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Biomonitoring of firefighters' exposure to priority pollutant metal(loid)s during wildland fire combat missions: Impact on urinary levels and health risks

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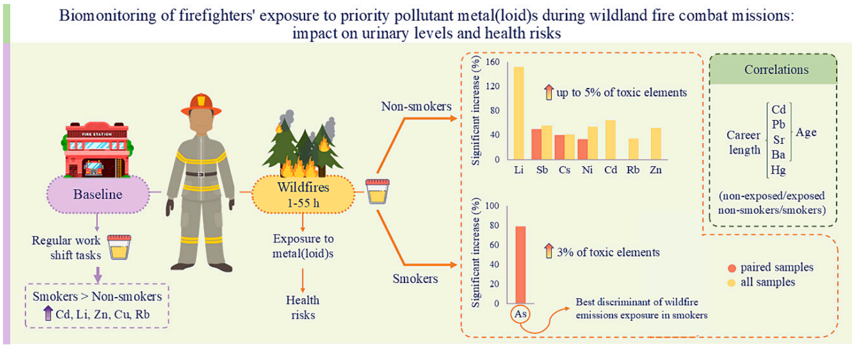
HIGHLIGHTS

- 18 urinary metal(loid)s were biomonitored pre- and post-wildfire combating.
- Smokers presented elevated baseline levels of Zn, Li, Cd, Rb, and Cu.
- The best discriminant of wildfire emissions exposure was As in smokers.
- Cd, Pb, Ba, Sr, and Hg were associated with career length and/or age.
- Urinary As, Zn, Cs, Ni, Sb, Cd, Pb, Tl, Hg, Cu, Co guidance values were exceeded.

GRAPHICAL ABSTRACT



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ABSTRACT

Wildland firefighters are exposed to metal(loid)s released during wildfires through vegetation combustion, which also promotes remobilization of accumulated anthropogenic metal(loid)s. Studies biomonitoring metal(loid)s exposure promoted exclusively by wildfire suppression activities are lacking. This work aimed to characterize, for the first time, the impact of real-life wildland firefighting operations on urinary levels of priority pollutant metal(loid)s [14 included in ATSDR, 11 in USEPA, and 4 in Human Biomonitoring for Europe Initiative priority lists] in firefighters. Spot urines were sampled pre-exposure (105 non-smokers, 76 smokers) and post-exposure to firefighting activities (20 non-smokers, 25 smokers); among those, paired samples were collected from 14 non-smoking and 24 smoking firefighters. Smokers displayed significantly higher baseline levels of zinc (28 %), lithium (29 %), cadmium (55 %), rubidium (13 %), and copper (20 %) than non-smokers. Following wildfire suppression, the concentration of the WHO potentially toxic metal(loid)s rose from 2 % to 3 % in smokers and 2 % to 5 % in non-smokers (up to 4 % for all firefighters and up to 5 % in paired samples). Levels of nickel (33–53 %), antimony (45–56 %), and cesium (40–47 %) increased significantly post-exposure in non-smokers (in all firefighters and in paired samples), whose urinary concentrations were generally more impacted by wildfire emissions than those of smokers. Arsenic (80 %) displayed the only significant increase post-exposure in smokers, being the best discriminant of exposure to wildfire emissions in these subjects. Significant positive correlations were found for age and/or career length with cadmium, lead, barium, strontium, and mercury, and for body mass index with arsenic. The reference/guidance values were exceeded for arsenic, zinc, cesium, nickel, antimony, cadmium, lead, thallium, mercury, copper, and cobalt in 1–90 % of firefighters suggesting augmented health risks due to wildfire combating and emphasizing the need of mitigation strategies. This study also provides biomonitoring data to help setting reference values for the occupationally exposed part of population.

1. Introduction

Given their natural existence and industrial application, metals and metalloids (metal(loid)s) are ubiquitous in the environment (Aprea et al., 2018). Anthropogenic sources include emissions released from the industry, waste incinerators, mining, tire attrition, among others (Fortoul et al., 2015). Organisms are not capable of decomposing metal(loid)s due to their nonbiodegradability, so they accumulate in the body, with some of them presenting a threat to human health (Ali et al., 2023). The priority substances/pollutants lists compiled by the Agency for Toxic Substances and Disease Registry (ATSDR), the United States Environmental Protection Agency (USEPA) and the Human Biomonitoring for Europe Initiative (HBM4EU) currently include 24, 13, and 5 metal(loid)s, respectively. Some, like arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) or their compounds are classified by the International Agency for Research on Cancer (IARC) as known/possible/probable carcinogenic to humans (IARC, 2023a). Many other health effects besides cancer can result from exposure to metal(loid)s, especially to those with higher toxicity, namely metabolic syndrome, hypertension and cardiovascular diseases, diabetes, increased risk of hypothyroidism, different types of anemia, respiratory problems, kidney damage, skin conditions, interference with the nervous system, infertility, low birth weight, fetal mortality, and preterm birth (Chen et al., 2023;; Kiran and Sharma, 2022; Mitra et al., 2022; Yang et al., 2022).

The occupational exposure as a firefighter was recently reevaluated by IARC and, due to its associated risk of developing mesothelioma and bladder cancer, was changed from "possibly carcinogenic to humans" (Group 2B) to "carcinogenic to humans" (Group 1) (IARC, 2023a). Firefighters are at high risk being exposed to several metal(loid)s since fire combating activities involve the protection of manmade structures and industrial products (IARC, 2023b; Li et al., 2023). Wildfires also result in metal(loid)s' exposure since trees and other vegetation absorb these elements from soil and water (Gonçalves et al., 2010), which are then released during combustion (Li et al., 2023). In addition, wildfires can also remobilize anthropogenic depositions of metal(loid)s accumulated in forests (Li et al., 2023). Firefighters' exposure to metal(loid)s released from fires occurs mainly through inhalation and, in a lower degree, via dermal contact (personal protective equipment (PPE) cross-contamination) or ingestion (Barros et al., 2023). Following exposure to smoke and fine particulate matter (PM), accumulation of metal(loid)s in human fluids is to be expected even if firefighters wear wildland PPE (during fire combating, training exercises, or controlled burns) since it does not include breathing apparatus (contrary to structural firefighters), which may compromise complete protection against chemical exposure (Hassan et al., 2023). In addition to being released during fires, metal(loid)s are ubiquitous (food, drinking water, dust, released through tobacco smoke, among other anthropogenic sources) (Fortoul et al., 2015; Vogel et al., 2021). Thus, employing a biomonitoring approach to assess the total body burden is essential, as exposure to metal(loid)s also takes place during regular shifts at the fire stations (without fire exposure) and outside any occupational activities. To the best of our knowledge, the determination of urinary levels of metal(loid)s among firefighters has been limited to a total of four studies with only two reporting information after real-life combating scenarios (not training or controlled fires) (Table 1). The FOX project (USA) and the study by Gündüzöz et al. (2018) (Turkey) collected the urine samples primarily during routine medical examinations, and there was no differentiation between firefighters recently exposed to fire emissions and those who were not. Engelsman et al. (2023) grouped together Australian firefighters recently exposed to different types of real-life fire scenarios (structural fires, vehicle fires, among others), firefighters exposed to fire training, and firefighters not recently exposed, providing a broad and realistic perspective on the general experienced exposure. Lastly, Wolfe et al. (2004) biomonitored USA firefighters who participated in the combat of a large vegetative fire. However, this fire extended to residential structures, samples were collected >2 weeks after fire combating, and the results of exposed and non-exposed firefighters are presented together. Thus, to the best of the author's knowledge, there are currently no studies that characterize urinary metal(loid)s exposure solely due to wildfire combating. Hence, the present study contributes to fill this gap by biomonitoring the exposure of Portuguese firefighters to 18 metal(loid)s during real-life wildfire combating, thus contributing to data collection, characterization of pre- (baseline levels) and post-exposure levels, and to establish reference values as intended by the Human Biomonitoring for Europe Initiative (HBM4EU., 2020). Since both non-smoking and smoking participants were enrolled, the individual and combined impacts of wildfire emissions and tobacco consumption were evaluated for the first time in firefighters, and correlations were estimated between the metal(loid)s' concentrations and factors related to exposure.

2. Materials and methods

2.1. Study location and characterization of the study population

Urine sampling in the present study involved 188 firefighters (Table 2), from July 2021 to July 2022, from 14 (out of 15) fire stations of the district of Bragança (North of Portugal; area of 6599 km^2; GEE, 2019). Portugal is constantly affected by wildfires, due to its Mediterranean climate (Meneses et al., 2018; Oliveira et al., 2020). The 530 wildfires that occurred in this district in 2021–2022 burned a total of 7914 ha, with at least four of them being large fires (255–3310 ha) (ICNF, 2021, 2022). An informed consent form approved by the Ethic

Table 1

Review of the existing studies reporting concentrations of urinary metal(loid)s in firefighters.

