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Elemental composition of household dusts extracted in simulated body fluids and their impact on culturable pathogenic bacteria responses

Asli Baysal · Sevilay Zora · Hasan Saygin

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Abstract In the last decade, a great deal of research has focused on the determination of potential toxic elements by total concentration and identification the microorganisms in dust. However, determining bio-relevant (e.g., inhalable) forms of elements instead of total contents in acids is necessary for human health. Moreover, examination of the behavior of micro-organism under these bio-relevant conditions and revealing the interaction between elements and pathogens is vital and necessary for deeper understanding. However, previous studies have ignored these topics. Therefore, the present study aimed to (i) investigate elements in household dusts extracted in simulated lung fluids, (ii) examine the total concentration of culturable bacteria and their biochemical responses with exposure to bio-fractions of household dusts, and (iii) assess their relations and risks using the model approaches by inhalation. Here, settled dusts were collected in 25 houses, and extracted in four simulated body fluids to determine bio-fractions of elements. Moreover, total count of potentially pathogenic and heterotrophic bacteria, and four clinically important culturable pathogens were incubated in the presence of household-dusts extracted in simulated body fluids. The activity, biofilm, biochemical and oxidative responses of pathogens were measured following household-dust exposures. Afterward, the relationship between elements and pathogen responses were evaluated, and model and derived approaches were used for risk assessments of elements and pathogens. The higher daily intake of elements obtained in artificial lysosomal fluid fraction of household dust mimicking the inflammatory condition compared to other body fluids. Moreover, bacterial responses were mainly influenced from bio-fractions of household dusts and their elemental contents.

Keywords Metals · Settled indoor dust · Home · In vitro · Microorganism

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Asli Baysal, Hasan Saygin have contributed equally to this work.

A. Baysal (✉)
Department of Chemistry, Faculty of Science and Letter, Istanbul Technical University, 34469 Maslak, Istanbul, Turkey
e-mail: asli.baysal@itu.edu.tr

S. Zora
Health Services Vocational School of Higher Education, Istanbul Aydin University, Sefakoy Kucukcekmece, 34295 Istanbul, Turkey

H. Saygin
Application and Research Center for Advanced Studies, Istanbul Aydin University, Sefakoy Kucukcekmece, 34295 Istanbul, Turkey
e-mail: hasansaygin@aydin.edu.tr

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1 Introduction

There has been increasing concern on indoor air quality as people spend up to most of their time (approximately 80–90%) in indoor environments such as homes, schools, and offices (Kurt-Karakus, 2012; Tan et al., 2016), and length of stay at their homes has been increased with the COVID-19 pandemic. In the meantime, it is known that indoor air is more contaminated in comparison with outdoor air (Gohain & Deka, 2020; Tan et al., 2016). Infiltration of outdoor air containing suspended particulate matter, incomplete and inefficient burning of solid fuels, wind-driven or traffic-related suspension of road, soil, and mineral dust, sea salt and biological materials, heating, cooking are causing the settled household-dusts that include the organic and inorganic contaminants. Among varied sources of indoor air pollution, settled dust in houses might be an important source of indoor contaminants including elements, organics and bio-based substances (bacteria, fungi, etc.) (Kurt-Karakus, 2012; Tan et al., 2016; Doyi et al., 2020; Wang et al., 2020; Ruiz-Gil et al., 2023). Accumulation of a variety of settled indoor dust has vital role on microbial growth (Hameed et al., 2024). Based on previous detection studies, dominant isolated genera of opportunistic pathogenic bacteria in various indoor air and dusts were identified as *Escherichia coli (E. coli), Staphylococcus, Bacillus sp. and Pseudomonas sp*. (Guo et al., 2020; Kumar et al., 2021; Singh et al., 2022; Thompson et al., 2021). Occurrences of bacteria influence to various factors including temperature, humidity, ventilation type, occupant activities, indoor and outdoor chemical composition and contamination sources (Guo et al., 2020). It is also known that occurrence and high levels of microorganisms in indoor air also causes respiratory infections, immunomodulatory reactions, and dermal problems (Kumar et al., 2021). Humans might expose to major and minor elements and pathogens via inhalation, ingestion, or dermal contact of indoor dust and, eventually, they enter the human body (Gohain & Deka, 2020; Tan et al., 2016; Turner, 2011). Epidemiological, animal toxicological and in vitro (simulated biological) studies have indicated that inhaled particles play a major role in human health due to their chemical composition, as well as the physical presence. Once they inhaled, the fraction of contaminants was readily released into the lung fluid that appeared to be accessible and toxic to cells (Boisa et al., 2014; Liu et al., 2019). Therefore, household-dust has a significant potential impact on the human health and indoor air quality by chemical and bio-based components.

