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Chemical and genotoxic characterization of bioaccessible fractions as a comprehensive in vitro tool in assessing the health risk due to dust-bound contaminant ingestion

Rebecca Castel^{1,2} · Virginie Tassistro² · Stéphanie Lebarillier¹ · Nathalie Dupuy² · Yves Noack³ · Thierry Orsière² · Laure Malleret¹

Abstract

In the last two decades, awareness grew on the matter of the impact of environment on human health. Contaminants sorbed onto soil and settled dust can be ingested and thus represent a hazard, particularly to young children, who play on the ground and bring their hands and objects to their mouth. Metal(loid)s and polycyclic aromatic hydrocarbons (PAHs) are of concern as they are both carcinogenic to humans and ubiquitous in outdoor environments. The present study aims to assess the total and bioaccessible fractions of PAHs and metal(loid)s present in settled dust of four preschools located in industrial, urban, and suburban areas. On the one hand, children's incremental life cancer risks (ILCR) were calculated according to ingestion pathway. On the other hand, the genotoxicities of the bioaccessible dust-bonded contaminants were determined on gastric cells. PAH concentrations ranged from 50.9 to 2267.3 ng/g, and the bioaccessible fraction represented 10.7% of the total in average. Metal(loid) concentration ranged from 12,430 to 38,941 µg/g, and the mean bioaccessibility was of 40.1%. Cancer risk ranged from $2.8 \cdot 10^5$ to $8.6 \cdot 10^5$, indicating that there is a potential cancer risk for children linked to the ingestion of settled dust. The inorganic bioaccessible fraction induced little DNA (<20% TailDNA) and chromosomal damages (30% increase in micronuclei), whereas the organic bioaccessible fraction induced higher DNA (17–63% TailDNA) and chromosomal damages (88% increase in micronuclei). Such experimental approach needs to be deepened, as a tool complementary to cancer risk calculation, since the latter only lays on a set of targeted contaminants with known toxicity values.

Keywords Environmental exposure · Genotoxicity · Incremental lifetime cancer risk (ILCR) · Metal(loid)s · Oral bioaccessibility · Outdoor settled dust · Polyaromatic hydrocarbons (PAHs)

Introduction

Nowadays, children below 6 years old are exposed to thousands of different chemicals in their environment. Because of their immature physiology — where organs are still developing, physiological barriers are more permeable, and their

intake per body weight is higher than in adults — and their exploratory behaviors — where they are still learning to walk, often play on the ground, and frequently bring their hands and objects to their mouth — children are the most susceptible population to be exposed to settled dust (Walker-Smith and MacDonald 1989; Moya et al. 2004; Trasande and Thurston 2005; Lu and Rosenbaum 2014). They can be exposed by dermal and respiratory pathways, but these are considered as minor in front of the oral pathway, since children may ingest up to 100 mg of settled dust per day (EPA 2017).

Settled dust regroups heterogeneous particles, of various kinds and of different sources, and may act as a sink for environmental chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and metal(loid)s (Maertens et al. 2008b; Plumejeaud et al. 2016). Both are ubiquitous compounds, measured in all environmental compartments. Both can be

produced or transported by natural phenomena (volcano eruptions, wildfires, erosion, water cycle). PAHs can be formed by natural pyrogenic sources (incomplete combustion of biomass) and petrogenic sources (leaks from natural deposits of petroleum), and they can be emitted by several outdoor anthropogenic activities, such as petrochemical industries, thermal motors emissions, or wood or coal heating and controlled burnings on farms (ATSDR 1995). Metal(loid)s are naturally found in the terrestrial crust, and human activities also cause important inputs of metal(loid)s in outdoor environments. These activities include mining industries, smelting plants, vehicular non-exhaust emissions (tires, brakes), and farming among others (Galażyn-Sidorczuk et al. 2008; Alloway 2013). Their environmental levels can be influenced by the seasons consecutively to weather (e.g., photolysis) and human behaviors (e.g., heating in winter) and by the location since human activities depend on the usage of a specific territory (Putaud et al. 2010; Longhin et al. 2013; Dergham et al. 2015). Overall, PAHs and metal(loid)s have many different outdoor sources and both are major contributors to pollution in metropolitan areas, such as the ones investigated in the present study.

Their ubiquity makes environmental exposure to these compounds inevitable, whereas causing numerous adverse health outcomes. Metal(loid)s may induce dermal, cardiovascular, hepatic, and neural toxicities (Tchounwou et al. 2012). PAHs increase the risk of respiratory and cardiovascular diseases as well as cognitive development delays (World Health Organization 2010). Moreover, As, Cd, Cr(VI), Ni, Th, and benzo(a)pyrene (BaP) are considered as carcinogens by the International Agency for Research on Cancer (IARC 2022), and several metal(loid)s and PAHs are registered as possible or probable carcinogens. Most carcinogenic compounds are also genotoxic and/or mutagenic, and it is possible to assess the genotoxicity and mutagenicity of such compounds with *in vitro* assays (DeMarini 2019). A regulatory methodology to assess the hazard of environmental contaminants is to calculate ecotoxicological or toxicological risk on the basis of toxicological values, which are available for several regulated PAHs and metals. More specifically, the incremental (or excess) lifetime cancer risk (ILCR) is a calculation approach for estimating the risk of occurrence of an adverse health outcome, namely cancer, due to the intake of carcinogens, such as metal(loid)s and PAHs, in a settled dust ingestion scenario (U.S. EPA 1989). Although widely applied, ILCR does have some limitations. It is based on the total quantity of a contaminant in a sample, without considering its bioavailability. Furthermore, it does consider only the carcinogenic potential of the measured contaminants having known toxicity values. All the other compounds present in the sample, i.e., those not analyzed and those whose toxic effects are unknown, are ignored in this risk assessment approach.

Bioavailability is the quantity of a contaminant, which is contained in a sample and will be absorbed by the organism after exposure. It is dependent on the type of matrix and the exposure route. For instance, contaminants in settled dust have a higher oral bioavailability than in soils (Rasmussen et al. 2008; Gloennec et al. 2012). Other parameters, such as the sample granulometry or the total organic carbon, can also influence bioavailability (Ruby et al. 1999; Raffy et al. 2018). Determining bioavailability involves a costly and complex procedure, since it is measured *in vivo*, either on animals or on volunteers. Oral bioaccessibility is the quantity of a contaminant that is released from a matrix in the gastro-intestinal lumen. Thus, the bioaccessible fraction is relevant in health risk assessment, as it represents the maximal quantity absorbable by an organism and it can be used as a proxy for bioavailability (Koch and Reimer 2012). The bioaccessible fraction is also of interest for ethical consideration, since it does not involve experiments with humans. Bioaccessibility methods aim to mimic digestion *in vitro* and are often based on the use of physiological-like fluids and parameters (temperature, shaking). These methods are cheaper, faster, and above all, more ethical, as they do not require animal experiments, unlike bioavailability studies (Denys et al. 2012; Beriro et al. 2016; Raffy et al. 2018). However, many different types of methods were proposed, ranging from chemical methods (vortexing a sample in a solvent) to cumbersome physiological-like implementations (multiple extractions with fluids containing enzymes, use of cells to represent uptake, use of a microbiome simulator, representation of a fed state) (Ruby et al. 1999; Oomen et al. 2002; Cave et al. 2010). To date, due to the lack of comparison with *in vivo*, no consensus has been reached for the *in vitro* determination of bioaccessibility, except for one test, i.e., the Unified BARGE Method (UBM). The UBM was validated in an inter-laboratory trial against an *in vivo* model, for As, Cd, and Pb (Denys et al. 2012). Simplified versions of the UBM were then developed, and good correlations with the original UBM were demonstrated (Pelfrêne et al. 2020). The acidic aqueous media used for metal(loid)s is however limited for the recovering of PAHs and other organic contaminants. Therefore, a set of chemical and physiological methods were developed for organics (Collins et al. 2015; Raffy et al. 2018). But only a few studies compared their results to *in vivo* models (James et al. 2011; Duan et al. 2014; Kang et al. 2018). To this end, a simple extraction with butanol demonstrated a good correlation ($r^2 = 0.75$, $p < 0.01$) with the swine model, the most representative model of the human digestive system (Duan et al. 2014). Nonetheless, strategies involving bioaccessibility remain scarcely applied in environmental risk assessment, even though the bioaccessible fraction should be more relevant to consider than the total content. These methods can also provide a representative mixture extract for bioassays,

containing a set of bioaccessible compounds extracted simultaneously, thus offering a more faithful representation of the actual exposure.

