Indoor air in a school: PAHs determination and untargeted approach for the chemical characterization by GC-orbitrap

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1 Introduction

Nowadays the approach of combining untargeted with targeted analysis for indoor air monitoring is increasingly frequent. Iit is fundamental to understand what other classes of compounds, in addition to those traditionally monitored, we are exposed [1,2]. It is well known from literature that indoor environments represent sinks of substances of different origin, including outdoor one, that accumulate inside [3,4]. Each environment can be considered unique, given that the parameters and factors that influence indoor pollution are often variable depending on the type of activity and action undertaken by users, the intensity of the air exchange, the ventilation system, the purification of the air supplied to the building, the temperature and relative humidity, the emissions from materials and equipment, the presence of external sources of emissions, as well as the formation of secondary pollutants [5–7]. Indoor air quality has a significant impact on the extent of human exposure to aerial pollutants, since people spend most of their time indoors. This aspect is particularly relevant to children, who are considered to be among the most vulnerable group in the population [8]. In fact, several works have studied pollutants in the particulate matter (PM) of schools [9–12]. This work is part of a larger project carried out in collaboration with the Italian Ministry of the Ecological Transition. The project involved monitoring indoor air quality in public (schools, hospitals, and universities) and private (homes) environments throughout the Italian national territory. In this paper, results from PM indoor/outdoor samplings of the case of study of a middle school in the city of Rome, Italy are reported. Schools are environments in which students and staff spend 5 to 8 hours a day and the sensitive target of students requires much more in-depth research. The aim of this study was to assess exposure to PAHs in the population (particularly the youngest) during winter (a polluted period of the year in Italy) and to better understand exposure to indoor pollutants through the analysis of untargeted compounds.

2 Materials and Methods

2.1 Sampling

An intensive measurement campaign was carried out in a middle school in the southeast area of Rome between November 21 and December 5, 2019. The site is located approximately 100 meter from a busy road and a large urban park. During the campaign period, the institute held 18 classes for a total of about 280 students. Parallel indoor and outdoor samplings were carried out on a daily basis. Indoor sampling was performed by collecting PM2.5 on Teflon membranes at a flow of 10 L/min in the rooms of the library on the ground floor (B) and in a corridor on the second floor (C). The windows were closed most of the time and the heaters turned on Monday to Friday 8 h a day. On a terrace on the first floor, outdoor PM10 samples (O) were collected at a flow rate of 38.3 L/min. The climate was characterized for the first half of the year by abundant rainfall which progressively decreased. The temperature was above the seasonal average.

2.2 Extraction, Clean-up

PM samples were grouped into weekdays (students and staff present) and weekend groups when the school was empty. The groups were extracted in an ultrasonic bath with dichloromethane:acetone (DCM:ACE) 4:1 (v/v) after the addition of a standard solution containing 50 ng of perdeuterated PAHs. The clean-up was performed with an alumina column eluting with solvents of increasing polarity, i.e. trimethylpentane (TMP) (non-polar fraction), TMP:DCM 3:2 (semipolar fraction containing PAHs), DCM:ACE 1:1 (polar fraction) [13]. The second fraction was analyzed for both the quantitative analysis of PAHs and for the qualitative evaluation of the unknown compounds.

Table 1: PM group details: groups, dates, place, denomination and average PM10 values (Avg PM) in $\mu g/m^3$.

Group	Dates	Library	Corridor	Outdoor	Avg PM
1	23-24/11/2019	B-1	C-1	O-1	16
2	25-29/11/2019	B-2	C-2	O-2	24
3	30/11-1/12/2019	B-3	C-3	O-3	42
4	2-6/12/2019	B-4	C-4	O-4	34
5	7-8/12/2019	B-5	C-5	O-5	62
6	9-13/12/2019	B-6	C-6	O-6	29
7	23-24/11/2019	B-7	C-7	O-7	49

2.3 PAHs analyses and unknown compound screening

The analyses were performed in high resolution mass spectrometry through GC-Orbitrap with He at a flow of 1ml/min. Quantities of PAHs were performed by collecting chromatograms in SIM mode with a resolution of 30000 using a Rxi-5ms, 30 m, 0.25 mmID and 0.25 μm. The calibration curves consist of 5 points with the native PAH congeners in the range 0.01 ng/μl-5 ng/μl and the labeled at 1 ng/μl [13].

