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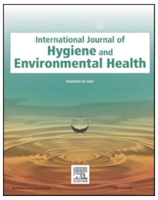

Particle and metal exposure in Parisian subway: Relationship between exposure biomarkers in air, exhaled breath condensate, and urine

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ABSTRACT

Subway particulate toxicity results from *in vitro* and *in vivo* studies diverge and call for applied human research on outcomes from chronic exposures and potential exposure biomarkers. We aimed to (1) quantify airborne particulate matter (PM) concentrations (mass and number) and metal concentrations in exhaled breath condensate (EBC), urine, and PM; (2) investigate their associations (EBC vs. PM vs. urine); and (3) assess the relevance of EBC in biomonitoring. Nine subway workers in three jobs: station agents, locomotive operators and security guards were monitored during their 6-h shifts over two consecutive weeks. Six-hour weighed average mass concentrations expressed as PM10, PM2.5 and their metal concentrations were determined. Urine and EBC samples were collected pre- and post-shift. Ultrafine particle (UFP) number concentrations were quantified in PM and EBC samples. Metal concentrations in urine and EBC were standardized by creatinine and EBC volume, respectively, and log-transformed. Associations were investigated using Pearson correlation and linear mixed regression models, with participant's ID as random effect. PM concentrations were below occupational exposure limits (OEL) and varied significantly between jobs. Locomotive operators had the highest exposure (189 and 137 $\mu g/m^3$ for PM10 and PM2.5, respectively), while station agents had the highest UFP exposure (1.97×10^4 particles/cm^3). Five metals (Al, Fe, Zn, Cu, and Mn) in PM2.5 and three (Al, Fe, and Zn) in PM10 were above the limit of quantification (LOQ). Fe, Cu, Al and Zn were the most abundant by mass fraction in PM. In EBC, the metal concentrations in decreasing order were: Zn > Cu > Ni > Ba > Mn. Security guards had the highest EBC metal concentrations, and in particular Zn and Cu. Urinary metal concentrations in decreasing order were: Si > Zn > Mo > Ti > Cu > Ba ≈ Ni > Co. All urinary metal concentrations from the subway workers were similar to concentrations found in the general population. A statistically significant relationship was found for ultrafine particle number concentrations in PM and in EBC. Zn and Cu concentrations in post-shift EBC were associated with Zn and Cu concentrations in PM10 and with post-shift urinary Zn and Cu concentrations. Therefore, EBC appears a relevant matrix for assessing exposure to UFP in human biomonitoring when inhalation is a primary route of exposure. We found different temporal variation patterns between particle and metal exposures in three matrices (PM, urine, EBC) quantified daily over two full weeks in subway workers. These patterns might be related to metal oxidation, particulates' solubility and size as well as their lung absorption capabilities, which need to be further explored in toxicological research. Further research should also focus on understanding possible influences of low chronic exposures to subway particulates on health in larger cohorts.

1. Introduction

Subways (also called metros, undergrounds or underground railways) are the most commonly used mode of public transportation in large cities (Wen et al., 2020). Subways are low-carbon transport modes and crucial in meeting climate goals. Public authorities worldwide have adopted clean air policies since 2000 and interventions on public transport systems were shown effective (Burns et al., 2020) in reducing particulate matter (PM) emissions (EEA., 2020). The toxicity of suspended particles is mainly due to aerosolized particles with a diameter of less than 10 μm. PM concentrations with a median diameter of less

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Abbreviations

BMI	Body mass index
EBC	exhaled breath condensate
GV	indoor air quality guide values
ICP-MS	inductively coupled plasma mass spectroscopy
LDSA	lung deposited specific area
LEM	Laboratory of Essays and Measurements
LOD	limit of detection
LOQ	limit of quantification
NTA	nanotracking analysis
OEL	occupational exposure limits
PBZ	personal breathing zone
PM	particulate matter
PNOS	particles not otherwise specified
ROS	reactive oxygen species
RTV	reference toxicological value
SD	Standard deviation
WHO	World Health Organization

than 10 μm are reported as PM10 and those less than 2.5 μm as PM2.5. Many countries have reported PM concentrations in subway air exceeding the guidelines for indoor air quality from the World Health Organization (WHO) currently set at 50 and 25 μg/m^3 for PM10 and PM2.5, respectively, for 24 h exposure (WHO, 2005). Poor subway air quality might present a potential health risk for regular subway users and in particular, for workers (Smith et al., 2020; Loxham et al., 2013). Environmental exposure assessments and guidelines for interpreting PM2.5 and PM10 concentrations are used for subway users, while occupational exposure assessments and regulations use total inhalable and respirable dust with median particle diameter of less than 100 μm and 4 μm, respectively. Inhalable and respirable dust concentrations are regulated with occupational exposure limits (OELs). The OEL set for dust exposures among subway workers is 'particles not otherwise specified' (PNOS).

Environmental exposure studies consistently document a very specific physical-chemical composition and size distribution of subway PM. Subway PM is highly ferruginous, with up to 67% iron oxide in PM2.5 (Seaton et al., 2005) and up to 50% in PM10 (Park et al., 2012). Subway PM also contains trace metals (Mg, Al, Si, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Ba, and Pb), some of which have known adverse health effects. Subway PM toxicity results from *in vivo* studies are inconclusive and diverge from those of *in vitro* studies. The latter show that subway PM generates more reactive oxygen species (ROS) and oxidative stress related outcomes compared to other PM (Loxham and Nieuwenhuijsen, 2019), although a direct evidence for the clinical significance of ROS generation *in vivo* is limited (Loxham and Nieuwenhuijsen, 2019). The controversy between *in vitro* and *in vivo* studies may be due to disparities between *in vivo* exposures and *in vitro* models, and differences in exposure doses, as well as lack of statistical power in *in vivo* studies of chronic exposures. Future research recommendations are to focus on outcomes from chronic *in vivo* exposures and understanding mechanisms and potential biomarkers of exposure (Wen et al., 2020; Loxham and Nieuwenhuijsen, 2019).

Following this recommendation, we launched a Franco-Swiss epidemiological research project, called "ROBoCoP" for the Respiratory disease Occupational Biomonitoring Collaborative Project. ROBoCoP aimed at assessing occupational exposures to airborne PM, exposure biomarkers related to PM exposures, and early respiratory effects among subway workers employed by the Parisian urban transport company (RATP) (Guseva Canu et al., 2021).

In this study, we present 6 h-weighed particle mass concentration assessed over two work-weeks in subway workers using both environmental and occupational exposure assessment approaches. Moreover, we determined metal concentrations in three matrices: exhaled breath condensate (EBC), urine, and PM (PM2.5 and PM10). We also assessed the particle number concentrations in EBC and PM in air with direct-reading instruments. Our aims were to (1) quantify particulate matter (PM) concentrations (mass and number) and metal concentrations in exhaled breath condensate (EBC), urine, and PM; (2) investigate their associations (urine vs. PM vs. EBC); and (3) assess the relevance of EBC in biomonitoring.

2. Material and methods

2.1. Study design, setting and participants

This six-week occupational pilot-study was conducted at the Parisian urban transport company (RATP) in France according to a registered research protocol (Guseva Canu et al., 2021). Collected samples were analyzed at Unisanté in Switzerland and RATP (LEM laboratories). Workers in three different jobs were included: subway station agents, locomotive operators, and security guards. Station agents oversee passenger information and ticket sale. They operate the ticket counters and have a mobile activity checking ticket distributors in the stations' concourses and controlling purchased tickets among travelers. Each station agent work on one assigned subway line during a work shift. Locomotive operators run the subway trains and spend the majority of their work shift inside the train cabin physically separate from the passenger rail cars. Security guards patrol stations on demand, constantly moving from one to another across all subway lines. We selected subway line 7 for our study, as it is underground, has no mechanical ventilation and therefore represents a worst-case scenario in terms of PM exposures. The convenience sample approach included nine (three per job or professional type) non-smoking subway workers of both sexes. We collected samples by job type: two weeks per type of subway professionals from October 7th to November 15th' 2019. Moreover, all participants filled in a standardized epidemiological questionnaire describing factors that may influence exposures and biological sample analysis (Guseva Canu et al., 2021).

