecules present at the surface of the lung was addressed using a validated *in vitro* model (Zielinski et al. 1999). Performing these experiments at equal doses enabled a comparison of the relative particle toxicity and clarifies whether the exceedences in the air quality directives at Manor Road are likely to pose a significant additional health risk.

Filters were placed into sterile (pre-weighed) 50mL falcon tubes containing 20mL of HPLC-grade methanol and vortexed for 20 minutes. A further 10mL of methanol was then added and the vortexing step repeated. Following this procedure the sample was sonicated at 15 microns for 60 seconds. The filter was then removed and the methanol extract dried down under a stream of nitrogen at 37^{o}C. The tube was then reweighed to establish the mass of extracted material. Parallel extractions were performed on unused Teflon filters, to generate a filter-blank. The extracted material was then resuspended at a set concentration in a volume of ultra-pure Chelex-resin treated water containing 5% methanol. The water was pre-treated with resin to remove any contaminating metal ions, which might interfere in the assay. The methanol was included to ensure that any relatively non-volatile organic material on the particle surface is extracted.

Antioxidant solutions, containing equimolar concentrations (200 μM) of ascorbate, urate, and reduced glutathione (the major antioxidants present at the surface of the lung) were exposed to PM_{10} resuspended in ultra-pure water at a dose of 50 μg/mL selected to reflect realistic concentrations likely to occur at the lung surface in active subjects breathing PM for periods of 4-8 hours at 50μg/m^{3}. Co-incubations of PM_{10} and antioxidants were then performed for 4 hours at 37^{o}C and at a

physiological pH of 7.4. At the end of this period, reactions were quenched by sample acidification, and particles removed by centrifugation prior to determination of the remaining antioxidant concentrations. In this model oxidant activity reflects the capacity of these particles to deplete these antioxidant molecules.

To ensure intra-assay standardisation between experiments control samples were run. These consisted of the antioxidant model without particles (auto-oxidation control). In addition to these, inhibition experiments were performed in order to identify whether the oxidant activity observed was related to the metal content of the PM_{10} and involves the production of highly reactive free-radicals. Incubations of particles with antioxidants were performed in samples treated with 0.2µM diethylenetriaminepentaacetic acid, which binds Fe^{3+} and Cu^{2+} to establish the role these metals play in the observed particle activity.

All samples were analysed for ascorbate and urate using reverse phase high pressure liquid chromatography (hplc) with electro-chemical -detection in the ampermetric mode (Iriyama, k., et al. *Anal. Biochem.* 1984; 141:238-243). Reduced and oxidised glutathione was determined using the enzyme recycling method of Tietze modified for use on a plate reader (Baker, M. et al. *Anal Biochem.* 1990; 190:360-365). Modifications made to these assays for the determination of PM-induced antioxidant depletion rates are described in Zielinski H et al. 1999;

3.4 Metals Analysis

Total filter metals analysis

This work was sub-contracted to EMC Environmental Ltd. Samples were digested in nitric acid / peroxide and the digests analysed by ICP/MS. The method used followed an EMC in-house procedure based on EPA Compendium Methods IO 3.1 and IO 3.5.

Water leachable (soluble) iron pool

This pool of iron is more likely to drive the oxidation reactions seen in those PM samples with high oxidative activity. To determine the water leachable iron pool we established a new assay making use of the Fe^{2+} chelator bathophenantroline disulphonate (BPS). BPS binds ferrous iron (Fe^{2+}) to form a complex that absorbs strongly at a wavelength of 535nm permitting the quantification of the soluble ferrous iron (Nilsson et al., , 2002). In addition, by incubating PM suspensions in the presence of the reductant ascorbate at high concentrations (10mM) ferric iron is converted to the ferrous form and thus becomes measurable by BPS to give a total soluble iron pool concentration. This method was used on the particle suspensions remaining after the antioxidant incubation experiments. Due to the scarcity of the material these analysis were only possible on 8 (Mon, Weds, Thurs and Sat from both sites) of the 14 filter samples.

4 Results and Discussion

4.1 PM_{10} Sampling

The tables below contains the exposure details for each filter. Seven contemporaneous filter pairs and one pair of blank filters were selected for oxidant activity and metal analysis. Filters were selected to provide one pair for each weekday and to represent a range of elevated PM_{10} at Manor Road. Filters selected for analysis are highlighted in the tables below.

The ACCU at Greenwich 4 developed a recurring fault which resulted in an unstable flow delivered to the filter collector on some days. Despite repeated visits from the equipment service unit this fault reoccurred in the later rounds of the study. Since the calculated collected mass required this volumetric flow to be accurately known the filters effected by this fault, noted as Flow Fault in the table above, were eliminated from the study. Similarly days when the monitoring was aborted during the course of a day, due to faults, were also eliminated.

For each day during the study the daily average mass of PM_{10} particulates per cubic metre of air at each site was calculated from the 15 minute mass concentrations recorded by the TEOM. Multiplying this daily average by the volumetric flow delivered to each filter on each day then derived the calculated collected mass.

Manor Road Round 1: 5th June – 2nd July 2002 inc.

Day of Week	Calculated Collected Mass µg	Notes
Monday	1786.95	
Tuesday	2082.73	
Wednesday	1826.21	
Thursday	2478.79	
Friday	2315.96	
Saturday	1615.09	
Sunday	1063.43	Analysed
Blank		

Greenwich 4 Round 1: 5th June – 2nd July 2002 inc.

Day of Week	Calculated Collected Mass µg	Notes
Monday	1165.34	
Tuesday	1230.23	
Wednesday	1145.60	
Thursday	744.89	Flow Fault
Friday	1192.81	
Saturday	1163.42	
Sunday	1017.75	Analysed
Blank		

Manor Road Round 2: 5th July – 23rd July 2002 inc.

Day of Week	Calculated Collected Mass µg	Notes
Monday	2272.86	
Tuesday		Aborted
Wednesday	1636.37	
Thursday	1573.59	Analysed
Friday	1823.25	Analysed
Saturday	2148.57	
Sunday	1044.39	
Blank		

Greenwich 4 Round 2: 5th July – 23rd July 2002 inc.

Day of Week	Calculated Collected Mass µg	Notes
Monday	676.71	Flow Fault
Tuesday		Aborted
Wednesday	679.05	
Thursday	655.34	Analysed
Friday	826.66	Analysed
Saturday	873.32	Flow Fault
Sunday	811.83	Flow Fault
Blank		

Manor Road Round 3: 31st July – 30th August 2002 inc.		
Day of Week	**Calculated Collected Mass µg**	**Notes**
Monday	1651.67	Analysed
Tuesday	3312.35	Analysed
Wednesday	3178.73	Analysed
Thursday	4126.53	
Friday	3060.27	Aborted
Saturday	3140.90	Analysed
Sunday	1432.64	
Blank		

Greenwich 4 Round 3: 31st July – 30th August 2002 inc.		
Day of Week	**Calculated Collected Mass µg**	**Notes**
Monday	923.38	Analysed
Tuesday	1206.89	Analysed
Wednesday	1265.93	Analysed
Thursday	833.72	Flow Fault
Friday	1567.37	Aborted
Saturday	1551.33	Analysed
Sunday	926.33	Flow Fault
Blank		

Other studies undertaken for London Borough of Bexley have sought to differentiate those days where marked local PM_{10} has had a marked effect on the PM_{10} concentration at Manor Road. This is largely a qualitative assessment; simply being a day when the PM_{10} particulates recorded at Manor Road was markedly elevated from that recorded at Greenwich 4 for some or all of the day. For the analysed filters the following table shows the number of locally polluted days at Manor Road. Most analysed filters were drawn from the second and third rounds of sampling since these covered the period of highest pollution at Manor Road during the study period.

Day of Week	Sampled Days	Locally Polluted Days
Monday	3	2
Tuesday	3	3
Wednesday	2	2
Thursday	2	1
Friday	3	2
Saturday	4	3
Sunday	4	0

4.2 PM oxidative activity: Comparison of Manor Road and Greenwich Sites

PM oxidative activity was assessed by its ability to deplete three antioxidants ascorbate, reduced glutathione and urate from lung lining fluid.

4.2.1 Ascorbate Depletion

The degree of ascorbate depletion seen after incubation with each PM sample is a direct index of the oxidant activity of the particles in that sample. We have previously demonstrated that such losses of ascorbate are predominately, though not exclusively, due to the presence of the transition metals. Ascorbate depletion is driven mainly by iron and but also copper.

