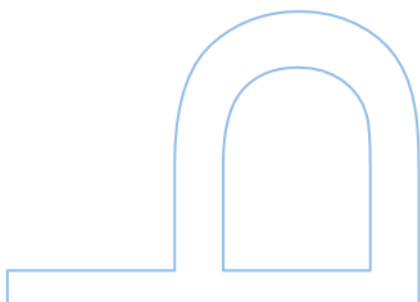




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Evaluation of firefighters' occupational exposure
and associated early health risks

Gabriel Guedes de Sousa



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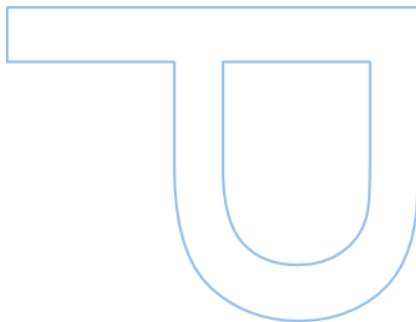
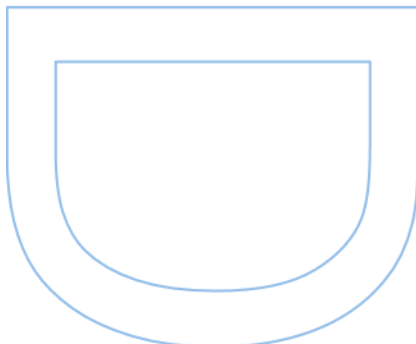
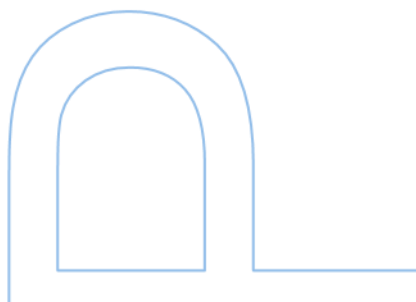


U. PORTO



Evaluation of firefighters' occupational exposure and associated early health risks

Gabriel Guedes de Sousa
Doctoral Program in Sustainable Chemistry
Department of Chemistry and Biochemistry
Faculty of Sciences of the University of Porto
2026



Evaluation of firefighters' occupational exposure and associated early health risks

Gabriel Guedes de Sousa

Thesis carried out as part of the Doctoral Program in
Sustainable Chemistry
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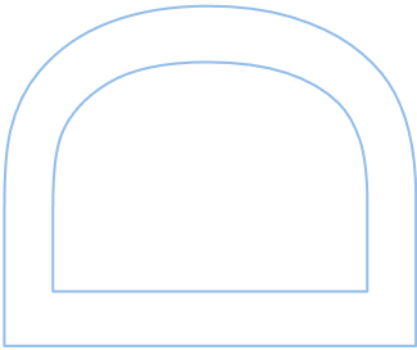
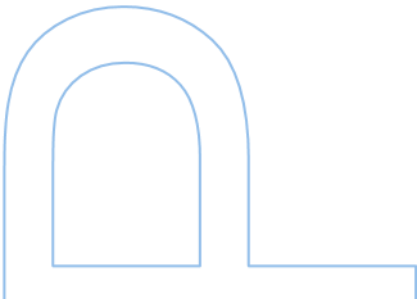
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All the corrections determined by the jury, and only those, were made.

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Resumo

O aumento da frequência e intensidade de incêndios florestais devido ao aquecimento global coloca crescentes desafios ambientais e de saúde pública. Em países do sul da Europa, como Portugal, já propensos a um maior risco de incêndio, as alterações climáticas estão a agravar este fenómeno. Estes incêndios libertam grandes quantidades de matéria particulada (MP) e poluentes tóxicos, incluindo metaloides e compostos orgânicos voláteis (COVs) e semivoláteis, como os hidrocarbonetos aromáticos policíclicos (HAPs), com riscos para os ecossistemas e a saúde humana. Os bombeiros, como profissionais de primeira linha, enfrentam uma maior exposição ocupacional a estas emissões, não só durante o combate ativo aos incêndios, mas também nos quartéis. Compreender a exposição dos bombeiros durante o combate aos incêndios e em ambiente de quartel e os principais riscos para a saúde é crucial para desenvolver medidas de proteção eficazes e mitigar os riscos para a saúde. Consequentemente, os objetivos desta tese foram (i) avaliar a exposição ocupacional de bombeiros portugueses através da amostragem do ar em diferentes cenários de combate a incêndios, para compreender a sua composição; (ii) recolher amostras biológicas para realizar estudos de biomonitorização e bioacessibilidade; (iii) e propor recomendações e medidas preventivas para promover a saúde e segurança dos bombeiros.

Primeiro, investigaram-se as propriedades gravimétricas, morfológicas e químicas das partículas finas ($PM_{2.5}$, PM_{156nm}) e ultrafinas ($PM_{54.5nm}$, $PM_{30.8nm}$) nos quartéis de bombeiros e durante dois tipos de incêndios: incêndios estruturais controlados (CSF) e queimadas prescritas (PFF). Nos quartéis, os níveis cumulativos de PM ($55.75\text{--}191.0\text{ }\mu\text{g}/\text{m}^3$) estavam abaixo dos limites de exposição ocupacional, mas foram detetadas concentrações mais altas nas salas de armazenamento dos equipamentos de proteção individual e garagens, com Cu, Zn e Cr a serem os metais predominantes. Elementos carcinogénicos, como Ni, Cd, Sb e Pb, foram identificados tanto nas PM finas como nas ultrafinas. Durante os cenários de incêndio ativo, os níveis de PM foram superiores nos CSF ($11.13\text{ mg}/\text{m}^3$) em comparação com as PFF ($1.232\text{ mg}/\text{m}^3$), sendo a $PM_{2.5}$ a fração predominante. A análise morfológica revelou a presença de matéria orgânica, compostos cristalinos e partículas ricas em metais, incluindo $PbCl_2$ e PbO nos CSF, e partículas de aço inoxidável nas PFF. O mapeamento elementar e a Espectrometria de Massa com Plasma Acoplado Indutivamente (ICP-MS) confirmaram a predominância de Zn, Cr, Cu e Ni, com metais carcinogénicos a representar 28–33% do conteúdo metálico total. Estes resultados sublinham a heterogeneidade das emissões dos incêndios e destacam a necessidade de mais investigação de modo a estabelecer diretrizes internacionais para a exposição a partículas ultrafinas.

Em seguida, foram realizados estudos de biomonitorização que avaliaram os efeitos das emissões dos incêndios e do consumo de tabaco na saliva dos bombeiros, caracterizando o perfil desta matriz em metais (e.g., Pb, Cd, Co, Sb) e HAPs (e.g., naftaleno, fenantreno, pireno). Os bombeiros expostos a emissões de incêndios apresentaram níveis de Pb até 45 vezes superiores após o incêndio, com os fumadores a apresentarem níveis ainda mais elevados. A exposição combinada a emissões de incêndios e hábitos tabágicos aumentou ainda mais a exposição a elementos tóxicos como o Cd e Ba. Os níveis de metais na saliva correlacionados com os anos de serviço, sugerem que a exposição crónica contribui para a bioacumulação. Além disso, as concentrações de metais na saliva estão positivamente correlacionadas com o aumento do índice de massa corporal (IMC). Os ensaios de toxicidade *in vitro* não evidenciaram citotoxicidade imediata. Contudo, as potenciais implicações a longo prazo para a saúde continuam a suscitar preocupação, particularmente devido ao risco de absorção sistémica. Os estudos destacaram a utilidade da saliva como ferramenta de biomonitorização, mas notaram limitações, como a variabilidade inter- e intra-individual e a necessidade de amostragens com um maior número de participantes. Estudos futuros deverão focar-se na avaliação de estratégias de prevenção da exposição ocupacional e promoção da higiene e segurança no trabalho, na expansão da biomonitorização a bombeiros e outras profissões de elevado risco ocupacional, e na análise de matrizes biológicas adicionais. A biomonitorização dérmica, por exemplo, deve ser explorada mais aprofundadamente, uma vez que estudos têm demonstrado que bombeiros e outros profissionais em situação precária estão expostos a poluentes como metais e HAPs através da pele. Alguns destes poluentes conseguem ser absorvidos/difundidos pela pele, sendo depois metabolizados e disseminados pelo organismo, podendo causar danos a proteínas e ao ADN. Nos bombeiros, as regiões do corpo mais afetadas são o rosto, os pulsos e o pescoço, as quais correspondem às zonas de intersecção dos EPIs. Além disso, procedimentos inadequados/insuficientes de limpeza dos EPI agravam a bioacumulação das emissões dos incêndios devido ao contacto com a pele. Existe, no entanto, uma grande dissimilaridade no que respeita à amostragem dérmica e aos métodos analíticos, pelo que futuras investigações devem centrar-se no aperfeiçoamento dessa vertente.

Este trabalho destaca a complexa interação entre as emissões dos incêndios, a exposição ocupacional e a absorção biológica nos bombeiros. A caracterização das PM sublinha a sua composição em poluentes gerados nos incêndios, enquanto a biomonitorização da saliva e contacto dérmico confirma a exposição ocupacional e fornece informações críticas sobre as vias de exposição, dinâmicas e os riscos para a saúde. Enfrentar estes desafios exige esforços interdisciplinares para reduzir a exposição ocupacional, refinar protocolos de proteção e segurança, reduzir a presença de emissões dos incêndios dentro dos quartéis de

bombeiros e expandir estratégias de biomonitorização para proteger a saúde dos bombeiros numa era de riscos crescentes de incêndio.

Palavras-chave: Emissões de incêndios; partículas ultrafinas; metais e metaloides; hidrocarbonetos aromáticos policíclicos; biomonitorização; saliva; pele;

Abstract

The increasing frequency and intensity of wildfires due to global warming pose significant environmental and public health challenges. In countries from southern Europe such as Portugal, which are already susceptible to increased risk of fire, climate change is exacerbating this phenomenon. These fires release vast amounts of particulate matter (PM) and toxic pollutants, including metal(loid)s, and semi- and volatile organic compounds (VOCs) such as polycyclic aromatic hydrocarbons (PAHs), with risks to ecosystems and human health. Firefighters, as first responders, face heightened occupational exposure to these hazardous emissions, not only during active firefighting but also at fire stations. Understanding the occupational exposure of firefighters during firefighting activities and at fire station and associated health risks is critical for developing effective safety measures and mitigating health risks. Consequently, the objectives of this thesis were (i) to assess the occupational exposure of Portuguese firefighters through air sampling in different firefighting scenarios, in order to understand its composition; (ii) to collect biological samples to carry out biomonitoring and bioaccessibility studies; (iii) and to propose recommendations and preventive measures to promote the health and safety of firefighters.

Firstly, the team investigated the gravimetric, morphological, and chemical properties of fine ($PM_{2.5}$, PM_{156nm}) and ultrafine ($PM_{54.5nm}$, $PM_{30.8nm}$) PM in fire stations and during two types of fires: controlled structural fires (CSF) and prescribed forest fires (PFF). In fire stations, cumulative PM levels ($55.75\text{--}191.0\text{ }\mu\text{g}/\text{m}^3$) were below occupational exposure limits, but elevated concentrations were detected in personal protective equipment storage rooms and garages, with Cu, Zn, and Cr being the predominant metals. Notably, carcinogenic elements such as Ni, Cd, Sb, and Pb were identified in both fine and ultrafine PM. During active fire scenarios, PM levels were significantly higher in CSF ($11.13\text{ mg}/\text{m}^3$) compared to PFF ($1.232\text{ mg}/\text{m}^3$), with $PM_{2.5}$ constituting most of the emissions. Morphological analysis revealed organic matter, crystalline compounds, and metal-rich particles, including $PbCl_2$ and PbO in CSF, and stainless-steel particles in PFF. Elemental mapping and Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) confirmed the prevalence of Zn, Cr, Cu, and Ni, with carcinogenic metals accounting for 28–33% of total metal content. These findings underscore the heterogeneity of fire emissions and highlight the need for further research to establish international guidelines for ultrafine PM exposure.

Then, biomonitoring studies assessed the effects of fire emissions and tobacco consumption on firefighters' saliva, revealing elevated concentrations of metals (e.g., Pb, Cd, Co, Sb) and PAHs (e.g., naphthalene, phenanthrene, pyrene). Firefighters exposed to fire emissions

exhibited up to 45 times higher Pb levels post-fire, with smokers showing exacerbated accumulation. Combined exposure to fire emissions and tobacco further increased toxic elements like Cd and Ba. Salivary metal levels correlated with years of service suggests chronic exposure contributes to bioaccumulation; also, salivary metals' concentrations are positively correlated with increasing BMI. *In vitro* toxicity assays indicated no immediate cell damage, but long-term health implications remain a concern due to the potential for systemic absorption. The studies emphasized saliva's utility as a biomonitoring tool but noted limitations such as inter- and intra-individual variability and the need for larger sample sizes. Future research should explore protective measures, extend biomonitoring in firefighters and other high-risk professions, and explore other matrices. Dermal biomonitoring, for example, should be explored further since studies have shown that firefighters and other precarious professionals are exposed to pollutants such as metals and PAHs through the skin. Some of these pollutants are able to be absorbed/diffused by skin barriers, and then metabolized, and spread through the system, potentially causing protein/DNA damage. In firefighters, the most affected body regions are the face, wrists, and neck, which correspond to the intersections of the PPE. Also, inadequate/insufficient cleaning procedures of the PPE further exacerbate bioaccumulation due to contact with the skin. There is, however, a lot of dissimilarity concerning dermal sampling and analytical methods, so future research should focus on refining that edge.

This work highlights the complex interaction between fire emissions, occupational exposure, and biological uptake in firefighters. The characterization of PM underscores its hazardous composition, while salivary and dermal biomonitoring provides critical insights into the route of exposure, dynamics and health risks. Addressing these challenges requires interdisciplinary efforts to reduce occupational exposure, refine protection and safety protocols, reduce the presence of fire emissions within fire stations, and expand biomonitoring strategies to protect the health of firefighters in an era of increasing fire risks.

Keywords: Fire emissions; ultrafine particles; metal(loid)s; polycyclic aromatic hydrocarbons; biomonitoring; saliva, skin

List of Publications

Scientific articles in international peer reviewed journals

- 1) Gabriel Sousa, Joana Teixeira, Cristina Delerue-Matos, Bruno Sarmento, Simone Morais, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, 2022. "Exposure to PAHs during firefighting activities: a review on skin levels, *in vitro/in vivo* bioaccessibility, and health risks" *International Journal of Environmental Research and Public Health*, 19(19):12677. <https://doi.org/10.3390/ijerph191912677>.
- 2) Joana Teixeira, Gabriel Sousa, Simone Morais, Cristina Delerue-Matos, and Marta Oliveira, 2023. "Assessment of coarse, fine, and ultrafine particulate matter at different microenvironments of fire stations". *Chemosphere*, 335, 139005. <https://doi.org/10.1016/j.chemosphere.2023.139005>.
- 3) Gabriel Sousa, Joana Teixeira, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Simone Morais, Xianyu Wang, Francisca Rodrigues, Maria Freitas, and Marta Oliveira, 2024. "Gravimetric, morphological, and chemical characterization of fine and ultrafine particulate matter inside fire stations", *Building and Environment*, 255, 111403. <https://doi.org/10.1016/j.buildenv.2024.111403>
- 4) Gabriel Sousa, Joana Teixeira, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, 2024 "Characterization of firefighters' exposure to breathable coarse, fine, and ultrafine PM, PM-bound polycyclic aromatic hydrocarbons and metals during prescribed fires". *Toxics*, 12(6), 422. <https://doi.org/10.3390/toxics12060422>
- 5) Gabriel Sousa, Joana Teixeira, Maria João Bessa, Cristina Delerue-Matos, Bruno Sarmento, Marta Oliveira, and Francisca Rodrigues, 2025. "Impact of fire emissions and tobacco consumption on the salivary content of metal(loid)s and PAHs: Exposure levels and assessment of oral and intestinal *in vitro* toxicity". *Biological Trace Element Research*. <https://doi.org/10.1007/s12011-025-04868-8>.
- 6) Gabriel Sousa, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Simone Morais, Xianyu Wang, Francisca Rodrigues, Maria Freitas, and Marta Oliveira, 2026. "Morphological and Elemental Characterization of Fine and Ultrafine Particulate Matter Generated from Fires". *Environmental Research*, 288(2), 123281. <https://doi.org/10.1016/j.envres.2025.123281>

7) Gabriel Sousa, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira. Unveiling Exposure Pathways at the Oral Cavity during Firefighting Work: Salivary Metal(loid)s and Polycyclic Aromatic Hydrocarbons, Occupational and Biological Determinants, and Exploratory Health Risks. Article accepted and waiting publication ([10.1016/j.envpol.2026.129288](https://doi.org/10.1016/j.envpol.2026.129288))

Book chapters (indexed in Scopus)

1) Gabriel Sousa, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, Chapter 1 - An introduction to occupational exposure. In F. Rodrigues, S. Morais, M. Oliveira (Eds.) In An Essential Guide to Occupational Exposure, F. Rodrigues, S. Morais, M. Oliveira (Eds.), Series Safety and Risk in Society, Nova Science Publishers, Inc., New York, USA, June 2022. ISBN: 978-1-68507-819-5. <https://doi.org/10.52305/VGMC6738>.

2) Gabriel Sousa, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, Marta Oliveira, "Potential of saliva for biomonitoring of occupational exposure: collection of evidence from the literature". In: PM Arezes, et al. (Eds.) *Occupational and Environmental Safety and Health IV, Studies in Systems, Decision and Control*, Cham, Switzerland: Springer Nature Switzerland, 2022. https://doi.org/10.1007/978-3-031-12547-8_47.

3) Gabriel Sousa, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, "Characterization of metal content in the saliva of firefighters: a preliminary study". In: PM Arezes, et al. (Eds.) *Occupational and Environmental Safety and Health V, Studies in Systems, Decision and Control*, Cham, Switzerland: Springer Nature Switzerland, 2023. [10.1007/978-3-031-38277-2_25](https://doi.org/10.1007/978-3-031-38277-2_25)

4) Gabriel Sousa, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, "A review on skin metal concentrations in occupational environments". In: PM Arezes, et al. (Eds.) *Occupational and Environmental Safety and Health VII, Studies in Systems, Decision and Control*, Cham, Switzerland: Springer Nature Switzerland, 2025. Accepted.

List of Communications

Oral communications in (inter)national conferences

1) Gabriel Sousa, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira. "Potential of Saliva for Biomonitoring of Occupational Exposure: Collection of

Evidence from the Literature". *International Symposium on Occupational Safety and Hygiene 2022* (SHO 2022), September 8th 2022, Porto, Portugal.

2) Gabriel Sousa, Maria Freitas, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, "Morphologic and elemental characterization of particulate matter from different firefighters' working environments". *Congresso Doutoral em Engenharia (DCE 2023)*, June 15-16th 2023, Porto, Portugal.

3) Gabriel Sousa, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, "Characterization of metal content in the saliva of firefighters: a preliminary study". *International Symposium on Occupational Safety and Hygiene 2023* (SHO 2023), July 20-21st 2023, Porto, Portugal.

4) Gabriel Sousa, Joana Teixeira, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, "Avaliação da exposição ocupacional de bombeiros portugueses a metais e PAHs por ingestão, através da saliva" Online workshop on *Exposição ocupacional no combate a incêndios e efeitos precoces na saúde das forças operacionais*, March 3rd 2025.

Poster communications in (inter)national conferences

1) Gabriel Sousa, Joana Teixeira, Cristina Delerue-Matos, Simone Morais, and Marta Oliveira. "Firefighters' personal exposure to airborne polycyclic aromatic hydrocarbons during controlled forest fires". *XXVI Encontro Galego Português de Química*, November 16 – 18th 2022. Galiza, Spain.

2) Gabriel Sousa, Joana Teixeira, Cristina Delerue-Matos, Bruno Sarmiento, Simone Morais, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira, "Contamination of firefighters' skin during firefighting activities and associated health risks". *International Wildfire Fire Conference*, May 16 – 19th 2023. Porto, Portugal.

3) Gabriel Sousa, Joana Teixeira, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira. "Assessment of firefighters' occupational exposure to metals on the oral cavity". *2024 International Firefighter Cancer Symposium*, February 22 – 23rd 2024, Miami, Florida.

4) Gabriel Sousa, Joana Teixeira, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira. "Composition of PM_{2.5} and PM_{0.1} on metals and polycyclic aromatic hydrocarbons released during prescribed fires". *2024 International Firefighter Cancer Symposium*, February 22 – 23rd 2024, Miami, Florida.

5) Gabriel Sousa, Joana Teixeira, Maria João Bessa, Cristina Delerue-Matos, Bruno Sarmiento, Francisca Rodrigues, Marta Oliveira. "Saliva as a promising matrix for biomonitorization of firefighters' occupational exposure to polycyclic aromatic hydrocarbons". *8th International Conference in Food Digestion*, April 9-11th 2024, Porto, Portugal.

6) Gabriel Sousa, Joana Teixeira, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Alice Santos-Silva, Francisca Rodrigues, and Marta Oliveira. "Characterization of wildland firefighters' exposure to coarse, fine, and ultrafine particles, polycyclic aromatic hydrocarbons and metal(loid)s, and estimation of associated health risks". *Encontro Ciência 2024*, July 2-5th 2024, Porto, Portugal.

7) Gabriel Sousa, Joana Teixeira, Rui Azevedo, Agostinho Almeida, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira. "Method development for the quantification of metals in firefighters wipe samples". *Analítica 2024*, March 25-26th 2024, Porto, Portugal.

8) Gabriel Sousa, Joana Teixeira, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, Maria Freitas, and Marta Oliveira. "Characterization of fine and ultrafine particulate matter released during structure and forest fires". *9th Portuguese Young Chemists Meeting*, April 28-30th 2025, Faro, Portugal.

List of Awards

1) The publication "Gravimetric, morphological, and chemical characterization of fine and ultrafine particulate matter inside fire stations" published in the scientific journal *Building and Environment* received a merit of certificate for articles published in journals that appear in the top 10% of their respective fields. This prize was assigned by the Polytechnic Institute of Porto.

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List of Abbreviations

Ahr	ARYL HYDROCARBON RECEPTOR
ATCC	AMERICAN TYPE CULTURE COLLECTION
ATSDR	AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY
B(a)P	BENZO(A)PYRENE
BMI	BODY MASS INDEX
CACO-2	HUMAN INTESTINAL EPITHELIAL CELL LINE
CALUX	CHEMICALLY ACTIVATED LUCIFERASE EXPRESSION
CSF	CONTROLLED STRUCTURE FIRE
CYP450	CYTOCHROME P450 ENZYMES
DALYs	DISABILITY-AJUSTED LIFE YEARS
DMEM	DULBECCO'S MODIFIED EAGLE'S MEDIUM
DMEM/F12	DULBECCO'S MODIFIED EAGLE MEDIUM/NUTRIENT MIXTURE F-12
DNA	DEOXYRIBONUCLEIC ACID
DMSO	DIMETHYL SULFOXIDE
EC20	EFFECTIVE CONCENTRATION AT 20%
EC50	HALF MAXIMAL EFFECTIVE CONCENTRATION
EDS	ENERGY DISPERSIVE SPECTROSCOPY
EPA	UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
EU-OSHA	EUROPEAN AGENCY FOR SAFETY AND HEALTH AT WORK
FBS	FETAL BOVINE SERUM
FS	FIRE STATION
GC-MS	GAS CHROMATOGRAPHY-MASS SPECTROMETRY
HBSS	HANK'S BALANCED SALT SOLUTION
HS	HEALTHY SALIVA
HSC-3	HUMAN TONGUE CARCINOMA CELL LINE
HT29 MTX	HUMAN INTESTINAL MUCUS-SECRETING CELL LINE
IARC	INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
ICP-MS	INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY
ICP-OES	INDUCTIVELY COUPLED PLASMA – OPTICAL EMISSION SPECTROSCOPY

KOW	COEFFICIENT OF OCTANOL/WATER PARTITION
LOD	LIMIT OF DETECTION
LOQ	LIMIT OF QUANTIFICATION
MTT	3-(4,5-DIMETHYLTHIAZOLYL-2)-2,5-DIPHENYLTETRAZOLIUM BROMIDE
NC	NEGATIVE CONTROL
NCBI	NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION
NEAA	NON-ESSENTIAL AMINO ACIDS
N/S	NOT SPECIFIED
NSE	NON-SMOKERS EXPOSED
NSNE	NON-SMOKERS NON EXPOSED
OH-PAHs	HYDROXYLATED POLYCYCLIC AROMATIC HYDROCARBONS
OSHA	OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION
PAHs	POLYCYCLIC AROMATIC HYDROCARBONS
PC	POSITIVE CONTROL
PCA	PRINCIPAL COMPONENT ANALYSIS
PFF	PRESCRIBED FOREST FIRE
PM	PARTICULATE MATTER
PPE	PERSONAL PROTECTIVE EQUIPMENT
PS	PAH-CONTAMINATED SALIVA
PVC	POLYVINYL CHLORIDE
REP	RELATIVE POTENCY
RNA	RIBONUCLEIC ACID
ROS	REACTIVE OXYGEN SPECIES
SCBA	SELF-CONTAINED BREATHING APPARATUS
SD	STANDARD DEVIATION
SE	SMOKERS EXPOSED
SEM	SCANNING ELECTRON MICROSCOPY
TEER	TRANSEPIHELIAL ELECTRICAL RESISTANCE
TEM	TRANSMISSION ELECTRON MICROSCOPY
TR146	HUMAN BUCCAL CARCINOMA CELL LINE
UV	ULTRAVIOLET
VOCs	VOLATILE ORGANIC COMPOUNDS
WHO	WORLD HEALTH ORGANIZATION

List of Symbols

Al	ALUMINIUM
As	ARSENIC
Ba	BARIUM
Cd	CADMIUM
Co	COBALT
Cr	CHROMIUM
Cu	COPPER
Fe	IRON
Hg	MERCURY
Li	LITHIUM
Mn	MANGANESE
Mo	MOLYBDENUM
Ni	NICKEL
Pb	LEAD
Pt	PLATINUM
Sb	ANTIMONY
Se	SELENIUM
Sr	STRONTIUM
Tl	THALLIUM
Zn	ZINC

Preface

1. Relevance and motivation

Portugal faces increasingly severe forest fires due to climate change, land-use shifts, and flammable monocultures like eucalyptus (EC, 2024). These ever-increasing climatic hazards are specially concern in rural environments, while urban/structure occurrences are more prone to happen in urban or industrial areas. These fires release hazardous pollutants, including particulate matter (PM) (coarse/fine/ultrafine PM), polycyclic aromatic hydrocarbons (PAHs) (e.g. naphthalene, and benzo(a)pyrene), and toxic metal(loid)s (e.g., arsenic, cadmium, and lead), posing significant risks to exposed populations (Reid et al., 2016). Firefighters are occupationally exposed to these pollutants and the International Agency for Research on Cancer (IARC) classified their activity as carcinogenic to humans (Group 1) due to the increased risk of developing mesothelioma and bladder cancer (Demers et al., 2022). Furthermore, chronic exposure to PM (especially ultrafine fractions), PAHs (e.g., benzo[a]pyrene) and heavy metals promote respiratory, cardiovascular, and neurological diseases (Barros et al., 2021; Loomis et al., 2013, Marval and Tronville, 2022). Although Portugal is a high-risk country in what concerns to wildfires, the characterization of fire emissions and their impact on human health, particularly in occupationally exposed workers, remains poorly characterized. Studies reporting the collection and analysis of PM released during fires mostly focused on fine PM (e.g. PM_{2.5}), even though the inhalation of ultrafine particles is increasingly recognised as a major burden for respiratory human health (OSHA, 2017). Most of the human biomonitoring studies focused on measuring urinary biomarkers (e.g., OH-PAHs and metal(loid)s) to determine the total internal exposure, whereas some routes of exposure remain poorly characterized, particularly dermal and salivary pathways (Barros et al., 2023). This work is critical to exploring and safeguarding the health of exposed firefighters on the frontlines of environmental disasters, filling the gaps in occupational safety and health policies.

2. Thesis objectives

This thesis aims to answer the following questions:

1. What is the composition of PM from fire stations, and forest and structure fire emissions and their main differences?

2. How does exposure to fire emissions affect the levels of xenobiotics in biological samples of firefighters? Can new biomonitoring strategies be included in future occupational exposure and health surveillance?

To answer these questions, the main objective is to characterize firefighters' occupational exposure and associated health risks. The following specific objectives were addressed:

- I To collect signed informed consents for voluntary participation, select and characterize firefighters' exposure to fire emissions, collect personal (dermal wipe) and biological (saliva) samples.
- II To collect and characterize the elemental composition and morphology of coarse, fine, and ultrafine PM inside fire stations and during forest and structure fires.
- III To characterize the composition of biological samples (e.g. saliva and dermal wipe samples) and assess occupational exposure to 18 PAHs and 16 metal(loid)s in smoking and non-smoking firefighters involved in firefighting activities.
- IV Determine early cytotoxicity effects through *in vitro* bioaccessibility assays in human mouth (TR146) and tongue (HSC-3) carcinoma cells, as well as Caco-2 and HT29-MTX intestinal cells.
- V To estimate the impact of firefighters' occupational exposure to health relevant pollutants in various work environments.
- VI To contribute to the implementation of safety and hygiene measures to protect firefighters and general population affected.

The work related to the inclusion of humans, their selection and characterization, and participation in biomonitoring studies was revised and approved by the Ethics Committee of the University of Porto, Portugal (Report Nr. 105/CEUP/2020), under the scope of project 4FirHealth (<http://doi.org/10.54499/PCIF/SSO/0090/2019>).

3. Thesis structure and organisation

This thesis concerns the work developed under the scope and objectives of the approved PhD project (2021.08526.BD). The document is organized into three parts:

Part I – Chemical composition and morphological characterization of PM from firefighters' occupational environments

Part II – Characterization of exposure to fire emissions in firefighters' health through biomonitorization of Portuguese firefighters (smokers and non-smokers)

Part III – “Final remarks and suggestions for future work”.

There are 8 chapters, which are listed below along with a brief summary:

- **Preface** (present section)

In this section the relevance and motivation that allowed this work to develop will be presented, alongside the thesis' objectives, structure and organisation.

- **Chapter 1** - Introduction

This introductory chapter is structured in five parts: (i) a book chapter with an introduction to occupational exposure; (ii) a brief state of the art on the occupational exposure and biomonitoring of firefighters; (iii) a book chapter with literature review concerning biomonitoring using saliva; (iv) a book chapter with literature review concerning skin wipe samples to characterize occupationally exposed groups, and (v) a literature review article on the levels of PAHs measured in the skin of exposed firefighters, as well as *in vitro/in vivo* bioavailability assays and health risks.

Part I – Characterization of fire emissions from various occupational environments: Chemical composition and morphology

- **Chapter 2** – Gravimetric, morphological, and chemical characterization of fine and ultrafine particulate matter inside fire stations.

This paper investigates the levels, morphology, and elemental content of fine (PM_{2.5}, PM_{156nm}) and ultrafine (PM_{54.5nm}, PM_{30.8nm}) PM inside fire stations. It assesses firefighters' exposure in different microenvironments (common areas, garages, and PPE storage rooms). The study characterizes PM using electron microscopy and spectroscopy, aiming to support stakeholders in promoting air quality in work environments to promote occupational safety and health.

- **Chapter 3** – Morphological and elemental characterization of fine and ultrafine particulate matter generated from fires.

This study investigates fine and ultrafine PM from CSF and PFF. It uses advanced analytical techniques to characterize PM and identify the elemental composition of PM and predominant elements in each fire type.

- **Chapter 4** – Part I - Global conclusion

In this section a short synthesis with the main conclusions of chapters 2 and 3 is presented.

Part II – Characterization of exposure to fire emissions in firefighters' health through biomonitorization of Portuguese firefighters (smokers and non-smokers).

- Chapter 5 – Unveiling Exposure Pathways at the Oral Cavity during Firefighting Work: Salivary Metal(loid)s and Polycyclic Aromatic Hydrocarbons, Occupational and Biological Determinants, and Exploratory Health Risks.

This research investigates the levels of metal(loid)s and PAHs in firefighters' saliva, considering smoking habits and fire exposure. The findings highlight the combined impact of these factors on firefighters' health and underscore the need for strategies to reduce exposure and strengthen safety protocols.

- Chapter 6 – Impact of fire emissions and tobacco consumption on the salivary content of metal(loid)s and PAHs: Exposure levels and assessment of oral and intestinal *in vitro* toxicity.

This paper investigates the levels of PAHs and metal(loid)s in pulls of firefighters' saliva, grouped according to smoking status and presence in different firefighting scenarios. It also assesses the *in vitro* toxicity of saliva samples on buccal and intestinal cell lines to understand the health risks associated with exposure to these substances, exploring the combined impact of firefighting activities and tobacco consumption on salivary content.

- Chapter 7 – Part II - Global conclusion

In this section a short synthesis with the main conclusions of chapters 5 and 6 is presented.

Part III - Final remarks and suggestions for future work

- Chapter 8 – Final remarks and suggestions for future work

In this section the questions presented in the thesis objective are answered, alongside a compilation of the results gathered and suggestions for future research on the topics of this thesis.

- Attachments

In this section, the published supplementary materials and the authorizations to replicate the articles in the thesis will be presented.

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Chapter 1

Introduction

- 1.1. An introduction to occupational exposure
- 1.2. State of the art on the occupational exposure and biomonitoring of firefighters
- 1.3. Potential of saliva for biomonitoring of occupational exposure: collection of evidence from the literature
- 1.4. A review on skin metal concentrations in occupational environments
- 1.5. Exposure to PAHs during firefighting activities: A review on skin levels, *in vitro/in vivo* bioavailability, and health risks

Chapter 1.1.

An introduction to occupational exposure

Chapter 1

An Introduction to Occupational Exposure

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Abstract

Only a small fraction of the worlds' working force has access to services of occupational health to control/prevent injuries and diseases at the workplace. Every year millions of people suffer from work-related accidents, illness, and even death. Almost every job is prone to occupational risk and associated health-related injuries. This chapter presents a summary of all the possible occupational risks amongst all the work fields and their main health consequences to the safety, health, and wellbeing of workers.

During regular working activities, workers are exposed to several occupational risks, namely physical, chemical, biological, ergonomic, psychological, and psychosocial risks. Workers from low- and middle-income countries are at a higher risk of exposure to occupational injuries and diseases due to poor working conditions, lack of implemented occupational hygiene and safety working programs, and deficient or even inexistent workers' health surveillance. There is a continuous demand to reduce the number of occupational risks across different working environments through the implementation and/or improvement of effective preventive measures that will promote the health of workers. Also, connections and cooperation between the developed and the still-

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developing nations are of utmost importance to mitigate as much as possible the existing poor labor conditions amongst the workers who are at higher risk of exposure to occupational hazards and associated health disorders.

Keywords: occupational risks, safety at work, occupational health

Introduction

The world is changing, and it is changing at a very high rate. The world population continues to grow and is progressively older and more consuming. To address the rapid population growth and the higher amount of consumption, the world is constantly adapting, and productivity became the watchword. In this so-called technological revolution or fourth industrial revolution the work is being redesigned to be more efficient, flexible, and to yield more in a shorter amount of time. Mankind owns this growth in productivity to automation and computerization. However, what cannot be resorted to machinery has to be done by someone. Although the world's jobs are evolving to be more knowledge-based, that is, more engineering and decision-making tasks, there are many other occupations that need to be done, and those are predominantly left to the non-wealthy and less educated workers.

Almost half of the world's population are workers and in the last decades, the workforce is more diverse than ever with a higher inclusion of women, migrants, immigrants, and older workers (ILC, 2006; WHO, 2001). Unsafe working conditions, occupational injuries, and illness are responsible for 4-6% of gross domestic product annual economic loss (WHO, 2001). About 2 million people lose their lives every year resulting of unsafe working conditions. In the south-eastern and west-pacific parts of Asia, the percentage of occupational deaths was between 2.1% and 3.3% of the total annual deaths, whereas in Europe this value varies between 1.3% and 1.8%, for low- and high-income countries respectively (Päivi Hämäläinen, 2017; Wolf et al. 2018). In the USA alone, work-related injuries and illnesses reach 3 million cases annually. Although, just like in the other regions, this value only represents a fraction of the real number since in some cases the health issues one might have are not associated with occupational causes (Azaroff et al. 2002; BLS, 2019; Spieler and Wagner 2014). The high work-related mortality and morbidity rates prove that more efforts need to be done to improve occupational safety and health. The disability-adjusted life years (DALYs)

associated with occupation varied between 2.4% in North America and 4.1% in the west-pacific area, which shows that work-related morbidity accounts for a significant percentage of total DALYs. Although the occupational deaths and DALYs percentage in Africa (2.0% and 2.2%) and the low-income areas of the Middle East (1.0% and 1.5%) is lower, the number does not represent the true amount since most cases are not documented (Päivi Hämäläinen, 2017; Wolf et al. 2018). Although some preventive and protective measures are continuously being implemented, the percentage of people with access to occupational safety and health services remains too low. Approximately 70% of the workforce do not have any type of insurance that can support medical costs related to occupational risks and accidents (WHO, 2001). Also, only 15% of the global working population, mostly from developed countries, has access to work protection. In underdeveloped countries the numbers are even lower, e.g., in African countries, only 5% of employees working from major establishments have access to occupational health services (Masekameni et al. 2020).

All individuals when performing their work are subject to some risks, the so-called occupational hazards that depend on the tasks executed and the place where the work is performed. Occupational risks will strongly contribute to the occurrence of accidents as well as to promote the development of work-related diseases and/or the aggravation of already diagnosed diseases. This chapter aims to present the most common occupational risks that workers from a wide range of activities can be exposed to, highlighting the need for adequate occupational hygiene and safety surveillance to promote the health of workers.

Occupational Risks

Assessment of occupational exposure is difficult in population-based studies and each profession can be affected by different risks, depending on the nature and task of the work activity. Naturally, there are activities in which risks can be more dangerous for the safety and health of workers (e.g., mining sector, agriculture, aquaculture, and construction). Due to the nature of the world's evolution, some of those activities are associated with the level of countries' development and the importance given, by the higher regulatory entities, to that working group. Workers from underdeveloped countries have worse working conditions and thus are exposed to a higher number of occupational risks than subjects from more developed nations. Also, the poorest groups of the population and immigrant workers are frequently exposed to precarious

working conditions due to their social and economic vulnerability (Arici et al. 2019; Ezzati, 2004; Moyce and Schenker 2017; Palacios Nava et al. 2018).

Occupational hazards can be grouped into six different categories, namely physical, chemical, biological, ergonomic, psychological, and psychosocial. Physical risks are related to the elements of the environment like thermal stress, radiation, and noise. Chemical risks are due to close contact with dangerous chemical substances such as particulate matter (PM), irritant gases, volatile organic compounds (VOCs), metals and organometallic compounds, organic solvents, pesticides, asphyxiants and anesthetic substances. Also, biological infections are caused by direct contact with living or biological active organisms (e.g., viruses, bacteria, parasites, fungi, industrial fungal enzymes). The risks can also be of ergonomic nature induced by repetitive tasks, improperly designed work tools, or excess of heavy work. Psychological and psychosocial problems are stress-driven hazards caused by high demand and less response capability by means of the employee (WHO, 2001; Wolf et al. 2018).

Physical Risks

Physical risks are associated with exposure to hazards present at the workplace that can harm the human body without existing any type of touching interaction. There are three main physical risks: thermal stress, radiation, and noise.

Thermal stress occurs when the human body presents difficulties in controlling its core temperature. Factors such as air temperature, humidity, work rate, and clothing will strongly contribute to thermal stress. The human body responds to heat by increasing blood flow to the surface of the skin and through sweating, radiation, and convection. This process is divided into three stages. Firstly, in a vasomotor control stage, the blood is pumped to the skin and less to the vital organs which can cause headaches, sleepiness, and lack of concentration. Then, an evaporative cooling stage occurs, and the body starts to sweat trying to reach the core body temperature. In this phase, the body starts to lose important minerals and dehydrate. The predominant symptoms include fainting, muscle cramps, severe thirst, heat rash, and exhaustion. The last stage of heat stress happens when the hypothalamus is unable to regulate the temperature through sweating and it begins to fail, called heatstroke. Heatstroke is a dangerous stage where the human body is hot and with dry skin, and the person may present confusion, convulsions, loss of

consciousness, and in the most severe cases, thermal stress can result in death. Heat stress has been reported in subjects working at some industries (e.g., glass and rubber manufacturing plants, ceramic and power plants, foundries and smelting operations), construction services, agricultural activities, firefighting, mining, bakeries, and catering' kitchens (Cheung et al. 2016; WHO, 2001).

Regarding exposure to cold, the human body presents an opposite reaction, with the reduction of blood flow to the extremities of the body, thus delaying the decrease of core body temperature. Cold stress exists when the environmental temperature is lower than the body temperature causing peripheral vasoconstriction of the skin vessels. Vascular injuries alongside chilblains and gangrene are some of the problems associated with low temperatures. And in more extreme cases hypothermia, failure of vital organs, or even death. Occupational cold stress has been associated with deep-sea fishing, food manufacturing, butcher and fishmongers, and petroleum extraction (Cheung et al. 2016). To prevent thermal stress various measures can be applied at the workplace, including protection with proper shielding, insulation, and adequate ventilation, periodic exams, replacement of lost fluids and minerals, and making use of working shifts and resting periods (WHO 2001; Wolf et al. 2018).

Exposure to solar radiation might be considered the most ancient occupational hazard considering that every outdoor labor has solar exposition (Modenese et al. 2018). Radiation can be divided into two types, non-ionizing (e.g., ultraviolet and infrared) and ionizing radiation (e.g., X rays, gamma rays, etc).

Non-ionizing radiation is part of the solar radiation that gets filtered by the ozone layer and reaches the surface of the planet – 45% of infrared and 5% of ultraviolet (Modenese et al. 2018). Exposure to infrared radiation is more prone to occur in workers from the glass industry, blacksmithy, furnace work, and the manufacture of metal components. The main health risks associated with exposure to infrared radiation include cataracts and corneal affection. The simple act of wearing protection goggles is enough to prevent exposure to these hazards (WHO 2001; Wolf et al. 2018). Ultraviolet radiation, although being a small percentage of the solar radiation that reaches the surface is way more toxic to the human race than infrared radiation and it is one of the highest causes of cancer (Modenese et al. 2018). Exposure to ultraviolet radiation may occur in welding and metal cutting industries. Prolonged exposure can be the cause of skin (e.g., skin erythema, burns, and hyperpigmentation) and eye (e.g., conjunctivitis, and corneal ulceration) diseases. The adequate use of face

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and ocular protection are key components for the prevention of injuries related to exposure to ultraviolet radiation (WHO 2001; Wolf et al. 2018).

Ionizing radiation is much more dangerous than non-ionizing radiation, being characterized by high-energy particles that interact with matter by displacing electrons and breaking chemical bonds, thus destroying them (Massalha et al. 2019). Ionizing radiation can be characterized as electromagnetic, for example, X-ray, and gamma-ray radiation, which are used in X-ray machines, or subatomic radiation e.g., alpha, beta, and neutrons that are descendants from radioactive isotopes. Working areas that can use this type of radiation are medicine, agriculture, mining, research activities, and some industrial complexes such as nuclear power plants (Kang et al. 2012; Stam and Yamaguchi-Sekino, 2018). Different ionizing radiations have distinct powers of penetration within the human skin and tissues, being the gastrointestinal and hemopoietic systems the most sensitive tissues. Therefore, ionizing radiation can have several acute and severe implications on workers' health. Leukemia is the most diagnosed disease associated with this type of exposure, it is most common in survivors of atomic accidents and patients treated X- and gamma-rays (Ezzati, 2004). The adequate use of personal protective equipment and regular frequency training sessions, as well as the maintenance ventilation systems, are key actions to prevent occupational risks from exposure to radiation (WHO, 2001; Wolf et al. 2018).

Noise at work is one of the most common occupational hazards that continue to increase, principally in crowded environments and due to more industrialized and modernized societies. Continuous exposure to any kind of unwanted or not tolerable sound while performing regular working activities will definitely injury exposed workers. Indeed, daily exposure to high intensity and/or high-frequency noise can be very hazardous. In order to minimize noise-related risks the level should not exceed 80 dB, noises between 85 dB and 90 dB are moderately high and noises above 90 dB are considered very high (Ezzati, 2004). Occupational exposure to noise can induce hearing loss and later on can lead to hearing impairment, reduction in the workers' ability to function. Hearing loss can be very slight, around -20dB, or severe, around -50dB, depending on the level of exposure and consequent damage to the ear mechanisms (Ezzati, 2004). Occupational noise can be the cause of annoyance, sleeping difficulties, and stress which may result in an increased number of work-related accidents and lower productivity (Imel Nelson et al. 2005; Themann and Masterson, 2019; WHO, 2001). The most common jobs associated with noise vulnerability are factory jobs, mainly manufacturing that requires heavy machinery like ship and aircraft building, construction as well

as road, traffic policemen, airport workers, textile mill weavers, military, and gardeners. To control this problem it is recommended the use of proper personal protective devices in the form of earplugs, muffs or/and helmets together with proper maintenance of machinery and the use of soundproof materials (Themann and Masterson, 2019). Working by shifts is also frequently used in subjects who are regularly exposed to noise at work.

Other physical hazards include poor illumination, vibration, and pressure variation.

Chemical Risks

A great number of occupations expose workers to a countless number of hazardous pollutants that can harm their health after short and/or long periods of work. Occupational exposure to chemical risks occurs in the most various forms – solids, liquids, gases, mists, fumes, and vapors – and can be moderate to extremely toxic to the human body. Direct contact with these substances occurs predominantly through inhalation and direct skin contact (dermal absorption) and to less extent by ingestion.

Air pollution in work environments is one of the most studied themes, emphasized by the nocuous effects it can have on the health of workers. The International Agency for Research on Cancer (IARC) classified more than 100 chemicals known as possible/probable/known carcinogens to humans (IARC, 2019). Moreover, IARC already released a report with recommended priorities for IARC Monographs during 2020-2024 (IARC, 2019). Therefore, people can be exposed to several pollutants in their working environments, such as PM, vapors including irritant gases, volatile organic compounds, metals, and organometallic compounds, organic solvents, pesticides, asphyxiants, and anesthetic substances (WHO, 2001).

PM is among the most hazardous pollutants that a worker can be exposed to. (Kim et al. 2015). PM consists of a complex heterogeneous mixture of solid and/or liquid particles that vary in shape, size, and chemical composition, depending on the source. Other pollutants can be found absorbed/adsorbed to PM, namely nitrates, sulfates, organic and elemental carbon, metals, and metalloids (e.g., arsenic, copper, iron, nickel, cadmium, lead, mercury, zinc, etc.), organic compounds (e.g., polycyclic aromatic hydrocarbons (PAHs), and biological compounds (e.g., virus, bacteria, spores, or cell fragments) (Oliveira et al. 2019). PM can be originated from both natural and/or anthropogenic sources. Natural causes of PM can be forest fires, volcano dust,

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sea spray, vegetation dust-like pollen, fibers, and spores, or animal waste (Oliveira et al. 2020). Anthropogenic sources of PM are the combustion of fossil fuels and biomass, industrial and agricultural activities, urban fires, traffic emissions, road wear caused by the traffic, and abrasion of the vehicle's brakes and tires (Oliveira et al. 2019). Firefighters, miners, construction workers or farmers, and aquaculturists are some of the most exposed workers to PM (Kim et al. 2015; Liu and Liu, 2020; WHO, 2005; Wolf et al. 2018). PM is classified according to the aerodynamic diameter since it influences its ability to penetrate inside the respiratory tract. Coarse particles, PM with more than 10 μm of aerodynamic diameter, are usually deposited in the upper airways (nose, throat, and mouth). Fine particles, PM with less than 2.5 μm , can penetrate and reach the most sensible parts of the lungs (e.g., alveolar region). The smallest fractions of PM are known to be the most dangerous for human health (Kim et al. 2015). Occupational exposure to PM promotes the development and/or aggravation of several respiratory diseases (e.g., allergies, asthma, etc.) (Alif et al. 2016; Oliveira et al. 2019; Omland et al. 2014; WHO, 2001). Exposure to other hazardous agents such as anthrax (caused by bacterial spores) and endotoxin, especially silicosis and asbestosis, is caused by inhalation of crystalline silica in quarries and mines and the inhalation of asbestos fibers, respectively (Liebers et al. 2020; Wolf et al. 2018). The last one is of extreme importance since it was used in the widest variety of products and only recently was demonstrated its carcinogenic potential (ILO, WHO, 2007). Safety measures that can be used to prevent exposure to PM and dust are proper ventilation combined with the use of personal protective equipment, regular and adequate cleaning activities, use of isolated eating spots in the workplace, and periodic health surveillance routine exams (WHO, 2001).

Human exposure to irritant gases interacts with the mucous membranes of the respiratory system, causing irritation and inflammation. Ammonia (NH_3), formaldehyde (HCHO), chlorine (Cl_2), sulfur dioxide (SO_2), and nitrogen oxides (NO_x) are some of the most common irritant gases which are frequently found in industrial complexes and chemical industries. Depending on the water solubility of these pollutants, these compounds will act in the upper airways, if highly soluble or in the lungs, if less soluble (WHO, 2001; Wolf et al. 2018).

VOCs are compounds with low boiling points caused by their high vapor pressure. These compounds are easily emitted from solids and liquids to the air close to the source and can have short- or long-term consequences to the health of exposed workers. Paints, cleaning products, glues and adhesives, cosmetics, pharmaceuticals, pesticides, and building materials are some of the

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sources that contain VOCs and can have occupational implications in the areas that interact with those products (e.g., agriculture, industry, and cleaning services). Some examples of the most commonly found VOCs at workplaces are benzene, toluene, ethylbenzene, xylene, ethane, chloroform, acetone, and trichloroethene Ezzati 2004; IARC, 2019; WHO, 2001).

Occupational exposure to metals and organometallic compounds occurs predominantly in the mining sector, production of ink and metallic alloys, and in the steel, petrol, and chemical industries (Huat et al. 2019; WHO, 2001). Continuous exposure to heavy metals and some metalloids can be extremely dangerous. For example, arsenic and cadmium are proved to be carcinogenic (IARC group 1); lead, antimony, and mercury compounds are possible/probable carcinogens (IARC groups 2A and 2B); chromium (VI) and aluminum are toxic to human health (Ezzati, 2004; IARC, 2021). Organometallic compounds like arsine (AsH_3), nickel carbonyl [$\text{Ni}(\text{CO})_4$], and anesthetic vapors like diethyl ether [$(\text{C}_2\text{H}_5)_2\text{O}$], chloroform (CHCl_3), or nitrous oxide (N_2O) are other examples of toxic gases that should be taken into account in some workplaces. Occupational health risks associated with continuous exposure to metals can have serious consequences for the health of workers, e.g., intestinal colic (lead), personality changes (mercury), Parkinson's disease (manganese), skin and lung cancer (arsenic), short-term memory loss (aluminum), and kidney dysfunction (cadmium) are just some examples of the problems associated with exposure to toxic metals. (Rehman et al. 2018; Que et al. 2008; WHO, 2001). Some of the control measures that are frequently used in work environments to minimize occupational exposure include the use of proper ventilation and adequate maintenance, an increase of mechanization to reduce workers' direct contact with hazardous components, isolation of eating and resting areas from the workplace to prevent food/water contamination and the implementation of periodic occupational safety and health surveillance (WHO, 2001).

Other group of chemical compounds that can cause occupational harm to the health of workers are organic solvents, chemicals derived from aliphatic, aromatic, and carbonyl compounds. Exposure to these solvents can be hazardous due to their high volatility and inflammability which requires adequate procedures to use and/or store in working environments. Organic solvents are widely used in the food, chemical and pharmaceutical, ink, and textile industries (WHO, 2001). Occupational exposure to organic solvents may cause eyes, throat, and skin irritation as well as injury in the nervous, gastrointestinal and respiratory systems of workers (Wolf et al. 2018).

Pesticides are frequently used in agriculture to help control pests and to increase the productivity of crops (Cole et al. 2009; Mostafalou and Abdollahi 2017). Overall, pesticides can be divided into three classes – insecticides, herbicides, and fungicides. Nowadays the most frequently used pesticides are organophosphates, carbamates and thiocarbamates, pyrethroids, and organochlorine (Mamane et al. 2015). The most common symptoms related with exposure to pesticides are nausea, dizziness, irritability, headaches, and respiratory problems (Ye et al. 2013; WHO, 2001). In order to occur the safe handling of pesticides, workers need to have full personal protective equipment and proper handling knowledge, as well as the right first aid treatments and the medications needed to revert symptoms if intoxication occurs in the work environment. Additionally, pesticides need to be tested and licensed by the government, for lower toxicity and higher biodegradability to prevent risks to the environment and to the workers. However, in some cases employers tend to choose some of the more unsafe pesticides because of their lower prices (Amoatey et al. 2020).

Occupational exposure to asphyxiants substances can also occur in some specific work environments. Once absorbed in the blood stream, asphyxiants, e.g., methane, nitrogen dioxides, carbon monoxide and dioxides, hydrogen sulfide, and hydrocyanic acid, are compounds that can cause asphyxia by replacing oxygen in the respiratory tract (Ezzati 2004; WHO, 2001). Exposure to these asphyxiants is common in jobs performed in enclosed environments like mines, sewers, caves, and places where there are combustion processes happening. Also, refinery workers, farmers, and firefighters might also be exposed to these compounds.

Some of these chemical agents are odorless which can promote continuous occupational exposure if monitorization surveillance actions are not performed to identify sources of exposure. Preventive actions to minimize exposure to chemical risks are usually simple since the correct use of personal protection equipment, including eyes protection, gloves, and mask, as well as adequate ventilation, and daily cleaning of work environments can drastically reduce exposure to these risks (WHO, 2001). However, special health surveillance needs to be considered in workers who already have a diagnosed-related disease.

Biological Risks

Occupational exposure to biological agents is classified as unwanted contact with the hazardous living and/or biological active organisms including viruses, bacteria, parasites, fungi, industrial fungal enzymes, among other agents at the workplace (Meima et al. 2020). Health care professionals working in hospitals, health centers, nursing homes, and related facilities are daily exposed to these biological agents. Also, workers from laboratories of clinical analysis, veterinary hospitals/clinics, zoo centers, exotic/wild animal natural reserves, as well as those working at animal husbandry, wool and leather industries, and in sewage and waste treatment facilities are at a higher risk of exposure to biological agents (Moodley et al. 2018; Myers, 2010; Wolf et al. 2018). Work-related exposure to biological agents is related to several diseases, including infectious diseases, cancer, and allergies (Meima et al. 2020).

Over the last two years, a completely new and unknown virus appeared and caused a global pandemic. A proximal contact with people infected with SARS-CoV-2 became one of the highest biological occupational exposures, not only amongst the health care workers but also in all the other sectors. The virus impacted the world tremendously and forced the population to stop their working activities. Some occupations were able to adapt to telecommute and/or to a combined system of telecommuting and presential work to follow all the strict hygiene measures that were implemented to control the infection growth. Other commonly reported occupational diseases are tuberculosis in health care professionals (doctors, nurses, nurses' assistants, etc.) and veterinary workers, brucellosis and anthrax which affects veterinarians, animal creators, slaughterhouse workers, and people who work with animal parts (e.g., bones, fur/wool/leather, etc.), and hepatitis B, hepatitis C, and acquired immunodeficiency syndrome (AIDS) in health care workers and medical laboratory workers. Other diseases like malaria, some respiratory and intestinal infections, or dengue are other examples of biological hazards in the workplace that are more common in low-income countries (Ezzati 2004; Jean et al. 2019; Wolf et al. 2018).

Depending on the route of exposure to the biological agent (e.g., inhalation, dermal contact, ingestion), the workplace characteristics, and the type of biological risk, there are several preventive and protective actions already implemented in the different occupational groups. Therefore, all biological risks can be prevented, if adequate personal protective equipment is used by workers as well as adequate cleaning/disinfecting procedures are

correctly implemented at the workplace. Whenever available and recommended by health entities, workers should be vaccinated or have immunization against the specific pathogen. The correct use of personal protective equipment, workers' surveillance and periodic examination, and continuous training are essential to prevent exposure to biological risks (Ohlander et al. 2020; WHO 2001).

Ergonomic Risks

Ergonomics is the science dedicated to understanding the interactions between an individual and surrounding environment, including the workplace, in order to optimize human well-being and overall performance through the development/implementation of tools and the adjustment of environments to ensure the most comfortable, safe, efficient, and healthy working conditions. At the workplace, ergonomics aims to support workers with the most comfortable and stress-free conditions, thus keeping them motivated and productive. In a world progressively more developed and industrialized, workers need to be constantly adapting to recently implemented technologies. Also, the number of jobs that requires more computer work and repetitive tasks is drastically increasing, which strongly promotes workers' sedentary lifestyle. People staying on their feet and/or sitting/kneeling for long periods of time at the workplace performing repetitive tasks as well as those lifting heavy items, pushing, and pulling heavy loads, and reaching objects/tools overhead will be subjected to body fatigue and injuries. Also, workers exposed to vibration (segmental/whole body) and mechanical pressure concentrations with insufficient recovery time are at higher risk to develop occupational injuries (Ezzati 2004; Hulshof et al. 2021). Despite the improvement of working conditions, there are several jobs whose workers are at a high risk of suffering from musculoskeletal disorders (e.g., public/private transport drivers, administrative workers, farmers, fishermen, forestry, construction, education, and factory workers, collectors of garbage, etc.) (Hulshof et al. 2021; Lurati, 2018). Musculoskeletal disorders are injuries that can affect almost all tissues from the human body, principally the soft ones (e.g., muscles, tendons, ligaments, joints, and cartilage) and the nervous system (Jaffar et al. 2011; OSHA, 2009). Moreover, occupational exposure to these risks will contribute to higher rates of work injury and promote the aggravation/development of chronic diseases and cardio-metabolic problems (Lurati 2018; Thorp et al. 2012). To minimize the impact of these occupational

hazards, further studies on biomechanics are needed to reduce fatigue and musculoskeletal illness and to prevent accidents at work. However, workers should be encouraged to daily exercise and to have adequate health surveillance (WHO, 2001).