2.2. Air sample collection and analyses

RATP safety regulations do not allow any RATP professionals to wear any equipment other than those used for their regular work. Consequently, airborne PM were collected with appropriate equipment but carried by two or three RATP LEM technicians job-shadowing the RATP worker for the entire shifts. According to our protocol (Guseva Canu et al., 2021), the RATP LEM technicians would don the air sampling equipment in the personal breathing zone (PBZ); however, this was not physically possible in the cramped space for station agents and locomotive operators. Therefore, the RATP LEM technicians carried all air sampling equipment and instruments in a backpack (security guards) or placed this backpack close to the sitting workers.

The sampling train for measuring PM2.5 and PM10 was equipped with a filter (PTFE Membrane Filters (37 mm), Sigma-Aldrich, France) in a cassette holder (Personal Impactor H-PEM, BGI, USA) connected to a cyclone and attached with flexible tubing to a pump (GilAir Plus, Sensidyne, Germany) operating at 4L/min. Inhalable (PM100) and respirable (PM4) dust were collected actively (pump rate at 10 L/min) on foam using individual dust samplers CIP 10-I and CIP 10-R, respectively (Tecora, France). A particle counter ("DISCmini", (Testo, Mönchaltorf, Suisse) measured particles from 10 to 300 nm, particle number concentration (#/cm^3), and lung deposited specific area (LDSA) (recorded every 10 s). Both particle size and number concentrations are exposure metrics related to adverse health outcomes of ultrafine particles (UFP) (Schraufnagel, 2020). A second sampling train included the Mini-Particle Sampler, MPS® (INERIS, France) equipped with a transmission electronic microscopy (TEM) grid (Q310AR-14; Quantifoil R 1/4, 300 Mesh, Gold, Quantifoil Micro Tools GmbH, Germany) for microscopy analyses (R'mili et al., 2013).

Random grid surfaces were analyzed with a TEM (CM100 Biotwin, at 80 kV, Philips) and a scanning electronic microscopy (SEM) (Phenom ProX, at 15 kV, Thermo Scientific) coupled with Energy-Dispersive X-ray detector (EDX). Particle morphology, size and chemical composition were determined. Quantification of mass concentration was determined using standard gravimetric analysis for total inhalable and respirable dust and for PM2.5 and PM10. PM were also analyzed for 11 elements (Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) using two acidic digestion steps (95°C; HCl 30% for 25 min then HNO_3 65% for 15 min) and inductively coupled plasma mass spectroscopy (ICP-MS). The measured concentrations were integrated over sampling time, which was equal to the 6-h work-shift duration. All laboratories were accredited for regulatory analysis and used certified methods.

2.3. Exhaled breath condensate sampling and analyses

The pre- and post-shift EBC samples were collected daily over two consecutive weeks, using a portable collection device (Turbo-DECCS, Medivac, Parma, Italy) set at −10 °C. The recommendations of the American Thoracic Society (ATS) and the European Respiratory Society Task Force (ERSTF) (Horváth et al., 2005, 2017) were strictly applied. None of the participants declared drinking coffee an hour before EBC collection. A volume of 2–3 mL of EBC was collected from each participant (20 min). EBC samples were aliquoted and conserved at −80 °C until analysis. Biological sample collection, aliquoting and storage were operated in a closed clean room equipped for study purposes.

EBC metal concentrations were quantified by ICP-MS (ICap TQ, Thermo Scientific) at the Unisanté laboratory. The calibration curve was prepared by diluting a multi-element certified stock solution (Plasma Cal, SCP Science, France) with water to 0–50 μg/L range. The HNO_3 (40 μl, Plasma Plus pure, 67–70%, SCP Science, France) containing the internal standards Y, Rh, and Ir (100 μg/L) was added to 400 μl of calibration or EBC sample. The mixture was directly introduced in the plasma by aspiration. The QTegra vers 2.10 software was used for the signal acquisition and treatment was done using. All metal concentrations were standardized per EBC volume and expressed in μg/L. The observed limit of quantifications (LOQs) was 10.0 μg/L for Ba, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, V, 1 μg/L for Fe, Ti and Zn, 5.0 μg/L for Al, and 20.0 μg/L for Si.

The number of sub-micron particles in the EBC samples was quantified using the nanotracking analysis (NTA), which also determines the hydrodynamic size distribution with a diameter of approximately 40–1000 nm (nm) in liquid suspension (Sauvain et al., 2017). About 400 μl of EBC sample was introduced into the cell of a NTA instrument (LM10, Malvern Pananalytical, Malvern, UK), which adds a laser beam to excite the particles in Brownian motion and track their movements with a camera. A total of 5 videos of 60 s was recorded and analyzed using the NTA software (version 3.1) (Sauvain et al., 2017).

2.4. Urine sampling and analyses

The pre- and post-shift urine samples were collected daily over two weeks. Urinary metal concentrations were quantified at Unisanté laboratory using ICP-MS (ICap TQ, Thermo Scientific, Switzerland). Element LOQs were as follows: 12.50 μg/L for Al, 0.39 μg/L for Ba, 0.20 μg/L for Co, 2.80 μg/L for Cu, 0.33 μg/L for Cr, 25.00 μg/L for Fe, 0.08 μg/L for Mn, 4.80 μg/L for Mo, 0.26 μg/L for Ni, 1.42 μg/L for Pb, 0.28 μg/L for Sb, 400.00 μg/L for Si, 20.00 μg/L for Ti, 0.31 μg/L for V and 24.80 μg/L for Zn.

Urinary concentrations were standardized per gram of creatinine to remove the influence of urine dilution on exposure biomarkers measured in spot samples. Urinary creatinine concentrations were measured with the Jaffe method (Jaffe, 1986). Only urine samples with creatinine concentrations in the normal range (0.5–3 g/L) were included in the analysis.

2.5. Data management and statistical analysis

2.5.1. Modelling of particle and metal concentrations

We first examined graphically the distribution of continuous quantitative variables corresponding to the measured particle or metal concentrations and log-transformed the variables that were log-distributed. For some elements, a proportion of the measurements fell below the LOQ or in the interval between limit of detection (LOD) and LOQ. In that case, we used the multilevel mixed-effects interval regression models, where the dependent variable, can be left- or interval-censored and recorded using two variables corresponding to its lower and upper values (Gelman and Hill, 2006). Among independent variables we considered Job and Day of the week as fixed effect variables and Participant's ID as random effect variable to account for intra-cluster correlation when modelling the airborne PM and metal concentrations. The inter-subject variance was compared with intra-subject variance (corresponding to the residual variance in each model), and reported as intra-class-correlation (i.e., ratio inter-subject variance/total variance). When modelling particle (EBC) and metal concentrations (EBC and urine), the sampling time (pre-or post-working shift) alone and in interaction with Job were considered as additional fixed effect variables. For each concentration, we then predicted the marginal mean concentration (dependent on the covariate pattern) with associated 95%-confidence intervals ($CI_{95\%}$), in original scale. For metals where a large proportion of measurements fell below the LOQ, the mixed effects modelling may result in biased estimates of the fixed effects and variability (Morton et al., 2014). Therefore, we limited the analysis to the metals with less than 50% of measurements below the LOQ to minimize the bias arising from censored data.

2.5.2. Analysis of the relationship between different exposure metrics

We first explored the relationships between different exposure matrices for every metal and PM sizes (PM10 and PM2.5). We considered three types of variables for EBC and urinary exposure biomarkers: pre- and post-shift concentrations and the ratio post-shift to pre-shift concentration (unit-less), expressing the change in exposure biomarker concentrations over the work-shift. Moreover, we explored the values measured 24 h and 48 h before, notified as lag 1 and lag 2, respectively to assess temporal variations in the biomarkers. This exploratory analysis was based on the pairwise Pearson correlation coefficients. We performed no adjustment for multiple testing since we wanted to identify possible relationships rather than testing an initial set of hypotheses (Rothman, 1990; Bender and Lange, 2001). We analyzed further exposure matrices with significant correlation using multilevel mixed-effects models, adjusted for participant's age, sex, and micronutrient/vitamin oral supplementation. All analyses were performed with STATA statistical software, version 16 (STATA, College Station, TX, USA).