Country	Age (mean; years)	Tobacco consumption	Exposure	n	Metal (loid)	Units	Mean (range)	Median (25th–75th)	LOD	Reference
Australia	44	3.1 % smokers	94 % Current fire exposure; this exposure includes real fires (structural fires, external fires; overhaul; vehicle fires) and compartment fire behavioural training; <50 % of firefighters provided their sample 24 h within fire exposure.	73	Cu	μg/L	(<LOD-22.80)	4.64 (<LOD-65.00)	1.27	Engelsman et al. (2023)
					Ni		(<LOD-65.00)	<LOD (<LOD-2.49)	1.17	
					Se		(<LOD-157.89)	38.10 (<LOD-58.36)	31.58	
					Co		(<LOD-2.77)	<LOD (<LOD-< LOD)	1.18	
					Pb		(<LOD-12.43)	<LOD (<LOD-< LOD)	4.14	
				63	Hg		(<LOD-6.13)	<LOD (<LOD-< LOD)	4.01	
				73	Tl		(<LOD-1.11)	<LOD (<LOD-< LOD)	0.82	
				73	Cu	μg/g creatinine	(<LOD-49.57)	3.75 (<LOD-65.00)	1.27	
					Ni		(<LOD-36.84)	<LOD (<LOD-2.75)	1.17	
					Se		(<LOD-139.60)	37.76 (<LOD-50.95)	31.58	
					Co		(<LOD-5.21)	<LOD (<LOD-< LOD)	1.18	
					Pb		(<LOD-18.32)	<LOD (<LOD-< LOD)	4.14	
				63	Hg		(<LOD-17.73)	<LOD (<LOD-< LOD)	4.01	
				73	Tl		(<LOD-3.61)	<LOD (<LOD-< LOD)	0.82	
Turkey	36.5	Non-smokers	Samples were collected during a periodic medical examination; no recent exposure information is provided.	19	As	μg/L	(2.5–246)	15.65	n.r.	Gündüzöz et al. (2018)
USA	n.r.	n.r.	72 % exposed; large vegetative fire which also affected several residential structures (May 9–15, 2004). Sample collection: May 25–June 2, 2004.	92	Ni	μg/g creatinine	5.15* (n.r.-41.38)	n.r.	n.r.	Wolfe et al. (2004)
				79	Mo		41.41* (n.r.-323.62)	n.r.	n.r.	
				92	Co		0.26* (n.r.-0.93)	n.r.	n.r.	
					Cd		0.22* (n.r.-1.20)	n.r.	n.r.	
					Pb		0.51* (n.r.-1.38)	n.r.	n.r.	
					Hg		3.70* (n.r.-43.51)	n.r.	n.r.	
					Tl		0.15* (n.r-0.29)	n.r.	n.r.	
					As		4.62* (n.r.-109.77)	n.r.	n.r.	
				77	Sb		0.07* (n.r.-0.31)	n.r.	n.r.	
				92	Cs		4.08* (n.r.-12.07)	n.r.	n.r.	
					Ba		1.56* (n.r.-9.31)	n.r.	n.r.	
USA (FOX project)	42.8	10 % Tobacco cigars or chewing	Some samples were collected during an annual/biannual wellness examination, and some after firefighters were off duty. Firefighters off duty may not have participated in firefighting activities 7–10 days or longer prior to sample collection.	101	Cd	μg/g creatinine	0.145*	0.138 (<LOD-0.190)	0.105	Biomonitoring California, 2016
					Hg	μg/L	0.445*	0.447 (0.221–0.914)	0.158	
					As		10.8*	10.4 (6.05–21.0)	0.0522	

FOX: Firefighter Occupational Exposure; IQR: Interquartile range (25th–75th); LOD: Limit of detection; n.r.: Not reported; USA: United States of America.

[*] Data are presented as geometric mean.

Table 2
All male Portuguese firefighters' studied population and number of urine samples per characterized group.

	NSNExp	NSExp	SNExp	SExp
Total number of urine samples	105	20	76	25
Number of paired samples (before and after exposure)	14	14	24	24
(Bio)metric characterization of all participants				
Age (mean ± SD; 25th–75th; years)	40 ± 11 (32–46)	40 ± 10 (32–45)	33 ± 10 (24–40)	32 ± 9 (24–39)
Body Mass Index (mean ± SD; 25th–75th; kg/m^2)	27.7 ± 3.8 (25.5–30.0)	27.5 ± 2.9 (25.1–28.9)	27.1 ± 4.3 (24.1–30.3)	26.8 ± 3.5 (24.8–29.2)
Firefighter career length (mean ± SD; 25th–75th; years)	17 ± 10 (10–25)	18 ± 11 (7–25)	14 ± 10 (6–21)	13 ± 9 (6–19)
Daily consumed cigarettes per day (mean ± SD; 25th–75th)	n.a.	n.a.	16 ± 8 (10−20)	18 ± 7 (12−20)
Recent participation in wildland combat (mean ± SD; 25th–75th; h)	n.a.	13 ± 15 (3−20)	n.a.	12 ± 12 (2–18)

NSNExp: non-smoking and non-exposed to fire emissions; NSExp: non-smoking and exposed to fire emissions; SNExp: smoking and non-exposed to fire emissions; SExp: smoking and exposed to fire emissions; n.a.: Not applicable; SD: Standard deviation.

Committee of the University of Porto (Report Nr. 92/CEUP/2020) in accordance with the Declaration of Helsinki was signed by each participant who agreed to join this study voluntarily. Through a questionnaire, personal (e.g., age, gender, height, tobacco consumption), medical (e.g., chronic diseases, current medication, weight), and professional (e.g., corporation, number of years as a firefighter, time spent at the fire station and exposed to wildfires) information was obtained. Since some of the firefighters were active smokers and tobacco consumption is a source of metal(loid)s, samples were organized in four distinct groups based on this criterium and recent participation in wildland firefighting activities: non-smoking and non-exposed to fire emissions (NSNExp), non-smoking and exposed (NSExp), smoking and non-exposed (SNExp), and smoking and exposed (SExp). Subjects recently (<3 days) involved in the combat of urban fires at the time of sample collection were excluded, therefore only the firefighters participating in wildfires were included in the present study. Thus, the term "exposed" is used to refer to firefighters recently exposed to wildfires emissions (groups NSExp and SExp), and the term "non-exposed" to firefighters not exposed to wildfires emissions (groups NSNExp and SNExp, which served as the control groups).

A total of 181 pre-exposure spot urine samples were collected from 181 firefighters (105 NSNExp and 76 SNExp) after regular work shifts as long as they had not participated in any firefighting activity for at least 3 days. In addition, 45 post-exposure samples were retrieved from individuals (20 NSExp and 25 SExp) participating in wildfire combat activities as soon as they returned to the fire station. Among total number of urine samples, 14 non-smokers and 24 smokers provided paired samples (pre- and post-exposure to firefighting activity); no significant differences ($p > 0.05$) were observed in demographic and exposure data in comparison to data from all participants. The samples were collected in sterilized 100 mL containers of polycarbonate, and frozen at −20 °C until further analysis.

Across all groups, the participants were all males, aged 20–65 years, with 13–18 years of service as firefighters on average (Table 2), and the majority (72–88 %) spent at least 8 h at the fire stations daily; no statistical differences were observed between groups with all participants and those only with paired samples. Smoking participants before and after fire exposure had a similar mean number of smoked cigarettes per day (16 and 18, respectively). Regarding recent exposure prior to the sample collection, on average, non-smoking and smoking firefighters were involved in wildfire combat activities for 13 and 12 h, respectively, thus there was no significance difference in exposure type and duration between the groups.

2.2. *Urinary metal(loid)s and creatinine analysis*

Lithium (Li), beryllium (Be), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), As, selenium (Se), rubidium (Rb), strontium (Sr), molybdenum (Mo), Cd, antimony (Sb), cesium (Cs), barium (Ba), Hg, thallium (Tl), and Pb were analyzed in urine. Their determination in urine samples was performed using inductively coupled plasma mass spectrometry (ICP-MS) as previously referred by Azevedo et al. (2023). Limits of detection (LOD) and quantification (LOQ) were calculated as 3.3 and 10 times the standard deviation of the blank, respectively (Miller and Miller, 2000). LODs (Table S1, Supplementary Material) ranged from 0.0099 μg/L (Sb) to 35 μg/L (Rb), and the respective LOQs from 0.033 μg/L to 117 μg/L. Seronorm™ Trace Elements Urine L-1 and L-2 (SERO AS; Billingstad, Norway) were used as certified quality control materials. The analytical quality control analysis showed a precision (relative standard deviation) between 1 and 10 % for all the metal(loid)s except for Be (2–32 %). The accuracy varied between 87 and 120 % for all elements (detailed information in Supplementary Material – Section 1S). Jaffe's colorimetric method was employed to quantify the urinary creatinine levels for normalizing the metal(loid) concentrations (Kanagasabapathy and Kumari, 2000).

2.3. *Statistical analysis*

The SPSS software (IBM Statistics 29) and the Excel (v. 16.82, Microsoft Corporation, USA, with the XLSTAT extension v. 25.3.1, XLSTAT) were used to perform the statistical analysis. The concentrations below LOD were replaced by $\text{LOD}/\sqrt{2}$ to carry out statistical analyses (Hornung and Reed, 1990). Urinary concentrations are presented in μg/L throughout the main text and in μg/g creatinine in the Supplementary Material (Fig. S1). Normality was checked using the Kolmogorov-Smirnov test for all participants and the Shapiro-Wilk test for paired samples, which indicated non-normal distributions even when data was log-transformed ($p < 0.05$). Thus, using the untransformed data, the non-parametric Mann-Whitney U and Wilcoxon Signed-Rank tests were used to compare concentrations of independent (unpaired) and paired samples, respectively. Spearman correlation coefficients (r) were applied to evaluate the relationship between metal(loid) concentrations and the variables extracted from the questionnaires. Statistical significance was defined as $p \leq 0.05$.

