

# Redox characteristics of size-segregated PM from different public transport microenvironments in Hong Kong

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**Abstract** Exposure to airborne particulate matter (PM) has been associated with various adverse health effects, including severe pulmonary and cardiovascular effects. PM consists of different chemical components that vary with microenvironments in urban areas and pose challenges to assess personal exposure. In Hong Kong, more than 70% of the population commutes through roadway and railway public transport. This study aims to determine the oxidative potential and role of aerosol carbon and water-soluble metals in fine ( $d_p < 2.5 \mu\text{m}$ ) and coarse PM ( $2.5 < d_p < 10 \mu\text{m}$ ) in public transport systems including underground (UG) subway, above-ground (AG) train, and buses (BUS). Metals such as Fe, Cr, Mo, Pb, Ni, and V from UG, AG, and BUS routes exhibited much lower solubility compared with ambient PM. The cell toxicity of PM in these transport microenvironments was also analyzed *in vitro* and compared with urban ambient environments. Strong positive associations were observed for reactive oxygen species (ROS) with water-soluble metals (Cr, Cu, Fe, Mn, Ni, V, Mo;  $R > 0.70$ ) and organic and elemental carbon (OCEC) ( $R > 0.85$ ) for UG and AG routes. In addition, PM from UG and AG routes generated 3–4-fold (in  $\text{PM}_{2.5}$ ) and 40–50-fold (in coarse PM) less ROS compared to urban

sites, suggesting PM in these public transport microenvironments may not be intrinsically redox active than in urban ambient, and water solubility of metals seems to have played an important role in it.

**Keywords** Particulate matter · Public transport · Reactive oxygen species · Redox activity · Urban microenvironment exposure

## Introduction

Railway public transport is widely utilized in many major cities around the world (Nieuwenhuijsen et al. 2007). Various studies have indicated that particulate matter (PM) with aerodynamic diameter  $d_p < 2.5 \mu\text{m}$  ( $\text{PM}_{2.5}$  or fine PM) and  $2.5 \mu\text{m} < d_p < 10 \mu\text{m}$  (coarse PM) registered higher concentrations than the urban ambient environments in many subway systems around the world such as in Barcelona (Querol et al. 2012), Budapest (Salma et al. 2009), Buenos Aires (Murrini et al. 2009), Los Angeles (Kam et al. 2011a), Paris (Raut et al. 2009), Shanghai (Ye et al. 2010), and Tokyo (Furuya et al. 2001), with enriched concentrations of metals generated from subway-related sources. For example, increases of 200% Fe, 60% Mn, and 40% Cu were noted in the Helsinki subway system through personal exposure assessment (Aarnio et al. 2005). Similarly, higher concentrations of Mn, Cr, and Fe with 160–350 times greater than the outdoor ambient areas were observed in the New York City subway (Chillrud et al. 2004). However, some subway systems have reported lower PM levels relative to other transport modes (Chan et al. 2002a; Chan et al. 2002b), though results are not always directly comparable because of the differences in measurement methods, time, and duration of investigation. Other studies done with daily commutes on a diesel bus at

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Los Angeles and Brisbane bus routes reported higher concentrations of PM<sub>2.5</sub>, NO<sub>2</sub>, and PAHs as compared to buses powered by compressed natural gas (Sabin et al. 2005; Yip et al. 2006), showing the importance of transport modes on commuter exposure.

Although passenger exposure in public transport systems represents a small fraction of integrated daily exposure, high concentrations of the metallic composition and combustion of source-related pollutants might pose serious health implications. A widely applied hypothesis is that PM-induced toxicity is due to the interaction of PM with cells that leads to the generation of reactive oxygen species (ROS), which in turn changes the oxidative potential of the cells (Donaldson et al. 2002; Xia et al. 2004). Such intracellularly generated ROS can be quantified using fluorescent probes (Wang et al. 2013). Transition metals are reported to be potential airborne toxicants as they can generate ROS such as superoxide radical, hydrogen peroxide, and hydroxyl radical through oxidation-reduction reactions, causing homeostasis imbalance, which may lead to pulmonary inflammation and exacerbate diseases like asthma (Tao et al. 2003; van Klaveren and Nemery 1999). In addition, size-fractionated PM regulates the magnitude of ROS generation in cells (Cho et al. 2005; Hu et al. 2008). The largely different chemical characteristics of PM in public transport systems compared to ambient atmosphere represent an important source of uncertainty in the estimation of urban population exposure to particulate pollutants.

This study investigates on the chemical composition of fine and coarse PM collected in three different urban public transport systems including underground subway (UG), aboveground (AG) railway, and bus (BUS) routes of Hong Kong. The cell viability and oxidative potential of the size-fractionated PM in the different environments were also investigated using sensitive macrophage-based assays, and generated intracellular ROS (endogenous) was compared with non-cellular ROS (exogenous) and with that of urban ambient environment. In addition, non-cellular ROS (exogenous) was quantified and compared with endogenous ROS. The results highlighted the importance of understanding the microenvironment pollutant characteristics to mitigate the health risks due to exposure to air pollutants.