3. Results and discussion

3.1. Description of study sample

Nine subway professionals participated to our study, and are described in Table 1.

All participants completed the questionnaires and provided all required biological samples (100% participation). Creatinine concentration was within normal range in 86% (N = 144) of urine samples.

3.2. PM and metal concentrations in air

3.2.1. Particle concentrations

We present the 6-h weighed average PM concentrations in Table 2. PM concentrations irrespective of particle size were lowest among station agents and highest among locomotive operators (Table 2, Fig. 1). The ticket booth equipped with a general ventilation probably contributed to the low airborne PM concentrations for the station agents.

Table 1
Description of the study sample.

Characteristics	Station agents	Locomotive operators	Security guards
Number of participants (n (%))	3 (100%)	3 (100%)	3 (100%)
Sex	Women	Men	Men
Age (in years, Mean ± SD)	42.00 ± 10.10	49.86 ± 12.32	49.83 ± 6.35
Length of employment (years, Mean ± SD)	10.66 ± 12.42	15.00 ± 2.64	18.33 ± 1.15
General health score (on 8-point scale)	2.00 ± 0.00	3.33 ± 0.58	1.33 ± 0.58
BMI (Mean ± SD)	23.70 ± 1.94	27.68 ± 2.17	25.25 ± 0.55
Use of vitamins/supplementation (n (%))	0 (0%)	1 (33%)	0 (0%)
Home to work commuting (min., Mean ± SD)	58.33 ± 15.27	60.00 ± 47.69	64.16 ± 31.05
Use of motor vehicle for commuting (n (%))	0 (0%)	1 (33%)	1 (33%)
Use of bicycle for commuting (n (%))	0 (0%)	0 (0%)	0 (0%)
Commuting by foot (n (%))	1 (33%)	1 (33%)	1 (33%)
Follow-up period	7–18.10.2019	21–31.10.2019	4–15.11.2019

Station agents were exposed to the highest number concentrations of UFP, but converted to LDSA metrics, locomotive operators had the highest exposure (44.79 $\mu m^2/cm^3$, $CI_{95\%} = 39.36–50.21$) closely followed by station agents (37.79 $\mu m^2/cm^3$, $CI_{95\%} = 28.99–46.59$). Both metrics exhibited statistically significant time-dependence, varying not only from day to day, but also over a much shorter time span (Petremand et al., 2021).

PM10 exhibited statistically significant daily variation. In the Stockholm subway, the PM10 concentrations were correlated with hourly train frequencies and the number of rail cars (Tu and Olofsson, 2021). We did not find any particular pattern for these variables in our study (Table 2). Our PM10 concentrations are in line with PM10 values in underground stations reported in the scientific literature (Tu and Olofsson, 2021).

The PNOS concentrations were below current French OELs; 4 mg/m^3 and 0.9 mg/m^3 for inhalable and respirable particles, respectively (Guillou et al., 2020). The OEL set for PNOS are used in the instances where the metals do not have their own OEL such as for titanium dioxide, which has been classified suspected carcinogen (Guseva Canu et al., 2020). Workers exposed to titanium dioxide may therefore have an increased risk of cancer, which goes undetected. Metals with specific effects deserve specific exposure assessments.

3.2.2. PM2.5 and PM10 metal concentrations

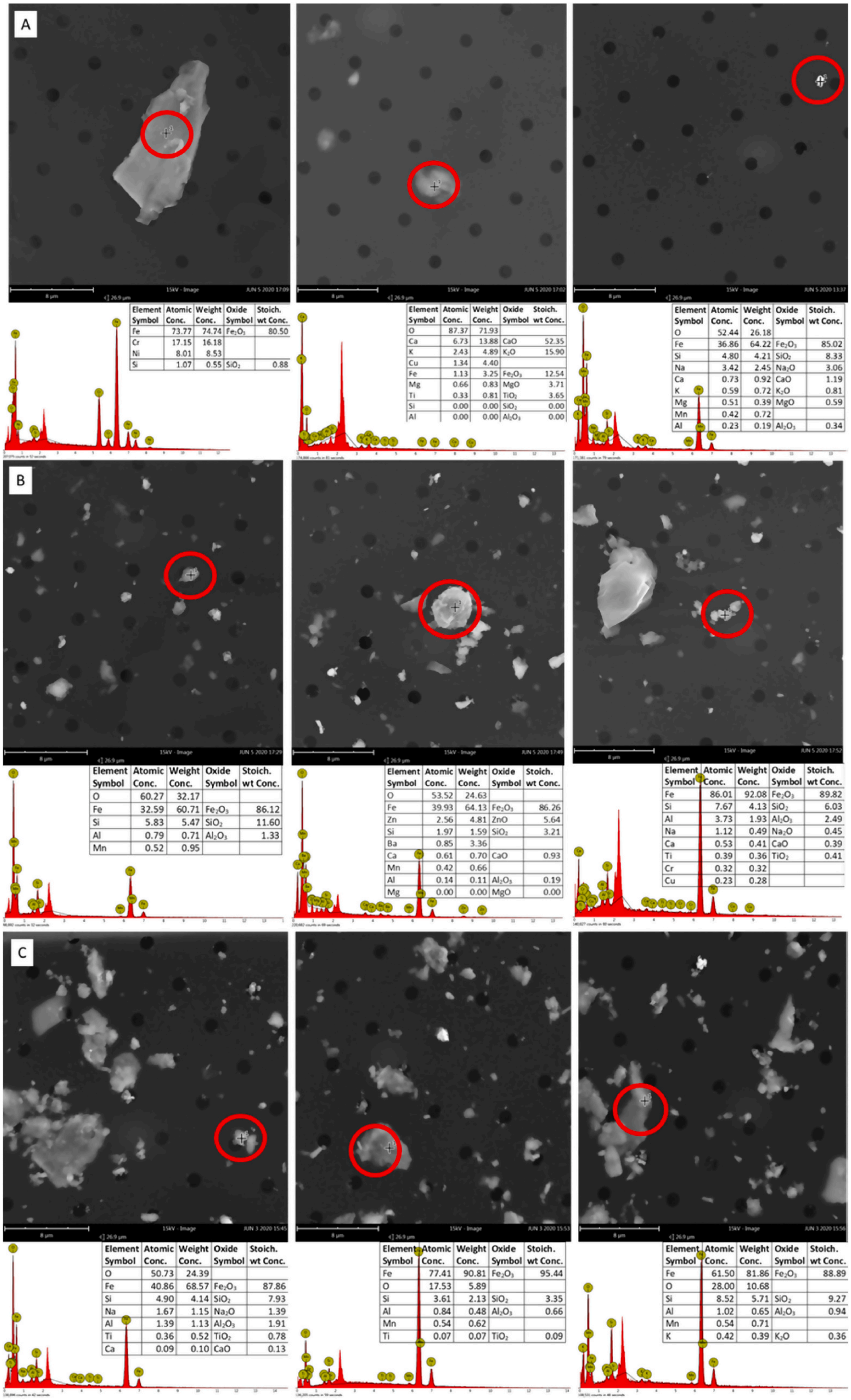
Metal concentrations in PM10 and PM2.5 are presented in Table 2. Fifty percent of the values for six (As, Ba, Cd, Cr, Ni, Pb) of the 11 metals quantified in PM10 were below LOQ. PM10 contained up to 40% Fe and 20% Al, and less than 2% Cu, Zn and Mn (results not shown) and varied significantly across jobs (Table 1). The locomotive operators had the highest exposure to Fe, Zn, and Mn, while the security guards had the highest exposures to Al, which was twice that of the other jobs for PM10 and almost twice for PM2.5 (Table 1). PM10 Cu concentrations were the same for locomotive operators and security guards, and three times greater than for station agents.

Table 2
PM and metal concentrations in the personal air samples of Paris subway workers.