3. Results and discussion

The organization of metals and metalloids into different groups was based on their inclusion in the priority substances/pollutants lists by ATSDR, USEPA, and HBM4EU (ATSDR, 2023; HBM4EU., 2020; USEPA, 2014). Group M1 (As, Pb, Hg, and Cd) is comprised of elements found consistently across the three lists mentioned, underscoring their universally recognized toxicological relevance. Group M2 (Zn, Sr, Se, Cs, Cu, Ni, Ba, Co, Tl, and Sb) consists of elements in at least one of the lists (ATSDR and/or USEPA), thus with varying degrees of priority across different agencies. Lastly, elements included in Group M3 (Rb, Mo, and

Li) are currently not featured in any of the lists mentioned. Be (classified as carcinogenic to humans, Group 1, by IARC (2023a)), although belonging to M2, is discussed separately due to its low detection rate (0.4 %) compared with the others (>92 %) (Table S1). This element was also quantified in a relatively low number of samples among adults from Germany [0–2 %; (Heitland and Koster, 2006; Schmied et al., 2021)] and Italy [18 %; (Aprea et al., 2018)]. Nisse et al. (2017) reported a median concentration for French adults of 0.01 μg/L Be, with 58.4 % of samples above LOD (0.0004 μg/L), and a 95th percentile of 0.15 μg/L, which is higher than the concentration of the only sample above LOD of this study (0.040 μg/L). Thus, the exposure of Portuguese firefighters to Be appears to be in line with that of adults from other European countries. In addition, non-detectable samples (LOD: 0.025 μg/L; Table S1, Supplementary Material) and the only detected sample were well below 1.4 μg/L, a limit that indicates an individual has likely been occupationally exposed to Be, according to Safe Work Australia (2020).

3.1. Metal(loid)s pre-exposure levels and impact of tobacco smoking

3.1.1. Comparison with reference and guidance values

The concentrations of the elements included in M1, M2, and M3 for the non-exposed non-smoking and smoking firefighters are presented in Table 3 and Fig. S2A, B, and C, respectively. Rb, belonging to group M3, was by far the element with the highest concentration in all groups of firefighters (1968 μg/L in NSNExp and 2218 μg/L in SNExp), followed by the M2 elements Zn and Sr (Zn: 323 μg/L in NSNExp and 414 μg/L in SNExp; Sr: 115 μg/L in NSNExp and 110 μg/L in SNExp), which emerged as the second and third most prevalent elements herein quantified. Sb (group M2; 0.0703 μg/L in NSNExp and 0.0741 μg/L) presented the lowest concentration, followed by Cd (group M1; 0.177 μg/L in NSNExp and 0.274 μg/L). The remaining elements ranked as follows: Mo > Li > As > Se > Cs > Cu > Ni > Ba > Pb > Hg > Co > Tl for non-smokers and Li > Mo > Se > As > Cs > Cu > Ni > Ba > Pb > Hg > Co > Tl.

Table 4 presents a list of reference/guidance values for the determined elements, along with an overview of how many firefighters exceeded those values. The median concentrations of metal(loid)s from M1 group are below their respective reference values, except for As in NSNExp and SNExp firefighters, which exceeded the German RV_{95} reference value (Table 4). Individual levels of As surpassed the Canadian, German, and Taiwanese RV_{95} reference values in 2–68 % of firefighters, while surpassing the accepted normal range according to ATSDR in 5–10 % of firefighters, and the Biological Exposure Index (BEI) in 33–36 % firefighters by 1.1–40 times. BEIs do not represent a defined threshold between a safe and hazardous concentration in a biological matrix, but the levels most likely to be observed in these matrices among healthy workers repeatedly exposed via inhalation of chemicals without the occurrence of adverse health effects. The exceedance of the BEI for As suggests occupational exposure to it. While 31–38 % of the firefighters exhibited concentrations slightly above the BEI (up to 10 %), a noteworthy 28–32 % of them surpassed it by up to 17 times, indicating

Table 3

Concentrations [median (mean) range; μg/L] and statistical analysis of metal(loid)s in the urine of all participants: non-smoking non-exposed (NSNExp; $n = 105$), non-smoking exposed (NSExp; $n = 20$), smoking non-exposed (SNExp; $n = 76$) and smoking exposed (SExp; $n = 25$) firefighters.

Group	Metal (loid)	Non-smokers			Smokers		
		NSNExp ($n = 105$)	NSExp ($n = 20$)	p value*	SNExp ($n = 76$)	SExp ($n = 25$)	p value*
Included in the ATSDR, USEPA, and HBM4EU priority pollutants lists	As	23.9 (48.0) 2.17–592	27.4 (37.6) 10.5–90.0	0.336	20.3 (34.8) 3.50–235	32.0 (59.0) 3.74–425	0.214
	Cd	0.177 (0.226) 0.0183–1.07	0.290 (0.361) 0.0668–1.01	0.005	0.274 (0.336) 0.0261–1.14	0.237 (0.383) 0.0636–1.14	0.991
	Hg	0.367 (0.467) 0.0236–2.53	0.438 (0.497) 0.159–1.51	0.657	0.314 (0.530) 0.0306–9.88	0.254 (0.300) 0.0306–0.882	0.243
	Pb	0.704 (1.03) 0.0105–3.97	0.889 (1.05) 0.179–2.70	0.762	0.901 (0.976) 0.0100–3.24	0.808 (1.02) 0.120–2.68	0.937
Included in the ATSDR and/or USEPA, priority pollutants lists	Ba	1.81 (2.37) 0.0538–13.5	1.67 (1.99) 0.226–4.37	0.731	1.44 (2.16) 0.0844–11.9	1.30 (1.97) 0.115–7.00	0.869
	Co	0.278 (0.321) 0.0131–1.92	0.339 (0.368) 0.131–0.799	0.120	0.295 (0.405) 0.0131–5.10	0.382 (0.572) 0.0131–5.38	0.353
	Cs	11.3 (12.0) 1.27–27.3	15.9 (15.6) 7.80–25.1	0.015	11.7 (12.9) 2.52–45.0	11.4 (13.2) 1.17–50.0	0.626
	Cu	7.45 (8.30) 0.733–22.4	9.06 (9.52) 4.95–18.3	0.191	8.96 (9.73) 1.20–27.4	10.7 (10.5) 4.91–18.7	0.306
	Ni	2.14 (2.31) 0.102–8.13	3.28 (3.38) 1.20–6.29	0.005	2.19 (2.68) 0.0936–7.52	3.02 (3.15) 0.413–8.84	0.208
	Sb	0.0703 (0.0919) 0.0251–0.791	0.110 (0.142) 0.0364–0.387	<0.001	0.0741 (0.0883) 0.0312–0.445	0.0890 (0.107) 0.0314–0.421	0.241
	Se	22.4 (25.0) 0.645–64.9	22.5 (28.1) 4.86–82.7	0.772	26.1 (26.8) 0.645–59.3	24.7 (24.5) 0.645–45.3	0.320
	Sr	115 (124) 2.92–429	106 (117) 23.9–248	0.819	110 (130) 3.73–374	115 (138) 4.28–383	0.801
	Tl	0.255 (0.284) 0.0260–1.34	0.227 (0.257) 1.01–0.561	0.567	0.284 (0.288) 0.0250–1.23	0.239 (0.261) 0.0445–1.02	0.075
	Zn	323 (385) 58.0–1980	487 (618) 150–1500	0.017	414 (554) 32.3–2586	536 (622) 93.3–1796	0.205
Not included in the ATSDR, USEPA or HBM4EU priority pollutants lists	Li	36.7 (78.4) 8.08–613	92.2 (187) 40.4–1150	<0.001	47.5 (134) 8.98–1089	55.9 (115) 4.99–637	0.753
	Mo	37.6 (44.4) 4.23–149	44.2 (51.9) 14.5–153	0.572	39.1 (46.4) 4.26–113	33.4 (41.9) 2.73–106	0.341
	Rb	1968 (2049) 185–5457	2635 (2682) 1224–6592	0.034	2218 (2377) 467–4549	2330 (2366) 223–3894	0.844

ATSDR: Agency for Toxic Substances and Disease Registry; HBM4EU: Human Biomonitoring for Europe Initiative; USEPA: United States Environmental Protection Agency.; As: arsenic; Ba: barium; Cd: cadmium; Co: cobalt; Cs: cesium; Cu: copper; Hg: mercury; Li: lithium; Mo: molybdenum; Ni: nickel; Pb: lead; Rb: rubidium; Sb: antimony; Se: selenium; Sr: strontium; Tl: thallium; Zn: zinc.

* p value: Mann Whitney U test for independent samples.

Table 4
Reference values for the metal(loid)s included in the present study.