## Materials and methods

### Instrumentation

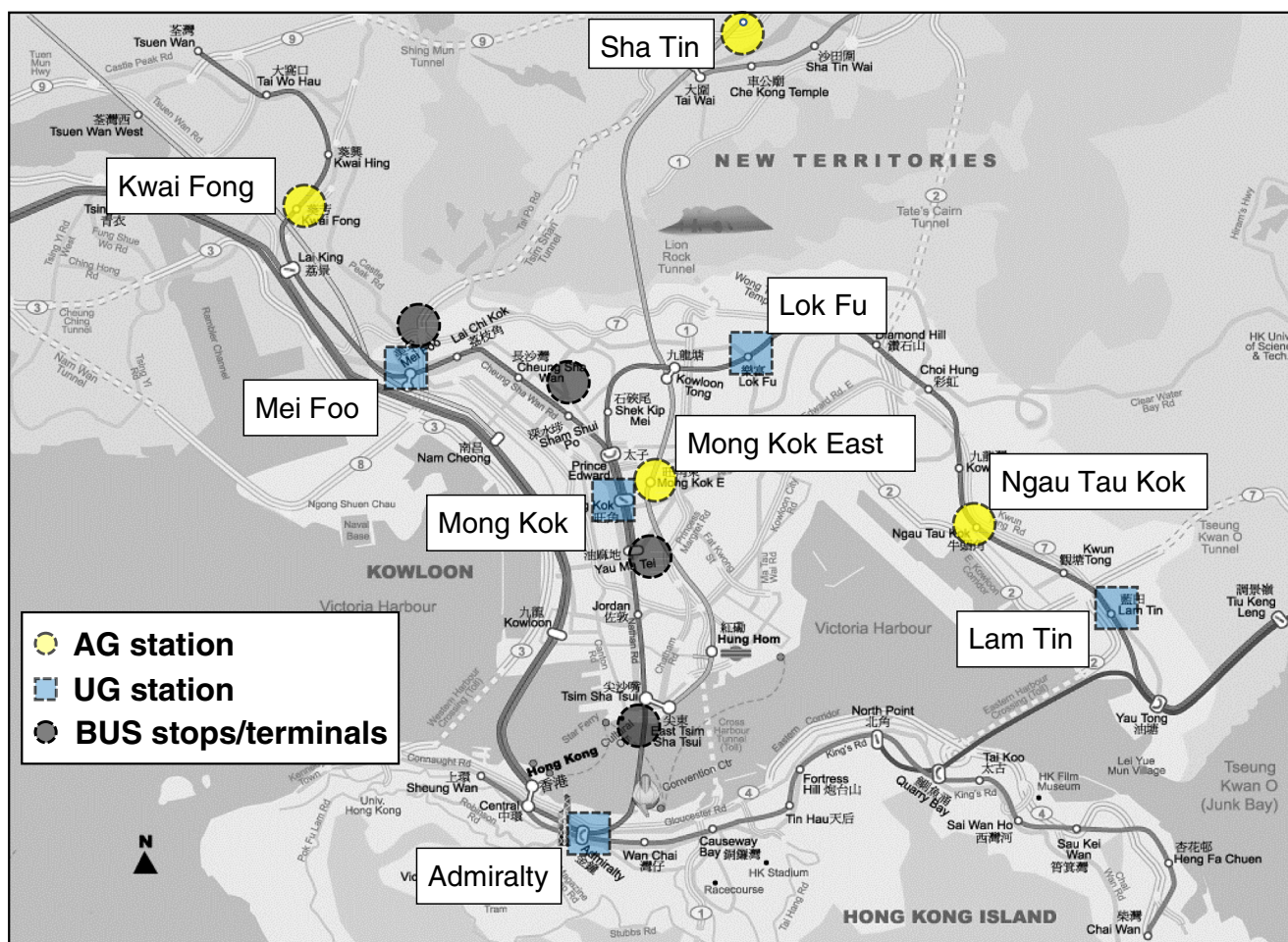
Four personal cascade impactor samplers (SKC Inc., Eighty-Four, PA, also referred to as PCIS) were installed in a suitcase with impaction stage at a cut point of 2.5 μm and after-filter stage to collect coarse (PM<sub>2.5</sub>–PM<sub>10</sub>) and fine PM (PM<sub>2.5</sub>), respectively. Two duplicate sets of PCIS

were loaded with 25-mm Zefluor filter (PALL Life Sciences, Ann Arbor, MI) for coarse PM and 37-mm PTFE filter (PALL Life Sciences, Ann Arbor, MI) for fine PM, while other two duplicate sets of PCIS were loaded with 25 and 37 mm quartz filters (Whatman Inc., Maidstone, UK). All PCIS was run throughout Leland Legacy Pump (SKC Inc., Eighty-Four, PA) at a standard flow rate of 9 l per minute. The flow rates were checked and calibrated by a Drycal flow meter (Bios International Corp., Pompton Plains, NJ) regularly before and after each sampling. Gravimetric measurement was performed by a microbalance (ME-5, Sartorius Inc., Germany). All filters were stored in plastic petri dishes sandwiched with baked aluminum foil and sealed with Teflon tape at –20 °C. A 24-h filter equilibration in a temperature (21 ± 2 °C) and humidity (35 ± 5%)-controlled room was allowed prior to the gravimetric measurement. Weighing was repeated at least three times to ensure the gravimetric uncertainty within ±3 μg.

### Sampling route

This study is part of a comprehensive investigation campaign on public transport-related air pollutants in Hong Kong. The real-time measurement of pollution concentration in different microenvironments has been reported elsewhere (Yang et al. 2015). This study reports the results of time-integrated filter sampling. All filter samples were collected during October 2013 to April 2014. Based on the real-time measurement campaign (Yang et al. 2015), three filter sampling routes were selected with representative characteristics including MTR aboveground route, MTR underground route, and bus route (Fig. 1). The design of individual sampling routes followed the earlier approach (Kam et al. 2011b) with combined time activities of waiting in MTR stations or bus stops and train or bus riding inside cabins. Hong Kong commuters spend on average 40 min on a daily basis in public transport systems with approximately 30% in transit and 70% in outside environment (HKTD 2014). This study adopted the same time distribution to represent the typical commuting profiles in different transport systems.

AG route covered four aboveground stations: Mong Kok East, Sha Tin, Kwai Fong, and Ngau Tau Kok, all of which were similar in built environment and pollutant characteristics, at either ground or elevated level that are directly open to the ambient atmosphere. UG route contained five underground stations: Lam Tin, Lok Fu, Mei Foo, Mong Kok, and Admiralty, all of which were enclosed at underground level with full-height screen doors separating the rail tracks and platforms. Bus route contained typical bus transport microenvironments with busy/secondary roadside stops and open/enclosed terminus (Yang et al. 2015). These MTR stations and bus stop/terminus were chosen to represent the typical



**Fig. 1** Sampling route map. Three routes were selected during sampling campaign including MTR aboveground (AG) route, MTR underground (UG) route, and bus route. MTR AG route contained four AG stations: Mong Kok East, Sha Tin, Kwai Fong, and Ngau Tau Kok, all of which

were built at ground level or elevated and directly open to the atmosphere. MTR UG route contained five UG stations: Lam Tin, Lok Fu, Mei Foo, Mong Kok, and Admiralty

daily commuting route between residential to urban/commercial areas and are continuous in their respective routes. Each route of sampling consisted of 4–6 days to collect enough PM mass and was repeated for three times. Between sampling days, the filter samples were stored at  $-20^{\circ}\text{C}$  as per standard procedures (Kleeman et al. 2000; Ning et al. 2007; Pakbin et al. 2011; Rees et al. 2004).

### Water and acid extraction

One of the two duplicate Teflon filters of  $\text{PM}_{2.5}$  sample was cut into halves after gravimetric analysis, and one half was used for acid extraction while the other whole duplicate sample was used for water extraction. For Teflon filters of coarse PM sample with limited mass, one of the two duplicate filters from three replicate runs were grouped together for water extraction, while one half of the duplicate filters from the replicate runs were grouped for acid extraction.