Parameter measured	Fixed effects (p-value)		Intra-class correlation	Geometric Mean [95% Confidence Interval]*		
	Day of the week	Job		Station agents	Locomotive operators	Security guards
Ultrafine particles <300 nm (10^4#/cm3)	<0.001	<0.001	0.49	1.97 [1.48 ; 2.46]	1.59 [1.39 ; 1.80]	0.95 [0.82 ; 1.08]
Ultrafine particles <300 nm (µm2/cm3)	< **0.001**	< **0.001**	0.49	37.79 [28.99 ; 46.59]	44.79 [39.36 ; 50.21]	25.45 [22.19 ; 28.71]
Respirable particles (mg/m3)	0.69	**0.05**	0.49	0.07 [0.06 ; 0.08]	0.10 [0.07 ; 0.12]	0.09 [0.07 ; 0.11]
Inhalable particles (mg/m3)	0.43	< **0.001**	0.48	0.06 [0.05 ; 0.08]	0.20 [0.15 ; 0.26]	0.14 [0.10 ; 0.18]
PM2.5 (µg/m3)	0.73	< **0.001**	0.44	44.49 [25.47 ; 63.52]	136.83 [70.04 ; 203.62]	47.79 [22.22 ; 73.37]
PM10 (µg/m3)	**0.03**	< **0.001**	0.46	54.20 [36.37 ; 72.03]	188.50 [101.93 ; 275.07]	79.71 [43.95 ; 115.46]
Al in PM2.5 (µg/m3)	0.61	0.28	0.44	5.76 [3.77 ; 7.76]	4.24 [2.08 ; 6.41]	7.74 [3.73 ; 11.75]
Fe in PM2.5 (µg/m3)	0.49	< **0.001**	0.47	1.48 [0.98 ; 1.97]	15.65 [9.03 ; 22.26]	5.11 [2.60 ; 7.63]
Zn in PM2.5 (µg/m3)	0.38	< **0.001**	0.49	0.40 [0.34 ; 0.46]	0.64 [0.50 ; 0.78]	0.40 [0.31 ; 0.49]
Al in PM10 (µg/m3)	0.66	**0.01**	0.43	5.20 [3.38 ; 7.02]	6.61 [2.97 ; 10.26]	13.22 [6.27 ; 20.17]
Fe in PM10 (µg/m3)	0.93	< **0.001**	0.47	2.49 [1.23 ; 3.75]	48.11 [21.72 ; 74.50]	12.32 [3.40 ; 21.25]
Zn in PM10 (µg/m3)	0.61	< **0.001**	0.48	0.61 [0.49 ; 0.73]	1.08 [0.76 ; 1.39]	0.42 [0.30 ; 0.54]
Cu in PM10 (µg/m3)	0.51	< **0.001**	0.46	0.05 [0.03 ; 0.07]	0.15 [0.08 ; 0.21]	0.15 [0.08 ; 0.21]
Mn in PM10 (µg/m3)	0.82	< **0.001**	0.48	0.06 [0.05 ; 0.08]	0.45 [0.32 ; 0.59]	0.13 [0.09 ; 0.17]

Statistically significant results are shown in bold.

[*] Marginal mean concentration predicted by the model.



Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
Fe	73.77	74.74	Fe_2O_3	80.50
Cr	17.15	16.18		
Ni	8.01	8.53		
Si	1.07	0.55	SiO_2	0.88

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	87.37	71.93		
Ca	6.73	13.88	CaO	52.35
K	2.43	4.89	K_2O	15.90
Cu	1.34	4.40		
Fe	1.13	3.25	Fe_2O_3	12.54
Mg	0.66	0.83	MgO	3.71
Ti	0.33	0.81	TiO_2	3.65
Si	0.00	0.00	SiO_2	0.00
Al	0.00	0.00	Al_2O_3	0.00

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	52.44	26.18		
Fe	36.86	64.22	Fe_2O_3	85.02
Si	4.80	4.21	SiO_2	8.33
Na	3.42	2.45	Na_2O	3.06
Ca	0.73	0.92	CaO	1.19
K	0.59	0.72	K_2O	0.81
Mg	0.51	0.39	MgO	0.59
Mn	0.42	0.72		
Al	0.23	0.19	Al_2O_3	0.34

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	60.27	32.17		
Fe	32.59	60.71	Fe_2O_3	86.12
Si	5.83	5.47	SiO_2	11.60
Al	0.79	0.71	Al_2O_3	1.33
Mn	0.52	0.95		

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	53.52	24.63		
Fe	39.93	64.13	Fe_2O_3	86.26
Zn	2.56	4.81	ZnO	5.64
Si	1.97	1.59	SiO_2	3.21
Ba	0.85	3.36		
Ca	0.61	0.70	CaO	0.93
Mn	0.42	0.66		
Al	0.14	0.11	Al_2O_3	0.19
Mg	0.00	0.00	MgO	0.00

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
Fe	86.01	92.08	Fe_2O_3	89.82
Si	7.67	4.13	SiO_2	6.03
Al	3.73	1.93	Al_2O_3	2.49
Na	1.12	0.49	Na_2O	0.45
Ca	0.53	0.41	CaO	0.39
Ti	0.39	0.36	TiO_2	0.41
Cr	0.32	0.32		
Cu	0.23	0.28		

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	50.73	24.39		
Fe	40.86	68.57	Fe_2O_3	87.86
Si	4.90	4.14	SiO_2	7.93
Na	1.67	1.15	Na_2O	1.39
Al	1.39	1.13	Al_2O_3	1.91
Ti	0.36	0.52	TiO_2	0.78
Ca	0.09	0.10	CaO	0.13

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
Fe	77.41	90.81	Fe_2O_3	95.44
O	17.53	5.89		
Si	3.61	2.13	SiO_2	3.35
Al	0.84	0.48	Al_2O_3	0.66
Mn	0.54	0.62		
Ti	0.07	0.07	TiO_2	0.09

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
Fe	61.50	81.86	Fe_2O_3	88.89
O	28.00	10.68		
Si	8.52	5.71	SiO_2	9.27
Al	1.02	0.65	Al_2O_3	0.94
Mn	0.54	0.71		
K	0.42	0.39	K_2O	0.36

Fig. 1. SEM of an aerosol sample from A) a station agent at the ticket sale counter, station *Porte de la Vilette*, line 7; B) a locomotive operator in the subway cabin when driving between *Chatelet* and *Pont-Marie* stations, line 7; and C) a security guard on the subway platform at the *République* station - Direction *Créteil*, line 8. Red circles indicate particles analyzed with EDX for elemental composition (emission spectrum indicated under the picture). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Our metal concentrations are in line with previously published subway studies and have multiple origins. For instance, CuO emissions usually arise from short circuits incurred when a catenary wire is attached to a pantograph to provide discharge or from draft lines abrasion (Li et al., 2018). Fe, Mn, and Cr contents are mainly generated by sparks from the brakes, wheels, rails, and electric cabin tracks (Johansson and Johansson, 2003). Emissions of trace elements Ba, Zn, Sb and Cu are attributed to brake abrasion, although their concentration depends on brake type. The Ba and Zn concentrations are higher in frontal brake pads whereas Sb and Cu are higher in lateral brake pads (Moreno et al., 2017). The origin of Al and Si is to soil materials and construction material deterioration. The interaction between both pantographs and catenaries supplying electricity and by the mechanical wear–tear and friction processes due to brake–wheel–rail contact generate Fe, K, Mn, Ba, Ca, Zn, Cr, Ni, Cu and Si, along with resuspension caused by the piston effect (Cartenì et al., 2020).