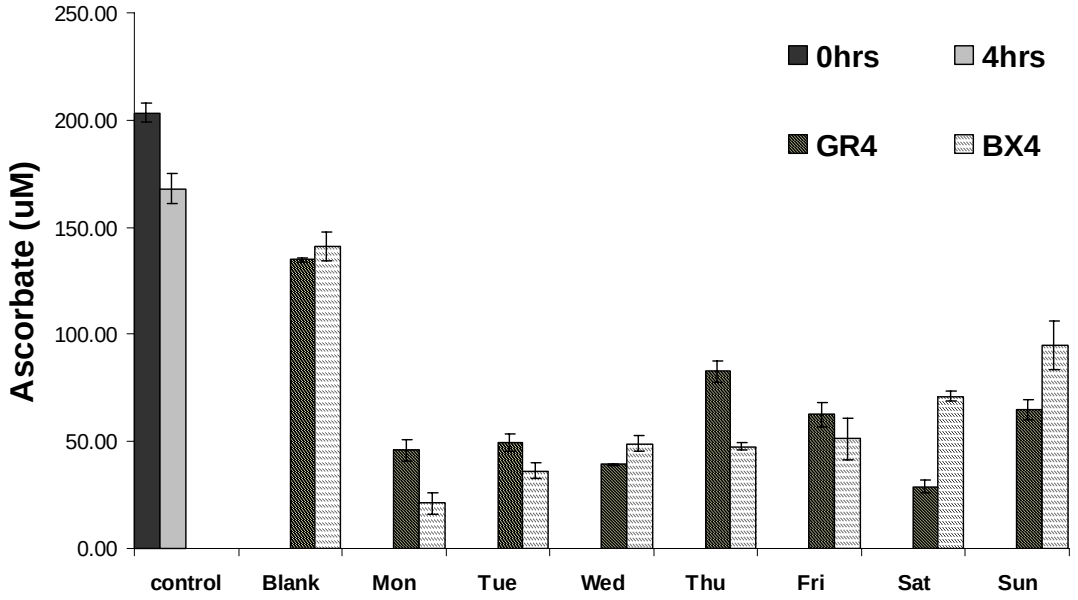


Figure 2: Loss of ascorbate from a composite antioxidant solution incubated for 4h with PM (50μg/ml) extracted from Teflon filters collected daily (Mon-Sun) at the Bexley, Manor Road and Greenwich 4 sites. Particle-free antioxidant controls are illustrated at time 0 and after 4h incubation at 37^{o}C. Blanks correspond to the supernatants obtained from 'clean' filters diluted to a similar extent to the PM samples. All filter extracts were run in triplicate.

Summary of observations

- A small amount of auto-oxidation is seen in the control samples (chelex treated [*transition metal free*] H_2O + antioxidants, minus particles) after the 4 hour time point – illustrated above as the grey bar. This was expected and allows us to control for this in the PM samples.
- The filter 'blanks' from both sites show only slight activity compared to the 4 hour control, suggesting mild contamination on the filter substrate. It should be noted that it was not simple to perform a mass per mass comparison of the blank filters with the extracted particles. Consequently, if anything we might have tended to over estimate the reactivity of the filter blanks.
- Weekday PM samples, Monday, Tuesday, and Thursday all show the Manor Road samples to be *more oxidative* on a mass basis than the PM collected from the Greenwich site. Both sites had similar activities on the Wednesday and Friday.

- In contrast, the weekend samples showed the Greenwich site to be more reactive than the Manor Road site on both days, suggesting a change in the composition of the PM. The Manor Road Sunday PM sample was particularly unreactive and there was a clear reactivity profile between the full working days (Mon-Fri), the half-working day (Sat), and the rest day (Sun) suggesting that changes in PM oxidative activity are related to industrial and commercial activity at this location.

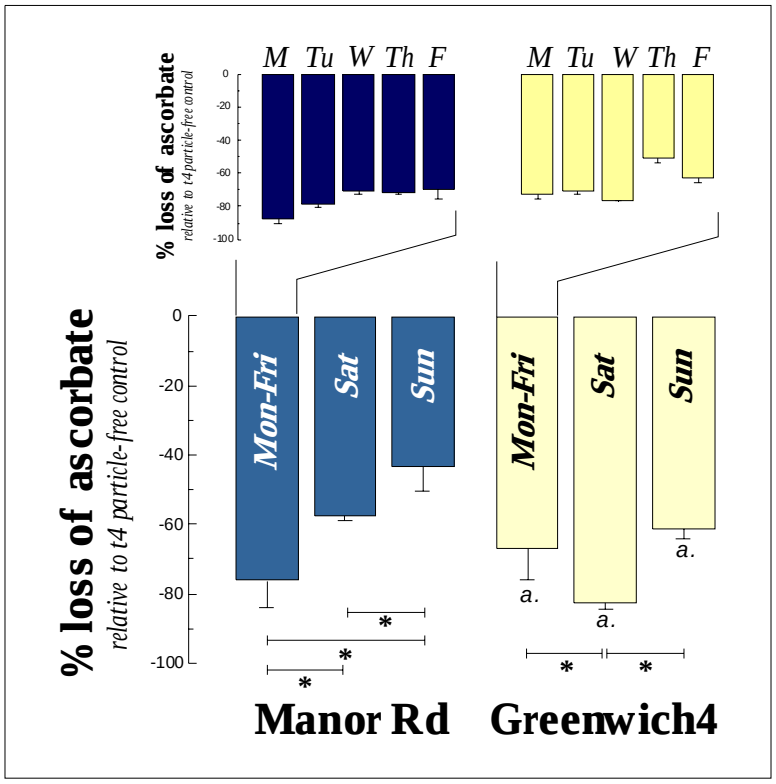


Figure 3: Percentage loss of ascorbate relative to the 4h particle free control concentration in antioxidant solutions incubated with Manor Road and Greenwich 4 filter PM samples. Comparison is made between the weekday samples (n=5x3), with those obtained on Saturday (the half working day at Manor Road) and Sunday. All data is illustrated as mean±SD. Statistical analysis was performed using a one-way ANOVA with post-hoc analysis performed using the Student-Newman-Kuels test. '*' illustrates significant differences between filter reactivities at a given site; 'a' signifies a significant difference in the % loss observed between the site across each of the sampling intervals.

4.2.2 Glutathione Depletion

Glutathione depletion also reflects the oxidant activity of PM due to the presence of transitional metals, mainly copper, and the magnitude of the response is usually less marked than that seen with ascorbate.

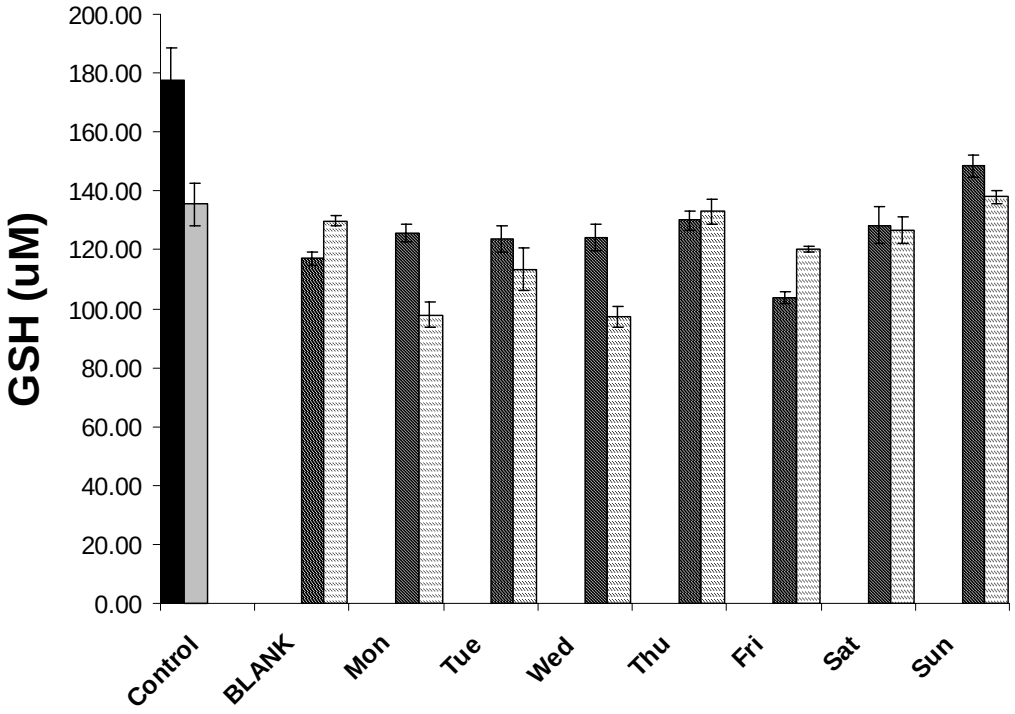


Figure 4: Loss of reduced glutathione from a composite antioxidant solution incubated for 4h with PM (50mg/ml) extracted from Teflon filters collected daily (Mon-Sun) at Bexley, Manor Road and Greenwich 4. All details are as outlined in the legend to Figure 2.