Psychological and Psychosocial Risks

Nowadays, the concern with occupational safety and health not only emphasizes physical health problems but also mental health and emotional balance. Work-related mental health disorders are becoming to be acknowledged. Psychological/psychosocial issues might be the ones that affect the most variety of areas since every job has its sources of stress. Stress can be related to career development, having too much responsibility, discrimination, lack of respect, noise and ergonomic factors, or in more extreme cases, sexual harassment or workplace physical and verbal violence (Ezzati 2004; WHO, 2001). Mental health problems are usually intangible, hindering the workers to recognize the symptoms, which in some cases can be quite severe. The symptoms of poor mental health are also felt physically and emotionally, so it is vital to recognize the importance of these problems to human health (Dalmaso et al. 2021; Wolf et al. 2018).

Recently, the state of doubt, anxiety, and fear caused by the pandemic SARS-CoV-2 virus affected the mental health of a great number of workers from all working areas (Sanghera et al. 2020). The perfect example is health care workers, mainly the nurses and doctors that interacted with patients infected with the virus in the early-days, when there was little to no information about the subject. Besides the extremely high biological risks, naturally associated with this job, the social demand and responsibility to respond to the pandemic emergency, physical and emotional exhaustion, forced these workers to experience a huge psychological pressure that might have health consequences for the next decades. Due to their high risk of occupational exposure to SARS-CoV-2, several working groups had to perform extended work shifts, several times without the expected resting periods, and in some cases being unable to spend time with family and friends. Some short-term health consequences associated with these occupational risks include insomnia, anxiety, post-traumatic stress disorder, depression, burnout, and acute stress reaction. (Cocker and Joss 2016; Dalmaso et al. 2021; Sanghera et al. 2020).

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Psychological occupational health is getting more attention from society and employers, however, that attention is far from being enough. The social support needs to act on providing and incentivizing the workers with counseling/medical services and general support, in developing the conditions the workers are subjected to since in some cases they are not adequate and need more attention and funding. The working schedules should also consider proper resting of the professional, which should be adjusted to the working periods (day *versus* night) and physical, mental, or psychological demands of each occupation. More research is required in the mental health area (Cooklin et al. 2017; WHO, 2001).

Occupational Hygiene, Safety and Health

Occupational hygiene is the working tool that allows the anticipation and recognition of occupational risks with further evaluation and control of those risks in the working environment, thus promoting the protection of workers' health and their well-being. Therefore, adequate occupational hygiene surveillance will identify hazardous agents at the workplace and implement actions to control their occurrence. Occupational safety is mainly focused on the prevention of accidents and injuries caused by the execution of specific work tasks that can directly or indirectly harm the health of workers. Occupational health is an area of work that pursues the highest degree of physical and mental health for all workers in all occupations. Reduction of occupational exposure requires not only etiologic studies but effective interventions (Cooklin et al. 2017; Romero et al. 2019). Those interventions are primarily accomplished through the mitigation of exposures to hazardous components. Some occupational exposures are preventable with simple measures that are feasible, cheap and everyone has access to. In 2013, the World Health Organization published a report dedicated to the *Global Plan of Action on Workers' Health (2008-2017)* (WHO, 2013), and urged all Member States to pursue the following actions:

- To enhance the skills and knowledge related to occupational health and safety, amongst primary health workers – nurses and general hospital staff, environmental and public health technicians, and community health workers – to empower them with basic occupational health advice and recommend the monitorization of workers from small companies, rural areas, the informal sectors,

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including migrants, to be able to identify common diseases related to their occupations.

- To improve the existing occupational health services in medium and big-sized businesses as well as in industrial zones by expanding the coverage of these services and the skills and knowledge of the specialized personnel. The focus is on improving the organization and environment of the workplace as well as working on the early detection of diseases.
- To develop strategies with the objective of facilitating the treatment of occupational illness as well as helping workers that were absent for a long time, get back in the society and the professional world.
- To work towards developing a proper occupational health system that can be able to provide companies good health surveillance and working conditions for them to prosper. All of this should not depend on the public health system.
- To include occupational health training in dealing with respiratory, skin, and neurological diseases, as well as cancer and musculoskeletal diseases in all the first-row health professionals and other specialists, is a must.
- To establish collaborations between institutions, national health programs, and human resources capabilities to raise funds and develop programs and guidelines to deal with the concern of occupational exposure – prevention of occupational diseases and injuries, communicable and non-communicable diseases, health, and exercise promotion as well as mental health and environmental health.
- To improve the cooperation and collaboration between developed and developing societies with the objective of implementing actions regarding the subject.

The most successful societies have proved that combining occupational hygiene and safety with health promotion improves the working conditions, productivity, and mainly the wellbeing of workers. There is a tremendous amount of practical knowledge regarding the subject of occupational exposure and the risks associated with it. That information can and should be used and transmitted in several educational events that promote cooperation of national/international agencies dedicated to the evaluation of occupational health and safety with the objective to promote the health, safety, and wellbeing of all workers. Although the attention given to occupational

hygiene, safety, and health is growing it is still not enough, principally in low and middle-income countries. Despite all the knowledge acquired there is much more to be discovered. With further funding and research much more will be unveiled, and that extra knowledge will be used in the creation of further hazard control measures and technologies, giving a brighter future for occupational health, safety, and hygiene.

Conclusion

The world working force is continuously exposed to several occupational risks and despite the progressive improvement of hygienic and safety conditions, occupational exposures and hazards are far from being fully minimized and more risks are continuously emerging. The six most common types of hazards present in the workplace are the chemical and biological source, as well as ergonomic, physical risks, and psychological/psychosocial risks. These occupational risks will significantly contribute to partial or complete disability of workers, by affecting their safety at work which will have implications for their health and wellbeing. Moreover, the most affected workers are the ones from low and middle-income countries that perform the most precarious jobs. The lack of occupational surveillance, principally in the most sensitive groups of workers, contributes to devastating outcomes related to the increased number of accidents, injuries, and development/aggravation of occupational diseases. To counterbalance those numbers, some measures have to be considered in different working areas. Occupational risks can many times be significantly reduced with easy-implemented and simple measures such as the adequate use of personal protective equipment and regular training sections for workers, frequent inspections at working place to identify risks and to monitor the most critical working areas as well as investing in the education of occupational health professionals. Nowadays, the world has all the means to adapt and to develop national/international regulations and recommendations that would reduce the burden of unhealthy workplaces and consequently promote the health of workers.

Despite workers, employers, and regulators being more conscious of occupational risks and associated health implications, together with occupational recommendations and directives already under supervision by regulatory entities, there is still a long way to go to ensure risks are clearly identified and preventive/protective measures are adequately implemented,

principally in the less characterized (e.g., public service, dentistry or cleaning services) and emerging occupations.

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Chapter 1.2.

State of the art on the occupational exposure and
biomonitoring of firefighters

State of the art on the occupational exposure and biomonitoring of firefighters

Over recent decades, the risk of forest fires in Europe has escalated, with the Mediterranean region emerging as a particular hotspot. Several converging factors contribute to this intensification, most notably climate change (Bolan et al., 2025; Gerberding and Schirpke, 2025). Rising average temperatures, prolonged heatwaves, and shifting weather patterns have increased the frequency and severity of droughts across Europe, resulting in reduced fuel moisture levels that leave vegetation more susceptible to ignition (Bolan et al., 2025; EC, 2024; De Rigo et al., 2017; Gerberding and Schirpke, 2025). Projections indicate that, under high-end climate warming scenarios, the number of days with extreme fire risk could increase dramatically in Southern Europe, including the Iberian Peninsula, southern France, Italy, the Balkans, and Turkey. Earlier onset of fire seasons and longer periods of high temperatures further exacerbate the scope and impact of wildfires in the region (van Daalen et al., 2022). Fire emissions are a major source of air pollution, releasing a complex mixture of particulate matter (PM), gases, and toxic pollutants into the environment. The principal pollutants include PM (e.g. fine and ultrafine), carbon monoxide (CO), methane (CH₄), nitrogen oxides (NO_x), volatile organic compounds (VOCs), metal(loid)s, and secondary pollutants such as ozone (O₃) (Reid et al., 2016). The acute and chronic health risks associated with exposure to fire smoke are diverse and significant. One of the most concerning components of fire emissions are fine particles, especially PM_{2.5} (particles ≤ 2.5µm in diameter) and ultrafine particles PM_{0.1} (≤ 0.1µm), which are capable of bypassing the body's natural respiratory defences and reach the deepest regions of the lungs (Chen et al., 2021; Chu et al., 2019; Marval and Tronville, 2022; Moreno-Ríos et al., 2022; Ohlwein et al., 2019; Schraufnagel, 2020; WHO, 2006). PM_{2.5} has been directly linked to increased rates of respiratory and cardiovascular disease, hospitalizations, and premature deaths, even at relatively low concentrations and short-term exposures (Chen et al., 2021; Chu et al., 2019). Ultrafine particles (PM_{0.1}) are even more concerning due to their ability to cross alveolar membranes, entering the bloodstream and reaching vital organs. Once inside the body, these particles can incite systemic inflammation, oxidative stress, and endothelial dysfunction, which might cause cardiovascular events, hypertension, diabetes, and even neurological disorders (Marval and Tronville, 2022; Moreno-Ríos et al., 2022; Ohlwein et al., 2019; Schraufnagel, 2020). Critically, these particles also serve as carriers for various adsorbed toxic/carcinogenic substances, including heavy metals and organic carcinogens, such as PAHs that are a class of organic compounds formed during incomplete combustion of organic matter (e.g. wood, food, gas, and oil) (Barros et al., 2021, 2023, IARC, 2002, 2010, 2025; Loomis et al., 2013; Marval and Tronville, 2022;

Moreno-Ríos et al., 2022; Schraufnagel, 2020; WHO, 2006). Notable PAHs include benzo[a]pyrene and dibenz[a,h]anthracene, both of which are recognized for their mutagenic and carcinogenic properties (IARC, 2002, 2010, 2025). PAH exposure can lead to DNA damage, oxidative stress, immune system suppression, and a variety of adverse health outcomes, including cancer (especially lung and skin), respiratory diseases, cardiovascular problems, and liver or kidney damage. Inhalation represents a critical route of exposure, with chronic health effects ranging from asthma-like symptoms to increased incidence of various cancers (ATSDR, 1995; Kamal et al., 2014; Patel et al., 2020). Other toxic components present in fire emissions are metal(loid)s such as Ni, As, Cd, and Pb. Specially the latter two require extra attention (Ebrahimi et al., 2020; IARC, 2012, 2025). Released through the combustion of vegetation and anthropogenic materials (e.g. paint, electronic devices, plastic, or waste), these metals are highly persistent and bioaccumulative. Prolonged exposure can result in neurotoxicity, carcinogenicity, and multi-organ damage (ATSDR, 2012, 2020; IARC, 2012; Jaishankar et al., 2014; Kim et al., 2015). Cd, for example, is known to accumulate in kidneys and disrupt the renal function, while Pb exposure in children is linked to cognitive impairment and behavioural delays (ATSDR, 2012, 2020). These metals induce the formation of reactive oxygen species (ROS), leading to DNA damage, inflammation, and immune system dysfunction. Chronic exposure contributes to heightened risks of kidney, respiratory, and cardiovascular diseases, as well as cancer (Barros et al., 2021, 2023; See et al., 2007).

Firefighters are subject to unique occupational risks due to their repeated, intense, and prolonged exposure to wildfire pollutants. Apart from acute respiratory symptoms and reduced lung function, firefighters also face heightened risks for cardiovascular effects. Heat stress, dehydration, and physical exhaustion further compound these risks (Esteves et al., 2025; Navarro et al., 2022; Reid et al., 2016; Schraufnagel, 2020). Epidemiological evidence increasingly supports associations between firefighting and long-term health outcomes such as elevated incidence of certain cancers (e.g., bladder cancer), mesothelioma, diabetes, and neurodegenerative diseases (Barros et al., 2021; Demers et al., 2022; IARC, 2023; Oliveira et al., 2017). The IARC has recently classified occupational exposure as a firefighter as carcinogenic to humans (Demers et al., 2022). The cumulative nature of these exposures, along with the physically demanding working conditions, underscores the need for enhanced preventive measures and support for firefighter health. Human biomonitoring is crucial for assessing firefighters' exposure to hazardous compounds encountered during firefighting activities. Traditionally, urinary and blood biomarkers have provided valuable insights, enabling detection of exposure to harmful substances such as PAHs, metal(loid)s, and other combustion byproducts (Barros et al., 2021, 2023; Hwang et al., 2021; IARC, 2010; Sousa et al., 2023, 2024; WHO, 2015). These assays not only reflect the internal dose of contaminants

but also help in understanding the associated physiological responses, such as oxidative stress or inflammatory changes. However, relying solely on these matrices can be limiting, given the complexity of exposure routes. Expanding biomonitoring to other matrices, particularly saliva and dermal analysis, offers substantial benefits. Saliva can provide non-invasive, rapid assessments of certain biomarkers reflecting both systemic and local exposures (Humphrey & Williamson, 2001; Milanowski et al., 2016; Michalke et al., 2015; Sousa et al., 2023, 2024). However, the information related to the use of saliva in human biomonitoring of occupationally exposed individuals is still scarce (Koh et al., 2003; Shawahna et al. 2021; Sousa et al., 2023, 2024). Dermal analysis directly evaluates the skin contamination, capturing a critical exposure route often overlooked (Araviiskaia et al., 2019; Abolhasani et al., 2021). Integrating multiple matrices would complement urine/blood-based biomonitoring, yielding a more comprehensive profile of firefighter exposure, ensuring protective measures and health surveillance.

Effective assessment of the health effects of fire emissions hinges on comprehensive monitoring of air pollution, public health indicators, and individual exposures. Real-time air quality monitoring networks provide essential data to track pollutant concentrations, support public health advisories, and inform emergency responses. Continuous biomonitorization of exposed populations, alongside environmental monitoring, allows the identification of health threats and helps to shape preventive and mitigation strategies. Such efforts are vital not only for reducing the immediate health impacts to firefighters during fire events but also for safeguarding the longevity of these individuals', as well as the general population's well-being.

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Chapter 1.3.

Potential of saliva for biomonitoring of
occupational exposure: Collection of evidence
from the literature

Potential of Saliva for Biomonitoring of Occupational Exposure: Collection of Evidence from the Literature



Gabriel Sousa, Cristina Delerue-Matos, Xianyu Wang, Francisca Rodrigues, and Marta Oliveira

Abstract *Introduction* workers are regularly exposed to several potential hazards including occupational pollutants that may affect their health. Occupational biomonitoring surveys predominantly use the collection of blood and/or urine. *Objectives* This work provides a compilation of available biomonitoring studies including the collection of saliva in occupationally exposed individuals. *Method* Literature research was performed in different online databases and, after applying the inclusion criteria, a total of 23 studies were selected. *Results* Available evidence demonstrates the use of saliva to assess recent exposure to metals and heavy metals (e.g., 3.03–129.0 µg/L of lead, 0.14–59.7 µg/L of cadmium, and 2.0–11.1 µg/L of mercury), volatile organic compounds (8.5–28.2 µg/L of styrene, 7.09 µg/L of toluene, 0.530–3.30 µg/L of phenanthrene, 0.437–1.48 µg/L of fluorene, 0.05–1.45 µg/L of acenaphthylene, 0.210–1.10 µg/L of fluoranthene, 0.230–0.860 µg/L of pyrene, 0.270–0.430 µg/L of naphthalene, and 0.199–0.329 µg/L of anthracene), and pesticides (0.047–4130 µg/L of atrazine, diazinon, and ethion) in workers. *Conclusion* Saliva has the potential to be used in occupational biomonitoring surveys. However, future health surveillance studies should include the collection of saliva to better characterize workers' recent exposure to occupational pollutants, explore its correlation with other biological fluids (e.g., serum, plasma, and urine), and improve occupational health risk assessment.

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1 Introduction

On a daily basis and depending on the work environment, workers can be subjected to several harmful hazards originating from various sources. Depending on the task performed, workers can be exposed to physical, ergonomic, and psychological/psychosocial risks, but also to chemical (e.g., particulate matter, volatile organic compounds, heavy metals, etc.) and biological (e.g., fungi, parasites, or viruses) hazards (WHO 2002). These occupational risks can have short- and long-term consequences on workers' health and will contribute to higher rates of morbidity and mortality. Regular occupational exposure to chemical hazards, e.g., particulate matter, heavy metals, and volatile organic pollutants including polycyclic aromatic hydrocarbons (PAHs) will promote the development/aggravation of cardiovascular and respiratory diseases and higher cancer incident rates (Kim et al. 2015; Oliveira et al. 2019; WHO 2006). Occupational exposure to biological active organisms has been associated with the development of allergies, infectious diseases, and cancer (EU-OSHA 2020). Human biomonitoring is a precious tool that allows programmed occupational surveillance, i.e., workers' exposure to pre-selected chemical risks, thus allowing the evaluation of the impact of preventive actions and procedures implemented in the occupational environment to improve workers' safety and health. Several biomonitoring surveys have been performed in workers from different occupational environments to assess their total internal levels of health-relevant pollutants through the direct quantification of pollutants or their main metabolites in biological fluids (Oliveira et al. 2020, 2021; Wang et al. 2015). Blood and urine are the most characterized and reliable matrices for human biomonitoring. However, over the last decade, other biological matrices (e.g., hair, skin, and saliva) have been considered in human biomonitoring studies (Liu et al. 2016; WHO 2015).

Saliva is an acidic oral secretion produced by the parotid, submandibular and sublingual salivary glands and is composed of 97–99% of water and some lipids, peptides, proteins, enzymes, electrolytes (e.g., sodium, potassium, calcium, and magnesium bicarbonates and phosphates), and immunoglobins with antibacterial activities (Almeida et al. 2008). This mucoserous exocrine secretion embodies several biological functions such as the taste, digestion, lubrication, buffering, maintenance of teeth integrity, clearance actions, antibacterial regulation, and protection (Humphrey and Williamson 2001). Saliva has been used to assess emotional, hormonal, neurologic, and immunologic status, nutritional, and metabolic conditions and to evaluate exposure to environmental and occupational hazardous pollutants (Humphrey and Williamson 2001; Milanowski et al. 2017; Michalke et al. 2015). This matrix has a good potential for being used in human biomonitoring studies because its collection is simple, non-invasive, inexpensive, and can be done by the

worker without the help of a health professional; contrary to blood collection, the risks of infection, anemia, or thrombosis are inexistent (Milanowski et al. 2017).

Studies that report the use of saliva in human biomonitoring assays to evaluate occupational exposure date back to the 1970s and are getting more popular in the last decades (Michalke et al. 2015). Inorganic elements such as metals and heavy metals as well as pesticides, organic solvents, and PAHs are some of the compounds detected in these occupational exposure bioassays. This work aims to compile available data found in the literature related to the use of saliva in biomonitoring assays performed in different working environments.

2 Materials and Methods

Literature research was conducted in different online databases, namely ISI Web of Knowledge, Science Direct, PubMed, Google Scholar, Scopus, and Scielo. Information was searched by combining two or more of the following keywords: occupational exposure, occupational health, saliva, and biomonitoring. The following inclusion criteria were applied: (i) to be an original study published in indexed and peer-review scientific journals, (ii) to include the determination of organic and/or inorganic compounds in saliva, and (iii) to be performed in an occupational environment. All the studies published till December 2021 were abstract screened and after the elimination of duplicates and verification of inclusion criteria, a total of 23 studies were selected.

3 Results and Discussion

The selected literature reports the quantification of inorganic ($n = 14$) and organic ($n = 9$) compounds in the saliva of workers. Due to the limited information, studies including the development of methodologies to determine organic/inorganic compounds in saliva were also included. Information from all the selected studies is summarized in Table 1.

3.1 Inorganic Elements

Metals, metalloids, and heavy metals are amongst the most common and dangerous chemical hazards present in the workplace. Workers in the mining sector, foundries, metallic alloy, and chemical industries are some of the most exposed individuals to inorganic compounds, thus posing additional risks for their health. Some of these compounds are possible/probable carcinogenic to humans, can cause kidney and intestinal disfunctions and may lead to the development of brain disorders (e.g.,

Table 1 Available literature that used saliva as a human biomonitoring tool in occupational environments

Human biomonitoring with saliva in occupational environments			
Organic pollutants		Inorganic pollutants	
Nigg et al.	1993	El-Sadik and El-Dakhakhny	1970
Denovan et al.	2000	Gervais et al.	1981
Lu et al.	2006	Brodeur et al.	1983
Hines et al.	2006	Örtendahl et al.	1989
Semple et al.	2007	Zaichick et al.	1995
Ferrari et al.	2008	Menegário et al.	2001
Wang and Lu	2009	Koh et al.	2003
Bonanni et al.	2015	Wang et al.	2008
Santos et al.	2019	Chaari et al.	2009
–	–	Cowan et al.	2009
–	–	Gil et al.	2011
–	–	Thompson et al.	2012
–	–	Staff et al.	2014
–	–	Shawahna et al.	2021

Parkinson disease) (Huat et al. 2019; IARC 2021; WHO 2002). Available biomonitoring studies assessed the levels of lead (Pb), cadmium (Cd), manganese (Mn), mercury (Hg), copper (Cu), iron (Fe), cesium (Cs), chromium (Cr), nickel (Ni), and zinc (Zn) in the saliva of a wide variety of workers from different occupational environments (Table 2).

A total of seven studies determined the salivary lead content in workers from different industries, namely from a factory manufacturing lead based on polyvinyl chloride stabilizer (7.7 µg/L; Koh et al. 2003), aluminum, plastic, detergents and paint factories (7.9 µg/L; Shawahna et al. 2021), a Brazilian battery factory (18.8 µg/L; Menegário et al. 2001), Chinese welding factories (24.9 µg/L; Wang et al. 2008), and from a Palestinian automobile and cell phone repair store (32.7 µg/L; Shawahna et al. 2021) (Table 2). Moreover, Staff et al. (2014) performed a study with 105 British workers with history of being occupationally exposed to lead across a variety of different industries (not specified by the authors) and reported mean salivary lead values of 40.2 µg/L (43.5 µg/L in smokers and 36.9 µg/L in non-smokers). Three studies included the quantification of cadmium, a well-known carcinogenic heavy metal, in the saliva of workers (Gervais et al. 1981; Gil et al. 2011; Wang et al. 2008). Gervais et al. (1981) reported a mean concentration of 59.7 µg/L in the saliva of 35 workers (occupation not specified) exposed to cadmium dusts and fume. In a study performed with 49 Chinese welders, Wang et al. (2008) reported cadmium mean values of 0.36 µg/L, while Gil et al. (2011) reported levels ranging between 0.14 and 1.90 µg/L in 178 Spanish workers from a steel industry. The presence of inorganic mercury, in the saliva of occupationally exposed workers was described by three studies (Chaari et al. 2009; El-Sadik and El-Dakhakhny 1970; Örtendahl

Table 2 Concentrations (mean or range; $\mu\text{g/L}$, unless indicated otherwise) of metals and metalloids in the saliva of workers

Country	Occupation	Concentration	Reference
Lead			
Canada	Battery recycling industry	129	Brodeur et al. (1983)
Brazil	Battery factory	18.8	Menegário et al. (2001)
Singapore	Lead based PVC stabilizer factory	7.7	Koh et al. (2003)
China	Welding factory	24.9	Wang et al. 2008
Spain	Iron and steel industry	3.03	Gil et al. 2011
Great Britain	N/S	40.2	Staff et al. 2014
Palestine	Aluminum, plastic, detergent, and paint factory	7.9	Shawahna et al. (2021)
	Cell phone repair shop	32.7	
Cadmium			
Canada	N/S	59.7	Gervais et al. (1981)
China	Welding factory	0.36	Wang et al. (2008)
Spain	Steel industry	0.14–1.90	Gil et al. (2011)
Mercury			
Egypt	Sodium hydroxide production plant	4.1–6.0	El-Sadik and El-Dakhakhny (1970)
Sweden	Navy divers performing underwater electrical cutting	2.0–4.0	Örtendahl et al. (1989)
Tunisia	Dentists and assistants	11.1	Chaari et al. (2009)
Manganese			
China	Welding factory	44.5	Wang et al. (2008)
China	Ferroalloy factory	22.3–31.3	Cowan et al. (2009)
Spain	Steel industry	0.12–70.47	Gil et al. (2011)
Copper			
Sweden	Navy divers performing underwater electrical cutting	10.0–200.0	Örtendahl et al. (1989)
China	Welding factory	28.5	Wang et al. (2008)
Zinc			
China	Welding factory	191.0	Wang et al. (2008)
Iron			
China	Ferroalloy factory	139.0–141.0	Cowan et al. (2009)
Nickel			
Spain	Steel industry	0.12–188.50	Gil et al. (2011)
Chromium			
Spain	Steel industry	0.10–98.71	Gil et al. (2011)
Cesium			

(continued)

Table 2 (continued)

Country	Occupation	Concentration	Reference
Russia	Cleaning operations at the Chernobyl nuclear power plant	0.082 ^a	Zaichick et al. (1995)

^a Value presented in $\mu\text{g/g}$

N/S Not specified

et al. 1989). El-Sadik and El-Dakhakhny (1970) reported mean mercury values of 6.0, 4.1, and 5.7 $\mu\text{g/L}$ in 52 workers from an Egyptian sodium hydroxide producing plant, respectively, with less than 6 months, between 6 months and 3 years, and with more than 3 years of exposure to mercury. Örtendahl et al. (1989) reported mercury concentrations ranging between 2.0 and 4.0 $\mu\text{g/L}$ in 5 Swedish navy divers performing electrical cutting under water. Also, Chaari et al. (2009) found a total of 11.1 $\mu\text{g/L}$ of mercury in the saliva of 52 Tunisian dentists and assistants. Regarding manganese, a total of three studies were found in the literature. Wang et al. (2008) reported a mean value of 4.45 $\mu\text{g/L}$ in the saliva of Chinese welders. Other authors determined the salivary concentration of manganese in 95 high—(mean of 31.3 $\mu\text{g/L}$) and 122 low—(mean of 22.3 $\mu\text{g/L}$) exposed workers from a Chinese ferroalloy manufacturer (Cowan et al. 2009). More recently, Gil et al. (2011) detected manganese in the saliva of 178 workers from a Spanish steel industry with values varying between 0.12 to 70.47 $\mu\text{g/L}$. Despite the scarce information available, some authors found other metals and/or metalloids in the saliva of workers: 10.0–200.0 $\mu\text{g/L}$ of copper in navy divers (Örtendahl et al. 1989) and 28.5 $\mu\text{g/L}$ in Chinese welders (Wang et al. 2008); mean concentration of 191 $\mu\text{g/L}$ of zinc in Chinese welders (Wang et al. 2008); 141 $\mu\text{g/L}$ and 139 $\mu\text{g/L}$ of iron, respectively for workers with high- and low-exposures in a Chinese ferroalloy manufacturer (Cowan et al. 2009); 0.12–188.50 $\mu\text{g/L}$ (mean value of 7.8 $\mu\text{g/L}$) of nickel and 0.10–98.71 $\mu\text{g/L}$ (mean value of 3.1 $\mu\text{g/L}$) of chromium in Spanish workers from a steel industry (Gil et al. 2011); 0.082 $\mu\text{g/g}$ (dry weight) of cesium in workers involved in cleaning operations at the closed Chernobyl nuclear power-plant (Zaichick et al. 1995).

Available evidence points saliva as a biological fluid with potential to be included in future occupational biomonitoring assays to monitor workers' recent exposure to health-relevant inorganic pollutants (Brodeur et al. 1983; Gervais et al. 1981; Gil et al. 2011; Staff et al. 2014; Wang et al. 2008; Zaichick et al. 1995). Saliva half-life time for some metals e.g., cadmium (50 months in mice saliva) and lead (5–7 days) are expected to be much lower than in blood (up to 16 years for cadmium and 19–28 days for lead), which makes saliva a more reliable fluid to evaluate recent exposures at working environments (Brodeur et al. 1983; Järup et al. 1983; Matsubara-Khan 1974; Rabinowitz et al. 1976). Some authors highlighted that higher water content in saliva (<99.4%) contributes for increased levels of heavy metals and metalloids up to 10 times more concentrated in dry saliva than in the blood (Zaichick et al. 1995). Additionally, saliva is a useful fluid to determine metal toxicity due to its aqueous stability for metal analysis (Wang et al. 2008). However, the presence of some dental

diseases (e.g., ulcers and/or oral candidiasis) can be a limiting factor, since these diseases may alter the metal contents in saliva (Wang et al. 2008).

3.2 Organic Compounds

In the literature, there are nine biomonitoring studies evaluating the presence of volatile and semi volatile organic compounds (VOCs; $n = 5$), and pesticides ($n = 4$) in the saliva of workers (Table 3).

Exposure to VOCs is frequent in different working environments, including in food, pharmaceutical, paint, glue, and textile industries, as well as in agriculture and firefighting activities, among many others (Barros et al. 2021; Lamplugh et al. 2019). VOCs are compounds with low boiling points that tend to evaporate easily at room temperature and to be absorbed by the human body, mainly via eyes and respiratory tract. These pollutants are known because of their toxicity and potential/known carcinogenic properties, being associated with eyes and skin irritation, allergies, skin disorders, and with the development/aggravation of respiratory diseases (WHO 2002). So far, only five study evaluated the occupational exposure to VOCs through the collection of saliva (Table 3). In 2008, Ferrari et al. measured the salivary concentration of toluene in 36 Italian workers from a synthetic leather manufacturer and reported mean post-shift levels of 7.09 $\mu\text{g/L}$. An identical study was performed by Wang and Lu (2009) with 34 Chinese workers from synthetic leather manufacturers and salivary concentrations of different organic solvents were determined: N-methyl formamide (3840 $\mu\text{g/L}$), dimethyl formamide (1400 $\mu\text{g/L}$), 2-propanol (1310 $\mu\text{g/L}$), acetone (540 $\mu\text{g/L}$), and methyl ethyl ketone (330 $\mu\text{g/L}$). Bonanni et al. (2015) assessed the concentrations of styrene in the saliva of 58 workers from a fiberglass reinforced plastic manufacturer, with concentrations ranging between 8.5 and 28.2 $\mu\text{g/L}$ (mean of 19.1 $\mu\text{g/L}$). More recently, Santos et al. (2019) described the development of a fast, effective and sensitive methodology based on a new liquid–liquid extraction with-programmed temperature vaporizer gas chromatography with mass spectrometry that was used to measure seven PAHs (naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene) in the saliva of firefighters in different moments of exposure to fires: immediately after, 6 h, and 12 h after firefighting; unexposed volunteers were included as a control group. All the seven PAHs were detected in the saliva samples collected immediately after the fire event, being phenanthrene (0.530–3.30 $\mu\text{g/L}$) and fluorene (0.437–1.48 $\mu\text{g/L}$) the most predominant compounds, followed by acenaphthylene (0.05–1.45 $\mu\text{g/L}$), fluoranthene (0.210–1.10 $\mu\text{g/L}$), pyrene (0.230–0.860 $\mu\text{g/L}$), naphthalene (0.270–0.430 $\mu\text{g/L}$), and anthracene (0.199–0.329 $\mu\text{g/L}$) (Santos et al. 2019). Fluorene and phenanthrene were the only compounds detected in the saliva of subjects from the control group, with values varying between 0.160–0.230 $\mu\text{g/L}$ and 0.202–0.750 $\mu\text{g/L}$, respectively. The salivary concentrations of the samples obtained 12 h after the fire events were comparable to the levels found in the control group (Santos et al. 2019).

Table 3 Concentrations (mean or range; $\mu\text{g/L}$) of organic compounds in the saliva of workers

Country	Occupation	Concentration	Reference
Toluene			
Italy	Synthetic leather factory	7.09	Ferrari et al. (2008)
N-methyl formamide			
China	Synthetic leather factory	3840	Wang and Lu (2009)
Dimethyl formamide			
China	Synthetic leather factory	1400	Wang and Lu (2009)
2-propanol			
China	Synthetic leather factory	1310	Wang and Lu (2009)
Acetone			
China	Synthetic leather factory	540	Wang and Lu (2009)
Methyl ethyl ketone			
China	Synthetic leather factory	330	Wang and Lu (2009)
Styrene			
Italy	Fiberglass reinforced plastic factory	8.5–28.2	Bonnani et al. (2015)
PAHs ^a			
Spain	Firefighters	0.05–3.30	Santos et al. (2019)
Cotinine			
Scotland	Non-smoker bar workers	0.3–3.4	Semple et al. (2007)
	Smoker bar workers	218.2–234.1	
Atrazine			
USA	Pesticide applicators	0.57–150	Denovan et al. (2000) Hines et al. (2006)
Diazinone			
USA	Pesticide applicators	0.047–0.061	Lu et al. (2006)
Ethion			
Nicaragua	Pesticide preparators	907.0	Nigg et al. (1993)
	Pesticide applicators	4130	

^a Naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene

Additionally, to the above-mentioned studies, Semple et al. (2007) conducted a study with 371 bar workers to measure the effects of the implementation of smoke-free legislations in Scottish bars. The authors measured the levels of cotinine in the saliva of bar workers, since it is one of the compounds found in tobacco but mainly because it is the main metabolite of nicotine (stimulant and anxiolytic found naturally in tobacco) and a biomarker of exposure to tobacco smoke. The mean reported values were 3.4 versus 0.3 $\mu\text{g/L}$ for non-smokers and 234.1 versus 218.2 $\mu\text{g/L}$ for smokers, respectively before and after the implementation of the legislation (Semple et al. 2007).

Pesticides can be divided in three categories: insecticides, herbicides, and fungicides. These compounds are widely used in agriculture to increase productivity by controlling and eliminating pests (Hines et al. 2006; Lu et al. 2006; Nigg et al. 1993). Atrazine, a common herbicide, was the pesticide determined in a field study with post-event salivary values ranging between 0.57 and 150 $\mu\text{g/L}$ in 15 male pesticides' applicators from the USA (Denovan et al. 2000; Hines et al. 2006). Those authors suggested saliva as a very sensitive, easy, and convenient matrix for the monitorization of recent exposure to atrazine. Also, diazinon (mean of 0.047 and 0.061 $\mu\text{g/L}$ after 12 and 30 h post pesticide application) and ethion (mean of 907.0 $\mu\text{g/L}$ in the worker that prepared the pesticide for application and 4130 $\mu\text{g/L}$ for the pesticide' applicant), both well-known commercial insecticides, were found in the saliva of North American and Nicaraguan workers (Lu et al. 2006; Nigg et al. 1993).

In summa, available information suggests saliva as an adequate and reliable fluid to assess recent occupational exposure to health-relevant inorganic and organic pollutants which will contribute to a better characterization of a subjects' total exposure at the working environment. This biological fluid can complement data obtained from conventional human biomonitoring surveys performed with urine and blood analysis. However, the main disadvantages of saliva as a biological fluid are related to its complex and dynamic (bio)chemical composition, water affinity, and the inter- and intra-individual variability. Therefore, more studies are urgently needed to better characterize workers' exposure through ingestion via saliva.

4 Conclusions

Information concerning the use of saliva as a complementary biological matrix in addition to urine and blood has been slowly emerging over the last decades. Despite limited, available studies demonstrated recent occupational exposure to metals and metalloids (Pb, Cd, Mn, Hg, Cu, Fe, Cs, Cr, Ni, and Zn), several VOCs (including PAHs), pesticides (atrazine, diazinon, and ethion), and tobacco smoke. Nevertheless, there are no occupational exposure limits defined for the presence of those pollutants in saliva. Biomonitoring through saliva not only demonstrates occupational exposure to health-relevant pollutants but also represents the fraction of xenobiotics that is available to be assimilated on the buccal cavity and to be distributed and/or bioaccumulated in the tissues of the human body. However, biomonitoring studies performed with saliva need to account for its dynamic (bio)chemical composition, the inter- and intra-individual variability, and the existence of dental and/or buccal diseases which may interfere with the levels of some xenobiotics. Therefore, future biomonitoring studies should include workers' biomonitorization through the collection of saliva and evaluate its potential for occupational safety and health surveillance.

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Chapter 1.4.

A review on skin metal concentrations in occupational environments

A review on skin metal concentrations in occupational environments

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Abstract

Introduction: Workers are exposed to various hazards in work environments. Human biomonitoring through dermal contact has been used to evaluate occupational exposure to health-relevant pollutants. However, dermal exposure to metals is overlooked. **Objectives:** This work assesses existing literature on dermal exposure to metals in occupational settings, identify sampling and analytical methods, and highlight associated health risks. **Methods:** Literature review was conducted using databases, focusing on studies, until January 2025 related to metal exposure in occupational environments via skin. After a full screen of literature, a total of 10 studies were compiled. **Results:** The dermal exposure to chromium, cobalt, nickel, and platinum has been documented across various occupational groups, varying according to the occupation. In the different studies, the skin deposition ranged from 0.00020 to 28 $\mu\text{g}/\text{cm}^2$ for cobalt; 0.0018 to 7.16 $\mu\text{g}/\text{cm}^2$ for nickel; 0.000040 to 0.58 $\mu\text{g}/\text{cm}^2$ for chromium; and 0.00030 to 0.577 $\mu\text{g}/\text{cm}^2$ for platinum. **Conclusion:** Dermal biomonitoring is valuable for assessing recent metal exposure at work and additional studies are needed to explore its contribution to total uptake. The assessment of dermal exposure has some challenges, and this non-invasive approach can be a useful tool to complement (blood/urine) biomonitoring, contributing to refine the exposure assessment strategies and improve the workplace safety and health.

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Keywords Occupational exposure; Human biomonitoring; Dermal contact; Skin; Occupational safety.

1. Introduction

Workers are daily exposed to various hazards originated from multiple sources that include physical, ergonomic, psychological/psychosocial, biological, and chemical risks. Chemical hazards, such as particulate matter, volatile organic compounds, and metals, are particularly concerning due to their potential to cause both short- and long-term health effects (WHO, 2002). Workers performing tasks that include handling and managing of metal-containing materials, such as dust, fumes, paints, wastes, solutions, *etc.*, face risks related to occupational exposure through different routes (Larese *et al.*, 2007). The occupational exposure to metals is most often associated with inhalation and ingestion pathways, however the dermal exposure also represents an important but underestimated route (Chamani *et al.*, 2023; Jaishankar *et al.*, 2014).

Human skin is the main protective barrier against external agents and the exposure through this route has attracted the attention of scientific community (Abolhasani *et al.*, 2021; WHO, 2015). The degree of dermal absorption depends on factors such as the chemical properties of the metal, the duration and frequency of exposure, the workplace hygiene, the environmental conditions as well as the individual skin state (Engebretsen *et al.*, 2016; Hostynek, 2003; Seurat *et al.*, 2021). The dermal contact with metals occurs when handling metals or metal products either directly or by deposition of the metal-containing PM/dust, leading to possible local irritation and allergic reactions (Jaishankar *et al.*, 2014; Kim *et al.*, 2015). For example, nickel (Ni) and chromium (Cr) are well-known sensitizers that can penetrate the skin and cause allergic reactions, even at low concentrations (Ahlström *et al.*, 2019; Bregnbak *et al.*, 2015). The systemic absorption of certain metals through

skin contribute to cumulative body burden, making essential to monitor the occupational exposure effectively and, if needed, support the enforcement of protective measures. Prolonged or repeated dermal exposure to metals, including lead (Pb), cadmium (Cd), Cr, cobalt (Co), and Ni compounds, increase the risk of chronic conditions such as allergic contact dermatitis, neurotoxicity and cancer development (Chamani *et al.*, 2023; Filon *et al.*, 2004).

Human biomonitoring is a valuable tool for assessing exposure to occupational hazards, including chemical risks, as well as to evaluate the effectiveness of preventive measures and safety protocols. Traditionally, biomonitoring assays relies on the assessment of biological fluids such as blood and urine for internal dose measurements, which reflect the total internal burden (Hopf *et al.*, 2024; Julander *et al.*, 2010; Oliveira *et al.*, 2020; WHO, 2015). Human biomonitoring through skin exposure has been used to evaluate the dermal levels of hazardous pollutants, such as volatile organic compounds, including polycyclic aromatic hydrocarbons, heavy metals, flame retardants, phthalate esters and particulate matter (Abolhasani *et al.*, 2021; Araviiskaia *et al.*, 2019; Giovanoulis *et al.*, 2018; Lv *et al.*, 2024; Sousa *et al.*, 2023). Some authors also use dermal exposure to evaluate the correct use and effectiveness of work protective equipment (Banks *et al.*, 2021; Engelsman *et al.*, 2020). Due to that, there is a growing interest in monitoring the dermal exposure in different occupational environments such as metal refineries, waste recycling units, chemical industry, and firefighting scenarios (Julander *et al.*, 2010; Lv *et al.*, 2024; Sousa *et al.*, 2022; WHO, 2015). Regarding exposure to metals, different sampling methods have been described in literature, including tape stripping, skin dipping, and skin wipe sampling, which are able to collect particles and metal residues present on skin surface (Therkorn *et al.*, 2024).

This work aims to review the existing literature related to dermal exposure to metals in occupational settings, with a focus on exposure assessment strategies, skin contamination levels, and associated health risks.

2. Materials and Methods

A literature review was conducted using various online databases, namely ISI Web of Knowledge, Science Direct, Google Scholar, and PubMed. The search focused on identifying studies related to dermal exposure to metals in occupational settings. The keywords "occupational exposure", "dermal exposure", "skin", "metals", and "biomonitoring" were combined in different configurations by using the term "AND" to ensure a more complete search. The following inclusion criteria were applied: i) original research articles published in indexed, peer-reviewed scientific journals, ii) studies involving the assessment or measurement of metal exposure through dermal contact, and iii) research conducted within occupational environments. Studies published up to January 2025 were screened based on abstracts, duplicates were eliminated, and a total of 10 research articles were retrieved from

the literature characterizing workers dermal exposure to metals. Since the information on this subject is scarce, articles with distinct collection, analytical methods, and measurement units were included. Additional studies were also selected to support the discussion of results.

3. Results and discussion

The available literature characterizes the dermal exposure with metals in North American ($n=1$), European ($n=7$), Indonesian ($n=1$), and South African ($n=1$) workers; no information is available for other geographic locations. The collection methods used across all the mentioned studies were similar and mostly consisted of acid wipe sampling ($n=6$), dry wipe sampling ($n=1$), wet wipe sampling ($n=1$), finger immersion ($n=1$), and sampling using a mixed cellulose ester filter ($n=1$). The collection of samples was performed during the shifts and/or immediately after the end of shift. The quantification of metals on skin samples were inductive coupled plasma – mass spectrometry ($n=8$), inductively coupled plasma – optical emission spectroscopy ($n=1$), and X-Ray fluorescence ($n=1$) (Day *et al.*, 2009; Jensen *et al.*, 2011; Julander *et al.*, 2010; Kettelarij *et al.*, 2018, 2016; Klasson *et al.*, 2017; Lidén *et al.*, 2008; Linde *et al.*, 2018; Oginawati *et al.*, 2022; Staton *et al.*, 2006).

Table 1 presents the data, organized by element, on workers' skin contamination with metals extracted from available studies. Overall, studies reported the workers skin contamination with Cr ($n=4$), Co ($n=6$), Ni ($n=6$), manganese (Mn) ($n=1$), iron (Fe) ($n=1$), copper (Cu) ($n=1$), zinc (Zn) ($n=1$), Pb ($n=1$), and platinum (Pt) ($n=1$), noting that most of the studies presented dermal concentrations values for at least 2 or more metals (Table 1).

Table 1. Concentrations (minimum – maximum) of workers' skin contamination with metals, organized by element and expressed as $\mu\text{g}/\text{cm}^2$, excepted indicated otherwise.

Country	Occupation (n)	Skin levels	Reference
<i>Chromium</i>			
Sweden	Gas turbines/space propulsors production workers (24)	Forehead: 0.000040 – 0.0030*	Julander <i>et al.</i> , 2010
		Back of hand: 0.00016 – 0.030*	
		Palm: 0.00069 – 0.085*	
		Thumb: 0.0021 – 0.47* (index/middle) fingers: 0.0015 – 0.58*	
Sweden	Carpenters, locksmiths, cashiers, and secretaries (18)	Hands: 0.0002 – 0.146	Lidén <i>et al.</i> , 2008

USA	Cemented tungsten carbide workers (41)	Hands: 0.5 – 101** Neck: 0.3 – 39**	Day <i>et al.</i> , 2009
Sweden	Dental technicians (13)	(index finger): 0.012 – 0.19	Kettelarij <i>et al.</i> , 2016
<i>Manganese</i>			
Indonesia	Batik industry workers (Canting, Stamping, and Dyeing processes) (30)	Hand: 0.00346 - 0.00678	Oginawati <i>et al.</i> , 2022
<i>Iron</i>			
Indonesia	Batik industry workers (Canting, Stamping, and Dyeing processes) (30)	Hand: 0.0295 - 0.1326	Oginawati <i>et al.</i> , 2022
<i>Cobalt</i>			
Sweden	Hard metal production workers (40)	(index) finger: 0.0019 – 28	Kettelarij <i>et al.</i> , 2018
Sweden	Gas turbines/space propulsors production workers (24)	Forehead: <LOD – 0.013* Back of hand: <LOD – 0.051* Palm: 0.00054 – 0.26* Thumb: 0.0040 – 4.5* (index/middle) fingers: 0.0025 – 2.5*	Julander <i>et al.</i> , 2010
Sweden	Carpenters, locksmiths, cashiers, and secretaries (18)	Hands: 0.0002 – 0.011*	Lidén <i>et al.</i> , 2008
Sweden	Hard metal production workers (72)	Hand: 0.046 – 100*	Klasson <i>et al.</i> , 2017
USA	Cemented tungsten carbide workers (41)	Hands: 4.1 – 4285** Neck: 0.5 – 693**	Day <i>et al.</i> , 2009
Sweden	Dental technicians (13)	(index) finger: 0.032 – 1.6	Kettelarij <i>et al.</i> , 2016
<i>Nickel</i>			
United Kingdom	Coin handling in various occupations (44)	Fingers: 0.0018 – 7.16	Staton <i>et al.</i> , 2006

Sweden	Gas turbines/space propulsors production workers (24)	Forehead: <LOD – 0.092* Back of hand: <LOD – 0.037* Palm: 0.0033 – 5.3* Thumb: 0.025 – 8.3* (index/middle) fingers: 0.021 – 15*	Julander <i>et al.</i> , 2010
Sweden	Carpenters, locksmiths, cashiers, and secretaries (18)	Hands: 0.003 – 2.070	Lidén <i>et al.</i> , 2008
Indonesia	Batik industry workers (Canting, Stamping, and Dyeing processes) (30)	Hand: 0.00184 - 0.00211	Oginawati <i>et al.</i> , 2022
USA	Cemented tungsten carbide workers (41)	Hands: 1.6 – 488** Neck: 0.2 – 137**	Day <i>et al.</i> , 2009
Denmark	Psychiatric nurses, industrial workers, dress-makers, sandwich makers, and carpenter/house painters (6)	(index) finger: 0.047 – 0.29 Palm: 0.0097 – 0.037	Jensen <i>et al.</i> , 2011
Sweden	Dental technicians (13)	(index) finger: 0.0076 – 0.27	Kettelarij <i>et al.</i> , 2016
<i>Copper</i>			
Indonesia	Batik industry workers (Canting, Stamping, and Dyeing processes) (30)	Hand: 0.00083 - 0.00929	Oginawati <i>et al.</i> , 2022
<i>Zinc</i>			
Indonesia	Batik industry workers (Canting, Stamping, and Dyeing processes) (30)	Hand: 0.00941 - 0.1023	Oginawati <i>et al.</i> , 2022
<i>Lead</i>			
Indonesia	Batik industry workers (Canting, Stamping,	Hand: 0.00588 - 0.00691	Oginawati <i>et al.</i> , 2022

and Dyeing processes) (30)			
<i>Platinum</i>			
South Africa	Metal refinery workers (40)	Total: 0.0003 – 0.577	Linde <i>et al.</i> , 2018

*Concentration of metal expressed as $\mu\text{g}/\text{cm}^2$ per hour of work; **Mass of metal (μg) in the skin of worker.

Julander *et al.* (2010) measured the total Cr levels in the skin of Swedish workers from a gas turbines/space propulsors production plant, reporting values between $0.000040 \mu\text{g}/\text{cm}^2/\text{h}$, in the forehead of a tool sharpening worker, and $0.58 \mu\text{g}/\text{cm}^2/\text{h}$ in the index and middle fingers of an individual working with space propulsion components. In this case, the workers were asked to not wear gloves during the 2-hours exposure period, justifying the increased levels of this metal in their fingers and palm. Lidén *et al.* (2008) measured the skin levels of Cr in Swedish individuals from various occupations, reporting concentrations between $0.0002 \mu\text{g}/\text{cm}^2/\text{h}$ in the hands of cashiers to $0.146 \mu\text{g}/\text{cm}^2/\text{h}$ in locksmiths (Lidén *et al.*, 2008). In another study, Day *et al.* (2008) analysed the mass of Cr present in hands and necks of USA citizens working in cemented tungsten carbide factory and verified that the values varied from 0.3 to 101 μg , in the neck of workers from the forming/machining process and the hands of production control workers, respectively. Also, Kettelarij *et al.* (2016) monitored the skin deposition of Cr in Swedish dental technicians that handled a CoCr alloy and reported levels between 0.012 and $0.19 \mu\text{g}/\text{cm}^2$.

Regarding the dermal exposure to Co, Kettelarij *et al.* (2016, 2018) assessed the skin contamination in workers from two different occupational groups. In Swedish dental technicians exposed to CoCr alloy, the concentrations ranged from 0.032 to $1.6 \mu\text{g}/\text{cm}^2$ (Kettelarij *et al.*, 2016). The authors also evaluated the hard metal production workers and found Co concentrations in the range between $0.0019 \mu\text{g}/\text{cm}^2$ in the skin of office workers to $28 \mu\text{g}/\text{cm}^2$ in the individuals working in the raw materials section (Kettelarij *et al.*, 2018). Regarding workers enrolled in gas turbines/space propulsors production, some authors observed Co concentrations between $0.00054 \mu\text{g}/\text{cm}^2/\text{h}$ in the palms of a worker from the space propulsion components unit to $4.5 \mu\text{g}/\text{cm}^2/\text{h}$ in the thumb of a tool sharpening worker (Julander *et al.*, 2010). Lidén *et al.* (2008) characterized the skin contamination with Co and reported values varying from $0.0002 \mu\text{g}/\text{cm}^2/\text{h}$ in the hands of carpenters and locksmiths and $0.011 \mu\text{g}/\text{cm}^2/\text{h}$ in cashier subjects. In a group of workers from a hard metal production plant, Klasson and coworkers (2017) measured Co skin concentrations and attested values ranging between $0.046 \mu\text{g}/\text{cm}^2/\text{h}$ in workers from the charging/discharging sector and $100 \mu\text{g}/\text{cm}^2/\text{h}$ in those working at pressing departments. A study performed with USA workers from a cemented tungsten carbide factory assessed the Co skin exposure and reported mass values varying between 0.5 and 4285 μg per hand, in administration and production control workers (Day *et al.*, 2009).

In what concerns to the dermal exposure to Ni, Staton and colleagues (2006) characterized workers from several occupations commonly associated with handling coins (shop assistants, store workers, hairdressers, bar staff, nickel refinery workers, and nickel platers) in the United Kingdom. The authors found Ni skin levels varying between $0.0018 \mu\text{g}/\text{cm}^2$ in the hands of a hair stylist to $7.16 \mu\text{g}/\text{cm}^2$ in workers from Ni-chrome plater (Staton *et al.*, 2006). Julander *et al.* (2010) performed measurements of Ni in the skin of Swedish workers attending a gas turbines/space propulsors production plant and reported concentrations ranging from 0.00051 and $15 \mu\text{g}/\text{cm}^2/\text{h}$ in the back of the hands of a worker from space propulsion components department and reference finger from a worker in the department of thermal application of surface layers. A study performed in the hands of Swedish secretaries and cashiers also found Ni skin levels varying from 0.003 to $2.070 \mu\text{g}/\text{cm}^2/\text{h}$ (Lidén *et al.*, 2008). In Indonesian Batik production workers, the hands were contaminated with Ni at levels ranging between 0.00184 (stamping process) and 0.00211 (dyeing process) $\mu\text{g}/\text{cm}^2$. USA subjects working in a cemented tungsten carbide factory presented Ni skin values ranging between $0.2 \mu\text{g}$ in the neck of administration workers and $488 \mu\text{g}$ in the hand of maintenance workers from the metal separation department (Day *et al.*, 2009). Jensen *et al.* (2011) measured the levels of Ni in the skin of Danish workers (psychiatric nurses, industrial workers, dressmakers, sandwich makers, and carpenter/house painters) with diagnosed Ni allergy-associated hand dermatitis. The authors reported Ni levels varying between 0.0097 and $0.29 \mu\text{g}/\text{cm}^2$ in the palm and index finger of a dressmaker, respectively (Jensen *et al.*, 2011). Swedish dental technicians managing CoCr alloy presented hand Ni concentrations varying from 0.0076 to $0.27 \mu\text{g}/\text{cm}^2$ (Kettelarij *et al.*, 2016). To the best knowledge of these authors, skin contamination with Mn, Fe, Cu, Zn, and Pb were only characterized by Oginawati *et al.*, (2022) at values ranging from 0.00346 (stamping) - 0.00678 (dyeing) $\mu\text{g}/\text{cm}^2$, for Mn; 0.0295 (stamping) - 0.1326 (dyeing) $\mu\text{g}/\text{cm}^2$, for Fe; 0.00083 (stamping) - 0.00929 (dyeing) $\mu\text{g}/\text{cm}^2$, for Cu; 0.00941 (canting) - 0.1023 (dyeing) $\mu\text{g}/\text{cm}^2$, for Zn; and 0.00588 (canting) - 0.00691 (stamping) $\mu\text{g}/\text{cm}^2$, for Pb. Also, only one study assessed the skin contamination with Pt in South African workers from a metal refinery (Table 1). Linde *et al.* (2018) found Pt concentrations varying between $0.0003 \mu\text{g}/\text{cm}^2$ in a worker from the precious metals department and $0.577 \mu\text{g}/\text{cm}^2$ in subjects attending the crushing and ignition department. Prolonged exposure to this metal can cause skin dermatitis, eczema, urticaria, *etc.* (Heederik *et al.*, 2016).

Some heavy metals and metal(loid)s rank among the most prevalent and hazardous chemical dangers found in the workplace (WHO, 2002). Individuals working in mining, metal and chemical industries face heightened exposure to these pollutants, which significantly increases the potential for the development of short- and long-term health risks. Some elements are classified as known (arsenic (As), Cd, Cr (VI), or Ni) or probable (Pb, or Co) human carcinogens, and the exposure can contribute to kidney and intestinal dysfunctions as well as neurological conditions, such as Parkinson's disease (Huat *et al.*, 2019; WHO, 2002; IARC, 2021). Regarding skin

exposure, three metals stand out, namely Cr, Co, and Ni, which are commonly released during metal working processes. These metals are known to cause skin lesions, such as irritant and allergic dermatitis, ulceration, chronic eczema, and itching/rashes (Jaishankar *et al.*, 2014; Julander *et al.*, 2010). In fact, Jensen *et al.* (2010) characterized individuals that had previously experienced Ni allergy, while Linde *et al.* (2018) worked with one patient (out of the 40 that participated) that had minor eczema-type symptoms from exposure to Pt in the past (Jensen *et al.*, 2011; Linde *et al.*, 2018).

The data available suggest that occupational biomonitoring assays to monitor workers' recent exposure to metals can be achieved through wiping/cleansing of workers exposed skin areas. The skin levels of metals can provide a reliable indicator of the short-term exposure when compared to systemic fluids like blood, where half-lives for some metals [~ 60 h for Ni, ~ 3 d for Co, ~ 17 h for Cr(III), and ~ 39 h for Cr(VI)] are significantly longer (Kerger *et al.*, 1996; Min *et al.*, 2024; Tvermoes *et al.*, 2014). Dermal biomonitoring presents some challenges that explain the scarce information available in the literature. The human skin absorption variability is very complex due to the differences in the skin type and the structural composition of skin, including its lipidic and protein contents that vary substantially between skin areas (*e.g.*, neck, hands, and wrists) and from person to person. Factors such as temperature, humidity, and duration of dermal contact can significantly impact dermal exposure (Naylor *et al.*, 2021). Moreover, variability in skin hydration, integrity (*e.g.*, abrasions or dermatitis), occupational contact with different contaminants, or interference from each individuals' personal hygiene (*e.g.* how frequently workers wash their hands) and habits (*e.g.* hand-to-mouth transfer) can influence the distribution and concentration of metals, posing potential challenges to standardizing skin-based biomonitoring methods (Engebretsen *et al.*, 2016; Seurat *et al.*, 2021). These challenges make it difficult to draw generalized conclusions. Therefore, dermal biomonitoring is most effective when integrated with other biological matrices (*e.g.*, blood, urine, hair), as it provides a comprehensive assessment of total exposure rather than focusing solely on localized metal exposure. Understanding these dynamics is crucial for developing effective biomonitoring protocols and risk management strategies, such as improved personal protective equipment, proper hygiene practices, routine dermal biomonitoring to calculate day-to-day average results instead of sparse monitoring episodes, and development of guidelines for skin metal concentrations in workplaces.