Group	Metal (loid)	Type of reference value	Value	Units	Firefighters above reference value (%)				Reference
					NSNExp	NSExp	SNExp	SExp	
Included in the ATSDR, USEPA, and HBM4EU priority pollutants lists	As	BEI[a]	35	µg/L	36	45	33	48	ACGIH, 2019
		Commonly accepted normal range	100	µg/L	10	0	5	3	ATSDR, 2007
		RV$_{95}$ for Canadian adults (20–79 years old)[b]	27	µg/L	44	25	39	36	Saravanabhavan et al., 2017
		RV$_{95}$ for German adults that did not eat fish 48 h prior to sample collection (18–69 years old)[b]	15.0	µg/L	64	90	68	84	Schulz et al., 2011
		RV$_{95}$ for Taiwanese adults (20–97 years old)[b]	370	µg/L	2	0	0	4	Liao et al., 2023
		Reference value for patients at risk from occupational or environmental exposure[c]	2.0	µmol/L	4	0	3	8	RCPA, 2019
			40	nmol/mmol creatinine	34	25	21	32	
	Cd	HBM-I[d]	1	µg/L	2	5	1	8	UBA, 2023
		HBM-II[e]	4	µg/L	0	0	0	0	
		RV$_{95}$ for Canadian adults (20–79 years old)[b]	1.3	µg/L	0	0	0	0	Saravanabhavan et al., 2017
		RV$_{95}$ for German non-smoking adults (18–69 years old)[b]	0.8	µg/L	2	10	4	20	Schulz et al., 2011
		RV$_{95}$ for Taiwanese adults (20–97 years old)[b]	2.21	µg/L	0	0	0	0	Liao et al., 2023
		BEI[a]	5	µg/g creatinine	0	0	0	0	ACGIH, 2019
		Trigger level for medical surveillance in occupationally exposed employees	3	µg/g creatinine	0	0	0	0	OSHA, 2004
		Reference value for patients at risk from occupational or environmental exposure[c]	40	nmol/L	0	0	0	0	RCPA, 2019
			3	nmol/mmol creatinine	0	0	0	0	
	Hg	HBM-I[d]	7	µg/L	0	0	1	0	UBA, 2023
			6	µg/g creatinine	0	0	1	0	
		HBM-II[e]	25	µg/L	0	0	0	0	
			20	µg/g creatinine	0	0	0	0	
		RV$_{95}$ for German adults without dental fillings (18–69 years old)[b]	1.0	µg/L	8	10	8	0	Schulz et al., 2011
		Reference interval for patients at risk from occupational or environmental exposure[c]	0.1	µmol/L	0	0	0	0	RCPA, 2019
			10	nmol/mmol creatinine	0	0	0	0	
		Trigger level for medical surveillance in occupationally exposed employees	40	µg/g creatinine	0	0	0	0	HSE, 2002
	Pb	RV$_{95}$ for Canadian adults (20–79 years old)[b]	1.9	µg/L	14	10	11	8	Saravanabhavan et al., 2017
		RV$_{95}$ for Taiwanese adults (20–97 years old)[b]	5.75	µg/L	0	0	0	0	Liao et al., 2023
		Reference value for patients at risk from occupational or environmental exposure[c]	0.4	µmol/L	0	0	0	0	RCPA, 2019
			21	nmol/mmol creatinine	0	0	0	0	
Included in the ATSDR and/or USEPA, priority pollutants lists	Ba	BE based on the RfD by USEPA[f]	192	µg/L	0	0	0	0	Poddalgoda et al., 2017
			246	µg/g creatinine	0	0	0	0	
			246	µg/g creatinine	0	0	0	0	
	Co	RV$_{95}$ for Taiwanese adults (20–97 years old)[b]	1.73	µg/L	1	0	1	1	Liao et al., 2023
		BEI[a]	15	µg/L	0	0	0	0	ACGIH, 2019
	Cs	RV$_{95}$ for the general Canadian population (3–79 years old)[b]	12	µg/L	44	70	45	48	Saravanabhavan et al., 2017
	Cu	RV$_{95}$ for Canadian adults (20–79 years old)[b]	25	µg/L	0	0	1	0	Saravanabhavan et al., 2017
		RV$_{95}$ for Taiwanese adults (20–97 years old)[b]	22.8	µg/L	0	0	3	0	Liao et al., 2023
		Reference value for patients at risk from occupational or environmental exposure[c]	1	µmol/L	0	0	0	0	RCPA, 2019
	Ni	Range for healthy adults	1–3	µg/L	22	65	36	52	ATSDR, 2005
		RV$_{95}$ for the general Canadian population (3–79 years old)[b]	4.4	µg/L	7	15	12	16	Saravanabhavan et al., 2017
		RV$_{95}$ for German adults (18–69 years old)[b]	3	µg/L	22	65	36	52	Schulz et al., 2011
		RV$_{95}$ for Taiwanese adults (20–97 years old)[b]	6.17	µg/L	2	5	7	4	Liao et al., 2023
	Sb	RV$_{95}$ for the general Canadian population (3–79 years old)[b]	0.17	µg/L	7	20	4	12	Saravanabhavan et al., 2017

(*continued on next page*)

Table 4 (*continued*)

Group	Metal (loid)	Type of reference value	Value	Units	Firefighters above reference value (%)				Reference
					NSNExp	NSExp	SNExp	SExp	
	Se	RV_{95} for Canadian adults (20–79 years old)[b]	120	μg/L	0	0	0	0	Saravanabhavan et al., 2017
		RV_{95} for Taiwanese adults (20–97 years old)[b]	102	μg/L	0	0	0	0	Liao et al., 2023
	Sr	RV_{95} for Taiwanese adults (20–97 years old)[b]	451	μg/L	0	0	0	0	Liao et al., 2023
	Tl	HBM-I[d]	5	μg/L	0	0	0	0	UBA, 2023
		RV_{95} for Canadian adults (20–79 years old)[b]	0.61	μg/L	4	0	1	4	Saravanabhavan et al., 2017
		RV_{95} for German adults without dental fillings (18–69 years old)[b]	0.5	μg/L	10	5	8	4	Schulz et al., 2011
		RV_{95} for Taiwanese adults (20–97 years old)[b]	0.49	μg/L	10	5	9	4	Liao et al., 2023
		Reference value for patients at risk from occupational or environmental exposure[c]	50	nmol/L	0	0	0	0	RCPA, 2019
			2	μg/g creatinine	1	0	0	0	
	Zn	RV_{95} for the general Canadian population (3–79 years old)[b]	1100	μg/L	1	15	9	8	Saravanabhavan et al., 2017
		BE based on the UL by IOM[f]	909	μg/L	7	25	14	20	Poddalgoda et al., 2019
			1170	μg/g creatinine	1	0	0	0	
		BE based on the RfD by US EPA[f]	481	μg/L	27	50	46	60	
			619	μg/g creatinine	7	0	3	2	
		BE based on the chronic MRL by ATSDR[f]	439	μg/L	30	50	49	76	
			564	μg/g creatinine	8	1	5	2	
		BE based on the UL by EFSA[f]	658	μg/L	12	45	29	36	
			847	μg/g creatinine	3	0	2	0	
		BE based on the PMTDI by JECFA[f]	1585	μg/L	1	0	4	4	
			2040	μg/g creatinine	0	0	0	0	
		BE based on the NOAEL by EU RAR[f]	1316	μg/L	1	10	7	4	
			1693	μg/g creatinine	0	0	0	0	
		BE based on the LOAEL by EU RAR[f]	3489	μg/L	0	0	0	0	
		RV_{95} for Taiwanese adults (20–97 years old)[b]	4488	μg/g creatinine	0	0	0	0	
Not included in the ATSDR, USEPA, or HBM4EU priority pollutants lists	Mo	RV_{95} for Canadian adults (20–79 years old)[b]	170	μg/L	0	0	0	0	Saravanabhavan et al., 2017
		BE based on the NOAEL adopted by Health Canada[f]	7156	μg/L	0	0	0	0	Faure et al., 2020
		BE based on the RfD by USEPA[b]	206	μg/L & μg/g creatinine	0	0	0	0	Hays et al., 2016
		BE based on the TDI by RIVM[b]	442	μg/L & μg/g creatinine	0	0	0	0	
		BE based on the UL by IOM[b]	1326	μg/L & μg/g creatinine	0	0	0	0	
		BE based on an oral repeated dose toxicity study reviewed by the OECD[b]	7516	μg/L & μg/g creatinine	0	0	0	0	

ATSDR: Agency for Toxic Substances and Disease Registry; BE: Biomonitoring Equivalent; BEI: Biological Exposure Index; EFSA: European Food Safety Authority; EU RAR: European Union Risk Assessment Report; HBM4EU: Human Biomonitoring for Europe Initiative; HBM-I and HBM-II: Human biomonitoring health-related guidance values derived by the German Human Biomonitoring Commission; IOM: Institute of Medicine; JECFA: Joint FAO/WHO Expert Committee on Food Additives; LOAEL: Lowest Observed Adverse Effect Level; MRL: Maximum Residue Limit; NOAEL: No Observed Adverse Effect Level; OECD: Organization for Economic Co-operation and Development; PMTDI: Provisional maximum tolerable daily intake; RIVM: National Institute for Public Health and the Environment; RfD: Reference Dose; TDI: Tolerable daily intake; UL: Tolerable Upper Intake Level; USEPA: United States Environmental Protection Agency; NSNExp: non-smoking and non-exposed to fire emissions; NSExp: non-smoking and exposed to fire emissions; SNExp: smoking and non-exposed to fire emissions; SExp: smoking and exposed to fire emissions; As: arsenic; Ba: barium; Cd: cadmium; Co: cobalt; Cs: cesium; Cu: copper; Hg: mercury; Mo: molybdenum; Ni: nickel; Pb: lead; Sb: antimony; Se: selenium; Sr: strontium; Tl: thallium; Zn: zinc.