Previous studies focusing on PAHs and metal(loid)s in settled dust assessed health risks by regulatory calculation (Maertens et al. 2008b; Škrbić et al. 2018; Masto et al. 2019; Živančev et al. 2022; Castel et al. 2023a). Some other investigated the mutagenic potential of the total extracts by using the Salmonella Mutagenicity assay, the Ames assay, and the SOS Chromotest (Maertens et al. 2008a; Kang et al. 2011). Rare are the studies evaluating the *in vitro* cytotoxicity and genotoxicity of bioaccessible fractions (Wang et al. 2013b; Plumejeaud et al. 2016). These papers reported that the total or the bioaccessible fractions could be mutagenic, cytotoxic, and genotoxic. To date, there are still too few studies investigating the genotoxicity of bioaccessible fractions of different chemical families, by applying bioaccessibility methods specific to the different groups of contaminants considered. Yet, this type of approach could be a means of discriminating fractions according to their toxicity and guiding the analyst towards the most hazardous compounds.

Therefore, with this paper, we aimed at studying the health risk associated to dust-bound PAHs and metal(loid)s ingestion, by coupling bioaccessibility methods and genotoxicity assays as an *in vitro* tool complementary to health risk calculation. The total and the oral bioaccessible contents of metal(loid)s and PAHs were quantified in dust samples. Furthermore, the genotoxicity of the organic and inorganic bioaccessible fractions were evaluated on gastric cells. To this end, outdoor dust samples were collected at two seasons, in four preschools located in different settings: industrial, urban, and suburban. Data were interpreted by using principal component analyses, on the one hand, to discriminate the influent parameters in bioaccessibility release of metal(loid)s and PAHs and, on the other hand, to investigate if the measured contaminant concentrations can justify the biological damages observed. Finally, based on the total content of metal(loid)s and PAHs and the methodology designed by the US EPA, we calculated children's cancer risks associated with dust ingestion and discussed the results in comparison to the *in vitro* bioaccessibility and genotoxicity approach.

Experimental section

Study sites, dust sample collection, and preparation

Four preschools located in Bouches-du-Rhône, South-East France, were investigated (see map in supplementary information Figure SI-1.1).

The industrial preschool (site 1) is in Port-Saint-Louis-du-Rhône. This small city (8500 inhabitants) is situated in the west of the department, near the mouth of the Rhône River.

Less than 10 km away are about 30 SEVESO high-risk industries, with chemical, petrochemical, and steel industries. Less than 5 km away is the western part of the Marseille-Fos harbor (around 80 Mt of merchandise per year). This area is one of the biggest industrial areas in France. More than 110 children are enrolled in this preschool. The courtyard was paved and a part of it was covered. The playground area was covered with a shock-absorbing coating.

Two preschools were located in the city center of Marseille, the second biggest city in France. One preschool is in a district undergoing urban renewal with industrial wastelands nearby. The eastern part of the harbor of Marseille-Fos (over 3 million passengers per year) and the highway A55 are both 500 m away. Therefore, the preschool is in a complex urban/highway/port environment (site 2). There are about 140 children in the preschool. The courtyard was entirely paved, and the playground had a shock-absorbing coating. The other preschool is mainly urban (site 3), close to a park in Marseille and to the train station. Nearly 200 children are enrolled in this preschool. The courtyard was paved and had a few trees. There was also a covered area and a playground covered with shock-absorbing coating.

The suburban preschool (site 4) is in Trets, a city of 10 000 inhabitants, in the Arc basin (east of the department). The main local activity is agriculture and 53% of the town's land use are forests and seminatural environments. The highway A8 is about 3 km away. There are 165 children in the preschool. The courtyard was partly paved but present also an area with soil and grass. The playground was recently renewed and had a shock-absorbing coating.

Each preschool was sampled in 2020–2022, once in summer and once in winter. All samples were collected by using brooms and dust pans. Samples were dried at 40 °C for 24 h, weighed and then sieved < 250 µm, since particles bigger than 250 µm cannot be considered as dust. All the sieved fractions were weighed again and kept in aluminum trays until analysis (< 12 months). Additional information concerning the preschools and the sampling campaigns can be found in Table SI-1.2.

Chemical extractions and analyses

Total organic content

The total organic content (TOC) was calculated as the difference between the total carbon (TC) concentration and the total inorganic carbon (TIC) concentration. To measure the TC, samples were weighed before being inserted in an oven HT 1300 (Analytik Jena) and analyzed by the multi N/C 2100S TOC analyzer (Analytik Jena). For the TIC, samples were weighed and put in an Erlenmeyer flasks. The flasks were sealed and put under an O₂ flow, directly linked to the multi

N/C 2100 TOC analyzer (Analytik Jena). Once no more carbon was detected, samples were acidified with 2 mL of phosphoric acid at 40% and placed at 80 °C with a magnetic agitation of 330 rpm. The released carbon was directly injected in the multi N/C 2100 TOC analyzer (Analytik Jena). Results were shown in the multiWin version 4.05 software (Analytik Jena).

Extraction of the oral bioaccessible contents of metal(loid)s and PAHs from dust samples

Metals and metalloids were extracted by following the protocol using HCl, as designed by Pelfrène et al. (2020). For each dust sample, 90 mg was weighed and placed into a polyethylene tube. Then, 75 mL of 0.65% HCl was added and ultrasonicated for 15 min. Samples were then digested in an oven at 37 °C for 1 h (Pelfrène et al. 2020). Following digestion, samples were filtered <0.45 µm. Half of each sample was diluted with 5% HNO₃ and 9.35% HCl for subsequent chemical analysis. The other half was kept as it was for subsequent genotoxicity assays.

PAHs were extracted with butanol as adapted from Duan et al. (2014). For each sample, we performed twice the following protocol: 2 g of dust sample placed in 10 mL of butanol and vortexed for 50 s. Then, samples were let sit for 1 h at room temperature before collecting the supernatants. Both supernatants of each sample were pooled and filtered <0.45 µm through PTFE filters. The pool was then divided in two, one for chemical analysis and the other for genotoxicity assays. After dry concentration under air flow, 200 µL of ACN was added to one sub-sample before chemical analysis, and 200 µL of DMSO was added to the other sub-sample for subsequent genotoxicity assays.

Extraction of the total contents of metal(loid)s and PAHs from dust samples

The total content of metal(loid)s was obtained through digestion of dust samples (100 mg) with *aqua regia* (HNO₃ 65% and HCl 35%) for 12 h on a heating plate at 140 °C. Then, filtration (<0.45 µm) and acidic dilution (5% HNO₃ and 9.35% HCl) were performed before analysis. The total content of PAHs was extracted from the dust sample (200 mg) by pressurized fluid extraction (ASE 200, Dionex, Sunnyvale, CA, USA) with a mixture of dichloromethane (DCM, Quality Suprasolv, Merck) and methanol (Quality HyPer-solv, Merck) (9:1, v/v). Afterwards, a clean-up on silica gel cartridge, followed by reconcentration process and dilution with 200 µL of ACN, was performed before analysis.

Analysis of metal(loid)s and PAHs in dust sample extracts

The studied inorganic elements, i.e., Al, As, Cd, Ce, Co, Cr, Cu, Fe, Ga, La, Mg, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Th, Ti, U, V,

Zn, and Zr, were analyzed by inductively couple plasma-mass spectrometry (ICP-MS, NexION® 300 X, PerkinElmer, MA, USA) in the total and bioaccessible extracts of dust samples.

Fifteen PAHs out of the 16 US EPA priority PAHs, namely naphthalene (Nap), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (dBahA), benzo(g,h,i)perylene (BghiP), and indeno(1,2,3-dc)pyrene (IcdP), were quantified by ultra-high-performance liquid chromatography (UPLC, PerkinElmer Altus) with programmed fluorescence detector (PFD, PerkinElmer) (Vergnoux et al. 2011; Castel et al. 2023a).

