Environment Canada Environnement Canada

# Water Science and Technology Directorate

# Direction générale des sciences et de la technologie, eau



# NANOSCIENCE APPLICATION TO THE CHARACTERIZATION OF $PM_{10}$ GROUND-LEVEL AIR PARTICLES WHICH IMPACT ON URBAN SURFACE WATER

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WSTD Technical Note No: TN11-011



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# Nanoscience Application to the Characterization of $PM_{10}$ Ground-Level Air Particles Which Impact on Urban Surface Water

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# Abstract

In a joint study by NWRI and the City of Toronto, the Aquatic Ecosystem Management Research Division of the National Water Research Institute published reports in 2006 and 2007 on deposit fractions of ground-level air particles which impacted on urban surface waters. These reports presented case studies of Toronto street dust, with the former one including a spectromicroscopical characterization of the ultrafine ($PM_{10}$) fraction of the particles. The original focus was on the effectiveness of street sweeping in stormwater pollution source control, while the $PM_{10}$ characterization focused on the detection of particles bearing toxic metals. Then, a potential resuspension of ultrafine particles from street sweepers was investigated, with early findings igniting a search for potentially toxic nanoparticles. It soon became apparent that collecting ground-level air particles *en masse* at source (with a subsequent fractionation of the total sample) was highly problematic. This 2011 report outlines the technical problems and all the various attempts to cope with them, for the purpose of obtaining representative non-artifacted fractions. While the earlier results remain valid, in the qualitative sense that the individual types of $PM_{10}$ particles characterized did exist in the sample, it was deemed important to test all applicable methods to improve the quantitative aspect of the work. An exhaustive search of the literature and repeated experimental attempts to reduce the artifact problem were continued to gain as much understanding as possible about what can be learned from improved sample preparation. In 2009, fractionating the ultrafine particles at source was revealed to be the only approach permitting proper quantification. A recently improved (L.E. Murr group, University of Texas at El Paso, USA) thermal precipitation device can be used in the field for ultrafine air particle collection on an electron microscope grid. During sampling, the air particles are deposited on a transparent film (placed on top of the grid). Thus the largest particles of the $PM_{10}$, the smaller $PM_{2.5}$ and its constituent nanoparticles can be visualized as separate entities, without the large particles masking the small ones or aggregating with them. During efforts to overcome the earlier sampling problem, many electron microscopical preparatory techniques were refined for specific use in the nanoscale characterization of air particles. The major contribution of this report is found in its development of refined applications of standard techniques (originally designed for characterizing aquatic particles) which yield improved characterizations of air particles. The preparatory techniques and instrumentation usage detailed herein represent a useful primer for scientists wishing to maximize environmentally significant information from a spectromicroscopical investigation of the entire range of $PM_{10}$ particles. The extensive literature review highlights the current level of nanoscience application to the characterization of ultrafine environmental particles.

# Résumé

Dans le cadre d'une étude conjointe de l'INRE et de la ville de Toronto, la division de la recherche en gestion des écosystèmes aquatiques de l'Institut national de recherche sur les eaux a fait paraître des rapports en 2006 et en 2007 sur des fractions des dépôts de particules dans l'air au niveau du sol qui polluent l'eau de surface en milieu urbain. On y trouve des études de cas à partir de la poussière de rue à Toronto. Le premier de ces rapports comprend une caractérisation spectromicroscopique de la fraction ultrafine ($PM_{10}$) des particules. À l'origine, on s'intéressait particulièrement à l'efficacité du balayage de la chaussée pour lutter à la source contre la pollution des eaux pluviales, alors que la caractérisation des $PM_{10}$ était axée sur la détection des particules portant des métaux toxiques. Ensuite, une remise en suspension possible des particules ultrafines par les balayeuses de chaussée a été envisagée; les premiers résultats ont lancé une recherche de nanoparticules potentiellement toxiques. Il est vite devenu apparent que la collecte en masse de particules d'air à hauteur du sol à la source (suivie d'un fractionnement subséquent de l'échantillon total) était une opération très complexe. Le présent rapport, de 2011, fait un survol des problèmes techniques, et de toutes les tentatives antérieures pour les régler, en vue d'obtenir des fractions représentatives et sans artefact. Les premiers résultats demeurent valides, au sens qualitatif selon lequel les types individuels de $PM_{10}$ caractérisées existaient bel et bien dans les échantillons, mais on a trouvé important de tester toutes les méthodes applicables en vue d'améliorer le volet quantitatif du travail. Un dépouillement systématique de la littérature et des expériences répétées visant à atténuer le problème des artefacts ont été réalisés pour nous procurer le plus de compréhension possible de ce qu'on peut apprendre à partir d'une meilleure préparation des échantillons. En 2009, on a montré que le fractionnement à la source des particules ultrafines constituait la seule démarche permettant d'obtenir une bonne quantification. Un dispositif de précipitation thermique récemment perfectionné (équipe de L.E. Murr, Université du Texas à El Paso, É.-U.) se prête à la collecte sur le terrain de particules atmosphériques ultrafines sur une grille de microscope électronique. Au moment de l'échantillonnage, les particules se déposent sur une pellicule transparente (placée au-dessus de la grille). Donc les particules de plus grand diamètre de la fraction des $PM_{10}$, les $PM_{2.5}$ de plus petit diamètre et leurs nanoparticules peuvent être observées comme des entités distinctes sans que les plus grosses particules ne masquent les plus petites ou s'y agrègent. Dans le cadre des tentatives pour surmonter les problèmes d'échantillonnage, beaucoup de techniques de préparation de microscopie électronique ont été optimisées en fonction de la caractérisation des particules atmosphériques à l'échelle nanométrique. La principale contribution du présent rapport porte sur le développement d'applications avancées de techniques classiques (axées au départ sur la caractérisation de particules aquatiques) permettant de mieux caractériser les particules atmosphériques. Les techniques de préparation et l'emploi de matériel décrits de façon détaillée dans ce rapport constituent un guide utile à l'intention des scientifiques souhaitant maximiser l'utilité de l'information importante sur le plan environnemental découlant d'un travail de recherche spectromicroscopique sur toute la plage des $PM_{10}$. L'importante recherche documentaire met en exergue le présent degré d'application de la nanoscience à la caractérisation des particules ultrafines trouvées dans l'environnement.

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# FOREWORD

This report presents a continuation of previous studies (Leppard & West, 2006; Rochfort et al., 2007) on the characterization of ultrafine ($PM_{10}$) ground-level air particles which impact on urban surface water. These studies formed part of the Urban Water Management Project (UWMProject) within the Water Science and Technology Directorate of Environment Canada. The particle collection technology used at the time presented considerable difficulty for doing quantitative analyses on particles in the ultrafine range, a problem which has been worrisome for over a decade (Chow, 1995; McMurry, 2000). While electron microscopy provides the only "per particle" analytical approach that yields both morphological and compositional information on the entire size range of ultrafine particles, quantitative analysis is made problematic by artifacts created by sample collectors normally used in the field. In the case of Leppard & West (2006), collection of the $PM_{10}$ fraction by sieving caused the nanoparticle subfraction to adhere to larger particles, thus yielding an unexpectedly small nanoparticle fraction in terms of particle number. There were two obvious artifacts leading to diminished counts in the lowest portion of the $PM_{10}$ range. When samples were added to an electron microscope grid, (1) large particles would fall on top of smaller ones so that the smaller ones were hidden, and (2) it was difficult to distinguish between a rough-surfaced larger particle and a larger particle with nanoparticles adhering to it. For nanoscale particle analyses in the aquatic sciences, there is a well developed technology to cope with such problems (Lienemann et al., 1998; Mavrocordatos et al. 2007), but it was not developed with atmospheric particles in mind.

Transmission electron microscopy (TEM and its associated spectroscopy and electron diffraction capability) has long been identified as the premier nanoscience approach to the analysis of atmospheric ultrafine particles, when detailed morphological and chemical characterizations must be extended into the lower limit of the nanoscale (Buseck et al., 2000). Earlier, TEM (with associated apparatus) was acknowledged as providing the best and most complete view of $PM_{10}$ gross structure, morphology, size, internal differentiation, crystallinity and chemical composition. However, the application of TEM progressed slowly until recently, because it is labour intensive, time consuming and requires expensive equipment. Currently, the need for TEM-based information is great enough to justify the difficulties.

Our earlier examination (Leppard & West, 2006) of ultrafine ground-level air particles provided interesting information on the nature of the particles (including biologicals), but was mainly qualitative. In this work, we seek a field sampling technique which allows for quantitative analysis and we assess the application of biomedical technology for supplementing the information content beyond what is normally found in the literature. This research presents a primer for those who wish to maximize the nanoscale information content from TEM-based analyses of $PM_{10}$. This primer draws heavily on biomedical preparatory techniques, as opposed to being limited by standard materials science techniques designed for investigating rigid materials only.

# ACRONYMS

## Microscopy and Spectromicroscopy: Major Instruments and Accessory Apparatus

AEM - analytical electron microscopy, the use of an electron microscope plus attachments to perform an *in situ* chemical analysis of a microscopic entity selected from a field of view; more precisely, AEM refers to the collecting of spectroscopic data in a STEM instrument, based on various signals generated following the inelastic interaction of the incident electron beam with a selected portion of the sample (Botton, 2007; Mavrocordatos et al., 2007)

EDS – energy dispersive spectroscopy, a microanalysis technique (used in conjunction with an electron microscope) which employs a beam of electrons, focused on a selected part of an image, to generate X-rays which provide information on the element composition of the selected target (Chandler, 1977; Warley, 1997)

EELS – electron energy-loss spectroscopy, a microanalysis technique (used in conjunction with an electron microscope) which directs a beam of electrons, focused on a selected part of an image, into a high-resolution electron spectrometer that separates the electrons according to their kinetic energy and produces an electron energy-loss spectrum (Egerton, 1996, 2003; Mavrocordatos et al., 2000)

ESEM – environmental scanning electron microscope, which provides a topographical image of a hydrated environmental sample at a resolution intermediate between that of a light microscope and a TEM (Danilatos, 1988, 1993; Ebert et al., 2002)

ETEM – environmental transmission electron microscope, which provides a transmission image of a hydrated environmental sample at the resolution of a TEM, and which permits analysis at the relative humidity extant at the time of aerosol sampling (Wise et al., 2005, 2007)

HRTEM – high resolution transmission electron microscope, a TEM capable of imaging a selected portion of a sample at sub-nanometre resolution (Botton, 2007)

SAED – selected area electron diffraction, which uses TEM to provide diffraction patterns from very small volumes of structural entities selected from the field of view (Beeston, 1973; Misell & Brown, 1987)

SEM – scanning electron microscope, which uses reflected electrons to form a topographical image, typically with greater depth of field and lesser resolving power than a TEM (Goldstein et al., 2003)

STEM – scanning transmission electron microscope, which combines features of both TEM and SEM, thus providing a high resolution image in one mode and a capacity in the other mode to examine selected features on a point-by-point basis (permitting element mapping when coupled to EDS) (Mavrocordatos et al., 2007)

TEM – transmission electron microscope, which uses transmitted electrons to form a high resolution image, with a resolution limit of ca 0.001 μm for sectioned particles (Egerton, 2005; Botton, 2007)

TPD – thermal precipitation device, a thermophoretic precipitator based on aerosol physics and thermodynamics, utilized for atmospheric particle collection on a grid, for subsequent examination by TEM (Bang & Murr, 2002; Bang et al., 2003, 2004)

# INTRODUCTION

## 1. Precursor Studies

Earlier studies of ground-level air particles (Leppard & West, 2006; Rochfort et al., 2007) were undertaken to examine deposit fractions on a well-trafficked road in the City of Toronto. A subsequent study was published recently (Rochfort et al., 2009). Since the particles were likely to be diverse and variable in amount and nature from one sampling time to another, we sought to define them in terms of multiple criteria, and not just by size range alone. Since this goal is best served by analytical electron microscopy, the use of electron microscope-based spectromicroscopy (Mavrocordatos et al., 2007) was explored for characterizing the major components of the $PM_{10}$ fraction. The earlier goals were to learn more about the test road site on Markham Road (see **Appendix 1** = Fig. 2.1 of Rochfort et al., 2007, showing the paired test catchment areas) and to search for nanoparticles within a $PM_{10}$ fraction which had been generated by street sweepers. Extensive background information on the site can be found in Rochfort et al. (2007), who produced a detailed study of the composition, contaminant levels and toxicity of particles collected by street sweepers. Earlier, to address pollution source control issues in Toronto, Ontario, a field study of street sweeping effectiveness had been initiated in 2004 (Kelman & Crowther, 2005). At that time, it had been determined, from public health considerations, that the particle fractions of interest were $PM_{10}$ and $PM_{2.5}$ (Kelman & Crowther, 2005). For the characterization of individual particle types within the $PM_{10}$, adaptations had to be made to existing electron microscope-based spectromicroscopy (Mavrocordatos et al., 2007) and the work had to be left at a semi-quantitative level pending technical improvements being developed elsewhere. This 2011 report contains a guide to the latest technologies available for extending the research; it shows their direct application to analyzing the Markham Road street samples and it outlines a future strategy for quantification of the nanoparticle fraction (a fraction whose remarkable toxicological properties give it a pre-eminent consideration). The technology development described herein focuses on transmission electron microscopy, because it is a primary tool of the rapidly emerging field of environmental nanoscience (Leppard, 2008, 2009). Specifically, we seek to maximize the analytical use of STEM-EDS, or scanning transmission electron microscopy coupled to energy dispersive spectroscopy.