For water extraction, filters were soaked in 8 mL of Milli-Q water in 15 mL metal-free centrifuge tubes and extracted by a multi-tube vortex mixer (Model X-2500, VWR) for 12 h. After extraction, the extracts were filtered with  $0.22\ \mu\text{m}$  filter membranes and stored at  $-20^{\circ}\text{C}$ . These water extracts were used for metal, anion, and toxicity analyses. Prior to metal analysis, water extracts were acidified to 2%  $\text{HNO}_3$  in order to prevent the loss of metals from solutions. Acid extraction was carried out in a microwave digester (ETHOS EZ, Milestone, Sorisole, Italy). Acid digestion matrixes were prepared in 6 mL Teflon inserts with 16 N  $\text{HNO}_3$  and 12 N  $\text{HCl}$  in a volume ratio of 3:1. Three Teflon inserts were put in a 100-mL Teflon vessel with 8 mL Milli-Q water and 2 mL  $\text{H}_2\text{O}_2$  to prevent the high temperature of the inserts. The parameters were set to ramp up to  $130^{\circ}\text{C}$  within 15 min, hold for 5 min, increased to  $180^{\circ}\text{C}$  within 10 min, and kept at  $180^{\circ}\text{C}$  for 10 min to finish the digestion process. The system was cooled down and ventilated automatically in 2 h. Acid-extracted samples

were not employed for toxicity experiments and hence omitted from discussion. After acid extraction, all the sample solutions were diluted four times, then stored at 4 °C before analysis.

### Metals analysis

The concentrations of Ca, Fe, Mg, K, and Na were determined using inductively coupled plasma–optical emission spectroscopy (ICP-OES). The analysis was carried out using an Optima 2100 DV system in scanning mode with ion lenses tuned for maximum sensitivity. The plasma flow was set at 15 L/min, auxiliary flow at 0.3 L/min, and nebulizer flow at 0.8 L min<sup>-1</sup>. The RF power was set at 1300 W and flow rate of pump at 1.0 mL min<sup>-1</sup>. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the trace metal concentrations of Al, Cd, Cr, Co, Cu, Pb, Mn, Mo, Ni, V, and Zn with a 7500cx system (Agilent, USA). Parameters were set at RF power (W) 1600, carrier flow at 0.93 L/min, makeup gas flow at 0.24 L/min, sample pump rate at 1.0 rps, and nebulizer pump rate at 0.2 rps. The modified procedures and detailed instrumental process were followed from methods of Jiang et al. (2014). Multi-element standard solutions for inductively coupled plasma (ICP) were purchased from Fluka (Mo, USA). Linear calibration standards from 0.005 to 5 mg/L was prepared from stock solution. Nitric acid (65%) and hydrochloric acid fuming (37%) were obtained from Merck (Germany). The argon used in ICP with purity ≥99.995% was obtained from Hong Kong Linde Ltd.

### OCEC analysis

Organic and elemental carbon were analyzed directly on quartz filter sample using an organic and elemental carbon (OCEC) analyzer (Model 4, Sunset Laboratory Inc.) following NIOSH 5040 protocol (Birch and Cary 1996), which operates by converting solid-phase carbon into equivalent CO<sub>2</sub> gas for their quantification. The thermal desorption of organic and inorganic compounds was initiated by gradual ramping of temperature inside the oven, and pyrolyzed compounds are quantified using non-dispersive infrared (NDIR) sensor following oxidation with MnO<sub>2</sub> to its equivalent CO<sub>2</sub>. The OCs and ECs of each sample are thus determined based on the ratio of their peak areas with internal calibrated methane gas peak. A known concentration of sucrose (2, 6, 12, 14, and 20 mg/L impregnated on baked quartz filter) was employed (before sample analysis) in an OCEC analyzer as a multipoint carbon calibrator to ensure measurement accuracy. Correlation between measured and injected carbon was maintained 1:1 with  $R^2 \sim 0.99$ .

### Reactive oxygen species and cell toxicity analysis

#### *Macrophage cell culture*

RAW 264.7 murine monocytic-macrophage cell line (ATCC, USA) was cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS, Gibco, USA). The cells were grown at 37 °C and 5% CO<sub>2</sub> (370 series, ThermoFisher Scientific, Marietta, Ohio) till a confluence of 80% was attained. Second-generation cells were used for the study. Cell counts and viability were determined on a hemocytometer (0.1 mm depth, Hausser Scientific, Horsham, PA) employing Trypan blue dye, and 10<sup>4</sup> cells (devoid of 10% FBS) were used for the study.

#### *Trypan blue exclusion*

Cell density and viability were determined using a hemocytometer. A solution of 0.4% Trypan blue (Invitrogen, USA) was prepared in phosphate buffer saline, pH 7.2.0. One milliliter of Trypan blue stock was added to 1 mL of cells and loaded on to the hemocytometer using a capillary tube and observed under a microscope (CKX41SF, Olympus Corp. Tokyo) to count the stained (takes up the dye and will be blue color) and unstained (live cells) cells. The aliquots with live cells were used for plating in cell viability and ROS experiments.

#### **Cell viability assay**

Cell viability was analyzed employing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT, Abcam, HK) (Yue et al. 2010). MTT is a chromogenic indicator used widely for rapid and large-scale assays for the determination of cell growth rates. The water-soluble MTT is metabolized into insoluble formazan by intracellular NAD(P)H oxidoreductase enzyme, when the cell is alive (Mosmann 1983). A reaction volume of 250 μL contained varying concentrations of PM, 10 μL cells, and 20 μL MTT dye. The final volume was made to 250 μL with PBS, pH 7.2. Cells at a number of 10<sup>4</sup> were seeded per well in a 96-well plate and incubated with different volumes of PM extracts (10, 50, and 100 μL) for 4 and 24 h at 37 °C and 5% CO<sub>2</sub>. The yellow MTT dye was then added to samples and untreated cell control at a final concentration of 0.45 mg/mL and allowed for 4 h of incubation at culture conditions, followed by replacement of 100 μL supernatant with DMSO to solubilize the formazan, which was spectrophotometrically (Spectramax M2e, Molecular Devices) recorded at 570 nm and compared with untreated controls (positive control) to determine percentage of cell

viability. PM along with the dye served as negative control. The result from this assay was used in assessing the safe PM dose to carry out ROS activity.