QA/QC

For PAH analyses, the calibration curves ($r^2 > 0.99671$) were drawn by dilution in the acetonitrile of the Standard Reference Material 1647e (SRM 1647e from Sigma-Aldrich) from the National Institute of Standards and Technology (NIST). Recoveries from dust were established and checked for each batch of sample extraction with NIST SRM 1649b urban dust (Sigma-Aldrich). Mean repeatability (expressed as RSD%, $n = 5$) was <20%. Recoveries were between 78 and 112%, except for Nap, Ace, and Flu (about 35%), because of losses by volatilization during the reconcentration steps. The calculated concentrations were corrected by this factor. In each batch of analysis sequence, duplicates of SRM 1649b were run and a blank and a calibration standard were run every six samples.

For metal(loid)s, NIST SRM 2583 indoor dust standard (Merck) was used. It was prepared and analyzed using the same procedure as the samples. The recoveries ranged between 80 and 120% and precision was <20%. For all samples' series, an acid blank (HNO₃ 65% and HCl 35% Merck Suprapur) was routinely prepared and analyzed.

More details about the QA/QC are given in the supplementary information (Tables SI-1.4 and SI-1.5).

Genotoxicity

Cell viability

Adherent human adenocarcinoma gastric cells (AGS) and the supplemented Dulbecco's modified Eagle's medium (DMEM) were furnished by Cell Line Service (Eppelheim, Germany). AGS cells were maintained in DMEM at 37 °C with 5% of CO₂. The supplemented DMEM had 4.5 g/L of glucose, 2 mM of L-glutamine, 10% of fetal bovine serum,

and 1% of penicillin–streptomycin. To assess cytostasis, AGS cells were exposed for 4 h to inorganic and organic bioaccessible extracts separately (10% for inorganic extracts and 2% for organic extracts) and to positive controls.

Cell viability was determined with the cytokinesis-block proliferation index (CBPI), when performing a cytokinesis-block micronucleus (CBMN) assay. This index (see Eq. 1), assessed on 500 cells, allows to calculate cytostasis (see Eq. 2).

$$CBPI = \frac{[No. Mononucleated \zeta + (2 \times No. Binucleated \zeta) + (3 \times No. Multinucleated \zeta)]}{Total No. \zeta} \quad (1)$$

$$\%cytostasis = 100 - 100 \times \left(\frac{CBPI_{exposed} - 1}{CBPI_{control} - 1} \right) \quad (2)$$

Alkaline comet assay

To assess primary DNA damage, we performed an alkaline comet assay (Tice et al. 2000; Castel et al. 2023b). Negative controls were media with 10% HCl 0.65% for inorganic fractions and media with 2% DMSO and 10% S9 mix (Xenomatrix, Allschwill, Switzerland) for organic fractions. A positive control consisting of 125 μ M of H₂O₂ for 4 min at 4 °C was performed after spotting cells onto slides. The details of the methods were described elsewhere (Castel et al. 2023b) and in the supplementary material (Text SI-1.6).

Briefly, cells were seeded at 25.10³ cells/well 24 h before a 4-h-long exposure to, on the one side, the inorganic and, on the other side, the organic bioaccessible extracts (10% for inorganic extracts and 2% for organic extracts), obtained by using protocols described in the “Extraction of the oral bioaccessible contents of metal(loid)s and PAHs from dust samples” section, for each preschool at each season separately. Then, cells were trypsinized, pelleted, and resuspended in low melting point agarose and spotted onto precoated slides with normal melting point agarose. After, lysis and denaturation were performed before doing an electrophoresis at 0.9 V/cm and 300 mA for 20 min at 4 °C. Neutralization was then done, and slides were dehydrated in methanol. The staining agent was propidium iodide, and the analysis was done with a fluorescence microscope. The Zeiss Axio Imager A.2 microscope ((Zeiss SAS, Marly le Roi, France) was equipped with a filter combination and a black and white camera CCD Andor Zyla (Andor Bioimaging, Belfast, UK). The software was Komet 6.0 (Andor™ Technology, Belfast, UK). For each slide, 100 cells were analyzed. All results are presented as %TailDNA.

Cytokinesis block micronucleus assay

Chromosomal damage was evaluated via a CBMN assay (Fenech 2000; Castel et al. 2023b). Seeding occurred at 25.10³ cells/chamber in LabTek™ 4 chamber slides (Lab-Tek™ II Nalgene Nunc International, Villebon sur Yvette,

France) 24 h before exposure. Cells were exposed, on one side, to the inorganic and, on the other side, to the organic bioaccessible extracts (10% for inorganic extracts and 2% for organic extracts), obtained by using protocols described in the “Extraction of the oral bioaccessible contents of metal(loid)s and PAHs from dust samples” section, for each preschool at each season separately. The negative controls were media with 10% HCl 0.65% for inorganic fractions and media with 2% DMSO and 10% S9 mix (Xenomatrix, Allschwill, Switzerland) for organic fractions. Positive controls included mitomycin C (MMC, clastogenic; 30 nM) and colchicine (aneugenic; 25 nM) for MMs fractions or benzo(a)pyrene (BaP; 7.5 μ M) with 10% S9 mix for organic fractions.

Briefly, cells were exposed for 4 h to the bioaccessible extracts (10% for inorganic extracts and 2% for organic extracts). Medium was then renewed. Exposure to 6.25 μ M of cytochalasin B was done 20 h later. After 24 h, medium was renewed for cells to rest. Two hours after, cells were washed with PBS and fixed with 4% of paraformaldehyde. Cells were permeabilized with PBS, 0.5% Triton X-100, and 2% BSA for 15 min. After rinsing, cells were incubated with 0.74 μ M of phalloidin-TRITC for 30 min to stain the cytoplasm. Cells were once again rinsed before a 10-min incubation with DAPI (diluted to 1/50,000) to stain the nuclei. Finally, slides were mounted in Prolong™ Gold antifade and stored at 4 °C until analysis. The fluorescence microscope Zeiss Axio Imager A.2 (Zeiss SAS, Marly le Roi, France) was equipped with a filter combination and a Nikon camera (Melville, USA); the software was NIS-Elements. Scoring criteria were in accordance with the protocol described by Fenech 2000). Results are expressed as the frequency of binucleated cells with micronuclei per 1000 binucleated cells.

Data analysis

Bioaccessible fraction

The bioaccessible fraction (BAF) of contaminants in the dust sample corresponds to the ratio of the concentration of the contaminant in the bioaccessible extract (obtained by following the protocol described in the “Extraction of the oral bioaccessible contents of metal(loid)s and PAHs from dust samples” section) divided by the concentration of the

contaminant contained in the total extract (obtained by following the protocol described in the “Extraction of the total contents of metal(loid)s and PAHs from dust samples” section) and is expressed as a percentage (Eq. 3) (Oomen et al. 2002).

$$BAF\% = \frac{[contaminant]_{bioaccessextract}}{[contaminant]_{totalextract}} \times 100 \quad (3)$$

Cancer risk assessment

Incremental lifetime cancer risk (ILCR) assessment was performed in a scenario of ingestion of settled dust by children below 6 years old, following the US EPA guidelines (U.S. EPA 2017). Currently, inhalation and dermal routes are considered as negligible (French National Agency of Food, Environment & Work Safety (ANSES) 2019). A composite adjustment factor was used since cancer risks are higher from childhood exposure according to the US EPA (U.S. EPA 2005). Cancer slope factors were obtained from the Risk Assessment Information System (RAIS) (Oak Ridge National Laboratory (ORNL) et al. 2023). All the details and the cancer slope factors are given in the supplementary materials (Text SI-1.7 and table SI-1.8).

Statistical analysis and principal component analysis

Data from the comet assay were submitted to a nonparametric statistical test (Kruskal–Wallis), whereas the data from the CBMN assay were submitted to χ^2 test. Statistical analyses were done with GraphPad Prism 9 (GraphPad Software, Inc., La Jolla, USA).