[a] Most likely concentration to be observed in the urine of healthy workers repeatedly exposed via inhalation to chemicals without the occurrence of adverse health effects.

[b] Reference values derived based upon the 95 % confidence interval of the 95th percentile of the concentration of a given element in the urine of a reference population. RV_{95} do not serve to assess health risks, but instead represent the upper margin of the current background exposure of the reference population to a certain element.

[c] Results within this interval exclude recent, clinically significant exposure.

[d] Concentration at and below which there is no risk of adverse health effects.

[e] Concentration at and above which adverse health effects are possible, demanding a reduction of exposure and biomedical advice.

[f] Biomonitoring equivalent value derived based upon and an existing exposure guidance value and that represents a concentration in urine consistent with that guidance value.

concentrations that raise concern. The reference interval for monitoring patients at risk from occupational or environmental exposure by the Royal College of Pathologists of Australia (RCPA), which indicates As exposure, was also exceeded in 3–4 % of firefighters (2 to 4 fold with unadjusted units) and by 21–34 % of firefighters (6.0 to 14 fold with creatinine adjusted units) (RCPA, 2019). Table S2 provides a list of relevant sources and health effects for the elements analyzed in this study. Relevant sources of As include drinking water (particularly well water), food (especially seafood and seafood based supplements; Table S2). Besides being carcinogenic to humans (IARC, 2023a), As induces oxidate stress and can result in several complications like diabetes and neurological problems (Kiran and Sharma, 2022; Table S2). Regarding Pb, 11–14 % of non-smoking and smoking firefighters were up to 109 % above the RV_{95} for Canadian adults, but none of the firefighters exceeded the limits related to occupational exposure (Table 4). Individual concentrations of Cd in 2 % of non-smokers and 1 % of smokers, and Hg in 1 % of smokers were between the HBM-I and HBM-II health-related guidance values derived by the German Human Biomonitoring Commission. This means that for these individuals adverse health effects cannot be excluded with enough certainty and a follow-up measurement should be conducted (Apel et al., 2017). Moreover, 2–10 % of firefighters surpassed the German RV_{95} reference values for Cd by up to 43 % and for Hg by 888 %, meaning firefighters had higher concentrations of these metals in comparison to the upper margin of the current background exposure of this population. These highly toxic elements that are found in food among other relevant sources (e.g. tobacco smoke for Cd) induce oxidative stress, carcinogenesis and other serious health complications (Table S2).

Median concentrations of metal(loid)s from M2 group are below their respective guidance values, regardless of the firefighters' smoking habits, except for Zn in the SNExp firefighters which surpassed two biomonitoring equivalents (BE; Table 4). Individual levels of Zn (especially with unadjusted units), Cs, and Ni, stood out for their exceeding levels by up to 5.9 times in 1–30 %, 44 %, and 2–22 % of non-smokers and 2–49 %, 45 % and 7–36 % of smokers, respectively. Tl and Sb levels listed in Table 4 were up to 4.7 times surpassed by 1–10 % and 1–9 % of non-smoking and smoking firefighters, respectively. Overall, most firefighters had urinary concentrations of Sr, Se, Cu, Ba, and Co below their respective reference values, except for 1 % of non-smoking and smoking firefighters for Co (up to 2.9-fold), and 1–3 % of smoking firefighters for Cu (up to 1.2-fold). While these metals result from multiple sources of exposure, food is the main source of Zn, Ni, Co, and Cu, and a common source for Cs, Tl, and Sb (Table S2). Excess Zn and Sb intake can lead to the development of diabetes mellitus and cardiovascular diseases, among other problems (ATSDR, 2005; Lai et al., 2022; Qu et al., 2020; Table S2). Ni and Tl can induce reduced lung function and cause damage to the kidneys, and other complications (Dai et al., 2019; Kiran and Sharma, 2022; Table S2). Meanwhile, knowledge about the health effects of Cs is limited, but animal studies suggest it has low toxicity (ATSDR, 2004).

Concerning M3, all firefighters had Mo concentrations below its reference values (Table 4), and for Rb or Li no reference values were found.

3.1.2. *Impact of tobacco smoking*

Concerning the effect of tobacco smoke on the levels of M1 elements, smoking increased the urinary median concentration of Cd by 55 % ($p < 0.001$) (Fig. S2A) in comparison to the concentration in non-smokers. French adult smokers had significantly higher Cd concentrations than non-smokers, while As and Hg were higher in non-smokers (Nisse et al., 2017), which is in agreement with the attained results in Portuguese firefighters. A mixture of cigarettes of popular brands in Portugal had a content of Cd > Pb > As with 0.79, 0.55, and 0.14 μg/g dry weight, respectively (Hg was not included in the study) (Pinto et al., 2017). The median Cd concentrations of the NSNExp and SNExp groups were 61 % and 83 % higher than those reported by the HBM4EU initiative for Portuguese non-smoking (0.11 μg/L) and smoking (0.15 μg/L) adults, respectively (no information exists for the other toxic elements characterized) (HBM4EU., 2021). In contrast to As, Pb, and Hg, Cd was moderately associated with the number of smoked cigarettes per day ($r = 0.458$; $p < 0.001$) among SNExp firefighters.

Regarding M2, Zn (28 %; $p = 0.006$) and Cu (20 %; $p = 0.02$) showed statistically higher concentrations in smoking than non-smoking individuals (Fig. S2B). Hoet et al. (2013) also found significantly higher concentrations of Zn and Cu in Belgium smokers compared to non-smokers, as well as Nisse et al. (2017) in French adults for Zn. Pinto et al. (2017) found that the 20 best-selling cigarette brands in Portugal had a high mean content of Zn (25.2 μg/g dry weight) compared to Tl, Co, and Ni (0.074–2.10 μg/g dry weight), which might explain the significant differences between urine concentrations of Portuguese smokers and non-smokers. While the composition of metal(loid)s in tobacco depends on several factors, such as the manufacturing processes and the soil, which varies geographically (Dahlawi et al., 2021), Cu content in tobacco cigarettes varied between 0.81 and 13.85 μg/g dry weight in other studies (Ajab et al., 2008; Dahlawi et al., 2021). Hence, Cu content in the tobacco smoked by Portuguese firefighters may have contributed to the significant increase in urine, while remaining below (60–86 %) the reference values in Table 4. Smoking has also been known to alter metal homeostasis in the human body (Pasupathi et al., 2009), for example, Cd has been associated with intra-cellular zinc depletion (Renu et al., 2022), while Cu levels are increased in tobacco consumers due to its impact on Cu metabolic functions (Esad et al., 2022; Satarug et al., 2018), thus supporting the higher urinary Zn and Cu found in smoking firefighters. Of all the metal(loid)s, Ba had the highest content in the most popular cigarette brands in Portugal (123.0 μg/g dry weight), but the reported rate transfer from tobacco to cigarette smoke is only 0.5–1.6 % (Pinto et al., 2017), unlike what was observed for elements like Cd (81–90 %) and Zn (13–21 %), supporting the present findings.

Smoking increased the median urinary Li and Rb (the most concentrated element) levels compared to non-smokers by 29 % and 13 %, respectively ($p \leq 0.04$; Table 3; Fig. S2C). Notably, Rb SNExp levels were associated with the number of daily smoked cigarettes ($r = 0.267$; $p = 0.007$).

In both non-exposed groups, most associations between the elements were positive and significant (93 % in NSNExp and 79 % in SNExp), strongly suggesting common sources (Table S3). Moreover, these associations might also reflect their interactions in the human body and shared mechanisms of metabolism and excretion (Jomova et al., 2022; Witkowska et al., 2021). These firefighters belong to corporations from the same region (district of Bragança having an area of 6599 km^2; GEE, 2019), which likely results in a similar environmental exposure (through air, water, and diet) to these elements. Additionally, these firefighters spend a significant amount of their time at fire stations (72–88 % of non-exposed firefighters spend 8 or more daily hours), i.e., at the same or similar microenvironments. When comparing the correlations between all the elements in the NSNExp group with those observed in the SNExp group, their strength increased for those affected by tobacco smoke, i.e. Li and Rb (0.333 for SNExp versus 0.200 for NSNExp; $p \leq 0.04$), Cu and Zn (0.511 versus 0.456; $p < 0.001$), Cu and Cd (0.522 versus 0.409; $p < 0.001$), Cu and Pb (0.570 versus 0.358; $p < 0.001$), Pb and Zn (0.561 versus 0.481; $p < 0.001$), and Cd and Pb (0.578 versus 0.529; $p < 0.001$), thus reinforcing tobacco smoke as a dominant common source for these elements.