Some elements can enter an underground subway station through vents and as dust generated by the cabins or transported on the clothes and shoes of passengers. Such elements can also access the passenger cabins as air is exchanged when passengers enter and exit the subway (Park et al., 2012). Microscopy analysis revealed that the particles' size, shape, and composition differed by job (Fig. 1), and visualized with the TEM images (Fig. 2). The shape and size of subway particles are useful in understanding sources. Abrasions of train vehicles, wheels and rail are sources of flake particles (Fig. 1B and C), while thermal processes due to the heating created by mechanical braking and wheel-rail contact generate spherical and semi-spherical particles (Loxham et al., 2013). UFP are generated by high temperature friction phenomena followed by vaporization or by electric arcs on the power supply system (Loxham et al., 2013). The EDX analysis of sub-micron particles (Fig. 1A, right picture) showed that they contain mostly iron. EDX analysis uses Fe_2O_3 as a standard (Fig. 1) and does not allow specification of iron species. We were therefore not able to distinguish Fe_2O_3 (hematite) from Fe_3O_4 (magnetite). Several studies have reported that Fe usually exists as Fe_3O_4 in the subway environments and as Fe_2O_3 in atmospheric PM (Karlsson et al., 2005; Eom et al., 2013; Moreno et al., 2015; Loxham et al., 2020), while others have reported the opposite (Smith et al., 2020; Querol et al., 2012). Iron oxides influence the ROS-related outcomes and thus may have an influence on respiratory diseases (Loxham et al., 2020). We believe that iron specification in PM from the Parisian subway deserves further attention.

3.3. PM and metal concentrations measured in EBC

3.3.1. Particle number concentrations and sizes

Particle number concentrations in EBCs were dependent on sampling time point (higher in pre-shift EBC than in post-shift) and on the day of the week (Table 3). Job did not have an effect per se on particle number concentrations, but acted as an effect modifier in interaction with the sampling time. This has been observed previously (Fireman et al., 2017).

The median particle size was independent of job, sampling time, or day of the week, though the latter was of borderline significance (Table 3). Locomotive operators had the highest concentration of particles in EBC compared to other subway professionals. NTA is a recent tool; we could therefore not find NTA data to compare our findings with others. Sauvain et al. reported EBC particle number concentrations in Brazilian workers processing crystal and quartz (20.1 ± 4.6 10^7 #/ml) or soapstone (4.2 ± 1.9 10^7#/ml) and compared these to university administration workers (2.8 ± 1.6 10^7 #/ml). The EBC particle concentrations found in our study were an order of magnitude lower than the Brazilian administration workers. Furthermore, Sauvain et al. found that the particle size distributions in the EBC were similar for all three groups (Sauvain et al., 2017). EBC particles smaller than 100 nm can be both of endogenous (e.g., lipidic bilayered vesicles or exosomes) and exogenous sources (Sauvain et al., 2017). Therefore, analysis of the EBC elemental content might help to understand particle nature and origin.

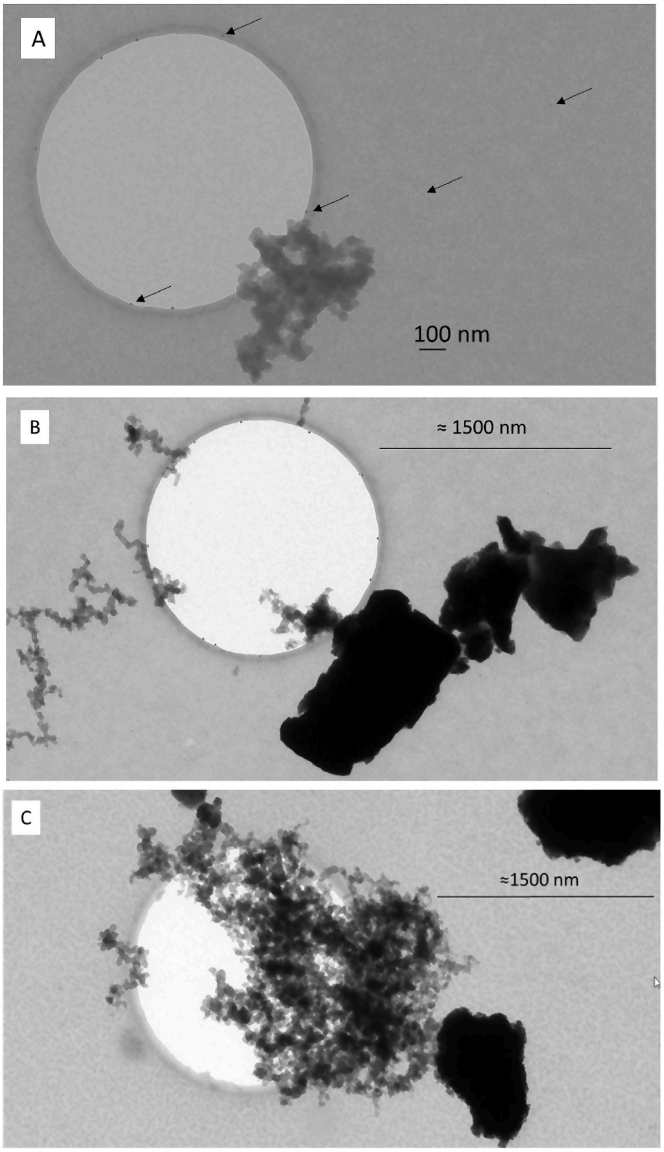


Fig. 2. TEM of an aerosol sample from A) a station agent in the ticket booth, station *Porte de la Villette*, line 7; B) a locomotive operator in the subway cabin when driving between *Chatelet* and *Pont-Marie* stations, line 7; and C) a security guard on the subway platform at the *Gare de Lyon* station - Direction *Boissy*, RER A. Arrows indicate non agglomerated nano-sized particles (median diameter 14–17 nm).

3.3.2. Metals

Among 15 metals quantified in EBC, six metal concentrations (Al, Fe, Mo, Si, Ti, and V) had more than half of the measurements below LOQ. Consequently, the statistical analyses were restricted to the other metals (Table 3). It is worth noticing that Fe and Al concentrations were below LOQ. This was unexpected given their high concentrations in subway PM. There is no data about respiratory clearance mechanisms of these metals in humans. However, our finding is consistent with the published literature on metals in EBC. Hulo et al. found that only 24% of Al concentrations in EBC in unexposed controls (67% smokers) were >LOQ (0.1 μg/L) (Hulo et al., 2016). The number of values for Al in EBC below LOQ in our study could be due to the 5-fold higher LOQ compared to Hulo et al. Other studies have also quantified metals in EBC but LOQs nor the numbers of values > LOQ were reported (Marie-Desvergne et al., 2016; Hulo et al., 2014; Ghio et al., 2018a).

The very low Fe levels in EBC can indicate a strong sequestration of this metal, as it is an essential element for life. Guio et al. suggested that

Table 3
Particle and metal concentration in the exhaled breath condensate (EBC) of Paris subway workers.