The principal component analysis (PCA) is a mathematical method that transforms a large set of n possibly correlated variables into a smaller set of new variables, named principal components. These principal components represent most of the variance in the dataset, and by analyzing the principal components in simple biplots, it is possible to visualize the correlations between the original variables. To investigate the influence of dust parameters on the compounds’ bioaccessibility, the %bioaccessibility and physicochemical parameters (granulometry and TOC) of the settled dust were correlated by PCA. To study the correlation between the observed biological damages and the analyzed compounds, PCA was applied to correlate the content of metal(loid)s in the bioaccessible fraction of inorganics with the DNA and chromosomal damages induced by this fraction. The same was done for the content of PAHs in the organic bioaccessible fraction with the DNA and chromosomal damages induced by the organic fraction. The software was Unscrambler X 10.3.

Results and discussion

Total contents of metal(loid)s and PAHs in outdoor dust samples

The sum concentrations of metal(loid)s (Σ MMs) ranged from 12 430 to 38 941 $\mu\text{g/g}$, and the sums of carcinogenic metal(loid)s (Σ MMcarc) ranged from 31.83 to 166.3 $\mu\text{g/g}$ (Table SI-2.1). The site 2 was the preschool with the highest concentrations of Σ MMs and Σ MMcarc. The site 4 was the preschool with the lowest concentrations of both Σ MMs and Σ MMcarc. In all preschools, Al, Fe, and Mg were the main elements followed by Cu, Zn, Mn, Sr, and Ti. Spatial differences could be noted between the preschools (Figure SI-2.2). The site 1 had the highest contribution of Cd, Co, Cr, Mn, Mo, and Ni. The site 4 had the highest contributions of Al, Ce, Cu, La, Th, Ti, U, and Zn. The site 3 had the highest contributions of Pb and Sb. The site 2 had the highest contributions of Sn and Zr. Seasonal variations were observed in sites 1 and 4, but not at the Marseille’s preschools (Figure SI-2.2). Metal(loid)s levels were lower than the ones measured in soil in kindergartens in Armenia and in street and road dust in Canada (Rasmussen et al. 2001; Nazzal et al. 2013; Tepanosyan et al. 2017). When comparing similar metal(loid)s with those measured in street dust in Serbia and in India, we observed that levels in the preschools were 1.5 to 3 times higher than in Novi Sad, Serbia (185 $\mu\text{g/g}$), whereas levels in India were similar to the levels measured in the sites 1, 3, and 4, but lower than in the site 2. However, the mean carcinogenic load in the French preschools was lower than in the street dusts (Škrbić et al. 2018; Mastro et al. 2019).

The sum of the 15 PAH concentrations (Σ PAHs) ranged from 50.91 to 2267.3 ng/g (Table SI-2.1). The highest concentration was measured in the site 1 in summer, and the lowest concentration was measured in the site 4 in summer. Dusts were dominated by Fla and Pyr in most micro-environments. PAH profiles were dominated by four- to five-ring PAHs whatever the season was, except for site 4. In summer, the profile of this latter site was dominated by the five-ring PAHs, whereas in winter, the three-ring PAHs were the most abundant, certainly because of inputs from heating sources (wood burners or oil-fired). PAH levels in site 4 were lower than the ones in other studies globally. It could be attributed to low traffic density and little industrial activities in the vicinity of the preschool from site 4. In Marseille, the maximal levels of PAHs were similar in both preschools, but the highest level was measured in winter for site 2 (1657.7 ng/g) and in summer for site 3 (1569.9 ng/g). The PAH concentrations in sites 1, 2, and 3 were all lower than the levels measured in dusts from China, India, Nigeria, and the USA, but higher than those

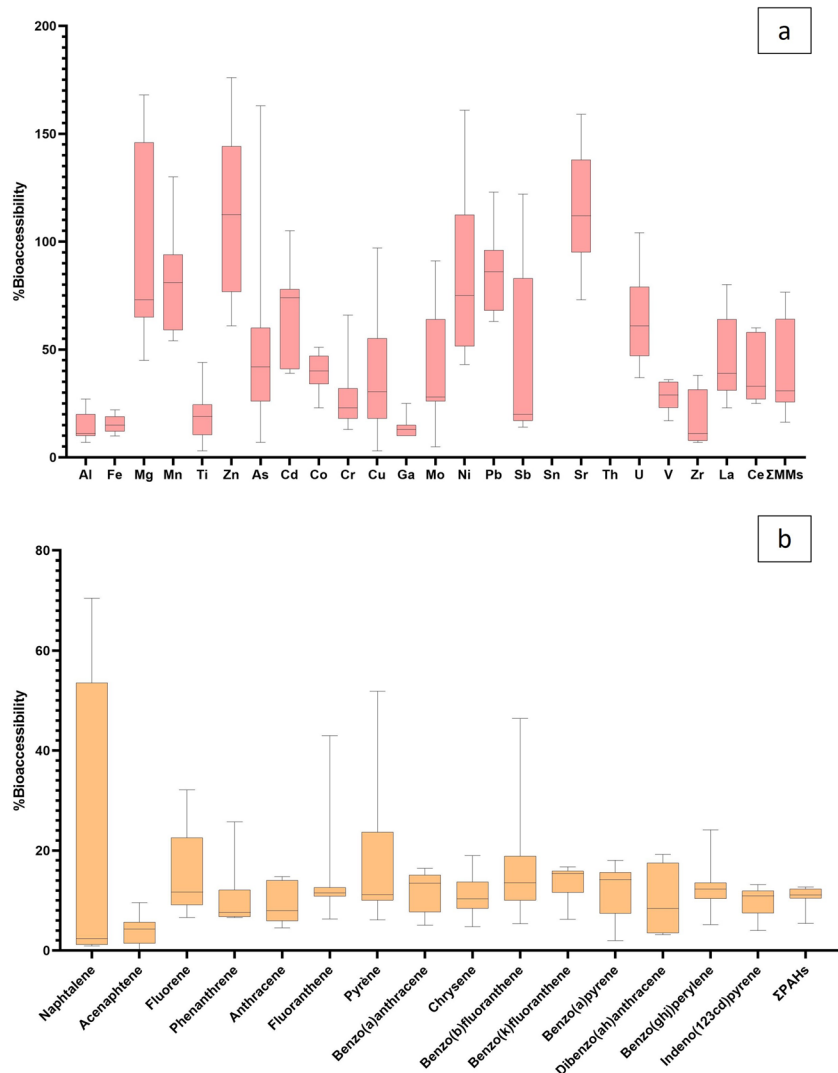
measured in street dusts from Egypt and Serbia (Hassanien and Abdel-Latif 2008; Škrbić et al. 2019; Masto et al. 2019; Ossai et al. 2021; Shen et al. 2022).

Oral bioaccessibility contents of metal(loid)s and PAHs in outdoor dust samples

The concentrations of metal(loid)s in the inorganic bioaccessible fraction (BAF) ranged from 2947.8 to 14,612.7 $\mu\text{g/g}$, with a mean of 9017.1 $\mu\text{g/g}$ (Tables SI-2.3). About 40.1% of the metallic load was bioaccessible (Fig. 1a). The lowest BAF was quantified in site 1 (16.3% in summer and 22.9% in winter) and the highest in site 3 (64.1% in summer and 76.6% in winter). However, for carcinogenic metal(loid)s, the mean BAF was estimated at 56.3%, indicating that these metals were in the high percentages of oral absorption. Metal(loid)s could be divided into four groups: Mg, Mn, Ni, Pb, Sr, and Zn were very highly bioaccessible (> 75%); As, Cd, and U were highly bioaccessible (53.5 to 65.2%); Ce, Co, Cr, Cu, La, Mo, Sb, and V were moderately bioaccessible (27.5 to 46.4%); and Al, Fe, Ga, Ti, and Zr were barely bioaccessible (< 15.6%).