Moreover, microorganisms (e.g., pathogens, allergens, bio-based toxins) in indoor dust are more likely to introduce human lungs, triggering serious health conditions (Wu et al., 2021). Characteristics and metabolic pathways of pathogens or microbial communities in indoor dust are affected by the chemical composition of indoor dusts and their availability in the media; however, their availability and viability responses under realistic conditions mainly underestimated in the literature, and only few studies examined the behavior of specific microorganisms and their interactions with chemical components. Furthermore, previous studies have indicated that the indoor dust exposure affects the growth, biofilm formation, oxidative stress, and virulence of microorganisms (Suraju et al., 2015; Bado et al., 2017; Brągoszewska & Biedroń, 2018; Brągoszewska et al., 2020; Pompilio & Di Bonaventura, 2020; Novak et al., 2020; White et al., 2020). Unfortunately, these studies are conducted under controlled conditions, little is known about the behavior of microorganisms with exposure to real samples, specifically samples from residential areas, and physiological impact of dust exposure on microorganisms. Although intracellular microorganisms are responsible for human diseases with significant morbidity and mortality (Lagier et al., 2015).

Moreover, various studies evaluated the risk of major and minor elements in household-dusts using the model approach by the United States Environmental Protection Agency (USEPA) (Tan et al., 2016). These studies have shown that elements have potential adverse effect on human health owing to their oxidative characteristics. On the other hand, the impact of elements in indoor dusts on human health are not fully understood since the available studies mostly determined the total concentration of the tested elements in the dusts. However, humans cannot take the total amount of elements owing to their partly solubility in biological fluids, and the total element content might not be a reliable parameter for assessing the exposure risks of elements in air or dust (Hu et al., 2018; Zheng et al., 2020; Dias da Silva et al., 2015; Gao et al., 2018; Huang et al., 2018). Therefore, risk assessment based on total concentrations of elements cannot reflect the potential health risks of elements

in household-dust on human, and assessing the bio-relevant concentration of elements, instead of their total concentration, can be more realistic approach to find their potential risks, and needs extra attention in indoor settled dust under realistic conditions and the associated health risk assessment (Gohain & Deka, 2020).