Summary of observations

- Some auto-oxidation of GSH is seen in the 4 hour control sample compared to the 0 hour control (black bar).
- The filter blanks have little or no activity towards the glutathione compared to the 4 hour control.
- A similar trend is apparent to that seen for ascorbate depletion with weekday Manor Road samples tending to have more reactivity than the Greenwich 4 samples (Monday, Tuesday and Wednesday). The Thursday collections show similar activity between sites. The Greenwich 4 Friday sample however, has greater activity than Manor Road Friday sample.
- The weekend PM collections appear relatively inert towards glutathione.

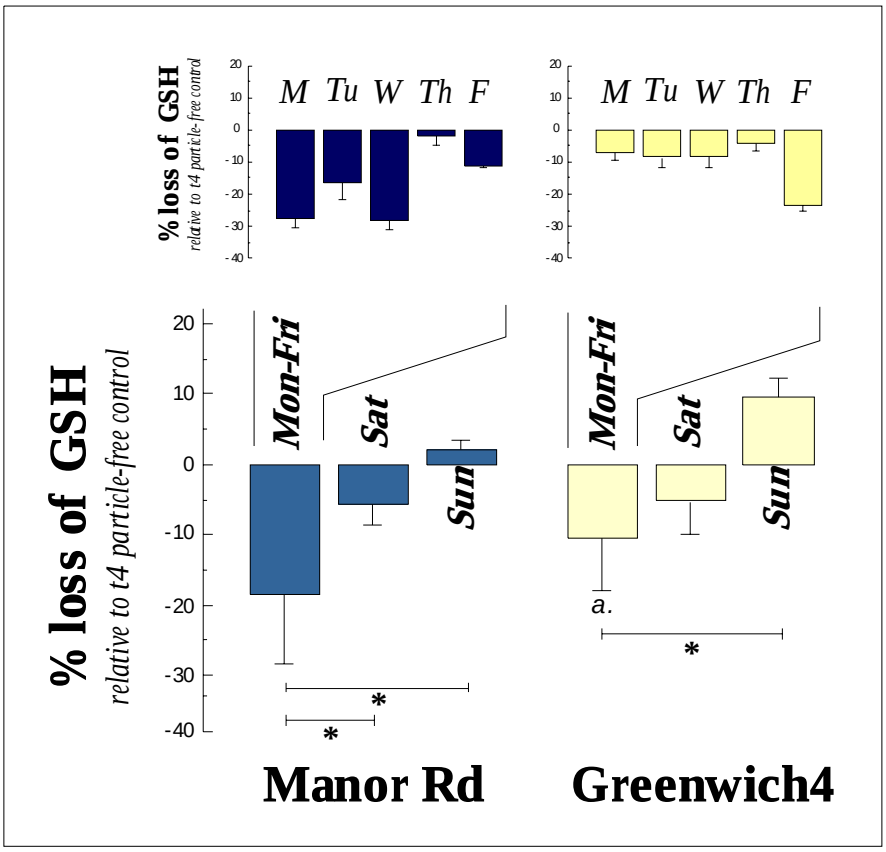


Figure 5: Percentage loss of reduced glutathione relative to the 4h particle free control concentration in antioxidant solutions incubated with Manor Rd and Greenwich 4 filter PM samples. Comparison is made between the weekday samples (n=5x3), with those obtained on Saturday (the half working day at Manor Road) and Sunday. All data are illustrated as mean±SD. Statistical analysis was performed using a one-way ANOVA with post-hoc analysis performed using the Student-Newman-Kuels test. '*' illustrates significant differences between filter reactivities at a given site; 'a' signifies a significant difference in the % loss observed between the site across each of the sampling intervals.

4.2.3 Urate Depletion

Urate is very sensitive to depletion through the generation of hydroxyl radicals; very reactive free-radical species often generated in metal-catalysed reactions. Examining its reaction, or lack of it, with PM help us to define the nature of the reaction(s) between the PM and the respiratory tract lining fluid antioxidants.

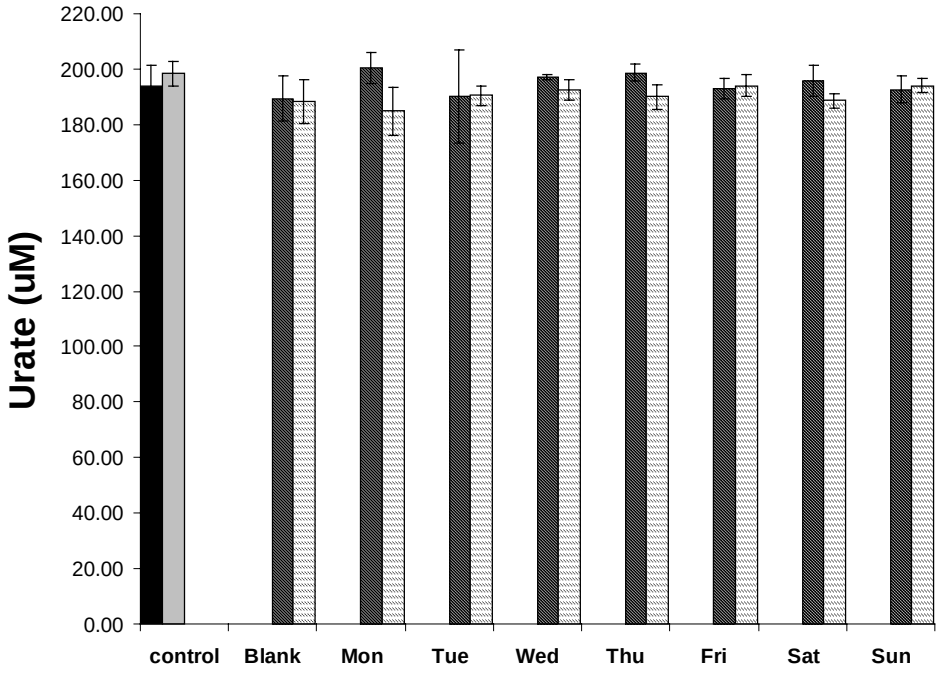


Figure 6: Loss of urate from a composite antioxidant solution incubated for 4h with PM (50mg/ml) extracted from Teflon filters collected daily (Mon-Sun) at Bexley, Manor Road and Greenwich 4. All details are as outlined in the legend to Figure 2.

Summary of observations:

- None of the samples collected from either site show any activity toward urate. This in activity is expected in transition samples where activity is limited to that from transition metals

4.2.4 Relative Ascorbate and Glutathione Depletion

Manor Road samples exhibited greater PM reactivity during the working week (Mon-Fri) per unit mass, with a fall off in PM reactivity on Saturday and the lowest activity observed in the Sunday sample. The activity of the weekday sample from Manor Road was significantly greater than the parallel Greenwich 4 sample (*Ascorbate*: -70.5±10.3 [GR] vs. –75.5±7.4% [Manor Rd], $P<0.05$; *Reduced glutathione*: -10.5±7.6 [GR] vs. –18.6±9.7% [Manor Rd], $P<0.05$; One-way ANOVA). Notably, there was considerable variation in the daily (Mon-Fri) activity of PM from both sites. For example, ascorbate losses ranged from –50.7 to –72.7% at GR vs. –69.5 to –87.3% at Manor Rd (c. figures 2 and 4).

Whilst weekday samples tended to be more reactive at the Manor Road site in terms of ascorbate depletion, the losses observed with the weekend samples obtained from Greenwich 4 were significantly greater than those seen with the Manor Road samples. This contrasted markedly with the reduced glutathione results where the reactivity's observed on Saturday and Sunday were broadly comparable, with no detectable activity in the later samples.

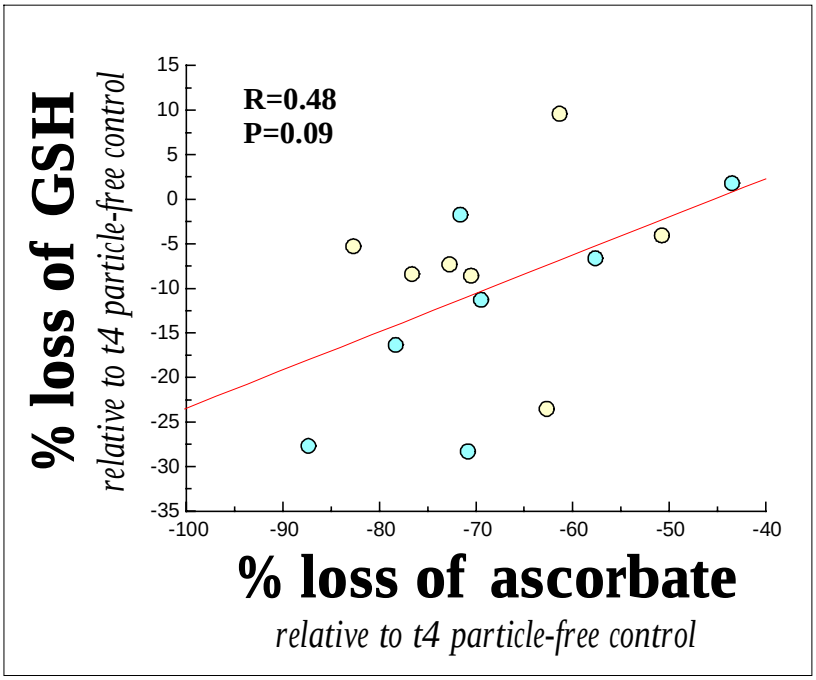


Figure 11: Relationship between the loss of reduced glutathione and ascorbate in composite antioxidant solutions incubated with PM (50µg/ml) from Manor Road (blue) and Greenwich 4 (yellow) sites. The strength of association between these two measures of oxidative activity was assessed using Pearson Correlation Coefficient. The fitted line illustrates the best fit linear regression through the data set.