In a previous study on indoor dust in Portugal, bioaccessibility of metal(loid)s was estimated with the Unified BARGE Method (UBM), validated for As, Cd, and Pb (Denys et al. 2012; Plumejeaud et al. 2016). The BAFs of As, Cu, Cr, and Co were similar to the ones calculated hereby, but were higher for Cd, Ga, Pb, and V and lower for Mn, Mo, Ni, and Sb than in the present study. In another study comparing bioaccessibility of Cu, Ni, and Zn extracted with diluted HCl from dust and soil in Canada, the authors observed that the BAF was higher in dust than in soil (Rasmussen et al. 2008). In dust, Zn had the highest BAF (65%) and Ni and Cu had similar BAFs (44%). These values are close to ours, except for Ni, having a lower BAF than the one measured here. In another study carried out on household dust, the bioaccessibility of the metal(loid)s was assessed with a simulated gastric solution (pepsin in diluted HCl). The BAFs for Al, Cu, and Fe (70%,

Fig. 1 Bioaccessibility of contaminants in settled dust ($n = 8$): **a** percentage of bioaccessible metal(loid)s determined in outdoor dust, by an extraction with HCl 0.65%. ΣMMs = sum of the 24 metal(loid)s; **b** percentage of bioaccessible PAHs determined in outdoor dust by an extraction with butanol. ΣPAHs = sum of the 15 PAHs



80%, and 35%, respectively) were considerably higher than the ones from our study, whereas for Cd, Mn, Ni, and Pb, the BAFs (55–100%) were in the same range as the ones from our study (Turner and Simmonds 2006). Finally, the BAFs, determined by the UBM in soils, ranged from 10.6 to 82% for Pb, from 8.4 to 87.7% for Cd, from 3 to 74.5% for As and from 2.8 to 14% for Sb, showing that the BAFs can be spanned over a wide range depending on the sample, even if the same extraction procedure is employed (Denys et al. 2012). Except for Sb (BAF of 47.8%), the BAFs determined in our study fall within the ranges determined by Denys et al.

The range of PAH in the organic bioaccessible fraction went from 6.49 to 282.65 ng/g, with a mean of 114.52 ng/g (Tables SI-2.4). The mean percentage of bioaccessible PAHs was of 12.73% and the same for carcinogenic PAHs (12.95%) (Fig. 1b). The percentages of bioaccessibility for the various PAHs were therefore more homogenous but quite lower than the ones calculated for MMs. The lowest BAF was found in the site 1 in winter (5.43%), whereas the highest BAF was found in the site 4 in summer (12.75%). Overall, high molecular weight PAHs (≥ 4 rings) had higher median BAFs than low molecular weight PAHs (< 4 rings). BkF had the highest median BAF (15.4%), followed by BaP (14.2), BbF, and BaA (13.5–13.6%). Nap had the lowest median BAF (2.4%) followed by Ace (4.44%). In one sample, we did observe an anomaly, where Nap had a bioaccessibility of 70.5%. It was assumed to be due to the presence of plant residues in the sample. In a study by Kang et al. on air conditioning filter dust, the bioaccessibility of PAHs was measured with a physiologically based extraction test (Kang et al. 2011). They found that BaA, Chr, B(b + k)F, BaP, IcdP, and dBahA had bioaccessibilities ranging from 1.3% (IcdP) to 17% (BaA), with a mean of 7%. Other studies were performed on PAH in soils. Juhasz et al. estimated the bioaccessibility of PAHs in creosote-contaminated soils with the fed organic human simulation test (FOREhST) and found that Fla, Pyr, BaA, Chr, BbF, BkF, and BaP had BAFs $< 4\%$ (Juhasz et al. 2014). The bioaccessibility of PAH in soils, determined with the latter method, ranged from 0.1 to 57%, with a mean of 25.1% (Cave et al. 2015).

It was shown that soil properties, and especially the carbonate content, modify the bioaccessibility of PAHs, thus partly justifying the differences in BAF determined in the different studies (Cave et al. 2015). The BAF of PAHs may also depend on the TOC of the sample, on the size of the particles and on the chemical properties of PAHs (Raffy et al. 2018). Similarly, the bioaccessibility of metal(loid)s was shown to highly depend on the nature of the particles (% clay, % silt, % organic matter), as silt may retain metal(loid)s more than organic matter, which can represent a high proportion in dust (Rasmussen et al. 2008; Pelfrène et al. 2020).

Hence, principal component analysis (PCA) was performed to determine possible correlations between

components in the bioaccessible fractions of settled dust regarding the physicochemical parameters (particles size, TOC) of the dust (Table SI-2.5) (Budak et al. 2018; Sena et al. 2002). The first data matrix used to carry out PCA was composed of 20 inorganic elements, and the two first components represented about 60% of the variance (Figure SI-3a). The PCA indicated that the size fractions of the particles were significant, with the fractions $< 50 \mu\text{m}$ and $50\text{--}100 \mu\text{m}$ being both anticorrelated to the fraction $100\text{--}250 \mu\text{m}$. Only the largest particles ($100\text{--}250 \mu\text{m}$) were correlated to the bioaccessibility of some inorganic elements such as Mn and Pb. Our results are different than the ones of Li et al., as they showed that the bioaccessibility of inorganic elements increased when the particle size decreased (Li et al. 2020). TOC was correlated with a few inorganic elements (V, Ni, Ti, Mg among others) and anticorrelated with As and Mo. Wang et al. found also positive correlations between organic matter content and the bioaccessibility of some inorganic elements (Cd, Pb, and Zn) (Wang et al. 2023). The second data matrix used to carry out PCA was composed of the 15 PAHs, and the two components represented more than 60% of the variance (Figure SI-3b). According to PCA, the size fractions were again significant and the smaller fractions ($< 50 \mu\text{m}$ and $50\text{--}100 \mu\text{m}$) were anticorrelated with the biggest size fraction, but the size fractions were not correlated with the bioaccessibility of PAHs. Our results differ from that of Wang et al., since they observed that the bioaccessibility of the three- and four-ring PAHs (Ace, Flu, Phe, Fla, and Pyr) decreased with increasing size particles (Wang et al. 2013a). The TOC was significant and correlated with the bioaccessibility of Nap and anticorrelated with the bioaccessibility of Ace, Pyr, BaP, and DBahA (Figure SI-3b). TOC is considered as an important parameter for the bioaccessibility of organic compounds (Collins et al. 2015), yet in our study, TOC was only important for a couple PAHs. Raffy et al. mention that organic matter's aromaticity and polarity could be more important for the bioaccessibility of organic compounds than only its content (Raffy et al. 2018). Further work should be done to elucidate the influence of the physicochemical properties of dust on the bioaccessibility of dust PAHs and MMs.

Genotoxicity of bioaccessible fractions

Cytostasis

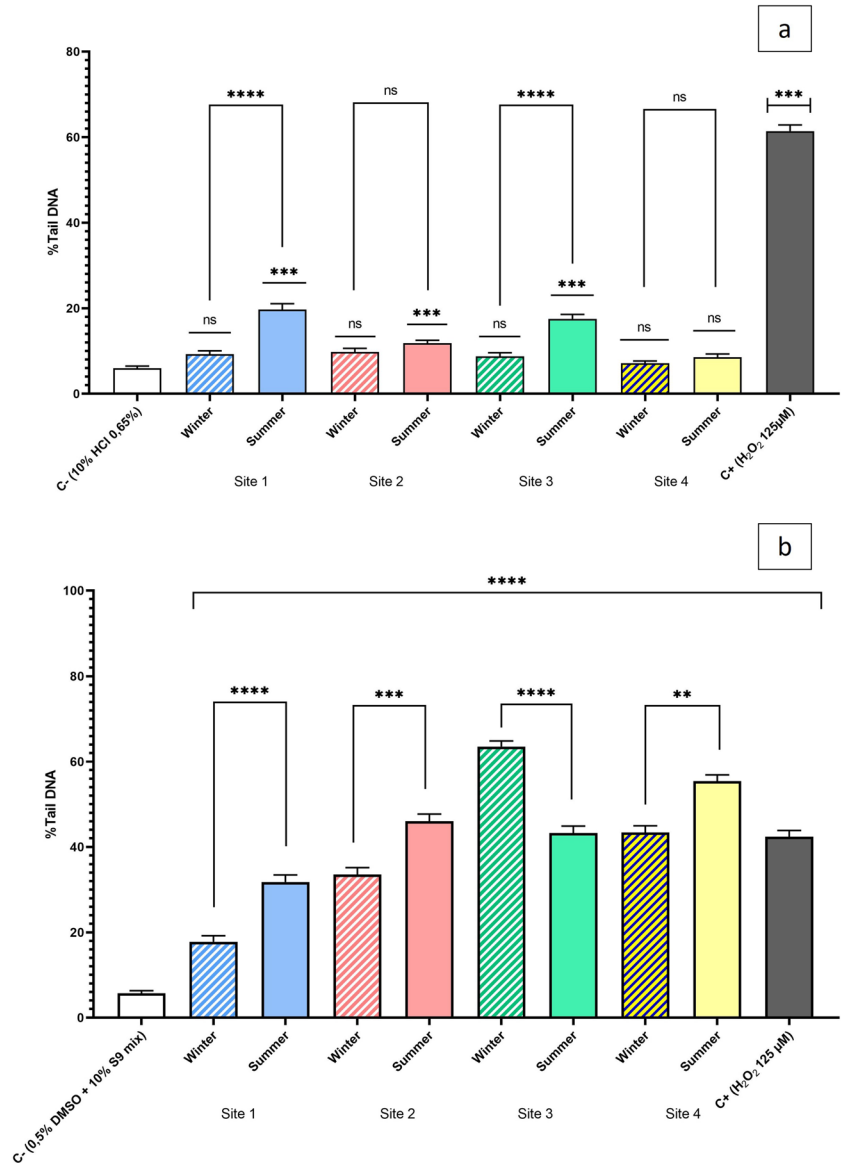
Inorganic oral fractions induced less than 5% of cytostasis. For organic oral fractions, cytostasis was assessed in the absence and in the presence of S9 mix, a metabolic activator since PAHs are carcinogenic once metabolized. Organic extracts induced between 20 and 60% of cytostasis without S9 mix, but less than 20% of cytostasis in presence of S9 mix. This implies that there must be contaminants other than PAHs