4. Conclusions

The information on dermal exposure to metals remains limited, with a small number of studies measuring the metal concentrations on the skin of workers handling materials containing Cr, Co, Mn, Fe, Ni, Cu, Zn, Pb, and Pt. Despite the data scarcity, the available studies demonstrate the relevance of skin as a direct exposure route to

metals in different occupational environments. Dermal biomonitoring offers distinct advantages, including its non-invasive nature and capacity to reflect recent and localized exposure. Therefore, it can be used as a complementary tool to traditional biomonitoring assays that evaluate blood and/or urine, allowing the generation of knowledge that can support dermal health risk analysis. However, different challenges need to be explored, including the variability in skin composition, hydration, and contamination, to improve reliability and comparability of results. Additional studies are needed to better characterize workers' exposure to metals through dermal contact. These studies will contribute to refining dermal exposure assessment strategies, standardize collection and analytical methods, and define occupational guidelines to protect workers from dermal absorption-related health risks. Integrating dermal biomonitoring into comprehensive exposure assessment frameworks will enhance occupational safety and workers health surveillance.

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Chapter 1.5.

Exposure to PAHs during firefighting activities: A review on skin levels, *in vitro/in vivo* bioavailability, and health risks



Review

Exposure to PAHs during Firefighting Activities: A Review on Skin Levels, In Vitro/In Vivo Bioavailability, and Health Risks

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Abstract: Occupational exposure as a firefighter is a complex activity that continuously exposes subjects to several health hazards including fire emissions during firefighting. Firefighters are exposed to polycyclic aromatic hydrocarbons (PAHs), known as toxic, mutagenic, and carcinogenic compounds, by inhalation, dermal contact, and ingestion. In this work, a literature overview of firefighters' dermal exposure to PAHs after firefighting and data retrieved from skin in vitro/in vivo studies related to their dermal absorption, bioavailability, and associated toxicological and carcinogenic effects are reviewed. The evidence demonstrates the contamination of firefighters' skin with PAHs, mainly on the neck (2.23–62.50 ng/cm²), wrists (0.37–8.30 ng/cm²), face (2.50–4.82 ng/cm²), and hands (1.59–4.69 ng/cm²). Concentrations of possible/probable carcinogens (0.82–33.69 ng/cm²), including benzo[a]pyrene isomers, were found on firefighters' skin. PAHs penetrate the skin tissues, even at low concentrations, by absorption and/or diffusion, and are locally metabolized and distributed by the blood route to other tissues/organs. Lighter PAHs presented increased dermal permeabilities and absorption rates than heavier compounds. Topical PAHs activate the aryl hydrocarbon receptor and promote the enzymatic generation of reactive intermediates that may cause protein and/or DNA adducts. Future research should include in vitro/in vivo assays to perform a more realistic health risk assessment and to explore the contribution of dermal exposure to PAHs total internal dose.

Keywords: dermal exposure; firefighters; polycyclic aromatic hydrocarbon; fire combat; dermal absorption; skin diseases

1. Introduction

Human exposure to air pollution is recognized as the single biggest threat to human health by causing millions of deaths and loss of healthy life annually [1]. Human environmental and occupational exposure to airborne pollutants through inhalation have been extensively characterized over the last decades [2–6]. At the interface with the air, skin, the largest organ of the human body, is the first-line barrier of protection against health-hazardous compounds. The dermal contact with pollutants may activate cell metabolism and promote oxidative stress and inflammatory processes; however, their impact on skin remains poorly explored [7–11].

Skin comprises three layers, namely, the epidermis, dermis, and hypodermis, and its normal barrier function can be altered through a disturbance of lipid metabolism at the surface layers. This organ can be damaged by exposure to different external factors, such as solar ultraviolet (UV) radiation, microorganisms, dehydration, and air pollutants (e.g.,

particulate matter, volatile organic compounds including polycyclic aromatic hydrocarbons (PAHs), carbon monoxide, and ozone) [7–9,11,12]. After dermal contact, air pollutants can be absorbed and/or dissolved on the outermost layer of the epidermis containing different proportions of sebum and sweat. Keratinocytes represent more than 90% of the epidermis cells and have an important role in antioxidant defense, since they can enzymatically metabolize organic pollutants, being directly involved in the response to dermal toxicity and inflammation [13–15]. The dermis and hypodermis can also be contaminated with pollutants assimilated by other routes of exposure, e.g., inhalation and ingestion, and afterwards, xenobiotics are transported within the blood system (systemic contamination) and distributed to the different tissues and organs [7].

PAHs are formed during the incomplete combustion of organic matter and can be released from natural (e.g., volcanoes and forest fires) and anthropogenic (e.g., traffic emissions, production of energy from fossil fuels, agricultural activities, heating, and cooking) sources [16]. These health-relevant persistent organic pollutants are well known for their toxic, carcinogenic, and mutagenic properties [17–19]. PAHs are lipophilic compounds, particularly those with high molecular weight, and they tend to accumulate in fat tissues. Besides this, PAHs are also known as potential endocrine disruptors [16,20]. The exposure to PAHs has been associated with reproductive, developmental, and hemato-, cardio-, neuro-, and immunotoxicity effects [16]. Evidence related to the interaction between PAHs and the skin has been slowly emerging during the last decade. The normal barrier function of the skin can be affected by disorders on the lipid metabolism in the stratum corneum layer, dysfunction of the deoxyribonucleic acid (DNA), and/or disorders of the skin's protein composition [8]. Dermal contact with PAHs represents an additional risk to skin tissues; however, the link between topical concentrations and cutaneous inflammation remains poorly characterized [13,21,22]. PAHs can permeate the skin barrier and promote the formation of reactive oxygen species (ROS), which stimulate oxidative stress, being responsible for the development and/or exacerbation of inflammatory skin diseases (e.g., atopic and contact dermatitis, eczema, psoriasis, acne, and skin aging) [7–9,11,21]. The formation of ROS is triggered by the aryl hydrocarbon receptor (AhR)-dependent induction of CYP1 isoforms, since PAHs are AhR activators [9,10,23]. During the enzymatic metabolism of PAHs on topical tissues, different intermediate compounds, such as epoxides and diols, are formed [24]. These reactive intermediates can induce mutagenesis and the formation of tumors by binding to cell proteins and DNA, which might contribute to the development of skin cancer [8,9]. Some authors already described a synergetic effect between the dermal exposure to PAHs and UV radiation and the acceleration of skin diseases, including cancer [7,25].

In June 2022, firefighters' occupational exposure to PAHs was classified as known carcinogen to humans (Group 1) by the International Agency for Research on Cancer (IARC) [26]. The available literature demonstrated sufficient evidence for the development of mesothelioma and bladder cancer in humans and limited evidence for the development of melanoma, colon, prostate, and testicular cancers, and non-Hodgkin's lymphoma [26–28]. During fire combat, firefighters are regularly exposed to PAHs, known/possible/probable carcinogens [18,19], via inhalation, dermal contact, and ingestion [28–33]. Dermal contact with the PAHs released from fires occurs not only during firefighting, despite the correct use of personal protective equipment (PPE), but also during the donning and/or doffing of PPE and contact with contaminated firefighting materials and vehicles [30]. Information has been slowly emerging during the last decade [29,34–39]. Despite the contamination of firefighters' skin with PAHs released from firefighting being demonstrated, scarce information is available relating to the transcutaneous penetration of PAHs, dermal bioaccessibility and bioavailability, and the consequent health risks. Therefore, this work aims to: (i) extract data relating to dermal exposure to PAHs after firefighting events from the available studies, and (ii) collect the information retrieved from dermal *in vitro*/*in vivo* studies that determine parameters conditioning the topical uptake of PAHs, rates of permeability, bioavailability, and toxic and carcinogenic effects after dermal exposure to PAHs. Further studies concern-

ing this issue should be addressed, since a broad multidisciplinary knowledge is urgently needed to better understand the topical assimilation of PAHs and the metabolic processes that occur within skin tissues. These studies will generate knowledge that would contribute to a more realistic health risk assessment, to define the critical levels for firefighters' dermal exposure, and to evaluate the efficiency of the available dermal preventive measures.

2. Methods

A literature search was conducted using several online databases: ISI Web of Knowledge, Science Direct, PubMed, Google Scholar, Scopus, and Scielo. Occupational exposure, firefighters, dermal exposure, PAHs, skin in vitro/in vivo studies, bioavailability, and bioaccessibility were the keywords used in the search. All of the available studies published up to 2021 were considered. In total, 13 studies reporting the concentrations of PAHs on the skin of firefighters after firefighting were selected for analysis. Regarding the in vivo/in vitro studies, all of the reports that described the dermal exposure to PAHs using animal and/or human skin models were considered. A total of 19 studies were retrieved from the literature, with eight characterizing dermal exposure to PAHs, four addressing bioavailability, and seven concerning toxicological and carcinogenic potential of PAHs on skin tissues.

3. Concentrations of PAHs on the Skin of Firefighters

3.1. Levels of Total PAHs

A total of 13 studies assessed the firefighters' dermal exposure during firefighting activities at structural, controlled, and training fire exercises; only two studies evaluated skin contamination from live fire scenarios [34,40]. However, one study [34] presented data in $\mu\text{g/wipe}$, a concentration unit that is not comparable with the results reported in other studies. The available studies characterized the exposure of Nordic (50%), North American (42%), and Australian (8%) firefighters and represented periods of exposure to fire emissions up to 30 min. The eligible studies evaluated skin contamination on the neck ($n = 7$), wrist and/or arm ($n = 4$), hand, including fingers ($n = 4$), face and/or forehead ($n = 2$), calf ($n = 1$), scrotum ($n = 1$), and back ($n = 1$) of firefighters.

Overall, the concentrations of total PAHs on the skin of firefighting forces ranged from 4.00 ng/cm^2 , in a Swedish study with 20 subjects during training exercises [37], to 1200 ng/cm^2 , during training activities performed by 19 firefighters at a fire house containing conifer plywood [41]. Keir et al. [36] reported significantly increased concentrations of total PAHs (pre- to post-exposure change of 3.3 times: 0.5 versus 1.6 ng/cm^2) on the neck, wrist, and forehead skin of 28 firefighters, after the suppression of an emergency fire. Sjöström et al. [29] determined the levels of PAHs on the skin of the wrists and neck of seven firefighters performing three different roles in fire events: team leaders responsible for the supervision of fire simulations, who stood far from the fire line; fire starters who initiated the fire exercise; and firefighters who fought the fire. The authors found significantly increased concentrations of total PAHs on the skin of the fire starters (20.42 ng/cm^2) than on the skin of the smoke divers (7.39 ng/cm^2) and team leaders (7.00 ng/cm^2). In the study performed by Andersen et al. [40], nine firefighters were enrolled in small emergency fire calls to an outdoor fire in a car and a fire in an electrical installation of a basement. The level of skin exposure to total PAHs reported after the events was, on average, 18.1 ng/cm^2 ; no differences were observed between pre- and post-shift levels. In 2014, Fent and coworkers reported lower concentrations of total PAHs on the skin regions (arm, neck, scrotum, hand, and face) of subjects participating in round 1 compared to those participating in round 2 controlled structural burns [35]. In that study, the round 1 fire burns were slightly longer than in round 2, and distinct fire scenarios were considered. In the round 1 fire scenario, the firefighters were stagnant, and the timber-framed room with a drywall interior was more than the double that of the round 2 metallic room. These changes contributed to different fire emissions and a less-concentrated smoke layer was observed in the first round. Moreover, subjects of the second round of controlled burns used brand new hoods that were approximately 7.5 cm longer [35]. In another study, Laitinen et al. [41] evaluated the

dermal exposure of 19 firefighters during fire training exercises with a sampler located under the PPE and on the surface of the chest and the back skin of participants. The authors reported mean values of dermal total PAHs of 1200 ng/cm² for the fire house containing conifer plywood, 760 ng/cm² for the exercise in the container with conifer plywood, and 30 ng/cm² in event with a gas simulator.

Figure 1 presents the levels of total PAHs on different skin regions of firefighters after firefighting. The evidence demonstrates that the neck/collarbone was the most characterized body site, with concentrations ranging from 2.23 ng/cm², during a 15 min controlled compartment fire with diesel [42], to 62.50 ng/cm², in a training exercise lasting up to 10 min [43] (Figure 1). Some authors reported increased values of total PAHs on the neck of exposed firefighters compared to other body regions [35,37,42,44]. Moreover, Fent et al. [35] concluded that neck is the most likely region to be contaminated with fire emissions, since other body parts are usually covered with multiple layers of clothes and/or the PPE. However, in a Swedish study performed by Sjöström et al. [29], higher values of PAHs were found on the wrist skin compared with the neck, across all of the firefighters participating on a training exercise: 16.07 versus 4.35 ng/cm² for fire starters, 4.25 versus 3.14 ng/cm² for the smoke divers, and 4.69 versus 2.31 ng/cm² for the team leaders. Andersen et al. [43] reported the highest concentrations of total PAHs on the necks of firefighters during different training exercises, principally during Campaigns 1 and 2 with the burning of wood pallets, while Campaigns 3 and 4 contained the burning of electrical cords and mattresses (Figure 1). Banks et al. [42] reported lower concentrations of PAHs (2.23 versus 3.06 ng/cm²) on the neck skin of Australian firefighters participating in a diesel-based fire than in a wood conglomerate simulated fire. The authors attributed the observed differences to the composition of the chipboard material burned: wood particles (>85%), glued with melamine/urea/formaldehyde resin (<13%), paraffin wax (<2%), and formaldehyde (0.0001%) that might release higher levels of PAHs. Similar results were also reported by Wingfors et al. [37].

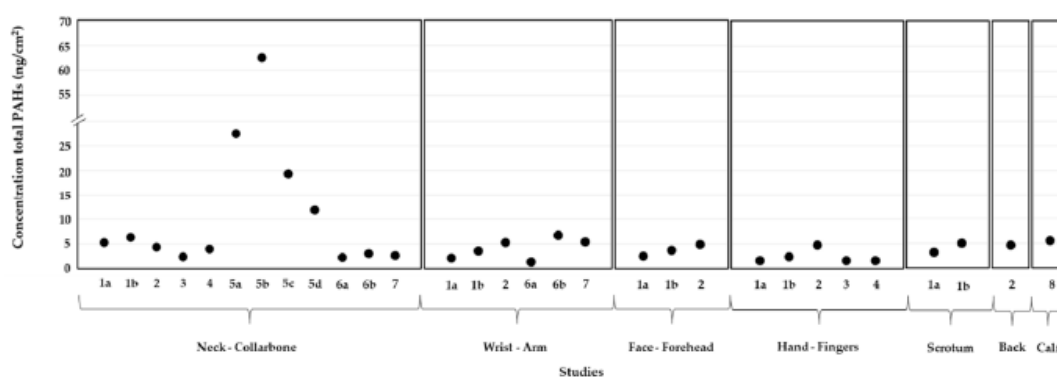


Figure 1. Concentrations of total PAHs on the skin of firefighters after firefighting activities (1—Fent et al. [35] (a—Round 1 fire; b—Round 2 fire); 2—Fernando et al. [45]; 3—Fent et al. [44]; 4—Wingfors et al. [37]; 5—Andersen et al. [43] (a—Campaign 1; b—Campaign 2; c—Campaign 3; d—Campaign 4); 6—Banks et al. [42] (a—diesel pan fire; b—particleboard fire); 7—Strandberg et al. [46]; 8—Beitel et al. [32]).

The levels of total PAHs were also reported on the wrist/arm (0.37 to 8.30 ng/cm²), face/forehead (2.50 to 4.82 ng/cm²), and hands/fingers (1.59 to 4.69 ng/cm²) of exposed firefighters (Figure 1). Only one study assessed the levels of PAHs on the skin of the scrotum of 15 firefighters involved in two controlled fires that lasted between 15 and 30 min [35]. The concentrations of total PAHs on the skin of the scrotum were increased in round 2 (5.04 ng/cm²; intermodal metal room with 15 m³ and mobile firefighters) than in round 1 (3.14 ng/cm²; 33 m³ timber-framed burn room and stationary subjects) (Figure 1).

Moreover, the authors observed increased levels of PAHs on the skin of the neck, wrist, face, and hands of firefighters involved in the burns of round 2 (Figure 1). A study performed by Fernando et al. [45] with 28 volunteer firefighters attending a fire with untreated wood (≈ 30 min) reported total PAH values of 4.58 ng/cm^2 on the skin of the back (Figure 1). Beitel et al. [32] determined the levels of total PAH (mean of 5.58 ng/cm^2) on the calf of 11 subjects involved in a controlled indoor fire that lasted 14 min.

Among all of the PAHs reported in the available literature, phenanthrene, fluoranthene, and pyrene were the main contributors to the total PAH concentrations found on the skin of the firefighters, regardless of the type of fire and the body site monitored [29,37,42,43,45].

3.2. Levels of Pyrene

Figure 2 presents the concentrations of pyrene, a biomarker of PAHs exposure, determined on the skin of firefighters after fire combat. Overall, the total levels of pyrene on the skin ranged between 0.70 ng/cm^2 , in a training exercise with 20 Swedish firefighter students [37], and 7.46 ng/cm^2 , in the second campaign of a training exercise with 53 Danish firefighters [43]. Banks et al. [42] determined the concentrations of pyrene on the skin of the neck and wrist of 25 subjects after firefighting at simulated compartment fires. Skin contamination with pyrene was lower after a diesel-based fire than after a particleboard fire event (Figure 2). Moreover, the reported wrist values of pyrene (1.3 ng/cm^2) were significantly higher at the particleboard fire than the levels (0.10 ng/cm^2) determined during the diesel pan fire; no differences were observed in the concentrations of pyrene on the neck skin (0.74 ng/cm^2) [42]. Sjöström et al. [29] reported increased levels of pyrene on the skin of fire starters (total of 3.68 ng/cm^2 , 2.97 on the wrists and 0.71 ng/cm^2 on the neck) than for fire combatants (1.25 ng/cm^2 : 0.76 and 0.49 ng/cm^2) and team leaders (1.09 ng/cm^2 : 0.78 and 0.31 ng/cm^2) after participation in training exercises. Fernando et al. [45] assessed the levels of pyrene in five different skin regions of 28 firefighters (total of 1.50 ng/cm^2) involved in fire training exercises with untreated wood (Figure 2). The authors found that the skin on the fingers presented the highest levels of pyrene (0.41 ng/cm^2), followed by the neck (0.34 ng/cm^2), forehead (0.30 ng/cm^2), back (0.23 ng/cm^2), and wrist (0.22 ng/cm^2).

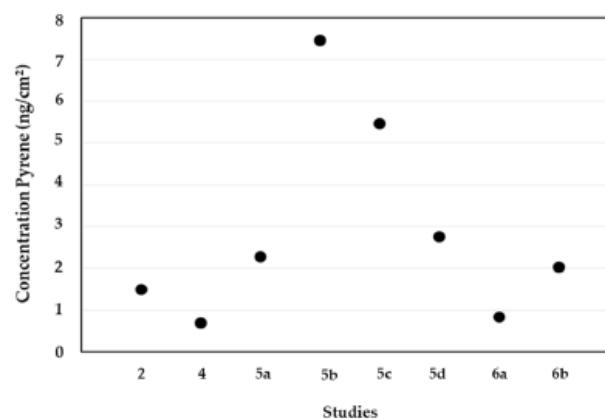


Figure 2. Concentrations of pyrene on the skin of firefighters after firefighting activities (2—Fernando et al. [45] (total of neck, wrist, back, forehead, and fingers); 4—Wingfors et al. [37] (neck); 5—Andersen et al. [43] (a—Campaign 1; b—Campaign 2; c—Campaign 3; d—Campaign 4) (neck); 6—Banks et al. [42] (a—diesel pan fire; b—particle board fire) (total of neck and wrist)).

3.3. Levels of Carcinogenic PAHs

Benzo(a)pyrene is the only PAH classified as a known carcinogen to humans by the IARC [18]. The same agency classified some PAHs, namely, naphthalene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene,

indeno(1,2,3-c,d)pyrene, and dibenz(a,h)anthracene, as possible/probable carcinogens to humans [18,19]. The available studies included the abovementioned compounds, with exception of Fent et al. [35] (due to some matrix interferences caused by the use of corn oil at sampling collection). However, only some authors reported the individual concentrations of possible/probable carcinogenic PAHs [29,42,43,45]. Figure 3 presents the concentrations of total carcinogenic PAHs on the skin regions of firefighters after fire combat. Overall, the concentrations ranged from 0.82 ng/cm², on the skin of 25 Australian firefighters attending a controlled diesel pan fire, to 33.69 ng/cm², in the second campaign of a fire with wood pallets (Figure 3).

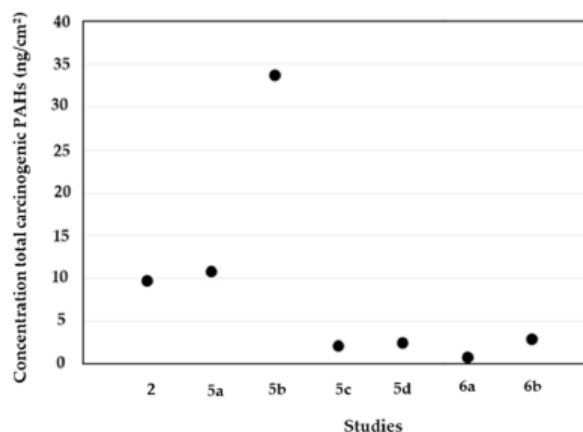


Figure 3. Concentrations of total carcinogenic PAHs (benzo(a)pyrene, naphthalene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, indeno(1,2,3-c,d)pyrene and dibenzo(a,h)anthracene) on the skin of firefighters after firefighting activities (2—Fernando et al. [45] (neck, wrist, back, forehead, and fingers); 5—Andersen et al. [43] (a—Campaign 1; b—Campaign 2; c—Campaign 3; d—Campaign 4) (neck); 6—Sjöström et al. [29] (neck); 6—Banks et al. [42] (a—diesel pan fire; b—particleboard fire) (neck and wrist)).

Banks et al. [42] presented lower values of carcinogenic PAHs on the skin (neck and wrist, respectively) of firefighters after a diesel fire (0.69 ng/cm² and 0.13 ng/cm²) than at a particleboard fire (2.28 ng/cm² and 0.81 ng/cm²). In a Swedish report, Sjöström et al. [29] assessed the levels of carcinogenic PAHs on the neck and wrist skin of firefighters after their participation in a training exercise. The participants of the study were tested while acting as fire starters, fire combatants, and team leaders (standing outside the burning house). According to the reported findings, the fire starters presented increased skin levels of carcinogenic PAHs compared to the other firefighters (2.84 ng/cm² versus 1.16 ng/cm² in fire combatants and 0.92 ng/cm² in team leaders), which can be attributed to their increased proximity to the fire front [29]. In addition, the authors observed that the wrist skin presented levels of carcinogenic PAHs that were more than five times higher than the neck skin for fire starters (2.40 versus 0.44 ng/cm², respectively), almost three times higher for smoke divers (0.86 versus 0.30 ng/cm², respectively), and two times higher for team leaders (0.62 versus 0.30 ng/cm², respectively). Overall, it was concluded that fire starters' wrists were the most contaminated skin region [29]. Fernando et al. [45] reported higher concentrations of carcinogenic PAHs on the forehead skin (2.16 ng/cm²), followed by the back (2.07 ng/cm²), the wrist (1.87 ng/cm²), the fingers (1.81 ng/cm²), and the neck (1.76 ng/cm²).

Only three studies were able to detect benzopyrene isomers, benzo(a)pyrene and benzo(e)pyrene, on the skin of the neck, wrists, forehead, fingers, and back of firefighters [29,42,45]. Recently, Banks and coworkers [42] reported significantly increased levels

of benzo(a)pyrene isomers on the skin of the neck and wrists of Australian firefighters after particleboard simulated fires compared to diesel pan burns (four times higher: 0.86 versus 0.20 ng/cm²) (Figure 4). Regarding the skin regions studied, the authors also found higher concentrations of benzo(a)pyrene isomers on the skin of the wrists (0.62 ng/cm²) than on the neck (0.23 ng/cm²) after the wood conglomerate fire. However, contrary results were obtained from the diesel fire simulation (0.027 ng/cm² in the wrists versus 0.17 ng/cm² in the neck) [42]. Sjöström et al. [29] also described higher values of benzo(a)pyrene isomers on the skin of the wrists than on the neck of exposed fire starters (0.35 versus 0.08 ng/cm²), smoke divers (0.18 versus 0.05 ng/cm²), and team leaders (0.09 versus 0.05 ng/cm²). Moreover, Fernando et al. [45] reported dermal concentrations of benzo(a)pyrene isomers (0.64 ng/cm²), with increased levels on the skin of the forehead (0.20 ng/cm²); similar values (0.11 ng/cm²) were found on the remaining skin regions (neck, wrist, back, and fingers).

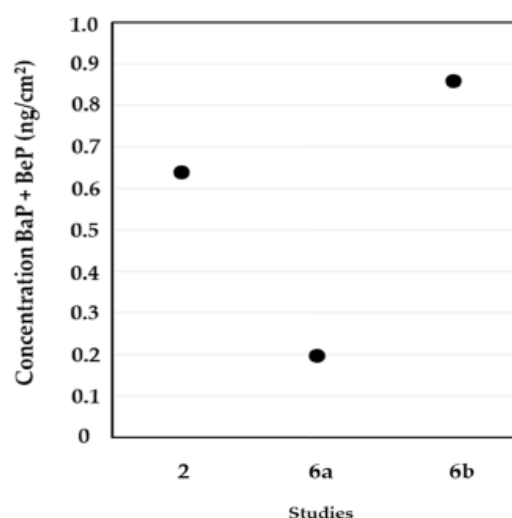


Figure 4. Concentrations of benzo(a)pyrene and benzo(e)pyrene on the skin of firefighters after firefighting activities (2—Fernando et al. [45] (neck, wrist, back, forehead and fingers); 6—Banks et al. [42] (a—diesel pan fire; b—particleboard fire) (neck and wrist)).

Other possible/probable carcinogenic PAHs were found on the skin of the firefighters after fire combat. Naphthalene was determined in two studies, at levels varying between 1.11 ng/cm² and 25.6 ng/cm² on the neck skin of subjects participating in training exercises [43,45]. Levels of benz(a)anthracene were reported in four studies, with values ranging from 0.04 ng/cm², on the collarbone skin of team leaders (firefighters controlling the fire from outside the burn house; Sjöström et al. [29]), to 33.69 ng/cm², on the neck skin of subjects attending a wood pallet fire [43]. Moreover, Andersen et al. [43] highlighted that benz(a)anthracene was the predominant PAH with carcinogenic properties on the skin of firefighters. In the study performed by Banks et al. [42], the skin values of benz(a)anthracene and chrysene are reported collectively: 0.038 ng/cm² (at the wrist) and 0.050 ng/cm² (at the neck) after a diesel pan fire, and 0.25 ng/cm² (at the neck) and 0.51 ng/cm² (at the wrist) after a particleboard fire. So far, only four studies have described the levels of chrysene on the skin of exposed firefighters, with concentrations ranging between 0.04 ng/cm² on the collarbone skin of team leaders [29] and 3.06 ng/cm² on the neck skin of subjects participating in a training fire [43]. Concentrations of benzo(b)fluoranthene isomers, benzo(b)fluoranthene, benzo(j)fluoranthene, and benzo(k)fluoranthene have been reported on the skin of firefighting forces [29,42,45]. The highest levels of benzo(b)fluoranthene was found on the skin of fire starters' wrists (total of 0.41 ng/cm² [29]), while 0.03 ng/cm² of benzo(j)fluoranthene was the lowest value

determined on the skin of the neck, forehead, back, and fingers of firefighters participating in a training exercise with untreated wood as fuel [45]. Recently, Banks et al. [42] also included the analysis of benzo(b)fluoranthene and benzo(k)fluoranthene and observed predominantly increased skin concentrations after particleboard fires (0.33 ng/cm^2 for the neck and 1.00 ng/cm^2 for the wrists) than after a diesel-based fire (0.47 ng/cm^2 on the neck and 0.069 ng/cm^2 on the wrists). Baxter et al. [34] also highlighted the predominance of benzofluoranthene isomers on the skin of the face and neck of North American firefighters after five fire events. Dermal concentrations of indeno(1,2,3-c,d)pyrene varied between 0.04 ng/cm^2 , on the skin of the collarbones of firefighters working at the fire front [29], to 5.15 ng/cm^2 , on the neck of the subjects involved in a fire with wood pallets, mattresses, and electrical cords [43]. Regarding dibenz(a,h)anthracene, only two studies were able to quantify the levels of this compound on the skin of the firefighters. Banks and coworkers [42] reported values of 0.14 ng/cm^2 of dibenz(a,h)anthracene on the wrists of firefighters who fought a particleboard fire; this compound was not detected on the neck of those subjects. Sjöström et al. [29] reported lower levels of this PAH (0.06 ng/cm^2) on the neck of fire starters after participation in a training exercise. The available literature demonstrates the contamination of firefighters' skin with PAHs released during firefighting activities regardless of the use of PPE. Most of the studies reported the use of PPE during fire combat; however, it is known that firefighters tend to partially remove their protective gear when the fire is under control, including during the overhaul phase, and/or when a reduced risk of exposure to fire emissions is predicted. Recent studies suggest the use of particulate-blocking hoods as a more effective piece of firefighter PPE to reduce the exposure on the neck and face of the subjects [47,48]; however, more studies are warranted. Dermal exposure to fire emissions may also occur due to the limitations of PPE design and the equipment's fit on the body of the firefighters [26]. Additionally, post-fire cleaning measures as well as regular PPE maintenance procedures vary widely, which will also contribute to the contamination of firefighters' skin [49–51].

4. Dermal In Vitro/In Vivo Studies

Once on the human skin, PAHs can be transdermally assimilated through passive diffusion and/or absorption and be metabolized by cytochrome P450 enzymes (CYP450). Different in vitro/in vivo studies performed with animal and/or human skin models have characterized the PAHs' permeation levels, transdermal flux rates, dermal absorption and bioavailability, and association with toxicological and carcinogenic risks.

4.1. Dermal Absorption

The dermal absorption of PAHs is dependent on their transdermal diffusion and/or absorption rates, followed by metabolism via CYP450 monooxygenase in epidermic cells and their conversion into more hydrosoluble compounds [52]. Nevertheless, the diffusion through the nonviable stratum corneum, the outer skin layer, is the rate-limiting step in the dermal absorption process. Once absorbed, PAHs and/or their metabolites can reach the blood circulation in the dermal layer and be distributed through other tissues and organs of the human body [52]. The rate and extent of dermal absorption are important parameters for the determination of the total internal dose of PAHs dermally absorbed [53].

Table 1 summarizes the available information related to the dermal absorption of PAHs. Some studies were conducted in animal models (pig [13], guinea pig [54,55], rat [54], and monkey skin [56]), while others were performed in human skin models [52,53,56,57]. A model based on synthetic human skin, with sweat and sebum, was described by Luo et al. [14].

Table 1. Data collected from in vitro/in vivo studies assessing dermal absorption of PAHs.

Model (Dosing Vehicle)	PAHs	Units	Results	Main Conclusions	Reference
Hairless guinea pig (acetone)	Phe	Mean $\pm$ SEM (%)	<p>(a) Permeation in vitro flow-through cells (balanced salt solution, 6.6 $\mu\text{g}/\text{cm}^2$) Receptor fluid: (6 h) 2.4 ± 0.5; (12 h) 6.4 ± 1.2; (18 h) 8.3 ± 1.3; (24 h) 8.7 ± 2.0 Dose remaining in skin: 59.7 ± 2.0; Total dose: 68.5 ± 2.0; Skin wash: 10.3 ± 2.0; Recovered dose: 78.8 ± 2.1</p> <p>(b) Permeation in vitro flow-through cells (balanced salt solution, bovine serum albumin, 6.6 $\mu\text{g}/\text{cm}^2$) Receptor fluid: (6 h) 38.9 ± 8.1; (12 h) 63.6 ± 8.8; (18 h) 73.4 ± 8.5; (24 h) 78.0 ± 8.6 Dose remaining in skin: 11.8 ± 0.1; Total dose: 89.7 ± 8.7; Skin wash: 3.5 ± 1.2; Recovered dose: 93.3 ± 8.4</p> <p>(c) Permeation in vitro flow-through cells (water, bovine serum albumin, 15.2 $\mu\text{g}/\text{cm}^2$) Receptor fluid: (6 h) 20.8 ± 0.5; (12 h) 41.4 ± 0.9; (18 h) 52.1 ± 2.5; (24 h) 57.4 ± 2.7 Dose remaining in skin: 8.8 ± 1.0; Total dose: 66.2 ± 3.3; Skin wash: 2.2 ± 0.2; Recovered dose: 68.4 ± 3.5</p> <p>(d) Permeation in vitro flow-through cells (balanced salt solution, bovine serum albumin, 15.2 $\mu\text{g}/\text{cm}^2$) Receptor fluid: (6 h) 33.7 ± 7.3; (12 h) 58.1 ± 7.7; (18 h) 67.2 ± 6.5; (24 h) 71.3 ± 5.7 Dose remaining in skin: 7.8 ± 2.8; Total dose: 79.1 ± 3.6; Skin wash: 1.6 ± 0.4; Recovered dose: 80.7 ± 3.2</p>	In vitro percutaneous absorption of Phe; Penetration through pig skin controlled more by the passive rate of diffusion than by metabolism.	[55]
Rat, guinea pig, and human abdomen skin (acetone)	BaP	<p>Mean $\pm$ SD (%)</p> <p>($\mu\text{g}/\text{cm}^2$)</p> <p>($\mu\text{g}/\text{cm}^2/\text{h}$)</p> <p>(%)</p>	<p>(a) In vitro recoveries after topical applications with BaP Activity (radioactive soap, water washes, 24 h): (Rat) 5.3 ± 0.32; (Guinea pig) 45.9 ± 3.95; (Human, 32 y) 74.0 ± 6.94; (Human, 50 y) 42.9 ± 5.05 Activity (methanol extracts, 48 h): (Rat) 21.1 ± 5.53; (Guinea pig) 4.9 ± 1.47; (Human, 32 y) 13.5 ± 2.11; (Human, 50 y) 35.2 ± 8.11 Activity (skin digest): (Rat) 22.9 ± 5.24; (Guinea pig) 18.2 ± 4.16; (Human, 32 y) 6.6 ± 2.55; (Human, 50 y) 7.3 ± 0.81 Activity (methanol extract, skin digest): (Rat) 44.0 ± 10.35; (Guinea pig) 23.1 ± 2.76; (Human, 32 y) 20.1 ± 4.62; (Human, 50 y) 42.5 ± 8.71</p> <p>(b) In vitro cumulative absorption of BaP topical application (48 h) (Rat) 5.6 ± 0.10; (Guinea pig) 3.7 ± 0.18; (Human, 32 y) 0.3 ± 0.07; (Human, 50 y) 0.1 ± 0.05</p> <p>(c) In vitro maximum rate of skin permeation (Rat) 0.38 ± 0.028; (Guinea pig) 0.42 ± 0.031; (Human, 32 y) 0.02 ± 0.009; (Human, 50 y) 0.01 ± 0.003</p> <p>(d) In vivo total (urinary, fecal, and tissue) dermal absorption of BaP (Rat) 69.4 ± 7.59; (Guinea pig) 67.8 ± 9.33</p>	BaP is well absorbed through animal and human skin; Assessment of total exposure to BaP should consider the dermal route.	[54]

Table 1. Cont.

Model (Dosing Vehicle)	PAHs	Units	Results	Main Conclusions	Reference
Monkey skin (acetone)	8 PAHs	Mean $\pm$ SD (cm/h)	Permeability constants Naph: $(5.12 \pm 2.88) \times 10^{-3}$; Ace: $(6.33 \pm 4.81) \times 10^{-3}$; Flu: $(6.26 \pm 4.74) \times 10^{-3}$; Phe: $(1.96 \pm 1.14) \times 10^{-3}$; Ant: $(3.44 \pm 3.09) \times 10^{-3}$; Pyr: $(1.69 \pm 1.36) \times 10^{-3}$; Chry: $(0.22 \pm 0.14) \times 10^{-3}$; BaA: $(0.15 \pm 0.08) \times 10^{-3}$	K_{OW} values correlated with the permeability constant ($r = 0.90$, $p < 0.001$) and the lag time ($r = 0.81$, $p < 0.01$); A multiple linear regression model between permeability constants, K_{OW} , and water solubility was reported ($p < 0.0001$).	[56]
Human back skin (6% aqueous solution of polyoxyethylene 20 oleyl ether)	18 PAHs	Mean $\pm$ SD (ng/cm ² /h) % (ng/cm ² /h)	Directly measured values for flux: Ant 6.5 ± 0.9 ; Fln/Pyr 1.8 ± 0.3 ; 3–6 ring PAC: 120 ± 30 Applied dose absorbed: Ant 5.3; Fln/Pyr 3.3; 3–6 ring PAHs: 1.8 Dermal penetration flux values: Naph: 24; Acen: 0.094; Ace: 11; Flu: 37; Phe: 20; Ant: 6.5; Fln: 1.5; Pyr: 1.1; B(a)A: 0.23; Triphenylene: 0.16; Chry: 0.21; B(b)F: 0.035; B(k)F: 0.0044; BeP: 0.062; BaP: 0.016; Ind: 0.0013; DB(a,h)A: 0.0023; B(ghi)P: 0.0075	High molecular weight compounds presented a reduced dermal penetration flux value.	[53]
Human breast skin (acetone)	11 PAHs	Mean $\pm$ SD (%)	In vitro dermal absorption rates in Bronaugh flow-through diffusion cells (24 h skin soap washes) (a) without soil: Phe 88.3 ± 4.83 ; Fln 82.6 ± 8.09 ; Pyr 82.5 ± 7.32 ; BaA 82.3 ± 8.70 ; Chry 81.5 ± 11.44 ; B(b)F 77.6 ± 7.49 ; B(k)F 81.0 ± 9.67 ; BaP 70.8 ± 5.73 ; DB(a,h)A 76.7 ± 15.36 ; B(ghi)P 75.2 ± 9.53 ; Ind 78.3 ± 7.17 (b) with soil: Phe 60.8 ± 7.75 ; Fln 49.9 ± 3.70 ; Pyr 48.7 ± 5.63 ; BaA 26.4 ± 8.09 ; Chry 33.5 ± 12.40	High molecular weight compounds presented reduced dermal absorption.	[57]
Synthetic human skin (simulated artificial sweat and sebum mixture)	4 PAHs	Value or Range (μ g/L) %	Skin absorption rates (up to 9 h post-exposure): Naph 0.22–1.84; Phe 0.24–2.30; Pyr 0.32–0.92; BaP 0.05–0.08 Intracellular levels: Naph 0.26, 0.76; Phe 0.47, 0.92; Pyr 0.45; BaP 0.08, 0.13 Residual levels: Naph 0.61, 1.13; Phe 0.63, 1.45; Pyr 1.13, 1.14; BaP 0.12 Total dermal penetration: Naph 76.4, 79.9; Phe 72.6, 73.3; Pyr 52.2, 38.7; BaP 8.30, 9.07 Total dermal absorption: Naph 84.6, 87.5; Phe 82.0, 82.5; Pyr 61.2, 43.2; BaP 10.9, 9.87 Loss ratio: Naph 6.19, 1.25; Phe 5.44, 3.00; Pyr 16.2, 46.4; BaP 96.7, 88.9	Low molecular weight PAHs were more easily absorbed by skin cells than heavier compounds; Dermal permeabilities were increased in 2–3 rings PAHs; BaP metabolism was affected by the levels and duration of exposure and the age of skin.	[14]

SEM—standard error of the mean; SD—standard deviation; K_{OW} —coefficient of octanol/water partition; Ace: acenaphthene; Acen: acenaphthylene; Ant: anthracene; BaA: benz(a)anthracene; BaP: benzo(a)pyrene; B(b)F: benzo(b)fluoranthene; BeP: benzo(e)pyrene; B(g,h,i)P: benzo(g,h,i)perylene; B(k)F: benzo(k)fluoranthene; Chry: chrysene; DB(a,h)A: dibenz(a,h)anthracene; Flu: fluorene; Fln: fluoranthene; Ind: indeno(1,2,3-cd)pyrene; Naph: naphthalene; Phe: phenanthrene; Pyr: pyrene.

Roy and coworkers used structure activity relationship models based on the partition coefficient and the percentage of the applied dose dermally absorbed to estimate the dermal absorption of the different PAHs [58,59]. The results obtained by the authors suggest that low-molecular-weight PAHs presented increased dermal absorption rates (37 ng/cm²/h for fluorene, 24 ng/cm²/h for naphthalene, 20 ng/cm²/h for phenanthrene, and 11 ng/cm²/h for acenaphthene) compared to the values reported for high-molecular-weight compounds (1.1 ng/cm²/h for pyrene, 0.23 ng/cm²/h for benz(a)anthracene, 0.016 ng/cm²/h for benzo(a)pyrene, and 0.0013 ng/cm²/h for indeno(1,2,3-cd)pyrene) (Table 1). These findings are in line with the results reported by other authors who also observed that low-molecular-weight PAHs are more easily absorbed by the skin and have increased dermal permeabilities than heavier compounds [13,14,57]. Moody et al. [57] reported that naphthalene, phenanthrene, fluoranthene, pyrene, benz(a)anthracene, chrysene, and benzo(k)fluoranthene presented dermal absorption rates predominantly above 80% in synthetic human skin and human breast skin (Table 1). For other PAHs, lower total dermal absorption rates were described (e.g., benzo(a)pyrene, benzo(b)fluoranthene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-c,d)pyrene) (Table 1). Regarding studies performed in animals models, Alalaiwe et al. [13] and Sartorelli et al. [56] reported similar values for dermal naphthalene permeability rates in pig and monkey skin ($\approx 5\text{--}6 \times 10^{-3}$ cm/h), respectively. Similar permeability rates were also described for acenaphthene and fluorene (6.33×10^{-3} cm/h and 6.26×10^{-3} cm/h, respectively) (Table 1).

The dermal absorption of PAHs is affected by the physicochemical and structural properties of compounds. Sartorelli et al. [56] highlighted the importance of considering the octanol/water partition coefficient (K_{OW}) in skin models to better predict the permeability constant of each compound. These findings are supported by the results described by other authors [13]. PAHs with high molecular weights (>200 g/mol) and log K_{OW} presented lower dermal absorptions and skin permeability flux [13,14,53,56,57]. Ng et al. [55] concluded that the dermal absorption of phenanthrene is predominantly controlled by passive diffusion rather than by metabolism. In addition, some other factors need to be taken into consideration during dermal absorption studies, including the site of contamination application, the selected animal species, the temperature employed during the assay, the levels of skin hydration, and the solubility of the analyte [55]. A radioactive species of benzo(a)pyrene was used to measure the recovery of this carcinogenic PAH after 48 h of exposure and the rate of skin permeation in different animal tissues and human skin [54]. It was found that rat and guinea pig skin presented increased dermal permeation rates (maximum values of 0.38 and 0.42 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively) compared to the abdomen skin of 32- and 50-year-old individuals (0.02 and 0.01 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively) (Table 1).

Concerning studies evaluating the dermal absorption of PAHs over time, Luo et al. [14] reported that the permeability of four PAHs (naphthalene, phenanthrene, pyrene, and benzo(a)pyrene) on human synthetic skin reached an equilibrium state after 3 to 6 h post-exposure to low (5 $\mu\text{g}/\text{L}$) and high (10 $\mu\text{g}/\text{L}$) concentration values; a decrease on the permeability rates was observed up to 9 h post-exposure. The nature and duration of exposure are important parameters that influence the dermal absorption of PAHs. Higher exposure levels can contribute to the enhanced deposition of PAHs on the skin, which may result in increased dermal absorption fluxes. However, when the skin saturation level is reached, lower penetration rates can be observed [52]. Bourgart et al. [52] found that the fraction of total unmetabolized benzo(a)pyrene globally decreased with the exposure time, regardless of the dose applied. The authors also determined the levels of three benzo(a)pyrene metabolites, benzo(a)pyrene-tetrol, benzo(a)pyrene-7,8-diol, and 3-hydroxybenzo(a)pyrene, in ex vivo human breast skin, as well as in the culture medium used (DMEM/F12, a common culture medium for the growth of mammalian cultures). The concentrations of all metabolites increased over time, being only detectable 24 h post-exposure, which was related with the steps involved in the metabolic pathway [52].

Moreover, the percentage of unmetabolized benzo(a)pyrene remaining on the skin surface after application was reduced with increasing exposure time.

Regarding the selection of the model (animal versus human skin), other factors should be taken into consideration. Due to the restrictions of animal use, ex vivo human skin models are preferable and have demonstrated a satisfactory correlation between in vivo and in vitro studies. Ex vivo samples from freshly excised human skin serves as the best representative model to reproduce realistic cutaneous exposure in humans, since it eliminates interspecies extrapolation and overestimates the dermal penetration often observed with animal models [52]. Reduced costs as well as faster results are other positive aspects [55]. However, the limited access to in vivo/ex vivo human skin has motivated the choice of animal models (e.g., mice, rat, or guinea pigs). Moreover, studies using human skin tissues have some limitations that should be considered during assay planification. The applied dose has a great influence on the results, which could affect the use of in vitro data on in vivo models for human risk assessment. The inter-individual variability of different skin donors (e.g., ethnicity, gender, genotype, general health, local blood flow, and formation and duration of skin depot) will also impact the results. In a realistic scenario, the dermal exposure comprises skin contact with complex mixtures containing several PAHs, among other pollutants. Therefore, both human and animal in vitro/in vivo studies involving a limited number of pre-selected PAHs could bias the effective dermal absorption rates.

4.2. Dermal Bioaccessibility and Bioavailability

The dermal bioaccessible fraction of PAHs represents the amount of topical PAHs that are bioavailable to be assimilated by the skin and to be subjected to cutaneous metabolic processes, while dermal bioavailability refers to the fraction of bioaccessible PAHs that can reach systemic circulation and the specific tissues and organs of the human body and exert its biological effects. Unmetabolized PAHs are poorly bioavailable after dermal exposure, which is related to enhanced first-pass metabolism, conditioning the carcinogenicity of unmetabolized PAHs to the local contamination on the skin. PAH metabolites easily cross the human skin, since they are more polar and have a superior hydro-solubility than the respective unmetabolized compound [52].

So far, few studies have explored PAHs' dermal bioavailability through in vitro/in vivo assays performed with animal models and human skin tissues. Firstly, Kadry et al. [60] resorted to radioactive species to compare the oral and dermal bioavailability of phenanthrene, and reported slower dermal absorption (8.6 h versus 1.0 h half-life time, respectively) and faster elimination (16.1 h versus 28.0 half-life time) than gastrointestinal tract in rats. These findings are in line with previous studies [61,62] reporting the elimination of PAHs after only 8 h of exposure in rats [60]. After 96 h of post-dermal exposure to radioactive phenanthrene, the skin application site presented the highest levels of radioactivity (0.310–0.840% initial dose/g tissue), followed by the ileum (0.021–0.046% initial dose/g tissue) and duodenum (0.017–0.021% initial dose/g tissue); radioactivity was also found in untreated skin (0.007–0.023% initial dose/g tissue) [60]. Kadry and coworkers [60] explained the distribution of radioactivity to the intestinal tissues as being due to the biliary excretion of phenanthrene metabolites, which are later eliminated through the urine as phenanthrenequinone, 9,10-phenanthrenediol, phenanthrol, and phenanthrene. The authors were able to demonstrate that, after dermal exposure, phenanthrene become significantly available to skin tissues.

Some authors explored the dermal bioavailability of PAHs due to the exposure to contaminated soils [63]. Different skin absorption experiments were performed with human skin exposed to soils contaminated with PAHs during controlled periods, with the receptor fluids collected at different post-dose moments. The in vitro dermal absorption revealed a slow release/diffusion of benzo(a)pyrene (50–300 pg/cm²/h) and reached a steady-state flux within 48–98 h after dermal contact [63]. Roy et al. [58] reported a close range, but slightly higher benzo(a)pyrene dermal flux rates (0.0064 to 750 ng/cm²/h) also reached

a stationary state of diffusion 48 h post-exposure. The available evidence suggests that absorbed dermal rates only represent a small fraction of the total dose applied (maximum reported value of 12%), thus validating a stationary permeability state with the absorption of a constant concentration over time [63].

The dermal absorption fraction can be used to express the dermal bioavailability. Recently, Forsberg et al. [64] calculated the dermal absorption fraction for six PAHs in the skin of female mice 24 h post-exposure to soils contaminated with PAHs and reported mean values of 0.85–3.6% for benzo(b)fluoranthene, 0.52–1.4% for benz(a)anthracene, 0.58–1.3% for benzo(a)pyrene, non-detected to 0.46% for chrysene, and non-detected to 0.42% for benzo(k)fluoranthene; dibenz(a,h)anthracene was not detected. Some authors highlighted that human skin is less permeable to chemical substances, including PAHs, than the skin of primates [64,65]. Additional research is needed to characterize the dermal bioavailability of PAHs in human skin tissues.

4.3. Toxicological and Carcinogenic Dermal Risks

Most PAHs require bioactivation through a complex enzymatic metabolism to be biotransformed into reactive intermediates that might cause mutagenicity, teratogenicity, and carcinogenicity in targeted biological molecules (e.g., DNA and proteins). PAHs can activate AhR and promote the gene expression of metabolizing enzymes such as CYP450 (e.g., CYP1A1, CYP1A2, and CYP1B1) [23]. These enzymes catalyze the oxidation of pro-carcinogens to carcinogenic reactive intermediates, and it is believed that this is the first step in the metabolism of PAHs that will promote the activation of the carcinogenicity process. The predominant mechanism involved in the activation of PAHs carcinogenesis results from mutational miscoding caused by the enzymatic generation of sterically hindered bay- or fjord-region diol epoxides by epoxide hydrolase that will generate DNA adducts [66,67]. For benzo(a)pyrene, a potent skin carcinogen in different animal species, the ultimate carcinogen is the bay-region diol epoxide benzo(a)pyrene-7,8-dio-9,10-epoxide [66]. Other relevant mechanisms in the activation of carcinogenicity are the formation of radical cations (e.g., by electron oxidation via peroxidases) and/or the generation of *o*-quinones via dihydrodiol dehydrogenases [66,68,69]. The metabolism of PAHs causes the activation of AhR and is directly associated with alterations in cell signaling and proliferation, which will promote oxidative stress and thus contribute to the cells' toxicity and carcinogenicity [66,70]. Dermal exposure to PAHs contributes to local inflammation, atopic dermatitis, skin aging, and skin diseases, including cancer [8–10].

Studies assessing the toxicological and carcinogenic risks of PAHs on skin tissues have been slowly emerging in the literature (Table 2). Sivak et al. [71] evaluated the incidence of sores and scabs after the skin of mice had been exposed to different combinations of condensed roofing asphalt fumes, including three fractions containing known levels of benzo(a)pyrene (0.01%, 0.001%, and 0.0001%). The bioassay consisted of the dermal administration of the tested materials twice a week, for 104 weeks, on 40 groups of 30 mice. The strongest response was found after the treatment with the highest concentration of benzo(a)pyrene (0.01%), which affected the sensitivity to assess the cocarcinogenic activity due to a saturation effect. Overall, a significant number of tested individuals died (all for the highest level of benzo(a)pyrene contamination) and the presence of tumors and carcinomas was found in several animals (Table 2). When the treatment was performed with the lowest concentration of benzo(a)pyrene (0.0001%), the presence of tumors or carcinomas was not observed, although 50% of individuals also died [71]. Hall et al. [72] studied the stereoselectivity mechanism of benzo(a)pyrene in human skin obtained from reduction mammoplasty, mastectomy, or amputation through the determination of dihydrodiols and tetrols, including the enantiomeric composition of the four benzo(a)pyrene tetrols, since the stereoselectivity of benzo(a)pyrene may be an important factor for carcinogenicity. Different intermediates were found on skin extracts, with benzo(a)pyrene-(7,8)-dihydrodiol being the predominant compound (1.71–18.27 pmol/cm² skin), mostly in the form of enantiomer (-)-7R,8R (Table 2).

Table 2. Data collected from in vitro/in vivo studies assessing dermal toxicological and carcinogenic risks of PAHs.

Model (Dosing Vehicle)	PAHs	Units	Results	Main Conclusions	Reference
Human breast skin (acetone and tetrahydrofuran)	BaP dihydrodiols and tetrols	Range (pmol/cm ²)	Concentrations extracted from skin BaP(7,8)-dihydrodiol: 1.71–18.27; BaP(4,5)-dihydrodiol: 0.24–10.43; BaP(9,10)-dihydrodiol: 1.03–12.65; BaP(7,9,10/8) tetrol: 4.6–10230; BaP(7,9/8,10) tetrol: 16.5–2017.7; BaP(7/8,9,10) tetrol: 2.1–630.2; BaP(7,10/8,9) tetrol: 29–1015.8	Observed interindividual variations in the stereoselective metabolism of BaP, which will conditionate the individual susceptibility to PAH-induced skin carcinogenesis.	[72]
Male C3H/HeJ mice (acetone and cyclohexane)	BaP	50th survival (days) Number of death individuals (%) Counts (dimensionless)	Treatment with 0.01% BaP 50th survival: 464; Number of death individuals: 100; Total papilloma/group: 1; Total carcinoma/group: 28; Number of tumors: 27 Treatment with 0.001% BaP: 50th survival: 732; Number of death individuals: 50; Total papilloma/group: 2; Total carcinoma/group: 3; Number of tumors: 5 Treatment with 0.0001% BaP 50th survival: 727; Number of death individuals: 50; Total papilloma/group: 0; Total carcinoma/group: 0; Number of tumors: 0	Groups treated with BaP at 0.01% had such a strong response due to the BaP alone that the sensitivity for assessing cocarcinogenic activity was limited.	[71]
Female FVB/N inbred mice (toluene and 5% DMSO)	BaP, DB(a,l)P	Mean ± SD (adducts/10 ⁸ nucleotides)	Total DNA adducts BaP: 141 ± 37 DB(a,l)P: 45 ± 13	Exposure produced primarily papillomas followed by squamous cell carcinoma and carcinoma in situ; BaP caused over three times the level of total DNA adducts; DB(a,l)P carcinogenicity was much higher than predicted.	[73]
Human cells—HaCaT cells (0.1% DMSO)	BaP, DB(a,l)P, Chry	EC50 (μmol/L)	Chry EC 50: 3.8; 2.0 DB(a,l)P EC 50: 0.035	Chry caused strong cytotoxic effects in cell lines; BaP and DB(a,l)P up-regulated the levels of metabolites; CYP1A1 and CYP1B1 expression was significantly increased in some cell lines treated with BaP and Chry and the metabolites formed contributed to the observed metabolomic alterations.	[66]

Table 2. Cont.

Model (Dosing Vehicle)	PAHs	Units	Results	Main Conclusions	Reference
Pig skin (30% propylene glycol/phosphate buffer, pH 7.4)	Naph, Flr, Pyr, Chry, BaA, BaP	(dimensionless)	<p>Toxicological index of PAHs</p> <p>Ciclo-oxygenase-2</p> <p>Naph: 0.01; Flr: 0.09; Pyr: 0.05; Chry: 0.60; BaA: 0.91; BaP: 0.97</p> <p>Prostaglandin E2</p> <p>Naph: 0.07; Flr: 0.34; Pyr: 0.15; Chry: 2.21; BaA: 5.61; BaP: 4.43</p> <p>Chemokine (C-X-C motif) ligand</p> <p>Naph: 0.34; Flr: 0.81; Pyr: 0.31; Chry: 1.83; BaA: 4.00; BaP: 4.66</p> <p>Interleukin-8</p> <p>Naph: 0.08; Flr: 0.19; Pyr: 0.07; Chry: 0.47; BaA: 1.08; BaP: 1.84</p>	BaA and BaP were the compounds revealing the great skin inflammation and barrier function damage.	[13]
CALUX Bioassay (0.8% DMSO)	PAH metabolites	EC20, EC50 (mol/L) REP EC20, EC50 (dimensionless)	<p>Concentrations measured on the assay</p> <p>1-Hydroxynaphthalene: EC20, EC50 > 2.40×10^{-4}</p> <p>2-Hydroxynaphthalene: EC20: 3.54×10^{-5}; EC50: 7.50×10^{-5}</p> <p>REP EC20: 2.99×10^{-5}; REP EC50: 6.08×10^{-5}</p> <p>1-Hydroxyphenanthrene: EC20, EC50 > 1.00×10^{-5}</p> <p>2-Hydroxyphenanthrene: EC20: 3.93×10^{-5}; EC50: 8.11×10^{-5}</p> <p>REP EC20: 2.69×10^{-5}; REP EC50: 5.63×10^{-5}</p> <p>3-Hydroxyphenanthrene: EC20, EC50 > 1.00×10^{-5}</p> <p>4-Hydroxyphenanthrene: EC20: 1.83×10^{-6}; EC50: 7.75×10^{-6}</p> <p>REP EC20: 5.78×10^{-4}; REP EC50: 5.89×10^{-4}</p> <p>9-Hydroxyphenanthrene: EC20, EC50 > 1.00×10^{-5}</p> <p>2-Hydroxyfluorene: EC20, EC50 > 8.00×10^{-4}</p> <p>3-Hydroxyfluorene: EC20: 2.38×10^{-6}; EC50: 8.43×10^{-6}</p> <p>REP EC20: 4.44×10^{-4}; REP EC50: 5.41×10^{-4}</p> <p>4-Hydroxyfluorene: EC20, EC50 > 3.00×10^{-5}</p> <p>1-Hydroxypyrene: EC20: 1.09×10^{-4}; EC50 > 1.00×10^{-4}</p> <p>REP EC20: 9.70×10^{-6}</p> <p>3-Hydroxychrysene: EC20: 2.46×10^{-7}; EC50: 1.03×10^{-6}</p> <p>REP EC20: 4.30×10^{-3}; REP EC50: 4.43×10^{-3}</p> <p>6-Hydroxychrysene: EC20: 3.06×10^{-6}; EC50: 1.34×10^{-5}</p> <p>REP EC20: 3.45×10^{-4}; REP EC50: 3.40×10^{-4}</p> <p>3-Hydroxybenzo(a)pyrene: EC20, EC50 > 8.00×10^{-6};</p> <p>BaP: EC20: 1.06×10^{-9}; EC50: 4.56×10^{-9}</p> <p>REP EC20, REP EC50: 1.00</p>	Increased bioassay response with extracts from post-fire neck and calf wipe samples; Correlation between the bioassay response and urinary levels of PAH metabolites.	[32]

SD—standard deviation. BaA: 1,2-Benz(a)anthracene; BaP: benzo(a)pyrene; Chry: chrysene; DB(a,l)P: dibenzo(a,l)pyrene; Flr: fluoranthene; Ind: indeno(1,2,3-cd)pyrene; Naph: naphthalene, Pyr: pyrene; REP: relative potencies in relation to BaP; DMSO: dimethyl sulfoxide.