To understand the bio-fractions of substances, various approaches (e.g., in vivo and in vitro) can be applied since in vivo experiments is complex, expensive, time-consuming, and great variability on inter- and intra-species of experimental animals. In vitro (simulated biological) approaches are an alternative way to measure the bio-relevant forms of substances due to being the simple, rapid, reproducible, and economic (Hu et al., 2018; Kastury et al., 2017, 2018). Moreover, various biological conditions have been applied to understand the immunologic response of organic, inorganic and biological substances. Despite a growing interest over the last decades, there has lack of available information in the field of assessing the fraction of substances, e. g., elements, that are released from dust after meeting biological fluids and their comparison using various in vitro solution (Kastury et al., 2017; Ren et al., 2020). Various extraction techniques have been used to assess the bio-relevant forms of components during inhalation, ingestion, or dermal contact (Kastury et al., 2018; Mukhtar & Limbeck, 2013). For instance, water has traditionally been applied to reflect the fluid lining the human respiratory system during inhalation tests mainly due to its neutral pH and lack of interference during element analysis. Moreover, water-soluble forms of elements often show the greatest cellular uptake and toxicity for all exposure ways (Kastury et al., 2017). Specifically in inhalation, other extraction environments can be used for the element solubility that simulated fluids lining the lung epithelium. For instance; (i) Gamble's solution is neutral lung fluids to simulate the extracellular fluid composition in the skeletal muscle and simulated the interstitial fluid deep within the lung at normal health condition, (ii) Alveolar macrophage fluid called artificial lysosomal fluid (ALF) is simulated inside the lysosome of macrophages (phagolysosomes) and mimic to inflammatory conditions, (iii) Phagolysosomal fluid (PSF) is used to formulate the alveolar macrophage fluid that includes fewer organic components, and similar ionic concentration compared to other simulants of lysosome of macrophages (Kastury et al., 2017; Stefaniak et al., 2010; Stefaniak, 2005). Moreover, phagolysosomal conditions are important since macrophages generate oxidative species, which contribute to microbial inhibition in the phagolysosome (Liu et al., 2010).

Therefore, the present study was designed to see the levels of elements in household dusts using various simulated physiological extraction approaches, and to examine the activity, biofilm formation and biochemistry of pathogens associated household dusts extracted in simulated bio-relevant conditions to understand the metabolic activities of pathogens. Moreover, the relationship between elements and pathogen responses were examined, and risk assessment model approaches were applied and proposed for elements and pathogens. The results of this study can supply a more accurate assessment of the impact of elements on bacterial behavior under realistic approaches, as well as environmental quality in household dusts under various health conditions on children and adults.

2 Materials and methods

2.1 Sampling

A total of 25 household dust samples were collected in four urban districts in Istanbul-Turkey from February 2020 to March 2020 (Supplementary Fig. 1). Istanbul is a megacity with 13–17 million inhabitants and responsible for 40% of Turkey's industrial activities (Ozbek & Baysal, 2016; Saygin et al., 2023a). There are no social-economic differences between sampling areas, and these areas are both highly populated residential areas and have high industrial organizations. Dust samples were obtained from vacuum cleaner bags in regular use of participating volunteers for the purpose of cleaning homes, and details were presented our previous study (Saygin et al., 2023b). The information about the houses (e.g., floor, m^2, age of building) was recorded. The dust samples were air-dried (minimum 24 h), followed by manual removal of large particles, and then sieved to < 100 µm, stored in freezer for further analysis (Kurt-Karakus, 2012).