## 2. The $PM_{10}$ Fraction of Ground-Level Air Particles

On urban streets, deposits from the atmosphere occur continuously, from both natural processes and human activities, and they can be washed away in part after storms. Some of the ground level deposits bear pollutants, with some of these becoming resuspended in the air and some moving into surface waters. While source controls are used routinely to help protect aquatic ecosystems against pollution, source control measures include the prior removal of pollutant deposits from streets by street sweepers in the case of urban stormwater. The removal of the ground-level deposits may have a secondary benefit in addition to minimizing the storm-induced release of pollutants from streets to surface waters. The secondary benefit comes from a reduction in the amount of the finest particles re-entering the urban atmosphere through the actions of wind and traffic. This apparent minimization of toxic airborne particles is, however, a more complex situation than one might assume. Recent advances in street sweeping technology have resulted in the development of street sweepers which are capable of retaining particulate material down to 10μm ($PM_{10}$ certified). While doing so, can they also resuspend $PM_{10}$ particles, including its nanoscale subfraction? If such a resuspension occurs, science must address the very important questions of the amount and nature of $PM_{10}$ escaping from street sweepers into the urban atmosphere! To address pollution source control issues in a Toronto (Ontario) site, a field study was initiated in 2004 to determine street sweeping effectiveness in great detail. The Urban Water Management Project of the National Water Research Institute (Burlington, Ontario) was invited to participate. The research described herein addresses the various components of the $PM_{10}$ using electron-optical techniques for nanoscale characterizations. A companion research by Rochfort et al. (2006, 2007, 2009) examines the chemistry and toxicology of street sweeper particles; their overview is shown in **Fig.1**.

The $PM_{10}$ is defined as the fraction of particles determined by mass that passes a size selective inlet with a 50% cutoff efficiency at 10 μm aerodynamic diameter (Harrison, 2004). A detailed review of atmospheric aerosol measurements has been published by McMurry (2000), whose topics complement those of an earlier review by Chow (1995) which focused on regulatory compliance measurements and filter measurements. The $PM_{2.5}$ subfraction (aerodynamic diameter of $\leq$2.5 μm) has long been considered important in urban situations (Marcazzan et al., 2001; Harrison, 2004; Maynard, 2004), and its properties are strongly emphasized in the USA for setting air quality standards (Samet et al., 2000, 2005). The Royal Society of the UK was sufficiently concerned about "ultrafine" ($PM_{10}$ and its subfractions down to the nanoscale) particles in the atmosphere that they published an influential document on the topic (Brown et al., 2000). In Canada (CEPA, 1999), a plan was set in motion to examine respiratory human

health effects (both acute and chronic) for ambient concentrations, in addition to examining effects in regions where particulate air pollution is known to be high and adverse. It has been noted that particles within different subfractions of $PM_{10}$, in urban ambient air, tend to come from different sources (Marcazzan et al., 2001; Birmili et al., 2006), although there can be appreciable subfraction differences on a daily basis.

Clearly, the $PM_{2.5}$ can come from different sources than the fraction encompassed by '<$PM_{10}$ but >$PM_{2.5}$' (Marcazzan et al., 2001; Artinano et al., 2004). In a study involving 24-hour simultaneous samplings of $PM_{10}$ and $PM_{2.5}$, in the central urban area of Milan (Italy), the elemental composition was shown to be quite different for the two fractions. The fraction containing the largest particles within $PM_{10}$ tended to be crustal, whereas the $PM_{2.5}$ fraction had a reduced content of elements of crustal origin, while presenting an enrichment of anthropogenic elements having relevant environmental and health impacts (Marcazzan et al., 2001).

Detailed information on crustal particles is found in Chen (1998) and Huang et al. (1998), while a comprehensive environmental perspective of minerals in the air is provided by Buseck et al. (2000), who endorse TEM as the best means to provide information for aerosols on an individual particle basis (size, shape, composition, crystallographic structure, and extent of aggregation). The Buseck group has since focused on the nanoparticle component of the atmosphere (Buseck & Adachi, 2008). Nanoparticles are generally defined as particles smaller than 100 nm in least dimension (Hochella, 2002; Dionysiou, 2004). Much earlier, Beutelspacher and van der Marel (1968) had produced an electron microscopic picture atlas of clay minerals and their admixtures; this atlas serves well as a reference for the morphologies of abundant types of previously characterized crustal particles.

The connection between size fraction and source was documented recently by Birmili et al. (2006) for urban atmospheric particles at four sites in the United Kingdom and Ireland. These sites were selected to simulate extreme degrees of vehicular-traffic influence on the environment. Urban air trace metals revealed distinct types of size-fractionated behaviour (in size ranges extending from 7.2 μm down to < 0.5 μm), depending on the particle sources from which they originated. In the particle size range of 1.5 to 3.0 μm, the concentrations of copper, barium and iron correlated well in urban air, which was interpreted as evidence that the particles were released through abrasive vehicular emissions, particularly the wear of brake linings. For the same size range, the results indicated strongly a decoupling of iron and calcium, the former arising predominantly from vehicles and the latter from soil resuspension. In a study of street particles from eighteen different city streets adjacent to five different land uses (industrial, roads, multi-family residential, commercial and single family residential) in Santa Monica (CA, USA), the greatest

mass of pollutants was associated with particles in the 100-250 μm range (Lau & Stenstrom, 2005). However, nanoparticles could not be collected by their sampling technique and it is not clear if $PM_{2.5}$ was properly sampled. High volume sampling can lead to inefficient fractionation within $PM_{10}$ if high density particles are abundant or if the technique is predisposed by its nature to cause artifactual particle aggregation (Moreno et al., 2004). With regard to particles bearing organic contaminants, Lee et al. (2005) examined urban ground-level air particles in relation to PAHs (polycyclic aromatic hydrocarbons) in the Lake Biwa area of Japan. They characterized PAHs within different particle size fractions of deposited road particles.

Analyses of much smaller subfractions of $PM_{10}$ revealed (Zhu et al., 2002a) that nanoparticles of 1-100 nm least dimension were important in terms of particle number over a major highway near the Los Angeles National Cemetery (CA, USA). In a related study of a freeway chosen because >25% of the vehicles were heavy-duty diesel trucks, the motor vehicle emissions constituted a major contribution to the UFP (ultrafine particle) fraction, with atmospheric dilution and coagulation playing important roles in a rapid decrease of particle number concentration with distance away from the freeway (Zhu et al., 2002b). Also, the UFP fraction has been shown to be a carrier of heavy metals over a heavily-trafficked road, in a city of southern Taiwan; the traffic-related metals included Pb, Cd, Cu, Zn, Ba and Ni (Lin et al., 2005).

Regarding illnesses related to air pollution, it is increasingly evident that unregulated nanoparticles are potentially the most dangerous particles within the $PM_{10}$ range (Nel, 2005). Nanoparticle toxicity can be a function of the sheer smallness of the particles. Recently, an association was found between traffic-related air pollution in a large city and perinatal mortality (de Medeiros et al., 2009). An earlier study of air pollution effects on child mortality in the same city (Sao Paolo, Brazil) had implicated the $PM_{10}$ fraction in respiratory deaths (Conceicao et al., 2001). In a study of airborne particles (<7.2 μm) from various urban and industrial sites in the Greater Thessaloniki area (Greece), labile fractions of several heavy metals were documented in the particles, with labile fractions of Cd, Cu, Ni and Zn (and bioaccessible fractions of Mn, Ni and Zn) being higher in urban sites; such urban particles were considered as a potential respiratory health risk (Voutsa & Samara, 2002). Clearly, airborne particles from vehicles have a strong impact on roads, the nearby environment and human beings (Biswas & Wu, 2005), with the predominant peak in the mass distribution being in the lowest portion of the $PM_{10}$ range (<200 nm).

## 3. Relevance of Focusing on the Nanoparticle Component of Ground-Level $PM_{10}$

*Nanoparticle cycling.* A past and continuing focus has been placed on nanoscale scientific investigations of atmospheric nanoparticles (Brown et al., 2000; Anastasio & Martin, 2001; Biswas & Wu, 2005) and of aquatic nanoparticles which are abundant (Leppard & Buffle, 1998; Mavrocordatos et al., 2007; Leppard, 2008) and which have a potential to interact with ground-level air particles. There is a nanoparticle cycle in the atmosphere (Buseck & Adachi, 2008), as illustrated by **Fig. 2**. Specific nanoparticle cycles have been described for specific aquatic ecosystems (Leppard, 2008). Both known and suspected routes of exposure, uptake, distribution and degradation of environmental nanoparticles have been outlined in relation to air, water, soil, sediment and their interfaces (Oberdorster et al., 2005). The nanoparticle contribution to the total mass of airborne particulate matter can be in the range of 1% to 8% (EPA, 1996) and the biological activities of nanoparticles are of considerable concern (Handy et al., 2008; Klaine et al., 2008).

*Nanoparticle dispersal and stability.* There is an increasing focus on the behaviour of nanoparticles at air/water interfaces (Biswas & Wu, 2005; Oberdorster et al., 2005; Buseck & Adachi, 2008). A considerable stimulus for this focus has been provided by the research of Hyung et al. (2007), who are examining this problem in relation to carbon nanotubes. Carbon nanotubes are allotropes of carbon with a cylindrical nanostructure, each individual tube being a few nm wide; they occur in nature and are of increasing interest to manufacturers who synthesize them for a wide range of applications and who fear that usage may be limited by potential toxicity (Muller et al., 2005; Hyung et al., 2007). They can be detected readily in the lower atmosphere as a product of combustion (Murr et al., 2004a; Murr et al., 2006). For ground-level studies, one must consider that engineered nanoparticles, entering natural waters from the atmosphere, can interact with natural organic molecules in a water body so as to receive an enhanced stabilization (Hyung et al., 2007). Manufactured multi-walled carbon nanotubes were dispersed as an aqueous suspension in both model NOM (natural organic matter) solutions and a natural surface water; surprisingly, these suspensions remained stable for over one month, vastly longer than expected when considering data on nanotubes suspended in pure water. This result suggests that dispersal of carbon-based nanomaterials in a natural aquatic environment could lead to a type of dispersion in surface waters which follows a mechanism not previously considered in studies of environmental fate and transport (Hyung et al., 2007).