These findings confirm the metabolic activation of benzo(a)pyrene [72]. The concentrations of benzo(a)pyrene tetrols ranged between 2.1 and 10,230 pmol/cm² skin (Table 2).

Hall and coworkers [60] observed variations among individual mice in the stereoselective metabolism of benzo(a)pyrene, which will have implications for the individual's susceptibility to induced skin carcinogenesis due to the dermal contact with PAHs. More recently, Bourgart et al. [52] studied the metabolism of benzo(a)pyrene when higher, but realistic, doses (up to 22.11 nmol/cm²) of this carcinogenic PAH were applied to an ex vivo skin model prepared with freshly excised human breast skin. After 8 h post-exposure, benzo(a)pyrene vastly penetrated in the skin, with 3-hydroxybenzo(a)pyrene and 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene being the predominant metabolites found in the culture medium. The dermal absorption and metabolization of benzo(a)pyrene continued to occur over 48 h after topical application (Table 1). The dermal bioavailability of benzo(a)pyrene was limited, and the accumulation of unmetabolized benzo(a)pyrene in and on the skin as the applied dose increased during the biological assay; less than 3% of the applied dose remained in the culture medium (Table 1). Bourgart and coworkers [52] found that the benzo(a)pyrene tetrol metabolic pathway was rapidly saturated as the applied dose increased, probably due to the saturation of epoxide hydrolase [52]. The authors highlighted that benzo(a)pyrene carcinogenicity is located on the contaminated skin tissue, since unmetabolized benzo(a)pyrene is poorly bioavailable after dermal exposure due to an enhanced first-pass metabolism mediated by CYP450 and epoxidehydrolase. Alalaiwe et al. [13] explored the absorption of six PAHs (naphthalene, fluoranthene, pyrene, chrysene, benz(a)anthracene, and benzo(a)pyrene) through the dermal route and estimated their effect on the cutaneous inflammation of pig dorsal skin. The dermal deposition of PAHs increased with a superior lipophilicity and molecular size of compounds, while skin absorptions were heavily affected by the structures and physicochemical properties of the PAHs. Compounds with higher dermal absorption rates presented stronger interactions with stratum corneum lipids [13]. After in vitro skin permeation, cultured keratinocytes were used to determine the cytotoxicity and increased levels of cyclo-oxygenase-2, prostaglandin E2, chemokine (C-X-C motif) ligand 1, and interleukin-8 were released by exposed cells [13]. Benzo(a)pyrene, benz(a)anthracene, and chrysene caused the highest expression in the concentrations of cyclo-oxygenase-2 and prostaglandin E2, while naphthalene (2-ring PAH) promoted the lowest expression of these biomarkers (Table 2). Alalaiwe and coworkers [13] were able to demonstrate that particulate matter containing PAHs caused stronger cytotoxicity as well as skin barrier deficiency via the inhibition of the filaggrin and integrin β 1 expression (markers for the formation of skin barrier function) than particles containing heavy metals. Therefore, the permeation of PAHs into and across the animal skin was proven. These authors found a strong in vivo/in vitro correlation, with benz(a)anthracene and benzo(a)pyrene having a great potential to disrupt the skin barrier and to cause enhanced inflammation in in vivo models [13].

Potratz et al. [66] studied diverse signaling pathways related to the dermal exposure to chrysene, benzo(a)pyrene, and dibenzo(a,l)pyrene through the determination of several endogenous metabolites. This metabolomic approach was performed on an in vitro assay with human adult wildtype (HaCat WT) and AhR knockdown (HaCat AHR) keratinocyte cell lines. The authors reported changes in the levels of 24 metabolites (including biogenic amines, acylcarnitines, amino acids, phosphatidylcholines, and sphingomyelins), which represent alterations in the regular metabolic profile of the cell lines, suggesting the influence of PAHs on the energy and lipid metabolism [66]. More specifically, lower concentrations of the amino acid glutamine were found after the cell lines' exposure to benzo(a)pyrene and dibenzo(a,l)pyrene. However, Potratz et al. [66] highlighted that the depletion of amino acids can result from the glutathione synthesis. PAHs are lipophilic and can incorporate cellular membranes and disturb its biological function. Strong phosphatidylcholine changes were suspected to be associated with perturbations of membrane integrity [66]. Moreover, Potratz et al. [66] reported increased values of some sphingomyelins and associated these findings with the onset of apoptosis in the analyzed cell lines

48 h post-exposure [66]. The enzymes CYP1A1 and CYP1B1 catalyze the oxidation of benzo(a)pyrene and dibenzo(a,l)pyrene, respectively [66,71]. Cell lines exposed to chrysene presented strong cytotoxic effects (with EC50 values of 3.8 and 2.0 $\mu\text{mol/L}$ for HaCaT WT and HaCaT AHR cells, respectively), while the treatment with dibenzo(a,l)pyrene enhanced the cytotoxicity in HaCaT WT cells (EC50 of 0.0035 $\mu\text{mol/L}$); the exposure to benzo(a)pyrene caused limited cytotoxicity (Table 2). Cell lines exposed to carcinogenic PAHs at levels capable of activating AhR demonstrated a strong capacity to enhance the prototype death receptor CD95, the best-characterized member of the tumor necrosis factor superfamily of receptors [74]. Potratz et al. [66] successfully demonstrated the activation of AhR by PAHs and the consequent induction of CYP1A1 and CYP1B1 at the mRNA and protein levels. Their expression was significantly enhanced in cells treated with the highest levels (3.5 $\mu\text{mol/L}$) of benzo(a)pyrene and chrysene. Cell lines treated with dibenzo(a,l)pyrene, a potent carcinogen in mice, registered a considerable lack of gene induction; however, the concentrations of the formed metabolites remained virtually unaffected [66]. A possible explanation for these findings relies on the fact that HaCaT AHR might become insensitive and partially/totally lose their capacity to differentiate dibenzo(a,l)pyrene. As CYP1B1 is mainly responsible for the metabolic activation of this PAH, a decrease in the cellular level of this enzyme might explain the reduced response of HaCaT AHR against the potential toxicity of this compound. Moreover, the dibenzo(a,l)pyrene' AhR-dependent toxicity could be caused by the non-genotoxic AhR signaling alone. Despite modifications to the AhR target genes, CYP1A1 and CYP1B1, Siddens et al. [73] determined the changes in other genes of expression in the skin of mice. After 12 h post-treatment with PAHs, the exposure to benzo(a)pyrene caused the formation of three-fold change levels of total DNA adducts, biomarkers of predicted carcinogenicity, than dibenzo(a,l)pyrene (141 versus 45 adducts/108 nucleotides) (Table 2). The formation of PAH-DNA adducts in the skin is not sufficient to predict the final tumor response. Siddens et al. [73] observed and registered the tumor incidence on a weekly basis with 25-week promotion intervals after mice dermal exposure to benzo(a)pyrene and dibenzo(a,l)pyrene. The authors demonstrated that dermal exposure to benzo(a)pyrene and dibenzo(a,l)pyrene predominantly produced papillomas, followed by squamous cell carcinoma and in situ carcinoma in mice. The authors also highlighted the greater carcinogenicity of dibenzo(a,l)pyrene than the predicted values, based on the available relative potency factors [73].

Recently, Beitel et al. [32] used an in vitro PAH CALUX[®] bioassay to assess the overall AhR-mediated toxicity of PAHs present on dermal wipe samples and the urinary PAH metabolites collected by North American firefighters before and after participation in a controlled fire. The authors found greater concentrations of AhR-active compounds on the neck and calf of firefighters' post-fire, which demonstrates the deposition of PAHs and other AhR active compounds on the skin. These findings are aligned with the reported levels of total PAHs on the skin of exposed firefighters (Figure 1). This study successfully proved that the post-fire cleaning of the head and neck area with a baby wipe significantly decreased the majority of the AhR-active compounds on the skin of firefighters, contributing to a reduction in the time of dermal exposure to fire emissions. PAHs are photo-reactive and phototoxic airborne pollutants and, therefore, the simultaneous dermal exposure to sunlight and PAHs might have synergetic effects on the skin. Soeur and coworkers [75] studied the combined influence of dermal daily UV irradiation and exposure to benzo(a)pyrene and indeno(1,2,3-c,d)pyrene on a reconstructed skin model based on human epidermal keratinocytes. The cell viability, clonogenic efficiency, glutathione metabolism, and redox homeostasis were evaluated. The authors observed that low concentrations of PAHs caused high cellular stress, phototoxicity, and impaired keratinocyte clonogenic potential (subtoxic levels), as well as the generation of ROS and decreased intracellular glutathione levels within the cells after several hours post-exposure [75]. The effect caused by benzo(a)pyrene was increased in comparison to the response caused by topical indeno(1,2,3-c,d)pyrene [75]. Soeur et al. [75] highlighted that people experiencing photo-pollution stress (e.g., firefighting forces) may have alterations in their cutaneous

homeostasis and promote skin damage and aging. Regarding urine samples, Beitel and coworkers [32] found increased values of AhR-active compounds after 2–4 h of post-firefighting and enhanced potencies (compared to benzo(a)pyrene) were observed for some PAH metabolites: 3-hydroxychrysene (relative potency EC20 and EC50 of 4.30×10^{-3} and 4.43×10^{-3} , respectively), 4-hydroxyphenanthrene (5.78×10^{-4} and 5.89×10^{-4}), 3-hydroxyfluorene (4.44×10^{-4} and 5.41×10^{-4}), 6-hydroxychrysene (3.45×10^{-4} and 3.40×10^{-4}), 2-hydroxynaphthalene (2.99×10^{-5} and 6.08×10^{-5}), and 2-hydroxyphenanthrene (2.69×10^{-5} and 5.63×10^{-5}); 1-hydroxypyrene had a relative potency EC20 of 9.70×10^{-6} (Table 2). A correlation was found between the bioassay response and the urinary levels of PAH metabolites; however, the values of increased AhR-active compounds in post-fire urine were probably also due to the presence of other PAH-related compounds [32]. Unsubstituted, nitro, and methylated PAHs, known mutagens and/or carcinogens, also presented AhR-mediated activity, some with expected superior potency than parent PAHs [76]. Therefore, more studies evaluating the potential toxicological and carcinogenic risks caused and/or promoted by dermal exposure to PAHs are needed.

5. Conclusions

Information related to firefighters' dermal exposure to PAHs released during fires remains limited. A lack of uniformity was marked in the sampling and analytical methods used by different authors. However, the available data clearly demonstrate the presence of PAHs included in the United States Environmental Protection Agency's list of priority pollutants on the skin of exposed firefighters, even when the adequate use of firefighting PPE was reported. The neck, wrists, and face were the primary contaminated body regions, regardless of the use of PPE. Some PAHs were also detected on the calf, scrotum, and back of exposed firefighters. These findings are somewhat expected, since these are the less-protected areas by the PPE, mostly due to the limitations of equipment design and the pre-cleaning procedures.

The available in vitro/in vivo assays demonstrate that PAHs can reach deep skin layers, even at low concentrations, either due to passive diffusion and/or absorption into the epidermis, with further metabolism and systemic distribution to other tissues and organs of the human body. PAHs with low molecular weight present increased dermal absorption rates than high-molecular-weight compounds, being more easily absorbed by skin tissues and, thus, presenting increased topical permeabilities. There are several factors that affect the dermal absorption of PAHs, e.g., the physicochemical and structural properties of compounds, nature and duration of exposure, and factors related to the skin cell lines used in the assay (hydration, animal species, etc.). However, PAHs' dermal absorption and bioavailability rates remain poorly characterized, mainly in human skin tissues. Evidence highlights the potential of dermal exposure to PAHs to promote cytotoxicity, oxidative stress, topical inflammation, and to activate the carcinogenic process through the activation of AhR and the consequent enzymatic generation of reactive intermediates, that will generate protein and/or DNA adducts. The generated reactive intermediates will be transformed via CYP450 enzymes to expedite their excretion from the organism as PAH metabolites in biological fluids (e.g., urine, bile, feces, and milk) [2]. Firefighters' regular dermal exposure to PAHs promotes the development and/or aggravation of skin disorders, with limited evidence suggesting increased values of AhR-mediated toxicity and urinary PAH metabolites after participation in fires. Future research needs to continue characterizing the dermal absorption and bioavailability of PAHs and the associated toxicological and carcinogenic effects. The use of bioavailable concentrations, rather than dermal exposure levels, will allow a more realistic health risk assessment of human exposure to PAHs. The contribution of dermal exposure to the total internal dose of PAH remains unclear. Therefore, more studies are needed to explore the relation between the topical values of PAHs, their bioavailability, and the levels of their metabolites in biological fluids. The missing data will be determinant to define maximum levels for dermal exposure to PAHs, which is of extreme importance in heavily occupationally exposed groups such as

firefighting forces. It will also allow the evaluation of the preventive measures, e.g., the decontamination of skin and PPE procedures already implemented by some fire brigades, to minimize firefighters' occupational exposure through dermal contact.

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PART I

Chemical composition and morphological characterization of PM from firefighters' occupational environments

Chapter 2.

Gravimetric, morphological, and chemical
characterization of fine and ultrafine particulate
matter inside fire stations



Gravimetric, morphological, and chemical characterization of fine and ultrafine particulate matter inside fire stations

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ABSTRACT

Poor air quality inside buildings has direct implications for human health. The activity of firefighters causes cancer and promotes the development/aggravation of cardio-respiratory diseases. The indoor air quality inside fire stations remains poorly characterized. This work assesses firefighters' daily exposure to fine (PM_{2.5}, PM₁₀) and ultrafine (PM_{0.45}, PM_{0.18}) particulate matter (PM) inside three microenvironments of fire stations, and, for the first time, characterizes the morphology and elemental content of collected PM. Fine PM fractions were predominant in all microenvironments. Fine and ultrafine PM concentrations were increased in the personal protective equipment (PPE) storage room than in the common area and garage [11.84–103.5 µg/m³ versus 18.67–51.89 µg/m³ and 22.57–70.69 µg/m³; $p > 0.05$ and 4.78–46.02 µg/m³ versus 3.03–40.99 µg/m³ and 5.55–23.30 µg/m³; $p > 0.05$]; values were below the guideline proposed for occupational exposure to respirable dust (5.0 mg/m³). The morphological analysis revealed the presence of organic matter, microorganisms, crystals, minerals, metals, and other unidentified substances in fine/ultrafine PM. Exposure to total PM-bound metals was increased in the garage comparatively to the common area and the PPE storage room (2765.0–46586.0 pg/m³ versus 2414.2–4568.6 pg/m³; $p > 0.5$). Cu, Zn, and Cr were the predominant PM-bound metals while Sb, Cd, and Tl were the less abundant. Exposure to PM-bound possible/probable carcinogenic metals (Ni, Cd, Sb, and Pb) accounted for 7.81–10.72% and 0.342–13.07% of fine and ultrafine PM, respectively. The data generated can support regulatory agencies in proposing air quality guidelines that promote occupational safety and health.

1. Introduction

Exposure to pollution is among the preeminent risks for human health and 4.2 million people have lost their lives due to ambient air pollution [1–4]. Exposure to air pollution occurs indoors and outdoors and in both environmental and occupational settings, causing the development and/or exacerbation of several cardiovascular and respiratory diseases [4–7]. In fact, the World Health Organization (WHO) has predicted that almost 99% of the world population is daily exposed to ambient pollution levels that exceed the available guidelines [4].

Particulate matter (PM) is one of the most health-relevant air pollutants and consists on a heterogeneous mixture composed of a wide

variety of solid particles, such as dust, soot, smoke, and liquid droplets. PM is classified according to its aerodynamic diameter as coarse (PM > 2.5 µm), fine (PM ≤ 2.5 µm), and ultrafine (PM ≤ 0.1 µm) particles [4–6, 8–10]. PM originates from anthropogenic (e.g., cooking, heating, and energy production processes, traffic emissions) and natural (e.g., ashes from volcanos and wildfires, dust storms, etc.) activities [4–6, 8]. Despite the global human exposure to PM, there is a worrying incidence in specific types of careers/occupations (e.g., miners, workers in alloy-producing factories, firefighters, etc.) with heightened health concerns since exposure to ambient PM causes lung cancer (Group 1) [4, 11, 12]. Some studies have suggested that smaller particles are more prone to cause harsh health effects than the larger fractions [9, 10, 13,

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14]. Smaller particles, especially ultrafine fractions, remain suspended in the air for longer periods, which makes them more susceptible to reach the inner areas of the lungs, specifically the alveolar surface membranes through which it can reach other organs and promote toxicity and carcinogenicity [9,15,16].

Currently, the available literature mostly characterizes coarse and fine PM indoors and outdoors [17–22]; limited data exists for the levels of ultrafine PM. WHO recently updated the air quality guidelines for long- and short-term exposure to ambient PM₁₀ (15 µg/m³ and 5 µg/m³, respectively) and PM_{2.5} (45 µg/m³ and 15 µg/m³, respectively); no limits are defined for ultrafine PM [4]. Concerning work environments, the Occupational Safety and Health Administration (OSHA), states that the maximum inhaled total PM concentration should not exceed 5.0 mg/m³, in a period of 8 working hours [23].

Different studies have already characterized the levels of fine and ultrafine PM by gravimetric analysis in indoor spaces, mainly in residential areas, schools, and nurseries [17,20,24–29]. Literature relating to ultrafine PM remains limited and focuses mainly on particle counting [30–38]. The content, size, and morphological analysis that can be performed by electron microscopy, particularly with scanning and/or transmission electron microscopy (SEM/TEM), coupled with energy-dispersive spectroscopy (EDS) analysis, were reported in the characterization of PM 2.5–10 µm, mainly in schools and households [18,19,39–42]. Despite the highlight provided by different authors and WHO [4,9,10,16,43] studies focusing on the characterization of ultrafine PM remain limited [13,20,27,28,34]. Additionally, there is a great lack of information regarding exposure to ultrafine PM and its chemical content in occupational environments [31,35,44].

The occupational activity of a firefighter (e.g., fire combat, search and rescue missions, handling hazardous materials, etc.) is carcinogenic [45–47]. Moreover, firefighters spend a considerable part of their work shift inside the fire station. The indoor air of fire stations may contain a variety of pollutants originated either from the *in-situ* activities (e.g., equipment maintenance, firefighters' habits, cooking, etc.) or outside emissions (e.g., pollutants from firefighting that traveled in the vehicles and personal protective equipment (PPE), highly trafficked roads,

industries, etc.) [46,48]. The available literature remains limited and predominantly characterizes fine PM, flame retardants, and some volatile organic compounds, including polycyclic aromatic hydrocarbons (PAHs) in the indoor air of fire stations [44,49–52]. Moreover, the characterization of fine, and especially ultrafine PM inside fire stations is of utmost importance due to the paucity of currently available studies [21,31,44,50–53]. Aiming to contribute to generating the much-needed data on human exposure to fine and ultrafine PM, this work aims to: i) assess the concentrations of four fractions of fine (PM_{2.5}, and PM_{1.56nm}) and ultrafine (PM_{54.5nm}, and PM_{30.8nm}) PM in the common areas, garages, and PPE storage rooms of fire stations; ii) perform morphological characterization of collected PM fractions by SEM; and iii) elemental characterization by EDS complemented with the quantification of 18 elements. To the best knowledge of these authors, the morphology of fine and ultrafine PM collected inside fire stations was for the first time characterized.

2. Materials and methods

2.1. Identification and characterization of sampling sites

Three fire stations (FS1, FS2, and FS3) situated in Porto (North of Portugal) were selected by the district commander since they are among the fire corporations with the highest number of registered occurrences in the district (Fig. 1). The air sampling campaign was performed during regular working days at the fire stations between November 2021 and January 2022 (winter season). FS1 is located in a town with nearly 3000 inhabitants, in the interface of urban and rural backgrounds. FS2 and FS3 are situated in urban areas with 21,000 and 34,000 inhabitants, respectively. Three microenvironments were selected inside each fire station: (A) a common area, usually an office room where firefighters can perform their administrative work and/or a nearby living room used during breaks to socialize; (B) a garage where firefighting trucks, ladder vehicles, and some emergency ambulances are parked on; and (C) the PPE storage room where all turnout gear used by firefighters during forest and structure fires are kept (Fig. 1). The selected



Fig. 1. Sampling campaigns were performed in three microenvironments, A) common area, B) garage, and C) personal protective equipment storage room with a low-pressure impactor (D) over 24 h of a regular working day to collect four fractions of fine (PM_{2.5}, and PM_{1.56nm}) and ultrafine (PM_{54.5nm}, and PM_{30.8nm}) PM (E) inside three fire stations located in the Porto district (F; North of Portugal).

microenvironments present a similar layout structure, with cement walls and tile floors. The garages of FS1 and FS2 present a similar infrastructure design, with a gate on only one side of the garage while FS3, a recently reformulated fire station, has two gates on the opposing sides of the garage to facilitate the circulation of heavy vehicles and to promote ventilation. The garages of FS1 and FS3 were physically separated by a closed door from the remaining areas of the fire station while at FS2, the garage was downstairs from the main floor. The firefighting PPE was stored in closed lockers in a room adjacent to the garage (FS1 and FS3) the PPE storage room, and in lockers at the button of the garage (FS2).

2.2. Collection of fine/ultrafine PM and gravimetric analysis

The air sampling campaigns were performed, as previously described by Teixeira et al. [44]. Briefly, a low-pressure impactor (DLPI+, Dekati, Finland) able to collect particles with a cut-off diameter ranging from 16 nm to 10 µm, was used during regular working days at the fire station. Different fractions of fine and ultrafine PM were collected in the indoor air of the selected microenvironments for each fire station: PM with cut-off diameters of 2.5 µm (PM_{2.5}), 156 nm (PM_{156nm}), 54.5 nm (PM_{54.5nm}), and 30.8 nm (PM_{30.8nm}). A total of 36 samples were collected. The equipment was positioned 1.5 m above the floor, to sample the air close to the breathing area of the firefighters working at the station, and away from the room's walls and door. The humidity and temperature of each sampled site were monitored with a HygroLog SERIES HL20 device (Rotronic, Switzerland). Each microenvironment was sampled over 24 h, to simulate a full working day inside the fire station. The equipment was previously calibrated by the supplier for each PM fraction, i.e., D50 average particle size for each stage at a pressure of 40 mbar and a constant flow rate of 9.96 L/min. The particles were collected onto aluminium filters (Ø25 mm; Dekati®, Finland) that were covered with a thin layer of substrate grease (Apiezon®-L, Sigma-Aldrich) to avoid bouncing off particles. Each sample was stored in a closed cartridge and kept at room temperature. The mass of particles collected in each fraction was determined by gravimetric analysis using an analytical balance (MS205DU, Mettler Toledo) at controlled temperature and humidity conditions.

2.3. Electron microscopy and elemental analysis

A conductive carbon adhesive tape was used to cover the SEM stub and to coat the aluminium filter containing the collected particles. Quanta 400 FEG scanning electron microscope (SEM, FEI, Hillsboro, OR) was employed to characterize the collected PM fractions inside fire stations. The equipment operated at an accelerating voltage of 15 kV with a magnification 10X up to 150,000X, equipped with a secondary electron and a back-scattered electron detectors operating in low vacuum with an electron beam resolution of 3.0 nm. EDS spectra were acquired using the electron microscope coupled with an EDAX Genesis X4M Energy-dispersive Spectroscopy, with a detector type SUTW SAPHIRE analysis system of resolution 132.19. Electron Microscopy characterization was performed at CEMUP – Centro de Materiais da Universidade do Porto, Porto, Portugal.

The collected PM fractions were mixed with 10 mL of acetone and sonicated for 20 min at room temperature. The solution containing the collected particles was evaporated until dryness and 3 mL of nitric acid (>65%, Sigma Aldrich, Germany), and 1.5 mL of hydrogen peroxide (30%, Merck Millipore, USA) were added to the dried extract and digested for 30 min at 95 °C in a DigiPREP Jr apparatus (SCP Science, Canada). The digested solution was analyzed by ICP-MS using an iCAP™ Q (Thermo Fisher Scientific, Germany) and a Meinhard® (Golden, CO) TQ+ quartz concentric nebulizer, following a method adapted from Pinto et al. [54]. The elemental content of 18 elements [lithium (Li), beryllium (Be), chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), antimony (Sb), cesium (Cs), barium (Ba), mercury

(Hg), thallium (Tl), and lead (Pb)] was determined in the digested PM fractions, as described by Pinto et al. [55]. The limits of detection (LOD) were determined by multiplying the blank standard deviations by 3.3 and ranged between 3.43 ng/L (Be) to 6.057 µg/L (Zn) [55]. All samples and standards were analyzed in triplicate.

2.4. Data analysis

The concentration of each PM fraction was determined according to the specifications of the impactor supplier, namely the DLPI+ specifications and equipment calibration parameters as well as the temperature and the relative humidity of the sampled locations, as described in detail in a previous work of the research team [44]. Additionally, PM concentrations were normalized considering the atmospheric pressure and the ambient temperature of 21 °C. When elemental concentrations were below the LOD, the values were replaced by LOD/√2 [56]. The results did not follow a normal distribution, according to Shapiro-Wilk's test, therefore the concentrations are presented as median and range. The software Excel (v.16.0. Microsoft Corporation, USA) was used to accomplish statistical analysis. The statistical significance was defined as $p \leq 0.05$.

3. Results and discussion

3.1. Levels of fine and ultrafine PM

Table 1 presents the concentrations of fine and ultrafine PM at the selected microenvironments inside fire stations. The total PM levels of the four fractions analyzed were increased in the PPE storage room (55.75–191.0 µg/m³), followed by the common area (57.41–129.6 µg/m³), and the garage (77.98–113.8 µg/m³); no significant differences were observed ($p > 0.05$). The breathable PM levels monitored inside the fire stations were well below the occupational exposure guideline of 5.0 mg/m³ proposed by OSHA for respirable dust [23]. Fine PM, PM_{2.5} and PM_{156nm}, were the predominant fractions in all the monitored microenvironments (Table 1). The median concentrations of PM_{2.5} in the indoor air of fire stations ranged between 21.53 µg/m³ in the PPE storage room to 44.27 µg/m³ in the common area. Regarding PM_{156nm}, the monitored values varied between 23.23 µg/m³ in the common area and 55.26 µg/m³ in the PPE storage room. To the best knowledge of these authors, no benchmark or guideline values are available for occupational exposure to fine PM including PM_{2.5}. Recently, the WHO updated the limit for short-term exposure to ambient PM_{2.5} (15 µg/m³); however, no recommendation exists for the indoor levels of PM [4,12]. The median concentrations of ultrafine PM, PM_{54.5nm} and PM_{30.8nm}, ranged between 11.35 and 27.47 µg/m³ and from 5.98 to 12.49 µg/m³, being the highest values found in the PPE storage room (maximum of 46.02 µg/m³) and the common area (maximum of 40.99 µg/m³), respectively (Table 1).

The characterization of fine PM in fire stations remains limited in the literature. Baxter et al. [53] measured the PM_{2.5} concentrations in the kitchen, truck bay, and sleeping quarters of two North American firehouses (Cincinnati, USA), and reported values ranging between 18 µg/m³ at the truck bay and 155 µg/m³ at the kitchen of the firehouse, which are 37.3% lower and 250% higher than the PM_{2.5} levels obtained for the garages and common areas sampled in this study, respectively. Couch et al. [57] found an average of PM₁ of 34.0 and 29.0 µg/m³ for the living quarters and apparatus bay, respectively.

For PM_{2.5}, those authors described average concentrations that were 20.9% lower and 4.57% higher than the levels found inside the common area and the garage of Portuguese fire stations (35 and 30 µg/m³ versus 44.27 and 28.69 µg/m³) respectively. Oliveira et al. [58] reported levels of PM_{2.5} inside Portuguese fire stations that were 42.6–430.6% lower (0.05–1.04 µg/m³). Sparer et al. [52] assessed the PM_{2.5} concentrations in the truck bay of two fire stations situated in Boston, USA, and reported

Table 1
Concentrations of fine (PM_{2.5} and PM_{156nm}) and ultrafine (PM_{54.5nm} and PM_{30.8nm}) PM fractions (median and range; µg/m³) in the microenvironments of fire stations.

PM size		Common area	Garage	PPE storage room	Significance
Fine PM	PM _{2.5}	44.27 (20.96–51.89)	28.69 (23.95–70.69)	21.53 (11.84–103.5)	<i>p</i> > 0.05
	PM _{156nm}	23.23 (18.67–30.61)	27.77 (22.57–34.57)	55.26 (21.81–62.28)	<i>p</i> > 0.05
Ultrafine PM	PM _{54.5nm}	14.75 (6.14–23.60)	11.35 (9.40–23.30)	27.47 (14.07–46.02)	<i>p</i> > 0.05
	PM _{30.8nm}	12.49 (3.03–40.99)	5.98 (5.55–8.11)	8.03 (4.78–24.31)	<i>p</i> > 0.05

levels up to 58.8% higher (12.14 and 45.56 µg/m³) than in the garages of Portuguese fire stations. Kim et al. [59] also determined the PM_{2.5} concentrations inside two Korean fire stations and reported values up to 210.4% higher than in this study (25.2–137.4 µg/m³ versus 21.53–44.27 µg/m³). Recently, Rakowska et al. [21] performed a study in the garage of a Polish fire station and reported indoor PM_{2.5} concentrations up to 45.8–83.6% (31.4 and 81.3 µg/m³) higher than in the garages sampled in this study. Overall, the PM_{2.5} levels presented in this work are in close range and sometimes lower than the concentrations reported in the literature. However, it is important to highlight that PM_{2.5} values reported in the literature represent the cumulative PM ≤ 2.5 µm while the levels achieved in this work correspond to the concentrations of PM with 2.5 µm, not including lower fractions of particles.

Regarding the monitorization of ultrafine PM in fire stations, only two recent studies are available. Kellnerová et al., [31] measured the ultrafine PM concentrations at values varying between 10.67 and 25.18 µg/m³ in the changing room and chemical workshop of a fire station situated in Brno, Czech Republic. Recently, Teixeira et al. [44] assessed the levels of 14 PM fractions, including 4 ultrafine PM (cut-off diameter of 95, 54, 31, and 15 nm), in 4 microenvironments of 7 Portuguese fire stations. The reported total cumulative levels of ultrafine PM in the fire stations ranged between 41.3 and 78.2 µg/m³, with the highest levels being found in the PPE storage room (69.7 µg/m³) and the garage of heavy vehicles (52.6 µg/m³) [44]. Those authors reported that fine and ultrafine PM respectively accounted for 63.4–74.2% and 14.9–21.2% of total cumulative PM inside fire stations, with PM_{54nm} and PM_{95nm} presenting the highest levels [44]. Other studies, (although still limited) have also performed measurements of ultrafine PM, mostly related to particle counting, in other occupational environments such as offices (6503–18060 particles/cm³), copy centers (PM_{100nm}, 3670–33743 particles/cm³), fitness centers (PM_{20–100nm}, 474–88600 particles/cm³), or dental clinics (PM_{10–300nm}, 6602–9196 particles/cm³) [37,38,59–61]. Due to the paucity of studies available, additional studies are needed to characterize the levels of PM, particularly ultrafine PM and to estimate potential risks to human health. This much-needed data will be crucial for regulatory agencies to propose occupational exposure limits.

3.2. Morphological characterization of fine and ultrafine PM

The PM fractions collected in the selected microenvironments of fire stations were analyzed through electron microscopy. Morphological and size content from fine and ultrafine PM were evaluated using SEM images (Fig. 2). The acquired images correspond to a representative singular PM spot, since PM is homogeneously distributed in the sampled aluminum filter (Fig. 2). Specific materials of reduced dimensions (e.g., rust, crystals, minerals, etc.), with composition based on variable elements, were identified in PM. Also, some biological components with a microscale size were also observed.

Regarding fine PM, the lower magnetization images (250x, Fig. 2A1, B1, C1) evidenced that PM material from each sample size has similar diameters, being homogeneously dispersed in the characterized area, i.e., no agglomeration of particles was observed. The format in which the material is disposed in the filter is similar for both fine PM fractions, PM_{2.5} and PM_{156nm}. The unique characteristics that differentiate fine PM fractions are mainly observed at higher magnetizations. Fig. 2A2 presents a yeast integrated in the sampled material, demonstrating that microorganisms are present in breathable PM collected inside the

sampled fire stations. Additionally, a round-shaped particle with approximately 2 µm diameter, with angular edges, was clearly identified in Fig. 2A2, thus representing the collected PM_{2.5}. The microscopic images also present other interesting material of angular shape (similar to a pollen grain or spore) that can be observed in Fig. 2B2. Meanwhile, the image presented in Fig. 2C2 demonstrated the predominance of organic matter (darker contrast) due to the absence of non-conductive compounds (lighter contrast). The organic fraction of PM can vary depending on the source of emission, however, it is mostly characterized by the presence of elemental and organic carbon, as well as volatile and semi-volatile organic compounds such as PAHs [6,8,34,50,62–64].

Regarding the morphological characterization of collected ultrafine PM, Fig. 2 exhibits a single PM spot from PM_{54.5 nm} (images A3) and PM_{30.8nm} (image B3 and C3). In these images, it is possible to observe round PM spots that display two colors with light and dark contrasts. In image A3, the presence of an outer ring with lighter contrast and a darker inner dot is visible; the opposite is observed in for image B3.

These findings can be explained by the deposition of the least amount of non-conductive material (light contrast) and more conductive material (darker contrast) on the surface of the sampled filter. The SEM images demonstrate the presence of organic materials absorbed/adsorbed to the collected ultrafine PM inside fire stations. There are different sources of organic matter at the sampled microenvironments, including the penetration of ambient air from the surrounding areas of the fire station, fire emissions brought from firefighters' PPE and firefighting vehicles, consumption of tobacco, meal preparation, cleaning, and maintenance activities, etc. [46,48,50]. It is expected the presence of higher levels of fire emissions and vehicle exhausts in the PPE storage room principally due to its physical proximity to the garage of firefighting vehicles and the contamination of used PPE with fire emissions [46,48].

Although similarities can be noted between images C3 and B3 (Fig. 2), it is hypothesized that the former is characterized by a lighter contrast that could be related to elements from fire combat released during combustion and deposited in the firefighters' PPE. Furthermore, images A4, B4, and C4 apparently demonstrate a similar morphology of the analyzed PM fractions; however, in image A4 layers at different depths of PM can be distinguished, while in images B4 and C4 only a single layer is visible (Fig. 2). Overall, it can be highlighted that fine PM fractions are characterized for being much more heterogeneous than ultrafine PM, which is composed of particles that are more regular in shape. Other authors found similar findings when performing electron microscopy analysis of PM collected in a school from Singapore, with 60–70% of the PM sampled corresponding to coarse PM (i.e., PM > 8) [40]. Information regarding the morphological characterization of PM, especially the fine and ultrafine fractions, is still quite limited, and a more comprehensive characterization is needed.

3.3. Electron microscopy characterization of PM

3.3.1. Fine PM

Sampled fine PM (PM_{156nm} and PM_{2.5}), collected from the air of FS1's common room was fully evaluated through electron microscopy. SEM images and EDS spectra analysis were conducted, and the data collected (SEM - A_I; EDS - A_{II} and A_{III}, for PM_{2.5} and SEM - B_I, B_{II}, B_{III}; EDS - B_{IV}, B_{VI}, for PM_{156nm}) are illustrated in Fig. 3. The acquired SEM images reveal that besides the observed organic fraction, some components of

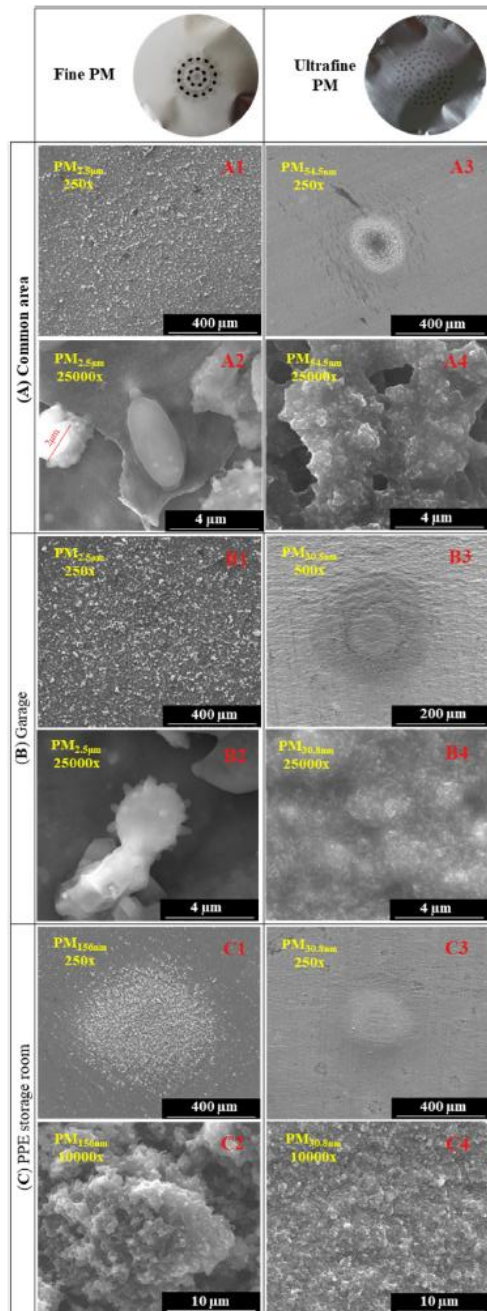


Fig. 2. Scanning electron microscopy analysis of fine ($PM_{2.5}$ and PM_{156nm}) and ultrafine ($PM_{5.5nm}$ and $PM_{30.8nm}$) PM collected on A) common area (A1–A4), B) garage (B1–B4), and C) personal protective equipment storage room (C1–C4) of fire stations.

interest were detected, with the full characterization by EDS spectra allowing a proper identification of the material content.

Regarding $PM_{2.5}$ (Fig. 3A_I), SEM depicted two highlighted zones (Z1 and Z2) that correspond to the respective EDS spectra (A_{II} and A_{IV}). In the image A_{II} (Z1 area) spectrum, among the noticeable peaks Ba, sulfur (S), aluminum (Al), and oxygen (O), only 3 of them are of interest (highlighted in blue dashed boxes), since the SEM imaging of the PM fractions was carried out directly on the aluminum filters used to collect the PM, and thus the Al peak will not be discussed. The identified peaks confirm the presence of barium sulphate ($BaSO_4$) in fine PM of fire stations. According to the literature, this salt is frequently used as an additive in cosmetics, and food packaging items, among other materials [64]. Accounting for the context of this analysis, the $BaSO_4$ found in the airborne PM of fire stations might be sourced from construction materials including paints (due to its hardening and pigment extender capabilities), products containing flame retardants, in primary health care equipment (as a sterile compound used in medical equipment, such as face masks, scrubs, or gloves), or even from products used in vehicle repair and maintenance [64].

Concerning Fig. 3A_{III} (Z2 area) image, a complex EDS spectrum was identified with the following compounds: (i) iron oxide (FeO , otherwise known as rust), which is among the most common compounds found in these environments, e.g., materials/equipment with heavy machinery that require regular maintenance and cleaning, which can release airborne particles; (ii) Ca (calcium), P (phosphorous), and Si (silicon) peaks that might be related to a mineral compound, since the obtained elements are major components of sand and crystals, such as fluorapatite, feldspar, or quartz; and (iii) Ti (titanium) which is commonly identified in its oxide (TiO) and dioxide (TiO_2) forms, being TiO_2 the most expected form in fire stations due to its usage in the ultraviolet (UV) radiation screens components present in paints [65,66].

Regarding PM_{156nm} , the SEM Fig. 3B_I (Z1 area) identifies the presence of a substance similar to a crystal. The EDS spectrum analysis (Fig. 3B_{II}) confirmed the presence of potassium sulphate (K_2SO_4) as the predominant substance in the composition of the crystal. This compound is majorly used in fertilizers, a possible source of contamination since the analyzed PM fraction was collected in a corporation located in a rural environment, or originated from construction materials [67]. It can be noticed that the identified crystals present a size that is larger than the cut-off diameter of the used impactor pores for this fraction (156 nm). This fact, along with the materials' arranged disposition (the crystals appear to have grown from the middle) can be related to a K_2SO_4 salt that entered the system dissolved in a water droplet and precipitated/crystallized in the aluminum filter. Conner & Williams [41] described the presence of particles larger than 2.5 μm in $PM_{2.5}$ fraction, which resembles this evidence taken from Fig. 3B_I (Table 1S of the Supplementary Material). Regarding the SEM image presented in Fig. 3B_{III}, which corresponds to the EDS spectrum B_{IV} (Z2 zone), distinct peaks stand out (Fe, Cr, and Ni), which can be related to the stainless-steel constitution, a common substance found in heavy machinery (typically present in fire stations). The identified Z3 zone (image B_V) presents a black aggregate (Fig. 3). Through the EDS spectrum (Fig. 3 B_{VI}) it is demonstrated the presence of organic matter in this aggregate of fine PM. Furthermore, the C (carbon), K (potassium), S, and Cl (chlorine) peaks were identified in this spectrum. Cl is a traditional constituent of cleaning products, being the possible source of this element in the collected PM [68]. There are still no electron microscopy studies performed on PM collected from fire stations, so comparison was made using studies that characterized PM from other indoor spaces and from PM released during woody biomass combustions (Table 1S). Evidence demonstrates similarities with the results obtained in this work, i.e., elements such as C, Cl, Fe, Cr, Ni, Cu, Zn, Sr, Ti, Ba, and Pb, were found in the indoor spaces of schools, houses, and public services [18,19,36,42,69]. Some authors also identified the presence of silicates in the fine PM collected from a school, a household, and a laboratory [19,40,70]. Additionally, chlorides and sulphates (e.g., Na, Mg, K, and Ca) were

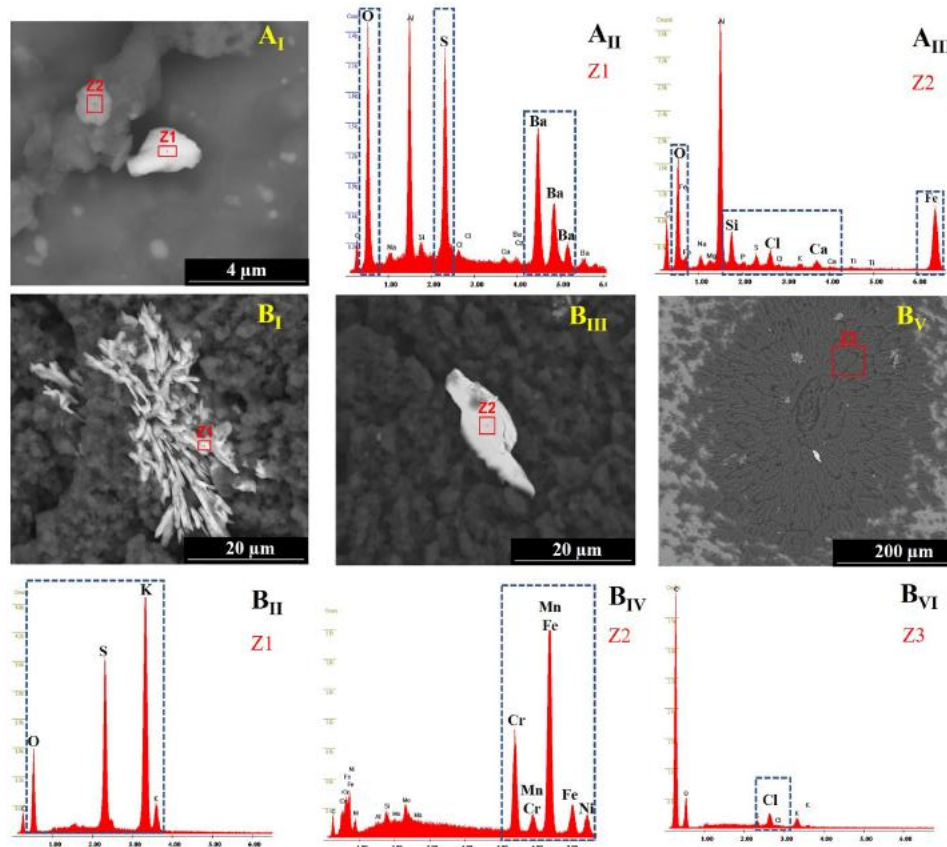


Fig. 3. Characterization of A) $PM_{2.5}$ and B) PM_{156nm} through scanning electron microscopy (A_I , B_I , B_{III} and B_V) and energy-dispersive spectroscopy (A_{II} , A_{III} , B_{II} , B_{IV} and B_{VI}) collected on the common area of fire station 1. Z1 to Z3 represent the amplified pre-selected zones of the collected PM fractions.

the most common compounds found in fine PM characterized during woody biomass burning [71–73] as well as in fine/ultrafine PM from indoor spaces of public services, and schools [18,40,70].

3.3.2. Ultrafine PM

SEM images and EDS spectra of ultrafine PM collected in the common room (images A–F) and the garage (image G) of FS1 are presented in Fig. 4 and identified according to the respective cut-off diameter of the filters: A–E and F–G represents the characterization of $PM_{5.4,50nm}$ and $PM_{30.8nm}$, respectively. Distinct zones/particles are highlighted (Z1–Z4) in Fig. 4A. As previously stated, K_2SO_4 is by far the most prevalent compound in this fraction (Z1). Z2 characterizes the black aggregate zone that corresponds to organic matter, according to the EDS spectrum (image A_{III}), where it is also possible to visualize a small chlorine peak. However, oppositely to the EDS spectrum observed in Fig. 3B_V, in this one, it is possible to see a large Al peak that is justified by the lower concentration of PM found in this fraction, which allows the electron beam to penetrate the sample and reach the filter layer. In Fig. 4B_I it is possible to perceive a rust (FeO) particle, identified as Z3. Besides the K_2SO_4 crystal present in Fig. 4C_I (Z1), one more zone was characterized (Z4). In the EDS spectrum of Z4, there are 3 main peaks of interest, namely Si, Al, and O. Together, these elements are the main constituents of aluminosilicates, one of the most common types of minerals. However, this compound might be a regular sand grain or a rust particle (Fe

and O are also present) since it is not possible to conclude if the source of Al is the filter used to collect PM or the elemental content of the PM fraction. In Fig. 3D_I, Z5 represents another stainless-steel particle (similar to Fig. 3B_{III}). The last image characterizing the $PM_{5.4,50nm}$ (Fig. 4E_I) shows a round-shaped particle that, according to the respective EDS spectrum, is identified as iron oxide. Contrarily to the previous times that this compound was identified, this one has a round regular shape, meaning that it was created under high heat and formed that shape after cooling down in the air, for example, a spark created by an angle grinder. These round-shaped compounds are also commonly known as fly ashes [74]. Fig. 4F_I and 4G_I represent $PM_{30.8nm}$ collected from the common room and garage of FS1, respectively. Although being from 2 different microenvironments of the same fire station, the fractions are identical since the only compound that the team was able to identify on the collected PM was K_2SO_4 (Z1 from Fig. 4F_{II} and 4G_{II}). Z2 from Fig. 4G_I represents the filter background and the organic matter, although the C peak in the EDS spectrum is much smaller than in the previous spectra, which is explained by the lower concentration of PM found in the filter of this fraction, in accordance with the values presented in Table 1. To the best of knowledge of these authors, only one study assessed the characterization of ultrafine PM by electron microscopy. Jiménez et al. [73] collected PM, including ultrafine fractions from oak tree, chestnut tree, and eucalyptus wood sawdust combustion. According to the EDS spectra, K, S, P, and Cl were the predominant

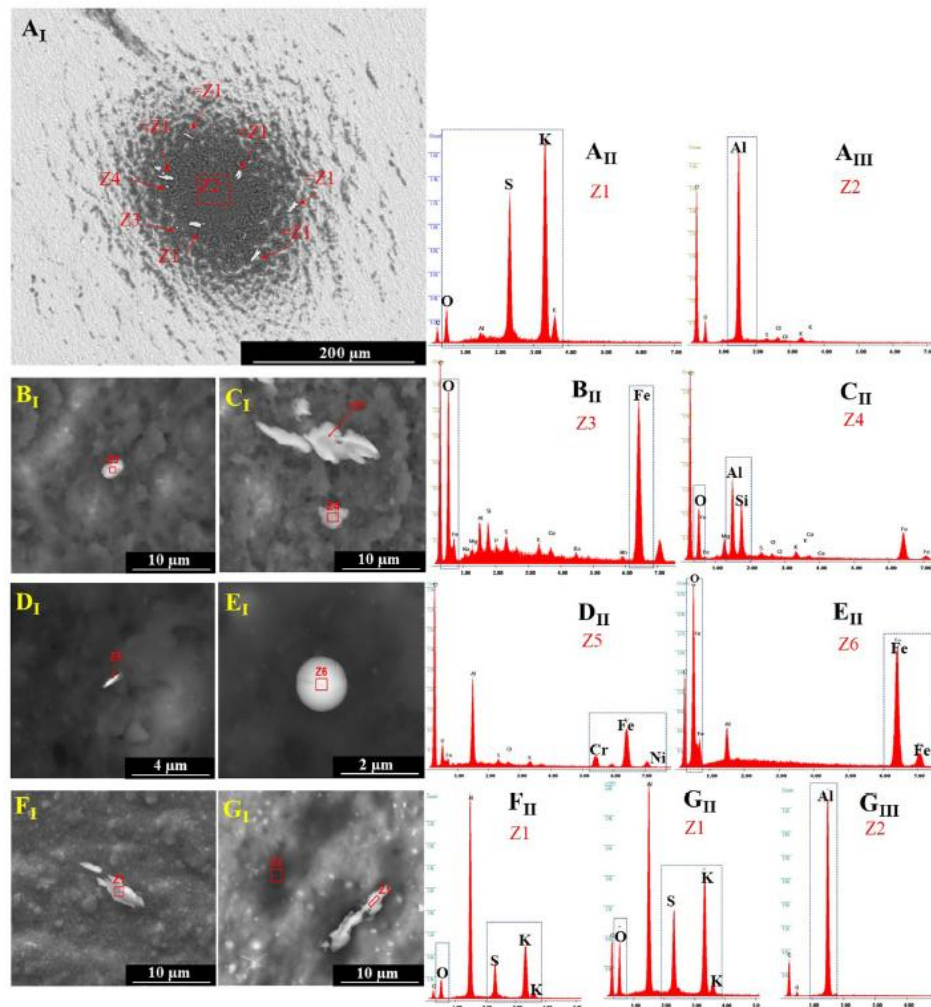


Fig. 4. Characterization of PM_{54.5nm} (A–E) and PM_{30.8nm} (F–G) through scanning electron microscopy and energy-dispersive spectroscopy on the common area (A–F), and the garage (G) of fire stations 1. Z1 to Z6 represents the amplified pre-selected zones of the collected PM fractions.

elements identified in ultrafine PM released from sawdust combustion (Table 1S). The presence of these elements was attributed to the identification of sulphates and chlorides salts, similar to what was observed in fine and ultrafine PM collected from the garage and common area of the sampled fire stations. The presence of these salts can be related to possible contaminants from fire emissions that travel with firefighters' equipment used during firefighting activities (Fig. 4). The paucity of studies regarding ultrafine PM characterization impedes the drawing of further conclusions. Thus, more studies characterizing fine and principally ultrafine PM in fire stations and other indoor environments are urgently needed to better characterize the morphology and chemical composition of PM from fire stations and PM in general.

3.4. Elemental analysis of fine and ultrafine PM

Overall, daily concentrations of total PM-bound metals were increased in the garage (2765.0–46586.0 pg/m³), followed by the

common area (2510.6–4568.6 pg/m³), and the PPE storage room (2414.2–4172.6 pg/m³); no significant differences were found ($p > 0.05$). The total metal content was predominantly higher in fine than in ultrafine PM of the sampled common areas (2127.3–2185.5 pg/m³ vs 325.1–2441.3 pg/m³) and PPE storage rooms (1369.9–3170.4 pg/m³ vs 1002.2–1044.3 pg/m³). Regarding the garage, a different tendency was observed with increased levels of total PM bound metals in the ultrafine (2289.5–46024.0 pg/m³) than the fine fractions (475.5–562.1 pg/m³). There are different sources of airborne metals inside fire stations, with fire emissions and diesel exhaust being the most relevant ones [75–82]. Out of the 18 analyzed metals, 7 elements (Li, Be, As, Se, Sr, Cs, and Hg) were not detected in the collected PM fractions. The metal distribution (%) of each individual metal in the fine and ultrafine PM, for the different locations of the fire stations analyzed, are presented in Fig. 5. Among the 11 metals detected, Cu (731.1–15944.9 pg/m³), Zn (1239.6–1692.3 pg/m³), and Cr (219.1–263.9 pg/m³) were the most predominant and accounted for 17.82–94.93 %, 3.96–66.45 %, and

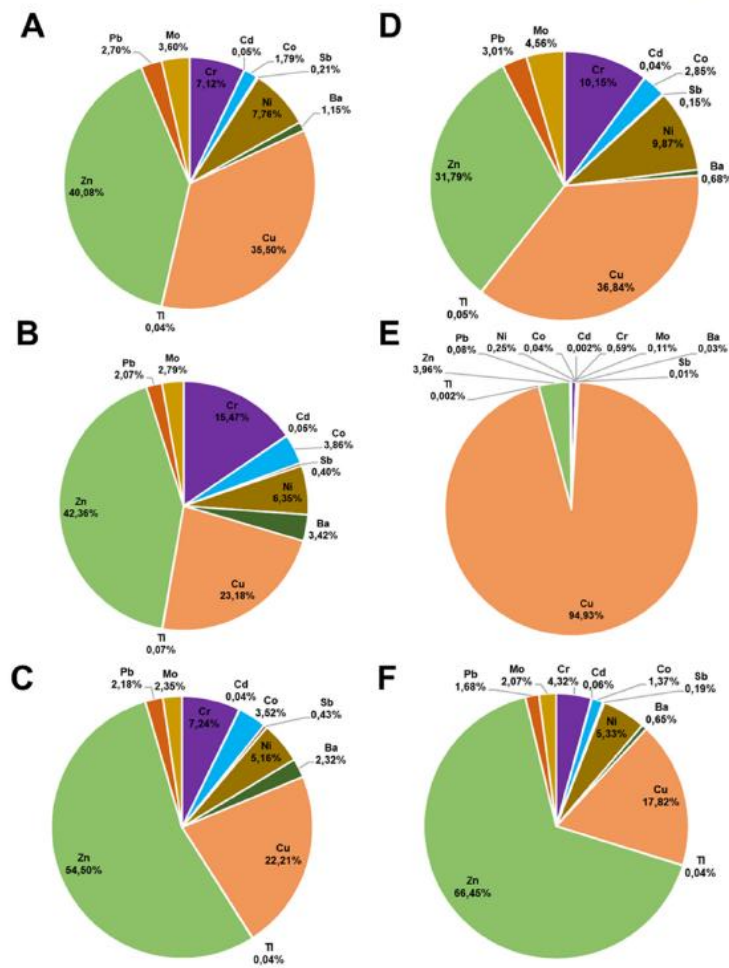


Fig. 5. Distribution (%) of PM-bound metals in the fine (A–C) and ultrafine PM (D–F) fractions collected in the common area (A and D), garage (B and E), and PPE storage room (C and F) of the analyzed fire stations.

0.59–15.47 % of the total metal content of fine and ultrafine PM in the entire fire stations, respectively. Sb (6.414–8.296 $\mu\text{g}/\text{m}^3$), Cd (1.283–1.369 $\mu\text{g}/\text{m}^3$), and Tl (1.218–1.362 $\mu\text{g}/\text{m}^3$) were the less abundant elements, representing 0.01–0.43 %, 0.002–0.06 %, and 0.002–0.07 % of the total metal content in the whole fire stations, respectively (Fig. 5). Elements such as Mg, K, Fe, Ni, or Zn are predominantly present in fumes from the combustion of biomass [67,73, 83–90], while Cu, Cd, Sb, or Pb are more frequently found in the fumes released during the combustion of plastics, paints, and electronic devices [83,91–93]. Also, firefighting tools, vehicles, and used PPE also bring fire emissions inside fire stations, principally to the garage and the PPE storage room [46,48].

Some of the metals that were detected have carcinogenic properties, namely Ni, Cd, Sb, and Pb, and their presence in the fire stations' air can contribute with potential risks for the respiratory health of firefighters [20,79,94–98]. Overall, the cumulative concentrations of these metals varied between 118.72 and 184.17 $\mu\text{g}/\text{m}^3$ in fine and from 67.20 to 157.66 $\mu\text{g}/\text{m}^3$ in ultrafine PM, accounting for 7.81–10.72% and 0.342–13.07 % of total PM-bound metals, respectively.