Biomonitoring studies with (non-recently exposed or exposed) firefighters are very limited and are exclusively focused on metal(loid)s from M1 group (Table 1). Data obtained herein were compared with findings from studies with firefighters that reported no recent exposure (Biomonitoring California, 2016; Gündüzöz et al., 2018). Although it is important to note that this comparison should be made with caution, as the urinary metal(loid) levels reported in these two studies (Biomonitoring California, 2016; Gündüzöz et al., 2018) were collected during periodic medical examinations and information about exposure is lacking (Table 1). The median Cd concentration in NSNExp firefighters was 20 % lower than that reported for a group of firefighters from California, USA, in the FOX project [some firefighters may not have been involved in firefighting activities in the previous 7 to 10 days, although this is unclear; (Biomonitoring California, 2016)], while the SNExp firefighters had a 24 % higher concentration. The median concentrations of Hg in the NSNExp and SNExp groups were 18 % and 30 % lower, respectively, than the one for the FOX project firefighters. Meanwhile, As levels were markedly higher, i.e. 53–130 % (NSNExp) and 29–95 % (SNExp), than the concentrations reported for the FOX project and Turkish firefighters (Gündüzöz et al., 2018). It is important to note that the emission and consequent exposure to metal(loid)s vary geographically (between countries and within the same country) and over time. Therefore, differences in exposure are to be expected among different populations.

3.2. Metal(loid)s post-exposure levels: Impact of wildfire emissions

To minimize the effect of background exposure to metal(loid)s when analyzing the impact of wildfire emissions, a comparison was drawn including only paired samples i.e., by analyzing the urine samples of the same subject before and after wildfire combating. Fig. 1A shows the median relative difference (%) between post- and pre-exposure for paired samples. Sb had the highest median relative increase post-exposure with statistical significance among non-smokers (50 %; $p = 0.04$), followed by Cs (40 %; $p = 0.04$), and Ni (33 %; $p = 0.02$) (Fig. 1A). Thus, only elements from group M2 were affected by wildfire exposure among non-smokers with statistical significance, and none from groups M1 or M3. Among smokers, As, from group M1 showed the highest median relative rise post-exposure (80 %; $p = 0.02$). These observed increases in Sb, Cs, Ni and As underscore the importance of promoting mitigation actions to minimize health risks in firefighters, especially since As is a proven carcinogenic to humans due to sufficient evidence for lung, urinary bladder, and skin cancer and Ni compounds for lung, nasal cavity, and paranasal sinuses cancer (IARC, 2023b). Moreover, Sb compounds are classified as probably or possibly carcinogenic to humans (IARC, 2023a).

On average, metal(loid)s represented 1.23 % and 0.91 % of the $PM_{2.5}$ and $PM_{2.5–10}$ mass, respectively, of smoke samples from several wildfires in the north and center of Portugal in 2009 (Alves et al., 2011). Additionally, Ni and As were quantified in the PM_{10} of wood smoke of trees grown in Portugal (Gonçalves et al., 2010). Sb was the most concentrated element quantified in the $PM_{2.5}$ air samples of controlled forest fires (technically also called prescribed burns) in Georgia, USA (Balachandran et al., 2013), although this was not observed in the $PM_{2.5}$ personal air samples from firefighters preforming controlled forest fires in Ohio (Wu et al., 2021), in which As was below detection limit. Meanwhile, Ni was not detected in the air samples of controlled forest fires in Georgia (Balachandran et al., 2013). Exposure depends on the wildfire PM composition, which can be highly variable geographically and over time, even within the same country. Smoke composition depends on several factors, including the burnt fuel (e.g. tree species) and its moisture content, soil characteristics, meteorological and wind conditions, fire conditions (e.g. temperature and duration), among others (Miranda et al., 2012; Wu et al., 2021). While in the north of Portugal the most common species are pine (28 %), eucalyptus (26 %), and cork oak (23 %) (ICNF, 2019), within the area where the controlled fires analyzed by Wu et al. (2021) took place, the species were mainly oak (63 %), maple (21 %), and cottonwood (9 %). When looking at potentially toxic elements according to WHO (M1 elements plus Li), their abundance increased in non-smoking from 2 % to 5 % (4 % if all firefighters are considered) and from 2 % to 3 % in smoking firefighters, underscoring the need to characterize firefighters' exposure to metal(loid)s, especially following fire combat missions. It is important to note that firefighters who smoke probably had a higher cigarette consumption before collection of the pre-exposure urine samples (i.e., no participation in firefighting activity for at least 3 days) than before the collection of the post-exposure samples, since they are less likely to smoke during fire combat activities. Hence, this may contribute to higher pre-exposure levels, fading the impact of wildfire emissions on the urinary levels of metal(loid)s among smoking firefighters.

Boxplots for the paired concentrations post- and pre-exposure of Ni, Sb and Cs (which showed statistical differences among non-smokers), and As (showed a statistical difference among smokers) are presented in Fig. 1B. Concerning non-smokers, the median concentration post-exposure compared to pre-exposure of Ni, Sb, and Cs increased significantly 39–47 % (Fig. 1B), which was also noted when all firefighters were included (41 % Cs, 53 % Ni, and 56 % Sb; $p \leq 0.02$; Fig. S3). In smokers, the median concentration of As post-exposure had a significant rise of 79 % compared to pre-exposure (Fig. 1B), which is in line with its respective median relative difference (Fig. 1A).

A comparison between the median concentrations of exposed and non-exposed firefighters was also conducted with all the participants (and not only with paired samples). Cd, which belongs to group M1 and is a proven carcinogenic for lung cancer (IARC, 2023b), showed a significant increase of 64 % ($p = 0.005$) (Fig. S2A). In group M2 (Fig. S2B), Zn, which was one of the most concentrated elements in smoke samples from wildfires occurred in Portugal, prescribed burns in the USA, and biomass burning in China (Alves et al., 2011; Huang et al., 2022; Wu et al., 2021) had an augmentation of 51 % ($p = 0.02$). Regarding group M3 (Fig. S2C), Rb had a rise of 34 % ($p = 0.03$), and Li had the biggest increase of all the elements, specifically 151 % ($p < 0.001$). It is worth noting that, despite not reaching statistical significance with paired samples, Li, experienced the most substantial increase pre- to post-exposure among non-smokers (199 % in all participants). Li, the 33rd most abundant element in Earth's crust (i.e., in rocks, soils, and waters), is widely distributed in nature primarily in compound forms (ILA, 2024), thus it can be readily remobilized during wildfires. While not included in ATSDR's, USEPA or HBM4EU priority substance/pollutant lists, and despite being used to treat bipolar disorder, Li is still a potentially toxic element according to WHO (1996), i.e., has no essential role in the human body, and excess intake can lead to complications like cardiac arrhythmia and convulsions (Shahzad et al., 2017). Given its notable increase in the urine of firefighters due to tobacco consumption and wildfire combating, this element might warrant further surveillance, especially in the context of firefighting. Among smokers in all participants, the increase of As was high, but did not reach statistical significance as seen with paired samples (58 %; $p < 0.05$).

When comparing the urinary median levels of the exposed groups (all exposed firefighters) with reference values, most elements showed similar tendencies to those observed with the non-exposed groups (Section 3.1.1). Exceptions were: i) As in the NSExp and SExp groups and Cs in the NSExp group exceeded the Canadian $RV_{95;}$ ii) Zn in smokers that was above two BE; iii) Ni in NSExp firefighters surpassed the range for healthy people according to ATSDR and the German RV_{95}. Furthermore, irrespective of smoking status, the number of firefighters whose individual metal(loid)s levels surpassed the set limits (Table 4) augmented at post-exposure to wildland firefighting in comparison to pre-exposure for: i) As: 45–90 % of firefighters at post-exposure (versus 33–68 % at pre-exposure) presented 1.1–28 times higher values than the BEI and German RV_{95} [and for smokers only (32 % (SExp) versus 21 % (SNExp)), concentrations were 1.1–3.0 times higher than the reference interval by RCPA]; ii) Cd: 5–20 % of firefighters at post-exposure (versus 1–4 % at pre-exposure) showed 1.1–1.4 times higher concentrations than the HBM-I and German RV_{95} values; iii) Zn (unadjusted units): 1–76 % of firefighters at post-exposure (versus 1–49 % at pre-exposure) displayed 1.1–4.1 times higher levels than all reference/guideline values; iv) Ni (unadjusted units): 4–65 % of firefighters at post-exposure (versus 2–36 % at pre-exposure) exhibited 1.1–2.9 times higher concentrations than the range for healthy adults and all reference RV_{95} values; v) 12–20 % of firefighters at post-exposure (versus 4–7 % at pre-exposure) for Sb, and 48–70 % of firefighters at post-exposure (versus