*Nanoparticle emissions.* In terms of number concentrations, urban areas and traffic are major contributors to atmospheric pollution by nanoparticles (Buseck & Adachi, 2008). In urban settings, nanoparticles are generated in significant numbers by road traffic and stationary combustion sources (Zhu

et al., 2002a, 2002b). Shi et al. (2001) showed, with measurements at an urban roadside and downwind from the traffic (more than 25 metres from the curb), that small nanoparticles (<10 nm diameter) accounted for 36% to 44% of the total particle number concentrations. Measurements designed to sample the plume of individual vehicles showed that both a diesel- and a petrol-fuelled vehicle generated nanoparticles of less than 10 nm diameter. Nanoparticle emissions from engines have been well documented in the past (Kittelson, 1998).

*Nanoparticle aggregation.* Aerosols can be present as complex branched clusters of nanoscale spherules. Nearly two decades ago, TEM was used to demonstrate that carbonaceous aggregates in the Phoenix (AZ, USA) urban aerosol had an irregular branched morphology (Katrinak et al., 1993). Fractal analysis methods applied to these branched clusters in ambient aerosols produced results yielding information on formation processes. Katrinak et al. (1993) postulated that, with sufficient characterization of emission sources, data on fractal dimensions could be useful in the apportionment of natural aerosols. More recently, Bang et al. (2004) employed TEM in conjunction with SAED and EDS to describe and compare the major kinds of airborne nanoparticles (individuals and aggregates) over El Paso (TX, USA). They described many kinds of aggregates in considerable detail, including carbon nanocrystal aggregates which contained nanotubes.

*Engineered nanoparticles.* Those nanoparticles resulting from the erosion or disintegration of novel recently-manufactured products (based on the nanotechnology explosion), and those nanoparticles which escape from manufacturer's laboratories, are anticipated to become (in some cases) an unavoidable airborne menace (Biswas & Wu, 2005; Muller et al., 2005; Nel et al., 2006). The great diversity of nanoparticles currently being developed for applications in medicine, pharmacology and biological research (Mallouk & Yang, 2009) are likely to add to the problem. Since engineered nanoparticles were not previously available to biota for evolutionary adaptation, it is unclear how biota will adapt.

*Nanoparticle toxicity.* Recent biomedical evidence shows that living cells can interact with manufactured nanoparticles in unexpected and deleterious ways (Geiser et al., 2005; Nel et al., 2006; Rothen-Rutishauser et al., 2006). Unexpected phenomena include a cytotoxicity which can be a function of nanoparticle ***shape*** (Jia et al., 2005). The respiratory toxicity of multi-walled carbon nanotubes is under intensive investigation (Muller et al., 2005) as is the connection between nanoparticles from vehicle exhaust and possible increased risks of cancer and cardiovascular and pulmonary diseases (Vinzents et al., 2005). Modest exposures to nanoparticles, while bicycling in road traffic, correlates with adverse biological effects in the cyclists (Vinzents et al., 2005). Using analytical TEM (AEM) for detection and characterization, atmospheric carbon nanotubes have been studied for potential health risks for some time

(Murr et al., 2004a). Generally, nanostructure-based phenomena in airborne particulate aggregates are currently being studied with regard to respiratory health effects (Murr et al., 2004b, 2006), the nanostructure *per se* (Murr et al., 2004c; Murr, 2008) and cytotoxic effects (Soto et al., 2007). Under development are rapid *in vitro* assays for cytotoxicity assessments of nanoparticulate materials, in relation to potential respiratory health risks in humans (Soto et al., 2007). In fact, a simple direct-contact cytotoxicity (*in vitro*) assay has been developed whereby nanoparticles and human cells are incubated together, followed by analyses for indicators of toxicity (Soto et al., 2008). Under way also are studies of the polycyclic aromatic hydrocarbon (PAH) contents of speciated atmospheric particles found in $PM_{10}$, accompanied by nanoscale observations on the primary particles and their aggregates (Shi et al., 2007). Recently, the behavior, fate, bioavailability and effects of nanomaterials in the environment have been critically reviewed (Klaine et al., 2008); the ecotoxicology of nanoparticles has also been reviewed (Handy et al., 2008) with regard to the current status, knowledge gaps, challenges and future needs. More recently, Scown et al. (2010) have posed the question, "Do engineered nanoparticles pose a significant threat to the aquatic environment?" Their review concludes that we must address significant gaps in our understanding of the fate and behavior of engineered nanoparticles, which can enter various environmental compartments via the atmosphere. Clearly, environmental and public health risks are presented by unintended and unanticipated transfers of nanoparticles (Theodore & Kunz, 2005; Hunt & Mehta, 2006). The concern over the risks posed by an increasing variety of nanoscale materials in the environment has become an increasingly urgent concern (Roco, 2005; Dunphy Guzman et al., 2006; Wiesner et al., 2006).

## 4. Environmental Significance of Deposit Fractions of Ground-Level $PM_{10}$ Air Particles

The adverse health effects of airborne particles (Pope et al., 1995; Holgate et al., 1999; Pope, 2000; Gwinn & Vallyathan, 2006) and the atmospheric deposition of contaminants into surface waters (Baker, 1997; Marsalek et al., 2008) are well-recognized environmental issues. Dry particle deposition of contaminants (e.g., excess nutrients, toxic chemicals) contributes to the degradation of aquatic ecosystems (Zufall & Davidson, 1997), and can account for a major fraction of the total atmospheric deposition to natural water bodies. In considering regions with an urban component, one finds similarities between urban watersheds and 'natural' watersheds, with regard to deposit fractions and their dispersion behaviour, but one also finds important dissimilarities. The complexity of anthropogenic factors within an urban ecosystem may result in substantial changes to sediment characteristics (physical, chemical, biological) during relatively

short transport distances and times (Droppo et al., 2006), in contrast to the more gradual changes in "natural' ecosystems. Also, remobilization of soil-stored toxic metals in close proximity to roads might significantly prolong the contamination of urban road systems, with a later impact on nearby water bodies (Sutherland & Tolosa, 2001).

For the cases of highway and urban pollution, the role of airborne pollutants is receiving increasing attention (Hamilton & Morrison, 2004). With toxic effects as a focus, scientists have begun fractionating airborne particles from urban areas into total, labile and bioaccessible fractions with regard to their heavy metal burden (Voutsa & Samara, 2002). In street sediments, a significant fraction of heavy metals can be considered as potentially bioavailable (Stone & Marsalek, 1996). Consequently, studies on bioavailability are now complementing earlier research on the correlation between toxicity and specific size fractions, with air quality standards in Canada, the USA and the United Kingdom being based on the $PM_{10}$ fraction.

Over the past decade, electron-optical technology has become the best means to obtain environmentally-relevant characterizations of individual kinds of $PM_{10}$ air particles (Harrison et al., 2000; Maynard, 2000; Bang & Murr, 2002; Bang et al., 2003, 2004; Murr et al., 2006; Mavrocordatos et al., 2007), with some electron-optical techniques being eminently applicable to the analysis of abundant airborne cellular material, including microbes (Jaenicke, 2005; Wittmaack et al., 2005). The reason that electron-optical means (and accompanying spectroscopical means) are the best for information content (sizing, morphology, ultrastructure, chemical composition) is that one can select and examine individual particles 'one at a time' (from a particle population displayed on an EM screen) for their specific characteristics, rather than being constrained by bulk information (e.g., average diameter, average chemical composition) pertaining to an entire particle population. The latest instruments can describe the distribution and chemical state of elements in a given nanoparticle, the three-dimensional structure of that nanoparticle and details of any crystalline portions within. Critical discussions of the collection and characterization of atmospheric particles have been available for some time (Harrison & Van Grieken, 1998; Buffat, 1999; Brown et al., 2000). Electron-optical techniques and instruments which became available recently, their optimal use and their quantitative application are described in Botton (2007) and in Mavrocordatos et al. (2007).

## 5. The Strengths and Limitations of TEM for Characterizing $PM_{10}$

The major advantage of analytical TEM is that it yields detailed morphological information on (and internal differentiation within) individual $PM_{10}$ particles covering ***the entire size range***, and can provide substantial chemical information on a "per particle" basis (Mavrocordatos et al., 2007). It is highly desirable to detect the different "species" of $PM_{10}$ particles present (including nanoparticles and their aggregates) and then characterize them on a "per species" basis. Achieving this level of detail, one might then employ the newly-emerging science of nanotoxicology (Oberdorster et al., 2005) to determine which ones are the particles of concern in a given environmental compartment. The overall contribution of analytical TEM is that it provides all the necessary information on ultrafine particles (size, shape, composition, crystallographic structure) for phase identification and determination of extent of aggregation, on an individual particle basis (Buseck et al., 2000). To emphasize this advantage, one can refer to certain situations found with ultrafine particle subfractions at the lowest part of the size range. For example, a nanoscale virus appears in a TEM image as a virus; with other sizing techniques, one might perceive just a carbon-rich nanoparticle of unknown nature. Another example involves clay micelles closely aggregated with Al-rich nanoparticles and Si-rich nanoparticles. Many techniques would perceive the aggregate as one of Al-Si-rich particles, and conclude that the aggregate was clay. Using spectromicroscopy at near-nanometer resolution, one documents the presence of three distinctly different particles, only one of which is a clay particle.

The drawbacks of TEM are: (1) considerable cost, especially when stringent quantification is required; (2) a large time requirement when biomedical preparatory protocols are used; (3) the need for a specialized dedicated technician; and (4) the limited number of facilities equipped and manned for the detection of $PM_{10}$ and their constituent nanoparticles in complex matrices. With the increasing need for the analysis of ultrafine particles, there will be a concomitant increase in availability of both research and commercial services.

## 6. A Need to Connect TEM-based $PM_{10}$ Particle Monitoring to Emerging Particle Characterization Techniques

Although using TEM-based techniques (principally STEM-EDS and SAED) for environmental monitoring can be cost-prohibitive, there are exceptional environmental situations, such as sites where water quality predictions go awry when based on water samples assessed by standard methods alone (Chanudet, 2008). Rapid and inexpensive techniques, selected from a diversity of quantitative colloid

size distribution methods (Wilkinson & Lead, 2007; Chanudet, 2008), are currently being adapted in university laboratories to pre-test environmental samples. The pre-testing, in which particle size tends to be inferred, allows the development of a cost-effective strategy for the use of TEM, namely using it to ***confirm*** inferred sizes and shapes of $PM_{10}$ particles as opposed to making the initial determination. Additionally, TEM contributes informative details and defines particle sub-populations. A brief outline of rapid cost-effective techniques currently applicable to water samples is found in Leppard (2008). Domingos et al. (2009) recently compared the most highly-considered methods for sizing and characterizing manufactured nanoparticles found in the environment. Their research supports the idea that a judicious cost-effective use of TEM for the detection and characterization of ultrafine particles (and their constituent nanoparticles), in certain critical but incompletely-defined samples, should help improve predictive models and an understanding of environmental mechanisms.

## 7. Characteristics of $PM_{10}$ Ground-Level Air Particles Found at the Toronto Site

The ultrafine particle fraction was described in considerable detail in our earlier report (Leppard & West, 2006). Salient details from that report and subsequent work are as follows. As expected, the larger particles within $PM_{10}$ tended to be crustal (in terms of mass), as determined by morphology and spectroscopy. With ESEM-EDS, clearly-defined peaks in spectral data on a "per particle" basis commonly showed major peaks for both Si and Ca; rarely, a spectrum showed either Fe or Ca as a single major peak. Minor peaks could be found on many spectra of $>PM_{2.5}$ particles for Na, Mg, Al, Si, S, Cl, K, Ca, Ti and Fe, with the S and Cl peaks tending to be trace peaks. Peaks revealed by STEM-EDS in ultrathin sections and whole mounts of $PM_{2.5}$ were those of Al, Ca, Cl, Cr, Fe, K, Mg, Mn, Na, P, S and Si. For the more intensively studied second year samples, the sites NBSW, NBUS, SBSW and SBUS were similar ***in general aspects***.

With regard to environmentally interesting metals in those particles of $PM_{10}$ which were larger than $PM_{2.5}$, the detectable metals were Ca, Ti and Fe, with not all necessarily in the same particle. For $PM_{2.5}$ particles, detectable metals found within individuals of the population were Ca, Cr, Fe and Mn. The nanoparticle population showed peaks in some individuals for Ca, Cr or Fe.