Table 2 summarizes the main findings related to the morphological and chemical characterization of collected fine and ultrafine PM inside Portuguese fire stations. Overall, the results gathered with the ICP-MS analysis complement the data collected from the SEM-EDS spectra analysis, by allowing the quantification of minor elements present in the collected fine and ultrafine PM fractions. The results presented in this work highlight the potential of combining these two methodologies for a more comprehensive characterization of fine and ultrafine PM-bound metals.

4. Conclusion

The concentrations of fine ($\text{PM}_{2.5}$ and $\text{PM}_{156\text{nm}}$) and ultrafine ($\text{PM}_{54.5\text{nm}}$ and $\text{PM}_{30.8\text{nm}}$) PM were determined in the common area, garage, and PPE storage room of Portuguese fire stations, with cumulative total PM levels (55.75–191.0 $\mu\text{g}/\text{m}^3$), being below the guideline proposed by OSHA for occupational exposure to total PM (5.0 mg/m^3 [23]). The PPE storage room was the microenvironment where the highest concentrations of total PM were observed, followed by the

Table 2
Main findings regarding the morphological and chemical characterization of fine and ultrafine PM collected inside fire stations.

	Analysis	Common area	Garage	PPE storage room
Fine PM (PM _{2.5} & PM ₁₀)	Electron Microscopy	Biological content (yeast) Crystals superior to cut-off diameter	Possible biological fraction (pollen/spore grain)	Homogeneous layer (organic carbon and organic compounds)
	Elemental characterization	Apatite mineral (Ca, P, and Si); BaSO ₄ , FeO, TiO ₂ , K ₂ SO ₄ crystal (>156 nm), stainless steel (Fe, Cr, and Ni), and Zn (40.1%), Cu (35.5%), Ni (7.76%), and Cr (7.12%)	Zn (43.4%), Cu (23.2%), Cr (15.47%), and Ni (6.35%)	Zn (54.5%), Cu (22.2%), Cr (7.24%), and Ni (5.16%)
Ultrafine PM (PM _{5.4.5} & PM _{3.0.8})	Electron Microscopy	Conductive and non-conductive material Overlapping layers Round shaped FeO fly ash	Conductive material Single layer	Non-conductive material Single layer
	Elemental characterization	K ₂ SO ₄ , aluminosilicate (Si, Al, and O), stainless steel (Fe, Cr, and Ni), and FeO (fly ash) Cu (36.8%), Zn (31.8%), Cr (10.2%), and Ni (9.87%)	K ₂ SO ₄ Cu (94.9%), Zn (3.96%), Cr (0.59%), and Ni (0.25%)	Zn (66.5%), Cu (17.8%), Ni (5.33%), and Cr (4.32%)

common area and garage, however, no significant differences were observed. The electron microscopy analysis provided insights into the morphological characteristics of the fine and ultrafine PM collected inside fire stations, revealing the presence of organic matter, microorganisms, rust, crystals, minerals, and several distinct metals among other unidentified substances. The morphological analysis of fine/ultrafine PM was complemented by the quantification of elemental content. Increased concentrations of total PM-bound metals were found in fine than in ultrafine PM fractions from the sampled common areas (2127.3–2185.5 pg/m³ versus 325.1–2441.3 pg/m³) and PPE storage rooms (1369.9–3170.4 pg/m³ versus 1002.2–1044.3 pg/m³), except for the garage (2289.5–46024.0 pg/m³ versus 475.5–562.1 pg/m³). The determined concentrations were increased in the garage of fire stations comparatively to the common areas and PPE storage rooms, being Cu, Zn, and Cr the predominant elements. Some possible/probable carcinogenic elements, e.g., Ni, Cd, Sb, and Pb, were found in fine and ultrafine PM at concentrations ranging between 184.17 and 118.72 pg/m³ and 157.66–67.20 pg/m³, respectively. Overall, these preliminary findings highlight the contribution of ultrafine particles for breathable total PM as well as the potential of combining the morphological characterization of (fine/ultrafine) PM with its metal content. The main limitation of this work was the limited number of fire stations and the reduced number of indoor microenvironments monitored in each sampling site. Therefore, additional studies including other fractions of fine/ultrafine PM and a higher number of sampling points collected on different seasons (e.g., winter and summer) are needed to corroborate the results of this work and to explore better morphology and composition of fine and ultrafine PM inside fire stations. Moreover, other techniques can be used in future studies to characterize PM, including TEM and dark field imaging [34]. The results achieved represent a spot view on the characterization of fine/ultrafine PM during some winter days inside fire stations, and it does not reflect the contribution of some firefighters' task, such as 1) firefighting activities that are more prone to occur on the hot season, and 2) assistance to the collapse of building and spillage of dangerous materials, which may change the morphology and composition of PM. This much-needed information will significantly move scientific knowledge forward by generating data that would be available to regulatory agencies and could be useful for the proposal of indoor air quality guidelines. It would also contribute to the implementation of preventive strategies that would reduce firefighters' occupational exposure inside fire stations and thus promote their occupational safety and health.

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CRediT authorship contribution statement

Gabriel Sousa: Writing – original draft, Investigation, Formal analysis, Data curation. **Joana Teixeira:** Visualization, Investigation, Formal analysis. **Rui Azevedo:** Investigation, Formal analysis. **Agostinho Almeida:** Visualization, Supervision, Resources, Formal analysis. **Cristina Delerue-Matos:** Visualization, Supervision, Funding acquisition. **Simone Morais:** Visualization, Resources, Funding acquisition. **Xianyu Wang:** Visualization, Supervision. **Francisca Rodrigues:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization. **Maria Freitas:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Marta Oliveira:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.buildenv.2024.111403>.

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Chapter 3.

Morphological and elemental characterization of
fine and ultrafine particulate matter generated
from fires



Morphological and elemental characterization of fine and ultrafine particulate matter generated from fires

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ABSTRACT

Fire emissions aggravate air pollution by releasing several hazardous pollutants, including respirable particulate matter (PM). Evidence on fires contribution to fine and ultrafine PM is scarce, principally in real fire scenarios. This study presents the first morphological and elemental composition of fine (PM_{2.5}, PM_{156nm}) and ultrafine (PM_{50.4nm}, PM_{30.8nm}, PM_{14.9nm}) fractions of PM released during three controlled structure (CSF) and two prescribed forest (PFF) fires.

Total median PM levels were 9 times higher in CSF emissions than in PFF [11.13 (1.245–228.5) mg/m³ versus 1.232 (0.5458–1.920) mg/m³], with fine PM being 13 and 3 times higher than ultrafine PM in CSF and PFF, respectively ($p > 0.05$). The morphological analysis revealed the prevalence of organic matter in fine/ultrafine PM, with several particles of bright contrast with distinct shapes and sizes, which were more prominent in PFF. The electron dispersive spectroscopy and elemental mapping analysis suggested the presence of sulphates, carbonates, and chlorides of sodium, magnesium, potassium, and calcium (biomass combustion), and stainless-steel particles associated with handling of heavy machinery in PFF. Also, particles of PbCl₂ and PbO (combustion of paints/solder) were observed in CSF. Zn, Cu, Cr, and Ni, as well as Co (in PFF), and Pb (in CSF) were the predominant elements in fire generated PM. Toxic and (possible/probable) carcinogenic elements represented up to 29.3 % and 33.1 % of fine and ultrafine fire generated PM, respectively. This work advances the knowledge on fire-generated PM highlighting the need for further studies on fire chemistry, PM behaviour, and realistic human exposure and health risk assessments.

1. Introduction

Environmental pollution negatively impacts societies and human health (IARC, 2015; Pozzer et al., 2023). The World Health Organization (WHO) reports millions of premature deaths, yearly, related to exposure to air pollution in indoor and outdoor environments and exacerbated cardiovascular and/or respiratory diseases (Douglas et al., 1993; Fromme, 2019; Garbarienė et al., 2022; IARC, 2015; Ozkaynak et al., 1996; Pozzer et al., 2023; Sang et al., 2022; WHO, 2021).

Particulate Matter (PM), one of the most health relevant pollutants, was recognized in 2013 as carcinogenic to humans by the International Agency for Research on Cancer (IARC) and contributes to the development or aggravation of cardiovascular and respiratory diseases including chronic obstructive pulmonary disease, hypertension, and lung cancer (Chu et al., 2019; Guo et al., 2022; IARC, 2002, 2010; Loomis et al., 2013; Marval and Tronville, 2022; Moreno-Ríos et al., 2022; Nezis et al., 2019; Ohlwein et al., 2019; Schraufnagel, 2020; WHO, 2006). PM have different emission sources, e.g., forest/urban

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fires, traffic emissions, cooking activities, among others and is categorized as coarse ($2.5 < PM < 10 \mu m$), fine ($PM \leq 2.5 \mu m$), and ultrafine ($PM \leq 0.1 \mu m$) particles (Chowdhury et al., 2024; Fromme, 2019; Karagulian et al., 2015; Loomis et al., 2013; Shaughnessy et al., 2015; Wang et al., 2020; WHO, 2021). Respirable PM ($PM \leq 2.5 \mu m$), particularly fine and ultrafine particles, poses a greater health risk due to its reduced size, since small particles remain more time suspended in the air and can penetrate in the deepest areas of the respiratory system by evading natural clearance mechanisms— the smallest particles can cross the alveolar surface membranes and translocate into epithelial tissue, interstitial, pulmonary endothelium, and even secondary organs via bloodstream or lymphatic system (Marval and Tronville, 2022; Moreno-Ríos et al., 2022). Respiratory PM cause inflammation, with finer particles potentially leading to more persistent inflammation and impairing macrophage function (Chen et al., 2020). Evidence demonstrate that ultrafine PM can cause mitochondrial damage, by inducing oxidative stress and potentiating mutagenic and genotoxic effects (Dubey et al., 2022; Li et al., 2003; Wei et al., 2021).

Large wildland fires across Europe burn an average of 3770 km² annually, displace 45,000 people, contribute to global air pollution, and cause economic losses of about EUR 2.5 billion each year. Controlled/prescribed burns, with forest and urban/structure background, are intentionally planned fires to secure land/environment management (e.g., fuel reduction, community and habitat protection, or ecological restoration), and serve as training firefighting scenarios (Beyene et al., 2023; CPFC, 2020; Fernandes, 2018; Kramer et al., 2023). Prescribed fires are regular practice across USA, Australia, and in the southern European countries to prevent the occurrence of large forest fires that pose major hazards to populations and first-responders. Fire emissions release PM and several organic and inorganic pollutants, some known toxic and/or possible/probable/known human carcinogens, are adsorbed/absorbed on the surface of PM (da Costa e Oliveira et al., 2019; IARC, 2002, 2010; Kwon et al., 2020; Loomis et al., 2013; Marval and Tronville, 2022; Moreno-Ríos et al., 2022; Schraufnagel, 2020; Xiao et al., 2016). Asbestos, metal(loid)s (e.g., As and Pb), and several persistent organic pollutants (e.g. dioxins and furans) including polycyclic aromatic hydrocarbons are among the pollutants found adsorbed/adsorbed in PM released during fires (Alshehri et al., 2023; Anttila et al., 2008; Barros et al., 2023; Boaggio et al., 2022; Engelsman et al., 2020; Huang et al., 2023; IARC, 2002, 2010; Lestari et al., 2024; Li et al., 2023; Loomis et al., 2013; Nestola et al., 2022; Scarpa et al., 2024; Yao et al., 2023).

The characterization (levels and composition) of fine and ultrafine PM has been reported in different scenarios, including dental clinics, kitchens, classrooms, auto repair shops, offices, and go-kart facilities (Alves et al., 2020; Chua et al., 2017; Luna-Carrascal et al., 2023; Okam et al., 2024; Sysoltseva et al., 2018; Tang et al., 2012; 2022). However, the characterization of fine and ultrafine PM released during fires is scarce. For real fire events, data is even more scarce since only three studies characterized PM_{2.5} (Costa et al., 2023; McNamara et al., 2012; Ward et al., 2006). Studies characterizing ultrafine PM from real fire events are still inexistent. To fill this gap, this study characterizes the morphological and elemental composition of fine (PM_{2.5} and PM_{1.56nm}) and ultrafine (PM_{54.5nm}, PM_{30.8nm}, and PM_{14.9nm}) PM collected during five controlled structure fires (CSF) and prescribed forest fires (PFF). Gravimetric analysis, scanning electron microscopy energy dispersive spectroscopy (SEM-EDS), and inductively coupled plasma-mass spectrometry (ICP-MS) analysis were performed to analyze the morphology and composition of fine and ultrafine PM. This work contributes to the characterization of human exposure to fine and ultrafine PM released during live fire scenarios, highlighting the associated human health risks.

2. Materials and methods

2.1. Fire events

In January 2022, two PFF were performed in Serra da Aboboreira, a mountain range stretching across Marco de Canaveze, Baião, and Amarante municipalities in the North of Portugal. A multidisciplinary team of local forest sapper firefighters, professional firefighters, and agents of emergency and civil protection planned the PFF by defining the perimeter of the area to be burned and the strategy to ignite and follow up the fire in approximately 2000 m² of wild area. PFF were designed to prevent the occurrence of large wildfires, by creating containment areas that prevent the spread of fire, and protect local fauna, flora, and a small village living in the mountains. *Calluna vulgaris*, *Cytisus scoparius*, *Ulex europaeus* and trees such as *Eucalyptus globulus* and *Quercus robur* were the predominant flora in the selected area. During PFF, there was no precipitation, the temperature varied from 7 to 13 °C, the relative humidity ranged between 32 and 45 %, and the wind was blowing north at 3.6–7.2 km/h.

Between March and April 2022, three CSF were planned by the Regiment of Sapper Firefighters from Porto, in the context of training new elements recently integrated into that operational force. The CSF occurred in a concrete three-floor firehouse situated in Baltar, Paredes (North of Portugal) and served as training structure firefighting exercises, search, and rescue simulations. The structure fires were started on the ground floor by igniting wooden (mostly pine) pallets with a mixture of diesel and gasoline (5:1 parts), and oil (residual; to produce more smoke).

2.2. PM sampling and gravimetric analysis

PM was collected with a low-pressure impactor (DLPI+, Dekati®, Finland) connected to an air vacuum pump (Leybold, Sogevac, Germany), as described by Sousa et al. (2024). The equipment, previously calibrated by the supplier, collected 120 samples, 90 during CSFs and 30 during PFF, with cut-off diameters of 2.5 μm (PM_{2.5}), 156 nm (PM_{1.56nm}), 54.5 nm (PM_{54.5nm}), 30.8 nm (PM_{30.8nm}), and 14.9 nm (PM_{14.9nm}). The DLPI+ was calibrated and verified by the manufacturer for each PM stage at a controlled constant outlet pressure and flow rate of 40 mbar and 9.96 L/min, respectively (Table S1, of the Supplementary Material) (Järvinen et al., 2014). During CSF, the sampling device was placed inside the burning house, protected from the fire ignition area, whereas during PFF, the equipment was placed in a safe spot pre-selected by the firefighters' chief in charge in the boarding line of firefighting activity (Fig. S1 of the Supplementary Material). Each sampling campaign collected PM during the entire duration of the fire event (60–120 min in CSF; 210 min in PFF), at a constant pressure and flow rate of 40 mbar and 9.96 L/min, respectively. PM collection was performed onto aluminum filters (Ø25 mm; Dekati®, Finland) covered with a thin layer of an inert grease (Apiezon®-L, Sigma-Aldrich) to prevent particles bouncing. Humidity and temperature were monitored by a HygroLog SERIES HL20 device (Rotronic, Switzerland). Gravimetric analysis was used to quantify PM by weighing each filter, before and after sampling in an analytical balance (MS205DU, Mettler Toledo), at controlled humidity and temperature conditions. The precision of these measurements varied between 0.010 % and 0.10 %. The sampled filters were stored at room temperature until morphological and elemental analysis.

2.3. PM morphological characterization

SEM characterization was achieved using a Quanta 400 FEG scanning electron microscope (SEM, FEI, Hillsboro, OR) in tandem with an EDAX Genesis X4M Energy-dispersive Spectroscopy system, following the procedures described in Sousa et al. (2024). The elemental mapping comprising carbon (C), sodium (Na), magnesium (Mg), silicon (Si), phosphorous (P), S, chlorine (Cl), K, calcium (Ca), chromium (Cr),

manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), barium (Ba), and lead (Pb), was achieved through EDS analysis. The morphological characterization of collected fine and ultrafine PM fractions during fires was performed at CEMUP – Centro de Materiais da Universidade do Porto (Porto, Portugal).

2.4. PM elemental analysis

Each collected PM fraction was digested using a mixture of nitric acid and hydrogen peroxide in a DigiPREP Jr system (SCP Science, Quebec, Canada), using the methodology described by Sousa et al. (2024). A total of 18 elements, lithium (Li), beryllium (Be), Cr, Co, Ni, Cu, zinc (Zn), As, selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), antimony (Sb), cesium (Cs), Ba, mercury (Hg), Ti, and Pb, were analysed via ICP-MS by using an iCAP™ Q (Thermo Fisher Scientific, Bremen, Germany) according to Teixeira et al. (2024). The limits of detection (LOD) and quantification (LOQ), obtained by multiplying the blank's standard deviations by 3.3 and 10 ranged between 3.43 ng/L (Be) and 7.54 µg/L (Zn), and from 10.4 ng/L (Be) and 22.8 µg/L (Zn), respectively. The accuracy of the method, expressed in percent error, was calculated using the reference material EnviroMAT (SCP Scienc, Quebec, Canada) and varied from 0.084 % to 18.6 % for Cd and Ba, respectively. Precision, expressed as the relative standard deviation, ranged from 0.73 % to 5.1 % for Pb and Mn, respectively. All samples and standards were analysed in triplicate.

2.5. Data treatment

PM levels were determined based on the specifications provided by the DLPI + supplier, including the equipment operating (temperature, humidity, and atmospheric pressure values) and calibration parameters, as described in detail by Sousa et al. (2024). Regarding the elemental characterization of each PM fraction, whenever the concentration of an element was below its LOD, the values were replaced by LOD/√2 (Hornung and Reed, 1990). Data are presented as median and range (minimum – maximum) values since normal distribution was not observed by Shapiro-Wilk's test.

3. Results and discussion

3.1. Concentrations of fine and ultrafine PM

The cumulative levels of PM_{2.5} (sum of all 5 fractions) were 9 times higher in CSF (11.13 mg/m³; 1.245–228.5 mg/m³) than in PFF (1.232 mg/m³; 0.5458–1.920 mg/m³) (Table S2 and Fig. S2 of the Supplementary Material). PM_{2.5} largely exceeded the daily guidelines settled by the national, European Union, and WHO air quality limits (25 µg/m³; not to be exceeded more than 18 times per calendar year) for ambient PM_{2.5} (República, 2010; EU - European Parliament and Council of the European Union, 2024; WHO, 2021).

Overall, the concentrations of fine PM (PM_{2.5} and PM_{156nm}) were 13 and 3 times higher than ultrafine PM (PM_{54.5nm}, PM_{30.8nm}, and PM_{14.9nm}), in CSF and PFF, respectively. CSF presented significantly increased PM levels than PFF in all fractions [PM_{2.5}: 7.774 mg/m³ (0.1194–223.6 mg/m³) versus 0.2429 mg/m³ (0.1153–0.3706 mg/m³), 69.83 % and 19.71 % of cumulative PM, respectively; PM_{156nm}: 2.552 mg/m³ (0.65–2.750 mg/m³) versus 0.706 mg/m³ (0.2745–1.137 mg/m³), 22.92 % and 57.24 % of total PM; PM_{54.5nm}: 0.4731 mg/m³ (0.0847–1.066 mg/m³) versus 0.1573 mg/m³ (0.0827–0.2319 mg/m³), representing 4.25 % and 12.75 % of total PM; PM_{30.8nm}: 0.2246 mg/m³ (0.0884–0.815 mg/m³) versus 0.0824 mg/m³ (0.03914–0.1257 mg/m³), accounting for 2.02 % and 6.69 % of total PM; PM_{14.9nm}: 0.1100 mg/m³ (0.03610–0.624 mg/m³) versus 0.04450 mg/m³ (0.03413–0.05486 mg/m³) corresponding to 0.98 % and 3.61 % of total PM] ($p < 0.05$; Fig. S2). PM_{2.5} and PM_{156nm} were the prominent fractions in CSF and PFF, respectively. Regarding ultrafine PM, a higher prevalence was found in

PFF comparatively to CSF (23.1 % vs 7.25 %; Fig. S2).

The characterisation of fine and ultrafine PM released during fire emissions remains limited in the literature. Evidence is typically focused on PM_{2.5}, with measurements performed during burning simulations in experimental conditions which are based on predictions, or measurements of background levels collected from geostationary satellites or monitoring stations in the surrounding of areas affected by fires (Afrin and Garcia-Menendez, 2020; Al-Naiema et al., 2015; Anttila et al., 2008; Barbosa et al., 2024; Boaggio et al., 2022; Costa et al., 2023; Alves et al., 2011; McNamara et al., 2012; Miranda et al., 2010; Oliveira et al., 2020; Price et al., 2016; Ward et al., 2006; Wu et al., 2021). To the extent of the team's knowledge, studies characterizing ultrafine PM concentrations during forest and structure fires are non-existent. With respect to forest fires, the concentrations of PM_{2.5} acquired in this study (0.2429 mg/m³) were aligned with the range of values (0.00100–100.7 mg/m³) reported in experimental and prescribed fires (Barbosa et al., 2024; Miranda et al., 2010; Price et al., 2016; Wu et al., 2021). The monitoring of real fire events has been rarely characterized in the literature, so far only PM_{2.5}, mostly due to the unpredictability of these events and the difficulties in the implementation of sampling campaigns. Ward et al. (2006) predicted PM_{2.5} concentrations varying from 0.0370 to 0.1368 mg/m³ in real forest fires that affected Montana, USA whereas McNamara et al. (2012) collected PM_{2.5} during wildfires in Washington, Oregon, and California (USA) at values ranging between 0.0088 and 0.0413 mg/m³. The PM_{2.5} concentrations described in those real events were significantly lower than the values found in the present study (0.2429 mg/m³). Recently, Costa et al. (2023) reported PM_{2.5} values (0.1360–140.0 mg/m³; mean: 27.21 ± 26.79 mg/m³) during a fire in the Amazon Forest (Rondonia, Brazil) that were in close range with our results (Costa et al., 2023).

Regarding structure fires, evidence describes the collection and analysis of total PM, at concentrations ranging from 0.137 to 560 mg/m³, during training fires and urban fires involving the burning of different materials (wood, paper, kerosene, PVC plastic, stuffed furniture, tenement, and rubbish, among other materials) (IARC, 2023). So far, only Baxter et al. (2014) evaluated the environmental concentration of PM_{2.5} in live firefighting overhaul events and reported concentrations ranging between 0.253 and 17.53 mg/m³, values up to 13 times lower than the levels measured inside the monitored CSF (1.245–228.5 mg/m³).

3.2. Morphological profile

The images, at increasing magnetizations, were representative of PM spots collected onto the aluminium filters. Fig. 1 presents SEM images of fine (PM_{2.5}: 1A₁–1A₃ and 1B₁–1B₃; PM_{156nm}: 1A₄–3A₆ and 1B₄–1B₆) and ultrafine (PM_{54.5nm}: 1A₇–1A₉ and 1B₇–1B₉; PM_{30.8nm}: 1A₁₀–1A₁₂ and 1B₁₀–1B₁₂) PM collected during PFF (A) and CSF (B), respectively. Interesting differences and similarities were observed between PM collected from PFF and CSF regarding the shape, size, and composition of fine and ultrafine PM.

3.2.1. Fine PM

Concerning PM_{2.5}, the main difference between PFF and CSF was the concentration of particles, which was considerably higher in CSF (Fig. S2), a well-visible difference at the lowest magnetization (250 ×, Fig. 1A₁ and 1B₁). For higher magnetizations (1000 ×, Fig. 1A₂ and 1B₂; 25000 ×, Fig. 1A₃ and 1B₃), some morphological similarities were observed between PM_{2.5} from both fire events. It was visible a mesh/agglomeration of fibres released upon the combustion of trees and bushes (Fig. 1A₂ and 1A₃), and wooden pallets (Fig. 1B₂ and 1B₃). Some fly ash, a key characteristic of emission from heat sources, was identified (Fig. 1A₃), in line with previous findings of (Karlfeldt Fedje et al., 2010). However, some differences were also identified among both events. A black agglomerate – conductive material representing carbonaceous matter and an area with particles of lighter contrast – non-conductive

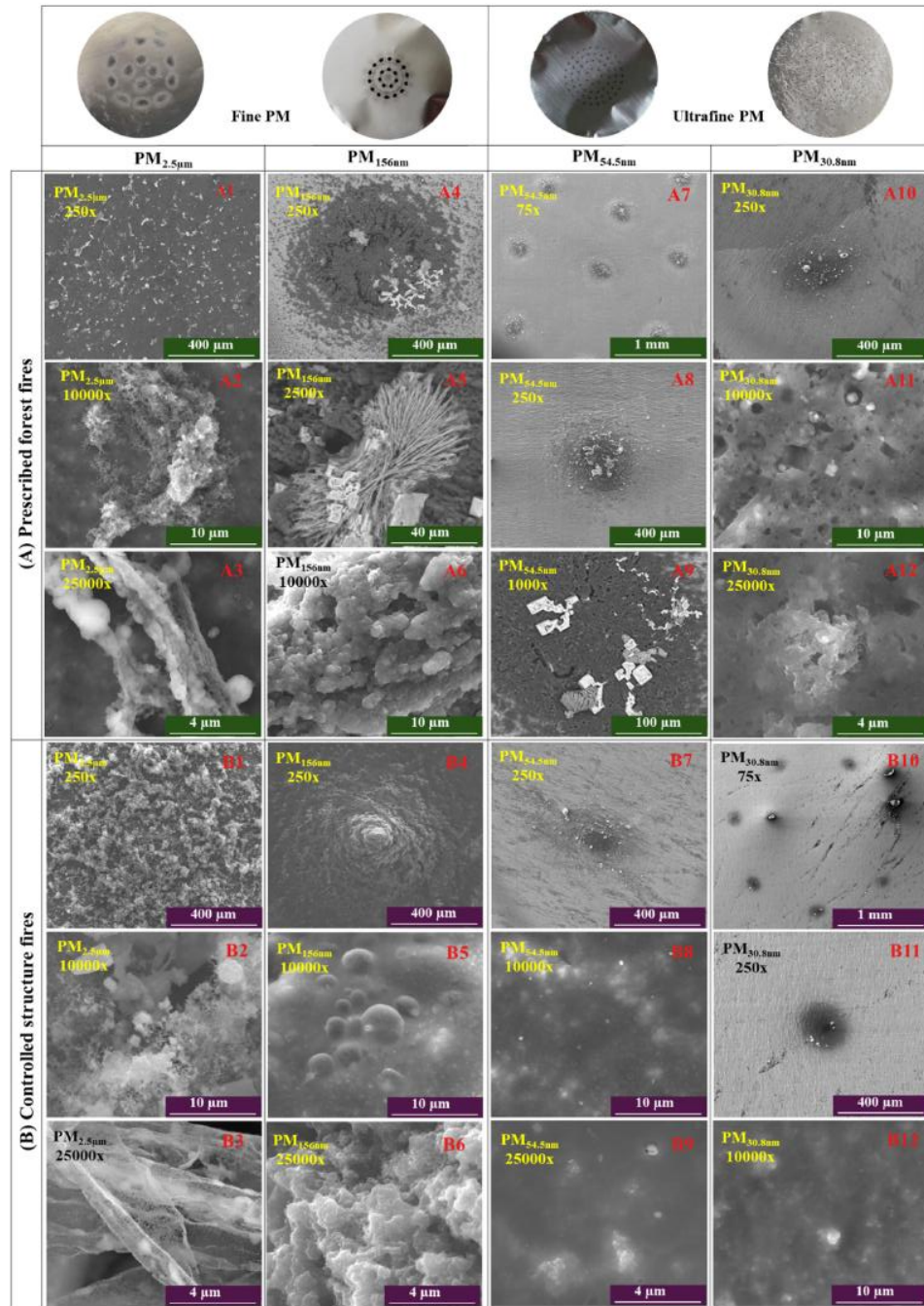


Fig. 1. SEM images from A) prescribed forest fires (PFF) and B) controlled structure fires (CSF), of fine (PM_{2.5} and PM_{10.0}; A1-A6, and B1-B6) and ultrafine PM (PM_{5.0}, PM_{10.0}, and PM_{14.9}; A7-A12, B7-B12) collected during.

material, like crystals deposited upon sampling were observed in PM_{156nm} from PFF (Fig. 1A₄). This heterogeneity was more visible at the highest magnetizations (2500x, Fig. 1A₅), allowing the identification of at least two types of crystalline structures in the carbonaceous background. In addition, the observed crystals were larger than the cut-off diameter (156 nm) of the LDPI + stage, suggesting that crystals were formed after sampling *i.e.*, inside the impactor while solubilized in small water droplets (Conner and Williams, 2004; Sousa et al., 2024). Also, PM_{156nm} from PFF exhibited a wide variety of particles compared to the same fraction collected from CSF (Fig. 1A₄ and 1B₄). Moreover, a central area with lighter contrast was observed in the samples from CSF comparatively to PFF (Fig. 1A₄ and 1B₄), which can be attributed to the depth of the image since upon PM collection, the particles tend to deposit in the centre of the filter. PM_{156nm} from CSF (Fig. 1B₄) presented a smoother surface than in the similar fraction from PFF (Fig. 1A₄), possibly resulting from the higher temperature inside the firehouse (400–600 °C). At a higher magnetization (10000x), PM_{156nm} presented distinct morphologies, with the sample surface (Fig. 1B₅) being smoother at CSF and containing small liquid droplets (*i.e.*, water from the fire extinguishing process) trapped below the surface; otherwise, it would have evaporated under the SEM vacuum conditions (Fig. 1A₆, and 1B₅) (Nadimi et al., 2024). On the other hand, a rugged surface was found in PM_{156nm} from PFF (Fig. 1A₆), allowing the identification of several layers of material at different depths. This rugged pattern was only visible in PM collected during CSF at the highest magnetization (25000x; Fig. 1B₆).

Literature related to the morphological characterization of fine PM generated during fire events are scarce. Available studies reported the collection of coarse and fine PM from experimental/controlled combustions of biomass (*e.g.* using coal or pellets), which can be extrapolated to comprehend emissions from live fire events. Studies characterizing fine PM from biomass burning reported carbonaceous agglomerates with particles of different shapes (typically spherical) and sizes (mostly below 2.5 µm), being aligned with our findings for CSF (Al-Naiema et al., 2015; Fernandes and Costa, 2012; Nestola et al., 2022; Wilczyńska-Michalik et al., 2020). Otherwise, ambient PM presents, besides the carbonaceous agglomerate, larger and irregular particles with terrigenous, biogenic, and/or crystalline structures, which are more similar to PM from PFF (Bahadar Zeb et al., 2018; Bora et al., 2021; Ferreira et al., 2013).

3.2.2. Ultrafine PM

PM_{54.5nm} collected during PFF presented more particles variety than the material from CSF (Fig. 1A₈ and 1B₇). Fig. 1A₈ (magnetization: 250x) and 1A₉ (magnetization: 1000x) exhibited several crystals (or other non-conductive materials) of different dimensions and shapes in a black agglomerate background, demonstrating the presence of organic/carbonaceous matter in ultrafine PM from PFF. Furthermore, the samples collected in CSF (Fig. 1B₇) revealed some similarities with Fig. 1A₈; however, some non-conductive material was observable in smaller and homogeneous sizes. Additionally, Fig. 1B₈ (10000x) and 1B₉ (25000x) representing PM from CSF, presented a black background with small round dots (fly ash) with brighter contrast. In the images corresponding to PM_{30.8nm}, a higher prevalence of crystals and other non-conductive material was observable in PM from PFF (Fig. 1A₁₀) and more carbonaceous matter in particles from CSF (Fig. 1B₁₁), as previously observed in fine PM fractions. Fig. 1A₁₁ exhibited round particles which are characteristic of emission from heat sources, resembling Fig. 1A₃, 1B₃, and 1B₉.

To date, there are still missing scientific studies that characterized the morphology, by electron microscopy, of individual ultrafine PM emitted from combustion sources (real or controlled fire events, coal combustion power plants, laboratory simulations). In a previous work of this research team, fine and ultrafine PM collected inside fire stations were characterized by electron microscopy (Sousa et al., 2024). Ultrafine PM originated from different indoor sources were identified as well

as from fire emissions. The PM morphology previously characterized inside fire stations resembles the one described in the present study. In both cases, a black background (carbonaceous matter) was prominent with the presence of conductive material (*e.g.* crystalline structures released from fire emissions and fly ashes). The morphological characterization of fine and ultrafine PM generated during PFF and CSF advances knowledge on fire emissions and highlights its presence inside fire stations, highlighting the potential associated adverse health effects (da Costa e Oliveira et al., 2019; Kwon et al., 2020; Xiao et al., 2016). However, additional studies are needed to corroborate these preliminary findings.

3.3. Electron microscopy characterization

3.3.1. Prescribed forest fires

To further characterize fire-generated PM, the EDS spectra of fine and ultrafine PM released during PFF was performed (Fig. 2). A highlighted zone (Z1) corresponding to a mesh/agglomerate of larger particles mainly constituted by C, O, Na, Mg, aluminium (Al), S, Cl, K, and Ca was identified in PM_{2.5} (Fig. 2A₁). The identified Al peak was attributed to the aluminium filter used to collect PM. The presence of C and O identified organic/carbonaceous matter as the main source of PM_{2.5}, which was expected in the emissions of biomass combustion. The other elements suggest the presence of sulphates, carbonates, or chlorides of sodium, magnesium, potassium, and calcium (Fig. 2A₁). However, the EDS spectrum analysis cannot undoubtedly confirm these findings because the highlighted area is a complex mixture of compounds. PM_{30.8nm} exhibited crystalline structures with distinct shapes and sizes (magnetization: 2500 × ; Fig. 2B₁). The EDS spectra of these structures confirm the presence of K₂SO₄ and Na₂SO₄ (Z1, Fig. 2B₁) and KCl (Z2, Fig. 2B₁). A higher magnetization of this ultrafine PM fraction allowed the identification of a particle (25000 × ; Z3, Fig. 2C₁). An interesting observation that was evident when combining the scale of the SEM images with the size of particles, Fig. 2B₁ (40 µm) and 2C₁ (4 µm), was that identified particles are larger than the cut-off diameter of LDPI + stage used to collect PM_{30.8nm}. Therefore, we hypothesize that sulphate and chloride crystals depicted in Fig. 2B₁ entered the system solubilized in water droplets during sample collection and later recrystallized in the aluminium filter before analysis. These findings were already corroborated by previous studies (Conner and Williams, 2004; Sousa et al., 2024). The EDS spectrum of collected PM revealed the presence of 3 main elements (Cr, Fe, and Ni) (Fig. 2C₁). These metals are the main components of a metal alloy known as stainless steel, frequently used in heavy machinery (*e.g.* fire trucks), and could have been released during the fire extinguishing process. Additionally, it was possible to identify a peak of Mo, an element also used as an additive in the composition of stainless steel and other Ni alloys, to enhance the resistance to pitting corrosion (Li et al., 2024). Similarly to our previous work, particles of K₂SO₄ and stainless steel were identified in ultrafine PM collected inside different areas of fire stations (Sousa et al., 2024). Although it was difficult to identify the source of stainless-steel in ultrafine PM, due to the limitation of measuring the depth of particles, it could have been released from working fire trucks and firefighting materials and tools. Another possible cause could have been the release of PM from the collision of particles with the inner walls of the impactor during the sampling process.

3.3.2. Controlled structure fires

Regarding fire generated PM during CSF, the EDS analysis of a black background area without visible particles with light contrast (Z2) showed the presence of C, O, Na, Cl, and K in PM_{54.5nm} (magnetization: 10000 × ; Fig. 3A₁). The C and O peaks suggested the presence of an organic/carbonaceous fraction, while Na, Cl, and K can be related to salts (*e.g.*, KCl, NaCl). K was also identified, and its presence in the elemental stage is usually an indicator of biomass burning (*e.g.*, wooden pallets) (Yu et al., 2018; Pachon et al., 2013).

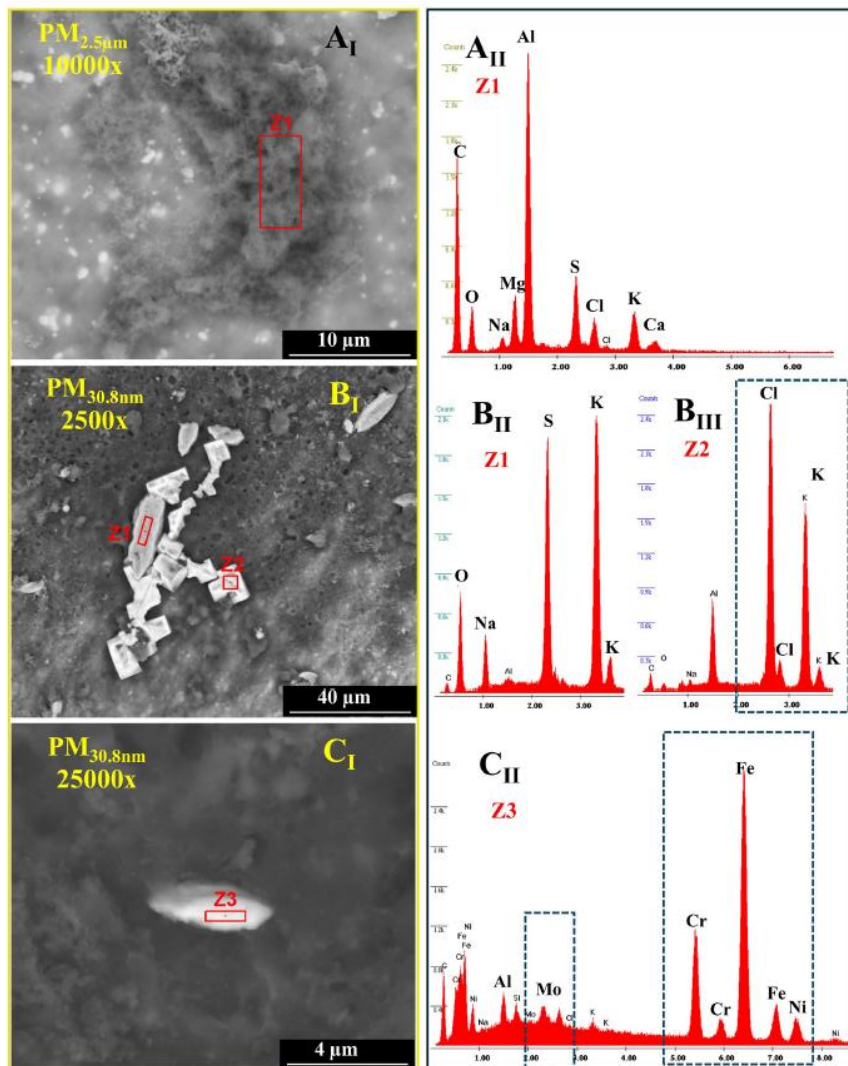


Fig. 2. SEM images and EDS spectra in identified zones (Z) of fine (A, $PM_{2.5}$) and ultrafine PM (B and C, $PM_{30.8nm}$) collected from PFF.

The image of $PM_{14.9nm}$ (magnetization: $10000 \times$, Fig. 3B_I), presented some areas with small ultrafine particles with light contrast, also identified in $PM_{54.5nm}$ (Fig. 3A_I). Also, the EDS spectrum of Z1 (Fig. 3B_{II}) allowed the identification of C, O, Na, Cl, K, and Pb. The presence of Pb, among other semi-volatile heavy metals, was present in fire emissions since these elements can be evaporated at high temperatures and will condense on PM under lower temperatures in its chloride ($PbCl_2$) and oxide (PbO) forms (Li et al., 2020; Zhang et al., 2017). Pb in these molecular forms is released after the combustion of coal, paints and solder, or solid waste (Li et al., 2020; Zhang et al., 2017). Additionally, within the EDS spectrum of Fig. 3B_{II}, it was possible to correlate the O, Cl, and Pb peaks' intensity to both molecular forms, Pb peak is superior to the Cl and O peaks, meaning that both molecules were present. These findings suggest that Na and K peaks detected in the EDS spectrum were representative of their elemental states (Fig. 3B_{II} and 3B_I). Furthermore,

at a higher magnification, $PM_{14.9nm}$ exhibited a small particle with light contrast ($25000 \times$, Fig. 3C_I) and its spectrum showed the presence of a $PbCl_2$ particle with the intensity of the Cl peak doubling the one of Pb (Fig. 3C_{II}). In this spectrum, the O peak was negligible suggesting that this particle was not representative of $PbCl_2/PbO$, unlike in the previous observations (Fig. 3B_I).

3.4. Elemental composition of fire-generated PM

3.4.1. Elemental mapping

The elemental characterization by EDS mapping was only performed in the ultrafine fraction $PM_{30.8nm}$ released from both PFF (Fig. 4) and CSF (Fig. 5), due to the limited budget. Six different particles or agglomerations, identified in Fig. 4 with small arrows and ellipses, were depicted for analysis. The elemental mapping of C confirmed the

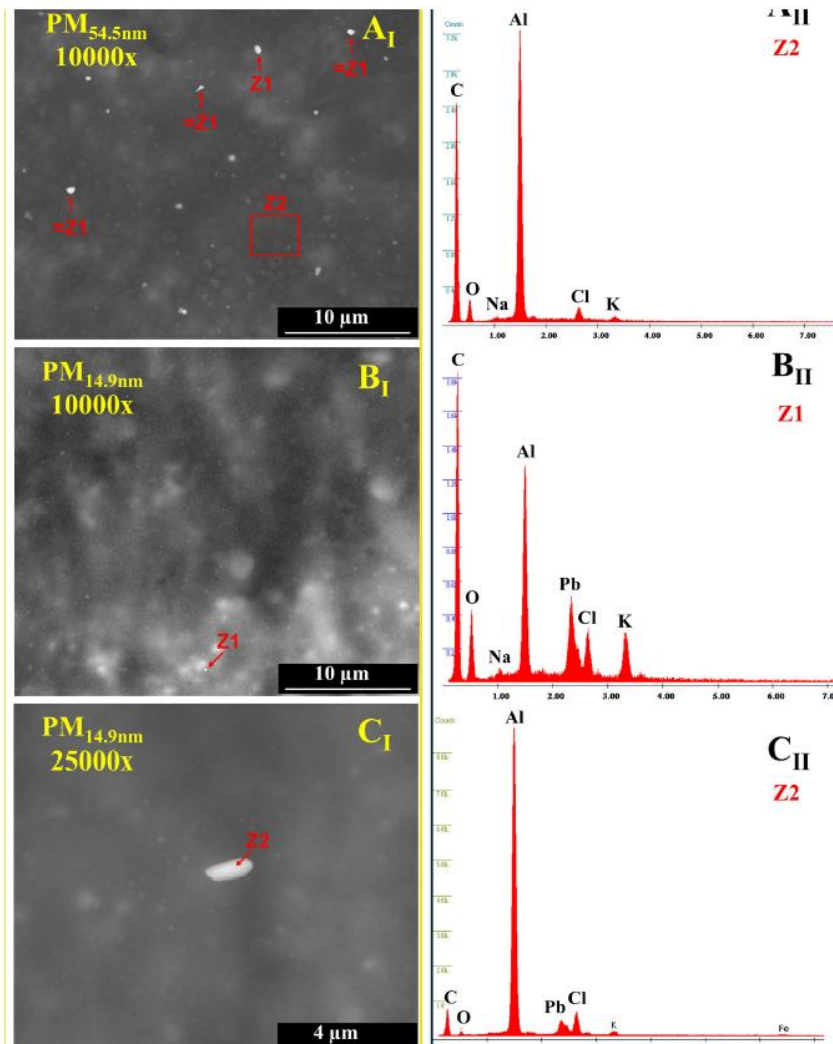


Fig. 3. SEM images and EDS spectra of identified zones (Z) in ultrafine PM (A, PM_{54.5nm}; B and C, PM_{14.9nm}) collected from CSF.

presence of an organic layer on the surface of the collected ultrafine PM. A small particle of Si, identifying a geogenic or terrigenous particle such as quartz, aluminosilicate particle (e.g. feldspar, mullite), or clay crystals was also identified (green arrow a, Fig. 4). Additionally, a larger particle of the assortment was highlighted (yellow ellipse b, Fig. 4) and its mapping images revealed the presence of P, Cl, K, and Ca. The size of this particle was close to 100 μm and corresponds to a mixture of two salts: KCl (higher contrast in the image) and Ca₃(PO₄)₂. Due to the size of this agglomerate, its presence in ultrafine PM can be explained by the solubilization in a water droplet at sampling time followed by recrystallization into the filter. Also, some particles (red ellipses c, Fig. 4) were identified in the images of P, S, K, and Ca, and represent a mixture of salts, that could form CaSO₄, K₂SO₄, and possibly phosphate of calcium and/or potassium. Other particles (pink arrow d, Fig. 4) suggest the presence of CaSO₄ and K₂SO₄; phosphate salts are not expected since these particles were not identified in the image of P. Small particles with

a noticeable bright contrast were also pointed out (blue arrows e, Fig. 4) and the mapping of these particles identified the presence of main components of stainless steel, Cr, Fe, Ni, and Mo. These findings are aligned with the identification of Mo in PM_{30.8nm} collected during PFF (Fig. 2CII). Mo acts as strengthening and hardening agent to avoid pitting and corrosion, also allowing for higher quenching temperatures. The lower percentage of this element in the composition of the alloy explains the lower visibility of the particles in the Mo mapping image. PM_{30.8nm} also exhibited two small particles that appeared in the elemental mapping of Fe (orange arrows f, Fig. 4), suggesting the presence of rust fly ashes (FeO), common materials near heavy machinery such as the fire trucks.

Fig. 5 exhibits the mapping images generated in the elemental analysis of PM_{30.8nm} collected during CSF. It was possible to observe two distinct zones in the image, which are demarked with red ellipses and green arrows (identified as a and b in Fig. 5, respectively). As previously

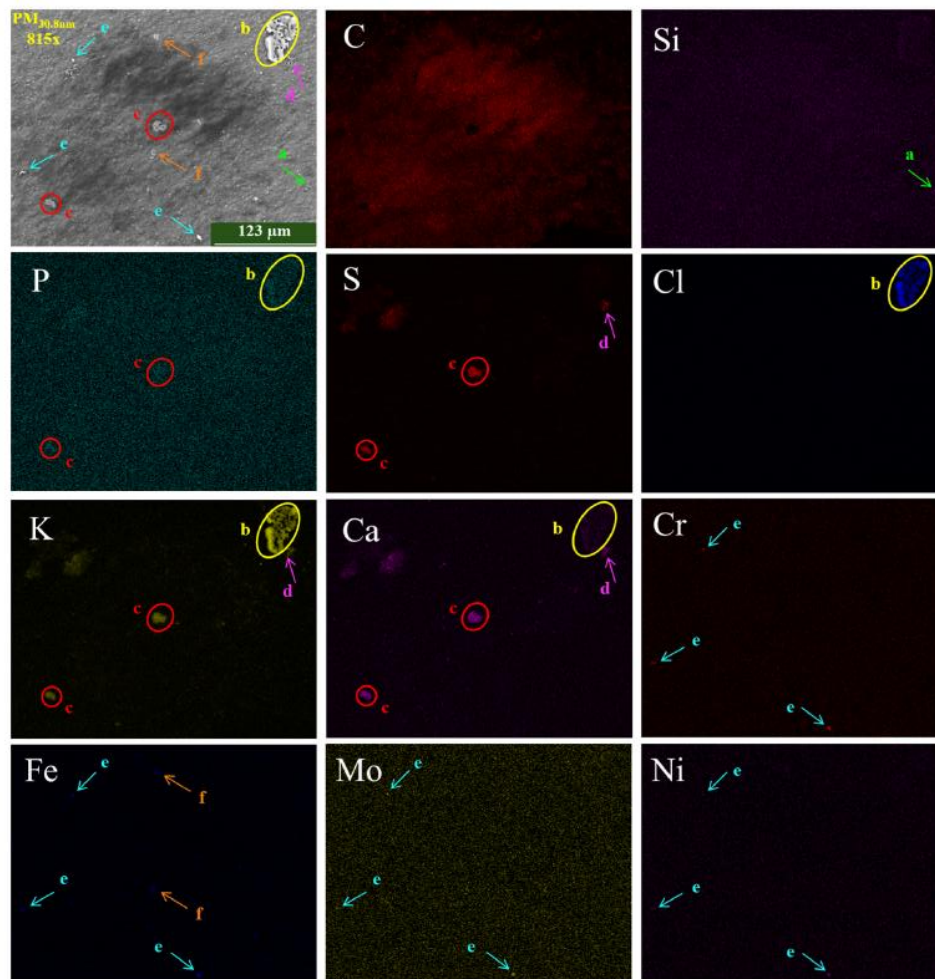


Fig. 4. Elemental mapping of 11 elements (C, Si, P, S, Cl, K, Ca, Cr, Fe, Mo, and Ni) identified in $PM_{30.8nm}$ collected during PFF and respective particles of interest detected (a–f).

observed in the SEM-EDS analysis, the variety of particles found in CSF samples was lesser than in PFF. From images analysis, two spots were visible in the elemental map of Mg, S, K, Ca, and Cd (red zone, Fig. 5). Pb was also visible, however, this element was not considered because there was an overlay with the S reading. This result suggests the presence of sulphates of magnesium, potassium, and calcium. Still, it does not explain the presence of Cd, which although being quite sparse, can be related to its elemental state. Also, in the zones where S (from sulphate) was present, the element Cl was absent. Furthermore, rather small particles were identified (green arrows b, Fig. 5) and confirmed the presence of Pb in ultrafine PM. This element was already observed in the EDS spectra of $PM_{14.9nm}$ Fig. 3B_{II} and 3C_{II}, attributed to $PbCl_2$ and/or PbO particles, which are additives used in solder and pigments used in paints or released during the combustion of electronic waste, or plastics frequently found in the firehouse or any other urban building. To the best knowledge of these authors, the microscopic elemental characterization of PM is limited in the literature, specially concerning PM from fire events. Despite previous authors have characterized $PM_{2.5}$ fractions

in different environments (Douglas et al., 1993; Ozkaynak et al., 1996), so far, the information is inexistent for ultrafine PM released during fire events. Therefore, additional studies are needed to expand current knowledge.

3.4.2. Elemental quantification

Fig. 6 presents the metal content of fine and ultrafine PM collected during PFF and CSF. Overall, the total metal content found in fine PM was 1.5 times higher than in ultrafine PM [293.4 ng/m^3 (287.9–301.1 ng/m^3) versus 196.2 ng/m^3 (186.0–201.5 ng/m^3)] for CSF. In the case of PFF events, the total metal content was 62.16 ng/m^3 (60.20–65.54 ng/m^3) in fine PM and 69.14 ng/m^3 (65.35–72.87 ng/m^3) in ultrafine PM (Fig. 6).

Out of the 18 metals that were analysed only Li was not detected in fine and ultrafine PM samples whereas Be, As, and Se were only detected in PM from CSF. The predominant elements in the PM released during CSF were Zn (54.2 %, fine PM; 37.0 %, ultrafine PM), Cu, Cr, Ni, and Pb (17.1 %, fine PM; 5.02 %, ultrafine PM). During PFF, the main elements

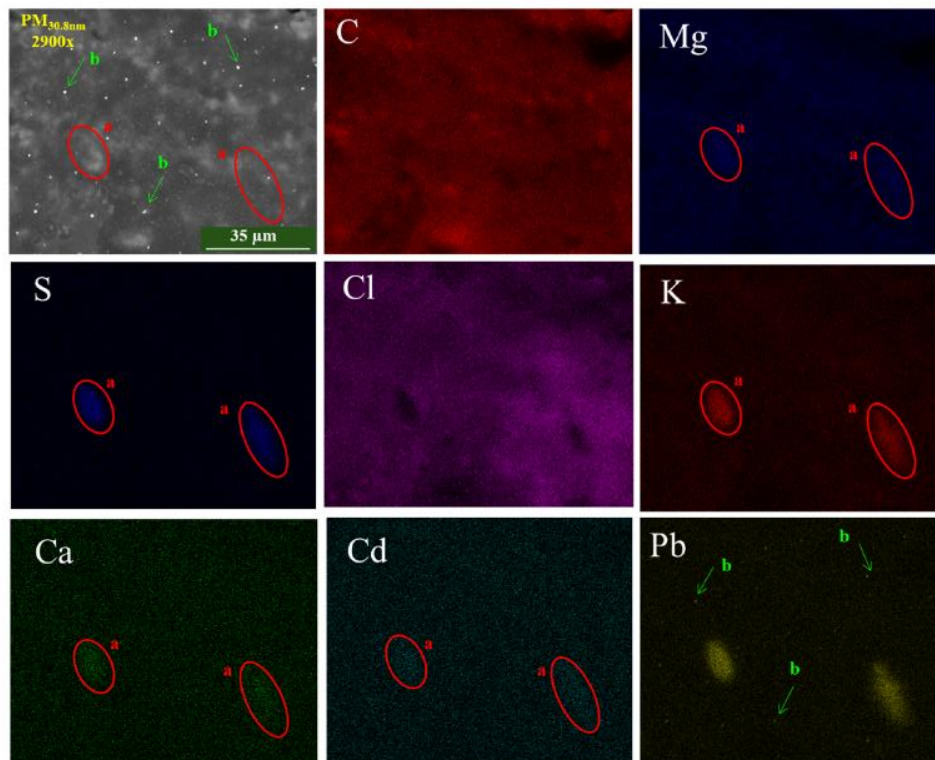


Fig. 5. Elemental mapping of 8 elements (C, Mg, S, Cl, K, Ca, Cd, and Pb) identified in $PM_{30.8nm}$ collected during CSF and respective particles of interest detected (a, and b).

found in PM were Zn (33.7 %, fine PM; 40.0 %, ultrafine PM), Cr, Cu, Ni, and Co (5.34 %, fine PM; 3.34 %, ultrafine PM) (Fig. 6). Toxic and (possible/probable) carcinogenic elements (Cr, Ni, Hg, As, Cd, Sb, Hg, and Pb) represented 28.4 and 30.6 % of total metal content found in fine and ultrafine PM generated during CSF, respectively. Regarding PFF, carcinogenic elements accounted for 29.3 % of fine and 33.1 % of ultrafine PM.

Elements such as Mg, K, Cl, and Zn can be found in the fumes of biomass combustion and have been associated with forest fire emissions (Alshehri et al., 2023; Breulman et al., 2002; Huang et al., 2023; Jiménez and Ballester, 2006; Nestola et al., 2022; Robinson et al., 2011; See et al., 2007; Yang et al., 2018; Yao et al., 2023). Cr, Ni, Cu, Zn, or Pb are present in the fumes released during the burning of materials containing plastics, paints, and electronic devices, commonly associated with urban fires (Alshehri et al., 2023; Engelsman et al., 2020; Li et al., 2023; Nzihou and Stanmore, 2013). Despite Cr, Ni, Cu, and Zn being the main elements found in PM from both fire events, they are not common in biomass combustion. Some authors previously characterized the composition of fire emissions from wild (Anttila et al., 2008), prescribed forest fires (Wu et al., 2021), and infrastructure fires (Boaggio et al., 2022), reporting the presence of several elements: Na, Mg, K, Ca, Fe, Cr, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Sb, Ba, and Pb. Anttila et al. (2008) reported concentrations for Zn (14.8–40.0 ng/m^3), Pb (5.87–10.70 ng/m^3), Cu (1.40–3.34 ng/m^3), Cr (0.33–1.86 ng/m^3), Ni (0.79–1.67 ng/m^3), As (0.33–0.90 ng/m^3), Cd (0.21–0.59 ng/m^3), and Co (0.07–0.25 ng/m^3), in PM_{10} from wildfire smoke. The elemental values measured in $PM_{2.5}$ from PFF in the present study were up to 23 times higher than the ones reported by Anttila et al. (2008) for Cr, Co, Ni, Cu,

and Zn; however, for Cd and Pb our concentrations were up to 24 times lower. Regarding the characterization of $PM_{2.5}$ during prescribed forest fires, Wu et al. (2021) reported the presence of Cr (168.8–9697 ng/m^3), Pb (26.00–5970 ng/m^3), Zn (105.2–4009 ng/m^3), Ba (74.10–1758 ng/m^3), Cu (5.100–1371 ng/m^3), Sb (1.700–338.1 ng/m^3), Ni (3.300–140.2 ng/m^3), Mo (0.9000–47.30 ng/m^3), and Cd (2.200–3.710 ng/m^3), at levels that were up to 2800 times above the values found in the present study. Also, a study characterizing PM from structure fires found Pb (1.47 ng/m^3) in $PM_{2.5}$, at values similar to the levels found in this study PFF (1.391 ng/m^3) and well below the value found for the CSF (50.22 ng/m^3) (Boaggio et al., 2022). Overall, the PM content in metals in both PFF and CSF was well below available air quality guidelines, for As (0.006 $µg/m^3$), Cd (0.005 $µg/m^3$), Pb (0.5 $µg/m^3$), and Ni (0.025 $µg/m^3$) (WHO, 2023).