Previously we have always tended to see a strong association between the extent of ascorbate and reduced glutathione from the composite antioxidant solution following incubation with model and environmental PM. One might expect this if the mechanism driving the loss of the antioxidants was the same, i.e. transition metal dependent. In this study we did not observe a strong correlation between these indices (c. figure 11). Interestingly, when the Manor Road samples (n=7) were considered on their own a weak association was apparent (r=0.74, P=0.06 – Pearson's Correlation, two-tailed), whilst there was clearly no underlying relationship in the Greenwich 4 samples.

4.2.5 Metal analysis

Total metal content following nitric acid digestion

Filters were assessed for the following metals: Al, As, Cd, Cu, Fe, Pb, Mg, Pt, W and Zn. Of these, platinum, tungsten and arsenic were below the limit of detection. Cadmium and copper were present at very low levels, and after subtraction of background filter concentrations, only measurable concentrations of Cu were seen in two of the 14 filters tested: BX-Mon (0.02ng) and BX-Weds (0.08ng). No Cd was seen in any of the Greenwich filters, but very low levels were detected in the Bexley samples (Mon-Sat). However as the background 'clean' filter concentration of cadmium varied 6-fold between the Bexley, Manor Rd and Greenwich 4 samples these data were considered unsuitable for inclusion in the full metal analysis. Therefore, detailed consideration is limited to Al, Fe, Pb, Mg, Ni and Zn. A total transition metal concentration was achieved by summing the concentration of each of these metals with the exception of Mg. All metal concentrations were standardized to the PM mass on the filter. These data are summarised in figure 7.

Major observations regarding filter total metal content:

- **Total Transition Metals:** No significant difference was observed between the overall transition metal concentration seen between the two sites: 17.27±14.42 vs. 16.27±10.28ng (standardised). Notably, there was relatively little metal in the Sunday sample from Manor Road and the Saturday and Sunday samples from Greenwich 4.

- **Aluminium**: Represents the second most abundant metal in the Manor Road filters and the third in the Greenwich filters. There was no significant difference in the overall concentrations (Mon-Sun) of Al between the two sites: 2.70±1.29ng [Manor Road] vs. 1.57±1.10ng [Greenwich 4], P=0.11. Notably, Manor Road filters contained almost 5-fold more Al across the weekend sampling than those obtained from the Greenwich 4 site: 1.91±0.002 [Manor Road] vs. 0.40±0.29ng [Greenwich 4].

- **Iron**: Fe was the most abundant metal in filters obtained from both sites. As with Al, there was no evidence for a significant difference in Fe concentrations between the two sites considering all the daily filters: 11.54±8.33ng [Manor Road] vs. 11.36±10.14ng [Greenwich 4]. Comparisons using only the working week samples also failed to reveal any significant difference. Interestingly, at the Manor Road site the Saturday sample had relatively high levels of Fe, 9.14ng compared with the parallel Greenwich 4 sample, 1.8ng. No Fe was detectable from either site in the Sun samples.

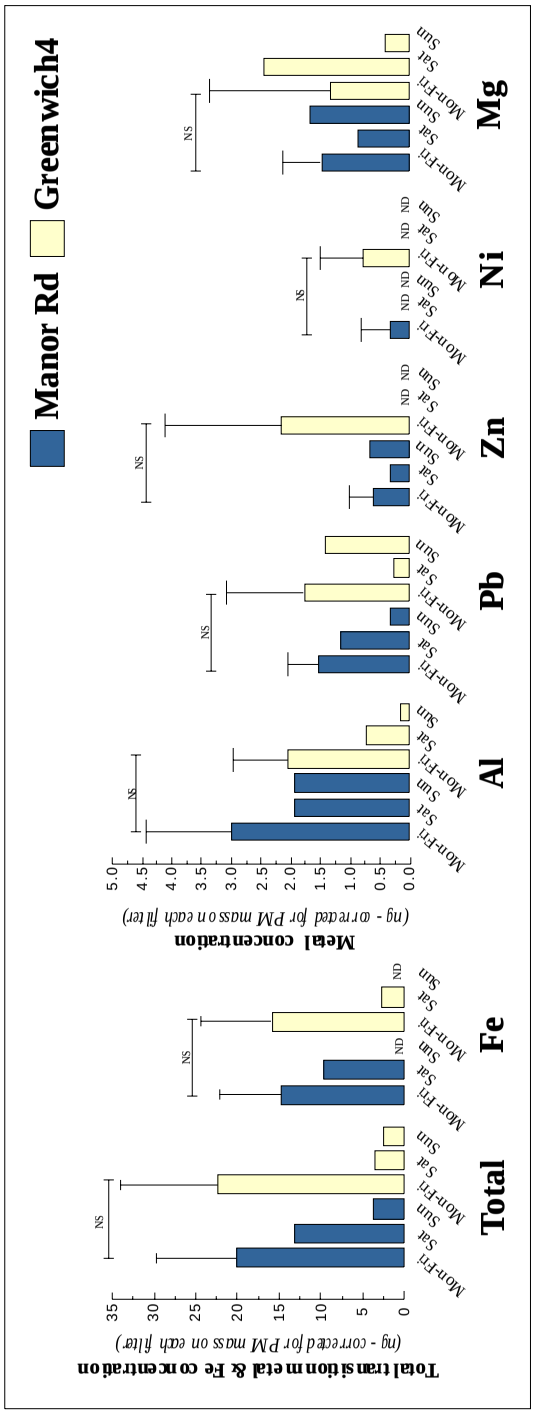
- **Copper**: concentrations were mainly below the limit of detection.

- **Zinc**: Zn concentrations were low in the Manor Road samples; with an overall weekly mean of 0.55±0.35ng, compared with a much higher concentration at the GR site, 1.52±1.91ng. This high mean value in the Greenwich 4 filters was primarily due to very high concentrations on the Mon and Thurs filters. Consequently, the difference in mean concentration between the two sites failed to reach statistical significance. No Zn was detected in the Manor Road sample over the weekend period.

- **Lead**: As with each of the previous metals considered there was no overall difference in the weekly mean lead concentrations between the two sites: 1.27±1.91ng [Manor Road] vs. 1.48±1.22ng [Greenwich 4]. At the Manor Road site there was clear evidence that the lead concentrations detected followed the pattern of work at this site, with higher concentrations on Mon-Fri, and intermediate concentrations on Sat and only trace levels on Sun.

- **Magnesium**: No difference in Mg concentration between sites across the studies week: 1.40±0.59ng [Manor Road] vs. 1.33±1.78ng [Greenwich 4].

- **Nickel**: Low concentrations of Ni were detected in approximately 50% of the collected filters.



Filter	Al	Cd	Cu	Fe	Pb	Mg	Ni	Zn	Total
M.rd Mon	5.224	0.030	0.024	25.939	2.318	2.354	0.264	0.937	34.736
M.rd Tues	2.103	0.050	0.000	10.546	1.350	1.151	0.000	0.651	14.700
M.rd Wed	3.612	0.040	0.000	13.905	1.592	1.913	0.000	0.937	20.086
M.rd Thur	2.285	0.020	0.000	16.192	1.019	0.770	1.136	0.000	20.652
M.rd Fri	1.734	0.030	0.000	5.044	1.198	1.114	0.000	0.378	8.385
M.rd Sat	1.908	0.020	0.000	9.138	1.136	0.833	0.000	0.296	12.497
M.rd Sun	1.911	0.000	0.000	0.000	0.294	1.654	0.000	0.643	2.848
GR-4 Mon	2.395	0.000	0.000	13.027	0.336	1.282	1.282	4.055	21.095
GR-4 Tues	1.701	0.000	0.101	18.457	3.738	0.000		0.893	24.890
GR-4 Wed	0.878	0.000	0.000	6.521	0.784	0.000	0.125	0.502	8.810
GR-4 Thur	3.355	0.000	0.000	29.240	2.037	4.853	1.438	4.494	40.565
GR-4 Fri	1.830	0.000	0.000	10.469	1.855	0.366	0.122	0.708	14.983
GR-4 Sat	0.690	0.000	0.000	1.804	0.239	2.427	0.000	0.000	2.733
GR-4 Sun	0.119	0.000	0.000	0.000	1.389	0.377	0.000	0.000	1.508

All metal concentrations quoted as ng per unit mass of PM

Figure 7: Total and individual metal concentrations in filters collected from the Bexley, Manor Road and Greenwich 4 sites. Individual filter data are illustrated in the inset table. The main figure collates the individual metal data across the Mon-Fri period at the two sites, with data expressed as the mean±SD. Comparison of the week day metal concentrations were performed using Student's unpaired t-test. All concentrations are standardized per mass of PM. ND = not determined. NS = not significant.