Parameter measured	Fixed effects (p-value)				Intra-class correlation	EBC sample	Geometric Mean [95% Confidence Interval]								
	Job	Shift	Day of the week	Job*Shift interaction			Station agents			Locomotive operators			Security guards		
Particles number concentration (10^6 #/mL)	0.29	**0.01**	**0.03**	**0.03**	0.38	pre-shift	31.34	[20.02;	42.66]	45.78	[29.5;	62.02]	39.85	[25.8;	53.9]
						post-shift	22.77	[14.4;	31.13]	33.25	[21.3;	45.23]	28.95	[18.5;	39.38]
Median particle diameter (nm)	0.69	0.38	**0.05**	0.55	<0.001	post-shift	138.25	[122;	154.5]	146.4	[130;	162.8]	150.3	[134;	166.6]
Metal concentration (µg/L)															
Cr	0.11	0.14	0.78	**0.01**	0.34	pre-shift	0.02	[0.01;	0.03]	0.04	[0.02;	0.05]	0.04	[0.03;	0.06]
						post-shift	0.03	[0.02;	0.04]	0.04	[0.03;	0.05]	0.04	[0.03;	0.06]
Mn	0.58	0.40	0.16	0.14	0.40	pre-shift	0.12	[0.08;	0.17]	0.12	[0.08;	0.17]	0.22	[0.15;	0.30]
						post-shift	0.14	[0.09;	0.19]	0.14	[0.10;	0.19]	0.18	[0.12;	0.24]
Co	0.99	0.72	0.70	0.94	0.39	pre-shift	0.04	[0.03;	0.04]	0.04	[0.03;	0.04]	0.03	[0.03;	0.04]
						post-shift	0.04	[0.03;	0.04]	0.04	[0.03;	0.04]	0.04	[0.03;	0.04]
Ni	0.35	**0.01**	0.11	**0.02**	0.34	pre-shift	0.41	[0.26;	0.57]	0.20	[0.14;	0.27]	0.31	[0.21;	0.41]
						post-shift	0.23	[0.15;	0.30]	0.30	[0.20;	0.39]	0.22	[0.15;	0.30]
Cu	0.34	0.24	0.31	**0.01**	0.36	pre-shift	0.98	[0.71;	1.25]	0.75	[0.57;	0.93]	1.38	[1.06;	1.70]
						post-shift	0.79	[0.60;	0.99]	1.01	[0.77;	1.25]	0.96	[0.71;	1.20]
Zn	0.28	0.36	0.97	**0.05**	0.38	pre-shift	8.65	[6.00;	11.30]	6.69	[4.95;	8.44]	11.68	[8.65;	14.72]
						post-shift	10.25	[7.47;	13.03]	7.58	[5.60;	9.56]	8.42	[6.08;	10.75]
Sb	0.87	0.85	0.87	0.75	0.34	pre-shift	0.02	[0.01;	0.03]	0.02	[0.01;	0.02]	0.03	[0.02;	0.03]
						post-shift	0.02	[0.01;	0.03]	0.02	[0.02;	0.03]	0.02	[0.02;	0.03]
Ba	0.08	1.00	0.17	**0.04**	0.30	pre-shift	0.21	[0.13;	0.29]	0.23	[0.15;	0.30]	0.37	[0.25;	0.48]
						post-shift	0.21	[0.14;	0.28]	0.20	[0.13;	0.26]	0.33	[0.21;	0.45]
Pb	**0.02**	0.34	**0.02**	**0.03**	0.32	pre-shift	0.06	[0.04;	0.08]	0.03	[0.02;	0.04]	0.05	[0.03;	0.06]
						post-shift	0.04	[0.03;	0.06]	0.06	[0.04;	0.07]	0.03	[0.02;	0.04]

Statistically significant results are shown in bold.

PM exposure induces changes in iron homeostasis Fe through the Fe complexion/chelation or displacement from pivotal sites in the cell, resulting in cellular Fe sequestration (Ghio et al., 2020).

Zn concentrations in EBCs were independent of sex, age, and smoking status and interestingly, 34-fold greater than the Fe concentrations (Ghio et al., 2018a). Similarly, we observed no effect of sex or job in our study on Zn concentrations in EBC. Our values were all below the values of Zn concentration in EBC reported in recent literature reviews (Ghio et al., 2018b; Corradi et al., 2009).

Cu concentrations in EBC were highest in security guards (Table 3) and greater than the median values reported for healthy adults (Corradi et al., 2009; Mutti et al., 2006)), but within the 25th and 75th percentile interval (0.30–1.80 µg/L) (Corradi et al., 2009). Ni concentrations in EBC were highest in station agents, but were affected by the sampling time as well as an interaction term between job and sampling time. Our results are in line with Ni concentrations in EBC reported for healthy adults (Ghio et al., 2018b). Ba concentrations in EBC were highest in security guards and remained stable across weekdays and work-shifts, which is consistent with its long clearance half-lives in experimental animals (EPA, 1998). Mn concentrations in EBC were also highest in security guards, and higher in pre-shift compared to post-shift EBC. However, the values measured are in line with values reported for healthy adults (Ghio et al., 2018b). All Cr concentrations in EBC were greater than the 75th percentile in healthy non-smoking adults (Corradi et al., 2009). Pb concentrations in EBC varied by job and weekday, but were in line with other studies (Corradi et al., 2009; Mutti et al., 2006). We found no effect of gender, job, weekday or shift for Co and Sb concentrations in EBC, and no literature values for comparison. The inter-class correlation was rather similar for all detected metals, ranging between 30 and 40% (Table 3).

3.4. *Metal concentrations in urine*

Among the 15 metals quantified in urine, seven metal concentrations (Al, Cr, Fe, Mn, Pb, Sb, and V) had more than half of the measurements below the LOQ. The statistical analyses was thus restricted to the eight metals above the LOQ (Table 4). The average creatinine-adjusted metal concentrations in urine were in decreasing order: Si $>$ Zn $\gg$ Mo $>$ Ti $>$

Cu > Ba ≈ Ni > Co, regardless of urine sampling time. As for EBC, iron I not found consistently in urine, suggestive of an effective sequestration in the body and a low excretion rate. Cu, Mo, Ba, and Si concentrations were greater post-shift compared to pre-shift. Zn was the only metal that had greater pre-shift than post-shift urine concentrations for all workers. For other metals (Ti, Ni, Co), the pre- and post-shift variations were limited to one or two jobs (Table 4). The highest inter-subject variability was observed for Zn and Co concentrations in urine (about 60% of total variance), and the lowest for Si and Mo (Table 4).

We relied on general population values from biomonitoring surveys when occupational biological limit values for urinary metal concentrations did not exist. The American Conference for Industrial Hygienists (ACGIH) has developed a biological exposure index for Co (30 μg/L) and several for Ni; metal (45 μg/L), Ni soluble salt (40 μg/L) and Ni insoluble salt (10 μg/L) (Hopf and Fustinoni, 2021). Our workers had urinary Co and Ni concentrations well below these as well as the 95th percentile reported for 40-59-year old adults in the French National Nutrition and Health Survey (FNNHS) (Fréry et al., 2017). The urinary Co concentrations in station agents and the urinary Ni concentrations in security agents appeared slightly above the central estimates of the creatinine-corrected concentration reported in FNNHS (Fréry et al., 2017). It is worth mentioning that the two- and tree-fold higher urinary Co concentrations we observed for station agents compared to the other professionals (Table 4), are likely a sex effect rather than a difference in occupational metal exposures. This difference is likely due to higher prevalence of iron deficiencies in females (Meltzer et al., 2010). Mo concentrations in urine from our workers were well below the 90th percentile reported for healthy non-smoking Swedish adults (Barregard et al., 2021). This was also the case for Cu concentrations in urine for males in our study, while female station workers' values were slightly higher than the Swedish values (Barregard et al., 2021), but lower than the Belgian (Hoet et al., 2013) and UK values (Morton et al., 2014). Urinary Cu concentrations were significantly higher in females than in males, which is consistent with our findings. This difference between the sexes can be due to the use of oral contraceptives (ATSDR, 2004). Moreover, the day-to-day variation in urinary Cu concentrations was low in comparison to the other metals (after adjusting for sex) (Morton et al., 2014).

In contrast to Cu and Co, urinary Zn concentrations were higher in males than in females. They were particularly elevated among locomotive operators being above the general population values reported for Swedish, Belgian, and UK adults (Morton et al., 2014; Barregard et al., 2021; Hoet et al., 2013) but below the biomonitoring equivalents for Zn concentrations in urine (Poddalgoda et al., 2019). Urinary Ba concentrations were highest in station agents followed by locomotive operators, who had twice the value compared to the security guards (Table 4). All values were below urinary Ba concentrations for the general population in Britain, Belgium, and France (Morton et al., 2014; Hoet et al., 2013; CDC, 2021; Goullé et al., 2005). Urinary Ti concentrations measured in our subway workers were all below British healthy non-smoking adults (Morton et al., 2014).

Si was the most abundant metal measured in the subway workers' urine (Table 4) with post-shift higher than pre-shift concentrations in station agents and security guards suggesting an occupational origin of this exposure. The urinary Si concentrations in security guards and particularly, in station agents were notably higher than those reported for Swedish healthy adults (Magnusson et al., 2020). Fe, Mn, Cu, Zn, and Mo are considered essential elements in human nutrition. As such, their concentration in the body is strictly regulated, thus diet and environmental factors have less effects on their concentrations (Morton et al., 2014). The excretion of these essential elements is essentially via bile (in feces); therefore, urine is not the most relevant biological matrix for biomonitoring. Notwithstanding, for Zn, the recent evidence suggests that in a health-risk context, urinary Zn is more reliable biomarker of exposure than blood due to homeostasis in blood (Poddalgoda et al., 2019).