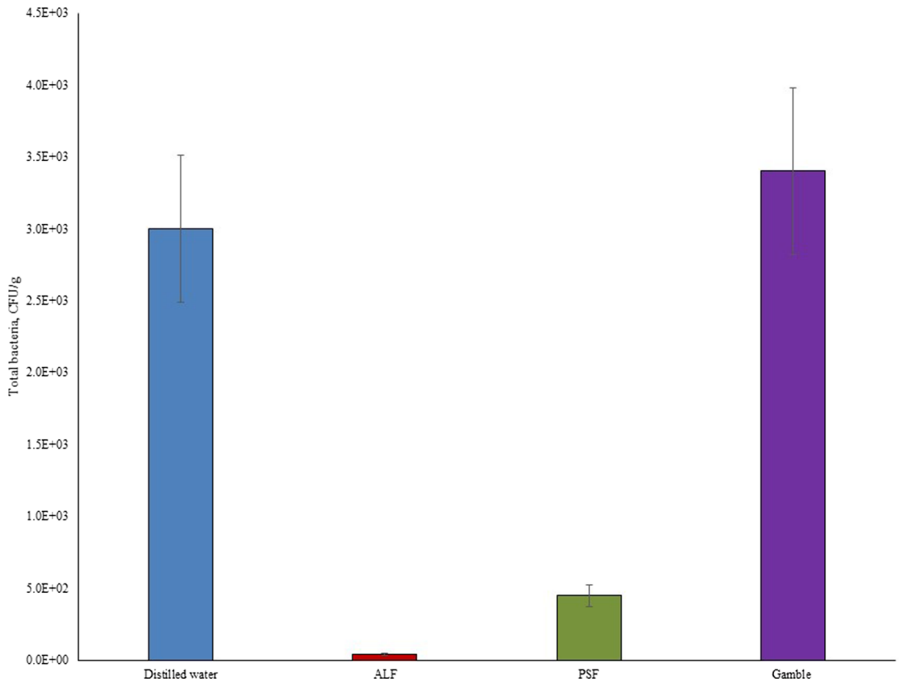

Fig. 1 Total bacteria count (CFU/g) in household-dusts extracted in simulated lung fluids

2.2 Simulated bio-fluids and analytical methods for extractions

The sieved and homogenized dust samples were divided into equal portions for the extraction with bio-relevant solutions (water, ALF, PSF, and Gamble's solution). The chemical compositions of the extraction solutions were shown in Supplementary Table 1. A 0.5 g portion in each dust samples were extracted in 50 mL (solid-to-liquid ratio was 1:100) in each simulated biological solution (water, ALF, PSF and Gamble's solution). Then the samples agitated during 24 h under 37 °C, centrifuged and filtrated with a 0.45 µm syringe filter successively (Kastury et al., 2017). Before filtration, a volume of extracts were removed and used for total bacteria count.

2.3 Determination of bio-fractions of elements

All the extracting solutions were stored at 4 °C before instrumental analysis with Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Spectro Blue- Spectro, Kleve-Germany). For ensuring the quality and accuracy in the sample preparation and measurements, blank measurements and triplicate measurements were taken. A external calibration was performed for each element prior to the analysis of samples, and the calibration curves with $R^2 > 0.9994$ were accepted for concentration calculation. Blank correction was applied before the calculation, and standard deviation reference material (SRM 2783) analysis was used and > 95% recoveries were obtained (Baysal et al., 2017). In any cases, we also determined the total concentrations of the elements in all household dust samples using acid digestion method.

2.4 Total *bacteria* in bio-fractions of household-dusts

After extraction in different simulated bio-fluids, household extracts were streaked onto plates, and the potentially pathogenic and heterotrophic bacteria were cultivated in Tryptone Soy Broth (TSB, Merck, Germany) containing 1.5% (w/v) agar at 37 °C for 24–48 h (Bouillard et al., 2005; Raisi et al., 2013). Total bacterial were counted as colony forming units per gram of dust (CFU/g).

2.5 Activity, biofilm formation, main biochemical and oxidative stress responses of pathogens

Escherichia coli (E. coli) (ATCC 35218), *Staphylococcus aureus (S. aureus)* (ATCC 25923),

Table 1 Classification for elements and pathogens

Risk characterization			Class	
For elements	Geoaccumulation ındex	Igeo	$Igeo \leq 0$	Practically uncontaminated
			$0 \leq Igeo \leq 1$	Uncontaminated to moderately contaminated
			$1 \leq Igeo \leq 2$	Moderately contaminated
			$2 \leq Igeo \leq 3$	Moderately to heavily contaminated
			$3 \leq Igeo \leq 4$	Heavily contaminated
			$4 \leq Igeo \leq 5$	Heavily to extremely contaminated
			$5 < Igeo$	Extremely contaminated
	Hazard quotients	HQ	$HQ \leq 1$	Unlikely the adverse health effect
			$HQ > 1$	Potential non-carcinogenic health effect
	Hazard ındex	HI	$HI \leq 1$	Unlikely the adverse health effect
			$HI > 1$	Potential non-carcinogenic health effect
	Cancer risk	CR	$CR < 1 < \times 10^{-6}$	No significant health effect
			$1 \times 10^{-6} < CR < 1 \times 10^{-4}$	Acceptable or tolerable cancer risks
			$CR > 1 \times 10^{-4}$	Carcinogenic risks
For pathogens	Pathogen risk	PR	$OD \leq ODc$	No risk
			$ODc < OD \leq 2 \times ODc$	Weak risk
			$2 \times ODc < OD \leq 4 \times ODc$	Moderate risk
			$4 \times ODc < OD$	Strong risk
	Contamination degree of pathogen	$CD_{pathogen}$	$CD_{pathogen} < 1$	Low contamination
			$1 \leq CD_{pathogen} < 3$	Moderate contamination
			$3 \leq CD_{pathogen} < 6$	Considerable contamination
			$CD_{pathogen} \geq 6$	Very high contamination