4. Conclusion

For the first time, fine ($PM_{2.5}$, PM_{156nm}) and ultrafine ($PM_{50.4nm}$, $PM_{30.8nm}$, $PM_{14.9nm}$) PM released during five PFF and CSF were characterized by complementary methodologies through gravimetric analysis, SEM-EDS, elemental mapping, and ICP-MS analysis. PM levels were increased during CSF than in PFF (11.13 versus 1.232 mg/m^3) with fine PM being more predominant than ultrafine PM. $PM_{2.5}$ accounting for 69.8 % and 19.7 % whereas PM_{156nm} represented 22.9 % and 57.2 % of total PM in CSF and PFF, respectively. Ultrafine PM fractions were increased during PFF than in CSF, with $PM_{54.5nm}$, $PM_{30.8nm}$, and $PM_{14.9nm}$ accounting for 4.25 %, 2.02 %, and 0.988 % of total PM during CSF and represented 12.8 %, 6.69 %, and 3.61 % of PM released from

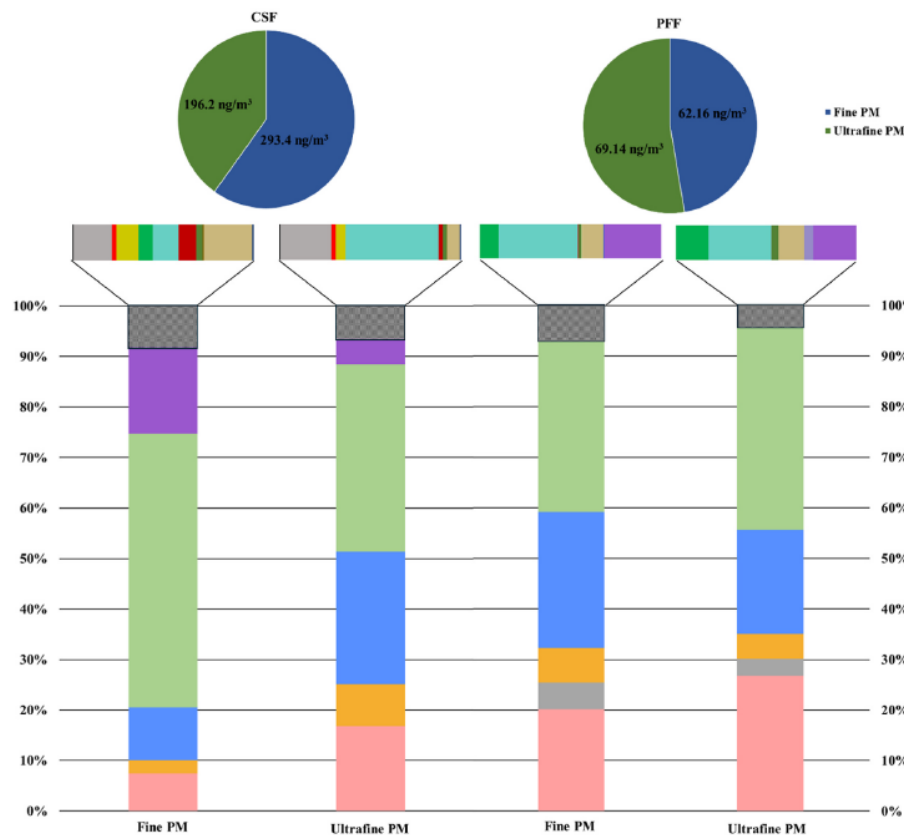


Fig. 6. Concentrations of total PM-bound metals content (ng/m³), and individual elemental distribution (%) in fine and ultrafine fractions collected during controlled structure fire (CSF) and prescribed forest fire (PFF).

PFF.

The morphological analysis exhibited a background with dark contrast, representing the presence of organic matter and smaller particles of non-conductive material, with varying shapes (spherical or irregularly shaped) and sizes. PFF exhibited a wider variety of bright contrast particles compared to CSF. In PFF most of these particles are suggestive of sulphate, carbonate, or chloride crystals of sodium, magnesium, potassium, and/or calcium. However, fly ash and small particles of stainless steel (Cr, Fe, Ni, and Mo) were also identified. PM released during CSF presented PbCl₂ and PbO as main compounds, which are common molecular forms of Pb released during fire emissions. Elemental characterization suggests the presence of small geogenic particles (Si), Ca₃(PO₄)₂, CaSO₄, and K₂SO₄, stainless steel, and rust particles (fly ash) in the PM from PFF; and particles of KCl and sulphates of magnesium, potassium, and calcium, alongside small particles of PbCl₂ and PbO, in the PM from CSF. Quantitative chemical analysis revealed the prevalence of Zn, Cr, Cu, and Ni in PM from CSF and PFF with (possible/probable) carcinogenic metals accounting for 28.4 % and 30.6 % of the total metal content in fine and ultrafine PM (CSF) and 29.3 % and 33.1 % in ultrafine PM (PFF). Data collected during the five controlled fires performed in Porto district, although insightful, may limit the comparison between PFF and CSF fine and ultrafine PM emissions, principally because PM samples were collected within the fire border line rather than outside the fire. Despite limited, these findings add valuable information to existing literature and contribute to a better

understanding of the chemistry of fire and air pollutants composition and behaviour. Due to the paucity of data available, additional studies assessing and characterizing fine, and principally ultrafine, PM released during fires are needed to corroborate the preliminary findings of this study. This missing valuable information would support the implementation of international recommendations for ambient ultrafine PM levels, support future studies on environmental toxicology, and contribute to a more realistic estimation of the impact of fire generated fine and ultrafine PM on human health.

CRedit authorship contribution statement

Gabriel Sousa: Writing – original draft, Investigation. Rui Azevedo: Methodology, Investigation. Agostinho Almeida: Resources. Simone Morais: Resources. Cristina Delerue-Matos: Visualization, Resources. Xianyu Wang: Visualization, Supervision. Francisca Rodrigues: Writing – review & editing, Supervision. Maria Freitas: Writing – review & editing, Methodology, Investigation, Conceptualization. Marta Oliveira: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marta Oliveira reports financial support was provided by Foundation for Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2025.123281>.

Data availability

Data will be made available on request.

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Chapter 4.

Part I conclusion

Part I conclusion

The work performed in Part I of this thesis explored and generated new data regarding firefighters' exposure in different occupational environments, namely different areas inside fire stations and during prescribed/controlled fire events.

PM concentrations regularly exceed current air quality standards, especially the PM_{2.5}. In Portuguese fire stations, fine and ultrafine PM concentrations were measured across multiple indoor microenvironments, being founded at higher concentrations in PPE storage rooms, followed by common areas, and garages. Although occupational exposure guidelines for total PM (*e.g.*, OSHA's 5.0 mg/m³) were not exceeded, the measured values frequently surpassed the recommended short- and long-term guidelines for ambient PM_{2.5}, highlighting the exposure risk for firefighters. By combining different techniques, namely SEM, EDS, and ICP-MS, distinct shapes, sizes and chemical composition were identified in fine, and ultrafine PM. SEM-EDS analysis revealed the presence of organic matter, recognizable biological debris (yeast, and pollen/spore grain), rust (fly ash), crystals (K₂SO₄), minerals (apatite, aluminosilicate), and metal alloys (stainless steel), among other unidentified substances. ICP-MS analysis revealed that Cu, Zn, Cr, and Ni were the main constituents of PM collected inside fire stations, and that the highest concentrations were found in garages, compared to PPE storage rooms or common areas. Toxic and carcinogenic metals, including Cr, Ni, Cd, Sb, and Pb, accounted for up to 11% and 13% of fine and ultrafine PM, respectively.

Likewise, PM concentrations observed during structure and forest fires exceed the European Parliament's ambient standard for PM_{2.5} (25 µg/m³) by a substantial margin, particularly during structure fires where levels could reach as high as 228.5 mg/m³. Fine and ultrafine PM collected from fires were mainly composed of organic/carbonaceous matter, as well as geogenic particles (Si), salts (predominantly sulphates and chlorides), and metallic particles (including stainless steel alloy). The controlled structure fires showed a higher prevalence of heavy metals compounds (*e.g.*, PbO, and PbCl₂) in PM comparatively to the samples collected during forest fires, which presented more crystalline and mineral salts possibly associated with biomass combustion (*e.g.* K₂SO₄, CaSO₄, or KCl). Moreover, PM-bound toxic and carcinogenic metals represented 28% and 30% of the total metal content found in fine and ultrafine PM released during PFF, and 29% and 33% of fine and ultrafine PM from CSF.

Human exposure to PM-bound metals has been associated with increased risks of cancer development (*e.g.*, lung, or bladder), cardiovascular and neurologic diseases

(e.g., strokes, Parkinson's), and exacerbation of many respiratory conditions (e.g., asthma and COPD). Specifically in the fire service, firefighters face chronic exposure to PM via different sources, including fire emissions on fire scene and brought to fire stations with firefighting materials and vehicles, pollution inside fire stations (e.g., vehicle maintenance and cooking), infiltration from outdoor sources, and contamination from used PPE, which underlines the need for improved occupational safety and health strategies. Currently, most air quality guidelines focus on PM₁₀ and PM_{2.5}, and there is a lack of (indoor/ambient) air quality recommendations for ultrafine PM. These findings offer a comprehensive assessment of the environmental and occupational risks posed by respirable particulate matter, emphasizing its direct impact on human health. They may also inform international regulatory bodies in the formulation of evidence-based air quality standards and guidelines on human exposure to fine and ultrafine PM to safeguard public health. In what concerns to occupational safety and health, this work also supports the need to reinforce the PPE decontamination protocols in fire service and ensure adequate ventilation and its systems maintenance inside fire stations.

PART II

Characterization of exposure to fire emissions in firefighters' health through biomonitorization of Portuguese firefighters (smokers and non-smokers).

Chapter 5.

Unveiling Exposure Pathways at the Oral Cavity during Firefighting Work: Salivary Metal(loid)s and Polycyclic Aromatic Hydrocarbons, Occupational and Biological Determinants, and Exploratory Health Risks

“Unveiling Exposure Pathways at the Oral Cavity during Firefighting Work: Salivary Metal(loid)s and Polycyclic Aromatic Hydrocarbons, Occupational and Biological Determinants, and Exploratory Health Risks”

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Abstract

Fire emissions are environmental contaminants with hazardous effects on the environment and human health. Human exposure to fire emissions occurs mainly via inhalation and dermal contact, while buccal exposure concentrations remain largely underexplored. This work characterizes, for the first time, exposure to metal(loid)s and polycyclic aromatic hydrocarbons (PAHs) at the oral cavity during firefighting work, accounting for five occupational and personal-life variables. Paired spot saliva samples were collected by 58 firefighters [organized in 4 groups: non-smoker non-exposed (NSNE), non-smoker exposed (NSE), smoker non-exposed (SNE), and smoker exposed (SE)] pre- and post-firefighting work. About 65–67% of firefighters showed increased metal(loid) and PAH levels after firefighting, with greater increments observed in smokers than in non-smokers. Σ metal(loid)s (18.7–28.6 $\mu\text{g/L}$) were up to 1.5x increase in SE than in NSNE, with Cu and Sr as the most abundant metals; similar findings were found for toxic/carcinogenic metal(loid)s (1.06–1.75 $\mu\text{g/L}$). Σ PAHs (1.19–1.40 $\mu\text{g/L}$) were slightly increased in NSE participants with seven detected compounds (naphthalene > phenanthrene > fluorene > anthracene > fluoranthene > pyrene) in all participants. Evidence suggests the potential bioaccumulation of metal(loid)s with the increase of BMI. Participants exposed to fires for more than 1h presented up to 1.8–2.1 higher salivary metal(loid)s compared to those exposed for less than 1h. NSNE firefighters with longer careers (> 10 years) presented 2.3x increased levels than those with less than 10 years. Salivary content represents a biomonitoring exploratory screening used in a toxicological screening based on conventional environmental risk assessment. Benzo(a)pyrene total toxicity equivalent concentrations and average daily potential dose were low with negligible non-carcinogenic (<1) risks and predominantly acceptable carcinogenic risks ($10^{-6} - 10^{-4}$). Overall, smoking and firefighting work increase salivary PAHs and meta(loid)s levels despite personal protective equipment use. These findings motivate targeted strategies to reduce exposure and promote long-term occupational and public health.

Keywords: Human exposome; Fire emissions; Saliva; Exposure to carcinogens; Health Risks

5.1. Introduction

Climate change and global warming are potentiating the occurrence of large wildland fires across Europe, including in wildland urban interfaces, burning an average of 3,770 km² of land annually, displacing 45,000 people (between 2008 to 2023), contributing to global air pollution, causing economic losses of about EUR 2.5 billion each year in damage to property and infrastructure, and affecting the health of exposed populations ([EEA, 2026](#); [JRC, 2026](#); Mimbrero and Bernal 2026). Fire emissions are a relevant source of environmental pollutants with hazardous effects to the environment and human health.

Amidst several professionals (e.g., mining, paints and petrol industry, agriculture, and kitchen workers), firefighters are among the most heavily exposed to occupational hazards, e.g., fire-generated pollutants (carbon monoxide, heavy metals, particulate matter, and volatile organic compounds including polycyclic aromatic hydrocarbons (PAHs)), high temperatures, biological hazards, and physical exertion and injuries ([Barros *et al.*, 2021, 2023](#); [Demers *et al.*, 2022](#); [Esteves *et al.*, 2025](#); [Hwang *et al.*, 2021](#); [IARC, 2023](#); [Oliveira *et al.*, 2017a, 2017b, 2020](#); [Sousa *et al.*, 2023, 2024](#); WHO, 2002). Firefighters are involved in complex tasks such as fire combat and investigation, containment of hazardous materials, medical assistance, search and rescue missions, training exercises, among others; all physically and/or psychologically demanding and with associated risks ([Barros *et al.*, 2021, 2023](#); [Demers *et al.*, 2022](#); [Hwang *et al.*, 2021](#); [IARC 2023](#)). Firefighters are also exposed to ultraviolet radiation, principally during firefighting of large wildfires, which cause skin lesions and may contribute to the development of skin diseases ([Demers *et al.*, 2022](#)).

Human exposure to fire emissions occurs primarily through inhalation and dermal contact, whereas exposure via the oral cavity has been rarely evaluated ([Santos *et al.*, 2019](#); [Sousa *et al.*, 2025](#)). Several authors have used biomonitoring assays to demonstrate the contribution of firefighting activities to total internal exposures to PAHs and metals mostly by using blood and urine analysis; limited evidence is available for other biological fluids ([Barros *et al.*, 2023](#); [IARC 2023](#); [Sousa *et al.*, 2024, 2023](#); WHO, 2015).

Saliva is an acidic oral secretion fluid that has several biological functions and can be used to assess hormonal and immunological status ([de Almeida Pdel *et al.*, 2008](#); [Humphrey & Williamson, 2001](#); [Michalke *et al.*, 2015](#)). The collection of saliva is simple, inexpensive, non-invasive, and can be performed by workers without the presence of a health professional ([Milanowski *et al.*, 2016](#)). Some studies have preliminarily explored

the potential of using saliva as a complementary biological fluid to widen the potential of human biomonitoring. Specifically, saliva has been used to assess short-term occupational exposure to pollutants in metals industry workers, welders, chemicals' production workers, firefighters, farmers/pesticide applicators, and synthetic leather producers ([Humphrey & Williamson, 2001](#); [Michalke et al., 2015](#); [Santos et al., 2019](#); [Sousa et al., 2024, 2023](#)). However, available data remains scarce and inconclusive ([Koh et al., 2003](#); [Shawahna et al., 2021](#); [Sousa et al., 2024, 2023](#)).

The present study advances current knowledge on human exposome by 1) assessing, for the first time, exposure pathways to the cumulative levels of 16 metal(loid)s and 18 PAHs, including 14 possible/probable carcinogens (Groups 1, 2A, 2B), in the saliva of firefighters directly involved in firefighting work, 2) evaluating the influence of three occupational – participation in firefighting activities, the time of exposure to fire emissions during firefighting work, the number of years working as a firefighter, and two personal-life – daily tobacco consumption and body mass index (BMI) factors on the salivary levels of metal(loid)s and PAHs, and 3) performing an exploratory estimation of the potential health risks due to exposure to PAHs and metal(loid)s in the oral cavity based on estimations derived from saliva and using a conventional environmental risk assessment model according to USEPA methodologies.

5.2. Materials and methods

5.2.1. Study participants and collection of saliva samples

A group of 58 firefighters and trainees attending eight fire stations from Porto district, north of Portugal, were enrolled in the study. Firstly, firefighters completed a self-reported questionnaire to collect sociodemographic information, including age, height, weight, years of experience as a firefighter, smoking and eating habits, regular use of medication, and overall health status.

The enrolled firefighters actively participated in different firefighting activities (structure and/or forest fires) between November 2021 and February 2023. During this period, firefighters collected paired spot non-stimulated saliva samples at the end of the work shift and whenever possible, immediately after participation in real and/or controlled firefighting work. Samples were collected by accumulating saliva in the oral cavity and spitting 5 consecutive spots of saliva into a sterile sample collection cup provided by the team. The procedure was repeated until a minimum volume of 2 mL was collected. The participants were asked not to eat or drink 60 minutes before sample collection. The procedure to collect the saliva was explained to each participant in a briefing meeting at

the beginning of the study. After collection, the samples were labeled and stored at -20°C until chemical analysis.

5.2.2. Analysis of metal(loid)s

Each sample was firstly thawed at room temperature, and immediately centrifuged during 10 minutes at 4000 rpm (Beckman Coulter, Microfuge 22R Centrifuge, California, USA), and 100 µL of supernatant was mixed with a diluent solution containing 2% (v/v) nitric acid (67-69% w/w TraceMetal® Grade, Fisher Scientific), 500 ppb of gold to stabilize elements prone to memory effects, 1.5 % (v/v) ethanol absolute anhydrous (Carlo Erba Reagents) to facilitate the formation of aerosol during quantification, and 10 ppb of an internal standard solution (Ga, Ir, and Rh in 5% ethanol). The mixture was homogenized and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) to assess its content in 16 metal(loid)s [lithium (Li), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), antimony (Sb), cesium (Cs), barium (Ba), mercury (Hg), thallium (Tl), and lead (Pb)]. An iCAP™ Q (Thermo Fisher Scientific, Germany) equipped with a Meinhard® (Golden, CO) TQ+ quartz concentric nebulizer, a Peltier cooled, high purity quartz, baffled cyclonic spray chamber and a de-mountable quartz torch with a 2.5 mm i.d. quartz injector was used for the analysis. The limits of detection (LOD) and quantification (LOQ), defined as the lowest concentrations of each metal measurable with signal-to-noise ratios of 3:1 and 10:1, respectively. LODs and LOQs varied between 0.0047 µg/L to 1.329 µg/L and from 0.015 µg/L to 4.39 µg/L for Tl and Cu, respectively. Samples were analyzed in triplicate and in a random order to avoid sequence effects ([Pinto et al., 2020](#)). Recovery rates varied from 89 ± 3.14% to 111 ± 1.33%, for Li and Co, respectively.

5.2.3. Analysis of PAHs

A 500 µL portion of defrosted saliva sample was combined with 200 µL of ultrapure water to decrease viscosity, 50 µL of methanol to cause protein precipitation, and 1500 µL of ethyl acetate to extract PAHs. The mixture was vortexed for 1 minute and centrifuged (HERAUS Megafuge 16R, Thermo Scientific, Massachusetts, USA) at 4000 rpm for 5 minutes. The supernatant was collected and the remaining extract underwent a second extraction with an additional 1500 µL of ethyl acetate. Both supernatants were combined and evaporated until dryness using a rotary evaporator (R-200 Büchi Rotavapor with a Büchi Vac V-500 pump, Flawil, Switzerland). The dried extract was reconstituted in acetonitrile, filtered (0.22 µm polytetrafluoroethylene filter) and stored at -20 °C until chromatographic analysis.

A Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) was utilized for the quantification of 18 PAHs, according to previous studies of the team ([Castro et al., 2009](#); [Sousa et al., 2025](#)). The chromatographic separation was performed using a C18 column (CC 150/4 Nucleosil 100-5C18 PAH, 150 × 4.0 mm; 5.0 µm particle size; Macherey-Nagel, Duren, Germany) at a controlled temperature of 25 °C. Anthracene and acenaphthylene were the compounds presenting the lowest and the highest LOD and LOQ (3.12 ng/L to 3.99 µg/L and from 10.4 ng/L to 13.3 µg/L, respectively). Three levels of contamination (20, 40, and 70 µL of a mixed standard solution) were added to saliva samples to evaluate the robustness of the methodology. These assays were performed in duplicate and the recovery rates obtained ranged from 41.1 ± 2.37% for benzo(b+j)fluoranthene to 105 ± 1.64% for acenaphthene. All samples were analysed in triplicate.

5.2.4. Health risk analysis

Salivary content was used to estimate potential health risks based on USEPA environmental risk assessment through the determination of benzo(a)pyrene total toxicity equivalent concentrations (TEQB(a)P), average daily potential dose (ADD), non-carcinogenic (THQ) and carcinogenic (TR) risks (Equations 1, 2, 3, and 4, respectively):

$$TEQB(a)P = \sum C \times TEF_n \quad (\text{Equation 1})$$

$$ADD = \frac{EFr \times ED \times IR \times C}{BW \times AT} \quad (\text{Equation 2})$$

$$THQ = \frac{EFr \times ED \times IR \times C}{RfD \times BW \times AT} \quad (\text{Equation 3})$$

$$TR = \frac{EFr \times ED \times IR \times C \times CSFo}{BW \times AT} \quad (\text{Equation 4})$$

The health risk screening was performed based on the variables, C – salivary concentration of each compound, TEF_n – toxicity equivalency factor relative to benzo(a)pyrene, EFr – exposure frequency, ED – exposure duration, IR – salivary swallowed, RfD – oral reference dose, CSFo – oral carcinogenic slope factor, BW – body weight, AT – number of days of exposure, retrieved from previous studies ([Tsai et al., 2004](#); [USEPA, 2024](#)) and listed in Table S1 of the Supplementary Material.

5.2.5. Statistical analysis

The data were analyzed using Microsoft Excel (v.16.0., Microsoft Corporation, USA) and results were expressed in µg/L saliva. If the concentration of a compound was below LOD, the value was replaced by $LOD/\sqrt{2}$ ([Hornung & Reed, 1990](#)). Normality was verified using the Kolmogorov-Smirnov test and the Shapiro-Wilk's test was used for paired samples, which indicated non-normal distributions. Therefore, the concentrations are presented as median, percentiles 25 and 75, and range. The tests Mann–Whitney *U* test and Kruskal–Wallis were used for intergroup and paired samples comparison. Significance was defined as $p \leq 0.05$, being the significant differences identified in the text as well as in the figures.

5.3. Results and discussion

5.3.1. Characterization of participants

The participants were grouped in 4 different groups according to their smoking habits and recent participation in firefighting activities: NSNE – non-smoker non-exposed group; NSE – non-smoker exposed group; SNE – smoker non-exposed group; SE – smoker exposed group. [Table 1](#) exhibits the main sociodemographic and occupational characteristics of the four groups of firefighters. Overall, participants were predominantly male (84-100%) with mean ages varying from 26.1 and 30.5 years old. The BMI, calculated as the subject's body mass (kg) divided by their height squared (m^2), ranged between 24.6 and 25.2 kg/m^2 . A total of 43.9% of participants were overweight as they presented BMI above 24.9 kg/m^2 ([WHO, 2010](#)). The conventional BMI formula allows broader comparability among groups; however, it may overestimate adiposity in athletic, physically active, and muscular individuals, including some firefighters. The variables required to determine body-composition indices such as fat mass index, fat-free mass index, and waist-to-height ratio were not available in this study and should be considered in the design of future studies to provide a more informative characterization of participants' adiposity and muscularity. Some participants are smokers and reported a mean daily number of cigarettes smoked of 14.1 and 11.8 for SNE and SE, respectively. Regarding the occupational activity as a firefighter, the participants reported a career length ranging between 1 and 45 years, with a mean of 1.48 to 1.60 hours per day in active (forest/structure) firefighting activities and/or training exercises during the sampling campaign period ([Table 1](#)).

Table 1. Characterization of firefighters based on the smoking habits and recent participation in firefighting activities [NSNE – non-smoker non-exposed group; NSE – non-smoker exposed group; SNE – smoker non-exposed group; SE – smoker exposed group]

Variable	NSNE	NSE	SNE	SE
n	31	23	19	15
Gender (%)	97% Male; 3% Female	96% Male; 4% Female	84% Male; 16% Female	100% Male
Age (mean ± SD; min–max; years)	30.5 ± 10.4 (22 – 57)	29.0 ± 10.5 (21 – 63)	28.1 ± 6.0 (22 – 45)	26.1 ± 2.6 (22 – 32)
BMI (mean ± SD; min–max; kg/m ²)	24.8 ± 2.6 (18.7 – 30.8)	24.6 ± 2.3 (18.7 – 27.7)	25.2 ± 6.9 (14.7 – 49.3)	25.1 ± 2.0 (22.0 – 29.4)
Number of cigarettes smoked in a day (mean ± SD; min–max; units)	n.a	n.a	14.1 ± 9.27 (4 – 40)	11.8 ± 5.72 (4 – 20)
Number of years as firefighter (mean ± SD; min–max; years)	7.8 ± 10.9 (1 – 40)	5.6 ± 10.7 (1 – 45)	6.4 ± 6.6 (1 – 27)	3.7 ± 3.8 (1 – 12)
Time of exposure in sampling day (mean ± SD; min–max; hours)	n.a	1.48 ± 1.65 (1 – 8)	n.a	1.84 ± 1.60 (1 – 8)

n.a – not applicable.

5.3.2. Impact of firefighting work and tobacco consumption

5.3.2.1. Metal(loid)s

Total cumulative metal content ($\sum$ Metal(loid)s) varied between 18.7 µg/L (4.99 - 73.6 µg/L) for NSNE group to 28.6 µg/L (11.8 - 137 µg/L) for SE ([Fig. 1A](#)). SE firefighters presented slightly increased $\sum$ Metal(loid)s content than NSNE subjects (increments up to 1.5 times), suggesting the cumulative contribution from firefighting activities and tobacco consumption. No changes were observed among $\sum$ Metal(loid)s for NSE and SNE groups [21.3 µg/L (5.01 - 55.9 µg/L) *versus* 19.0 µg/L (5.36 - 113 µg/L; $p>0.05$)] ([Fig. 1A](#)). All the 16 metal(loid)s were detected in the saliva samples and the most abundant metals were Cu > Sr > Mn > Li ~ Se ~ Cs with Cd, Hg, and Tl as the least abundant metals ([Table 2](#)).

IARC classified Cd and As as carcinogenic (Group 1), Pb as probable carcinogen (Group 2A), and Ni and Sb as possible carcinogens (Group 2B) to humans ([IARC, 2025](#)). Although Hg and Tl are not recognized as human carcinogens, both elements are highly toxic upon ingestion ([ATSDR, 2024a, 2024b](#)) and were therefore included in the toxicological analysis. The cumulative concentrations of toxic and carcinogenic metals ranged between 1.06 µg/L (0.434 - 8.36 µg/L) for the NSNE group to 1.75 µg/L (0.656 - 10.4 µg/L) for the SE group ([Fig. 1B](#)) and accounted for 5.66-6.84% of total metal content. The levels of toxic and carcinogenic metals were 1.6 times increased in SE comparatively to NSNE group ($p<0.05$). The distribution of these elements followed the distribution: Ni (0.287 – 0.607 µg/L) > As (0.303 – 0.443 µg/L) > Sb (0.0428 – 0.248 µg/L) > Pb (0.0423 – 0.190 µg/L) > Cd (0.00640 – 0.0979 µg/L) > Tl (0.0463 – 0.0768 µg/L) ≈ Hg (0.0536 – 0.0710 µg/L) ([Table 2](#)).

Fig. 1 Representation of A) total and B) carcinogenic cumulative concentrations (median, P25 – P75, and range, µg/L) of metals in the saliva of NSNE (non-smoker non-exposed), NSE (non-smoker exposed), SNE (smoker non-exposed), and SE (smoker exposed) firefighters.

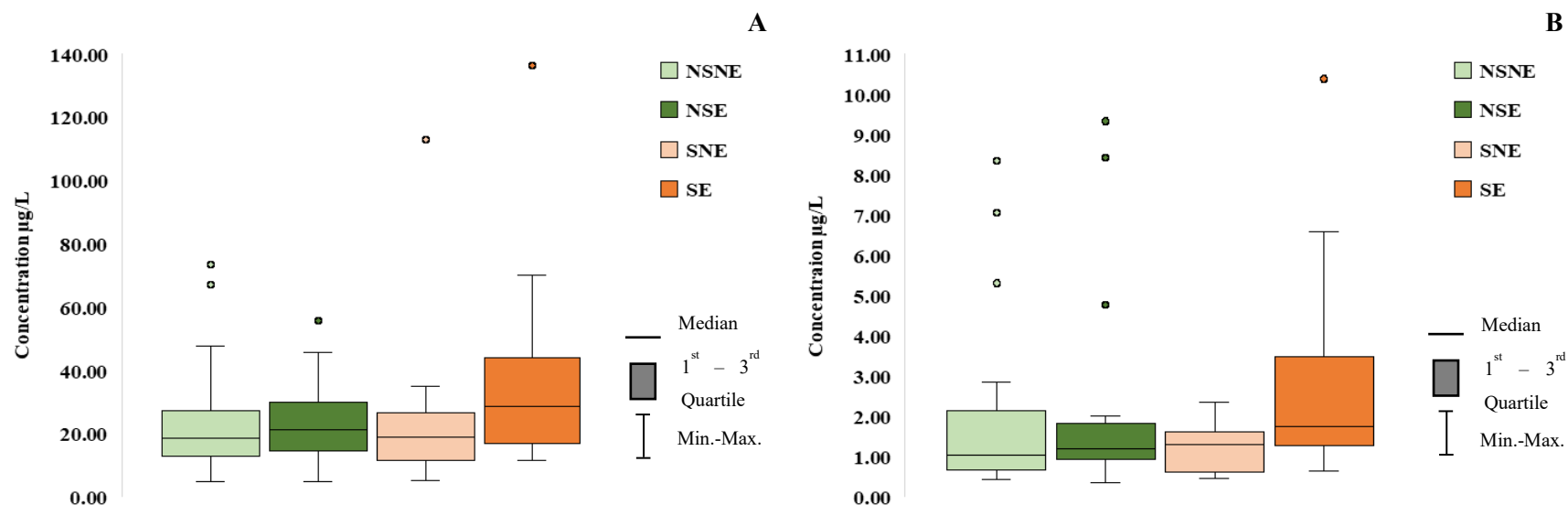


Table 2. Concentrations (median and range, µg/L) of metals and PAHs in the saliva of non-smoker non-exposed (NSNE), non-smoker exposed (NSE), smoker non-exposed (SNE), and smoker exposed (SE) firefighters.

ND – Not detected; Naph – naphthalene; Ace – acenaphthene; Flu – fluorene; Phe – phenanthrene; Ant – anthracene; Fln – fluoranthene; Pyr - pyrene

Pollutant	NSNE	NSE	SNE	SE
Li	1.67 (0.707 - 6.58)	1.40 (0.480 - 4.94)	1.45 (0.651 - 37.0)	1.97 (0.711 - 4.63)
Mn	2.73 (0.123 - 15.8)	2.56 (0.0962 - 9.18)	1.72 (0.223 - 4.84)	3.67 (0.237 - 21.8)
Co	0.0741 (0.0123 - 1.94)	0.0637 (0.0124 - 1.42)	0.185 (0.0155 - 2.03)	0.453 (0.0217 - 3.16)
Ni	0.380 (0.118 - 6.30)	0.287 (0.0821 - 4.76)	0.325 (0.121 - 1.35)	0.607 (0.107 - 5.81)
Cu	4.02 (1.18 - 35.1)	4.58 (0.503 - 40.1)	2.88 (0.781 - 52.4)	5.35 (0.964 - 79.2)
As	0.308 (0.125 - 1.24)	0.350 (0.118 - 0.865)	0.303 (0.0603 - 0.508)	0.443 (0.154 - 1.16)
Se	1.63 (0.724 - 4.36)	1.76 (0.305 - 4.09)	2.07 (0.787 - 4.17)	2.07 (1.29 - 4.44)
Sr	3.72 (0.524 - 12.3)	3.25 (1.06 - 18.4)	3.21 (0.876 - 12.7)	4.30 (1.69 - 19.5)
Mo	0.264 (0.0669 - 0.715)	0.264 (0.0388 - 0.886)	0.324 (0.0874 - 2.70)	0.342 (0.0773 - 1.54)
Cd	0.00979 (0.00180 - 0.188)	0.0177 (0.00145 - 0.117)	0.00640 (0.00259 - 0.127)	0.0210 (0.00500 - 0.104)
Sb	0.0428 (0.00968 - 1.01)	0.121 (0.0164 - 2.04)	0.0726 (0.00958 - 0.451)	0.248 (0.0402 - 2.86)
Cs	1.26 (0.523 - 13.5)	1.24 (0.175 - 25.3)	1.36 (0.508 - 2.55)	1.68 (0.389 - 6.26)
Ba	0.500 (0.151 - 1.16)	0.747 (0.144 - 4.95)	0.399 (0.0954 - 2.63)	0.969 (0.554 - 2.42)
Hg	0.0671 (0.0173 - 0.898)	0.0710 (0.0173 - 1.60)	0.0465 (0.0173 - 0.565)	0.0463 (0.0173 - 0.164)
Tl	0.0501 (0.0297 - 0.473)	0.0463 (0.0186 - 0.178)	0.0677 (0.0278 - 0.219)	0.0768 (0.0296 - 0.298)
Pb	0.0626 (0.0116 - 5.21)	0.107 (0.0231 - 6.72)	0.0423 (0.00631 - 0.969)	0.190 (0.0204 - 0.676)
Naph	0.921 (0.525 - 2.09)	1.17 (0.378 - 1.95)	0.910 (0.298 - 1.76)	0.899 (0.725 - 2.28)
Ace	ND	ND	ND	0.580 (0.463 - 0.698)
Flu	0.0872 (0.0317 - 0.490)	0.0865 (0.0317 - 0.201)	0.0898 (0.0317 - 0.250)	0.130 (0.0317 - 0.324)
Phe	0.143 (0.0682 - 1.50)	0.195 (0.0712 - 0.688)	0.115 (0.0700 - 1.06)	0.133 (0.0949 - 2.16)
Ant	0.0116 (0.00110 - 0.107)	0.0104 (0.00110 - 0.0233)	0.00247 (0.00110 - 0.0421)	0.0170 (0.00551 - 0.0763)
Fln	0.00515 (0.00515 - 0.195)	0.00515 (0.00515 - 0.0513)	0.00775 (0.00515 - 0.132)	0.00638 (0.00515 - 0.394)
Pyr	0.00348 (0.00348 - 0.0468)	0.00950 (0.00348 - 0.0158)	0.00693 (0.00348 - 0.0491)	0.00733 (0.00348 - 0.126)

Exposure to fire emissions in NSE group caused increments (1.5-2.8 times) in the salivary levels of Sb (0.0428 *versus* 0.121 µg/L), Cd (0.00979 *versus* 0.0177 µg/L), Pb (0.0626 *versus* 0.107 µg/L), and Ba (0.500 *versus* 0.747 µg/L) than in NSNE group; a slight increase was also observed for Cu, As, Se, and Hg ([Table 2](#); Fig. S1A). Available literature supports that tobacco smoke and recent exposure to fire emissions can significantly affect the salivary metal content ([Alshehri et al., 2023](#); [Breulman et al., 2002](#); [Engelsman et al., 2020](#); [Khabour et al., 2018](#); [Kim et al., 2010](#); [Li et al., 2023](#); [Nzihou & Stanmore, 2013](#); [Wu et al., 2021](#); [Zhai et al., 2023](#)). To the best knowledge of these authors, no information is available on the salivary metal content of firefighters performing different work tasks. The concentrations monitored in NSE firefighters were predominantly lower than the values reported in other occupational groups [Mn (0.0962 - 9.18 *versus* 0.12 - 70.5 µg/L), Ni (0.0821 - 4.76 *versus* 0.12 - 188.50 µg/L), Cu (0.503 - 40.1 *versus* 10.0 - 200.0 µg/L), Cd (0.00145 - 0.117 *versus* 0.14 - 59.7 µg/L), Hg (0.0173 - 1.60 *versus* 2.0 - 11.1 µg/L), and Pb (0.0231 - 6.72 *versus* 3.03 - 129 µg/L) in Spanish steel industry workers, Swedish navy divers, Tunisian dentists, and Canadian battery recycling workers] ([Brodeur et al., 1983](#); [Chaari et al., 2009](#); [Cowan et al., 2009](#); [El-Sadik & El-Dakhakhny, 1970](#); [Gervais et al., 1981](#); [Gil et al., 2011](#); [Menegário et al., 2001](#); [Ortendahl et al., 1989](#); [Shawahna et al., 2021](#); [Sousa et al., 2023](#); [Staff et al., 2014](#); [Wang et al., 2008](#); [Zaichick et al., 1995](#)). The SNE group presented increased concentrations of Co (2.5 times: 0.185 *versus* 0.0741 µg/L) and Sb (1.7 times: 0.0726 *versus* 0.0428 µg/L) comparatively to NSNE group. Some small increments (up to 1.35 times) were also observed for Ti, Se, Mo, and Cs ([Table 2](#); Fig. S1B). Based on the evidence retrieved from literature, the salivary content in Mn (0.223 - 4.84 and *versus* 2.26 - 3.40 µg/L), Sr (0.876 - 12.7 *versus* 1.85 - 3.46 µg/L), Mo (0.0874 - 2.70 *versus* 0.14 - 0.35 µg/L), and Ti (0.0278 - 0.219 *versus* 0.012 - 0.020 µg/L) were slightly increased in SNE firefighters than in other regular smokers; lower range values were reported for Cu (0.781 - 52.4 *versus* 1.39 - 172 µg/L), Cd (0.00259 - 0.127 *versus* 0.016 - 28.3 µg/L), and Pb (0.00631 - 0.969 *versus* 0.078 - 4.8 µg/L) ([Khabour et al., 2018](#); [Nizamani et al., 2019](#); [Talio et al., 2010](#)). Similar observations were observed for NSNE participants, who presented slightly increased values of Mn (0.123 - 15.8 *versus* 2.94 µg/L), Sr (0.524 - 12.3 *versus* 2.16 µg/L), Mo (0.0669 - 0.715 *versus* 0.29 µg/L), Ti (0.0297 - 0.473 *versus* 0.012 µg/L), and Pb (0.0116 - 5.21 *versus* 0.082 - 0.45 µg/L) and lower concentrations of Cu (1.18 - 35.1 *versus* 1.53 - 191 µg/L) and Cd (0.00180 - 0.188 *versus* 0.023 - 1.38 µg/L) than other non-smokers ([Khabour et al., 2018](#); [Nizamani et al., 2019](#); [Talio et al., 2010](#)). When recent firefighting work was combined with tobacco consumption (SE group), the salivary metal content presented increased levels of all

metal(loid)s, principally Co and Sb (6 times: 0.0741 *versus* 0.453 µg/L and 0.0428 *versus* 0.248 µg/L, respectively), Pb (3 times: 0.0626 *versus* 0.190 µg/L), Ba (2 times: 0.500 *versus* 0.969 µg/L), Ni and TI (1.5-1.6 times: 0.380 *versus* 0.607 µg/L and 0.0501 *versus* 0.0759 µg/L, respectively) comparatively to NSNE group; exception was found for Cd and Hg ([Table 2](#); Fig. S1C).

5.3.2.2. PAHs

Total cumulative concentrations of PAHs (Σ PAHs) found in firefighters' saliva varied according to the distribution: NSNE (1.19 µg/L; 0.654 – 3.06 µg/L) $\approx$ SE (1.21 µg/L; 0.911 – 4.95 µg/L) $\approx$ SNE (1.23 µg/L; 0.456 – 2.66 µg/L) $\approx$ NSE (1.40 µg/L; 0.504 – 2.49 µg/L); no differences were found between groups ($p > 0.05$) ([Fig. 2](#)). Among the 18 PAHs under study, including the 16 compounds listed in the USEPA and EU list of priority pollutants), only 7 were detected in the collected saliva samples: naphthalene > phenanthrene > fluorene > anthracene > fluoranthene > pyrene. Acenaphthene was only detected in the saliva of SE participants ([Table 2](#)); the remaining 11 PAHs were not detected in any saliva sample, reason why they were excluded from further analysis.

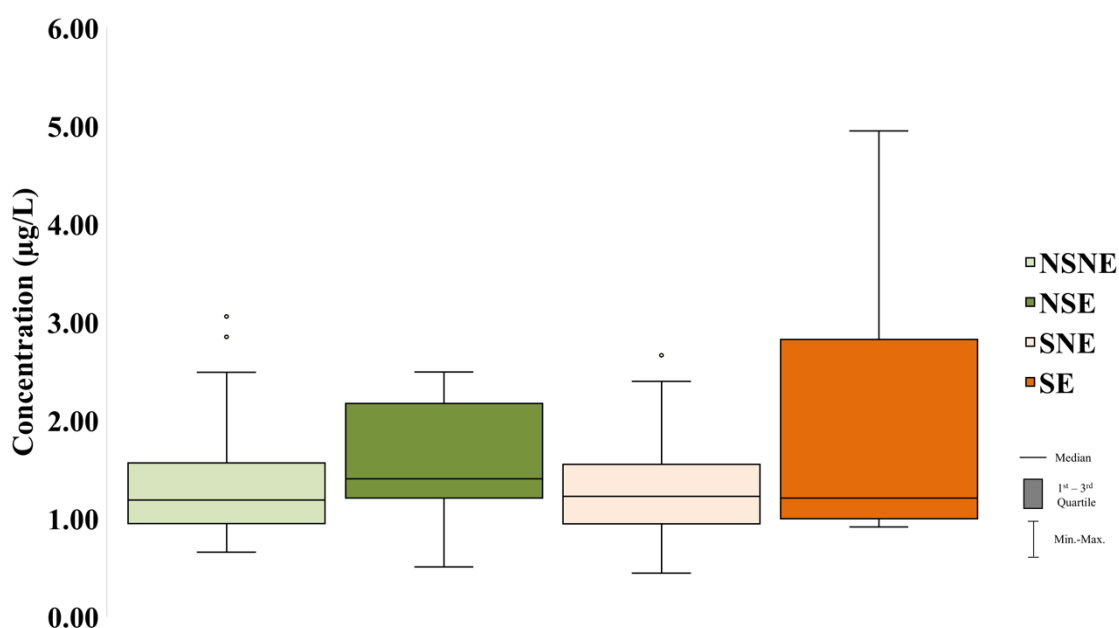


Fig. 2 Total cumulative concentration (median, P25 – P75, and range; µg/L) of PAHs in the saliva of NSNE (non-smoker non-exposed), NSE (non-smoker exposed), SNE (smoker non-exposed), and SE (smoker exposed) firefighters.

Biomonitoring studies characterizing occupationally exposed individuals through saliva are scarce. To best of our knowledge, only two studies assessed the levels of PAHs in the saliva of firefighters ([Santos et al., 2019](#); [Sousa et al., 2025](#)). Overall, both studies identified the presence of naphthalene, fluorene, phenanthrene, acenaphthene,

anthracene, fluoranthene, and pyrene in the saliva of Spanish and Portuguese firefighters, with the first three PAHs as the most prevalent compounds. These findings are aligned with our results ([Table 2](#)). NSE group presented slightly increased concentrations of naphthalene (1.3 times, 1.17 µg/L *versus* 0.921 µg/L), phenanthrene (1.4 times, 0.195 µg/L *versus* 0.143 µg/L), and pyrene (2.7 times, 0.00950 µg/L *versus* 0.00348 µg/L) than subjects from NSNE group. ΣPAH concentrations found in NSE group are predominantly higher than the values reported in Spanish firefighters immediately after firefighting activities for naphthalene (0.378 – 1.95 µg/L *versus* 0.27 – 0.43 µg/L) and slightly lower for fluorene (0.0317 – 0.201 µg/L *versus* 0.437 – 1.48 µg/L), phenanthrene (0.0712 – 0.688 µg/L *versus* 0.53 – 3.3 µg/L), and anthracene (0.00110 – 0.0233 µg/L *versus* 0.199 – 0.379 µg/L) ([Santos et al., 2019](#)). Regarding tobacco consumers (SNE group), slightly increased levels of fluoranthene (1.5 times, 0.00775 µg/L *versus* 0.00515 µg/L) and pyrene (2.0 times, 0.00693 µg/L *versus* 0.00348 µg/L) were found in saliva comparatively with NSNE participants ([Table 2](#)). So far, only 3 studies assessed the salivary levels of PAHs in smoking individuals ([Santos et al., 2020](#), [Peña et al., 2021](#), [Jouyban et al., 2020](#)). Available data revealed that smokers presented levels of naphthalene (0.0727 – 4.10 µg/L), and phenanthrene (0.130 – 0.608 µg/L) globally aligned with the values found in this study for SNE (0.298 – 1.76 µg/L and 0.0700 – 1.06 µg/L, respectively) ([Table 2](#)). However, the salivary content of fluorene (0.0420 – 0.800 µg/L *versus* 0.0317 – 0.250 µg/L), anthracene (0.0890 – 0.293 µg/L *versus* 0.00110 – 0.0421 µg/L), and fluoranthene (0.00400 – 0.521 µg/L *versus* 0.00515 – 0.132 µg/L) in smoking Spanish firefighters were slightly higher than the levels measured in SNE participants ([Table 2](#)). The SE group exhibited slightly increased concentrations of fluorene (1.5 times; 0.130 µg/L *versus* 0.0872 µg/L), anthracene (1.5 times, 0.0170 µg/L *versus* 0.0116 µg/L), fluoranthene (1.2 times; 0.00638 µg/L *versus* 0.00515 µg/L), and pyrene (2.0 times, 0.00733 µg/L *versus* 0.00348 µg/L) than the values obtained in NSNE group ([Table 2](#)). These findings suggest the contribution of firefighting activities as a source of salivary metal(loid)s and PAHs in occupationally exposed workers. Previously, other studies were able to demonstrate firefighters' occupational exposure via inhalation and skin contact to metal(loid)s and PAHs generated during fires ([Sousa et al., 2022](#); [Teixeira et al., 2024](#)). Regular tobacco consumption is an additional source of several health-relevant pollutants, including metal(loid)s and PAHs, and when combined with work activities (e.g., firefighting tasks), the workers' exposure levels through the oral cavity are predominantly increased ([Sousa et al., 2025](#)). These observations are supported by different biomonitoring studies which have demonstrated increased urinary

and blood levels of metal(loid)s and PAH metabolites after firefighting work ([Oliveira et al., 2017b, 2020](#); [Paiva et al., 2024a, 2024b](#)).

5.3.3. Impact of time of exposure to fires, career length, and body mass index

5.3.3.1. Time of exposure to fires

Occupational exposure during firefighting activities is primarily dependent on the time of exposure to fire emissions, *i.e.*, the duration of firefighting work and on the use of PPE. The firefighters with less than 1h of exposure were enrolled in firefighting work, and search and rescue missions during training structure fires whereas those exposed to more than 1h participated in an industrial fire (≈ 8 hours) that occurred in a paint production factory, and in forest fires (3-5 h). All participants reported the use of complete PPE during the entire fire events. Overall, both non-smoker and smoker firefighters presented 1.7 and 2.1 times increased levels of Σ Metal(loid)s after longer periods of exposure to fire emissions [36.9 $\mu\text{g/L}$ (31.1 - 42.7 $\mu\text{g/L}$) *versus* 21.0 $\mu\text{g/L}$ (11.8 - 136 $\mu\text{g/L}$), $p>0.05$; and 35.5 $\mu\text{g/L}$ (25.2 - 45.7 $\mu\text{g/L}$) *versus* 17.0 $\mu\text{g/L}$ (5.01 - 55.9 $\mu\text{g/L}$), $p>0.05$ in SE and NSE groups, respectively] ([Fig. 3A1](#)). A similar tendency was observed for Σ PAHs in the saliva of SE group with more than 1h of exposure to fires [1.7 times: 2.57 $\mu\text{g/L}$ (1.07 - 4.06 $\mu\text{g/L}$) *versus* 1.51 $\mu\text{g/L}$ (0.94 - 4.95 $\mu\text{g/L}$), $p>0.05$] ([Fig. 3A2](#)). Accounting for the nature of firefighting activity, our results suggest that salivary concentrations of Σ Metal(loid)s were slightly increased in forest fires (42.7 - 45.7 $\mu\text{g/L}$) than in training structure fires (25.2 - 31.1 $\mu\text{g/L}$). The respiratory protection used during firefighting activities may explain these results, since firefighters used a complete PPE with respiratory flash hood during forest fires and an airtight self-contained breathing apparatus (SCBA) during structure fires. Salivary PAH data were insufficient to explore the impact of firefighting activity and exposure duration in non-smoking firefighters. However, previous studies have already demonstrated that firefighting work increases firefighters' exposure to metal(loid)s and PAHs with higher levels of these fire-generated pollutants and their main metabolites in biological fluids ([Arinaitwe & McNamee, 2026](#); [Kim et al., 2022](#)).

5.3.3.2. Career length

Previous studies have suggested that mortality, development of health conditions, and aging are exacerbated in firefighters compared to the general population and especially in workers with longer careers ([Chun et al., 2025](#); [Pinkerton et al., 2019](#)). Our data suggests that firefighters with longer careers (mean of 23.8 years) presented 2.3 times increased Σ Metal(loid)s concentrations in saliva than those reporting a shorter (mean of

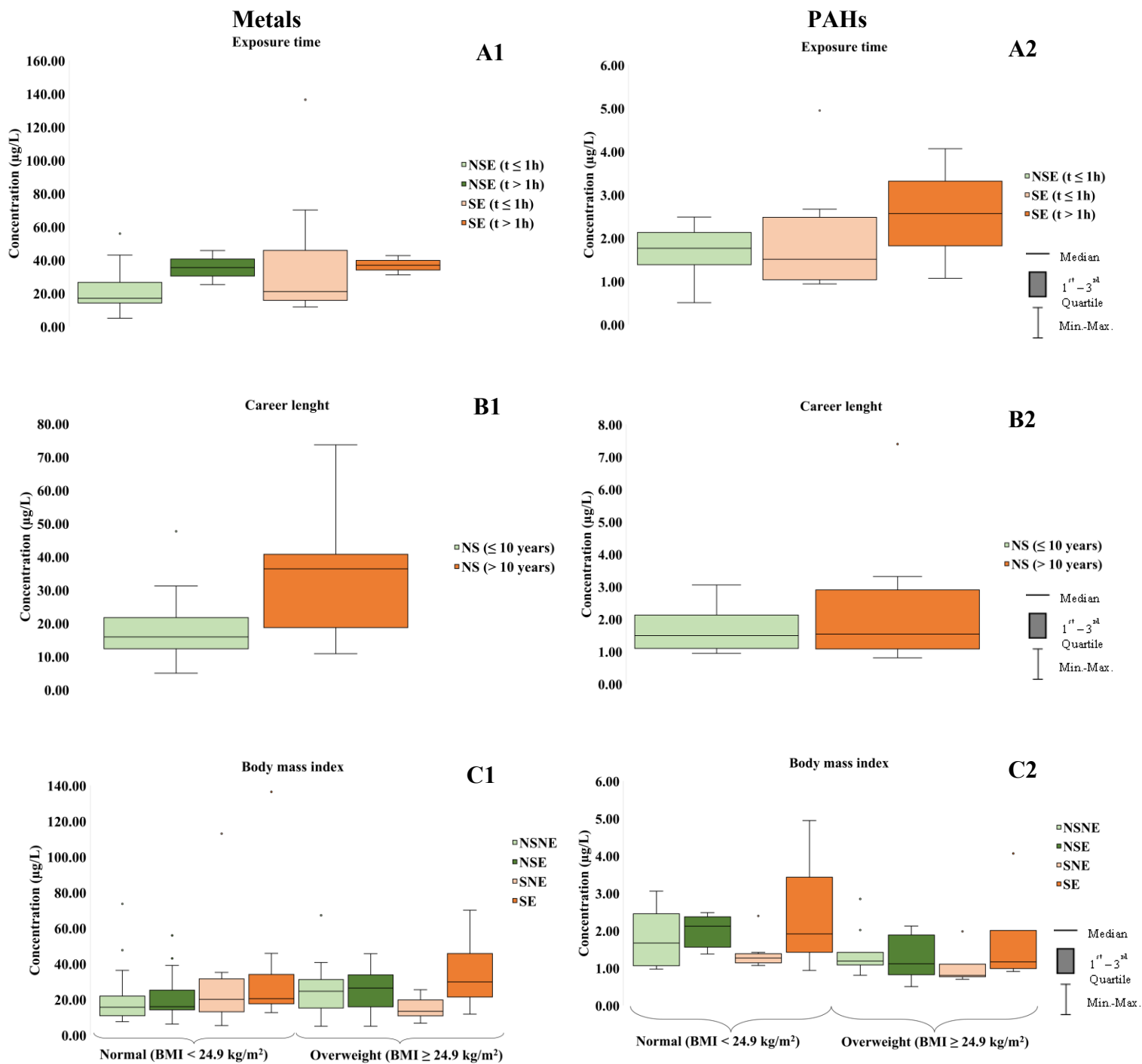


Fig. 3 Cumulative concentration (median, P25 – P75, and range, µg/L) of metals (1) and PAHs (2) according to (A) exposure time in non-smokers (NSE) and smokers (SE) recently exposed to fire emissions (t ≤ 1h; t > 1h) during firefighting work, (B) career length of firefighters (t ≤ 10 years; t > 10 years), and (C) body mass index in NSNE (non-smoker non-exposed), NSE (non-smoker exposed), SNE (smoker non-exposed), and SE (smoker exposed) firefighters. * Statistically significant differences at p≤0.05

1.8 years) career length [36.4 µg/L (10.9 - 73.6 µg/L) *versus* 15.9 µg/L (4.99 - 47.7 µg/L), $p \leq 0.05$ ([Fig. 3B1](#))]. Regarding Σ PAHs, a less pronounced increase was observed in firefighters with a longer career [1.54 µg/L (0.809 - 7.40 µg/L) *versus* 1.49 µg/L (0.945 - 3.06 µg/L), $p > 0.05$ ([Fig. 3B2](#))]. To date, no studies have assessed firefighters' occupational exposure to PAHs and metal(oid)s over the course of their career.

5.3.3.3. Body mass index

The participants of this study were grouped based on a normal ($<25 \text{ kg/m}^2$) and overweight ($\geq 25 \text{ kg/m}^2$) BMI, according to WHO criteria ([WHO, 2010](#)). Overall, 56% of firefighters presented a normal BMI whereas 46% were overweight. According to the data gathered, salivary content in Σ Metal(oid)s was slightly increased (1.5-1.7 times) in overweight firefighters when compared with those reporting a normal BMI [29.9 µg/L (11.8 - 70.1 µg/L) *versus* 20.4 µg/L (12.6 - 136 µg/L), for SE; 26.4 µg/L (5.01 - 45.7 µg/L) *versus* 15.9 µg/L (6.20 - 55.9 µg/L) for NSE; 24.6 µg/L (4.99 - 67.2 µg/L) *versus* 15.6 µg/L (7.55 - 73.6 µg/L) for NSNE], except for SNE group [16.0 µg/L (6.71 - 27.3

µg/L) *versus* 20.0 µg/L (5.36 - 113 µg/L); $p > 0.05$] ([Fig. 3C1](#)). These results suggest slightly increased concentrations of metal(oid)s in overweighted firefighters. Concerning Σ PAHs, a similar profile tendency (1.4-2.0 times fold increase) was observed for SE firefighters with a normal body weight comparatively with overweight individuals [1.91 µg/L (0.936 - 4.95 µg/L) *versus* 1.17 µg/L (0.911 - 4.06 µg/L) for SE; 2.12 µg/L (1.38 - 2.48 µg/L) *versus* 1.12 µg/L (0.504 - 2.13 µg/L) for NSE; 1.67 µg/L (0.973 - 3.06 µg/L) *versus* 1.19 µg/L (0.809 - 2.85 µg/L) for NSNE; 1.27 µg/L (1.07 - 2.39 µg/L) *versus* 0.804 µg/L (0.702 - 1.98 µg/L); $p > 0.05$ for SNE] ([Fig. 3C2](#)).

5.3.4. Individual profiles of firefighters

At an individual level, a total of 67% of firefighters presented 1.12 to - 3.7 times higher levels of Σ Metal(oid)s from pre- to post-firefighting work ([Fig. S2](#)). Increments were more frequent (90% *versus* 55%, respectively) and noticeable in smokers than in non-smokers, with pre- to post-fire ratios ranging between 1.0 to 3.7 in smokers and from 0.4 to 3.2 in non-smokers ([Fig. S3](#)). A similar tendency was found in the salivary content of toxic and carcinogenic elements (Ni, As, Cd, Sb, Hg, Tl, and Pb), with 73% of the individuals (90% smokers; 65% non-smokers) presenting 1.1 - 8.0 (smokers) and 1.0 - 2.3 times higher post-event concentrations than pre-fire values ([Fig. S3](#)).

Regarding Σ PAHs, a total of 65% of participants (88% of smokers; 50% of non-smokers) presented 1.0 - 2.6 (smokers) and 1.1 - 1.8 times (non-smokers) increased concentrations after firefighting work comparatively with pre-exposure levels ([Fig. S4](#)).

As previously observed for the individual profiles on salivary metal(loid)s content, the PAH increments were more pronounced in smokers (1.75 times; 0.86 – 2.6 times) rather than in non-smokers (1.0 times; 0.42 – 1.8 times). These results suggest that smokers experience higher salivary concentrations of fire emitted pollutants and the differences between *post*- and *pre*-fire scenarios are more noticeable in smoking firefighters. It is important to note that most of participants with paired samples participated in structure firefighting training exercises and the use of a full SCBA system was mandatory during the events. These findings may reflect that regardless of the use of SCBA system during firefighting activities, occupational exposure occurred and increased levels of metals and PAHs were found on the oral cavity of firefighters. It is also worth mentioning that exposure might have occurred during the firefighting work as well as during doffing of SCBA and turnout gear among other work tasks performed at the fire station.