### ROS activity

Endogenous ROS (endo-ROS) activity was measured by employing 2,7-dichlorofluorescein-diacetate probe (DCFH-DA, Sigma, USA) (Wang et al. 2013) with few modifications which are as detailed below. The non-fluorescent DCFH-DA probe due to its diacetate moiety diffuses into the cell, where it undergoes intracellular deacetylation by cellular esterase enzyme to form DCFH, which further on reaction with intracellular ROS becomes oxidized to DCF, whose fluorescence measured is equated to the amount of ROS generated (Wang et al. 2013). The reaction volume of 250  $\mu\text{L}$  contained varying concentrations of PM, 10  $\mu\text{L}$  cells, and varying volumes of PBS buffer. Cells ( $10^4$ ) were seeded per well in 96-well plates and incubated with PM extracts (three doses 10, 50, and 100  $\mu\text{L}$ ) at standard culture conditions for 4 and 24 h. A 140- $\mu\text{L}$  DCFH-DA (final conc. 20  $\mu\text{M}$ ) reagent was then added to the samples and controls and incubated for another 30 min at culture conditions. The final volume is made to 250  $\mu\text{L}$  with PBS. Untreated cells and cells with  $\text{H}_2\text{O}_2$  served as negative and positive controls, respectively. While untreated cells represent basal ROS generation in cells,  $\text{H}_2\text{O}_2$ -treated cells are used for preparing calibration curve. The increase in absorbance of DCF per minute was recorded at excitation and emission wavelength of 485 and 530 nm, respectively, and the measured light intensity is compared with calibration curve of standard  $\text{H}_2\text{O}_2$  prepared in the concentration range from 50 to 1500  $\mu\text{M}$ , and the ROS activity unit was defined as micromolar of  $\text{H}_2\text{O}_2$  produced per minute per cubic meter of air volume or per microgram of PM. PM-induced exogenous ROS (exo-ROS) was measured similarly without cells, and DCFH-DA is replaced with DCFH reagent. DCFH reagent is prepared freshly prior to the experiment by incubating DCFH-DA (50 mM) with NaOH (0.1 N) in 1:4 ratio, for 15 min, and diluted with HBSS to get a final concentration of 20  $\mu\text{M}$  DCFH. PM free sample served as DCFH control.

### Statistical analysis

All experiments were done in triplicates with three independent biological replicates, and data was presented as the mean  $\pm$  standard deviation (SD). One-way ANOVA test was used to compare the mean of groups, and significant changes were noted as  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$  accordingly.

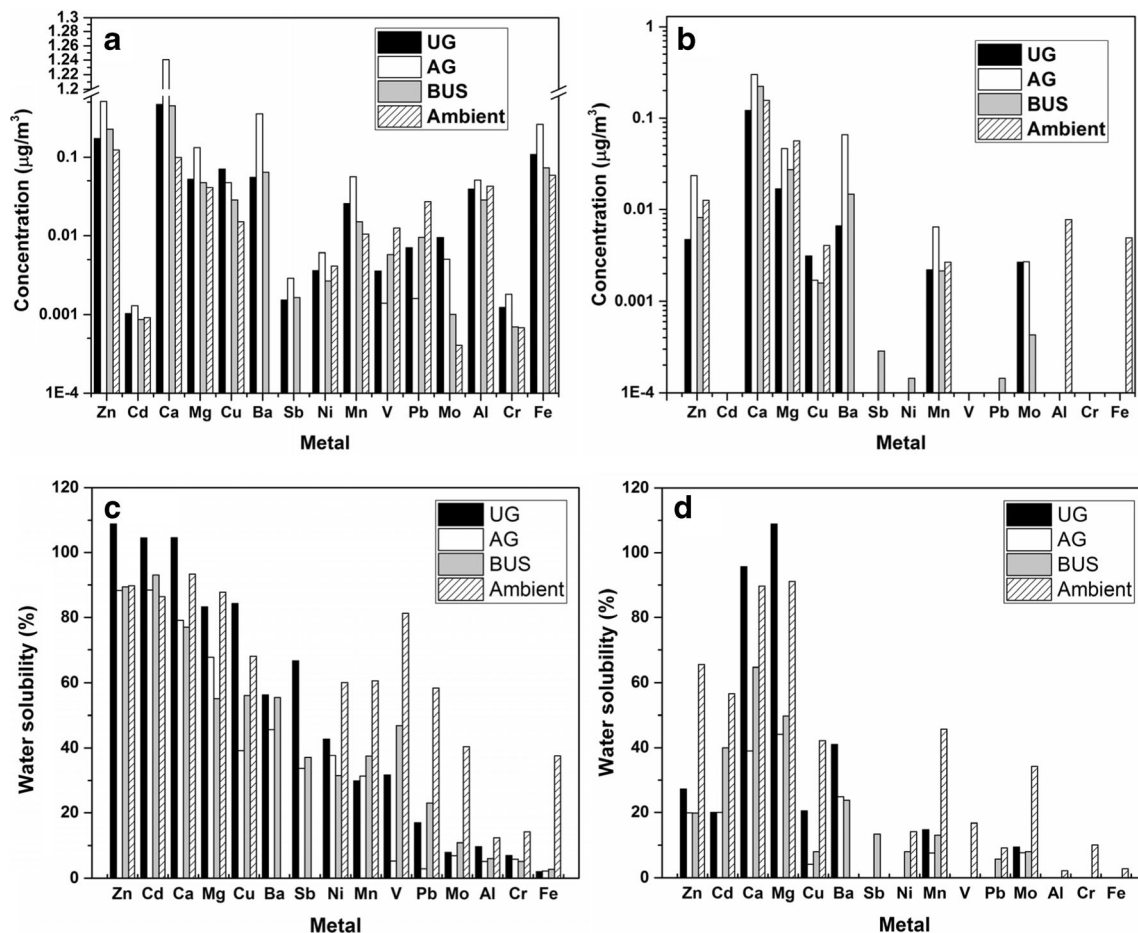
## Results and discussion

### Gravimetric PM mass measurements

The gravimetric PM concentrations across MTR routes were measured as shown in Fig. S1. The fine and coarse PM concentrations were estimated as the average of three replica filters for each MTR route. The filters were distributed for different experiments—metal and ion analysis, OCEC quantification, and toxicity analysis—and no significant difference in PM mass concentration was observed between them as shown in Fig. S1, even though PM mass was in the order  $\text{AG} > \text{UG} > \text{BUS}$  ( $n = 3$ ). However, a combined average (of three sets shown in Fig. S1) of fine PM concentration across UG, AG, and BUS routes was  $47.9 \pm 13.0$ ,  $86.8 \pm 12.4$ , and  $43.8 \pm 2.0 \mu\text{g}/\text{m}^3$ , respectively, showing the AG route has significantly ( $p < 0.001$ ,  $n = 9$ ) higher fine PM mass concentration compared to the UG and BUS routes. But, a combined coarse PM concentration was 4–5-fold less, recording  $10.0 \pm 4.7$ ,  $17.9 \pm 8.2$ , and  $11.1 \pm 1.3 \mu\text{g}/\text{m}^3$  for respective routes with no significant difference. Although high PM mass has been often reported in the underground subway stations (Kim et al. 2010; Loxham et al. 2013; Spagnolo et al. 2015) due to the confined space with railway emissions, the UG fine PM sample in this study did not show significant concentration due to the glass door feature in the subway systems in Hong Kong that effectively shield the platform from train passage. The contrast of relative abundance of PM in the different routes clearly evidenced the direct impact of source-specific emissions in microenvironments. The vastly different PM composition from the commute microenvironments implies the complex role of PM toxicity in the integrated exposure during commuting (Valavanidis et al. 2008). The following sections describe the oxidative potential of PM from different route samples and their relation with PM composition.