The unknown compounds study was carried out on both indoor and outdoor 4, 5 and 6 groups. The analyses were performed in fullscan mode with a resolution of 60000, AGC 1e6 and scan range 50 to 450 m/z. A DB-XLB column (60 m; I.D. 0.25mm; Film: 0.25 μm) was used and an oven ramp from 90 °C to 320 °C maintaining the AUX temperatures at 280 °C were applied. Chromatograms were collected in two batches, one for the indoor and one for the outdoor and each included a relative blank. The batches were processed with the TraceFinder 4.1 software for both deconvolution and the unknown function. The signals of the chromatograms with a TIC intensity threshold equal to at least 1000 were deconvoluted with an Ion overlap window of 98%, RT aligning of maximum 3 s, SI Threshold of 500. Once the retention times were aligned, including those of the blank, each single identification was screened. The retention index function has been applied and all the libraries for the cross search have been included. Two levels of reliability were defined: a) High level: it includes only compounds with score> 90% in all the samples of a batch and absent in the blank. The score represents the correspondence between the deconvolved spectrum and the library search and it is expressed in percentage units. b) medium level: it includes all compounds with an average score (AVG Score%) > 90% and absent in the blank. The AVG score of a compound is the weighted average by intensity of a compound's match score across the batch. Each compound of a) and b) lists were included in the definitive list only after the study of mass spectra, online database and literature research.

3 Results

3.1 PAHs

The analyzed PAHs were comprised between acenaphthene and benzo(ghi)perylene. Figure 1 shows the PAH concentrations in the three locations investigated. One peak of concentration was observed in the 30/11-1/12 weekend (group ?) in all the sites, and that corresponding values were not included for the calculation of the average concentration. Table 2 reports the average concentration of the total PAHs, of the sum of the seven carcinogenic PAHs, considered by the European legislation, and of benzo(a)pyrene as chemical index of PAHs toxicity in the atmospheric particulate ((Directive 2004/107/CE). Also, the average diagnostic ratio B(a)P/B(e)P is reported.

Table 2: Average concentrations (ng/m^3) of the sum of PAHs concentrations from acenaphthene to benzo(ghi)perylene, of the sum of the seven carcinogenic PAH concentrations and of benzo(a)pyrene concentrations. Values of ratio between benzo(a)pyrene and benzo(e)pyrene. Peak value concentrations are excluded from the calculation.

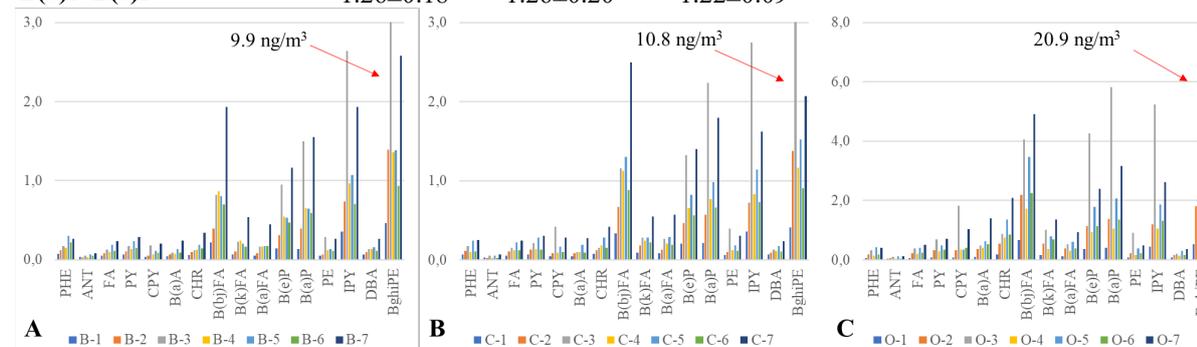|	Library (B)	Corridor (C)	Outdoor (O)	IN/OUT library	IN/OUT corridor
Total PAHs	6.0 ± 3.5	6.6 ± 3.7	13.3 ± 7.6	0.47 ± 0.09	0.52 ± 0.08
Cancerogenic PAHs	2.9 ± 1.9	2.3 ± 2.2	6.7 ± 4.1	0.45 ± 0.08	0.49 ± 0.09
B(a)P	0.66 ± 0.47	0.83 ± 0.53	1.57 ± 95	0.41 ± 0.11	0.53 ± 0.10
B(a)P/B(e)P	1.26±0.18	1.26±0.20	1.22±0.09		