Pseudomonas aeruginosa (P. aeruginosa) and *Staphylococcus epidermidis (S. epidermidis)* (ATCC 35984) were selected as a model pathogen approach and used during the experiments. The bacteria were cultivated and incubated at 37 °C for 24 h. Tryptic Soy Broth (TSB) medium was prepared and autoclaved at 121 °C for 15 min. Simulated bio-relevant-suspended household-dusts were diluted into TSB medium. Saturated cultures of *E. coli, S. aureus, P. aeruginosa, S. epidermidis* grown in TSB medium including simulated bio-relevant-suspended household-dusts at 37 °C for 24 h. For controls, only TSB and cultures in TSB medium was applied (Suraju et al., 2015). The optical density (OD) of the samples were measured at 600 nm 96 well plates for the pathogen activity. The biochemical pathways of the activity were examined through protein, carbohydrate, oxidative responses (antioxidant content and lipid peroxidation) according to our previous studies (Saygin & Baysal, 2022). Protein, carbohydrate, total antioxidant and lipid peroxidation contents of cells were determined by Bradford, the phenol sulfuric acid, CUPRAC and thiobarbituric acid methods, respectively (Saygin & Baysal, 2022; Saygin et al., 2023).

For the biofilm formation, the crystal violet (CV) method was applied using microplate (O'Toole, 2011; Suraju et al., 2015). After 24 h of incubation of saturated cultures of *E. coli, S. aureus, P. aeruginosa,* and *S. epidermidis* grown in TSB including simulated bio-relevant-suspended household-dusts at 37 °C for 24 h, the growth culture medium was collected, and a volume of each culture media (200 µL) were transferred to 96-well plates (Saygin et al., 2024). After incubation, the supernatant was removed from the plates, and plates were washed three times using normal saline. Then, 100 µL of 0.01% CV solution was added to wells and dried for 15 min. At the end of the period, excess CV was removed by washing with sterile water (Xu et al., 2016). Finally, the fixed CV was mixed with 95% ethanol, and mixture absorbance measured at 595 nm. Controls were similarly applied.

2.6 Model approaches for risk assessments

To evaluate the risks of the elements extracted in simulated bio-fluids, pollution indices and health assessments were applied. For the realistic approach, we used concentrations of bio-fractions of the determined elements for the calculation of pollution indices and health assessments.

To assess the anthropogenic impact of the bio-fractions of the elements, we used modified form of the geo-accumulation index (Igeo) (Baysal & Akman, 2018; Baysal & Saygin, 2022; Jayarathne et al., 2018; Li et al., 2019; Liu et al., 2019). Igeo evaluates the pollution level of elements in household-dust by comparing the present concentration with pre-industrial levels (Baysal & Akman, 2018; Baysal & Saygin, 2022; Jayarathne et al., 2018; Li et al., 2019; Liu et al., 2019). The Igeo was calculated using Eq. (1) and was classified according to Table 1:

$$\mathrm{Igeo} = \log2\left(C_{\mathrm{sample}}/1.5 \times C_{\mathrm{background}}\right) \quad (1)$$

where C_{sample} and $C_{\mathrm{background}}$ are the bio-relevant concentrations of the elements in household-dust sample and background, respectively. Factor 1.5 was applied as the background matrix correction value.