Microbes and microbial consortia could be visualized in ultrathin sections. Some microbes were found encased in mineral colloids. In whole mount preparations, rod-shaped bacteria could be seen with nanoscale mineral colloids on their surface. Nanoscale mineral colloids were also seen on the surfaces of extracellular polymeric substances (of macromolecular size) projecting from bacteria. One could also find examples of complex aggregated particles in which a single (submicrometer) bacterium is connected

to large and small $PM_{2.5}$ mineral particles. The nanoscale morphological information permitted precise identification of many kinds of ultrafine particles and valuable clues for pursuing further characterization by spectromicroscopy and SAED. Morphological clues to particle origin were readily found, a subject of considerable interest to atmospheric scientists. For distinguishing biologicals using biomedical preparatory techniques, a guide to TEM-based identification of viruses, bacteria and cell debris is available (Leppard, 1992a).

Nanoparticles were readily visualized in whole mounts, although their frequency was surprisingly low. Many appeared as granules, apparently showing a tendency to aggregate; the extent of this tendency needs to be explored experimentally (or a better, less-artifacted collection technique must be found). Clusters of the smallest nanoparticles presented peaks for Ca and Si. In views showing little evidence of dehydration-induced aggregation, clusters of nanoparticles and larger particles of $PM_{2.5}$ showed a mixture of granular, sharp-edged and fibrous entities. Additionally, granular calcium-containing nanoparticles were seen in association with bacterial surfaces. While a near-spherical shape was predominant, there were some fibrous and curved needle-shaped nanoparticles having extremely great length-to-diameter ratios, and also some baton-shaped nanoparticles (most likely bacterial extracellular appendages). In a size range bridging the lower end of $PM_{2.5}$ and the upper end of the nanoscale, there were many particles with apparently sharp edges, as evidenced in both ultrathin sections and whole mount views.

***The significance of the characterizations above is that they cannot be obtained by conventional averaging approaches.*** High resolution visualization is required for "per particle type" analyses of elemental composition, when the sample is a heterogeneous mixture of particle "species".

## MATERIALS AND METHODS

### 1. Sample Site and Sampling

The site and sampling approach of the first (2004) and second (2005) sampling years are described in **Appendix 2.** In the third sampling year, samples of air particles generated by street sweepers on Markham Road (Kelman & Crowther, 2005) in Toronto were collected in July and August of 2006. At that time, the test street site (see **Appendix 1**, for experimental layout in aerial view) had a traffic flow of 26,000 vehicles/day in each direction, northbound (NB) and (SB) southbound (Rochfort et al., 2007). Both swept (SW) and unswept (US) samples were taken from northbound and southbound lanes. Large

data collections for individual samples, on their chemical components and contaminants (total metals, PAHs and nutrients), as well as results from toxicity tests (Rochfort et al., 2007), were established by and are stored at NWRI (as collections of the UWMProject). The 2006 samples receiving extensive nanoscale analyses were July and August samples, with each representing NBSW, NBUS, SBSW and SBUS. **Appendix 3** presents an earlier précis of sampling strategy. **Appendix 4** presents a street sweeper description and operation, and **Appendix 5** details the sample fractionation. All five appendices appear in the main text of Leppard & West (2006).

## 2. Sampling Strategy and Coupling to Multidisciplinary Analyses

**Fig. 1** (from Rochfort et al., 2007) shows an overview of the sampling procedure and analyses, including toxicological and chemical analyses. The sampling strategy and coupling to multidisciplinary analyses (including TEM-based analyses) is described in **Appendix 3** (published earlier in Leppard & West, 2006). The details of the analytical electron microscopy approaches are described below in "Correlative Microscopy Approach", "Spectromicroscopy" and "Intermediate Resolution Microscopy". For environmental samples, the correlative AEM approach has been utilized seldomly but well for two decades (Leppard, 1992a, 1992b). For preparations of samples based on using biomedical techniques, the selected individual preparations for comparisons are used correlatively to overcome specific artifacts inherent to each one when used independently. For biological particles, the correlative approach allows one to detect, assess and (by preparation modification) minimize artifacts (especially artifacts of extraction and shrinkage). A given AEM sampling strategy often is specific to either atmospheric or aquatic particles; this is discussed in detail in Mavrocordatos et al. (2007) and succinctly in Perret et al. (2005).

## 3. Correlative Microscopy Approach

A multimethod analytical approach should be used for the characterization of individual particles within samples of ground level atmospheric particles, in the size range of 1 nm to 10 μm. Initially, particle populations from the entire sample are examined by conventional optical microscopy (COM) to obtain gross scale (ca. 0.25 μm resolution limit) structural information on all of the starting sample that can be visualized. Then an ESEM (Danilatos, 1993) is used in tandem to obtain structural information, at moderate electron-optical resolution (0.04 μm). Using the COM and the ESEM correlatively with a high

resolution (0.001 μm practical resolution) TEM permits one to obtain ultrastructural information approaching the molecular scale, in a cost-effective manner, and relate it back to the fresh sample. Samples are stabilized (chemically fixed) and prepared for TEM, with no storage step [see Leppard and Buffle (1998) for rationale], by the multi-preparatory approach designed by Liss et al. (1996), to yield ultrathin sections for analysis. As an alternative to ultrathin sections, a portion of an air particle sample can be added directly to a transparent film on an electron microscope grid so as to obtain an optimal coverage of the grid, a procedure whose artifacts are known but can be difficult to avoid when the particle size range is large (Lienemann et al. (1998). The analysis of entire particles sitting on a grid is the analysis of "whole mounts".

By using a multimicroscope approach, one can bridge the gap between "high-volume-but-low-resolution" information and "low-volume-but-high-resolution" information (Leppard, 1992b). The ultrastructural information is supplemented by element composition analysis on a "per individual particle" basis by EDS (Chandler, 1977), used with a STEM. The TEM component of the STEM produces the high quality image while the rest of the apparatus is used for spectromicroscopy of selected particles in the image. In this manner, the location of nanoscale agglomerates of individual metal-rich species can be spatially related to individual particles (and their substructures) whose image sometimes provides sufficient ultrastructural information to make sound inferences about biological and chemical attributes of a specific metal-binding particle (Beutelspacher & van der Marel, 1968; Leppard, 1992a, 1992b; Chen, 1998; Jackson & Leppard, 2002; Leppard et al., 2003; Mavrocordatos et al., 2007), including coatings on particles which can alter the metal-binding properties of those particles (Jackson et al., 2011). There is an abundance of cellular material in the atmosphere (Jaenicke, 2005); an overview of bioaerosols is available (Wittmaack et al., 2005).

For the preparation and analysis of ultrathin sections of fixed samples, the detailed protocols are below. The analysis of ultrathin sections can provide more detailed information and more profound characterizations than can the analysis of whole mounts, although the added time and cost has to be considered. Note that the biomedical preparatory approach allows for the identification of many biologicals, provided that they can be rehydrated sufficiently to regain most of their original morphology; achieving this level of rehydration may present extreme difficulty for examining some organic-rich materials (Leppard, 1992a, 1992b). As demonstrated earlier with aquatic samples, nanoscale organic coatings can be visualized and characterized for both organic and inorganic ultrafine particles (Jackson et al., 2011).

*Fixation of particle samples.* Portions of particle samples in containers were used for TEM analysis. The fixatives chosen were those developed for use with aquatic colloids; they were employed to preserve and identify biologicals in the heterogeneous sample (see Leppard and West, 2006, for earlier analyses of biologicals). The air particles were fixed and preserved in one of three solutions: (1) a hydrophilic melamine embedding resin having fixative properties (Frosch and Westphal, 1989), and either (2) glutaraldehyde or (3) glutaraldehyde with ruthenium red (GRR), with the latter two fixatives followed by a secondary fixation in an osmium tetroxide-based solution, prior to embedding in a hydrophobic resin (Pazin et al. (2005). Fixative solutions are designed to preserve as faithfully as possible the ultrastructure of a biological sample, exactly as that ultrastructure was, in its natural setting, at the instant before cells were killed by the fixative. There is no universal fixative for preserving all kinds of samples equally well, with regard to intracellular contents and extracellular structures, but the strengths and weaknesses for a given fixative are known. The detection, assessment and minimization of artifacts are described in a monograph by Mavrocordatos et al. (2007). The appropriateness of the fixatives and resins selected here is described in Liss et al. (1996).

*Rationale for fixative selection, regarding the biologicals.* To summarize briefly our use of the three selected preservation procedures, the images resulting from each procedure could be analyzed correlatively to ascertain specific artifacts inherent to each one when used independently. Thus we were able to construct an overall image by mentally combining the three related images. For examinations of ultrafine particle composition, in terms of the "species" present, the principal artifacts of concern were excessive extraction, shrinkage of hydrated structures and internal damage to cells. The use of a hydrophilic resin (Nanoplast FB 101, a melamine resin) as a combination fixative and embedding material (Frosch & Westphal, 1989) permitted one to omit the solvent dehydration step, thus minimizing extraction and shrinkage while having the defect of yielding minimal ultrastructural details of cell contents. Glutaraldehyde alone yielded excellent ultrastructural details of cell contents, while permitting considerable extraction of extracellular materials and consequent shrinkage of matrices comprised of extracellular polymeric substances. Glutaraldehyde plus ruthenium red minimized all three problems of concern, but presented a slightly grainy image which interfered with high resolution analyses.

*Sectioning and the proxy preparation.* Nanoscale observations were made on ultrathin sections (ca. 50-70 nm thickness) of samples prepared by the protocols of Pazin et al. (2005), which had been derived from the detailed protocols of Liss et al. (1996) and Leppard et al. (2003). As per the highly detailed protocol descriptions of Liss et al. (1996), a research focus was placed on one preparatory protocol which was found to produce representative images with low but known artifact content (see Liss et al., 1996, for

scientific rationale). This proxy preparation consisted of sample immersion (1:1) at room temperature in a solution of 0.1 M sodium cacodylate-buffered (pH 7.1) primary fixative containing both (freshly mixed) 3 % glutaraldehyde and 0.1 % ruthenium red (ammoniated ruthenium oxychloride). The wash steps and the secondary fixation (on ice, in a freshly mixed solution of cacodylate-buffered 1 % osmium tetroxide and 0.05 % ruthenium red), followed by washes and a solvent exchange series, leading to infiltration into and embedding by the hydrophobic epoxy resin of Spurr (1969), were done exactly as described in Liss et al. (1996). After polymerization of the resin in a mold, all samples were sectioned identically with a diamond knife mounted in an ultramicrotome (Leica Ultracut UCT ultramicrotome; Leica Mikrosysteme, Wien, Austria).

*Specimen contrast.* All ultrathin sections were mounted on Formvar-covered copper (electron microscope) grids. To maximize specimen contrast in the TEM, the epoxy resin sections were counterstained with a drop of concentrated uranyl acetate in 50 % ethanol for 10 minutes in the dark, and then rinsed with distilled water. The grids were then stained again for 5 minutes in Reynolds' lead citrate solution (Reynolds, 1963), in a carbon dioxide-free environment, rinsed again and allowed to dry. The counterstaining was carried out exactly as in Pazin et al. (2005). This combination of counterstains produces a high differential contrast for the documentation of nanoscale structures by TEM at high resolution (Lewis & Knight, 1977). For a thorough treatment of artifacts and their correction, with regard to ultrathin sections, consult Hayat (1970). For a spectromicroscopical mineralogical analysis of inorganic colloids whose native electron opacity is sufficiently great, there is an option; the staining can be omitted and one can proceed directly to both EDS analyses and selected area electron diffraction, on a "per colloid" basis (Chanudet & Filella, 2006).

*Nanoplast assessment.* To assess the proxy preparation for appropriateness (minimization of artifacts of dehydration and extraction), its major features were checked against a Nanoplast preparation (see "*Rationale for fixative selection, regarding the biologicals*" above). The preparation was done according to Liss et al. (1996). The natural situation of the air particles, being present as either individual particles or as aggregates which had previously been dehydrated by nature, meant that the shrinkage artifact was of lesser concern than is the case for aquatic particles produced naturally in water.