Table 4
Metal concentration in urine (μg/g creatinine) of Paris subway workers.

Metal	Fixed effects (p-value)				Intra- class Cor.	Urine sampling	Mean [95% Confidence Interval]								
	Job	Shift	Day of the week	Job*Shift interaction			Station agents			Locomotive operators			Security guards		
Co	0.14	**0.01**	0.51	< **0.001**	0.60	pre-shift	0.64	[0.09;	1.20]	0.24	[0.03;	0.44]	0.11	[0.01;	0.21]
						post-shift	0.49	[0.06;	0.92]	0.24	[0.03;	0.46]	0.14	[0.02;	0.27]
Ni	0.28	**0.05**	0.15	**0.01**	0.52	pre-shift	1.16	[0.54;	1.78]	0.74	[0.34;	1.13]	0.92	[0.43;	1.42]
						post-shift	0.92	[0.42;	1.42]	0.69	[0.31;	1.06]	1.28	[0.60;	1.97]
Cu	< **0.001**	0.88	0.90	< **0.001**	0.50	pre-shift	6.91	[5.47;	8.36]	4.27	[3.37;	5.17]	3.97	[3.14;	4.81]
						post-shift	6.97	[5.47;	8.47]	4.91	[3.86;	5.95]	3.92	[3.10;	4.74]
Zn	0.27	0.01	0.82	< **0.001**	0.57	pre-shift	137.69	[54.25;	221.13]	261.54	[102.90;	420.18]	233.74	[91.97;	375.51]
						post-shift	114.20	[44.56;	183.83]	232.95	[91.43;	374.48]	161.36	[63.54;	259.18]
Mo	0.39	0.22	0.97	0.42	0.44	pre-shift	18.41	[12.08;	24.74]	16.76	[10.91;	22.61]	26.62	[17.34;	35.91]
						post-shift	22.27	[13.99;	30.55]	17.73	[11.40;	24.06]	25.06	[16.38;	33.73]
Ba	< **0.001**	< **0.001**	0.18	< **0.001**	0.52	pre-shift	1.24	[0.47;	2.02]	0.91	[0.34;	1.49]	0.48	[0.18;	0.78]
						post-shift	1.93	[0.71;	3.16]	1.13	[0.42;	1.84]	0.35	[0.13;	0.57]
Ti	0.07	< **0.001**	0.80	**0.01**	0.54	pre-shift	22.70	[4.91;	40.49]	14.40	[3.06;	25.75]	15.09	[3.18;	27.01]
						post-shift	36.69	[7.44;	65.95]	12.50	[2.54;	22.46]	11.20	[2.35;	20.06]
Si	< **0.001**	< **0.001**	0.07	< **0.001**	0.45	pre-shift	6786.36	[5901.40;	7671.32]	4233.52	[3666.52;	4800.53]	5089.95	[4409.88;	5770.02]
						post-shift	8819.21	[7502.73;	10135.70]	3863.69	[3327.66;	4399.72]	6014.52	[5221.72;	6807.31]

Statistically significant results are shown in bold.

3.5. Relationship between different exposure metrics

The exploratory pairwise correlation analysis showed that UFP number concentrations in air and post-shift EBC were positively correlated and became significant when pre/post-shift ratio of the particle number concentration was used (Supplementary Material Table S1). Both post-shift Zn EBC and pre-post shift Zn ratio were negatively correlated with Zn concentrations in PM2.5 measured two days earlier. On the other hand, Zn concentrations in PM2.5 were positively correlated with urinary Zn concentrations measured the same day post-shift. The same was true for Cu concentrations in PM2.5. These results suggest a temporal effect, as Cu concentrations in PM2.5 the day before was positively correlated with the urinary Cu concentrations pre-shift. Cu concentrations in PM2.5 two days before was positively correlated with the urinary Cu concentrations 48 h later (post-shift). Post-shift urinary Cu concentrations was negatively correlated with pre-shift copper concentration in EBC. Negative correlations were observed between post-shift Zn concentrations in EBC and both, pre- and post-shift urinary Zn concentrations. These correlations were slightly higher with the work-shift change in Zn concentration in EBC. The same pattern of relationship was observed for Ni (Table S1). These findings, although exploratory by nature, suggest a complex interplay between different exposure metrics. The difference in temporal variation of measured concentrations depending on the biological matrix may reflect the mechanism of metal clearance after exposure trough inhalation, which remains poorly documented in humans, particularly for these metals.

In the multivariate analysis, we found a significantly positive relationship for Zn concentration between post-shift EBC and PM10, while this relationship was negative for Cu (Table 5). Moreover, the change in Zn concentrations in EBC over the work-shift was positively associated with the change in urinary Zn concentration, though the coefficient was of borderline statistical significance. The relationship between post-shift EBC and post-shift urinary Cu concentrations was positive and strong (Table 5). These results show an interdependence of metals in EBC and PM10 as well as EBC and urine, but not PM and urine.

3.6. Findings' biological relevance and implications

3.6.1. Metals in PM

Toxicological profiles for most metals, and particularly Zn and Cu, lack data on inhalation exposure and respiratory tract absorption (ATSDR, 2004; ATSDR, 2005) as well as short and long-term health effects. Environmental epidemiologists have identified inhalation of Cu, Fe, Ni, Si, K, V, and Zn in PM of particular health concern (Wolf et al., 2015; Chen et al., 2021). Zn, Si, Fe, Ni, V, and K in PM were associated with cardiovascular health effects (Yang et al., 2019), while Cu was associated with both cardiovascular and respiratory health effects (Rohr and Wyzga, 2012). For iron concentrations in PM, the evidence of adverse health effect is less conclusive than for Cu, Zn and Al (Rohr and Wyzga, 2012). The concentrations of these metals in PM2.5 and PM10 are rarely reported. Nevertheless, we recommend monitoring their concentrations in subway PM as long-term exposure to metals may trigger adverse health effects, especially for Ti, a suspected carcinogen, and V and K, which are associated with respiratory and cardiovascular morbidity (Chen et al., 2021; Yang et al., 2019). We did not detected V and K in these elements in EBC or urine. But a previous analysis of the RATP workers' mortality showed an excess of ischemic heart disease in males (Campagna et al., 2008). Unfortunately, our analytical methods were not able to quantify V and K concentrations in PM. Their airborne concentrations should be assessed in a future study.

3.6.2. Cu and Zn implication in oxidative stress mechanism

The fact that only Zn and Cu concentrations were >LOQ in all three types of samples, was surprising. Loxham et al. showed that exposing primary bronchial epithelial cells (a key site of PM deposition) for 6 h or 24 h to iron-rich ultrafine PM collected from a subway, yielded at both time-points an upregulation of metallothioneins with antioxidant activity (Loxham et al., 2020). The main function of these metallothioneins is the binding and homeostasis of Zn and Cu ions, but not Fe ions. Loxham et al. explained that *in vivo*, Fe(II) is unable to displace Zn(II) or Cu(I) from the metallothioneins binding sites. Therefore, it is possible that the metal sequestration by metallothioneins is relatively ineffective against direct toxicity from Fe-rich PM, and it may affect the homeostasis of other metals. Indeed, Zn-loaded metallothioneins appear less protective against iron-induced DNA strand breakage compared with Cu. Nevertheless, the presence of Fe can have a profound effect on metallothioneins (Cai et al., 1995). The metallothionein-Zn complex is able to reduce ferritin-bound Fe(III) to Fe(II), which results in release of redox active Fe(II) from complex with ferritin, and concomitantly oxidation of the metallothionein thiolate groups, resulting in release of Zn(II). This free Fe(II) is then able to participate in other ROS-generating reactions, thus increasing oxidative stress, while there may also be dysregulated Zn homeostasis (Krężel and Maret, 2017). It is unclear whether oxidative stress and/or the presence of ferritin–Fe(III) may have an impact on the sequestration of Cu by metallothioneins in the same way. If this were the case, and given the greater affinity of

Table 5

Results of the mixed multivariate models* of PBZ, urinary and EBC metal concentrations in Paris subway workers.