in the bioaccessible organic fractions, which can disrupt cell functions or dysregulates the cell cycle before metabolism. Suspected contaminant could be N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), used in rubber and tires and found in tire-wear particles, and 6PPD quinone, its derivative from a reaction between 6PPD and ozone, since both are ubiquitous (Chen et al. 2023). Studies have shown that 6PPD quinone was toxic to mice and different aquatic species (Fang et al. 2023; Chen et al. 2023). However, no in vitro studies have yet been carried out on human cells, despite a study showing that the general population, including children, has been exposed to these compounds (Du et al. 2022). These compounds were found along with PAHs in bioaccessible fractions of recycled tire crumb rubber, which are used in synthetic football fields or playgrounds (Armada et al. 2023).

DNA damage

For inorganic bioaccessible fractions, significant DNA damages were observed in the urban and industrial preschools in summer (Fig. 2a). For all preschools, the metallic ILCRs were above the acceptable threshold of 10^{-6} . Yet the winter samples and the site 4 samples did not induce higher DNA damage than the negative control. The highest DNA damage was observed for site 1 in summer (19.7%). Interestingly, despite a higher metallic ILCRs in the winter samples of sites 1 and 2, significant DNA damages were observed instead in the summer samples from both sites. At site 3, on the other hand, the metallic ILCR was higher in summer and, similarly, only the summer sample induced significant DNA damage. In another study on the genotoxicity of bioaccessible extracts of metal(loid)s from

Fig. 2 Comet assay on AGS cells, 4 h exposure to bioaccessible fractions: **a** bioaccessible inorganic fractions and **b** bioaccessible organic fractions. Site 1 = Port Saint Louis du Rhône preschool; site 2 = Ruffi preschool; site 3 = Longchamps preschool; site 4 = Trets preschool. ns, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

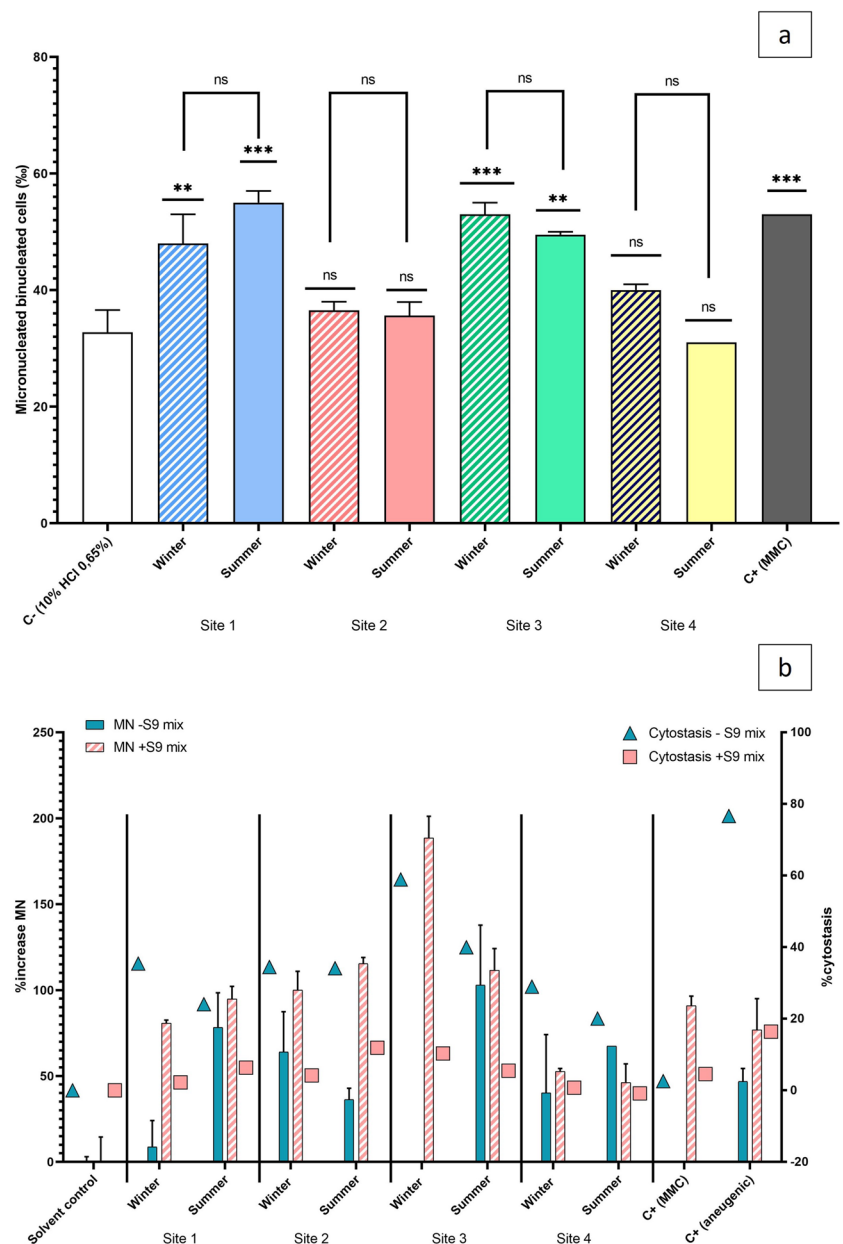


indoor dust, they observed significant increase in DNA damage for almost all their samples (Plumejeaud et al. 2016).