*Documentation.* Examination and documentation by TEM were done with a JEOL JEM 1200 EX TEMSCAN scanning transmission electron microscope (JEOL, Peabody, MA, USA), operated in transmission mode at 80 kV. The practical resolution for the epoxy-embedded sections was ca. 3 nm. The searches of TEM views of ultrafine particles, to select representative images of ultrastructural

features of specific types, were done systematically according to the protocol of Leppard et al. (2003). Representative ultrathin sections were searched initially using both low (4,000 x) and medium (15,000 x) primary magnifications to get an overview of the relative abundance and associations of the most common ultrastructural components, as defined by morphology and size. Observations and documentations were then made in greater detail at a primary magnification of 50,000 x. For examining the smallest nanoparticles, much higher magnifications (to 250,000 x) were used. To establish representativity, the common kinds of particles and their aggregates were defined, based on their relative abundances (Jackson & Leppard, 2002) following extensive observation.

In order to quantify the mean size of particles from each sampling site, weighed aliquots of dry particles were distributed onto the surface of SEM stubs (covered by double-sided carbon tape) and images for 10 representative areas were acquired for each sample type. MetaMorph image analysis software (Universal Imaging Corp., Downingtown, PA, USA) was used to quantify individual particle dimensions.

## 4. Spectromicroscopy for Determining Elemental Composition

*Instrumentation.* Spectromicroscopy was employed to confirm the identity of individual distinctive $PM_{10}$ particles. The scanning mode of the TEMSCAN instrument was used to generate a microprobe beam of electrons for EDS. Spectra were obtained using a Tracor Northern X-ray detector (Noran, Madison, WI, USA) and EDS 2004 microanalysis software (IXRF Systems, Inc., Houston, TX, USA). On a "per particle" basis, using a selection of individual particles seen in a field of view, EDS (Chandler, 1977) was employed to identify pertinent elements ($Z>10$). For those elements of environmental interest, the minimum detectable mass fraction in the electron beam "spot" (which locates the volume of section irradiated by the beam) is in the range of 0.1%-0.5%. For the purposes of this research, absolute quantification of elements by EDS was not necessary; instead, we determined the relative abundances (Jackson et al., 1999) of elements present in measurable amounts, and then examined element associations in precisely located particles. Particles selected for EDS were those made visible by native electron opacity (an indicator of moderate-to-large atomic number).

*Analysis of spectra and rationale for selection of particles for EDS.* A minor constraint on our capacity to detect and interpret relative abundances of elements in ultrathin sections (essentially the relative intensity of X-ray emission of each element from the same concentration, or "*P*" value as defined

by Russ, 1972) had to be taken into account. This constraint is revealed here by considering the Kα line at 80 kV. The "*P*" value differs for different elements. For each element that we were able to detect, the "*P*" value is as follows: Al, 58%; Ca, 94%; Cl, 87%; Cr, 100%; Fe, 98%; K, 93%; Mg, 47%; Mn, 100%; Na, 34%; P, 75%; S, 82%; Si, 67%; Ti, 99%. For a quantitative comparison of peaks, the total counts under a given peak were adjusted using the *"P"* value. For samples embedded in plastic, the plastic itself was tested for detectable amounts of Cl (typically nil). Not used herein but worthy of consideration is the "k-factor", introduced shortly after the "*P*" value. The Cliff-Lorimer "k-factor" was introduced as part of a procedure for the quantitative analysis of individual elements in thin specimens (Cliff & Lorimer, 1975). The calculation and use of "k-factors" is well described in Williams & Carter (1996).

## 5. Intermediate Resolution Microscopy (SEM and ESEM Protocols)

SEM and ESEM present topographical images of entire particles and particle aggregates. For particles larger than ca. 50 nm, TEM tends to display a section through a particle, recording differentially transmitted electrons which yield images at near nanometer resolution. TEM reveals the nanoscale structure within a particle while SEM and ESEM reveal the outer surface of a particle. With increasing diameter, the shape of an individual irregular particle becomes increasingly difficult to discern by TEM, unless one takes serial sections. With a conventional SEM and an ESEM, one can readily ascertain the overall shape of most kinds of particles in the $PM_{10}$ size range, provided that the EM employed can resolve the smallest particles. In this regard, an ESEM is limited by resolution constraints, but has the advantage of permitting control over the relative humidity in the specimen chamber. This latter feature is so important for some researches that an ETEM has been developed for them, to provide transmission images of hydrated environmental samples at TEM resolution (Wise et al., 2005, 2007).

Well-mixed populations of dry particles were placed (as whole mounts) on stainless steel stubs for observation and documentation with a conventional SEM (JEOL, JSM-840 SEM, Tokyo, Japan). Drops containing particles (dispersed in water) were placed on stainless steel stubs, which in turn were fitted into a Peltier stage, cooled to 1°C, and viewed in a Model 2020 ESEM (ElectroScan Corporation, Wilmington, MA, USA) operating at 20 kV. The chamber pressure was carefully monitored and altered to slowly lower relative humidity within the chamber, to remove enough surface water from the sample to allow observation of topographical views. Images of native hydrated particles and their aggregates were archived on disk as TIFF files; dynamic changes could be recorded on videotape.

## 6. Selected Area Electron Diffraction

Under an electron beam, the atoms of crystalline particles may cause electron beam scattering into a patterned array. The interplanar distances between diffraction pattern spots provides information on crystalline symmetry and aids in crystal identification. In the TEM, a selected area electron diffraction pattern is formed by the use of an intermediate lens aperture to limit the sample area exposed to the electron beam. In this way, a single isolated particle can be selectively exposed to the beam. The objective aperture is removed from the electron pathway and the user switches from imaging mode to diffraction mode. This alters the current in the first projector lens of the microscope so that the diffraction pattern formed at the selected area is viewed on the imaging screen (Misell & Brown, 1987).

## 7. High Resolution Transmission Electron Microscopy

HRTEM (Botton, 2007) analyses must await a proper quantitative collection of ultrafine particles and their size-range sub-populations. With HRTEM one could pinpoint the exact distribution of an element within a particle (e.g., within the core vs within a surface layer vs homogeneously distributed) using a resolution in the lowest part of the nanoscale range, near atomic dimensions.

## 8. Spectromicroscopy for Speciation of Molecules within a Particle

Scanning transmission X-ray microscopy, or STXM, can be used to quantitatively differentiate different molecular species within a given particle. It does not have a resolution as high as that of TEM, but it has been used correlatively with TEM to produce chemical composition maps of an individual particle which can be superimposed on a TEM image (Lawrence et al., 2003). When the molecules of interest are heavy metals, STEM-EDS can be employed to verify the presence of those heavy metals. The major TEM-based technology (STEM-EDS) resolves particle structure better than STXM does, but is confined to identifying specific elements rather than compounds of those elements. EELS can be used with TEM to produce information on particle chemistry (Mavrocordatos et al., 2000), but the chemical information is limited. Neither STXM nor EELS was used herein, for the same reason that HRTEM was not used.

## RESULTS

The major results are in technique refinements, rationales for technique selections and correlative analysis strategies to obtain information on ground-level $PM_{10}$ particles, via nanoscale examinations. Using this technical report as a primer, an experienced technician (dedicated to electron spectromicroscopy and related technology) can pursue ground-level air particle research quickly, without requiring a major preparation phase. Research scientists can now use our detailed literature survey and protocol outlines to begin their efforts quickly.

A routine analysis of a field of view of air particles, using STEM-EDS and SAED, is shown in **Fig. 3**. A quantitative automated size analysis, of particles selected from a field of view, is recorded in **Fig. 4**. Biomedical preparatory approaches to ultrafine particle analysis are outlined in Mavrocordatos et al. (2007) and in Leppard (2008).

In a special sense, our literature-based discovery of the spectromicroscopical research of Dr. L. E. Murr was a result. His overall approach is similar to ours (although he borrows less from biomedical preparatory techniques), but his skilled development of a sampling device for ultrafine particles in the field permits a level of quantification which we were not able to achieve with our size fractionation approaches. However, in our efforts to find a means to do quantitative research on ground-level $PM_{10}$ particles, we did learn valuable lessons about how to extract a maximum of information from individual particle types even though we could not discern for the smallest particles what proportion they represented of the total sample.

**Fig. 5** shows an analysis by STEM-EDS and SAED done by the Murr group (Bang et al., 2004). It resembles our **Fig. 3**, although the specific nature of the air particle sample was different.

## CONCLUSIONS AND DISCUSSION

A thermal precipitation device (TPD) has been modified and refined for use with TEM and developed for the quantitative analysis of fine and ultrafine air particles (Bang & Murr, 2002; Murr & Garza, 2009) by the L.E. Murr group in the University of Texas at El Paso, TX, USA. This specially designed TPD is based on simple concepts of aerosol physics and thermodynamics; it is shown in **Fig. 6**. The particles of major interest to atmospheric scientists can be very efficiently collected, with claims of collection efficiencies as high as 100% (Bang & Murr, 2002). The TPD uses characteristics of thermal-gradient-driven adsorption on the surface of a carbon-coated Formvar (polyvinyl formal) film on a copper (or nickel), 100 mesh, 3 mm TEM grid. An efficient collection regime is achieved by (1) varying the temperature of a tungsten resistively-heated (hot) wire placed in the entering air stream, (2) the cooling of

a copper (substrate) block into which the TEM grids were placed, and (3) adjusting the air flow rate. A simple metal baffle directs the airflow onto the hot wire and the cold substrate/grids. The substrate can be cooled conveniently using flowing ice water. Series of calibrations are readily developed.

While electron microscope-based spectromicroscopical methods currently provide the best means to both detect and analyze nanoparticles on an individual basis, it would be best to use them in tandem with less expensive, more rapid techniques which are complementary. Many of these are described in Mavrocordatos et al. (2007). An apparently reliable complementary approach is being developed by Wang et al. (2006) who have produced a nanoaerosol mass spectrometer for real-time characterization of individual airborne nanoparticles. More recently, Hu et al. (2009) used an electric vehicle mobile platform equipped with fast response instruments to measure (by inference) atmospheric $PM_{2.5}$ particles, including the nanoparticle component.

A more primitive technique than that of Bang et al. (2004) for collecting $PM_{10}$ particles on an electron microscope grid is that of Dye et al. (1997) who collected urban atmospheric aerosol on a porous carbon film sitting on a 200 mesh TEM copper grid. Its utility was tested by us and it can be effective, provided that the carbon films are not overloaded with particles and if particle size is minimized. Note that a film on a grid can be embedded and sectioned.

When the sample is inside the electron microscope chamber, constraints can be imposed on measurements, according to which kind of microscope is employed (e.g., TEM vs ESEM). Depending on both the nature of the aerosol and of the chamber, ultrafine particles can gain or lose water, with a concomitant change in dimensions. For some researches, it is important to adjust the relative humidity of the chamber, to obtain realistic dimensions. Ambient relative humidity can affect the dry depositional fluxes of aerosol particles which bear trace elements and heavy metals, especially submicrometer particles (Ondov et al., 1997). Posfai et al. (1998), using correlated information from atomic force microscopy and TEM, showed that the wet and dry sizes of atmospheric aerosol particles can be substantially different, being many times larger when hydrated. ESEM has been proposed as a technique to determine the hygroscopic behaviour of individual aerosol particles (Ebert et al., 2002). For certain samples and research goals, it should definitely be used.

Extending the idea of employing ESEM, Wise et al. (2005) were able to study phase transitions of single salt particles, using a transmission electron microscope equipped with an environmental cell (an ETEM). They used deliquescence relative humidity and efflorescence relative humidity values from the literature, for each salt, to calibrate the relative humidity in the environmental cell. Then they reliably and accurately measured the phase transitions and hygroscopic behaviour of inorganic particles. In 2007, they

extended this research to ETEM analyses of the hygroscopic behaviour of NaCl-bearing natural aerosol particles to generate information of environmental relevance for five kinds of particles (Wise et al., 2007).