Correlation Analysis

The relationship between the extent of antioxidant depletion and the concentration of metals on the PM filters were examined using Pearson's correlation. When all 14 filters were considered in a single analysis no significant associations between antioxidant depletion and any of the metals were observed. These analyses were than repeated considering the data obtained from the two sites separately. These data are summaraised in Figures 8 and 9 in the form of correlation matricies.

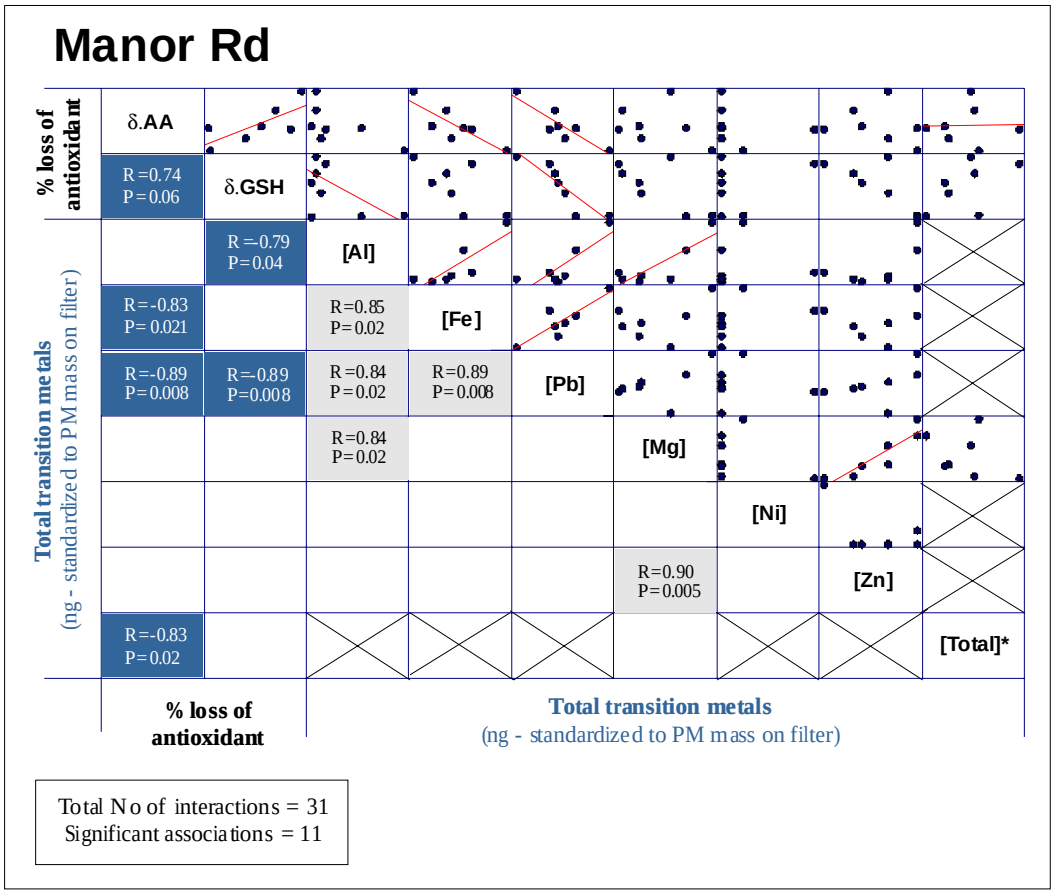


Figure 8: Correlations between filter PM oxidative activity and metal content in filters obtained from Manor Road. The results of the primary analyses are highlighted in blue, secondary interactions between metals in grey. Where significant interactions were observed scatter plots with trend lines are shown. Where matrix squares are crossed out correlation analyses were not performed, as individual metals were summed to obtain the total transition metal concentration.

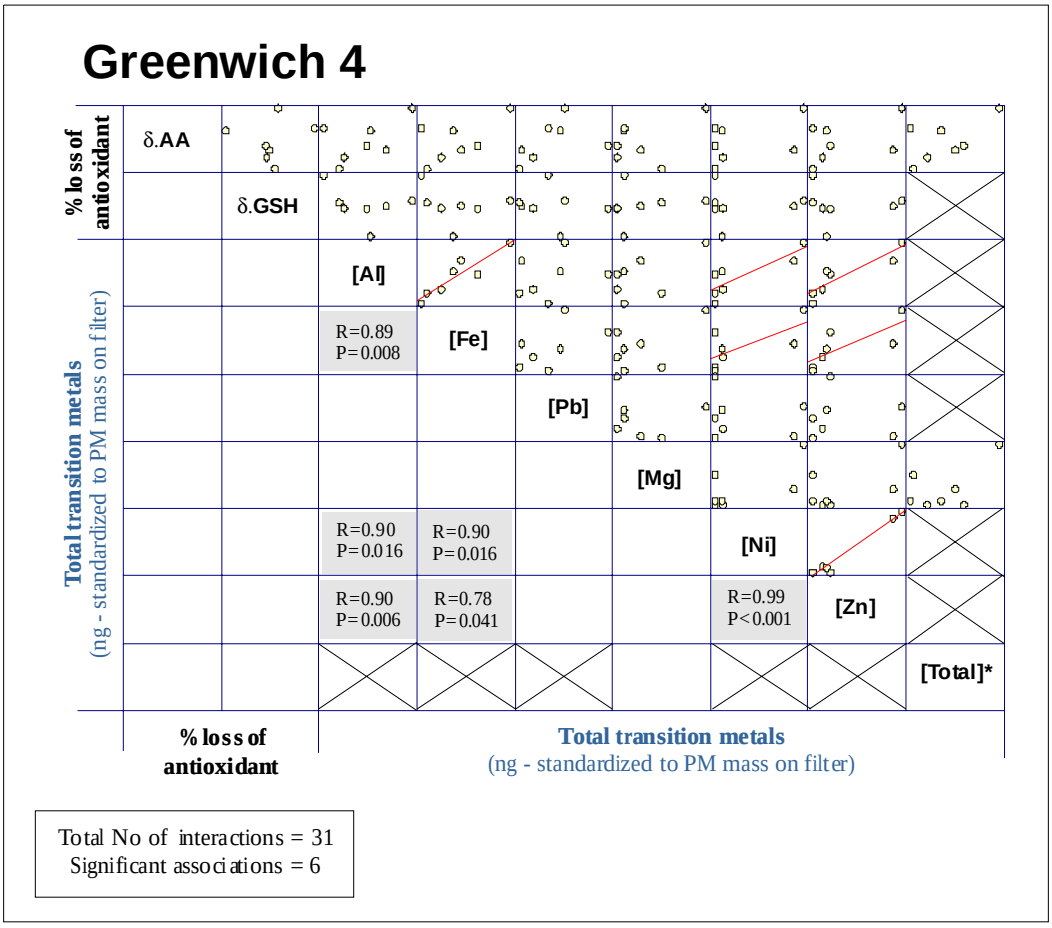


Figure 9: Correlations between filter extract oxidative activity and metal content in filters obtained from the Greenwich background site. All details as outlined in the legend to Figure 8.

When the Manor Road samples were analysed separately we observed significant associations between the degree of ascorbate loss (oxidation) and sample Fe, Pb and total transition metal concentrations. In contrast the loss of glutathione was not related to Fe, but rather to Al and Pb concentrations (c. figure 8). No such interactions were apparent in the Greenwich 4 samples (c. figure 9). Differences were also noted between the two sites between the underlying associations between the PM metals. In the Manor Road sample Pb was strongly correlated to both Fe and Al, whereas no such interactions were apparent in the Greenwich 4 samples. In contrast Zn and Ni concentrations were strongly associated in Greenwich 4 sample, as were Ni/Zn with Fe and Al.