Human exposure risk to household-dust contaminants is often estimated by comparing respiratory intake or inhalable dose with an acceptable dose (Liu et al., 2016, 2019). In this study, the daily exposure of elements (daily intake (DI)) for inhalation pathway was estimated using a modified version and bio-fraction concentration of elements in Eq. 2 (Liu et al., 2016).

$$\mathrm{DI}_{\mathrm{inh}} = C \times \frac{\mathrm{Inh}R \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{PEF} \times \mathrm{BW} \times \mathrm{AT}} \quad (2)$$

DI (mg/kg.day): dose contacted (daily intake) through inhalation ($\mathrm{DI}_{\mathrm{inh}}$). C (mg/kg) is the content of the elements in each bio-fluids. Input assumptions indicated in Supplementary Table 2.

According to USEPA's guideline on human health risk assessment of elements (USEPA, 1989; USEPA 2009), carcinogenic and non-carcinogenic risks of dusts were assessed using separate models. For estimating non-carcinogenic effects, USEPA has established reference dose (RfD) as indicated in Supplementary Table 2, an estimate of daily exposure of human population to contaminants that would unlikely cause adverse effects (USEPA, 2009). The non-carcinogenic risk of inhalation of dusts was assessed based on the Hazard Quotient (HQ) (Onat et al., 2020).

$$\mathrm{HQ}_{\mathrm{inh}} = \frac{\mathrm{DI}_{\mathrm{inh}}}{\mathrm{RfDI}_{\mathrm{inh}}} \quad (3)$$

The cancer risk (CR) is applied to estimate that an individual exposure to carcinogenic hazards during a lifetime.

$$\mathrm{CR} = \mathrm{DI} \times \mathrm{SF} \quad (4)$$

where DI is inhalation ($\mathrm{DI}_{\mathrm{inh}}$), and SF cancer slope factor for each element extracted in bio-relevant

Table 2 Concentration of elements in household dust with the extraction of various simulated bio-relevant fluids

Elements	Simulated bio-relevant fluids			
	Water	ALF	PSF	Gamble's solution
Al, µg/g	108.38 ± 74.46	316.36 ± 284.37	85.00 ± 37.63	40.40 ± 47.93
Cr, µg/g	3.71 ± 2.51	6.21 ± 7.57	5.06 ± 4.86	4.21 ± 3.81
Cu, µg/g	11.41 ± 8.40	28.75 ± 23.60	17.61 ± 15.28	13.12 ± 12.25
Mn, µg/g	6.90 ± 3.99	45.39 ± 24.35	31.91 ± 12.92	8.14 ± 4.31
Ni, µg/g	4.68 ± 4.08	8.62 ± 5.82	8.32 ± 10.37	3.82 ± 3.03
Zn, µg/g	45.61 ± 30.04	374.01 ± 231.31	243.66 ± 118.93	34.61 ± 17.07
Ca, mg/g	1.77 ± 1.20	22.81 ± 11.38	9.43 ± 3.54	2.05 ± 2.71
K, mg/g	2.89 ± 1.07	10.18 ± 16.41	1.75 ± 3.21	1.93 ± 2.18
Mg, mg/g	1.23 ± 0.89	1.35 ± 1.20	1.17 ± 0.87	1.36 ± 1.23
Na, mg/g	4.27 ± 2.26	3.15 ± 5.31	2.37 ± 4.96	0.23 ± 0.94
P, mg/g	0.11 ± 0.10	0.50 ± 0.27	0.13 ± 0.17	0.02 ± 0.06
Fe, %	6.10 ± 4.47	62.43 ± 91.50	8.28 ± 7.45	3.46 ± 3.27

solution indicated in Supplementary Table 2. HQ and CR classifications are given in Table 1.

To assess the pathogen risk respecting the simulated bio-relevant conditions, two models were derived and proposed. The first risk model approach was derived from biofilm formation classification by Christensen et al. (1985) classification and called Risk of Pathogen (PR). The risk assessment is based on the relationship between the OD response of the strain in the relevant simulated biological condition including household dust sample and the OD of their control medium (no household dust, OD_c).