Selective elimination must also be considered as presenting a constraint on measurement. Vaze & Chiew (2002), studying atmospheric particles >$PM_{10}$ deposited on an urban road surface, found that there could be an elimination of particles in a certain size range as a result of disintegration or fragmentation into smaller particles. Such selective elimination was reviewed earlier, as were the constraints caused by masking phenomena for size fractions containing a very wide range of sizes, and the problem of spontaneous nucleation of nanoparticles from the gas phase (Leppard & West, 2006).

## RECOMMENDATIONS

We recommend the use of this primer for technique refinements, rationales for technique selections and correlative analysis strategies. We recommend the use of a thermal precipitation device for collection of $PM_{10}$ air particles in the field. For certain sample types and certain research goals, we recommend the use of ESEM and ETEM. We recommend also the use of scanning transmission X-ray microscopy and biomedical preparatory techniques when applicable.

## SUMMARY

The $PM_{10}$ fraction of Toronto street dust at the Markham Road site was analyzed at the nanoscale over a three year period (2004, 2005, 2006), with year 2006 being the focus herein of attempts to characterize the major particle types by spectromicroscopy, both quantitatively and qualitatively.

Characterizations made on a "per particle" basis were successful, but our selection of fractionation techniques did not allow us to determine the relative amounts of each particle type in the lowest portion of the $PM_{10}$ size range. The nature of $PM_{10}$ particles changes from the top to the bottom of the size range. The lower part of the $PM_{2.5}$ fraction can be underestimated if particle collection is not carried out correctly and fastidiously.

A literature search pointed us in the direction of the TPD as a collection device for ultrafine particles in the field. We are convinced that a TPD must be employed in future work to quantify, by electron optical techniques, the numbers of each particle type for the smallest ultrafine particles. The TPD permits the collection of fractal aggregates of ultrafine particles; the structural characteristics of fractal aggregates are highly informative to atmospheric environmental scientists.

This technical report evolved into a compendium of the best and most cost-effective means to produce environmentally-relevant characterizations covering the entire ultrafine particle size range

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## FIGURE LEGENDS

Figure 1. Overview of sampling procedure and analyses. Figure 2.5 of Rochfort et al. (2007).

Figure 2. Schematic of the nanoparticle cycle in the atmosphere. Figure 4 of Buseck & Adachi (2008).

Figure 3. AEM analysis of ground-level $PM_{10}$ air particles, starting with (left) a TEM image of a field of view of particles, then a selection (upper left) of an aggregated particle for STEM-EDS analyses (upper right) of elemental composition, followed by (lower right) selected area electron diffraction at analysis site 2. The $PM_{2.5}$ aggregated particle revealed a strong signal for Al and Si in one subcomponent and a strong Ca signal in another. The diffraction diagram is a fingerprint which can subsequently be analyzed for mineralogical information. The air particles were portions of an SBSW sample taken in July, 2006. The copper signals came from the grid.

Figure 4. Comparison of mean particle size ratios for 8 sampling sites. The ratios were calculated from sizing all particles in SEM micrographs of 10 representative areas from aliquoted samples placed on SEM stubs.

Figure 5. This is Figure 3 of Bang et al. (2004) and is analogous to Figure 3 (above). The particles are $TiO_2$ nanocrystal aggregates collected by a TPD at various times and locations from the air over El Paso (TX, USA). (a) A TEM image, with SAED pattern insert and EDS spectrum. (b) Another TEM image and EDS spectrum, with SAED pattern insert. Note that the two samples have different elements associated with the $TiO_2$ nanocrystals.

Figure 6. From Bang et al. (2004), Figure 1a which diagrams the TPD (simplified version of the schematic), and Figure 1b which shows the field of view of its TEM grid and the collected air particles.

Figure 1

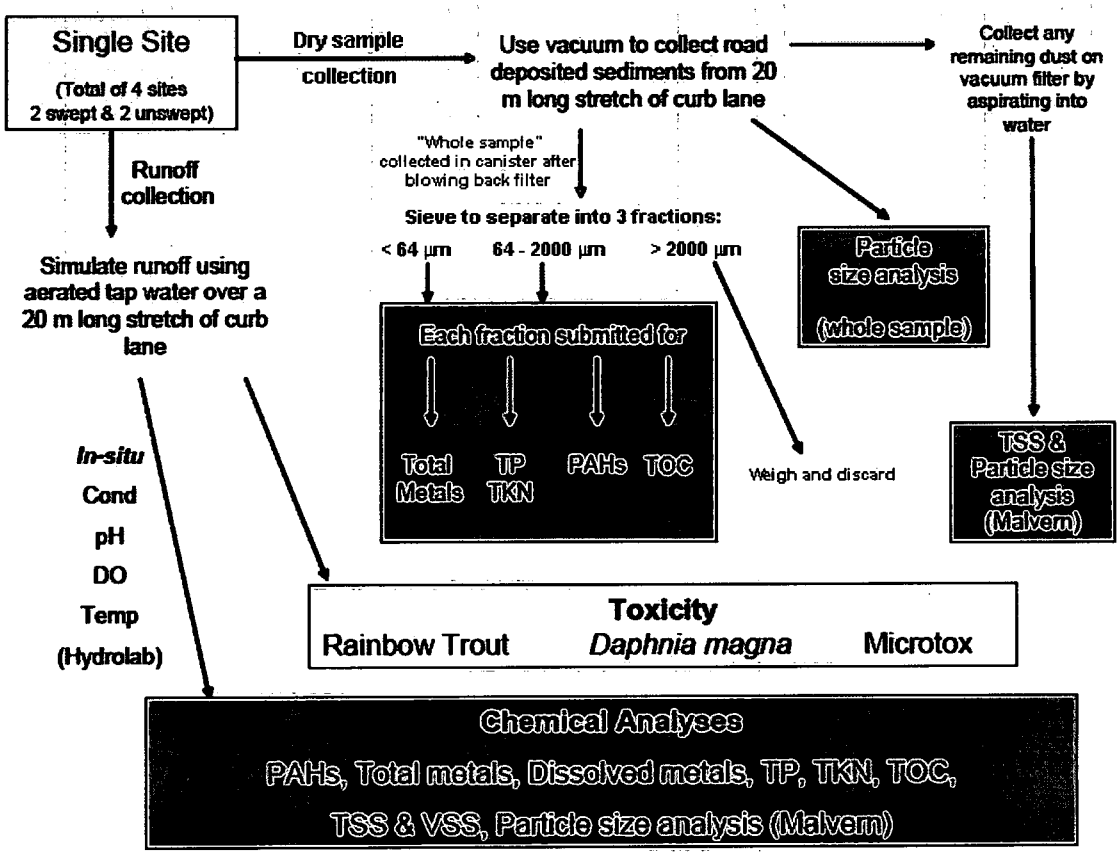

Single Site
(Total of 4 sites
2 swept & 2 unswept)
Dry sample collection
Use vacuum to collect road deposited sediments from 20 m long stretch of curb lane
Collect any remaining dust on vacuum filter by aspirating into water
"Whole sample" collected in canister after blowing back filter
Runoff collection
Sieve to separate into 3 fractions:
< 64 µm
64 - 2000 µm
> 2000 µm
Particle size analysis
(whole sample)
Simulate runoff using aerated tap water over a 20 m long stretch of curb lane
Each fraction submitted for
Total Metals
TP
TKN
PAHs
TOC
Weigh and discard
TSS & Particle size analysis (Malvern)
In-situ
Cond
pH
DO
Temp
(Hydrolab)
Toxicity
Rainbow Trout
Daphnia magna
Microtox
Chemical Analyses
PAHs, Total metals, Dissolved metals, TP, TKN, TOC,
TSS & VSS, Particle size analysis (Malvern)

Figure 2

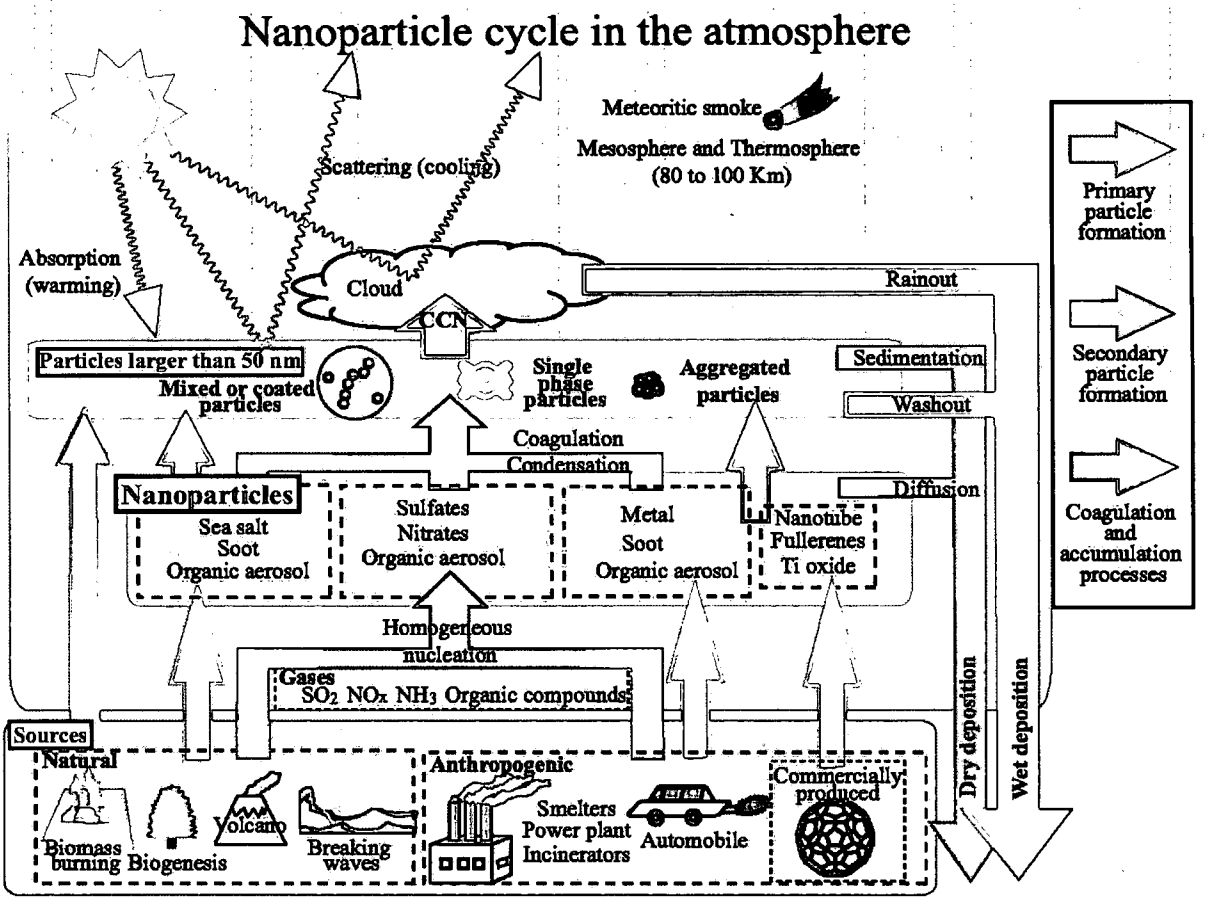

Nanoparticle cycle in the atmosphere
Meteoritic smoke
Mesosphere and Thermosphere
(80 to 100 Km)
Scattering (cooling)
Absorption
(warming)
Cloud
CCN
Rainout
Particles larger than 50 nm
Mixed or coated particles
Single phase particles
Aggregated particles
Sedimentation
Washout
Coagulation
Condensation
Diffusion
Nanoparticles
Sea salt
Soot
Organic aerosol
Sulfates
Nitrates
Organic aerosol
Metal
Soot
Organic aerosol
Nanotube
Fullerenes
Ti oxide
Homogeneous
nucleation
Gases
SO2 NOx NH3 Organic compounds
Sources
Natural
Biomass burning
Biogenesis
Volcano
Breaking waves
Anthropogenic
Smelters
Power plant
Incinerators
Automobile
Commercially produced
Dry deposition
Wet deposition
Primary particle formation
Secondary particle formation
Coagulation and accumulation processes