Dependent variable	Concentration in PM10 (µg/m3)				Concentration in PM2.5 (µg/m3)				EBC or Urine Concentration**			
	β	IC-inf		IC-sup	β	IC-inf		IC-sup	β	IC-inf		IC-sup
Urine pre-shift Zn (µg/g creatinine)	−0.06	−0.19	;	0.07	0.09	−0.09	;	0.26	−0.02	−0.12	;	0.08
Urine post-shift Zn (µg/g creatinine)	0.06	−0.19	;	0.31	−0.06	−0.43	;	0.31	0.03	−0.14	;	0.20
Urine post/pre-shift Zn ratio	0.17	−0.14	;	0.49	0.30	−0.12	;	0.72	0.06	−0.11	;	0.23
EBC pre-shift Zn (µg/L)	−0.21	−0.57	;	0.16	0.08	−0.41	;	0.56	−0.17	−0.91	;	0.58
EBC post-shift Zn (µg/L)	**0.52**	**0.04**	**;**	**1.01**	−0.72	−1.53	;	0.08	0.59	−0.15	;	1.34
EBC post/pre-shift Zn ratio	−0.21	−1.25	;	0.84	No convergence				***0.34***	***−0.94***		***1.61***
Urine pre-shift Cu (µg/g creatinine)	0.03	−0.08	;	0.15	−0.04	−0.50	;	0.42	0.00	−0.08	;	0.08
Urine post-shift Cu (µg/g creatinine)	0.12	−0.02	;	0.25	−0.02	−0.61	;	0.57	−0.02	−0.12	;	0.07
Urine post/pre-shift Cu ratio	0.08	−0.13	;	0.29	0.23	−0.50	;	0.95	0.07	−0.05	;	0.19
EBC pre-shift Cu (µg/L)	−0.31	−0.69	;	0.08	0.17	−1.02	;	1.36	0.08	−0.80	;	0.97
EBC post-shift Cu (µg/L)	**−0.67**	**−1.12**	**;**	**−0.21**	−0.12	−1.41	;	1.18	**0.81**	**0.03**	**;**	**1.58**
EBC post/pre-shift Cu ratio	No convergence				No convergence				0.12	−0.52		0.76

* All models are adjusted for age, sex, and vitamin or food supplement intake.

** β coefficient corresponds to EBC concentration (in µg/L) when urine concentration (in µg/g creatinine) is dependent variable and *vice versa*. Values in bold correspond to statistically significant estimates ($p < 0.05$); those in italic correspond to the estimates of borderline statistical significance.

metallothioneins for Cu compared to Zn, this may result in displacement of metallothionein-bound Zn, and thus further dysregulation of Zn metabolism (Loxham et al., 2020).

These results support our exploratory findings, calling for a further hypothesis-based investigation of subway PM Zn and Cu effect. Both EBC and urine concentrations of these metals should be better characterized. Currently, urinary Zn concentration is considered a reliable biomarker for oral zinc intake and not from inhalation (Poddalgoda et al., 2019). Zn concentrations in EBC might reflect daily airborne Zn exposure as we have shown here, but this is still debated (Monsé et al., 2021). Zn concentration in EBC and its ratio to EBC iron was shown varying between non-smokers, smokers and COPD patients, exhibiting the highest ratio in non-smokers (Ghio et al., 2018a). Regarding Cu, Mutti et al. reported that chronic oxidative stress may be associated with Cu depletion in EBC, the antioxidant responsive element, and that Cu levels in EBC may be of particular interest because of their positive correlation with lung function parameters in COPD patients (Mutti et al., 2006).

3.7. *Relevance of biomonitoring*

3.7.1. *EBC as biological matrix*

Our results suggest that EBC can be a proficient matrix to sample airborne exposures to UFP (particle number concentrations) and some metals (Zn and Cu). These metals in EBC were associated with their respective urine concentrations. Assessing the metal dose in the target tissue (lung) is an advantage as opposed to blood or urine metal concentrations. This study demonstrated that EBC as a biological matrix provides complementary data on internal particle and metal exposures, and some insights on their mechanism after inhalation of PM in the subway environment. Some researchers wish to extend its use (Corradi and Mutti, 2005), while others consider EBC analysis a “niche approach” in occupational health research (Maestrelli et al., 2020). The main EBC advantages are that it is safe, rapid, simple to perform, non-invasive compared to blood draws, and effort independent compared to spirometry. The potential for using EBC in biomonitoring is high, but depend on further characterization of human breath, as it contains upwards of 250 chemicals (Corradi and Mutti, 2005). We provide here nine metal concentrations in EBC, which adds to the almost non-existent EBC exposure data. Along with this, we have also shown that EBC can be used to determine exposures to UFP expressed as particle number concentrations. The main concerns with EBC use are the need for standardization of sampling and analytical methods (Maestrelli et al., 2020). The contamination from outdoor and indoor air and from devices and reagents used are additional issues that need to be considered carefully (Horváth et al., 2005; Hemmendinger et al., 2021). Thus, inter-lab comparisons are necessary in the near future.

3.7.2. *Biomonitoring result communication*

Communicating biomonitoring results to workers is a topic on its own. However, we would like to share some reflections from issues raised during our study. Under French regulations, the physicians are mandated to communicate results to the workers. Many physicians are uncomfortable with this, as the biomonitoring data are not readily interpretable. Biomonitoring studies provide metal concentrations in biological matrices (e.g., mg/g creatinine in urine), the exposure guidance values for toxicity, such as reference doses are reported as oral intake values (in mg mg/kg bw/day) rather than biological equivalents (available only for two of analyzed metals (Poddalgoda et al., 2019; Poddalgoda et al., 2017)). Only few biological limit values are available and when they are, many physicians have no training in how to interpret these as there is no direct clinical values (as opposed to cholesterol values), but are related to exposures (Fréry and El Yamani, 2020). Within this study, we discussed how the individual results should be interpreted and communicated to the study participants. Namely, we retrieved from the literature the metal concentrations in urine and EBC of general population and control groups of healthy unexposed workers, respectively, and when necessary harmonized units using appropriate unit conversion. For some biomarkers we also performed meta-analysis of available values (Graille et al., 2020a, 2020b; Hemmendinger et al., 2020; Shoman et al., 2020). Therefore, this study plea for a better standardization and generalization of exposure biomonitoring in occupational and environmental health research and practice.

3.8. *Study limitations*

The exploratory nature and therefore small sample size is likely limiting the statistical power, especially for the mixed multivariate models for EBC and urine relationships, and the results generalizability. Although the personal air sampling was performed as close as possible to the participants’ personal breathing zone, the logistical constraints encountered, prevented us from satisfying the conditions required to consider the samples taken in the breathing zone.

4. Conclusion

Particle and metal exposure in Parisian subway was assessed using a triple approach: in PM, EBC and urine of subway professionals. PM2.5 and PM10 concentrations were in compliance with the French guidance values. Fe, Al, Zn, and Cu were the most abundant PM constituents; however, only Zn and Cu were consistently quantified in EBC and urine. The relationships between both metal concentrations in EBC and PM, and in EBC and urine, as well as a correlation between UFP exposures and particle number concentrations in EBC confirm the interest to use EBC as a collection matrix in exposure assessments, especially when inhalation is a primary route of exposure.

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Ethics approval and consent to participate

The study protocols were approved by the French Personal Protection Committees South-Est II (N°2019-A01652 55), Declaration of conformity to the French National Commission for Computing and Freedoms (CNIL) N° 2220108. Written consent to participate was obtained from all study participants.

Authors’ contributions

CC, MH, AD, VJ, TBR, GS, SB, data collection; GS, MH, JJS, SB, lab analyses; IGC, PW: statistical analysis; IGC: drafted the manuscript; NH, CC, and SB critically reviewed it; All authors participated in the result interpretation, manuscript preparation and read and accepted its final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113837.

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