The second model approach was derived from Contamination Factor of elements (CF). In the pathogen related approach, contamination factor of pathogen which identified Contamination Degree of Pathogen ($CD_{pathogen}$) is the ratio between the OD of household-dust sample in each bio-relevant medium included strain to the control values in strain as explained in Eq. (5):

$$CD_{pathogen} = OD_{sample}/OD_{control} \quad (5)$$

For this purpose, $OD_{control}$ includes strain cultivated in each bio-relevant medium with no household-dust sample and OD_{sample} is contained strain cultivated in bio-relevant medium with household-dust sample.

2.7 Statistical analysis

The correlation coefficient (r) and the correlation significant t-test were determined. Pearson correlations, Student's t-test was used to estimate the significant difference between the mean concentrations of element, pathogen, and biochemical parameters.

3 Results and discussion

3.1 Characterizing inhalable bio-fractions of elements in household dusts

As indicated in Table 2, Al, Cr, Cu, Mn, Ni and Zn were determined in bio-fractions of household-dusts. The average bio-relevant concentrations of Al, Cr, Cu, Mn, Ni and Zn decreased according to the following sequence: ALF > water > PSF > Gamble's solution, ALF > water > Gamble's solution > PSF, ALF > water > PSF > Gamble's solution, ALF > water > Gamble's solution > PSF, ALF > water > Gamble's solution > PSF, and ALF > PSF > Gamble's solution > water, respectively. These results showed that the solubility of these elements was greater in the ALF conditions, which mimics inflammatory conditions, compared to other simulated bio-fractions of household-dusts. This can be explained that ALF is reflected inside the lysosome of macrophages (phagolysosomes) having a more acidic condition; thus, dissolution of element is generally higher in this condition compared to the neutral lung fluid (Kastury et al., 2018). Although PSF has same pH with ALF, which is 4.5 reflecting the alveolar macrophage and phagolysosomal fluid, the dissolution of the elements was not higher in PSF compared to ALF. This result can be explained by simpler composition of the PSF limiting the dissolution compared to ALF (Innes et al., 2021; Ren et al., 2020). Moreover, the solubility in ALF also depends on the chemical state of the element. For example, element oxides, carbonates, chlorides are known to be easily soluble in this media. This result suggested the elements in household-dust can be in the form of oxides, carbonates, and chlorides rather than sulfite, phosphate, and silico (Innes et al., 2021). However, there have been limited study to compare the findings since previous studies mostly conducted to find total amount of elements using different strong acid digestion, not to determine bio-accessible forms, and the studies mainly applied in outdoor and soil samples (Pelfrêne et al., 2017; Ren et al., 2020; Innes et al., 2021). Moreover, metals and their levels in household dusts vary due to various factors including climate, deposition, social habits, region, air pollution, composition, content, and digestion conditions; however, bio-accessible fraction of heavy metals in household dust mainly underestimated (Wu et al., 2024).

The results are assessed using major and trace elements. The results indicated that the average trace element concentrations of water and ALF fractions of household-dust are the order of Al > Zn > Mn > Cu > Cr > Ni whereas it was Zn > Al > Mn > Cu > Ni > Cr and Al > Zn > Mn > Cu > Ni > Cr for PSF and Gamble's fractions, respectively. The order showed that crustal element (e. g., Al) was the predominant element found in all bio-fractions of household-dusts; while, toxic elements such as Cu, Cr, and Ni were at lower