Figure 3

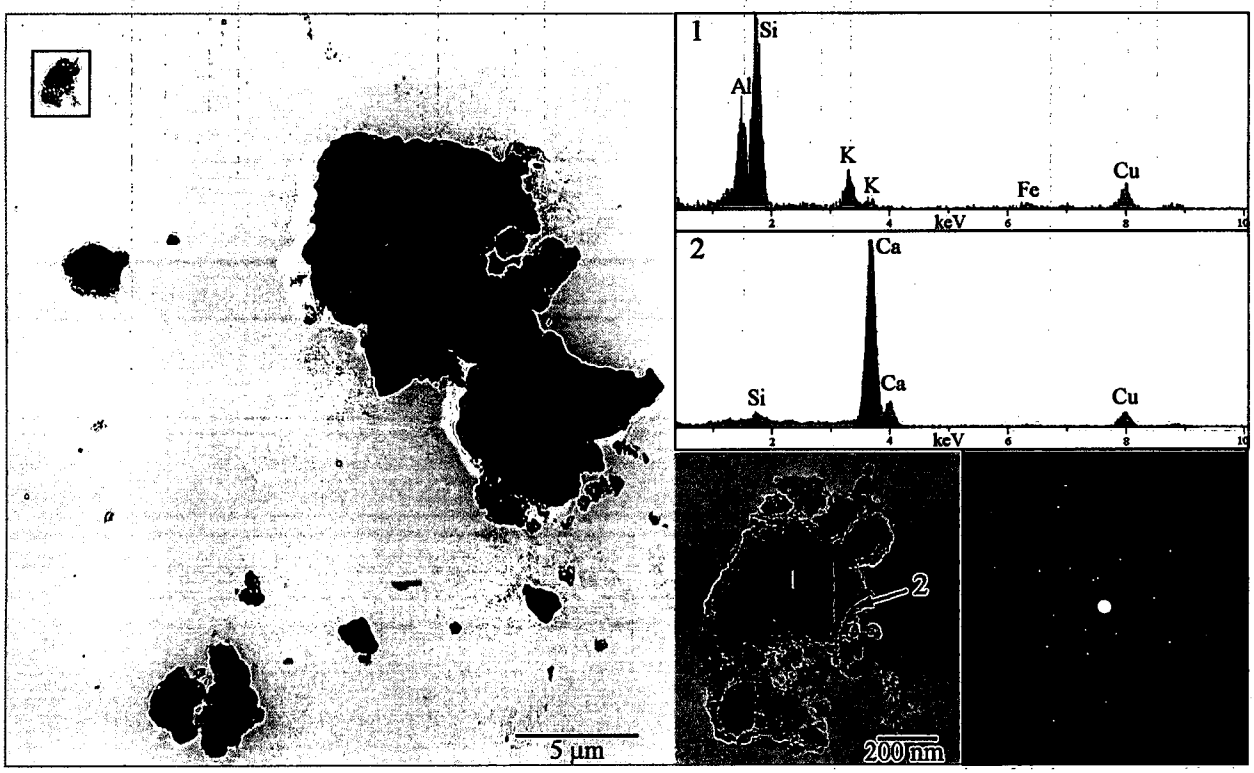


Figure 4

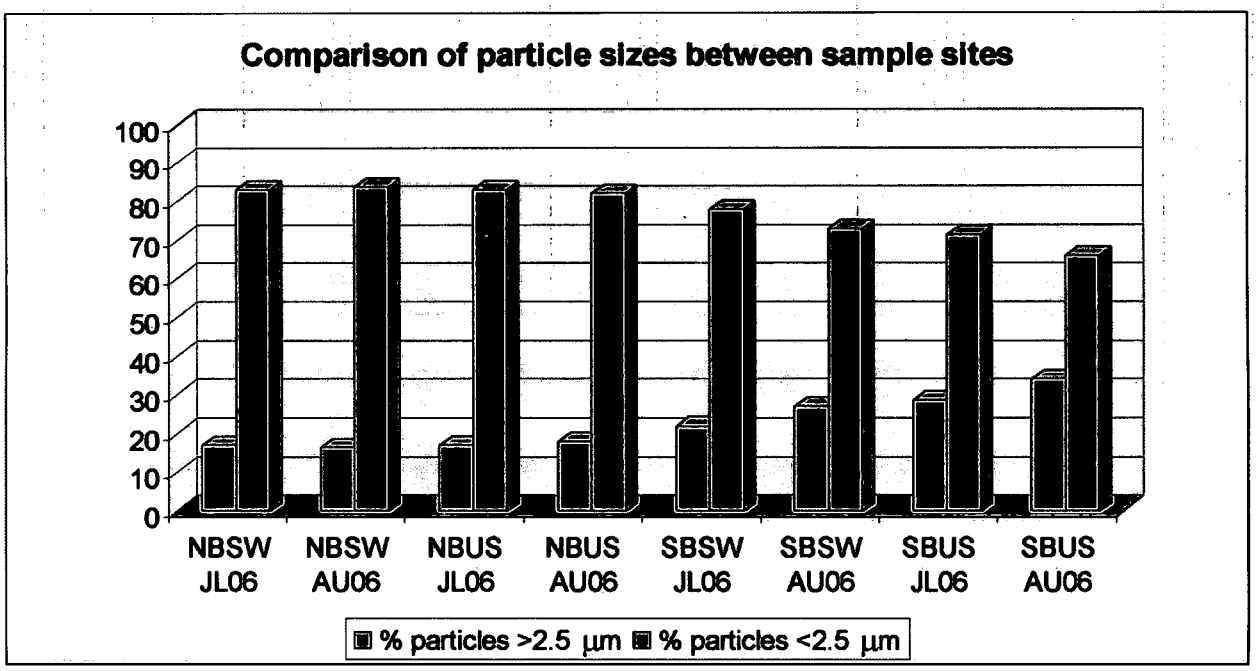

Comparison of particle sizes between sample sites
100
90
80
70
60
50
40
30
20
10
0
NBSW JL06
NBSW AU06
NBUS JL06
NBUS AU06
SBSW JL06
SBSW AU06
SBUS JL06
SBUS AU06
% particles >2.5 µm
% particles <2.5 µm

Figure 5

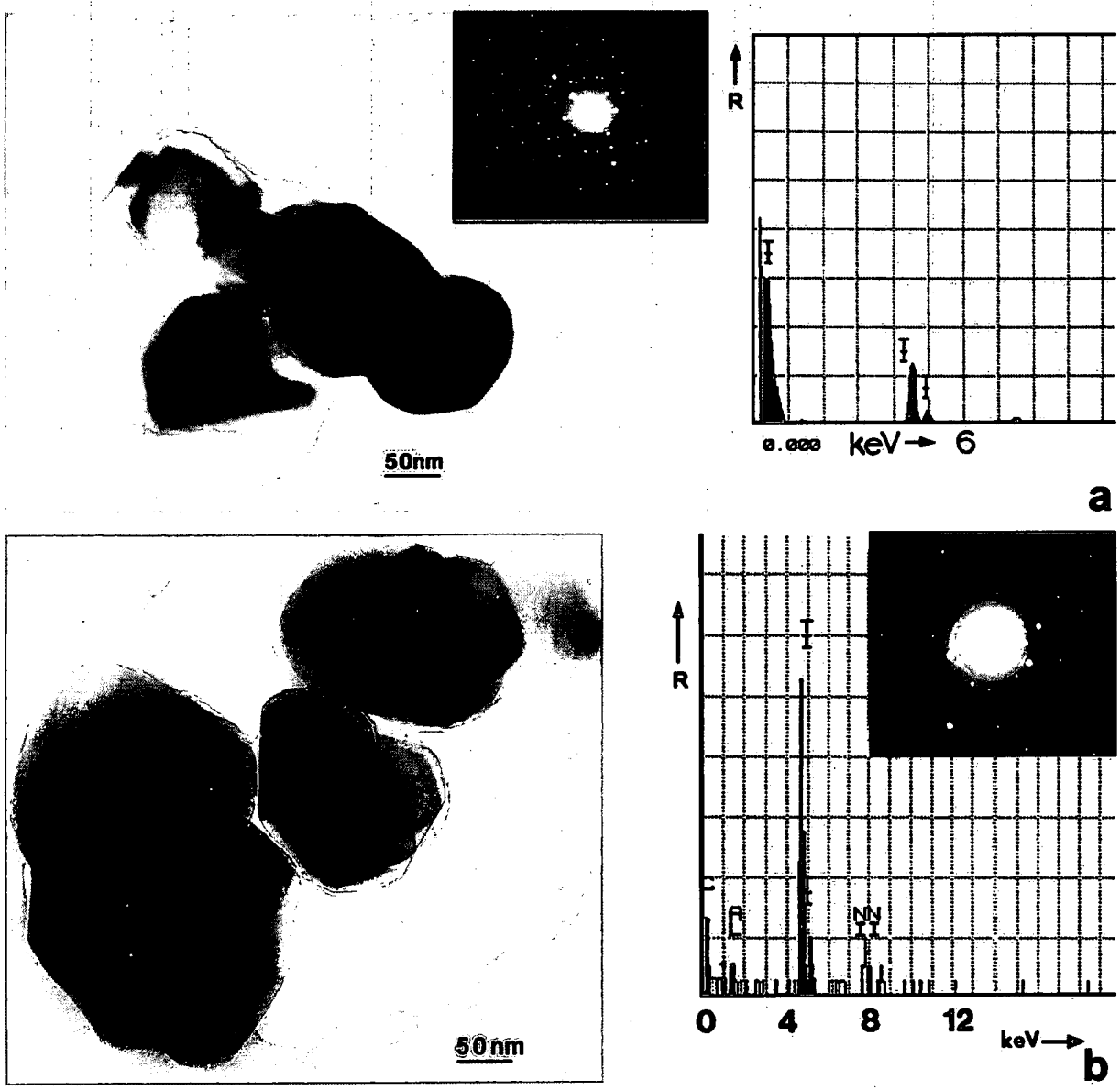
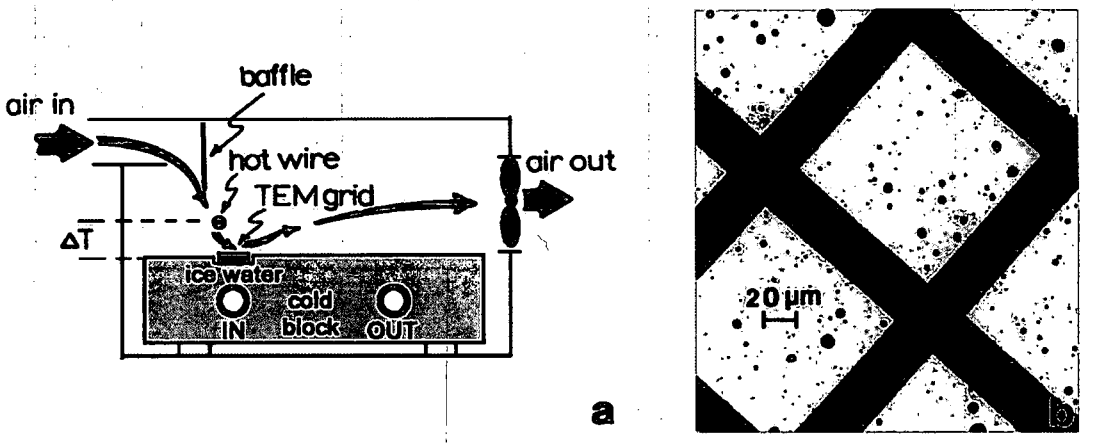