Water Leachable Iron

The metal concentration quoted for the filter PM above refers to the total metal pool following extensive acid extraction. In the lung, we feel that it is unlikely that all this iron (or indeed other metals) will be available to drive the biological reactions. In order to assess the water leachable (freely accessible and soluble) iron pool, we developed a new assay using the iron chelator, bathophenantroline disulphonate (BPS). BPS will bind ferrous iron (Fe^{2+}) to form a complex that absorbs strongly at 535nm permitting the quantification of the soluble ferrous iron. In addition, by incubating PM suspensions in the presence of the reductant ascorbate at high concentrations (10mM) ferric iron is converted to the ferrous form and thus becomes measurable by BPS to give a total soluble iron pool concentration. We used this method on the particle suspensions remaining after the antioxidant incubation experiments. Due to the scarcity of the material these analysis were only possible on 8 (Mon, Weds, Thurs and Sat from both sites) of the 14 filter samples. These data are summarised in Figure 10.

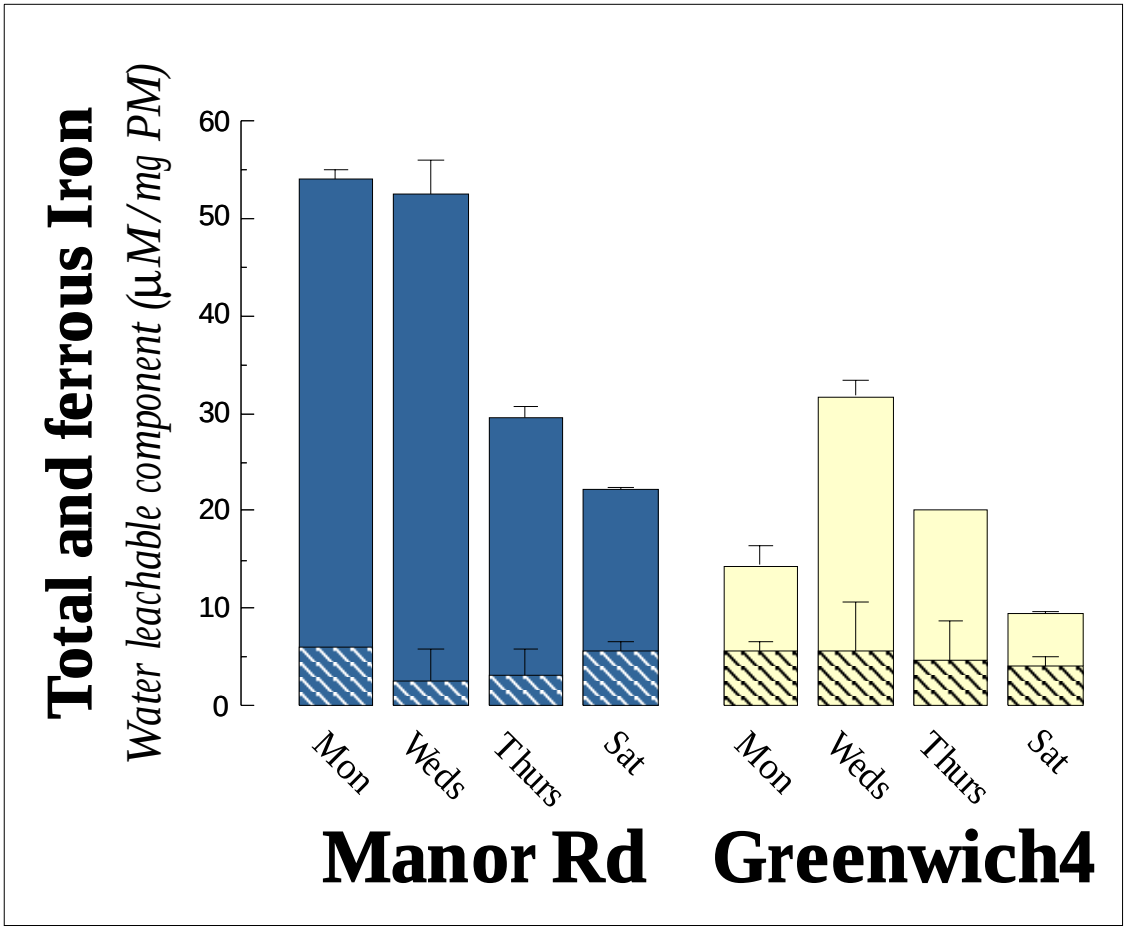


Figure 10: Total (solid bars) and ferrous (hatched bars) water-soluble iron concentrations in filters collected from the Bexley, Manor Rd and Greenwich 4 sites. Samples for these analyses were only available for 4 of the 7 daily samples.Based on previous experience we know that the loss of ascorbate from artificial respiratory tract lining fluid is partially explained by its oxidation during the reduction of ferric to ferrous iron. Given this, we expected samples with high ferric iron metal content to be the most reactive.

Based on this criterion, we would expect the Bexley samples to be the more active across the whole sampling period. However, clearly this was not the case at the weekend. Therefore these data do not explain the heightened PM reactivity that was noted in the Greenwich 4 Saturday sample, implying that some other PM component is driving this effect.

Given that only 8 samples were available for this type of analyses (4 from each site) it was not appropriate to attempt a correlation analysis between the soluble iron content and antioxidant depletion.

4.3 Metal Chelation Experiments

To demonstrate the causation between transition metal content of PM with oxidative activity, metal chelation experiments were performed. In these experiments PM were incubated (50μg/ml) in the composite antioxidant solution with or without the inclusion of the Cu^{2+} and Fe^{3+} metal chelator diethylenetriaminepentaacetic acid (DTPC at 200μM). Inclusion of this DTPC should effectively remove the oxidising activity of iron and cupper associated with the PM samples. Experiments were performed on pooled PM suspensions covering the period Mon-Fri, as well as on the separate Sat and Sun samples. In all cases incubation with DTPC completely blocked PM-dependent ascorbate and reduced glutathione oxidation. These data are summarized in the table below:

Site	Interval	%loss AA (-DTPC)	%loss AA (+DTPC)	%loss GSH (-DTPC)	%loss GSH AA (+DTPC)
Manor Road	*Mon-Fri*	-66.3±7.6	-3.7±1.9*	-31.1±6.5	-4.8±1.4*
	Sat	-55.0±5.5	-2.5±1.7*	-15.3±5.7	9.1±0.0*
	Sun	-45.6±2.6	-5.1±4.3*	-24.4±1.6	4.5±0.0*
Greenwich 4	*Mon-Fri*	-77.1±3.0	-3.2±2.8*	-22.9±2.5	-1.7±3.3*
	Sat	-82.3±3.6	-0.8±1.9*	-26.0 ±2.9	1.5±3.6*
	Sun	-55.3±2.3	-2.8±0.3*	-19.7±6.3	-0.4±3.6*

All values represent the mean of three separate experiments. The percentage loss of each antioxidant is expressed relative to the time zero control. This amendment was made, in contrast to other % losses, expressed as a percentage of the C4 particle free control as the incubation with DTPC also knocked out the background auto-oxidation seen in the particle free controls. '' indicates that DTPC significantly ($P<0.05$) protects against antioxidant oxidation.*

5 Conclusions

5.1 Comparison of Oxidant Activity at Manor Road and Greenwich 4

The purpose of the study was to examine the oxidative activity of PM_{10} collected at Manor Road compared to that at the nearby background site Greenwich 4. The study was built around the supposition that the local PM_{10} at Manor Road would have a different oxidant activity (and therefore toxicity) to that at a background locations. Assuming Greenwich 4 to be representative of background conditions at Manor Road the difference between the oxidative activity at the two sites would enable the oxidant activity of the additional local PM_{10} at Manor Road to be determined. This would allow the Manor Road PM_{10} concentrations to be placed in a health context.

The pattern of elevated local PM_{10} has been well established at Manor Road (Baker and Fuller 2001). Elevated PM_{10} being measured during the day time on each weekday and in the morning on Saturdays. No elevated PM_{10} is measured at Manor Road on Sundays. The study was designed to utilise this known pattern of local PM_{10} at Manor Road by comparing samples from each day of the week. Multiple filter exposures were used and a total of 26 collection days at each site were subjected to oxidant and metals analysis.

From the known pattern of local PM_{10} at Manor Road we expected distinct differences to be exhibited between weekday samples and Sunday samples, with those on Saturday lying between the two. However, on making these measurements we found the oxidant activity at the two sites varied considerably on a daily basis and it is therefore difficult to draw conclusions from daily comparisons between the two monitoring sites. However, the average weekday oxidant activity per unit mass in the Manor Road PM_{10} is greater than that at Greenwich 4. This is consistent for both ascorbate and glutathione. For the weekend samples the pattern is less clear with greater ascobate depletion being exhibited by the weekend samples at Greenwich 4 and no conclusive differences between the glutathione depletion. The results from the weekend samples suggest that the Greenwich 4 site may not be a good background for the Manor Road site.