Figure 2: PAHs profiles recorded during the measurement campaign: A) Library; B) Corridor C) Outdoor. PHE: phenanthrene; ANT: anthracene; FA: fluoranthene; PY: pyrene; CPY: cyclopentapyrene; B(a)A: benzo(a)anthracene; CHR: chrysene + triphenylene; B(bj)FA: benzo(b/j)fluoranthene; B(k)FA: benzo(k)fluoranthene; B(a)FA: benzo(a)fluoranthene; B(e)P: benzo(e)pyrene; B(a)P: benzo(a)pyrene; PE: perylene; IPY: indenopyrene; DBA: dibenzo(a,h)anthracene; BghiPE: benzo(ghi)perylene. The compounds below LOD are not reported.

3.2 Unknown screening

Once processed for deconvolution and unknown function, 2479 compounds were identified in the Indoor batch, and the outdoor batch counted 5623 compounds. Tables 2 and 3 show high and medium level of identification reliability, respectively.

Table 2: High level of identification reliability. Compounds common to the whole indoor batch. Compounds common to both indoor and outdoor batches are written in bold

Compound	CAS	Reference
1-(1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone	70987-81-4	
1,2-Benzenedicarboxylic acid, diphenyl ester	84-62-8	[14, 15]
2,4-Di-tert-butylphenol	96-76-4	[14]
2,6-Dichloro-4-(1,1-dimethylethyl)phenol		
2,6-Di-t-butyl-4-methylphenol acetate(ester)	29311-34-0	
2-Methoxy-4-vinylphenol	7786-61-0	[14,15]
4-tert-Butyltoluene	98-51-1	[15]
7-Hexadecene, (Z)-	35507-09-6	[16]
Benzene, 1,2,4,5-tetramethyl-	95-93-2	
Benzene, 1-ethyl-4-methyl-	622-96-8	[14]
Benzene, 1-methyl-3-propyl-	1074-43-7	
Hexanedioic acid, mono(2-ethylhexyl)ester	4337-65-9	
Octocrylene	6197-30-4	[17]
p-Hexylbenzaldehyde diethyl acetal	89511-01-3	
Phthalic acid, 6-methylhept-2-yl nonyl ester		
Phthalic acid, nonyl tridec-2-yn-1-yl ester		
Tonalid	21145-77-7	[15]
Undecane, 2,4-dimethyl-	17312-80-0	

Table 3: Medium level of identification reliability. Compounds common to the whole indoor batch. Compounds common to both indoor and outdoor batches are written in bold.

Compound	CAS	Reference
Benzeneethanol, .beta.-ethenyl-	6052-63-7	
Quinoline, 1,2-dihydro-2,2,4-trimethyl-	147-47-7	[15]
Phosphonic acid, [(4-carboxyphenyl)amino]-phenyl-methyl, dimethyl ester	37753-62-1	
Glutaric acid, 3-chlorophenyl 2,4-dimethylpent-3-yl ester		
Hexacosanoic acid, methyl ester	5802-82-4	

4 Discussion

4.1 PAHs

An increase in PAH concentrations is observed during the measurement campaign. This coincides with an increase in his outdoor PM10 concentration (see Table 1) and a decrease in precipitations. At the weekend of 11/30 - 12/12/2019 (group 3), there was a concentration peak accountable to an unidentified local event, recorded both indoor and outdoor. During this event, the greatest increase was observed in the five-ring PAHs generally associated with the combustion of petrogenic products [18]. The highest concentrations were measured outdoor, which suggests that the source was external, as expected in the lack of significant indoor sources.