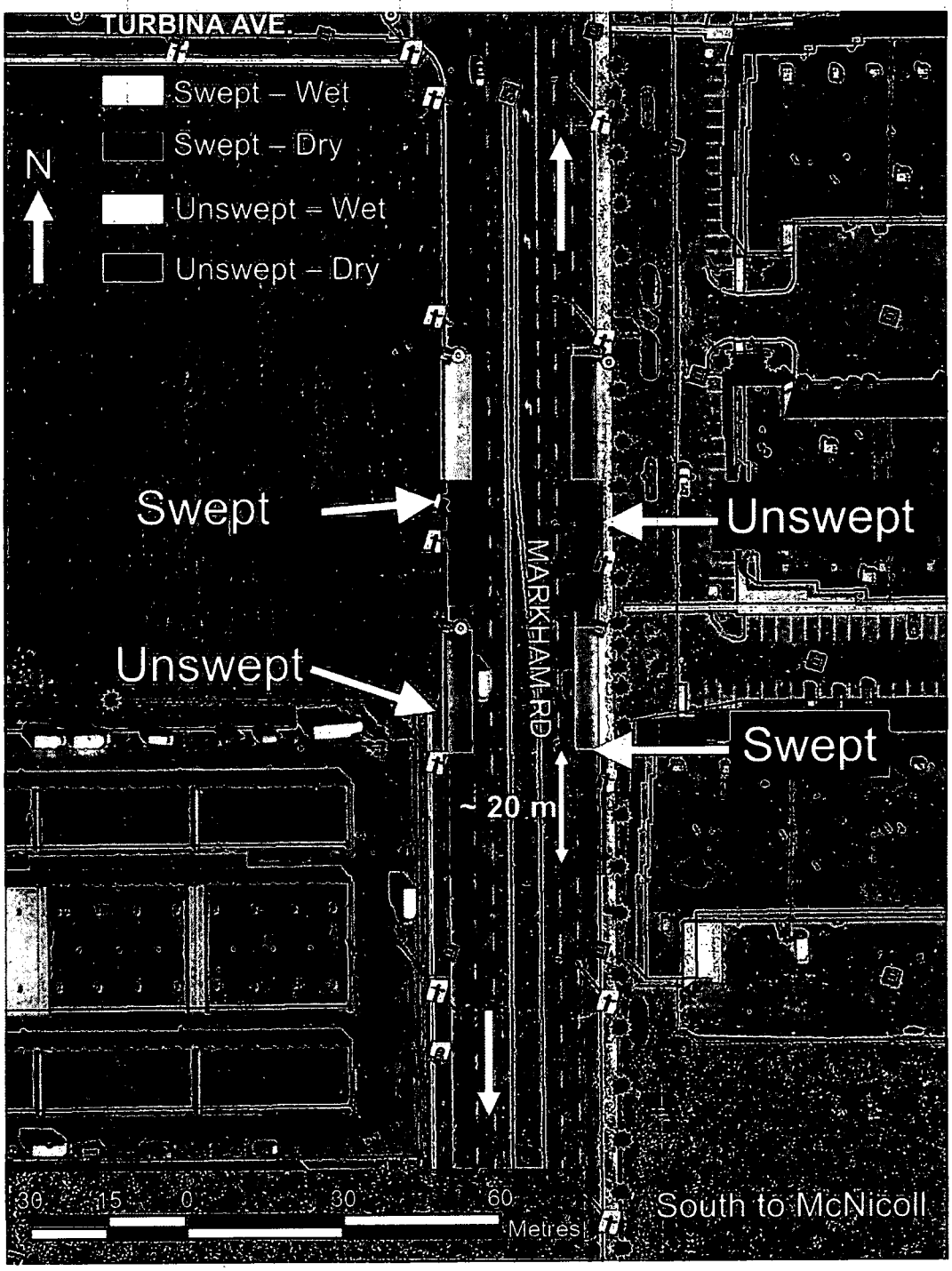
Figure 6


baffle
air in
hot wire
TEM grid
air out
ΔT
ice water
cold
block
IN
OUT
a
20 µm

# APPENDICES

## APPENDIX 1

Markham Road site paired test catchment areas; aerial view. Figure 2.1 of Rochfort et al. (2007).



## APPENDIX 2

### Sample Site and Sampling

***First year.*** Samples of air particles generated by street sweepers, on Markham Road in Toronto (Kelman & Crowther, 2005), were collected on 23 September, 2004 (northbound swept; NBSW). The test street site (see Appendix 1, for experimental layout in aerial view) has a traffic flow of 26,000 vehicles/day in each direction, northbound (NB) and (SB) southbound (Rochfort et al., 2006, 2007). The approach to field collection at the time described the 2004 sample size range as "2000 μm down to 3 μm", and "768.5 gr / 80m$^2$". A large data collection on its chemical components and contaminants (total metals, PAHs and nutrients), as well as results from toxicity tests (Rochfort et al., 2006, 2007), are stored at NWRI, by the UWMProject.

***Second year.*** In 2005, a more extensive sampling for particle characterization by electron-optical techniques (27 October, 2005; NBSW, NBUS, SBSW, SBUS) was done, with an emphasis on comparing swept (SW) and unswept (US) samples. These samples have been described extensively (since 27 October, 2005) by non-microscopical techniques (as per the 2004 samples), with their data collections also stored by the UWMProject (Rochfort et al., 2007). Experience gained after 2004 permitted a more intensive approach to characterizing the total particulate by EM in 2005; this included ready removal (by sieving, see below) of problematic particles larger than several tens of micrometers. This report on particle characterization is concerned mainly with the samples from 2005, and subfractions of them.

## APPENDIX 3

### Sampling Strategy and Coupling to Multidisciplinary Analyses

During the sampling period, the field site (Appendix 1) was neither swept nor washed by Toronto City works vehicles, except as directed during site visits for sample collection. Pollutants were allowed to build up on the road surface during the antecedent dry period, with at least seven days of dry weather preferred. For each test site, the dry sampling was initiated prior to the wet sampling, whenever possible, to ensure an optimal surface for dry vacuuming; the procedure is described in detail in Rochfort et al. (2007). *Note that wet sampling, not examined by electron-optical means in this report, was also done on a similar area to that of the dry sampling (20 m x 4 m), but located so that the wet sample would drain into the catch basin and be collected in a catch basin insert, according to the detailed methodology of*

*Rochfort et al. (2007).* Modifications to the sampling strategy imposed by nature and by the need to avoid traffic disruptions, accompanied by rationales for the modifications, are described in Rochfort et al. (2007). Outlines of the multidisciplinary analyses of the site properties, the initial particle fractionations (<64 μm, 64-2000 μm, >2000 μm), the pollutants associated with the initial fractions and the toxicity testing are also found in Rochfort et al. (2007). Samples for sieving (<38 μm) and further subfractionation, to be examined subsequently by analytical electron microscopy and associated spectromicroscopy, were brought back to NWRI in Burlington.

## APPENDIX 4

### Street Sweeper Description and Operation

***Street sweeper comparison.*** Toronto street sweeper machines, provided by the City of Toronto, collected air particles deposited at ground level. The northbound lanes were swept with "old technology regenerative air" sweepers (Air-Bear); the southbound lanes were swept with "conventional broom" sweepers (Johnston 4000). Regenerative air technology blasts a curtain of air onto the pavement, clearing dust from the cracks and blowing off loosened dirt into the vacuum, which then picks up the particulate, removing most particles via a cyclone, followed by filters. The old technology regenerative air sweepers have filters which are periodically blown off to ensure that the vacuum exerts a greater effect than the air blast, but this old technology can become problematic; the air coming through the filters is recycled back onto the pavement, so that when filters malfunction there can be an undesirable resuspension of particulate back into the nearby air (a potential health hazard). Analyses of function of the "new technology regenerative air" version, for future comparison of strengths and weaknesses regarding undesirable resuspension, uses improved filters and increased air volumes to scrub more material from the road. The "conventional broom" sweeper is simple, using a rotating nylon bristle brush to collect material in a hopper. For a more detailed description of street sweepers, consult Rochfort et al. (2009).

***Air particles retained by the sweepers.*** The swept air particles, which have a bearing on nearby air and surface water quality if they escape the sweeper and become resuspended in the nearby atmosphere, are of concern to this project. Until now, we have not examined the escaped particulate, since that topic is the domain of the City of Toronto Technical Services. Thus far, we have confined ourselves to the retained particulate which was subsequently sampled by a vacuum device for laboratory analysis; of great concern was the nature of $PM_{10}$ and $PM_{2.5}$ particles which can get entrained into the air by street sweepers (Kelman & Crowther, 2005). The device was a Nilfisk 2050 Series (Nilfisk-Advance

ID 2050 Cyclone) industrial vacuum cleaner used for dry vacuuming (Nilfisk-Advance, Radigan Bros. Ltd., Hamilton, ON). It produces a 24.5 kPa vacuum, at a flow rate of 75 L/s at the vacuum head; the total filter area for the 3 micron pleated filter is 14,400 $cm^2$. Laboratory experiments revealed that the Nilfisk-Advance industrial vacuum was capable of picking up particles in the 3-10 μm range at 97% efficiency, from concrete in good condition (Fralick, 2005).

## APPENDIX 5

### Sample Fractionation

General considerations. An initial examination of the "total" air particulate in the 2004 sample was carried out in its dry state. However, detailed examinations were feasible only on fractionated samples within the size range from tens of micrometers down to the lowest level of the nanoscale. Very large particles, visible by eye, were inappropriate for analysis by TEM; these should be collected separately and analysed for chemical composition by standard physical and chemical means. This early technical problem, namely fractionating away the very large particles, was not an issue in terms of research needs, since it had previously been determined, from public health considerations (Kelman & Crowther, 2005), that the particle fractions of interest in Toronto were $PM_{10}$ and $PM_{2.5}$. In a TEM view, such particles could be selected by eye (from the total particle population displayed) for subsequent dimensional, morphological and compositional analyses. After the initial experience with the 2004 sample, the detailed microscopical and spectromicroscopical analyses of fractions, representing the size range from 1 nanometer to the upper limit for $PM_{10}$, was based on the 27 October 2005 samples.

Sieving. Fractionation of dry air particles began with the removal of "large" particles (from 27 October, 2005, samples), using a <38 μm sieve, as defined by the Canadian Standard Sieve Series No. 400 sieve (W.S. Tyler Co. of Canada Ltd., St. Catharines, ON). This step was sufficient to remove particles large enough to interfere with TEM visualization of both nanoscale particles and particles in the range of 100-10,000 nm (0.1-10 μm). Starting with a <38 μm dry fraction, particle populations were prepared for electron-optical examination and characterization in three ways. Applied to particle populations, in a correlative manner (using portions taken from well-mixed starting samples), were (A,B) two different embedding procedures (followed by ultrathin sectioning) and also (C) the deposition of a representative population on a transparent film (with an optimal coverage, as defined by Lienemann et al., 1998). The TEM preparatory protocols, for sieved and subfractionated sieved samples, are found in Leppard & West (2006) in their section on 'TEM and STEM Coupled to EDS', which is expanded in this 2011 report.

Washoff technique for obtaining $PM_{2.5}$ and nanoparticles. Once the dry conventional-size particles had been poured out of the Nilfisk-Advance vacuum cleaner, the remaining particulate (fine material trapped on the pleated Teflon filter, and too fine to be poured out as a dry material) was washed out by wet vacuuming (the filter blowback material, wet vacuumed). This fine trapped particulate on the Teflon filter was vacuumed into a water filtration apparatus (Rochfort et al., 2007); this apparatus consisted of two flasks in series, each filled with one litre of distilled water. Airflow was set such that the air was discharged below the surface of the water in each flask, thus trapping the entrained particulate within the water matrix. This hydrated particle population was checked by TEM to verify the presence of $PM_{2.5}$ and its nanoparticle subpopulation, and to assess the extent of enrichment for them.

Subfractionation by dispersion in water, followed by differential sedimentation. The finest particulate material (the filter blowback material, wet vacuumed) could be examined *in toto* (using either ultrathin sections of embedded sample, or whole mounts of sample on a transparent film) or it could be examined after differential sedimentation (Marvin et al., 2004). For preliminary analyses of the fractionated finest particulate material, a simple gravitational sedimentation (in 'reverse osmosis water' at room temperature) for more than 4 hr was sufficient to yield a pellet, with a colloidal suspension above.

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