For organic extracts with S9 mix, all the samples induced a significant increase in DNA damage (see Fig. 2b). For sites 1, 2, and 4, there was a significant increase from winter to summer, whereas the opposite was observed in site 3. The winter sample of site 3 induced the most DNA damage (63.5%). The overall order of the preschools inducing the most DNA damage with the organic extract + S9 mix was the following: site 3 > site 4 > site 2 > site 1. Surprisingly, it is nearly the opposite from the PAH ILCR (site 1 > site 2 > site 3 > site 4). Sevastonya et al. also studied the effect of an organic extract of PAH from particulate matter samples (PM10) in three different European cities on HepG2 cells. They noticed a direct

correlation between BaP and the carcinogenic PAH concentrations and the number of DNA adducts formed in the cells (Sevastyanova et al. 2007). We did not observe such correlation for our data, hinting that PAHs alone are not sufficient to fully explain our results. In a study reporting the genotoxicity of an organic extract from PM2.5 on Beas-2B cells, the authors observed that the organic extract and the aliphatic, aromatic, and slightly polar fractions of the extract induced significant DNA damage at all the tested concentrations (Oh et al. 2011). Thus, aliphatic or slightly polar compounds could also be partly responsible for the genotoxicity observed in an extract. It is possible that compounds such as aliphatic hydrocarbons, plastic additives (phthalates, per- and polyfluoroalkyl substances, flame retardants), or aromatic amines like 6PPD

Fig. 3 CBMN assay on AGS cells, 4 h exposure to bioaccessible fractions: **a** micronuclei induced by the inorganic bioaccessible fractions; **b** cytostasis and increase in micronuclei induced by the organic bioaccessible fractions, with (+S9 mix) and without S9 mix (-S9 mix), aneugenic compounds: colchicine without S9 mix and BaP 7.5 μ M with S9 mix. Site 1 = Port Saint Louis du Rhône preschool; site 2 = Ruffi preschool; site 3 = Longchamps preschool; site 4 = Trets preschool. MMC, mitomycin C. ns, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



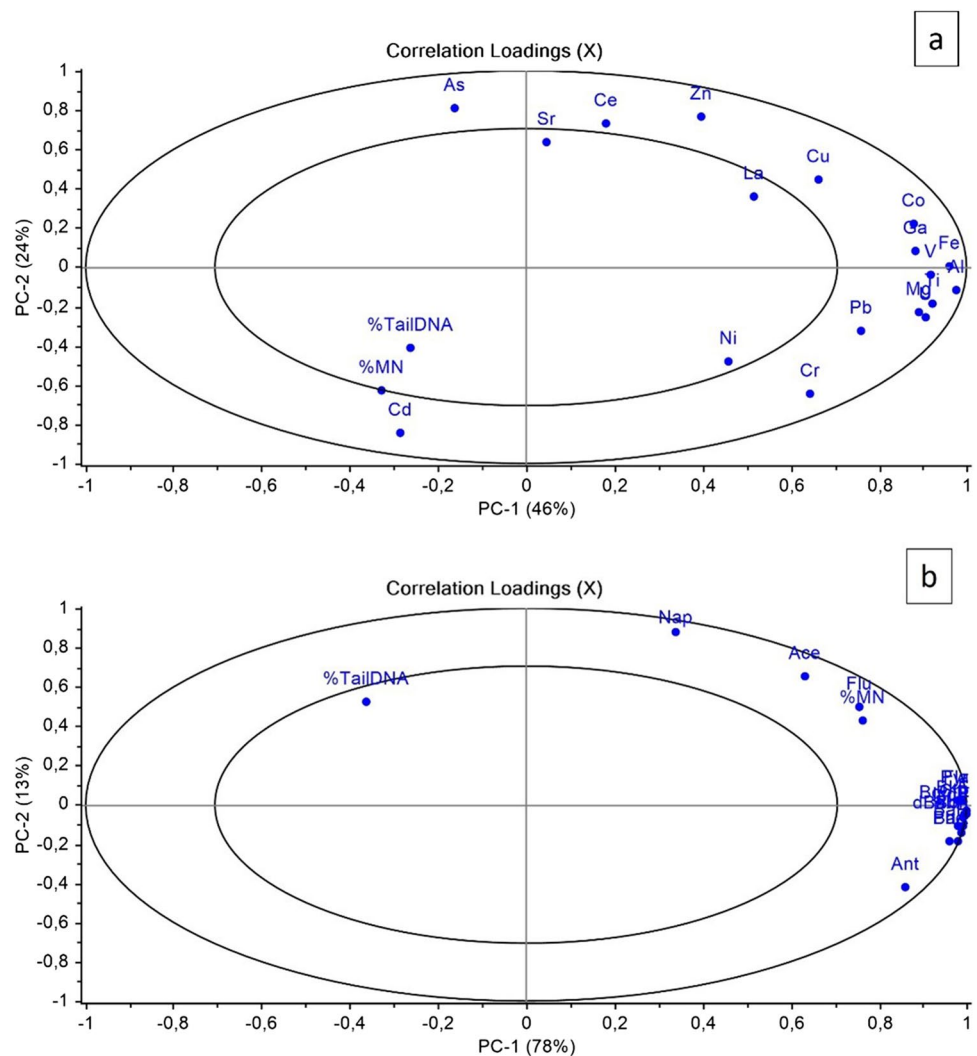
were also present in our own extracts (Sasaki et al. 2000; EFSA 2012; Temkin et al. 2020; Ojo et al. 2022; Chen et al. 2023). DNA damage could not be assessed for organic bioaccessible fractions in absence of S9 mix due to high cytostasis.

Chromosomal damage

For inorganic extracts (Fig. 3a), the samples from sites 1 and 3 induced a significant increase in the frequency of micronuclei at both seasons, somewhat similar to the results of the Comet assay (site 1 and site 3 both had the highest genotoxicity). But, it is different from the metallic ILCR (site 3 > site 2 > site 1 > site 4). In another study on the genotoxicity of gastric extracts of MMs from indoor dust, they observed an increase in chromosomal damage for all their samples (Plumejeaud et al. 2016). No seasonal differences between the samples were noticed, similarly to a Brazilian study on the genotoxicity of PM10 aqueous extracts from three sites at four different seasons (Palacio et al. 2016).

Nearly all the organic bioaccessible fractions induced a significant increase in chromosomal damage with S9 mix (Fig. 3b) except site 3 in winter, which could not be studied as the cytostasis was too high (58.9%). Overall, the chromosomal damages were similar in all the preschools, except for the site 4 (lower responses) and the winter sample of site 3 (the highest response, presence of plant residues in the collected outdoor dust). The fractions in the presence of S9 mix induced more chromosomal damage than in the absence of S9 mix, in accordance with the toxic mechanism driven by PAHs. Since chromosomal damage was also observed in the absence of S9 mix, then other contaminants in the organic fractions were expected to be genotoxic without requiring metabolic activation; such a mechanism may be observed in the presence of oxidated contaminants or contaminants with a heteroatom. As stated above, it is possible that aliphatic and/or slightly polar compounds are present in our organic extracts and partly cause the micronuclei formation, as reported on BEAS-2B cells exposed to aliphatic, aromatic, and slightly

Fig. 4 Principal component analysis with the bioaccessible content of contaminants and the genotoxicity of the bioaccessible fractions: **a** metal(loid)s in the inorganic bioaccessible fractions, DNA (%TailDNA) and chromosomal (%MN) damages, **b** PAHs in the organic bioaccessible fractions and DNA (%TailDNA) and chromosomal (%MN) damages



polar fractions of the organic extracts from PM_{2.5} (Oh et al. 2011). For the organic bioaccessible fractions, we noticed slight seasonal difference but only in site 3.

Linking chemical contents and health effects

Correlation between the contents of metal(loid)s and PAHs in the bioaccessible fractions and the in vitro genotoxicity

Principal component analysis (PCA) was performed to determine possible correlations between the pollutants quantified in the bioaccessible fractions of settled dust and their genotoxicity, in terms of DNA and chromosomal damage. The data matrices used to carry out the PCAs were either composed of 20 inorganic elements (Fig. 4a) or the 15 PAHs (Fig. 4b). For both matrices, the two first components represented at least 70% of the variance. Specifically, for metal(loid)s, PC-1 and PC-2 respectively represented 46% and 24% of the variance, whereas for PAHs, PC-1 and PC-2 respectively represented 78% and 13% of the variance.

For metal(loid)s, the chromosomal damage was significant and was mostly correlated to Cd, but anticorrelated to Zn (Fig. 4a). The DNA damage, while not significant, seemed also correlated to Cd. Other genotoxic metal(loid)s, like As and Pb, were not correlated to the chromosomal damage caused by the inorganic extracts. For PAHs, the sample from site 3 in winter was excluded from the analysis, because it had the highest genotoxicity and amongst the lowest concentration of PAHs, thus distorting the results from other samples. Moreover, this sample contained a lot of vegetation debris, which could have biased the results. The DNA damage was also not significant for the organic samples and seemed to not be correlated to PAHs (Fig. 4b). Conversely, chromosomal damage was correlated to most PAHs, especially to Flu.