Although the school was closed, the pollutants still penetrated inside as seen in Figure 1 but the indoor/outdoor ratios were slightly lower than the average of the other periods. In fact, the average indoor/outdoor ratio for the seven carcinogenic PAHs evaluated in the indoor location was about 25% lower than in the remaining part of the sampling. On the other hand, considering the average values of PAHs, excluding the peak weekend, benzo(a)pyrene only exceeded the limit concentration of 1 ng/m^3 outdoor. Over the entire period, the limit was also exceeded within the corridor (1.1 ng/m^3). B(a)P/B(e)P diagnostic ratio values greater than one indicate that the sources were nearby. All the diagnostic reports, however, did not reveal significant differences between indoor and outdoor, confirming the presence of a single source, most likely linked to vehicular traffic.

4.2 Unknown screening

Relevant compounds were identified, such as diphenyl phthalate classified by companies according to CLP Regulation criteria as very toxic to aquatic life, eye and skin irritant, and respiratory and skin sensitizers. Evidence has traced this compound to packaging, so the source could be attributable to polymeric plastic materials [14]. The 2,4-di-tert-butylphenol, a substance classified according to CLP criteria as very toxic to aquatic life with long-lasting

effects, is added to the list. It also causes serious eye damage and skin irritation and it is under assessment as endocrine disrupting substance under REACH Regulation. This product is found in fuel and is used industrially in packaging. The release to the environment can occur, for example, from coolants in refrigerators, electric oil heaters or, outdoors, such as hydraulic fluids in automobile suspensions or lubricants in engine oil [15].

In addition to the compounds emitted by polymeric plastic materials, substances associated with fragrances were also identified: 2-Methoxy-4-vinylphenol is a tobacco and food additive, and it is also found in packaging and toys. According to the classification provided by companies under CLP this substance causes severe skin burns and eye damage and may cause respiratory irritation. On the other hand, considering personal care, tonalide [19] and octacrylene used as a UV shield in sunscreens [1] were identified.

5 Conclusions

The reported results showed that in the peak periods of pollution, the concentrations of benzo(a)pyrene in the ambient air of Rome exceed the value of 1 ng/m^3, whereas they are lower within the school, with IN/OUT ratios for the 7 carcinogenic PAHs considered by European legislation equal to approximately 0.45.

Through the GC-Orbitrap, it was possible to carry out the first qualitative analysis of non-targeted compounds in schools (according to our knowledge).

The study highlighted the presence in indoor of compounds recognized as endocrine disruptors, irritants, allergens or compounds deemed harmful to the environment. The main classes were products of combustion, natural products, personal care, drugs, household cleaning products, solvents, paint components and adhesives. Furthermore, the library has highlighted the presence of a greater variety of chemical species in the suspended dust in the air, probably linked to the presence of the paper material and the wooden cabinets that contained it. It would be advisable to extend research on indoor pollutants in the future. The next step will be to validate the results obtained, through the use of analytical standards that will allow the certain and quantitative identification of the substances found [20].