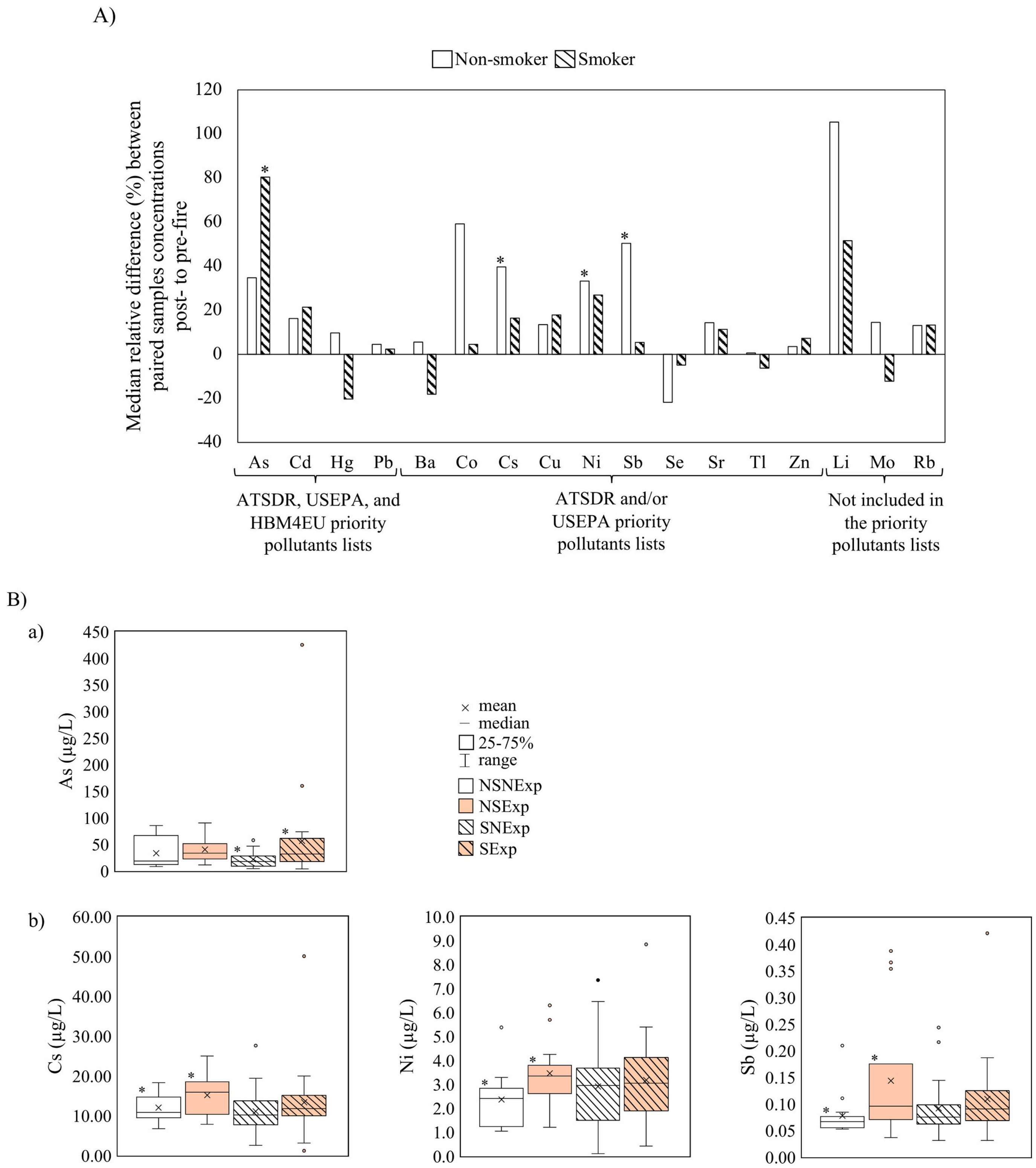

Fig. 1. A) Median relative difference (%) between the levels of post-exposure and pre-exposure to wildfire emissions among paired samples of non-smoking and smoking firefighters.

B) Concentrations (µg/L) of the of priority pollutant metal(loid)s a) included in the ATSDR, USEPA, and HBM4EU lists (As) and b) included in the ATSDR and/or USEPA lists (Cs, Ni, Sb) that showed the most relevant increases between the urinary concentrations post-exposure and pre-exposure to wildfire emissions among paired samples.

NSNExp: non-smoking non-exposed; SNExp: smoking non-exposed; NSExp: non-smoking exposed; SExp: smoking exposed.

Wilcoxon signed-rank test for paired samples: *Statistically significant differences at $p \leq 0.05$.

ATSDR: Agency for Toxic Substances and Disease Registry; HBM4EU: Human Biomonitoring for Europe Initiative; USEPA: United States Environmental Protection Agency; As: arsenic; Ba: barium; Cd: cadmium; Co: cobalt; Cs: cesium; Cu: copper; Hg: mercury; Li: lithium; Mo: molybdenum; Ni: nickel; Pb: lead; Rb: rubidium; Sb: antimony; Se: selenium; Sr: strontium; Tl: thallium; Zn: zinc.

44–45 % at pre-exposure) for Cs, exceeded the Canadian RV_{95} by 1.1–4.2 times (Table 4). Concentrations of Zn and Ni (and other metal(loid)s not determined in this study) were reported to be at several orders of magnitude higher in the firefighter ensemble, and surfaces inside vehicle cabins and fire stations from Australia than those measured in homes or offices (Engelsman et al., 2019). This might be related with the contamination of protective gear and vehicles during fire combating events, that are then transported back to the fire station, corroborating the release of Zn, Ni, and other metal(loid)s during fires.

Wolfe et al. (2004) characterized the exposure to metal(loid)s in American firefighters who fought a large vegetative fire, which also affected residential structures (72 % exposed; Table 1). However, it is important to acknowledge, as noted in the study's limitations (Wolfe et al., 2004), that the described levels could reflect background exposure instead of fire smoke exposure, given that samples were collected 2.5 weeks after fire combating. Compared to those American firefighters (Wolfe et al., 2004), the present data revealed higher geometric mean concentrations of As (242–312 %) and Cs (31–81 %), and, in contrast, lower values of Cd (33–34 %). Ni concentrations were also found to be 69–71 % lower than in firefighters from the USA (Wolfe et al., 2004), but simultaneously 83–180 % greater than the LOD in firefighters from Australia, whose median fell below this limit (94 % were under current fire exposure to urban and wildfires; 3 % smokers; Engelsman et al., 2019; Table 1), while Cu concentrations were 95–131 % higher than in these Australian firefighters. Lastly, Portuguese firefighters exhibited urinary concentrations of Se, Mo, Co, Pb, Hg, Sb, and Ba that were 7–97 % lower compared to firefighters from Australia and the USA, regardless of smoking habits (Engelsman et al., 2019; Wolfe et al., 2004).

The length of time firefighters are exposed to fire smoke influences their exposure. However, no significant positive associations were found between the number of hours firefighters spent combating wildfires and their urinary concentrations of metal(loid)s when all participants were examined. The only significant correlation was negative and observed for Se ($r = -0.623$; $p = 0.008$), which suggests a different source for this element, likely diet, its main source (often reflecting its levels in soil) (Genchi et al., 2023; Table S2). During wildfire combating, the position occupied/role during fire suppression, the type of vegetation, wind, smoke composition, PPE efficiency, among other factors, affect the exposure. Additionally, due to the challenging nature of sample collection after real life fire events, spot urine samples were collected right after the firefighters returned to the fire stations (the exact time between the end of wildfire suppression and sample collection is not known), which poses a limitation considering that each element has a different half-life varying from a few hours to several years (Mitra et al., 2022).

Age correlated positively with Cd (NSNExp and SNExp) and Pb (NSNExp and SNExp) ($0.318 < r < 0.506$; $p \leq 0.02$). Besides these elements, only Sr and Ba showed positive significant correlations with age (only in NSExp; $0.549 < r < 0.750$; $p \leq 0.01$). These elements, in addition to Hg, also showed positive correlations with firefighter's career length (Cd in NSExp and SNExp; Pb in NSNExp, NSExp, and SNExp; Sr and Ba in NSExp; Hg in SNExp; $0.269 < r < 0.796$; $p \leq 0.03$) suggesting an increasing bioaccumulation with age, potentially accompanied by continuous occupational exposure and lower elimination rates (Gasull et al., 2024). As was the only element significantly and positively correlated with BMI ($r = 0.395$; $p = 0.05$), although this was only observed in the SExp group.

The associations between elements for paired firefighters' samples post-exposure were generally lower and less significant than pre-exposure (60 % and 65 % of the correlation coefficients decreased among non-smokers and smokers, respectively). Still, the association between Li and As increased significantly post-exposure (0.705; $p = 0.005$ versus 0.407; $p > 0.05$).. Among smokers, no association between the elements with increased concentrations stood out. Additionally, unlike the predominantly positive significant associations observed between the elements in the non-exposed groups with all firefighters, the NSExp group exhibited only 13 % of positive and significant associations. This contrast between the groups indicates a notable change in the predominant sources of exposure following firefighting activities. Despite the lower correlation coefficients among NSExp firefighters, among the elements affected by wildfire emissions, the elements Zn and Sb (0.580 versus 0.434; $p \leq 0.01$), and Cd and Cs (0.448 versus 0.422; $p \leq 0.05$) had stronger correlations in the NSExp group compared to the NSNExp group. When comparing the correlations in the SExp group with the SNExp group, the change was not as pronounced, likely due to the impact of tobacco smoke in both groups.