### Water-soluble metals

Figure 2 shows the water-soluble metal concentration (Fig. 2a, b) and their solubility (Fig. 2c, d) in percentage for  $\text{PM}_{2.5}$  and coarse PM from the UG, AG, and BUS routes. The three different public transport microenvironments were also compared with the typical urban environment in Hong Kong (Jiang et al. 2014). As shown in Fig. 2a, b, the water-soluble metal concentration was presented in logarithmic scale, where Ca, Zn, Fe, etc. were found in high quantities in aboveground route compared to UG, BUS, and urban environments. The solubility ratio of metals varied significantly between  $\text{PM}_{2.5}$  and coarse PM among different routes. As shown in Fig. 2c, Zn, Cd, Ca, Mg, and Cu in  $\text{PM}_{2.5}$  have equivalent or slightly different solubilities in the urban ambient site compared with the three routes; however, significantly lower solubility was observed for other metals including Ni, Mn, V, Pb, Mo, Cr,



**Fig. 2** Concentration of metals in air for **a**  $\text{PM}_{2.5}$  and **b** coarse PM from UG, AG, and BUS routes in comparison with typical urban environment (Jiang et al., 2014) and its water solubility for **c**  $\text{PM}_{2.5}$  and **d** coarse PM

and especially Fe in the UG, AG, and BUS routes compared to the ambient site. It is worth noting that although the subway and ambient samples were taken at different time periods (the general ambient air quality data for different sampling periods are shown in Figs. S2 and S3), the distinct difference of metal solubility between the two environments provides important insight of their different chemical forms and sources. Among the three routes, AG appears to have more metals in  $\text{PM}_{2.5}$  with lowest solubility including Fe, Cr, and Al which can be attributed to their origins from electrically influenced surface wear and mechanical disintegration (Mugica-Alvarez et al. 2012; Salma et al. 2007) that can produce insoluble forms of elemental Fe or metal oxides such as  $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}_3\text{O}_4$ ,  $\text{Al}_2\text{O}_3$ , and  $\text{Cr}_2\text{O}_3$ , or as clathrates complexed with organic compounds as per earlier reports (Ghio et al. 1999).

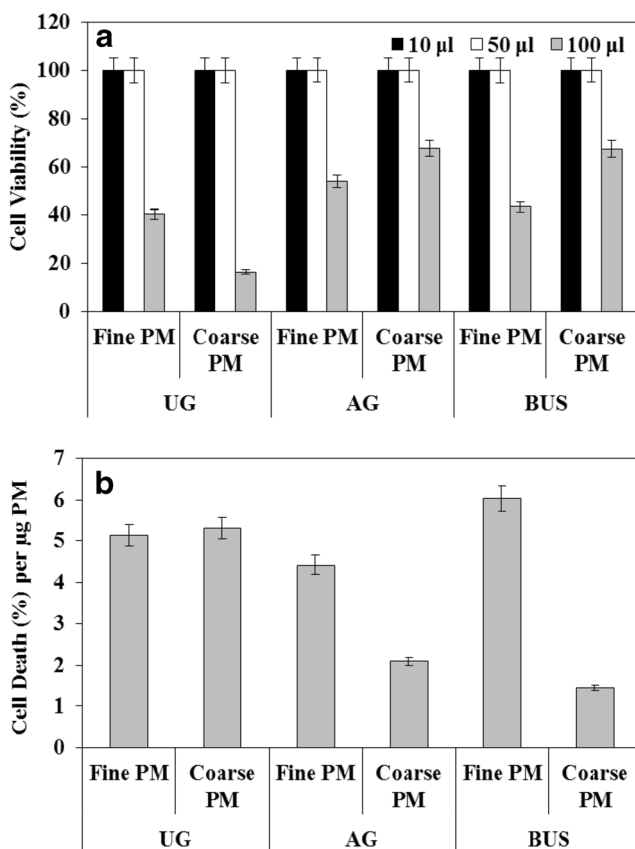
In comparison to fine PM, coarse PM metals exhibited substantially different solubility profile as shown in Fig. 2d. Other than Ca and Mg as major components of soluble sea salt particles, all other metals have less solubility by different degrees in coarse PM compared to fine PM, clearly demonstrating the different chemical forms and origins of metals such as friction of train wheels that generate coarse particles,

combustion of metal containing fossil fuel that is emitted with fine PM. For example, Zn and Cd in  $\text{PM}_{2.5}$  from AG have over 90% solubility but only 20% in coarse PM. The ambient coarse PM metals, although with less solubility than fine PM, still consistently had higher solubility than did coarse PM metals of the UG, AG, and BUS routes, except for Ca and Mg where UG noted higher solubility. For V, Al, Cr, and Fe, their soluble forms were not even detectable in coarse PM sample for all three routes. As transition metals, such as Fe and Cu, play an important role in generating free radicals through Fenton reactions in their ion forms, the vastly different solubility of PM metals in the public transport microenvironments compared with ambient indicates different health implications as discussed in the following sections.

### Cell toxicity analysis

#### *Effect of size-segregated PM on cell viability*

Figure 3 shows the effect of fine and coarse PM on macrophage cell viability on a dose-dependent manner for 4 h. As shown in Fig. 3a, cells that are exposed to 10 and 50  $\mu\text{L}$  PM