5.3.5. Potential health risks

Salivary concentrations can provide information about oral exposure, but the relationship is indirect and depends strongly on the substance and the exposure scenario. Assumptive $TEQ_{B(a)P}$ estimation (4.40×10^{-4} $\mu\text{g/L}$ for SNE to 5.20×10^{-3} $\mu\text{g/L}$ for SE) and ADD (2.63×10^{-7} mg/kg-day for SE to 1.32×10^{-5} mg/kg-day for SNE) showed a low potential toxicity concerning the concentrations of PAHs determined in the saliva (Fig. S5A and S5B, respectively). The determined total THQ values (1.03×10^{-4} for SE to 7.25×10^{-3} for NSNE) were well below the USEPA threshold ($THQ < 1$), indicative of non-carcinogenic negligible risks (Fig. S5C). Regarding TR values (6.99×10^{-6} for SE to 2.52×10^{-4} , for NSNE) were predominantly within the acceptable carcinogenic risk range (10^{-6} to 10^{-4}); exceptions were observed for some NSNE and NSE firefighters (Fig. S5D). Overall, increased risks were identified among NSNE and SNE subjects; however, these findings should be interpreted with caution, accounting for differences in exposure duration. For NSNE and SNE subjects, saliva was collected after an 8-h work shift at the fire station performing multiple tasks excluding firefighting activity. The mean fire-combat exposure duration reported for NSE and SE subjects (1.60 and 1.48 h, respectively) reflected only the time spent actively firefighting, since samples were collected immediately after fire combat; the contribution from other sources of exposure while working at the fire station were not considered in this study. Saliva composition is not necessarily constant throughout the day and can reflect not only direct oral contamination from multiple sources (e.g., inhaled particles, contaminated hands/PPE, food, or other materials) but also contaminants entering the mouth from the systemic circulation. Therefore, the estimated screening risks based on the salivary content of PAHs and

meta(loid)s may be underestimated and do not represent total oral exposure. Daily foods and drinks intake were not evaluated and participants were asked to not ingest any food and drinks at least 60 minutes prior sample collection. Firefighters' work is complex and exposure to fire-related contaminants is not limited to firefighting activity but may also occur during routine tasks performed throughout the working day at the fire station (e.g., cleaning and maintenance of PPR and vehicles).

Evidence demonstrates that firefighters are chronically exposed to complex mixtures of fire pollutants, including PAHs and metals through multiple pathways, principally inhalation and dermal contact, leading to increased internal dose of health-relevant pollutants ([IARC, 2023](#); Sousa *et al.*, 2022). With increasing the years of service, firefighters experience cumulative exposure to different toxic combustion by-products. Exposure via oral cavity potentiates the direct contact of pollutants (e.g., metals and PAHs) with the oral mucosa, facilitating oral absorption, which contributes to systemic total exposure and can progressively alter biological systems and affect organs ([Balali-Mood *et al.*, 2021](#); [D'Evelyn *et al.*, 2022](#); [IARC, 2023](#); [Jaishankar *et al.*, 2014](#); [Mitra *et al.*, 2022](#); Sousa *et al.*, 2022). Repeated exposures, often combined with incomplete and/or delayed decontamination procedures, heat stress, dehydration, disrupted sleep, and physical strain, may impair the body's ability to recover and eliminate the toxins and metabolites formed during the metabolic process of detoxification. Also, as workers age, natural physiological changes often lead to variations in BMI, including gradual weight gain, even in physically demanding occupations as in the activity of firefighters. Excessive weight has been associated with impaired immune response and reduced functional capacity ([Roa & del Sol, 2018](#)). Therefore, age-related BMI variations can play an important role in shaping disease risk and overall health status. Some lifestyle factors (e.g., consumption of tobacco and alcohol) also contribute to human exposome. Over time, all these factors potentiate chronic inflammation, oxidative stress, genotoxicity, epigenetic alterations, and early organ dysfunction, increasing the risk of cardiovascular, respiratory, renal, and cancer-related diseases, as already demonstrated by IARC ([IARC 2023](#)).

With respect to firefighters' exposure to metal(loid)s, despite some metals (Mn, Cu, Se, and Mo) being essential trace elements that only become toxic at elevated concentrations, others are known as toxic elements (e.g., As, Cd, Hg, Tl, and Pb) ([ATSDR, 2012, 2020, 2024a](#); [IARC, 2012](#)). When in contact with oral mucosa (e.g., via contaminated particles, dust, and soot), these elements can cause local irritation and lesions. Exposure to substances containing high levels of Ni, Co, Cu, Cd, Ba, and Pb

can cause irritation on skin tissues, gingival inflammation, ulcers, taste disturbances, or periodontal diseases (Senevirathna *et al.*, 2024; Zhu *et al.*, 2025). Based on their solubility properties, metal(loid)s can be locally absorbed by buccal tissues or be ingested and assimilated into the gastrointestinal tract, with later systemic absorption, and will contribute to neurodevelopmental deficits and renal and cardiovascular toxicity. Moreover, exposure to toxic metal(loid)s has been associated with increased cancer risk. (Jaishankar *et al.*, 2014; Kim *et al.*, 2015; Staff *et al.*, 2014; Senevirathna *et al.*, 2024; Zhu *et al.*, 2025). However, biological exposure indices of occupational exposure to metal(loid)s via ingestion and in saliva are still non-existing.

During the enzymatic metabolization process of PAHs in the human body, different reactive intermediates are formed and can interact with biological molecules to form DNA adducts and inducing oxidative stress and inflammation, promoting mutagenesis, toxicity, and carcinogenesis (Batbaatar *et al.*, 2025; Sayed *et al.*, 2025; Zhu *et al.*, 2025). Exposure to PAHs has been associated with the development and/or aggravation of several health disorders, including cardiorespiratory diseases and cancer (IARC, 2010; Sayed *et al.*, 2025).

5.3.6. Limitations of the Study

Our findings must account for the following limitations. As a biological fluid, saliva reflects combined exposure routes and does not represent a direct measure of oral intake (Esteban & Castaño, 2009; Han *et al.*, 2019; Michalke *et al.*, 2015). The relatively small sample size and limited number of participants enrolled in the study, particularly after stratification by smoking status and recent firefighting work limits statistical power and warrants cautious interpretation of intergroups comparison. Although participants were instructed to avoid food and beverages 60 minutes before each sample collection time-point, salivary concentrations may still have been influenced by daily dietary habits (e.g., ingestion of smoked foods, canned fish, *etc.*). Tobacco consumption, second-hand smoking, non-occupational (e.g., ambient traffic emissions, fireplaces or gas-heating systems) and occupational (e.g., diesel exhausts at the garage of fire service, cooking or maintenance of equipment) factors are other relevant sources of exposure to PAHs and metal(loid)s (de Almeida Pdel *et al.*, 2008; Humphrey & Williamson, 2001; Michalke *et al.*, 2015; Sousa *et al.*, 2024, 2023). At the biological level, the hydration status, salivary flow rate, and individual metabolic differences are factors that affect salivary composition (de Almeida Pdel *et al.*, 2008; Humphrey & Williamson, 2001; Michalke *et al.*, 2015; Sousa *et al.*, 2024, 2023). Moreover, the reported concentrations of PAHs and metal(loid)s in the saliva only reflect short-term changes associated with recent

firefighting activities when compared to the control group of firefighters and do not fully capture daily oral exposure nor temporal variability across multiple fire events.

5.4. Conclusion

This pioneer study advances a novel saliva-based framework for characterizing firefighters' exposure to PAHs and metal(loid)s during regular firefighting work, accounting for five occupational and personal-life factors. Total cumulative metal(loid)s and PAHs were up to 1.5 (SE group) and 1.2 times (NSE group) superior to NSNE group, respectively. Firefighting activities increased salivary levels of metal(loid)s and PAHs, with approximately 65–67% of participants showing post-exposure rises regardless the use of PPE. Exposure intensity (*e.g.*, duration >1 h), career length, and increased BMI further contributed to higher exposures, suggesting potential bioaccumulation effects. The consistent detection of multiple PAHs and toxic/carcinogenic metal(loid)s highlights the relevance of saliva as an underexplored complementary biological matrix. The toxicological screening based on the salivary concentrations of PAHs and metal(loid)s presented overall neglected risks.

Future studies should include larger cohorts characterizing daily dietary habits, use repeated sampling across multiple days, firefighting scenarios, and fire seasons complemented by conventional biomonitoring matrices to determine blood and urine concentrations of metal(loid)s and PAH metabolites to support a stronger exposure-to-dose framework. Additional body-composition measures *e.g.*, waist circumference, fat mass percentage, or lean mass (*e.g.*, waist circumference, fat mass, and lean mass) could also help clarify the influence of body composition on salivary contaminant profiles. Such multidisciplinary approaches are needed to validate our preliminary findings and advance current knowledge on the use of saliva as a complementary biological fluid in human biomonitoring studies by exploring its contribution to total internal dose, exposure kinetics, and related toxicological mechanisms.

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Chapter 6.

Impact of fire emissions and tobacco consumption on the salivary content of metal(loid)s and PAHs: Exposure levels and assessment of oral and intestinal *in vitro* toxicity



Impact of Fire Emissions and Tobacco Consumption on the Salivary Content of Metal(loid)s and PAHs: Exposure Levels and Assessment of Oral and Intestinal In Vitro Toxicity

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Abstract

Human exposome via ingestion, by assimilation of health-relevant pollutants in the oral cavity and/or intestinal tract, has been poorly explored, principally in heavily exposed professionals. This work assessed the salivary content of 18 polycyclic aromatic hydrocarbons (PAH) and 16 metal(loid)s in smoker/non-smoker firefighters enrolled in (forest/structural) firefighting activities and explores the associated in vitro toxicities on buccal (TR146; HSC-3) and intestinal (Caco-2; HT29 MTX) cell lines. Total PAH levels (1.95–3.61 µg/L) were predominantly increased after firefighting compared to control groups, with naphthalene (up to 1.9 times in forest fires), phenanthrene (up to 4.5 times in the industrial fire), and anthracene (up to 3.1 times in forest fires) presenting the highest increments. Total salivary content of metal(loid)s (23.6–90.9 µg/L) was increased in exposed firefighters, being the highest increments found in the samples collected after an industrial fire (Pb: up to 45 times; Ba: up to 4–6 times; Sr: up to 2.8–5.6 times; Hg: up to 1.7 times). The impact of tobacco consumption was globally less pronounced than firefighting work. The salivary concentrations of PAHs and metal(loid)s were positive and globally moderate to strongly correlated. A principal component analysis was constructed to evaluate source apportionment and allowed the differentiation of firefighting activities at forest, controlled structure, and industrial fires; the impact of tobacco consumption was not evidenced. An in vitro health risk assessment was performed, and no significant toxicity was found in buccal/intestinal cell lines. Additional studies are needed to corroborate these preliminary studies and more peely explore the human exposome via ingestion.

Keywords Human exposome · Biomonitoring studies · Firefighters · Saliva · Metal(loid)s · Polycyclic aromatic hydrocarbons

Gabriel Sousa, Joana Teixeira and Maria João Bessa contributed equally to this work.

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Introduction

In recent decades, global warming has increased the frequency and severity of fires worldwide, impacting pollutant emissions and posing environmental and health risks [1–7]. Firefighters, as frontline responders to natural and man-made disasters, face significant occupational hazards. Their exposure to hazardous pollutants, including carcinogens, oxidative stressors, and inflammation-inducing chemicals, places them at elevated risk for cancer and other health complications [8]. Therefore, biomonitoring is essential to mitigate the health effects of their work [9].

Biomonitoring studies have identified numerous exposure biomarkers in firefighters' blood and urine [8, 10–19]. Saliva is an interesting alternative biospecimen for biomonitoring of recent exposure to chemical hazards due to its non-invasive collection and ease of handling, being more easily accepted by the study population [20–22]. Additionally, *in vitro* toxicity studies provide valuable insights into the cellular mechanisms behind firefighting-related health effects [23, 24]. Recent advances in technology have led to more complex and physiologically relevant *in vitro* systems by using cell cultures of specific tissues, including lung, skin, and gastrointestinal tract, which are able to mimic the behavior of differentiated human cells [25]. However, most reported *in vitro* studies using cellular assays to assess exposure to chemicals emitted during fires and wood smoke have only focused on monoculture respiratory models [10]. Existing studies reported reduced cell viability, enhanced oxidative stress, increased pro-inflammatory cytokine levels, and cell death frequencies, mechanisms linked to the development of chronic respiratory diseases, lung impairment, and cancer [10, 26–29].

The ingestion route in occupational exposure remains poorly studied, particularly among firefighters [30–34]. To date, only one study characterized post-firefighting saliva, and no *in vitro* studies examined associated ingestion-related toxicity [32]. This study aims to (i) quantify the levels of 18 polycyclic aromatic hydrocarbons (PAH) and 16 metal(loid)s in the saliva of smoking and non-smoking firefighters enrolled in firefighting activities, (ii) evaluate associated *in vitro* toxicity in buccal and intestinal culture models, and (iii) explore the combined contribution from firefighting activities and tobacco consumption on the salivary content of PAH and metal(loid).

As far as these authors are aware, this is the first study evaluating the *in vitro* toxicity of firefighters' saliva samples during real-life occupational activities. The authors hypothesized that regardless of the use of personal protective equipment (PPE), firefighting exposes firefighters to fire hazardous fire pollutants, making those substances accessible for assimilation in the mouth or ingested through saliva.

Materials and Methods

Participants and Sampling Campaigns

This study enrolled 41 firefighters (north of Portugal) who voluntarily participated and signed a consent form validated by the Ethical Committee of the University of Porto (Report 105/CEUP/2020). All participants filled out a self-characterizing sociodemographic questionnaire (age, gender, weight, height, smoking and eating habits, years as a firefighter, and recent participation in firefighting activities).

Firefighters were requested not to eat or smoke for at least 2 h before collecting samples. Saliva collection followed a straightforward procedure that consisted of accumulating and spitting non-stimulated saliva for 5 consecutive minutes into sterile collection cups. A total of eight pull mixtures of saliva ($4 < n < 7$) were prepared: S1—non-smoking firefighters non-exposed to fires, S2—smoking firefighters non-exposed to fires, S3—firefighters enrolled in forest fires, S4—non-smoking sappers non-exposed to fires, S5—non-smoking sappers enrolled in training structure fires, S6—smoking sappers non-exposed to fires, S7—smoking sappers enrolled in training structure fires, and S8—firefighters enrolled in an industrial fire. Samples from groups S1 and S2 were collected during a regular work shift at fire stations. Samples from groups S4–S7 were collected immediately before and after participating in training structure fires (2.5–5 h), occurring in a two-story cement building with old furniture (a replicate layout of a real house). Wooden pallets were used as the burning material ignited with a mixture of diesel and oil. Samples from S3 and S8 were collected upon firefighters' arrival at the fire station, immediately after participation in a forest fire (2.5–5 h) and an industrial (8 h) fire in a paint manufacturing factory, respectively. All firefighters used the full PPE during firefighting. Samples were frozen (-20°C) until chemical analysis.

Quantification of PAHs

An aliquot (500 μL) of each sample was extracted with ultrapure water (200 μL), methanol (50 μL), and ethyl acetate (1500 μL), being vortexed (1 min), and centrifuged (4000 rpm; 5 min). The supernatant was collected, and the sample was re-extracted. The collected supernatant aliquots were joined, completely dried in a rotary evaporator, redissolved in acetonitrile, and filtered with polytetrafluoroethylene (0.22 μm). A Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a C18 column (CC 150/4 Nucleosil 100-5C18 PAH, 150×4.0 mm; 5.0- μm particle size; Macherey–Nagel, Duren, Germany) and in line photodiode array (SPD-M20A) and fluorescence (RF-10AXL) detectors quantified the 18 PAHs [acenaphthene, acenaphthylene, anthracene, benz(a)anthracene, benzo(a)

pyrene, benzo(b+j)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, dibenzo(a,l)pyrene, fluoranthene, fluorene, indeno(1,2,3-c,d)pyrene, naphthalene, phenanthrene, pyrene] at 25 °C [35]; a representative chromatogram is presented in Figure S1 of the Supplementary Material. The limits of detection (LOD) and quantification (LOQ), representing the minimum detectable amount of analyte with signal-to-noise ratio of 3:1 and 10:1, ranged from 3.12 ng/L to 3.99 µg/L and between 10.4 ng/L to 13.3 µg/L, for anthracene and acenaphthylene, respectively. Quality control involved human and artificial saliva samples, with recovery assays yielding 41.1 ± 2.37 to $105 \pm 1.64\%$, for benzo(b+j)fluoranthene and acenaphthene, respectively. All extracts were analyzed in triplicate.

Quantification of Metal(loid)s

Saliva samples were centrifuged (4000 rpm; 10 min), and a supernatant aliquot (100 µL) was mixed with a diluent containing nitric acid 2% (v/v), gold (500 ppb), absolute anhydrous ethanol 1.5% (v/v), and an internal standard (10 ppb of gallium, iridium, and rhodium). Samples were analyzed by inductively coupled plasma with mass spectrometry (ICP-MS) for 16 metal(loid)s [lithium (Li), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), antimony (Sb), cesium (Cs), barium (Ba), mercury (Hg), thallium (Tl), and lead (Pb)] using an iCAP™ Q instrument (Thermo Fisher Scientific, MA, USA). LODs and LOQs ranged from 4.66 ng/L to 1.33 µg/L and 15.5 ng/L to 4.43 µg/L, respectively. The recovery values varied from 89 ± 3.14 to $111 \pm 1.33\%$. To prevent sequence effects, samples were analyzed randomly in triplicate.

In Vitro Cell Models

Cell Lines Culture and Exposure Assessment

Human mouth (TR146) and tongue (HSC-3) carcinoma cells, as well as Caco-2 were obtained from American Type Culture Collection (ATCC, USA). HT29-MTX cell line was kindly provided by Dr. T. Lesuffleur (INSERM U178, Villejuif, France). Cells were cultured in Dulbecco's modified eagle's medium (DMEM) high glucose with stable glutamine and sodium pyruvate supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) non-essential amino acids (NEAA) and 1% (v/v) 100,000 U/mL penicillin–10 mg/mL streptomycin mixture and maintained at 37 °C, 5% CO₂. For exposure experiments, cells were seeded in 96-well plates (2.5×10^4 cells/well) and adhered for 24 h. The buccal model was created by culturing TR146 cells on Transwell® inserts (2.4×10^4 cells/cm²) for 30 days [36], while the intestinal co-culture used Caco-2 and HT29-MTX cells (9:1 ratio,

1×10^5 cells/cm²) for 21 days [37]. Medium changes followed standard protocols. Buccal/intestinal monocultures were exposed to firefighters' saliva for 24 h, with cell culture media as negative (NC) and 1% Triton-X100 as positive (PC) controls. In Transwell models, saliva exposure lasted 4 h, using HBSS as NC. Additional controls included human saliva (HS) and PAH-contaminated saliva (PS).

Cell Viability Assessment

Transepithelial electrical resistance (TEER) was measured in the advanced cell culture models with an EVOM Epithelial Voltmeter Instrument equipped with a chopstick electrode. Resistance (Ω) measurements were made every 2–3 days (before changing the medium) and during the entire permeability assay at each assessed timepoint by immersing the electrode between the apical and basolateral of the Transwell models, avoiding contact with cells.

Determination of Transepithelial Electrical Resistance in Culture Models

Cell viability was assessed using the 3-(4,5-dimethylthiazolyl-2)–2,5-diphenyltetrazolium bromide (MTT) assay by removing the samples and adding MTT reagent (0.5 mg/mL) (100 µL/well for 96-well plates, 500 µL/insert for 12-well Transwells) followed by incubation for 3 h at 37 °C in the dark. Formazan crystals were solubilized in DMSO, agitated for 10 min, and absorbance measured at 570 nm (reference: 630 nm) using a Synergy™ 2 Microplate Reader. Each sample was tested in triplicate.

Statistical Analysis

Data analysis was performed using Microsoft Excel (v16.0), SPSS (v28.0), and GraphPad Prism (v8.0). Values below the LOD were replaced by $\text{LOD}/\sqrt{2}$ [38]. Cell viability data were expressed as mean ± standard deviation and tested for normality (Shapiro–Wilk) and homogeneity of variances (Levene's test). Non-normal data were analyzed using the Mann–Whitney *U* test. Differences between concentrations and controls were assessed via one-way ANOVA followed by Dunnett's post hoc test; or Kruskal–Wallis for when data did not meet normality. Significance was set at $p \leq 0.05$. In figures/tables, * and ** indicate $p \leq 0.05$ and $p \leq 0.01$, respectively.

Results and Discussion

Characterization of Firefighters

Overall, most of participants were male, except for groups S2 (20% female) and S8 (60% female). The age and body

Table 1 Socio-demographic and occupational characterization of firefighters organized in eight different groups according to tobacco smoking habits and firefighting activity: S1—non-smoking professional firefighters non-exposed to fire emissions; S2—smoking professional firefighters non-exposed to fire emissions; S3—profes-

sional firefighters exposed to forest fires; S4—non-smoking sappers non-exposed to fire emissions; S5—non-smoking sappers exposed to training structure fires; S6—smoking sappers non-exposed to fire emissions; S7—smoking sappers exposed to training structure fires; S8—professional firefighters exposed to an industrial fire)

Characteristics	S1	S2	S3	S4	S5	S6	S7	S8
Firefighters (n)	5	5	4	5	7	5	5	5
Male (%)	100%	80%	100%	100%	100%	100%	100%	40%
Female (%)	n.a	20%	n.a	n.a	n.a	n.a	n.a	60%
Age (mean ± SD; min–max; years)	36.8 ± 9.95 (22.0–47.0)	40.2 ± 7.71 (26.0–49.0)	39.7 ± 16.5 (28.0–63.0)	28.8 ± 6.11 (25.0–41.0)	27.7 ± 5.55 (23.0–41.0)	24.8 ± 2.93 (20.0–29.0)	24.8 ± 2.93 (20.0–29.0)	27.0 ± 3.03 (24.0–32.0)
Work as a firefighter (mean ± SD; min–max; years)	16.4 ± 9.65 (4.50–27.0)	21.0 ± 7.46 (13.0–33.0)	18.7 ± 18.6 (4.50–45.0)	5.10 ± 8.20 (1.00–21.5)	3.93 ± 7.17 (1.00–21.5)	2.10 ± 2.20 (1.00–6.50)	2.10 ± 2.20 (1.00–6.50)	7.40 ± 3.44 (4.00–12.0)
BMI (mean ± SD; min–max; kg/m ²)	28.1 ± 1.96 (25.6–31.1)	25.3 ± 4.94 (20.3–34.8)	26.9 ± 3.26 (23.2–31.1)	25.8 ± 2.79 (22.1–30.1)	25.3 ± 2.47 (22.1–30.1)	24.0 ± 1.74 (22.0–26.2)	24.0 ± 1.74 (22.0–26.2)	25.4 ± 5.49 (20.3–34.1)
Participation in firefighting activity ^a	No	No	Yes	No	Yes	No	Yes	Yes
Type of fire	n.a	n.a	Forest fire	n.a	Training structure fire	n.a	Training structure fire	Structure fire
Time of exposure (mean ± SD; min–max; hours)	n.a	n.a	3.9 ± 1.1 (2.5–5.0)	n.a	5.4 ± 0.49 (5.0–6.0)	n.a	5.4 ± 0.49 (5.0–6.0)	8.0 ± 0.0 (8.0–8.0)
Smoking habits (%)	0	100%	25%	0%	0%	100%	100%	40%
Smoked cigarettes (mean ± SD; min–max; cigarettes)	n.a	15 ± 0.0 (15–15)	n.r	n.a	n.a	8.8 ± 6.0 (4.0–20.0)	8.8 ± 6.0 (4.0–20.0)	6.0 ± 7.3 (0.0–15.0)

n.a.—not applicable; n.r.—not reported; ^a—Firefighting activity reported during the last 3 days prior to the sampling campaigns

mass index (BMI) ranged from 24.8 to 40.2 years and between 24.0 and 28.1 kg/m², respectively (Table 1). About 56.1% of participants presented BMI values below the recommended normal index (25 kg/m²), whereas 43.9% exhibited values that may suggest overweight (> 25 kg/m²) [39]. The recent firefighting activity ranged from 2.1 years in early-career sappers to 21 years in seasoned professionals, with participants reporting involvement in forest (4.0 h), training structure (5.4 h), and industrial fire (8.0 h) combat activities (Table 1).

Salivary Content in PAHs

The median levels of total PAH (Σ PAHs) varied from 1.81 and 3.60 µg/L, with maximum levels reaching 3.88 µg/L. The distribution of Σ PAHs followed the sequence: S3 < S7 < S2 ≈ S5 < S8 < S1 < S6 < S4; no differences were observed between the eight groups of firefighters. Among

the 18 PAHs under study in the collected saliva samples, only five compounds, naphthalene, acenaphthene, fluorene, phenanthrene, and anthracene, were detected; the other 13 PAHs were never detected. Naphthalene (0.740–1.61 µg/L) was the predominant compound, representing 29.0 to 81.4% of Σ PAHs, followed by acenaphthene (0.66–2.34 µg/L; 6.27–65.0%), and fluorene (0.206–0.253 µg/L; 10.4–10.7%). Anthracene was detected in all groups, with values ranging between 0.0110 and 0.0336 µg/L (0.304–1.43% of Σ PAHs). Phenanthrene was found in groups S1, S5, S6, and S8 at levels ranging between 0.0115 and 0.120 µg/L (0.55–5.08% of Σ PAHs) (Table 2). The prevalence of low molecular weight PAHs was predicted due to their higher affinity to water matrices [40, 41]. Among the detected compounds, naphthalene and anthracene are classified by IARC as possibly carcinogenic (group 2B) to humans [42, 43]. However, no limits for human exposure to these PAHs are defined.

Active participation in firefighting activities increased the concentrations of some PAHs in firefighters saliva, namely naphthalene and anthracene in professional firefighters enrolled in forest fires [1.4–2.7 times higher, 1.44 µg/L (S3) versus 0.740 µg/L (S1) versus 1.03 µg/L (S4) and 0.0294 µg/L (S3) versus 0.0148 µg/L (S1) versus 0.0110 µg/L (S4), respectively] (Table 2).

Saliva samples collected by sapper firefighters immediately after training structure fires presented slightly increased levels of naphthalene (1.12 versus 1.03 µg/L), fluorene (0.231 versus 0.212 µg/L), and anthracene (0.0219 versus 0.0110 µg/L) in non-smoking subjects (groups S5); increased values of anthracene (0.0262 versus 0.0157 µg/L) were also observed in smoking and exposed sapper firefighters (group S7) than in the control groups S4 and S6 (Table 2). Concentrations of naphthalene (1.29 versus 0.740 and 1.03 µg/L), fluorene (0.253 versus 0.238 and 0.212 µg/L), phenanthrene (0.120 versus 0.0265 and 0.0091 µg/L), and anthracene (0.0336 versus 0.0148 and 0.0110 µg/L) were also increased in professional firefighters enrolled in an industrial fire (group S8) than in the control groups S1 and S4. Overall, the highest increments in the saliva content of firefighters participating in firefighting activities were observed in naphthalene (up to 1.9 times in forest fires), phenanthrene (up to 4.5 in the industrial fire), and anthracene (up to 3.1 times in forest fires). Smoking habits among professional and sapper firefighters contributed to slightly increased levels of naphthalene, fluorene, and anthracene in the collected saliva samples (Table 2).

The number of studies characterizing PAHs in human saliva is quite limited [21, 32, 44–48]. So far, only one study assessed the concentrations of PAHs in the saliva of workers, more specifically in firefighters [32]. This study detected seven (naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene) out of 16 PAHs in the saliva of 11 firefighters and characterized the concentrations of PAHs in 45 samples collected immediately after (0 h), 6, and 12 h after the fire events. These fire events consisted of a fire induced specifically for the purpose of practicing, a fire in a train and a fire in a supermarket. As observed in the present study (Table 2), naphthalene, fluorene, and phenanthrene were the predominant compounds found in the saliva of firefighters [32]. The concentrations reported by Santos et al. (2019) in the saliva of firefighters immediately after ($t=0$) firefighting activities were predominantly higher than the values determined in the saliva of Portuguese firefighters (0.207–0.253 µg/L versus 0.437–1.48 µg/L; 0.0091–0.120 µg/L versus 0.53–3.3 µg/L; and 0.0219–0.0336 µg/L versus 0.199–0.379 µg/L, for fluorene, phenanthrene, and anthracene, respectively), except for naphthalene (0.90–1.44 µg/L versus 0.27–0.43 µg/L). The PAH levels decreased as post-fire time passed and only acenaphthylene (0.43–1.42 µg/L), fluorene (0.338–0.78 µg/L), phenanthrene (0.15–1.2

µg/L), fluoranthene (0.20–1.06 µg/L), and pyrene (0.20–1.5 µg/L) were found after 6 h of exposure [32]. The saliva content in non-exposed firefighters were in close range with the values reported in this study for fluorene (0.205–0.238 µg/L versus 0.202–0.75 µg/L), but higher for phenanthrene (0.0091–0.0265 µg/L versus 0.16–0.23 µg/L).

To the best knowledge of these authors, only three studies assessed the levels of PAHs in the saliva of smoking individuals [44, 46, 47]. Smokers presented levels of naphthalene (0.073–4.10 µg/L) and fluorene (0.0420–0.80 µg/L) in close range with the values found in smoking firefighters (1.27–1.61 µg/L and 0.205–0.220 µg/L for naphthalene and fluorene, respectively, in groups S2 and S6; Table 2) [44, 46, 47]. Salivary content in acenaphthene reported in smokers was predominantly lower than the values found in this study (0.00460–0.170 µg/L versus 0.124–1.29 µg/L) [44, 46, 47]. However, the concentrations of phenanthrene (0.130–0.61 µg/L), anthracene (0.089–0.293 µg/L), and fluoranthene (0.00400–0.521 µg/L) were higher than the values determined in the saliva of smoking Portuguese firefighters (Table 2) [44, 46, 47].

Salivary Content in Metal(loid)s

The median concentrations of total metal(loid)s found in the saliva of firefighters varied from 23.6 to 90.9 µg/L (maximum of 93.3 µg/L) and were distributed according to the sequence: $S4 \approx S6 < S8 < S1 \approx S5 < S3 < S2 < S7$ (Fig. 1). Saliva has a complex biochemical composition, and the observed inter- and intra-individual variability can be explained through the contribution of different variables, including the time of exposure to fires, time between the end of firefighting and the sampling at the arrival to fire station, the hydration level of each participant, and the number of daily smoked cigarettes. Overall, firefighters enrolled in firefighting activities presented increased levels of metal(loid)s in the saliva, while the lowest values were predominantly observed in non-exposed subjects. More specifically, professional and sapper firefighters involved in the fire combat of a forest fire and training structure fires presented 1.5 (63.7 µg/L in group S3 versus 42.2 µg/L in group S1) and up to 3.54 (90.9 µg/L in group S7 versus 25.7 µg/L in group S6) times the metal content in saliva than non-exposed participants. Regarding the impact of tobacco consumption, only some smoking firefighters presented superior salivary concentrations of metal(loid)s than non-smoking individuals (71.6 µg/L in group S2 versus 42.2 µg/L in group S1; Fig. 1).

All the 16 metal(loid)s were found in the saliva of firefighters, with detection rates ranging between 79.2% (Cd) to 100%. Zn (10.4–61.3 µg/L; 39.7–67.4% of total metal(loid)s), Cu (2.80–10.7 µg/L; 10.4–15.0%), Sr (3.21–6.0 µg/L; 4.09–16.6%), Se (2.35–5.05 µg/L; 5.39–11.6%), and Mn

Table 2 Concentrations (median and range; µg/L) of PAHs, metals, and metalloids in the saliva of firefighters

Sample	S1	S2	S3	S4	S5	S6	S7	S8
PAHs								
Naphthalene	0.740 (0.675–0.765)	1.61 (1.59–1.66)	1.44 (1.42–1.45)	1.03 (1.01–1.04)	1.12 (1.05–1.16)	1.27 (1.22–1.30)	0.896 (0.827–0.902)	1.29 (1.29–1.38)
Acenaphthene	1.54 (1.27–1.56)	0.124 ^a	0.1239 ^a	2.34 (1.75–2.597)	0.690 (0.466–0.930)	1.29 (1.28–1.45)	0.905 (0.795–0.926)	0.657 (0.557–0.751)
Fluorene	0.238 (0.214–0.242)	0.205 (0.203–0.214)	0.214 (0.211–0.221)	0.212 (0.206–0.231)	0.231 (0.228–0.238)	0.220 (0.208–0.224)	0.207 (0.197–0.209)	0.253 (0.243–0.254)
Phenanthrene	0.0265 (0.0248–0.0281)	0.00910 ^a	0.00910 ^a	0.00910 ^a	0.0115 (0.00910 ^a –0.0139)	0.0143 (0.00910 ^a –0.0194)	0.00910 ^a	0.120 (0.113–0.126)
Anthracene	0.0148 (0.0144–0.0159)	0.0289 (0.0280–0.0290)	0.0294 (0.0290–0.0296)	0.0110 (0.0108–0.0110)	0.0220 (0.0218–0.0256)	0.0157 (0.0151–0.0178)	0.0262 (0.0244–0.0271)	0.0336 (0.0328–0.0352)
Metal(loid)s								
Li	1.231 (1.215–1.246)	4.196 (4.075–4.237)	1.613 (1.469–1.671)	1.381 (1.339–1.389)	1.078 (1.078–1.098)	0.9620 (0.9368–1.069)	0.7314 (0.7302–0.7615)	1.510 (1.506–1.520)
Mn	2.218 (2.189–2.248)	3.487 (3.454–3.570)	4.067 (3.157–4.597)	1.021 (1.016–1.039)	2.669 (2.653–2.701)	0.9473 (0.9298–1.537)	4.727 (4.649–4.806)	1.642 (1.600–1.643)
Cu	5.249 (5.249–5.875)	10.74 (10.49–10.91)	8.393 (7.073–8.678)	2.798 (2.797–2.801)	6.373 (6.261–6.480)	3.855 (3.722–4.401)	10.75 (10.68–11.30)	3.757 (3.697–3.903)
Zn	20.32 (20.04–20.58)	39.41 (39.37–39.75)	30.77 (25.81–31.16)	10.36 (10.28–10.50)	19.19 (18.77–19.28)	10.49 (10.48–12.41)	61.30 (60.75–62.71)	14.35 (14.11–14.84)
Se	4.224 (4.147–4.409)	4.068 (3.997–4.632)	5.048 (4.751–5.248)	2.347 (2.333–2.422)	3.441 (3.325–3.519)	2.970 (2.657–3.114)	4.904 (4.743–4.991)	3.266 (3.089–3.279)
Sr	4.731 (4.708–4.738)	4.414 (4.265–4.459)	5.135 (4.523–5.370)	3.211 (3.161–3.235)	5.963 (5.875–5.988)	3.376 (3.359–3.670)	3.715 (3.609–3.750)	6.012 (5.891–6.146)
Cs	1.233 (1.201–1.246)	1.729 (1.728–1.776)	1.558 (1.536–1.565)	0.8151 (0.8120–0.8358)	1.205 (1.190–1.235)	0.6743 (0.6722–0.7171)	1.298 (1.289–1.301)	0.7562 (0.7542–0.7756)
Co	0.08738 (0.07966–0.0887)	0.08327 (0.08180–0.09373)	0.1690 (0.1367–0.2091)	0.02662 ^a	0.07759 (0.07397–0.08165)	0.08472 (0.07142–0.08597)	0.1084 (0.1038–0.1146)	0.06066 (0.05695–0.06142)
Ni	0.7669 (0.7667–0.8126)	1.539 (1.524–1.645)	2.224 (1.855–2.398)	0.7292 (0.6932–0.7483)	0.9425 (0.9290–0.9447)	0.8172 (0.8140–0.9825)	1.424 (1.393–1.429)	0.6590 (0.6307–0.6797)
As	0.5757 (0.4851–0.6577)	0.4457 (0.4059–0.4839)	1.171 (0.6327–1.247)	0.2185 (0.1977–0.2920)	0.4076 (0.4020–0.4298)	0.3191 (0.3046–0.6033)	0.3655 (0.3302–0.3721)	0.3261 (0.2884–0.3641)
Mo	0.5427 (0.5139–0.6341)	0.3965 (0.3488–0.4181)	1.632 (0.6387–1.752)	0.2609 (0.2359–0.2755)	0.4461 (0.3993–0.4516)	0.3991 (0.3801–0.7567)	0.6913 (0.6648–0.7472)	0.2332 (0.2312–0.2592)
Cd	0.01972 (0.01861–0.02280)	0.02409 (0.02233–0.02705)	0.03421 (0.02145–0.03825)	0.01034 (0.006361 ^a –0.01219)	0.02419 (0.02416–0.02597)	0.006361 ^a (0.006361–0.01215)	0.05586 (0.05290–0.05615)	0.006361 ^a (0.006361 ^a –0.01006)
Sb	0.07911 (0.06320–0.08854)	0.09910 (0.09059–0.1036)	0.4412 (0.2845–0.4619)	0.04072 (0.03830–0.04156)	0.2705 (0.2685–0.2729)	0.06127 (0.05485–0.1082)	0.1863 (0.1780–0.1977)	0.2268 (0.2267–0.2367)

Impact of Fire Emissions and Tobacco Consumption on the Salivary Content of Metal(loid)s and...

Table 2 (continued)

Sample	S1	S2	S3	S4	S5	S6	S7	S8
Ba	0.2219 (0.2061–0.2610)	0.3664 (0.3407–0.3783)	0.4856 (0.3933–0.4865)	0.1507 (0.1377–0.1643)	0.4482 (0.4446–0.4939)	0.3644 (0.3184–0.3715)	0.2596 (0.2542–0.2800)	0.9182 (0.9098–0.9491)
Hg	0.4817 (0.4747–0.6216)	0.1826 (0.1445–0.1980)	0.6043 (0.3162–0.8016)	0.07373 (0.06221–0.09336)	0.09911 (0.08800–0.1106)	0.1637 (0.1602–0.3624)	0.2480 (0.1979–0.2575)	0.1267 (0.09200–0.1305)
Tl	0.1194 (0.1105–0.1219)	0.1163 (0.1161–0.1175)	0.1523 (0.1093–0.1986)	0.07682 (0.07340–0.08069)	0.1160 (0.1128–0.1178)	0.1204 (0.1174–0.1611)	0.09204 (0.08987–0.1085)	0.03770 (0.03688–0.03874)
Pb	0.05032 (0.04293–0.7512)	0.2654 (0.2633–0.2671)	0.2196 (0.09666–0.2312)	0.09345 (0.08891–0.09371)	0.1340 (0.1305–0.1407)	0.04420 (0.04399–0.08305)	0.09110 (0.09088–0.09211)	2.272 (2.254–2.287)

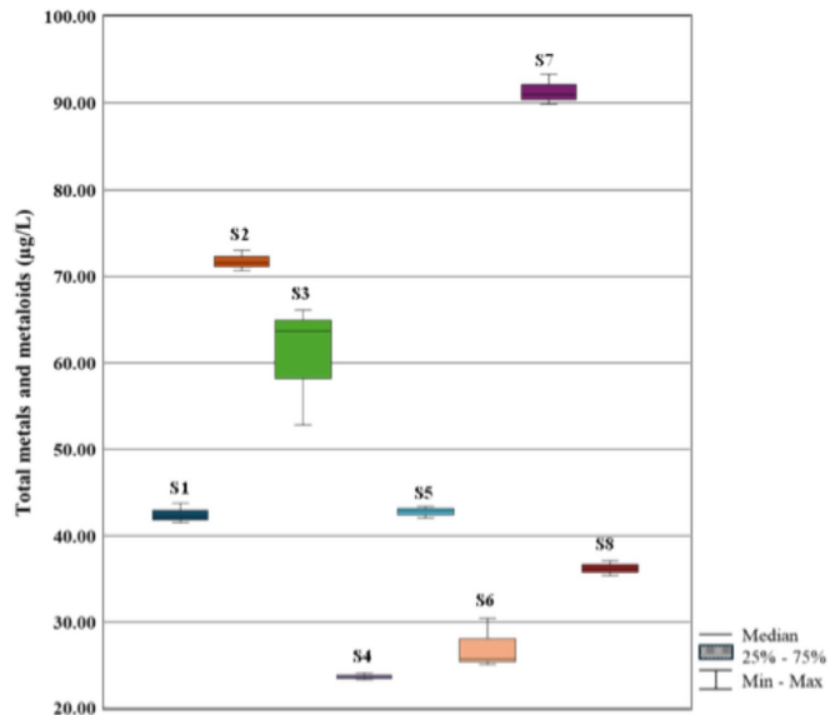
^aLOD/ $\sqrt{2}$ was used when the concentrations were below the LOD [38]

(0.95–4.73 $\mu\text{g/L}$; 3.7–6.4%) were the more predominant, while Pb (0.0442–2.27 $\mu\text{g/L}$), Sb (0.0407–0.441 $\mu\text{g/L}$), Tl (0.0377–0.152 $\mu\text{g/L}$), Co (0.0266–0.169 $\mu\text{g/L}$), and Cd (0.00636–0.0559 $\mu\text{g/L}$) were the less abundant. Among the elements under study, As and Cd are known (group 1) carcinogens, Sb trioxides and Pb are probable carcinogens (group 2A), and (organic) Hg is a possible (group 2B) human carcinogen [11, 49–53]. However, guidelines and salivary limits of exposure to these metal(loid)s are still absent.

The saliva of professional firefighters enrolled in fire-fighting activities during an industrial fire (groups S8) presented increased levels of Pb (up to 45 times; 2.27 $\mu\text{g/L}$ versus 0.0503 and 0.0867 $\mu\text{g/L}$), Ba (four–six times; 0.92 $\mu\text{g/L}$ versus 0.222 and 0.393 $\mu\text{g/L}$), Sb (2.8–5.6 times; 0.227 $\mu\text{g/L}$ versus 0.0791 and 0.0407 $\mu\text{g/L}$), and Hg (1.7 times; 0.127 $\mu\text{g/L}$ versus 0.074 $\mu\text{g/L}$) than non-exposed subjects (groups S1 and S4). The saliva of firefighters who were directly involved in the fire combat of forest fires (group S3) presented four-times higher concentrations of Pb (0.220 $\mu\text{g/L}$ versus 0.0503 $\mu\text{g/L}$), three-times increased values of Ni (2.22 $\mu\text{g/L}$ versus 0.767 $\mu\text{g/L}$) and Mo (1.63 $\mu\text{g/L}$ versus 0.543 $\mu\text{g/L}$), and up to two-times elevated levels of Mn (4.07 $\mu\text{g/L}$ versus 2.22 $\mu\text{g/L}$), Co (0.169 $\mu\text{g/L}$ versus 0.0874 $\mu\text{g/L}$), As (1.17 $\mu\text{g/L}$ versus 0.576 $\mu\text{g/L}$), Cd (0.0342 $\mu\text{g/L}$ versus 0.0197 $\mu\text{g/L}$), and Ba (0.486 $\mu\text{g/L}$ versus 0.222 $\mu\text{g/L}$) than firefighters performing regular tasks at the fire station (group S1). Concentrations of Cd (two–nine times; 0.0242 $\mu\text{g/L}$ versus 0.0103 $\mu\text{g/L}$ and 0.0559 $\mu\text{g/L}$ versus 0.0064 $\mu\text{g/L}$), Sb (three–seven times; 0.270 $\mu\text{g/L}$ versus 0.0407 $\mu\text{g/L}$ and 0.186 $\mu\text{g/L}$ versus 0.0613 $\mu\text{g/L}$), Mn (three–five times; 2.67 $\mu\text{g/L}$ versus 1.02 $\mu\text{g/L}$ and 4.73 $\mu\text{g/L}$ versus 0.947 $\mu\text{g/L}$), Cu (two–three times; 6.37 $\mu\text{g/L}$ versus 2.80 $\mu\text{g/L}$ and 10.7 $\mu\text{g/L}$ versus 3.85 $\mu\text{g/L}$), and Mo (1.7 times; 0.446 $\mu\text{g/L}$ versus 0.261 $\mu\text{g/L}$ and 0.691 $\mu\text{g/L}$ versus 0.399 $\mu\text{g/L}$) were also increased in the saliva of sapper firefighters after training structure exercises (groups S5 and S7) than non-exposed sappers (groups S4 and S6) (Table 2). During all stages of fire combat, firefighters use the full PPE, including respiratory protection (flash hood during forest fires and SCBA during structure fires). However, some authors have already demonstrated the contamination of turnout gear inner layers and firefighters' skin after firefighting [16, 54, 55]. Also, the removal of contaminated PPE may also contribute for short periods of exposure to fire emissions [16, 56, 57].

Emissions released from structure and industrial fires contain particulate emissions with bound organic and inorganic pollutants, including some metal(loid)s (e.g., As, Cr, Cd, Hg, Sb, and Pb), since these elements are present in plastics, electronic devices, paints, or treated woods [58–63]. As for emissions from biomass burning/wildland fires, the most common trace elements present are Mn, Fe, Ni, Cu, Zn, Cd, Hg, and Pb [63–67]. However, it is important to note that some elements, such as Pb, Cr, As, Cu, and Zn, are

Fig. 1 Concentrations of total metals and metalloids (median, P25–P75 and range; $\mu\text{g/L}$) determined in the saliva of (non-exposed/exposed; non-smoker/smoker) firefighters (S1–S8)



also common in anthropogenic emission sources and might enrich the particulate emissions from forest fires, principally those that occur at the wildland-urban interface [61].

Regarding the contribution of tobacco consumption to the salivary levels of metal(loid)s, it was found increased concentrations of Pb (5.3 times; $0.265 \mu\text{g/L}$ versus $0.0503 \mu\text{g/L}$), Li (3.4 times; $4.20 \mu\text{g/L}$ versus $1.23 \mu\text{g/L}$), Cu (2.0 times; $10.7 \mu\text{g/L}$ versus $5.25 \mu\text{g/L}$), Ni (2.0 times; $1.54 \mu\text{g/L}$ versus $0.77 \mu\text{g/L}$), and Zn (1.9 times; $39.4 \mu\text{g/L}$ versus $20.3 \mu\text{g/L}$) in smoking non-exposed firefighters (group S2) comparatively with non-smoking and non-exposed participants (group S1) (Table 2). So far, to our best knowledge, only four studies assessed the levels of metal(loid)s in the saliva of smoking individuals [68–71]. These studies evaluated the salivary concentration of Mg, Al, Mn, Fe, Cu, Zn, Rb, Sr, Mo, Cd, Tl, Cd, and Pb in smoking office workers from a dental hospital [68], non-occupationally exposed smokers [69], pulmonary tuberculosis patients [70], and Jordanian waterpipe smokers [71]. From the analyzed elements, only Al, Fe, and Rb were not measured in the present study. Overall, the elemental salivary values measured in unexposed smoking firefighters (groups S2 and S6) for Mn (0.95 – $3.49 \mu\text{g/L}$ versus 2.26 – $3.40 \mu\text{g/L}$); Cu (1.39 – $172 \mu\text{g/L}$ versus 3.85 – $10.7 \mu\text{g/L}$); and Zn (0.94 – $130 \mu\text{g/L}$ versus 10.5 – $39.4 \mu\text{g/L}$) were equivalent or comprehended between the values found in literature studies (Table 2) [68, 70, 71]. Levels of Sr

(1.85 – $3.46 \mu\text{g/L}$ versus 3.38 – $4.41 \mu\text{g/L}$) and Mo (0.12 – $0.35 \mu\text{g/L}$ versus 0.396 – $0.399 \mu\text{g/L}$) in unexposed firefighters that are smokers found in this study are slightly superior to the literature salivary values [68]. The salivary concentration of Cd (0.016 – $28.3 \mu\text{g/L}$ versus 0.0064 – $0.0241 \mu\text{g/L}$) and Pb (0.078 – $4.8 \mu\text{g/L}$ versus 0.0442 – $0.265 \mu\text{g/L}$) were lower in unexposed smoking firefighters than the values available for smokers in the presented literature [68–71]. However, the highest values of Cd and Pb found in the literature belong to Jordanian waterpipe smokers, which is a different method of smoking that might increase the overall intake of these pollutants. Also, Khabour et al. [71] compared the salivary concentration of Cd and Pb in smokers versus non-smokers. In non-smokers the measured values for Cd and Pb were, $0.64 \mu\text{g/L}$ and $2.8 \mu\text{g/L}$, respectively, which are also much superior to the values found in the present study for smokers, meaning that these participants might be influence by environmental factor such as water or air pollution [71].

A preliminary study of this research team characterized salivary metal content in non-smoking firefighters that were not recently enrolled in fire events [72]. The total elemental content in the saliva of those Portuguese firefighters (10.9 – $73.6 \mu\text{g/L}$) is aligned with the values measured in this study for the control groups (Groups S1 and S4) (23.6 – $42.2 \mu\text{g/L}$). Overall, the individual contribution of each metal is aligned across both studies except for Co (0.0307 – $1.94 \mu\text{g/L}$

versus 0.02662–0.084 µg/L), and Sb (0.0215–1.01 versus 0.0407–0.099 µg/L) that were found at considerably lower levels in the present study. Other studies also characterized the metal content in the saliva of occupationally exposed individuals, reporting the concentrations of Pb, Cd, Hg, Mn, Cu, Zn, Fe, Ni, Cr, and Cs [33, 73–82]. The elements Mn (0.120–70.5 µg/L versus 1.64–4.73 µg/L), Ni (0.120–188.5 µg/L versus 0.66–2.22 µg/L), Cu (10.0–200 µg/L versus 3.76–10.7 µg/L), Zn (191 µg/L versus 14.3–61.3 µg/L), Cd (0.140–59.7 µg/L versus 6.4×10^{-3} –0.0559 µg/L), Hg (2.00–11.1 µg/L versus 0.099–0.60 µg/L), and lastly Pb (3.03–129 µg/L versus 0.091–2.27 µg/L) (Table 2) were predominantly found at slight higher concentrations in Spanish workers from a steel industry, Swedish Navy divers performing underwater electrical cutting, Chinese welders, workers exposed to Cd (not-specified), Tunisian dentists and assistants, and Canadian battery recycling workers than in the saliva of occupationally exposed firefighters (groups S3, S5, S7, and S8) [31, 33, 75, 78, 81, 82]. It is important to note that most of the characterized workers perform tasks related to the manufacturing/welding/handling of metal and metallic products, thus contributing to the elevated salivary reported in the literature comparatively with the monitored firefighters. Nevertheless, firefighters also perform tasks that involve handling metal products when performing cleaning and maintenance of equipment (mostly trucks and ambulances) that could further increase their exposure [7, 11, 83].

A study performed by Bhowmick et al. [84] reported predominantly higher salivary content in individuals living near the Bengal delta plain (West Bengal, India) than in firefighters. Zn (182 µg/L versus 10.4–61.3 µg/L) and As (4.1 µg/L versus 0.218–1.17 µg/L); Pb (0.60 µg/L versus 0.0442–2.27 µg/L), Mn (2.60 µg/L versus 0.947–4.73 µg/L), and Ni (1.12 µg/L versus 0.659–2.22 µg/L) were in close range with the values found in this work. The authors reported that local groundwaters used in households to drink, bath, and wash foods presented As levels exceeding WHO guidelines (10 µg/L) [84, 85].

(Co)relation Between Salivary Content and Source Apportionment

A correlational analysis of the salivary content in metal(loid)s and PAHs was performed to explore the interdependency between the different pollutants present in the monitored firefighters. Overall, metal(loid)s were positively well correlated between each other ($-0.357 < r < 0.952$; $p \leq 0.01$), except for Sr, Ba, and Pb (Fig. 2a). Regarding PAHs, salivary levels of fluorene were strongly correlated with phenanthrene ($r = 0.903$; $p \leq 0.01$) and anthracene were moderately correlated with naphthalene ($r = 0.690$; $p > 0.05$) and phenanthrene ($r = 0.220$; $p > 0.05$). Positive and moderate to strong correlation were observed between salivary anthracene and

all metal(loid)s ($0.204 < r < 0.881$; $p \leq 0.01$), except for Mo and Tl (Fig. 2a). Based on these findings, anthracene can be proposed as a marker of the presence of metal(loid)s in human saliva. Moreover, a moderate to strong interdependency was found between naphthalene and some metals, Ni, Sb, Ba, and Pb ($0.381 < r < 0.714$; $p \leq 0.05$), and between fluorene and phenanthrene with Sr, and Ba ($0.333 < r < 0.619$ and $0.415 < r < 0.561$, respectively ($p > 0.05$)) (Fig. 2a). The demonstrated interdependency between some metals and PAHs in firefighters' saliva suggest co-exposure to common sources of these pollutants.

Envisaging to deepen the source apportionment, a principal component analysis (PCA) was performed with the salivary content with PAHs and metal(loid)s. The PCA model has a Kaiser–Meyer–Olkin sample adequacy of 0.570 based on the extracted two principal components with eigenvalues of 10.0 and 4.95, representing 71.52% of data variability (Fig. 2b). F1 represented 47.96% of the total variance and was heavily influenced by almost all the metal(loid)s under analysis (Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Cs, and Hg, with squared cosine values varying between 0.489 (Sb) and 0.878 (Se)) and acenaphthene (square cosine 0.501). The principal component F2 contributed with 23.56% of total data and was influenced by Sr, Ba, Pb (square cosines of 0.638, 0.791, and 0.630, respectively) and the PAHs naphthalene, and anthracene (squared cosine values of 0.385 and 0.547, respectively). This PCA model also generated two additional principal components with eigenvalues > 1 , F3 and F4 representing close to 20% of total variance, reason why they were not considered in the PCA analysis. The PCA model differentiate the groups of firefighters immediately after their participation in firefighting activities, S3 in real forest fires, groups S5 and S7 in controlled structure fires, and S8 in an industrial fire (Fig. 2b). This differentiation was based on the contribution of the variables with the highest and significant square cosine, which are supported by the increments found between non-exposed and exposed firefighters (the “Salivary Content in PAHs” and “Salivary Content in Metal(loid)s” Sections). It was observed that firefighters enrolled in non-controlled firefighting activities, groups S3 and S8, were even more isolated from the other two groups of exposed participants, because they presented the highest increments of some metal(loid)s and PAHs (Table 2). The PCA model isolated the non-exposed groups of firefighters, the non-smokers (groups S1 and S4) and the smoker group S6 in the negative quadrant of both F1 and F2; the group S2, also non-exposed smoking firefighters is the exception as it was positioned at the positive quadrant of F1 and F2 but presented low positive scores on F2 (Fig. 2b). Despite the differences observed in the salivary content of metal(loid)s and PAHs between smoking and non-smoking firefighters, the PCA model

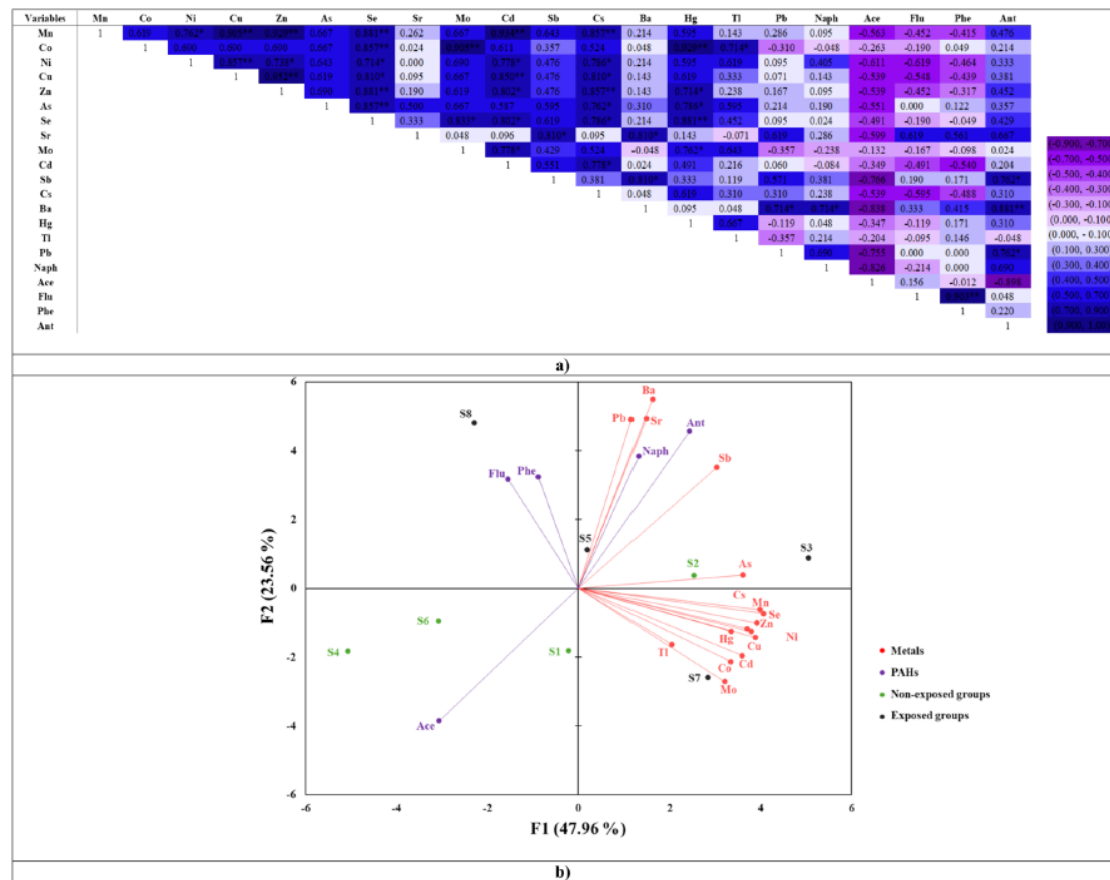


Fig.2 Representation of **a** Spearman's correlation matrix based on salivary content in metals, metalloids, and PAHs measured in firefighters (* $p \leq 0.05$; ** $p \leq 0.01$) and **b** Principal component analysis

(PCA) based on the salivary content of metals, metalloids, and PAHs in the eight groups of (non-exposed/exposed; non-smoker/smoker) firefighters

was not able to differentiate the contribution of tobacco consumption due to the prevalence of the impact of exposure to fire emissions.