6 References

1. Salthammer, T., 2020. Emerging indoor pollutants. Int. J. Hyg. Environ. Health 224, 113423.
2. Nehr, S., Hösen, E., Tanabe, S. ichi, 2017. Emerging developments in the standardized chemical characterization of indoor air quality. Environ. Int. 98, 233–237. https://doi.org/10.1016/j.envint.2016.09.020
3. Plaisance, H., Mocho, P., Desauziers, V., 2020. Comparative analysis of formaldehyde and toluene sorption on indoor floorings and consequence on Indoor Air Quality. Indoor Air 30, 1256–1267.
4. Tichenor, B.A., Sparks, L.A., White, J.B., Jackson, M.D., 1990. Evaluating Sources of Indoor Air Pollution. J. Air Waste Manag. Assoc. 40, 487–492.
5. Śmiełowska, M., Marć, M., Zabiegała, B., 2017. Indoor air quality in public utility environments—a review. Environ. Sci. Pollut. Res. 24, 11166–11176.
6. Ye, W., Zhang, X., Gao, J., Cao, G., Zhou, X., Su, X., 2017. Indoor air pollutants, ventilation rate determinants and potential control strategies in Chinese dwellings: A literature review. Sci. Total Environ. 586, 696–729.
7. Wang, B., Zhao, Y., Lan, Z., Yao, Y., Wang, L., Sun, H., 2016. Sampling methods of emerging organic contaminants in indoor air. Trends Environ. Anal. Chem. 12, 13–22.
8. De Gennaro, G., Dambruoso, P.R., Loiotile, A.D., Di Gilio, A., Giungato, P., Tutino, M., Marzocca, A., Mazzone, A., Palmisani, J., Porcelli, F., 2014. Indoor air quality in schools. Environ. Chem. Lett. 12, 467–482.
9. Slezakova, K., Oliveira, M., Madureira, J., Fernandes, E. de O., Delerue-Matos, C., Morais, S., Pereira, M. do C., 2017. Polycyclic aromatic hydrocarbons (PAH) in Portuguese educational settings: a comparison between preschools and elementary schools. J. Toxicol. Environ. Heal. - Part A Curr. Issues 80, 630–640.
10. Krugly, E., Martuzevicius, D., Sidaraviciute, R., Ciuzas, D., Prasauskas, T., Kauneliene, V., Stasiulaitiene, I., Kliucininkas, L., 2014. Characterization of particulate and vapor phase polycyclic aromatic hydrocarbons in indoor and outdoor air of primary schools. Atmos. Environ. 82, 298–306.
11. Sivanantham, S., Dassonville, C., Grégoire, A., Malingre, L., Ramalho, O., Mandin, C., 2021. Coexposure to indoor pollutants in French schools and associations with building characteristics. Energy Build. 252, 111424.
12. Seo, S.H., Jung, K.S., Park, M.K., Kwon, H.O., Choi, S.D., 2022. Indoor air pollution of polycyclic aromatic hydrocarbons emitted by computers. Build. Environ. 218, 109107.
13. Balducci, C., Cecinato, A., Paolini, V., Guerriero, E., Perilli, M., Romagnoli, P., Tortorella, C., Iacobellis, S., Giove, A., Febo, A., 2018. Volatilization and oxidative artifacts of PM bound PAHs collected at low volume sampling (1): Laboratory and field evaluation. Chemosphere 200, 106–115.
14. ECHA, 2022. [WWW Document]. URL https://echa.europa.eu/it/substance-information/-/substanceinfo/ (accessed 6.29.22).
15. PubChem, (2022). https://pubchem.ncbi.nlm.nih.gov/compound/ (accessed June 29, 2022).
16. Syamsudin, T.S., Hafsah, H., Iriawati, I., 2019. Data set on volatile compound of coffee flowers at different annual rainfall. Data Br. 26, 104418.
17. comptox.epa.gov, 2022. Octocrylene [WWW Document]. URL https://comptox.epa.gov/dashboard/chemical/details/DTXSID9025299 (accessed 6.29.22).

18. Alves, C.A., 2008. Characterisation of solvent extractable organic constituents in atmospheric particulate matter: An overview. An. Acad. Bras. Cienc. 80, 21–82.

19. Lamas, J.P., Sanchez-Prado, L., Garcia-Jares, C., Llompart, M., 2010. Determination of fragrance allergens in indoor air by active sampling followed by ultrasound-assisted solvent extraction and gas chromatography-mass spectrometry. J. Chromatogr. A 1217, 1882–1890.

20. Rochat, B., 2017. Proposed Confidence Scale and ID Score in the Identification of Known-Unknown Compounds Using High Resolution MS Data. J. Am. Soc. Mass Spectrom. 28, 709–723.