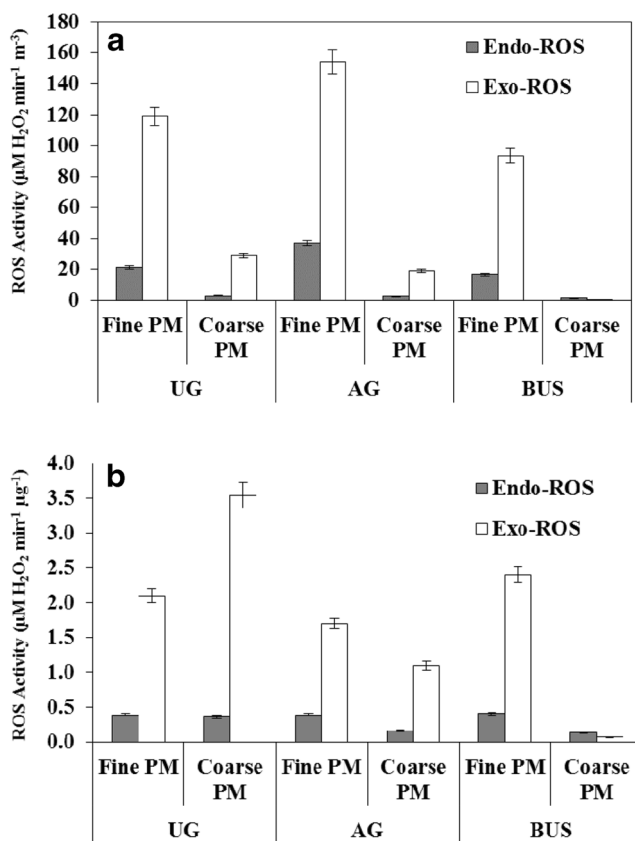
**Fig. 3** MTT-reduction assay for determining cell viability with UG, AG, and BUS route samples. **a** Dose-dependent PM exposure on  $10^4$  RAW 264.7 macrophages. **b** Mass equalized PM contribution for cell death with respect to 100 µL volume dose. Data presented are mean  $\pm$  SD of three independent determinations

extracts for 4 h retained 100% viability on air volume basis; however, 100 µL dose exposure resulted in reduced cell viability variably (32–83%). Beyond 4 h of exposure, at 24 h, cell loss was greater (>60%) for all the three doses of PM employed, and therefore, the study is limited to 4 h. The use of 24 h data is omitted from discussion, as this assay resulted in greater decrease in cell counts. It is also noted that coarse PM ( $p < 0.001$ ) in UG samples, and fine PM in AG ( $p < 0.010$ ) and BUS ( $p < 0.001$ ) sample exposure, resulted in reduced cell number compared to their size-segregated fine or coarse PM. Similarly, significant cell reduction was noted for fine and coarse PM at all three sampling routes. UG fine ( $p < 0.001$ ) and coarse PM ( $p < 0.001$ ) elicited higher toxicity than AG samples did, suggesting uniform UG environment-augmented PM-induced cell reduction without the interference of diversified surrogates. But, UG fine PM ( $p > 0.050$ ) did not result in significant change with BUS fine PM. However, BUS fine PM ( $p < 0.010$ ) registered notable reduction in cell number compared to AG fine PM. It is noted that the untreated control (with PM free) cells remained 100% viable. The negative control, PM, and dye without cells showed no absorbance of formazan.

On PM mass basis, fine PM of AG and BUS samples resulted in significant higher cell death ( $p < 0.001$ ) as shown in Fig. 3b, compared to their coarse PM. However, UG fine and coarse PM did not show comparable difference. Intercomparison between routes showed that BUS fine PM resulted in greater ( $p < 0.001$ ) cell loss followed by UG and AG fine PM. But, with coarse PM, the UG route dominantly increased cell death per microgram PM than did AG and BUS. It is also evident from the results that fine PM is much more toxic to cells than coarse PM is, which is in line with other findings (Kam et al. 2011b; Loxham et al. 2013), suggesting the existence of responsible agents for oxidative potential in fine PM as compared to coarse PM. However, both fine and coarse PM in UG route elicited similar toxicity, which may be due to the involvement of specific metals largely detected in underground railways as reported earlier (Loxham et al. 2013) and may be the direct inducer of cell toxicity, which however will be further investigated in the following sections of ROS analysis and its correlation with OCEC and metals.

#### Comparison of endogenous and exogenous ROS activities

Figure 4 shows quantitative measurement of endo- and exo-ROS on exposure to per cubic meter of air volume and per microgram of fine and coarse PM extracts. While endo-ROS represents change in oxidative potential inside the cell, exo-ROS quantifies ROS on the surface of PM (Lakey et al. 2016). The ROS results were expressed per cubic meter of air and per microgram of PM, because while the air volume-based unit represents the level of human exposure to the atmospheric PM-induced oxidative species, the mass-based unit better relates to the intrinsic oxidative potential of PM (Boogaard et al. 2012). Based on cell viability assay, ROS activity was limited to 50 µL PM dose and 4 h exposure even though ROS experiments were carried out at all three doses for 24 h exposure time, which otherwise could make it difficult to determine whether change in ROS activity is due to PM exposure or because of lowered cell counts. Also, the zero on the Y-axis of Fig. 4 represents the basal ROS activity of untreated controls (PM free samples). Overall, both endo- and exo-ROS activity per cubic meter of air was measured significantly higher ( $p < 0.001$ ) for fine PM compared to coarse PM at all the three routes studied—UG, AG, and BUS, as shown in Fig. 4a, and it also has to be noted that though the exo-ROS is 4–6-fold higher than its corresponding endo-ROS, both had similar trend of increased and reduced ROS activity. The three routes had comparable differences in endo- and exo-ROS, where ROS generation was in the order AG > UG > BUS. While the UG and BUS routes clearly represent railway and roadway environments, the AG route encompasses mixed-pollution sources from both typical railway emissions and traffic-related emissions, a possible reason that leads to higher



**Fig. 4** Endogenous and exogenous ROS activity of fine and coarse PM-induced ROS. **a** ROS activity per  $\text{m}^3$  of air and **b** ROS activity per microgram of extracted PM. Endo-ROS was measured by exposing  $50 \mu\text{L}$  PM dose to  $10^4$  RAW 264.7 macrophage cells for 4 h as per method. Data presented are mean  $\pm$  SD of three independent determinations

induction of multivariate toxic agents causing higher ROS generation.

In comparison, on PM mass basis, the cellular endo-ROS did not show any significant difference for fine PM as shown in Fig. 4b, with the BUS route resulting in higher ROS induction. Though exo-ROS is 3–5-fold higher than endo-ROS, both had comparable trends in UG route (Fig. 4b). Endo-ROS activity (Fig. 4b) is in good agreement with the cell viability assay (Fig. 3b), where ROS generation has corresponded with cell death. For example, greater cell death due to BUS fine PM sample corresponded with its higher ROS generation; fine and coarse PM of UG did not have comparable differences both in cell viability and endo-ROS; and importantly, trend of cell death and increased ROS generation was similar with the order  $\text{BUS} > \text{UG} > \text{AG}$ . It is in reverse trend to volume-based analysis suggestive of varied analytic modes (Boogaard et al. 2012). The correlation coefficient of determination ( $R^2$ ) between endo- and exo-ROS across all routes and PM size was observed to be 0.53 ( $n = 12$ ) and 0.71 ( $n = 12$ ) when analyzed per cubic meter of air volume and per microgram of PM, respectively, suggestive of

considering “normalized PM method,” a more appropriate mode of expressing ROS activity. It is unclear on how the fine and coarse PM in the UG route is different from those in the other two sampling routes. It can be presumed that there might be some common toxicological agents both in fine and coarse PM of UG route, which are not the same or in variable quantities with the other two routes (Loxham et al. 2013), where the role of soluble metal ions can be a limiting factor in eliciting cell toxicity, which can be better understood relating to ROS as discussed in further sections. Also, it should be noted that ROS generation alone cannot be translated into oxidative stress, and the role of secondary agents such as interleukins, TNF- $\alpha$ , etc. that are elicited through cell membrane should also be taken into account (Jia et al. 2017; Steenhof et al. 2011)