If Manor Road is however considered in isolation of Greenwich 4 a clearer pattern is seen in the oxidant activity with activity being greatest on weekdays, lower on Saturday and lowest on Sunday. This matches the pattern of local PM_{10} sources suggesting that the PM_{10} at Manor Road is more active when local sources making a contribution.

5.2 Mechanisms Driving the Oxidant Activity

Although samples from both sites exhibited oxidant activity in their reactions with ascorbate and glutathione they were relatively inert in their reactions with urate. Both ascorbate and glutathione reactions are determined by transition metals whereas urate is very insensitive to depletion through free radical generation from transition metals. Thus the findings with urate have an important physiological relevance, and ensure that the assay accurately mimics the main antioxidants in human lung lining fluid.

In previous studies with environmental particulate matter there has been a strong association between the glutahione and ascorbate depletions. This would be expected if the mechanism driving the loss of the antioxidants was the same, i.e. transition metal dependent. In this study we did not observe a strong correlation between these indices at the two sites. A weak association was apparent with the Manor Road samples but there was no underlying relationship in the Greenwich 4 samples. This may suggest that the mechanisms driving the oxidant activity are different at the two sites as would be expected due to the local PM_{10} sources at Manor Road, which do not affect Greenwich 4.

When however oxidant mechanisms driven by copper (specifically Cu^{2+}) and iron (as Fe^{3+}) were blocked, samples from both sites lost significant oxidant activity. This finding suggests that although the mechanisms driving the oxidant activity in the PM_{10} from both sites are different, the majority of the oxidant activity at both Manor Road and Greenwich 4 are due to transition metals.

5.3 PM_{10} Composition

To shed light on the differences in oxidant activity at Manor Road and Greenwich 4, samples were analysed for transition metals. Two methods were used. One method measured the total metal content while the second measured the water soluble iron content.

Correlations between total transition metals following nitric acid digestion and the oxidant activity in the Manor Road were considered. These samples showed significant associations between the ascorbate depletion and concentrations of iron, lead and total transition metals. In contrast the loss of glutathione was not related to iron, but rather to aluminium and lead concentrations. No such interactions were apparent in the Greenwich 4 samples although the Greenwich filters did contain considerable amounts of iron, aluminium and lead. Thus, these metals do not appear to be driving the antioxidant losses observed at Greenwich 4 . Further, at Manor Road we found that the concentration of lead was strongly correlated to both iron and aluminium suggesting that they have the same source, or sources. Total transition metals, iron and lead exhibit a significant difference in concentration between weekdays, Saturdays and Sundays following the established pattern of local PM_{10} at Manor Road. The source for these metals is likely to be partially responsible for daily pattern in the oxidant activity in the Manor Road PM_{10}. However, determining the sources(s) is more problematic since metals were also present in the Greenwich samples and no statistically significant difference was observed in the weekday concentration of these metals between the two sites.

Water-leachable iron content was considered for a sub set of the samples analysed. The water leachble iron is a better determinant of the iron that would be bio-available to react in the lung, and in the oxidant depletion tests, than total iron. The water leachable iron analysis can also differentiate between the iron ion oxidation states. The Manor Road samples were found to have more soluble iron than those from Greenwich 4 and far greater amounts of Fe^{3+}. The oxidative activity is partially explained by the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron and therefore samples with high ferric iron metal content are expected to be the most reactive. This approach revealed a difference between the composition of PM_{10} at Manor Road and Greenwich 4 with substantially greater concentrations of ferrous iron at Manor Road. Based on this criterion, we expected the Bexley samples to be the more active across the whole sampling period. However, clearly this was not the case at the weekend. Therefore these data do not explain the heightened PM reactivity that was noted in the Greenwich 4 Saturday sample, implying that another metal component other than leachable iron is driving this effect.

Leachable or bio-available iron is far more prevalent in Manor Road samples that those from Greenwich 4. This contrasts with the total iron content from the nitric digestion suggesting that the iron content of PM_{10} at Manor Road is in a different chemical form to that at Greenwich 4.

5.4 Assessing overall health risk

We have hypothesized that mass is not the only metric of PM_{10} toxicity, but that particle oxidative activity is an important determinant of potential health effects. The preceding analysis compares the oxidative activity on an equal mass basis. In order to compare health effects between the daily samples at Manor Road and to compare the health effects at Manor Road to those at Greenwich 4 we have to factor in the atmospheric concentration of PM_{10}. To achieve this, the atmospheric PM_{10} concentration has been multiplied by the sample oxidant activity (based on ascorbate depletion) to achieve an overall health effects index. This approach is shown in Figure 11.

The health effects index suggests that the PM_{10} burden at Manor Road may have a greater health effect than the PM_{10} burden at Greenwich 4. The health effects arising from the PM_{10} burden at Manor Road is greater on weekdays, than Saturdays, with lowest health effects arising from the Sunday PM_{10}. This matches the pattern of local PM_{10} at Manor Road and suggests that the local sources of PM_{10} make an additional contribution to the toxicity of PM_{10} at Manor Road.

To date, only limited information is available regarding oxidative activity of PM at different locations. Currently, the antioxidant depletion assay utilised in the study to measure oxidant activity is being employed in a comparative study of 6 sites in the UK (sponsored by the Medical Research Council) and 8 sites in Europe (supported under EU Framework V). Although these studies are still in progress

it can be said that PM collected in London (Lambeth Palace road) has higher oxidant activity than some other cities such as Cardiff, Sheffield, Munich, while Rome has PM with similar oxidant activity. The 'oxidative activity' per unit mass of PM collected at the Manor road for this study is similar to that measure for PM collected at Lambeth Palace Road. Given the higher masses of PM at Manor Road the overall oxidative burden must be considered to be high, even for London.

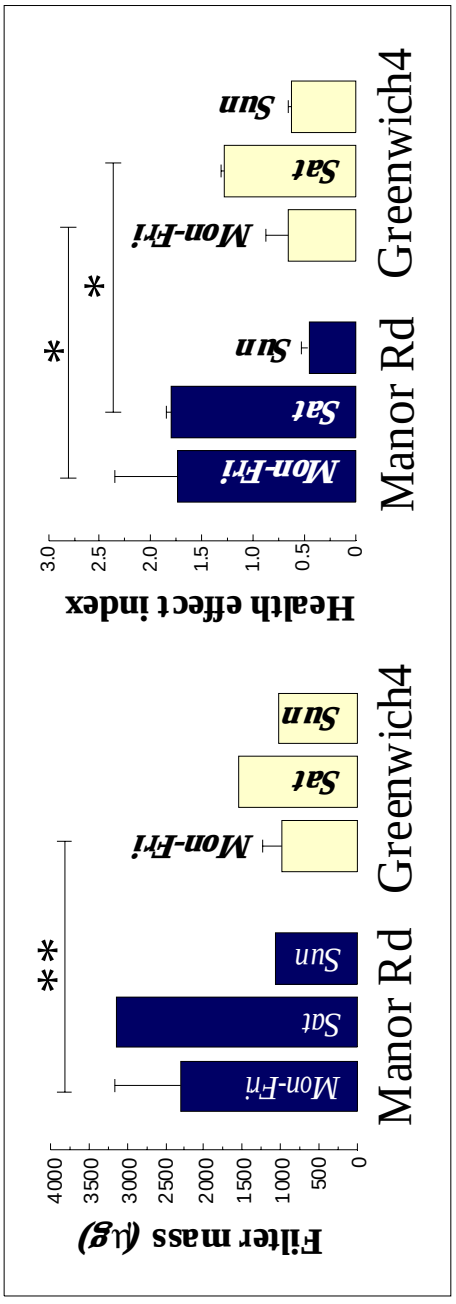


Figure 11: Filter masses obtained form Manor Road and Greenwich 4 with a derived health effect index derived from the mass, and oxidant activity.

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Appendix

Antioxidant concentrations remaining in the RTLF model following a 4h incubation (37°C, ph 7.4) with daily PM samples obtained from the Manor Road (BX44) and Greenwich (GR4) sites.

All antioxidant concentrations (AA-ascorbate; UA-urate & GSH-reduced glutathione) are expressed as mol/L. C0 refers to the antioxidant concentration at time zero, before the addition of particles; C4 the antioxidant concentrations after the 4h incubation in the particle free control.