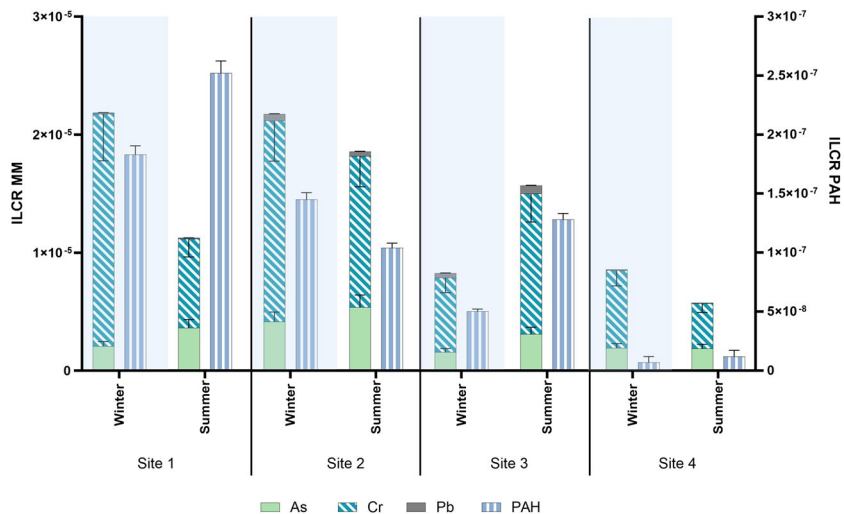
Thus, the genotoxic responses are explained in good part by the PAH concentrations in the organic fractions. There is

also a possibility that some interactions between the PAHs could influence the global genotoxicity, as it was observed in a recent study exploring toxicity of PAHs in mixtures (Castel et al. 2023b). These results are similar to previous ones (Bonney et al. 2017), studying the genotoxicity of PM_{2.5} extracts collected in the South of France. Similarly, they determined that chromosomal damage was not correlated to any of the measured metal(loid)s (Cd not included in their study) for their inorganic extracts, but chromosomal damage was correlated to most PAHs in their organic extracts.

Incremental lifetime cancer risk (ILCR) for children due to dust-bound PAHs and metal(loid) ingestion

Incremental lifetime cancer risks (ILCRs) were determined for carcinogenic elements (As, Cr, Pb) and for PAHs (Fig. 5; Table SI-4). The total ILCR (elements and PAHs) were above the 10^{-6} threshold, indicating there was a potential cancer risk linked to the ingestion of the settled dust at the preschools, but none was above the unacceptable limit of 10^{-4} . The lowest ILCR was found in site 4 in summer, and the highest was found in site 1 in winter. For most of the preschools, the cancer risks were higher in winter than in summer, except for site 3. Globally, the ILCRs calculated in the present work were higher than the previous ones estimated in soils in Armenia and in street dusts in Brazil, Spain, and Shanghai, China (Shi et al. 2011; Tepanosyan et al. 2017; Gabarrón et al. 2017; Franco et al. 2017). They were lower than the ones estimated in street dust in Serbia, Iran, and Hefei, China (Škrbić et al. 2019; Ghanavati et al. 2019; Shen et al. 2022). However, the latter ILCRs were performed either on PAHs or on metal(loid)s, conversely to our study summing the MM and PAH ILCR. In Hefei, China, Brazil, and Serbia, they only included PAHs. When compared to our own PAH ILCRs, they were 5 to 500 times higher than our average PAH ILCR, due either to higher

Fig. 5 Incremental life cancer risk (ILCR) estimated for both carcinogenic metal(loid)s and PAHs for outdoor dust samples. site 1 = Port Saint Louis du Rhône preschool; site 2 = Ruffi preschool; site 3 = Longchamps preschool; site 4 = Trets preschool



concentrations of PAHs in settled dusts and/or to a difference in the calculation methodology (Škrbić et al. 2019; Ghanavati et al. 2019; Shen et al. 2022). When looking at metal(loid)s, the ILCR in Armenia and Spain were 6 to 400 times lower than our metallic ILCR, due to much higher concentrations of metal(loid)s in our case (Tepanosyan et al. 2017; Gabarrón et al. 2017). The metallic ILCRs in Shanghai, China, were in the same range as the ILCRs of sites 3 and 4 (Shi et al. 2011).

Conclusion

Distinctive loads of PAHs (50.9 to 2267.3 ng g⁻¹) and metal(loid)s (12,430 to 38,941 µg g⁻¹) were measured in settled dusts, depending on the preschool. Specifically, PAHs and metal(loid)s contents were higher in urban and industrial contexts, likewise for the contents of carcinogenic PAHs and metal(loid)s. On the contrary, no clear trend in contaminant concentrations could be identified between winter and summer (heating, non-heating seasons).

A mean percentage of 20 to 40% of metal(loid)s was recovered in the bioaccessible inorganic fraction extracted with diluted HCl (at 0,65%), except in one preschool, where the bioaccessibility rose to 75%. The percentage of bioaccessible PAHs, extracted with butanol, ranged between 10 and 13%, but dropped down to 5% in one preschool. The genotoxicity of the organic and inorganic bioaccessible fractions was assessed separately on gastric cells for each preschool. The inorganic bioaccessible fraction induced little DNA (< 20% TailDNA) and chromosomal (30% increase in micronuclei) damages, unlike the organic bioaccessible fraction, which induced higher DNA (17–63% TailDNA) and chromosomal (88% increase in micronuclei) damages.

Good correlations were noticed between the observed genotoxicity and PAHs/metal(loid) contents, as the multivariate analyses (PCA) suggested that chromosomal damages were correlated to Cd in the inorganic bioaccessible fraction and to most PAHs in the organic bioaccessible fraction. This is in line with the remaining of these two chemical families among the priority toxics to study in environmental and health studies. Yet, some samples with low contents of metal(loid)s and PAHs induced higher genotoxicity than samples with higher contents of metal(loid)s and PAHs. Non-targeted contaminants co-extracted in the bioaccessible fractions were supposed to be partly responsible for the observed genotoxicity. Coupling of the comprehensive in vitro tool presented hereby with a non-targeted analysis could enable further investigation, especially in samples inducing higher-than-expected genotoxicity in relation to the compounds quantified.

Following regulatory risk assessment methodology, values of incremental (excess) lifetime cancer risk (ILCR) fell under the unacceptable limit of 1.10^{-4} , but were all above

the threshold of acceptable risk (10^{-6}), ranging from $2.8.10^5$ to $8.6.10^5$. Accordingly, a cancer risk potentially exists for children due to the ingestion of dust-bound PAHs and metal(loid)s in the studied environment. Of note, the ranking of preschools according to highest ILCR was not necessarily consistent with the ranking according to highest DNA or chromosomal damages. The comparison of experimental and regulatory approaches, such as the one described here, could help to advance the guidelines currently applied in environmental cancer risk assessment.

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Author contribution Rebecca Castel, Yves Noack, Thierry Orsière, and Laure Malleret contributed to the study conception and design. Material preparation, formal analysis, and investigation were carried out by Rebecca Castel, Virginie Tassistro, Stéphanie Lebarillier, and Yves Noack. Data treatment and visualization were performed by Rebecca Castel, Nathalie Dupuy, and Laure Malleret. Laure Malleret, Thierry Orsière, and Yves Noack contributed to funding acquisition, supervision, and administration of the project. The original draft of the manuscript was written by Rebecca Castel. Laure Malleret, Thierry Orsière, and Yves Noack participated in the final writing—review and editing of the manuscript. All authors read and approved the final version of the manuscript.

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Data availability All data generated and analyzed during our study are included in the main article or in supplementary information.

Declarations

Ethical approval The authors declare research did not involve human participants and/or animals. The submitted manuscript complies with all the ethical requirements of the journal following the rules of good scientific practice. No part of this paper has been previously published or is being considered for publication elsewhere. We report results from original completed research. No data, text, or theories by others are presented as if they were the author's own (“plagiarism”). We provide referenced information from studies from the authors' own works as background to the study carried out.

Consent to participate All authors agreed with the content that all gave explicit consent to participate and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out.

Consent for publication All authors agreed with the content and gave explicit consent to submit and publish and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out.

Competing interests The authors declare no competing interests.

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