### Role of water-soluble PM metals and OCEC in ROS activities

Table 1 shows the summary of correlation between metals and ROS activity of PM samples collected across UG, AG, and BUS routes. The correlation between water-soluble metals and endo-ROS activity was carried out across the routes for better determination. However, Table S1 presents the correlation coefficients for individual routes. On air volume basis as shown in Table 1a, the degree of correlation of endo-ROS varied largely with different metals, where Cr, Fe, Mn, and Ni in the fine PM registered significant and positive association with  $R$  values  $>0.85$  ( $n = 9$ ) across routes. In addition, metals such as Al, Ba, Ca, Mg, and Na had positive associations with  $0.70 < R < 0.75$  ( $n = 9$ ), while the other metals studied had either less correlation or negative association with PM-induced endo-ROS. Since the metals are involved in redox reactions in cell-free conditions as well, for example Fenton’s reaction, their correlation with exo-ROS will help determine its role in ROS change. In this study, the exo-ROS had good correlation with very few metals, while Ni had  $R = 0.83$  and Fe and Cr registered  $R = 0.68$  ( $n = 9$ ). In comparison, coarse PM-induced endo- and exo-ROS had positive association with only Mo ( $R > 0.93$ ,  $n = 3$ ) and Cu ( $0.63 < R < 0.81$ ,  $n = 3$ ) when observed across the routes. It should be noted that the sample size is limited ( $n = 3$  in this case) and does not provide a statistical significance. However, the earlier report shows high correlations with coarse PM-induced ROS with greater sample size (Kam et al. 2011b). However, it has to be noted that the validity of these correlations appears to depend on the variation in sampling routes, source, and bioavailability. Table S1a can show the relative correlation between the routes.

On PM mass basis, as shown in Table 1b, the number of fine PM metals that correlate with endo-ROS has drastically decreased, when analyzed across the routes. While Ni had  $R = 0.677$ , V observed  $R = 0.508$  ( $n = 9$ ). On the other hand,



**Table 1** Summary of cumulative associations between metals of fine PM and ROS of all three public transport routes

Metals	(a) R value on volume basis		(b) R value per mass basis	
	v Endo-ROS	v Exo-ROS	v Endo-ROS	v Exo-ROS
Al	0.773*	0.531	-0.176	-0.345
Ba	0.871**	0.426	0.065	-0.411
Cd	0.283	-0.040	-0.630	-0.437
Cr	0.883**	0.695*	0.237	0.023
Cu	0.543	0.434	0.223	0.097
Mn	0.857**	0.464	-0.174	-0.496
Mo	0.188	0.122	0.056	0.019
Ni	0.813**	0.831**	0.677*	0.653*
Pb	-0.340	-0.175	-0.591	-0.184
Sb	0.566	0.094	-0.370	-0.397
V	-0.251	0.270	0.508	0.857**
Zn	0.519	0.060	-0.052	-0.023
Ca	0.729*	0.309	-0.262	-0.345
Fe	0.894**	0.681*	0.197	-0.241
Mg	0.731*	0.300	0.031	-0.075
Na	0.819**	0.700*	0.500	0.526
K	0.303	-0.062	-0.693	-0.488

(a) per  $\text{m}^3$  of air volume, (b) per microgram PM. Actual  $R$  values were provided for the correlations, where  $n = 9$ , three data points each from MTR-UG, AG, and BUS

Significance in correlations are shown as \* $p < 0.05$ ; \*\* $p < 0.01$

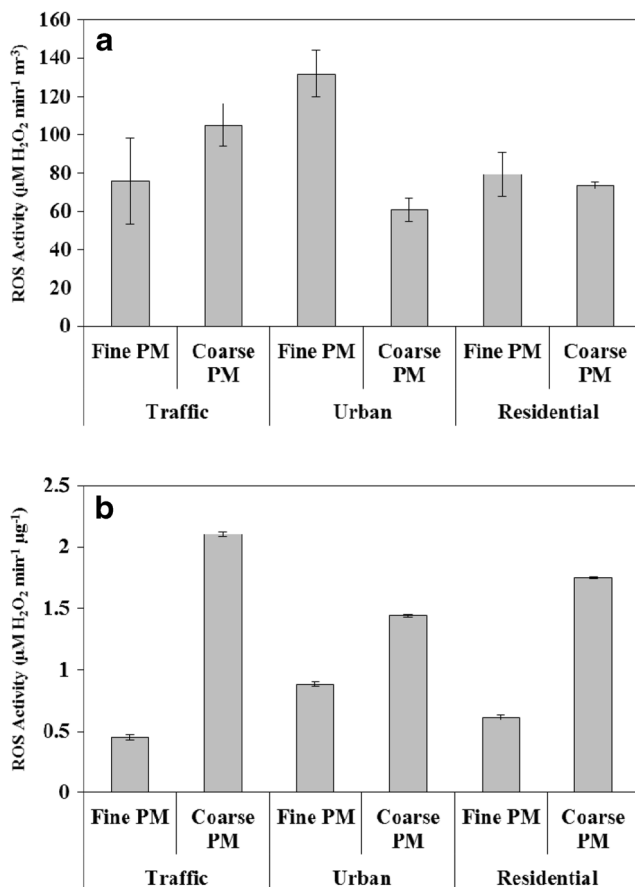
the rest of metals had either negative or weaker association including Fe, Cr, Cu, etc. The similar was in the case with exo-ROS, where Ni and V are of the same magnitude as that with endo-ROS. The strong correlation for Ni and V suggests that these elements, which may be components of stainless steel used for MTR tracks, may share a common source (Kam et al. 2011b), and components of heavy oil combustion (Cheng et al. 2015) and these two metals were reported to be associated with cardiovascular and respiratory issues (Zhang et al. 2009). Various cellular toxicity mechanisms mediated by metals of PM were reported in the literature (Araujo 2011; Roy et al. 2016). However, caution is required when identifying single surrogates for PM oxidative potential. Ni and V of fine PM from urban sites were also earlier reported to have strong correlation with endo-ROS ( $R > 0.76$ ,  $n = 32$ ) (Gali et al. 2015). In comparison, only Cu and Mo of coarse PM had good correlations ( $R > 0.89$ ) both with endo- and exo-ROS, despite the lower sample size ( $n = 3$ ). The weaker association across the routes could be due to the limitation of good correlation with a particular route, while the other routes exhibited bad correlations, where Table S1b may throw some interpretation.