In Vitro Toxicity

After assessing the salivary content in metal(loid)s and PAHs, an in vitro health risk analysis was performed. Cell lines from buccal or intestinal origin were cultivated in flat 96-well plate substrates to assess the cellular viability after 24 h of exposure to saliva samples collected by firefighters during daily regular activities in the headquarters and in fire-related scenarios. Figure 3 depicts the cell viability of buccal (TR146 and HSC-3) and intestinal (Caco-2 and HT29 MTX) epithelial cell lines. No major cytotoxicity was observed, with cell viability values being equal to or above 70% for

all samples. Overall, the observed outcomes were similar between buccal (TR146 and HSC-3; Fig. 3A1) and intestinal (Caco-2 and HT29 MTX; Fig. 3B1) cells tested. Apparently, saliva samples did not induce major detrimental effects on the cultivated cell lines. Additionally, advanced in vitro buccal and intestinal models were used to deeper explore the integrity of the cellular membrane. The acquired data revealed that no major effects were found on the barrier integrity of both buccal and intestinal cell models (Fig. 3A2 and B2). Also, as expected, no significant changes were observed in the cell viability of both cell lines after 4 h of exposure to firefighters' saliva (Fig. 3A2 and B3). The inexistent data in the literature limits the comparison of these results and additional studies are needed to corroborate these preliminary findings.

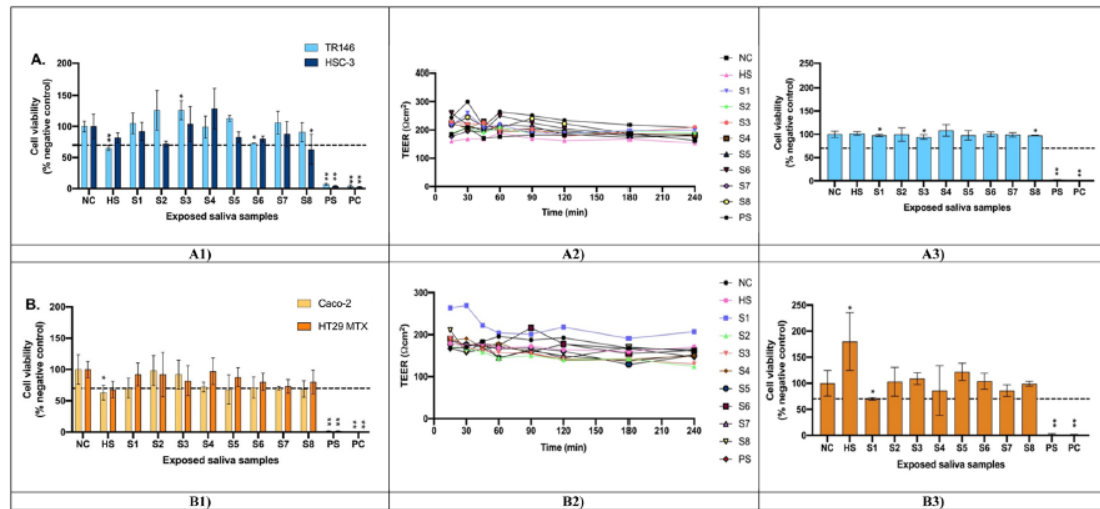


Fig. 3 Representation of cell viability of **A1** buccal (TR146 and HSC-3) and **B1** intestinal (Caco-2 and HT29 MTX) monocultures cell lines after 24-h exposure to saliva samples collected by firefighters during regular working tasks at fire stations and during firefighting activities, and characterization of Trans-Epithelial Electrical Resistance (TEER) measurements and cell viability of the **A2** and **A3** buccal and **B2** and **B3** intestinal Transwell models after 4-h exposure

to firefighters' saliva samples, assessed by the MTT assay. Values were normalized considering negative control (NC) as 100%; PC represents the positive control (Triton X-100 1% in serum-free medium, for monocultures, and HBSS with Triton X-100 1%, for Transwells); * $p \leq 0.05$; ** $p \leq 0.01$. NC: negative control (cell culture medium, for monocultures, and HBSS for Transwells); HS: healthy saliva; PS: healthy saliva contaminated with PAH

Conclusion

This study assessed the firefighters' exposure to metal(loid)s and PAHs through ingestion by analyzing saliva, investigating firefighting and tobacco contributions, and evaluating associated in vitro toxicity on buccal and intestinal cell models. Five PAHs were detected in saliva (1.95–3.61 $\mu\text{g/L}$), with naphthalene (0.740–1.61 $\mu\text{g/L}$) and anthracene (0.0110–0.0336 $\mu\text{g/L}$) being the most and least predominant compounds, respectively. Metal(loid)s levels ranged from 23.6 to 90.9 $\mu\text{g/L}$, with Zn (10.4–61.3 $\mu\text{g/L}$) being the most abundant and Cd (0.00640–0.0559 $\mu\text{g/L}$) the least one. Higher concentrations were found in exposed firefighting groups, with Pb levels increasing up to 45 times after an industrial fire. The tobacco consumption influenced the metal(loid)s levels, with Pb, Li, Cu, Ni, and Zn being increased in non-exposed smokers (S2) than in non-smokers (S1). The PCA model distinguished exposed from non-exposed firefighters, particularly in forest (S3) and industrial (S8) fire groups, though tobacco's contribution was masked by fire emissions. In vitro assays with buccal and intestinal cells revealed that no significant toxicity was observed, while the cell integrity of used cellular models was demonstrated. Future studies should cover the limitations of the present study by increasing the sample sizes and analyzing additional fire-emitted pollutants, including VOCs and

gases, to explore combined exposure effects. However, it is of utmost importance to develop standardized sampling and analytical methods to minimize variability with saliva analysis. Occupational exposure through ingestion should be explored in other high-risk professions (e.g., miners, pavement, and industrial workers) by implementing routine biomonitoring, developing stricter and enhanced protective measures and providing training for health personnel and workers, only then the risks are reduced.

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Author Contributions Conceptualization, M.O. and F.R.; Methodology, J.T., M.J.B. and G.S.; writing-original draft preparation, J.T., G.S., and M.J.B.; writing-review and editing, B.S., M.O., F.R., and C.D.-M.; Supervision: F.R. and M.O., Funding, F.R. and M.O.. All authors have read and agreed to the published version of the manuscript.

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Data Availability The datasets analysed during the current study are not publicly available to ensure data protection and anonymity of firefighters that agreed to participate; however, there are available from the corresponding author on reasonable request.

Declarations

Ethics Approval The study was revised and approved by the Ethical Committee of the University of Porto (Report 105/CEUP/2020).

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication The authors affirm that human research participants provided informed consent for publication of the present results.

Competing interests The authors declare no competing interests.

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Chapter 7.

Part II conclusion

Part II conclusion

The work presented in Part II of this thesis pioneered the characterization of firefighters' occupational exposure via saliva, demonstrating that firefighters exhibited higher salivary concentrations of specific metals (e.g., Zn, Pb, Cd, Ni, Cu) and some PAHs (e.g., naphthalene, phenanthrene, pyrene) after exposure to fire emissions. Tobacco consumption further exacerbates firefighters' exposure to these pollutants, with a synergistic effect leading to a more pronounced increase in toxic elements like Co, Sb, Pb, Cd, and Ba. Notably, the duration of exposure and the years of professional experience increases metal accumulation in saliva, suggesting a chronic exposure component even in the absence of recent fire events. Also, results highlighted that salivary metals' concentrations increase with increasing BMI; however, PAHs concentrations decrease with increasing BMI. These findings highlight the potential of using saliva in future surveillance of firefighters, allowing the evaluation of the efficiency of breathing apparatus that are part of the PPE, characterizing firefighters' recent exposure to fire emissions, or even identifying individuals that usually perform tasks where they are more exposed, suggesting workers to diversify tasks. This work suggests saliva's utility to measure not only recent and long-term exposure but also the bioavailable fraction of xenobiotics that can be systemically absorbed via the buccal cavity. For instance, the *in vitro* studies that were performed using buccal and intestinal cell models, though not showing significant acute toxicity in the tested scenarios, validate the use of these models for further investigating the biological impact of absorbed pollutants. PCA successfully distinguished between exposed and non-exposed individuals, as well as between smokers and non-smokers, further supporting the robustness and applicability of these biomonitoring tools for exposure assessment.

The integration of saliva biomonitoring into occupational health frameworks offers a non-invasive, complementary approach to traditional analysis that frequently uses blood and/or urine, which are more invasive and time consuming. This strategy provides a more holistic view of exposure, capturing both internal dose and recent exposures and with potential to characterize occupational exposure to other health-relevant pollutants (e.g., pesticides, dioxins/furans, or per- and polyfluoroalkyl substances). Moreover, the study acknowledges the limitations associated with saliva analysis, particularly the inter- and intra-individual variability observed among participants. This underscores the necessity for future research employing larger sample sizes and increased sampling frequency to validate the current findings. Subsequent studies should also investigate

the efficacy of diverse protective interventions and exposure mitigation strategies within firefighters.

PART III

**Final remarks and suggestions for future
work**

Chapter 8

“Final remarks and suggestions for future work”

This thesis provides a comprehensive assessment of the occupational exposure of firefighters to hazardous pollutants, namely fine and ultrafine PM, PAHs, and metals, through the (bio)monitoring of air and saliva in different work environments (fire stations and controlled forest and structure fires) that was performed to answer the research questions that motivated the planned work.

PM collected inside different microenvironments of fire stations, and during forest and structure fires were distinct in their composition and morphology. Overall, PM from these three work environments was constituted by carbonaceous matter, a variety of geogenic particles, mineral crystals, fly ash, and metal alloys. Besides these common elements, PM collected inside fire stations presented recognizable biological debris and Ba compounds, while CSF produced PM with higher concentrations of toxic and carcinogenic heavy metals such as Pb compounds (PbO and PbCl₂). On the other hand, forest fires generated PM that was richer in crystalline and mineral salts, like potassium and calcium sulphate, or potassium chloride, typically arising from biomass combustion. Elements found commonly in PM collected in firefighters' work environments included Cr, Co, Cu, Zn, Ni, and Pb. Total PM inside fire stations did not surpass established occupational limits; however, both types of fire emissions exceed regulated ambient PM_{2.5} levels by a considerable margin.

With respect to the human biomonitoring assay, the saliva analysis revealed that firefighters' exposure to fire emissions influenced the levels of xenobiotics detected, revealing elevated concentrations of metals, including Co, Ni, Sb, Mo, Hg, Ba, and Pb, and some PAHs (*e.g.*, naphthalene and anthracene), following exposure when compared with baseline values. Regular smoking habits magnifies these effects by further increasing the salivary levels of toxic elements such as Pb, Li, Cu, Ni, and Zn. Moreover, firefighters with prolonged exposure and years of service presented higher salivary metal concentrations which might imply cumulative risks and chronic exposure even when no recent fire combat has occurred.

These findings collectively advocate for the integration of saliva in biomonitoring strategies, complementing the use of urine and blood, into future occupational exposure and health surveillance protocols. The incorporation of saliva-based analysis represents a non-invasive and practical alternative/complement to traditional methods like blood or

urine sampling, offering a comprehensive overview of long and recent term exposures. This approach can also help evaluate the effectiveness of PPE, distinguish between different exposure scenarios, and identify individuals or tasks associated with higher exposure risks. Broadening the scope of biomonitoring will create a more robust methodological approach for assessing firefighters' exposure and health risk assessment, by aligning reinforced decontamination protocols, improved ventilation inside fire stations, and targeted occupational exposure limits. However, the analysis of this matrix has its challenges. Saliva's complex chemical composition causes intra- and inter-individual variability, meaning that variables such as exposure to environmental airborne pollutants, time of exposure, time between end of activity and sample collection, dietary habits, hydration level, and tobacco consumption can substantially alter the composition of saliva between individuals and even in the same individual at different time points. So, it is important to collect this information about the participants, in the form of self-characterizing questionnaires. By grouping individuals with similar characteristics together this variability gets diffused as opposed to characterizing single entities.

Ingestion is only one of the possible routes of exposure to health relevant pollutants. Dermal exposure represents a significant and often underestimated route in firefighters' exposure to hazardous substances, including metal(loid)s and PAHs. For firefighters, skin contamination occurs predominantly on less-protected areas such as the neck, wrists, and face, even when PPE is used correctly, due to design limitations and inadequate cleaning procedures. There is a scarcity of data on dermal absorption rates and bioavailability in human skin, coupled with a lack of standardised sampling and analytical methods. Some preliminary work was implemented in this research topic related to (i) the study of PPE contamination with PAHs and metal(loid)s and (ii) characterization of firefighters' skin contamination after firefighting activities and associated *in-vitro* toxicity. So far, the sampling campaigns were concluded and some chemical analyses are ongoing to analyse the presence of metal(loid)s and PAHs; however, the database is not completed for data treatment. Therefore, future research will give continuity to this work and focus on using bioavailable concentrations rather than exposure levels to conduct realistic health risk assessments.

Ultimately, the collective evidence underscores the urgent need to establish or update occupational and ambient exposure guidelines, in indoor and outdoor spaces, for fine and ultrafine PM. The latter is especially hazardous given its capacity to penetrate deep into the lungs, enter bloodstream, and spread internally. Despite the severity, there remains a regulatory gap concerning standards for ultrafine PM. Furthermore, the

findings advocate for the refinement of protocols for the collection, analysis, and interpretation of all kinds of biological samples, accounting for factors such as dietary and lifestyle habits, professional experience, and PPE use, to help overcome current limitations in data comparability and analytic reliability. Standardizing these processes will aid in creating exposure limits for matrices such as saliva which are still currently absent, despite the evidence of cumulative/chronic risk presented in this work. Regarding dermal monitoring, future studies should focus on characterizing rates of skin absorption and bioavailability of different contaminant classes, rather than solely quantifying skin deposition, as it helps attain a more holistic understanding of the actual exposure. Future research should focus biomonitoring efforts beyond the limited sampling, and geographic scope of this thesis; perform cross-seasonal approaches and expand to other high-risk professions, such as miners, industrial workers, cooks, and paint-manufacturing workers. This will strengthen the knowledge on exposure and health risk data that can be crucial for developing comprehensive safety guidelines and research into more effective PPE design, improved cleaning protocols, station ventilation to promote occupational hygiene, safety and health.

Attachments

Supplementary material – Chapter 2

Gravimetric, morphological, and chemical characterization of fine and ultrafine particulate matter inside fire stations

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Table 1S. Studies characterizing PM collected in indoor environments and woody biomass burning by electron microscopy.

PM size	Sampling	Analysis	Sampled matrix	Main findings	Reference
Electron microscopy analysis of PM from indoor spaces					
PM ₁₀ and PM _{2.5}	N/S	SEM, TEM, ICP- MS	Electric, petrol, and liquid-gas Go-kart facilities (Bavaria, Germany)	Fe concentration in electric go-kart facility was 3 times higher than petrol and liquid-gas; Cu, Zn, Sr, Ba, Al, Ca, Mn, Fe, and Ni were found in almost all facilities	[1]
SPM	Tapered element oscillating microbalance	SEM, AAS	Laboratory Jawaharlal, Nehru University (New Delhi, India)	Ca, Mg, and Fe are the common elements; Silicates and soot particles are the most dominant	[2]
SPM	Low-volume handy air samplers with paper filters	SEM- EDX, ICP- OES	School vs adjacent roadway (Madras, India)	Daily average indoor SPM concentrations in monsoon, winter and summer seasons were 158.18, 170.08 and 149.63 µg/m ³ ; Al, Ca, Fe, K, Mg, Na, and Ti appeared in higher percentages indoor and outdoor. Heavy metals appear but in minor concentrations	[3]
PM ₁₀ and PM _{2.5}	High-volume constant air flow samplers, combined with PM sampling heads	SEM-EDS	Cafeteria influenced by tobacco smoke vs single person apartment not	Tobacco smoke increased PM _{2.5} and PM ₁₀ by 320% and 250%, respectively. Chlorides were the common ions, followed by Fe oxides and alloys;	[4]

			influenced by tobacco smoke (Porto, Portugal)	Cu, Zn, Pb, Ni, and Cr (traffic emissions) and sulphates of K and Na (related to proximity of the sea) were found	
PM _{2.5}	Gs-3 multiple-inlet cyclone, fitted with polycarbonate membrane filter	SEM-EDS, ICP-MS	Area close to FeMn smelter, cement, and coal-fired starch factories. Indoor (households) vs outdoor (outside households) (Gauteng, South Africa)	Outdoor PM levels were 2.27 times higher than indoor PM values; Indoor PM is generally more regular than outside PM. However, both samples had similar morphologies; Si, Mn, and Fe were the most common elements in both the indoor and outdoor environments	[5]
PM _{2.5}	Automated Aerosol Sampling Device type ISAP1050e with glass microfiber filters	SEM-EDS, XRD	Living room and balcony in a minimally furnished house in an industrialized city (Galati, Romania)	Identification of heavy elements (Ti, Ba, Cr, Mn, Fe, Zn, Rb, and Sr) and Na, Ca, Mg, Al, K, S, and C	[6]
PM _{2.5}	ECHO PM sampler; MiniVol TAS sampler	SEM-EDS	4 kitchens and outside of respective dwelling (Aveiro, Portugal)	Carbonaceous constituents are 30-50% of PM _{2.5} content and represent most of the ultrafine soot; PM from kitchens with gas cooking apparatus are significantly different from the ones using electric hobs	[7]
PM _{2.5}	Personal environmental monitors with Teflon membrane filters	SEM-EDX	Apartment in retirement facilities as well as personal samples (Baltimore, USA)	Some particles larger than 2.5 µm were found in the filters of that fraction	[8]

PM ₃₅ – 0.25µm	Naneum Nano ID Select particle collector using clean-dry glass slides	ESEM- EDX	School (classroom and corridor) (Singapore city, Singapore)	Ratio of indoor/corridor PM ranged from 0.3 to 0.7; 60-70% of total PM corresponded to fractions from 35 to 8.1 µm; Smaller fraction had more regular shaped particles Si, Ca, and Al were the most predominant elements in larger fractions while C, Ca, S, and K, were common in smaller fractions.	[9]
Electron microscopy analysis of PM from woody biomass burning					
PM and residual ash.	Dust trap at the top of the burner	SEM- EDS, TEM, XRD, TGA	Sugarcane and forest leaves	Average size of PM from sugarcane burning was 37 nm while forest leaves were 35 nm	[10]
PM<10 and >10µm	Low-pressure impactor	ESEM- EDS	Rice husk, corn stalk, cotton stalk, and camphor wood combustion	PM from agricultural biomass combustion is generally under 1 µm and mainly composed of Na and K; In woody biomass, PM ₁₀₋₁ is dominant, with Mg and Ca as the main inorganic compounds	[11]
PM _{18nm} to PM _{16µm}	Berner type low-pressure cascade Impactor	SEM- EDS, TEM-EDS	Oak tree, chestnut tree and eucalyptus wood saw dust	K, S, P, and Cl, were the main elements; K sulphates and chlorides are the main salts found in finer PM	[12]

PM ₁	Electrical low-pressure impactor	SEM-EDS	Birch, spruce, pine, alder, and willow bark and stems	K ₂ SO ₄ , KCl, K ₂ CO ₃ , KOH, and organic material were the main components of PM ₁	[13]
PM ₁	Low-pressure impactor and electrical low-pressure impactor	SMPS, XRD, TEM	Spruce and typical Finnish deciduous tree species	The addition of reed canary grass to wood chips slightly increased both the PM ₁ mass and the particle number emission. The addition of straw to wood chips increased the PM _{1.0} µm mass emission substantially while decreasing the particle number emission	[14]
PM ₁₀	13-stage Berner impactor	SEM-EDX	Beech wood	Size range of 0.06 - 0.1 µm. PM ₁ represented 90% of total PM mass	[15]

AAS – Atomic absorption spectrometry; EDS – Energy-dispersive spectroscopy; EDX – Energy dispersive X-ray; ESEM – Environmental scanning electron microscopy; ICP-MS – Inductive coupled plasma – mass spectrometry; N/S – Not-specified; OES – Optical emission; SEM – Scanning electron microscopy; SMPS – Switched mode power supply; SPM- Suspended particulate matter; TEM – Transmission electron microscopy; TGA – Thermogravimetric analysis; XRD – X-ray diffraction.

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Supplementary material – Chapter 3

Morphological and Elemental Characterization of Fine and Ultrafine Particulate Matter Generated from Fires

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Table S1. Calibration data of Dekati DLPI+ Impactor, cut points (D50%) and downstream pressures of for each PM fraction, performed at 20.3 °C, 40 mbar, and a flow rate of 9.96 L/min.

PM fraction	D50% (μm)	Pressure (kPa)
PM _{14.9nm}	0.0149	4.00
PM _{30.8nm}	0.0308	9.81
PM _{54.5nm}	0.0545	22.59
PM _{156nm}	0.156	69.05
PM _{2.5}	2.47	101.18
Inlet		101.33

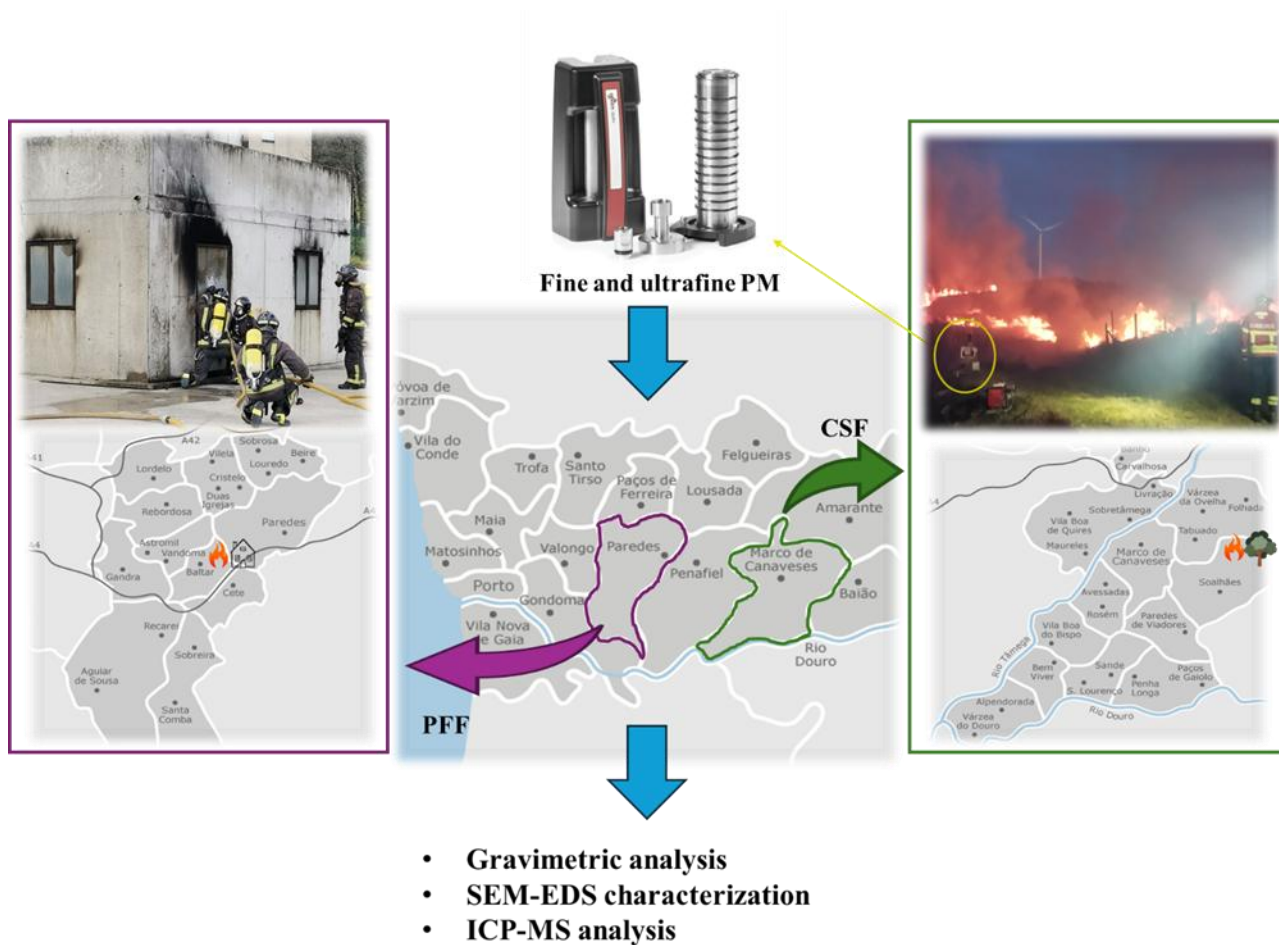


Fig. S1 - Representation of sampling campaigns to collect fine and ultrafine PM during prescribed forest fires (PFF) and controlled structure fires (CSF) performed in the North of Portugal.

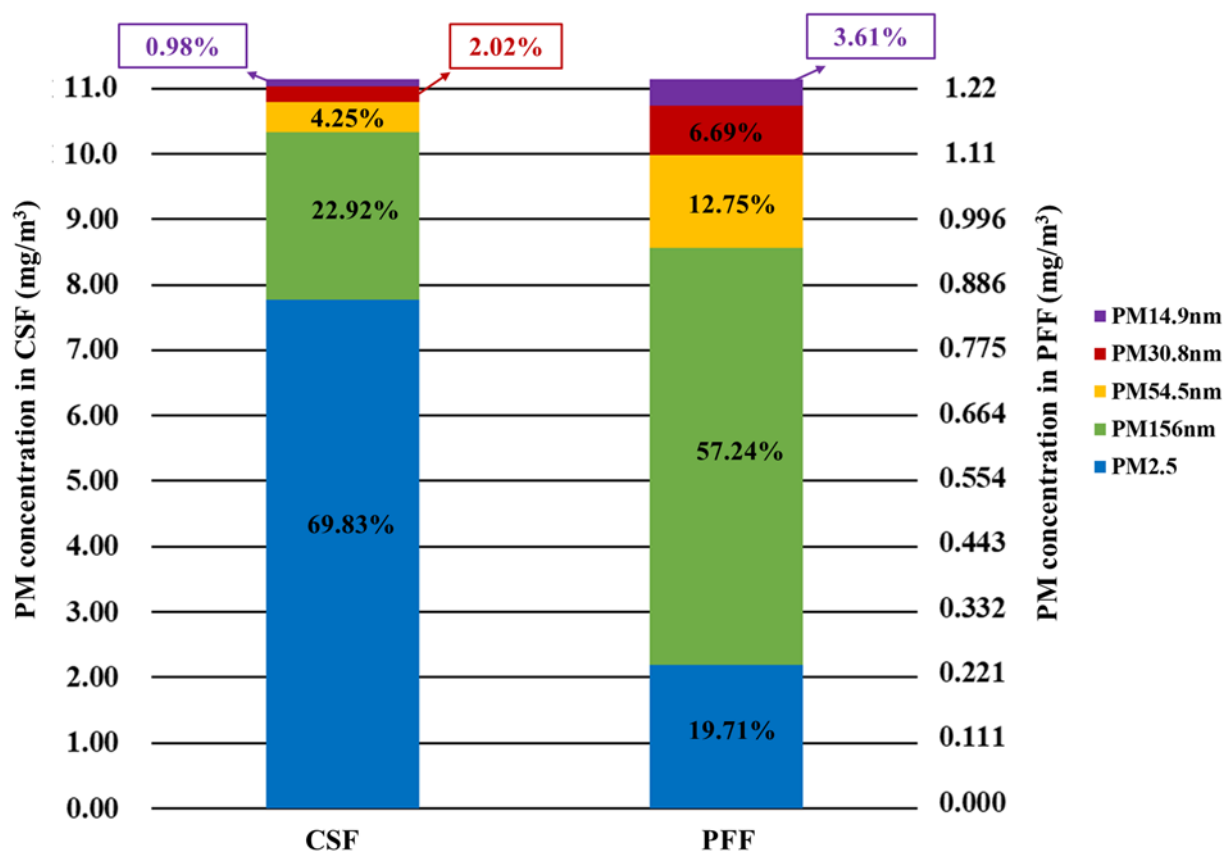


Fig. S2 - Concentration (mg/m^3) of cumulative PM and distribution (%) of fine ($\text{PM}_{2.5}$ and $\text{PM}_{156\text{nm}}$) and ultrafine ($\text{PM}_{54.5\text{nm}}$, $\text{PM}_{30.8\text{nm}}$, and $\text{PM}_{14.9\text{nm}}$) fractions collected during prescribed forest fires (PFF) and controlled structure fires (CSF).

Table S2 – Concentrations (median and range; mg/m³) of fine (PM_{2.5} and PM_{156nm}) and ultrafine (PM_{54.5nm}, PM_{30.8nm}, and PM_{14.9nm}) PM collected during controlled structure fires (CSF) and prescribed forest fires (PFF).

PM size		CSF (n=3)	PFF (n=2)
Fine PM	PM _{2.5}	7.774 (0.1194 – 223.6)	0.2429 (0.1153 – 0.3706)
	PM _{156nm}	2.552 (0.6568 – 2.750)	0.7056 (0.2745 – 1.137)
Ultrafine PM	PM _{54.5nm}	0.4731 (0.08472 – 1.066)	0.1573 (0.08270 – 0.2319)
	PM _{30.8nm}	0.2246 (0.08842 – 0.8147)	0.08243 (0.03914 – 0.1257)
	PM _{14.9nm}	0.1100 (0.03610 – 0.6238)	0.04450 (0.03413 – 0.05486)

Supplementary material – Chapter 5

Unveiling Exposure Pathways at the Oral Cavity during Firefighting Work: Salivary Metal(loid)s and Polycyclic Aromatic Hydrocarbons, Occupational and Biological Determinants, and Exploratory Health Risks

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Table S1. Identification of the variables and reference values used in the estimation of benzo(a)pyrene total toxicity equivalent concentrations (TEQB(a)P), average daily potential dose (ADD), non-carcinogenic (THQ) and carcinogenic (TR) risks

Variable (unit)	Values	Notes
TEFn Toxicity equivalency factor relative to benzo(a)pyrene (dimensionless)	Naphthalene: 0.001; Fluorene: 0.001; Phenanthrene: 0.001; Anthracene: 0.01; Fluoranthene: 0.001; Pyrene: 0.001	Tsai <i>et al.</i> (2004)
EFr Exposure frequency (days)	250 days per year	Days of work per year
ED Exposure duration (days)	NSNE: 0.33; NSE: 0.07; SNE: 0.33; SE: 0.06	8h of daily work at the fire station for NSNE and SNE; and duration of fire events for NSE (1.60 h) and SE (1.48 h)
IR Salivary intake (L/day)	1 L	Average daily salivary production/ingestion
C Salivary concentration of the target compound/element (µg/L)	Compound/element-specific measured value	-
RfD Oral reference dose (mg/kg-day)	Li: 0.002; Mn: 0.140; Co: 0.0003; Ni: 0.011; Cu: 0.040; As: 0.0003; Se: 0.005; Sr: 0.600; Mo: 0.005; Cd: 0.0001; Sb: 0.0004; Ba: 0.200; Tl: 0.00001; naphthalene: 0.02; fluorene: 0.04; anthracene: 0.30; fluoranthene: 0.04; pyrene: 0.03	USEPA (2024)
BW Body weight (kg)	70 kg	Average adult body weight
AT Number of days of exposure (days)	250 × 40	Career of 40 years of work
CSFo Oral carcinogenic slope factor (kg-day/mg)	Arsenic: 1.50 Naphthalene: 0.12	USEPA (2024)

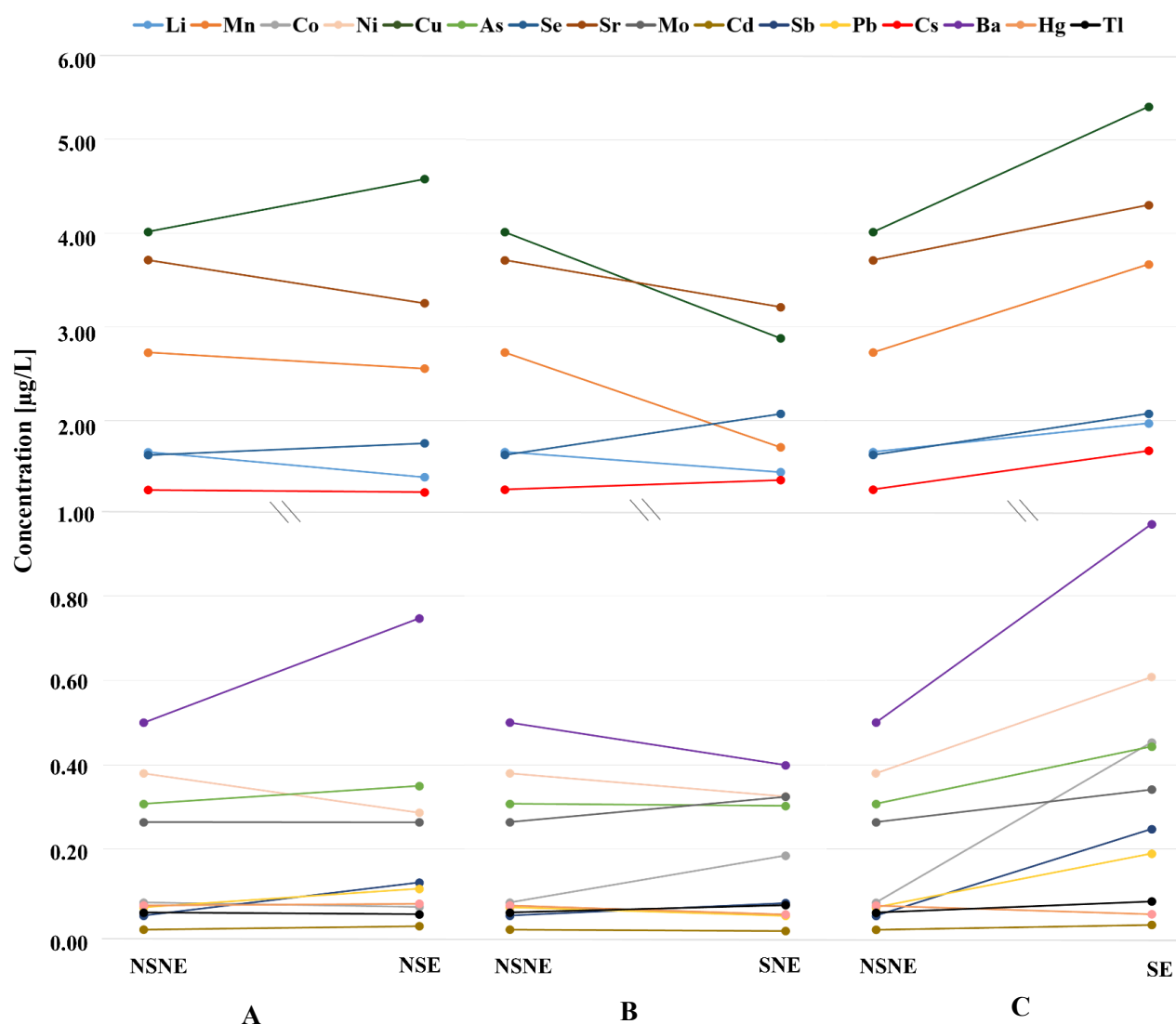


Figure S1. Representation of the changes in the salivary concentrations of each metal and metalloid among the different groups of participants taking into consideration A) the impact of firefighting work (non-smoker non-exposed *versus* non-smoker exposed), B) the impact of tobacco consumption (non-smoker non-exposed *versus* smoker non-exposed), and C) the cumulative effect of firefighting work and tobacco consumption (non-smoker non-exposed *versus* smoker exposed).

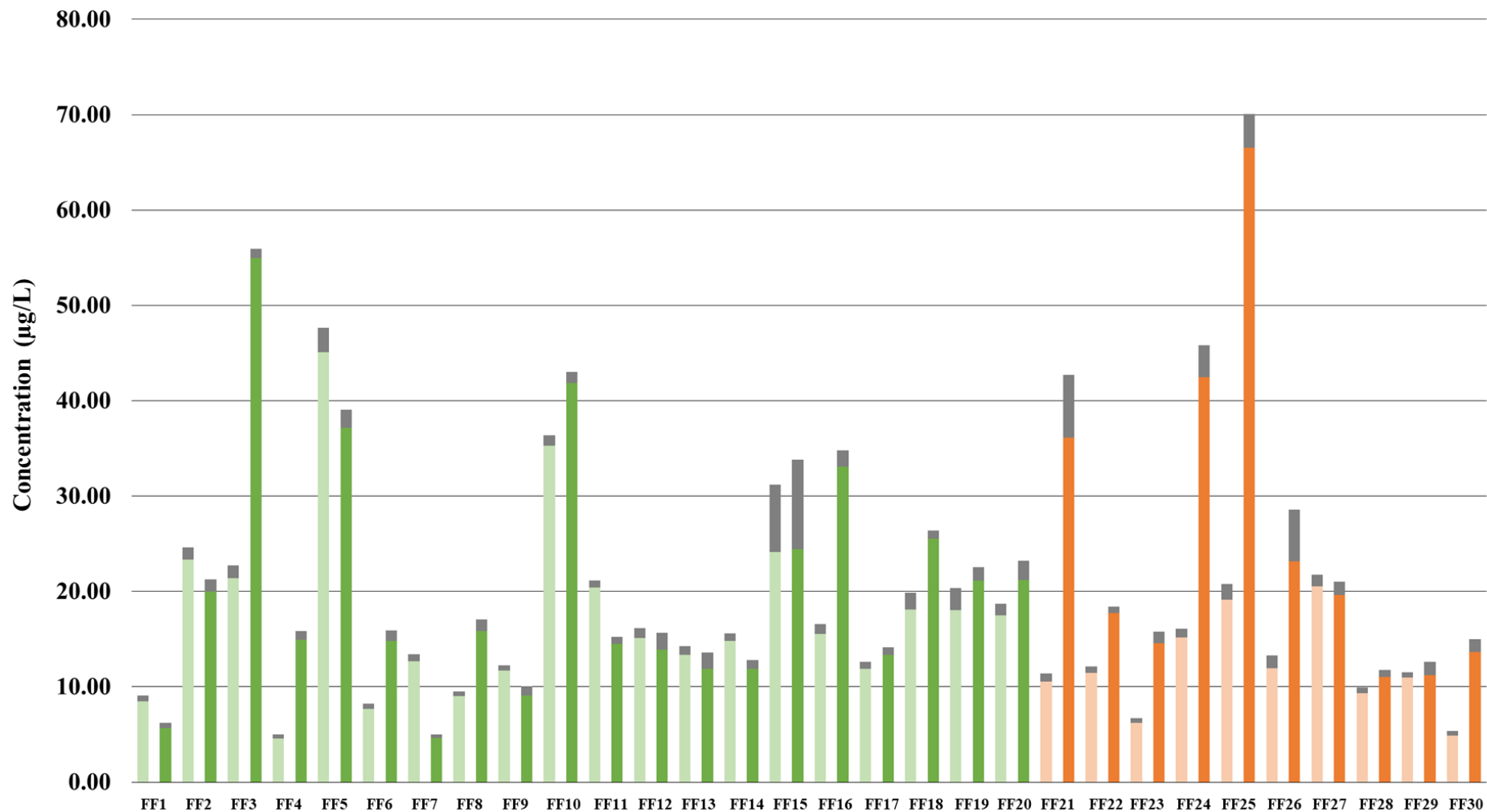


Figure S2. Individual profile of cumulative metals content (µg/L) in the saliva of non-smoker (green) and smoker (orange) firefighters, before (lighter color) and after (darker color) a firefighting intervention. The gray bars represent the levels of carcinogenic metals (Ni, As, Cd, Sb, Hg, Tl, Pb).

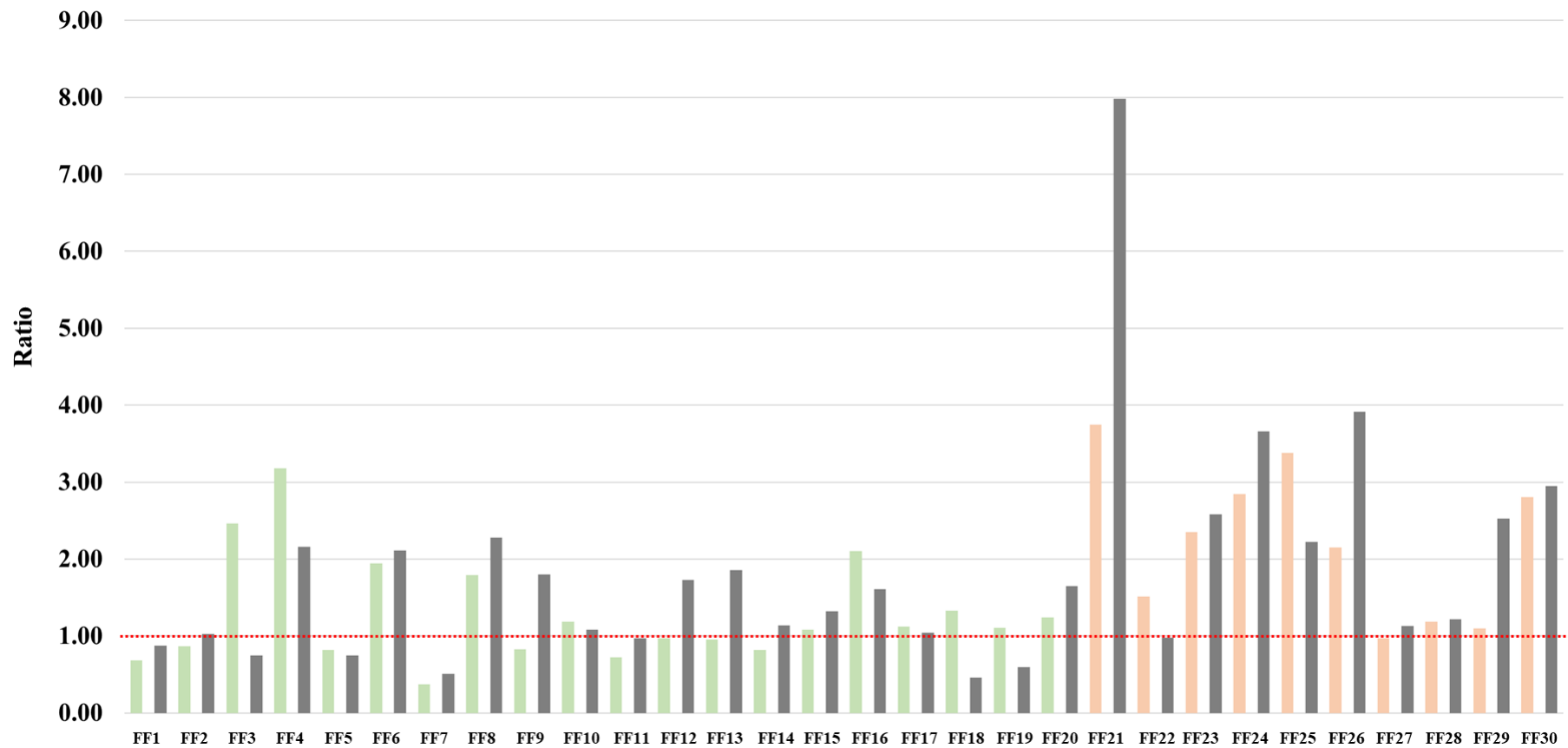


Figure S3. Individual profile of pre- to post-firefighting work ratios of total (green – non-smokers; orange – smokers) and carcinogenic (grey) cumulative metal(loid)s in the saliva of firefighters.

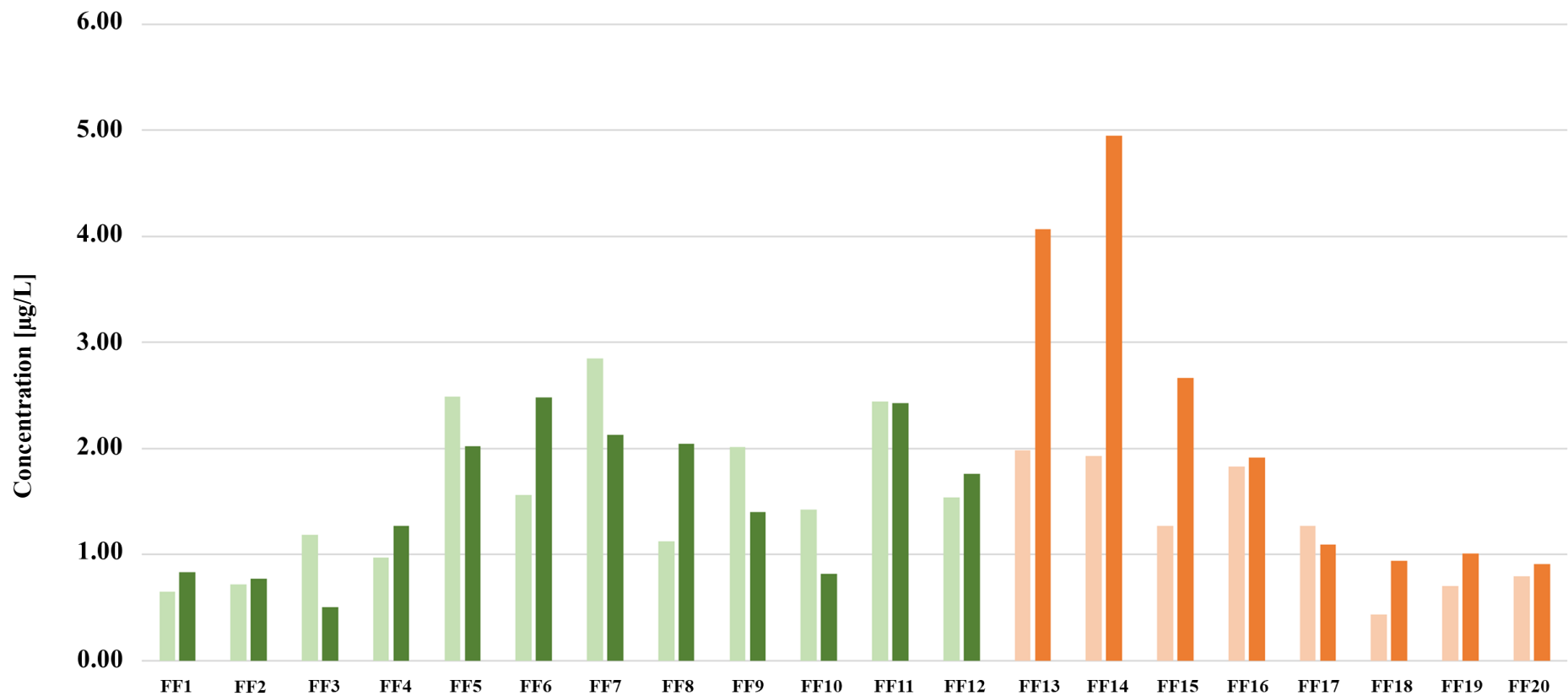


Figure S4. Individual profile of cumulative PAH concentrations (median, $\mu\text{g/L}$) in the saliva of non-smokers (green) and smokers (orange) firefighters, before (lighter color) and after (darker color) a firefighting intervention.

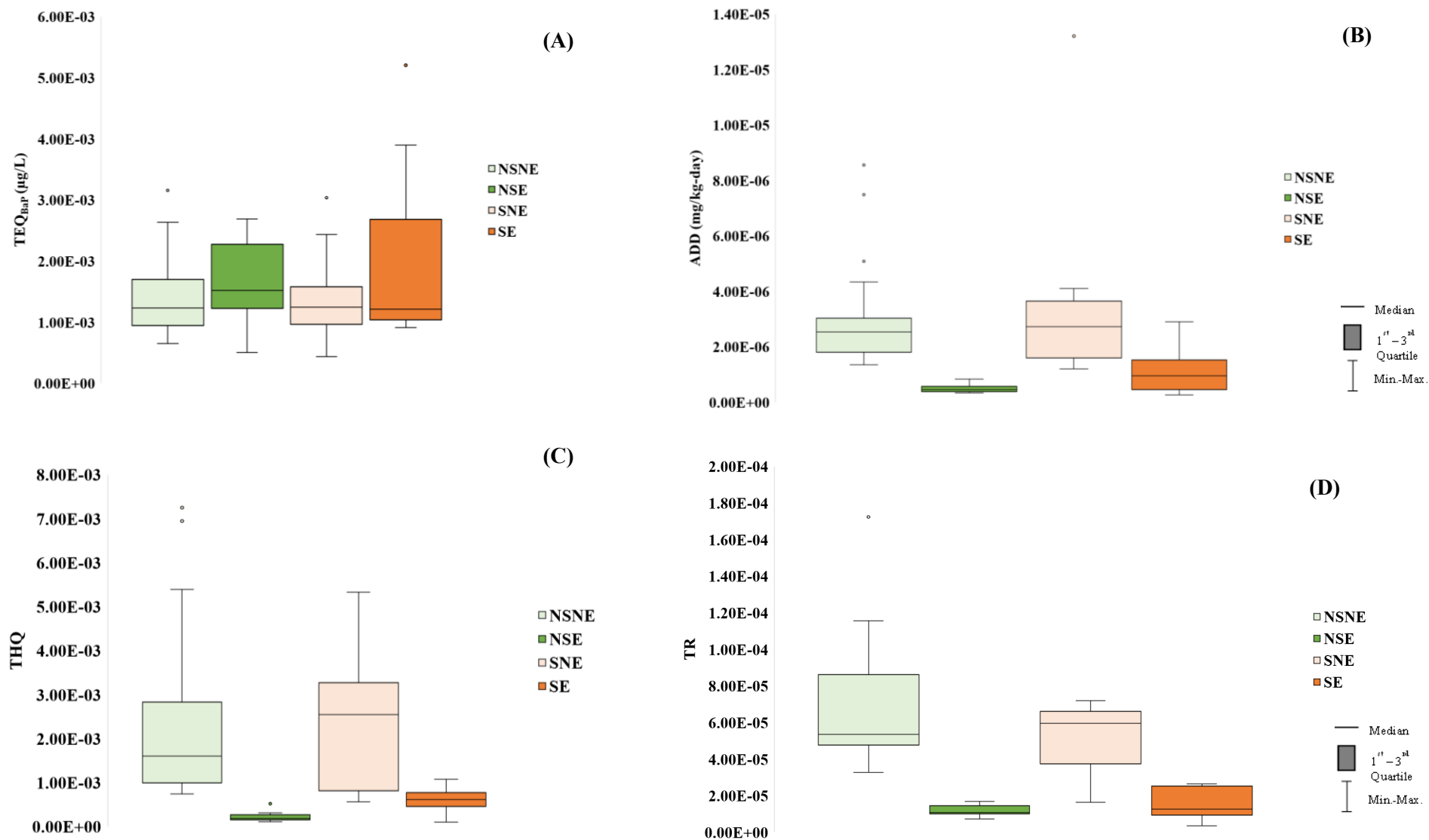


Fig S5. Potential health risks estimated for firefighters' oral exposure through the ingestion of saliva: benzo(a)pyrene total toxicity equivalent concentrations (TEQ_{B(a)P}; A), average daily potential dose (ADD; B), non-carcinogenic (THQ; C) and carcinogenic (TR; D) risks.

Supplementary material – Chapter 6

Impact of fire emissions and tobacco consumption on the salivary content of metals, metalloids, and PAHs: Exposure levels and assessment of oral and intestinal *in vitro* toxicity

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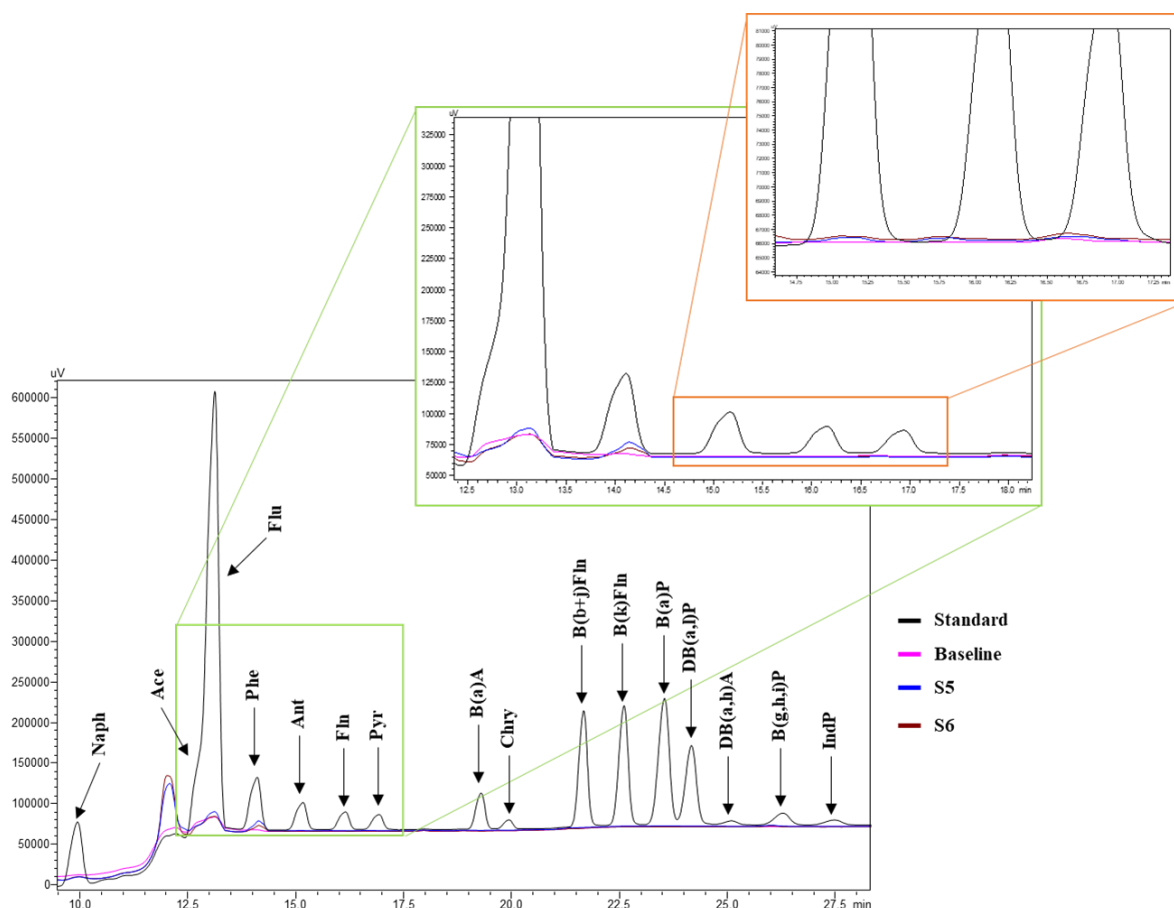


Figure 1S. Representative chromatograms of a standard solution containing a mixture of 18 PAHs [naphthalene (150.45 $\mu\text{g/L}$), acenaphthylene (600.0 $\mu\text{g/L}$), acenaphthene (150.45 $\mu\text{g/L}$), fluorene (29.68 $\mu\text{g/L}$), phenanthrene (15.01 $\mu\text{g/L}$), anthracene (15.03 $\mu\text{g/L}$), fluoranthene (29.59 $\mu\text{g/L}$), pyrene (14.53 $\mu\text{g/L}$), benz(a)anthracene (14.76 $\mu\text{g/L}$), chrysene (14.86 $\mu\text{g/L}$), benzo(b + j)fluoranthene (60.12 $\mu\text{g/L}$), benzo(k)fluoranthene (14.97 $\mu\text{g/L}$), B(a)P (15.04 $\mu\text{g/L}$), dibenzo(a,l)pyrene (30.00 $\mu\text{g/L}$), dibenz(a,h)anthracene (30.00 $\mu\text{g/L}$), benzo(g,h,i)perylene (29.85 $\mu\text{g/L}$), and indeno(1,2,3-c,d)pyrene (15.06 $\mu\text{g/L}$)], a baseline of chromatographic solvent, and a replicate sample of groups S5 and S6.

Annexes

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Gravimetric, morphological, and chemical characterization of fine and ultrafine particulate matter inside fire stations

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Morphological and elemental characterization of fine and ultrafine particulate matter generated from fires

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		URL	http://link.springer.com/journal/12011
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REQUEST DETAILS

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NEW WORK DETAILS

Title	Evaluation of firefighters' occupational exposure and associated early health risks	Institution Name	Faculdade de Ciências da Universidade do Porto
Instructor Name	Gabriel Sousa	Expected Presentation Date	2026-04-15

ADDITIONAL DETAILS

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Gabriel Sousa

REQUESTED CONTENT DETAILS

Title, Description or Numeric Reference of the Portion(s)	10.1007/s12011-025-04868-8	Title of the Article / Chapter the Portion Is From	Impact of Fire Emissions and Tobacco Consumption on the Salivary Content of Metal(loid)s and PAHs: Exposure Levels and Assessment of Oral and Intestinal In Vitro Toxicity
Editor of Portion(s)	Sousa, Gabriel; Teixeira, Joana; Bessa, Maria João; Delerue-Matos, Cristina; Sarmento, Bruno; Oliveira, Marta; Rodrigues, Francisca	Author of Portion(s)	Sousa, Gabriel; Teixeira, Joana; Bessa, Maria João; Delerue-Matos, Cristina; Sarmento, Bruno; Oliveira, Marta; Rodrigues, Francisca
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