Sample Code	AA	Mean	SD	UA	Mean	SD	GSH	Mean	SD
C0	208.04			202.32			178.78		
C0	202.63			192.22			166.45		
C0	199.35	203.34	4.39	187.69	194.08	7.49	187.76	177.66	10.70
C4	173.03			197.05			142.91		
C4	160.01			203.10			135.12		
C4	170.83	167.96	6.96	194.94	198.36	4.24	128.36	135.46	7.28
GR4 Mon	44.12			205.72			122.04		
GR4 Mon	41.99			200.46			126.95		
GR4 Mon	51.33	45.81	4.89	194.58	200.26	5.57	127.78	125.59	3.10
GR4 Tue	-			172.83			122.63		
GR4 Tue	46.77			206.56			128.86		
GR4 Tue	52.28	49.53	3.89	190.76	190.05	16.88	120.12	123.87	4.50
GR4 Wed	-			198.21			-		
GR4 Wed	39.45			197.03			120.91		
GR4 Wed	39.05	39.25	0.28	196.03	197.09	1.09	127.28	124.10	4.51
GR4 Thu	79.13			199.99			132.72		
GR4 Thu	86.35			201.01			130.52		
GR4 Thu	-	82.74	5.11	195.38	198.79	3.00	126.62	129.96	3.09
GR4 Fri	65.71			197.40			102.46		
GR4 Fri	56.25			191.25			102.41		
GR4 Fri	66.17	62.71	5.60	190.78	193.14	3.70	105.94	103.60	2.02
GR4 Sat	27.86			195.27			123.46		
GR4 Sat	26.81			190.51			126.07		
GR4 Sat	32.53	29.07	3.05	201.50	195.76	5.51	135.46	128.33	6.31
GR4 Sun	63.02			188.27			151.51		
GR4 Sun	70.46			197.68			144.31		
GR4 Sun	61.34	64.94	4.85	192.02	192.66	4.74	149.47	148.43	3.71
BX4 Mon	20.13			186.77			99.18		
BX4 Mon	26.85			192.65			93.38		
BX4 Mon	16.82	21.26	5.11	175.64	185.02	8.64	101.39	97.98	4.13
BX4 Tue	35.96			194.34			110.72		
BX4 Tue	40.37			188.26			121.56		
BX4 Tue	32.93	36.42	3.74	188.78	190.46	3.37	107.71	113.33	7.28
BX4 Wed	-			196.21			93.40		
BX4 Wed	51.62			188.84			100.54		
BX4 Wed	46.39	49.00	3.70	192.52	192.52	3.69	97.54	97.16	3.59
BX4 Thu	-			192.70			-		
BX4 Thu	48.73			192.54			133.13		
BX4 Thu	46.58	47.66	1.52	184.66	189.97	4.60	127.15	133.13	4.23
BX4 Fri	45.28			197.09			120.76		
BX4 Fri	45.99			195.46			119.60		
BX4 Fri	62.51	51.26	9.75	189.44	194.00	4.03	117.91	120.18	0.81
BX4 Sat	70.42			189.03			123.34		
BX4 Sat	69.27			190.76			129.65		
BX4 Sat	73.84	71.18	2.38	185.86	188.55	2.49	130.37	126.49	4.46
BX4 Sun	81.73			196.03			136.34		
BX4 Sun	100.54			194.82			139.49		
BX4 Sun	102.55	94.94	11.48	191.48	194.11	2.36	139.07	137.92	2.22

Antioxidant concentrations remaining in the RTFL model following a 4h incubation (37^0C, ph 7.4) with workday (Mon - Fri); half workday (Sat) and rest day (Sun) PM samples obtained from the Manor Road (BX4) and Greenwich (GR4) sites. incubations were either performed in the presence or absence of the Cu & Fe chelator DTPC.

All antioxidant concentrations (AA-ascorbate; UA-urate & GSH-reduced glutathione) are expressed as mol/L. C0 refers to the antioxidant concentration at time zero, before the addition of particles; C4 the antioxidant concentrations after the 4h incubation in the particle free control.

Sample description	AA	Mean	SD	UA	Mean	SD	GSH	Mean	SD
C0	240.51			195.45			175.06		
C0	232.66			202.45			190.00		
C0	236.34	236.17	3.99	154.39	197.43	4.38	176.15	180.40	8.33
C4	232.06			200.91			152.54		
C4	234.61			196.85			154.52		
C4	226.74	231.20	4.10	200.45	199.40	2.22	156.38	154.48	1.92
GR4 mon-fri	49.61			195.73			143.99		
GR4 mon-fri	50.40			193.44			138.31		
GR4 mon-fri	62.39	54.13	7.16	196.75	195.31	1.70	135.13	139.14	2.59
GR4 mon-fri +DTPC	233.78			198.65			182.83		
GR4 mon-fri +DTPC	221.14			192.85			170.94		
GR4 mon-fri +DTPC	231.00	228.64	6.64	196.76	196.09	2.96	178.13	177.30	5.99
GR4 sat	51.55			200.04			137.41		
GR4 sat	37.47			199.58			135.52		
GR4 sat	36.24	41.75	8.51	192.84	197.49	4.03	127.68	133.64	5.16
GR4 sat +DTPC	229.43			200.69			175.60		
GR4 sat +DTPC	237.94			193.00			187.21		
GR4 sat +DTPC	236.43	234.27	4.37	197.94	197.21	3.90	186.46	183.09	6.50
GR4 sun	102.04			193.50			131.76		
GR4 sun	102.97			196.99			150.18		
GR4 sun	111.86	105.62	5.41	192.40	194.97	3.53	152.35	144.76	11.31
GR4 sun +DTPC	229.12			194.20			186.60		
GR4 sun +DTPC	229.11			197.63			178.91		
GR4 sun +DTPC	230.24	229.49	0.65	191.52	194.45	3.06	173.65	179.72	6.51
BX4 mon-fri	86.57			194.97			127.44		
BX4 mon-fri	98.69			195.95			111.26		
BX4 mon-fri	92.35	79.21	18.00	188.02	192.98	4.32	134.06	124.26	11.73
BX4 mon-fri +DTPC	224.07			199.70			188.12		
BX4 mon-fri +DTPC	232.50			191.35			192.02		
BX4 mon-fri +DTPC	226.02	227.53	4.42	187.86	192.97	6.08	187.25	189.13	2.54
BX4 sat	116.46			189.51			140.98		
BX4 sat	110.64			194.34			159.68		
BX4 sat	91.75	106.28	12.91	193.45	192.43	2.57	157.88	152.85	10.32
BX4 sat +DTPC	239.00			196.97			196.74		
BX4 sat +DTPC	232.06			191.90			202.28		
BX4 sat +DTPC	225.70	230.25	3.97	194.10	194.32	2.65	214.57	204.53	9.13
BX4 sun	123.36			193.52			131.32		
BX4 sun	136.13			192.99			136.48		
BX4 sun	126.93	128.47	6.04	195.99	194.17	1.60	136.86	134.55	2.89
BX4 sun +DTPC	239.37			197.59			188.46		
BX4 sun +DET	213.18			195.82			208.88		
BX4 sun +DET	225.61	224.06	10.18	193.84	195.75	1.88	214.08	203.81	13.54

Ferric (Fe^{3+}) and total (Fe^{3+} and Fe^{2+}) iron concentrations in a selection of the daily PM samples collected from the two sites in this study. Individual values and mean ± SD are summarised.

All iron concentrations (ferric and total) are expressed as mmol per unit mass of PM.

Sample	Iron	Fe(umol/mg PM)				
		a	b	c	Mean	SD
BX4 (Mon)	Fe2+	6.20	6.20	6.20	6.20	0.00
BX4 (Weds)	Fe2+	0.00	1.55	6.20	2.58	3.23
BX4 (Thur)	Fe2+	0.00	4.65	4.65	3.10	2.68
BX4 (Sat)	Fe2+	6.20	6.20	4.65	5.68	0.89
BX4 (Mon)	Fe2+ + Fe3+	55.23	53.56	53.56	54.12	0.97
BX4 (Weds)	Fe2+ + Fe3+	55.23	48.54	53.56	52.45	3.48
BX4 (Thur)	Fe2+ + Fe3+	30.13	30.13	28.45	29.57	0.97
BX4 (Sat)	Fe2+ + Fe3+	21.76	23.43	21.76	22.32	0.97
GR4 (Mon)	Fe2+	6.20	4.65	6.20	5.68	0.89
GR4 (Weds)	Fe2+	0.00	7.75	9.30	5.68	4.98
GR4 (Thur)	Fe2+	0.00	7.75	6.20	4.65	4.10
BX4 (Sat)	Fe2+	3.10	4.65	4.65	4.13	0.89
GR4 (Mon)	Fe2+ + Fe3+	13.39	16.74	13.39	14.51	1.93
GR4 (Weds)	Fe2+ + Fe3+	33.48	30.13	31.80	31.80	1.67
GR4 (Thur)	Fe2+ + Fe3+	20.09	20.09	20.09	20.09	0.00
GR4 (Sat)	Fe2+ + Fe3+	10.04	10.04	8.37	9.48	0.97