The concentration of EC and OC from the PM samples of MTR UG, AG, and BUS are as shown in Table S2. Only their correlation coefficients with endo-ROS are discussed to

identify possible surrogates. The correlation coefficient ( $R$ ) varied largely for OC and endo-ROS when analyzed by volume and mass basis. While the volume-based analysis showed strong correlation between OC and endo-ROS, a weaker correlation was noted for mass-based analysis. The similar correlation was observed between EC and endo-ROS. For example, both EC and OC of UG and AG fine PM had strong positive association ( $0.81 < R < 0.99$ ,  $n = 3$ ) with endo-ROS on volume basis, but a weak association of  $0.28 < R < 0.49$  ( $n = 3$ ) was noted for UG fine PM on mass basis. At a sample size  $n = 3$ , UG had significant correlation ( $p < 0.05$ ) for EC, while AG had  $p < 0.05$  correlation with OC on volume basis. On both volume and mass bases, EC and OC of BUS route PM observed strong negative association ( $R < -0.68$ ,  $n = 3$ ) suggesting mixed roadway environment with fresh emissions may lead to multi-speciated agents capable of inducing cellular oxidative stress, and OC or EC may not be the appropriate surrogate for PM toxicity. Across the MTR routes, OC had a significant correlation with endo-ROS ( $R = 0.779$ ,  $n = 9$ ,  $p < 0.05$ ) on volume basis, but EC had no significant correlation. It should also be noted that correlation of water-extractable PM-induced endo-ROS with OC and EC is limited by their relative abundance of water-soluble and water-insoluble fractions. Although many studies in ambient environments have demonstrated good correlation of OC with ROS generation (Ntziachristos et al. 2007), the source of emissions, atmospheric aging, and processing all contribute to the variation of water solubility in OC. Lack of association for OCEC and ROS in BUS route sample may be attributed to the dominant primary vehicle emissions in roadside environment that are often hydrophobic in nature (Ho et al. 2003).

### Comparison of ROS generation from different environments

Figure 5 shows the PM-induced endogenous ROS for the UG, AG, and BUS samples against the urban and residential ambient sites in Hong Kong we reported earlier (Gali et al. 2015). Though the sampling periods are different for this study (October 2013–April 2014) and the urban ambient sites (November 2012–April 2013), similar patterns of PM and co-pollutants are shown as in Figs. S2 and S3. We present the comparison between the different environments here to provide an insight of implications in exposure in different microenvironments. On volume basis, as shown in Fig. 5a, fine PM accounts for more than 90% of ROS activity at UG, AG, and BUS routes; however, both fine and coarse PM of urban and ambient sites accounted for 40–50% of total ROS activity suggesting the varied distribution of PM components that lead to change in oxidative potential. It is interesting to note that ROS activity from the ambient sites was 3–4 (for fine PM)- and 40–50-fold (for coarse PM) higher than from the UG, AG, and BUS routes, while there is no such significant



**Fig. 5** Endogenous ROS activity of PM at ambient environments. The sites Hung Hom, Kowloon Tong, and Shatin represents traffic, urban, and residential sites, respectively (map in Fig. 1). **a** ROS activity per m<sup>3</sup> of air and **b** ROS activity per microgram of extracted PM. Ambient sites represent traffic, urban, and residential sites. Endo-ROS was measured by exposing PM to 10<sup>4</sup> RAW 264.7 macrophage cells for 4 h making 100% cell viability as point of ROS activity comparison. Data presented are mean ± SD of three independent determinations

difference in the PM mass concentrations among different samples. The similar trend is seen with the concentration of water-soluble metals like Fe, Cu, Ni, and V, suggesting them as possible inducers of increased ROS activity. On PM mass basis, as shown in Fig. 5b, while fine PM of UG, AG, and BUS accounted for 70% of ROS activity, the coarse PM of urban ambient sites resulted in 60–80% of ROS activity suggesting the method of measuring intrinsic properties of the collected particles discriminates the toxicological agents. A 1–2-fold (for fine PM) and 6–10-fold (for coarse PM) increase in ROS activities were noted for the ambient sites than for the MTR and BUS routes. Overall, while fine PM is a potential inducer of ROS at UG, AG, and BUS routes, coarse PM emerged as a dominant toxicity inducer at urban sites. It is well in line with cell viability assays, where fine PM caused 5–7% of cell death at all three routes. The effect of endotoxins in PM of MTR routes was negligible or sometimes has no effect on oxidative stress similar to that at urban ambient sites

(Gali et al. 2015). Although these measurements suggest that subway samples may not be much toxic compared to urban ambient sites, caution has to be taken for a more appropriate exposure assessment for better understanding of public health implications.

## Conclusions

The present study investigated the chemical and toxicological characteristics of PM<sub>2.5</sub> and coarse PM from different modes of public transport in comparison to urban environment in Hong Kong. Metals and OCEC showed different mass distribution in size-fractionated PM with much higher metal fraction in coarse PM in railway routes but higher abundance of carbon content in PM<sub>2.5</sub> of roadway routes, clearly demonstrating the different source contribution of railway emission and fossil fuel combustion emissions to the public exposure in different commute routes. Vast majority of the metals have significantly less solubility in railway-dominant transport microenvironments implying the different bioavailability of the potentially harmful metals. There exists different degrees of association between PM components and ROS when comparing different commute modes that is attributed to the different chemical characteristics. The findings strongly suggest the PM mass concentration alone may not be the best surrogate of dose and toxicity while assessing the public exposure, especially when crossing different microenvironments such as different commuter modes. We also found that PM-induced endogenous ROS for the public transport microenvironments were over 50-fold lesser than the typical ambient sites, implying very limited oxidative potential of PM in daily commuter exposure in public transport routes, and water solubility of the metals appears to be an important limiting factor of PM in the ability to elicit cytotoxicity that may be responsible to the contrast of ROS profiles in different environments.

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## Compliance with ethical standards

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**Conflict of interest** The corresponding author Zhi NING received the above-said grants and declares no conflict of interest with funding sources and others.

**Human and animal rights and informed consent** This study did not employ either human participants or animals for the experiments.

The authors declare that this manuscript is submitted solely to this journal and is not in consideration/in publication with any other journal.

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