A principal component analysis (PCA) was performed based on the paired samples of the non-smoking and smoking firefighters with the concentrations of Co, Cu, Ni, Li, As, Sr, Sb, and Cs, i.e., the elements that showed increases of at least 20 % post-fire exposure (Fig. 2). Together, PC1and PC2 explain 68 % of the original data, making a robust approximation (Table S4). PC1 and PC2 exhibit eigenvalues of 4.0 and 1.5, respectively, accompanied by a Kaiser-Meyer-Olkin sampling adequacy of 0.79. The included elements show varying degrees of correlation with each other, of which Co, Ni, and Sr displayed the strongest positive correlations (indicated by the small angle between their vectors; Fig. 2). Co, Ni, and Sr also showed relatively high Spearman correlation coefficients within the 4 groups with paired samples of firefighters ($0.464 < r < 0.940$; $p \leq 0.05$), except in the NSExp group for Ni and Co, and Ni and Sr. The majority of the NSNExp observations are located on the negative sides of both PC1 and PC2, while conversely, most NSExp observations are on the positive sides of PC1 and PC2, hence the PCA displays a clear separation between NSNExp and NSExp firefighters, in agreement with the 26–199 % increases observed for Co, Cu, Ni, Li, As, Sr, Sb, and Cs post-exposure among non-smokers. Separation also occurs between the paired samples of smokers, although not as evident as for non-smokers. While somewhat dispersed, SNExp observations are mostly on the negative side of PC2 (75 %), clustered on lower negative values (−5 to −3), and lower positive values (0–2) of PC1. Meanwhile, the bulk of the SExp observations are mostly dispersed toward positive values of F1 (−1 to 3). The observed separation among smokers is likely due to the post-exposure increase in As (79 %), Li (64 %) and Sb (20 %) that were previously observed (Fig. 1). A weaker separation of (non-exposed/exposed) smokers compared to non-smokers is in line with the previous results since Co, Cu, Ni, Sr, and Cs, did not show relatively high increases after exposure among smokers (−2 % to 15 %), unlike what was observed for non-smokers.

4. Conclusions

This study characterized the impact of real-life wildfires on the urinary levels of priority pollutant metal(loid)s among non-smoking and smoking Portuguese firefighters. Exposure to wildfire emissions affected both non-smokers and smokers, with lower increments on the latter group probably due to its higher baseline levels. Among non-smokers, wildfire emissions significantly impacted the urinary levels of Ni, Sb, and Cs among paired samples of firefighters. When all firefighters were taken into account, non-smokers' urine concentrations of Li, Cd, Sb, Ni, Zn, Cs, and Rb showed increase after exposure to wildfire emissions. Among smoking firefighters, only As was significantly affected by wildfire emissions in paired samples, while no element changed when all firefighters were considered. Thus, two elements included in the ATSDR, USEPA, and HBM4EU priority pollutants lists (As and Cd) along with some elements included in the ATSDR and/or USEPA priority pollutants lists (Ni, Sb, Cs, and Zn) showed significantly increased concentrations following wildfire combating, which is particularly relevant in terms of health risks considering their carcinogenic properties and/or potential to induce oxidative stress, among other health effects.

The urinary levels of Cu, Zn, Li, Rb, and especially Cd were significantly impacted by tobacco consumption. Across different groups, age and/or career length positively and significantly correlated with Cd, Pb, Ba, Sr, and Hg, while with BMI this was observed for As.

Several firefighters (up to 90 %) displayed individual levels of Zn, Ni,

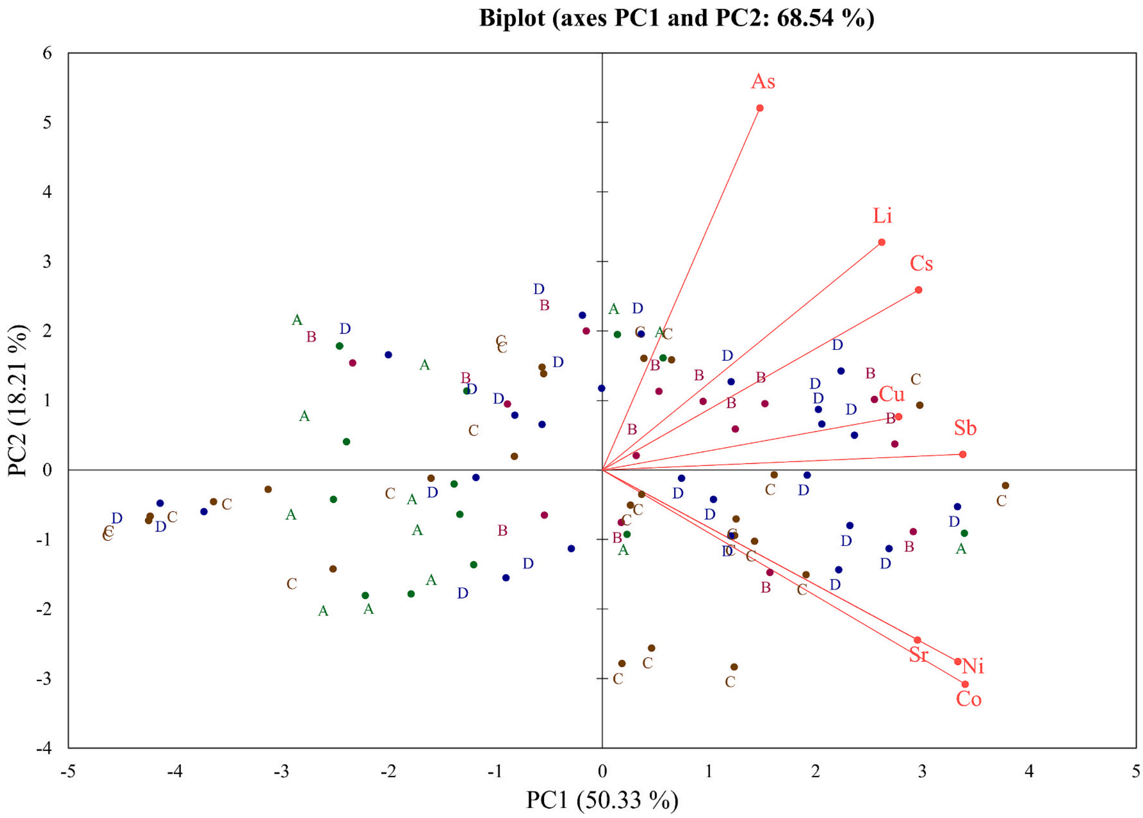

Fig. 2. Principal component analysis plots of the PC1 and PC2 based on the concentrations of Co, Cu, Ni, Li, As, Sr, Sb, and Cs in the urine of non-smoking non-exposed (A; green), non-smoking exposed (B; purple), smoking non-exposed (C; brown) and smoking exposed (D; blue) firefighters.
As: arsenic; Co: cobalt; Cs: cesium; Cu: copper; Li: lithium; Ni: nickel; Sb: antimony; Sr: strontium.

As, and Cs above their respective guidance values, as well as some firefighters (up to 20 %) for Cu, Co, Cd, Hg, Tl, Pb, and Sb, suggesting that health risks cannot be discarded. Additionally, non-exposed and exposed Portuguese firefighters appear to be more exposed to As than firefighters from other countries. A follow-up study is therefore suggested to be conducted, which should be based on a comprehensive characterization, including concurrent monitoring of fine PM fractions in the air and determination of metal(loid)s in skin, and PPE, if possible, as well as exploring possible associations with health risks, as their lack is a limitation of the present study. The collection of multiple urine samples post-fire combat to mitigate the different half-time life of the elements is also encouraged, however under real life fire scenarios, firefighters are often exhausted and dehydrated, which may pose an obstacle for this practice. Since diet (including supplement consumption) is an important source of metal(loid)s, it should be incorporated in the participant questionnaires and combined with the use of paired samples to minimize variability within each subject. The inclusion of a higher number of paired samples may also help to increase the detection of differences across firefighting exposure and work at fire station. The effect of potential variables common during firefighting activities (such as heat, exercise and sweating) and the lack of control of potential confounders measured in this study, namely age, career length, and BMI between the groups also constitute study limitations that would be interesting to further explore. Also, it might be beneficial to include a control group with participants from the general population and not just non-exposed firefighters, especially considering the lack of data regarding urinary metal(loid)s among the Portuguese population. Future studies should also prioritize exploring the impacts of wildland firefighting on urinary metal(loid) levels in women and delving into gender-specific effects, as there is a notable knowledge gap concerning this subject. Still, considering the scarcity of studies on the urinary levels of metal(loid)s, not only among firefighters worldwide, but also within the general European (including the Portuguese) population, the results of this study represent a valuable contribution toward setting reference values for these groups and raising awareness for better mitigation actions.

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Ethics approval

This work received approval for research ethics by the Accredited Ethics Committee of the University of Porto, Portugal, Report Nr. 92/CEUP/2020, under the project BioFirEx project: “A panel of (bio)markers for the surveillance of firefighter’s health and safety”.

CRediT authorship contribution statement

Ana Margarida Paiva: Writing – original draft, Methodology, Data curation, Conceptualization. **Bela Barros:** Writing – review & editing, Validation, Data curation. **Rui Azevedo:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Marta Oliveira:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Sara Alves:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Filipa Esteves:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adília Fernandes:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Data curation. **Josiana Vaz:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Maria José Alves:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Klara Slezakova:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Maria do Carmo Pereira:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization. **João Paulo Teixeira:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Solange Costa:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Agostinho Almeida:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Simone Morais:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, 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

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176105.

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