levels. Furthermore, Al and Mn oxides/hydroxides in soils are typically nanosized, possess large surface areas and internal porosities. These properties are the keys to the adsorption and transport of ions such as toxic elements, nutrients, etc. (Barron & Torrent, 2013; Liu et al., 2016). Moreover, the higher contents of Zn and Mn can be originated by the road and windblown dust, biomass burning and traffic activities (Eneji et al., 2015; Ogundele et al., 2018). These results correlated the study of Hu et al. (2018), who reported the in vitro inhalation/ingestion solubility of the airborne particle-bound elements in room air conditioners filters. In the study of Wu et al. (2024), trace elements in house-dusts were determined using simulated gastric and intestinal fluids. Similar with our study, higher concentrations of Zn, Cu, Mn, and Ni were determined in simulated gastrointestinal fluids at lower pH. The levels were also compared using soil reference values which is the standard of Chinese National Soil Quality Standards-National Environmental Protection Agency 1995 are employed to assess level of elements in dusts (Duzgoren-Aydin et al., 2006; Zhang et al., 2018). Because available international standards regarding indoor air have limit values for carbon dioxide, formaldehyde, particulate matter, nitrogen dioxide, carbon monoxide, and radon (Settimo et al., 2020; Paleologos et al., 2021; Morawska & Huang, 2022; Dimitroulopoulou et al., 2023). In addition, Türkiye has not any national standards related to indoor air. The concentration of Cu, Ni and Zn in the household dusts in various bio-fractions were considerably higher than those of Grade I soils, which highlights the impact of anthropogenic contribution. Moreover, Cr concentration were changed between 6.21 ± 7.57 µg/g and 3.71 ± 2.51 µg/g for the tested simulated bio-fluids (Table 2). These levels were also compared with World Health Organization permissible limit of Cr and it is 1.3 mg/kg for plants (World Health Organization, Permissible limits of heavy elements in soil and plants. Geneva, Switzerland, 1996). Cr concentration in all simulated bio-fractions was greater than this permissible limit. Thus, the potential human health effect from determined elements in bio-fractions cannot be ignored in the household-dust samples.

To understand source of trace elements, thee correlations were analyzed (Supplementary Table 3). The correlation analysis showed that there had strong correlation between Cu and Cr in ALF and Gamble's solution, and moderate correlation obtained between these elements in PSF representing the vehicular traffic-related emissions and paints in these conditions (Jadoon et al., 2021). These results showed that the occurrence of these potentially toxic elements had no strong relationship with adsorption and transporting the toxic elements with soil Al and Mn content. The weak positive correlation was found between Cr and Al and Mn in water, Cr and Mn in ALF, Cu and Ni with Al and Mn in PSF, and Cu and Zn and Al in Gamble's solution. The moderate positive relationship indicated between Cu and Mn in water, Cr and Ni and Al in Gamble's solution.

Moreover, to characterize the major element components related the biochemical reactions, Ca, Mg, Na, K, Fe and P were evaluated in these bio-fractions of household-dusts (Table 2). Similar with the potentially toxic elements, the levels of the major element components were greater in the ALF due to the acidic and complex environment of the solution compared to other tested bio-fractions (e.g., water, PSF, and Gamble's solution) (Innes et al., 2021). The order of the major elements in water, ALF, PSF and Gamble's solution were $Fe > Na > K > Ca > Mg > P$, $Fe > Ca > K > Na > Mg > P$, $Ca > Fe > Na > K > Mg > K$ and $Fe > Ca > K > Mg > Na > P$, respectively. The highest concentration of Fe compared to other major elements can be explained that Fe is an abundant element in the earth crust and has been transported via wind. It can be also associated with soil or street dust, and originated by coal burnt in outdoor environment (Eneji et al., 2015). The higher contents of Ca, Na, Mg, and K clearly showed the impact of soils, paints, and cements on household-dusts and their bio-fractions. Moreover, use of insecticides and chemicals for the general cleaning of the house might contribute to K levels (Christensen et al., 1985).

3.2 Total *bacteria* counts in bio-fractions of household-dusts

The mean count of bacteria in bio-fractions of household-dusts were 2981 ± 767, 43 ± 17, 375 ± 89, and 3438 ± 1109 CFU/g in water, ALF, PSF and Gamble's solution, respectively (Fig. 1). The higher content of bacteria in water and Gamble's solution can be explained by pH and chemical content of Gamble's solution. In addition, the lower levels of bacteria in

Table 3 Daily intake (DI), Non-carcinogenic and carcinogenic risks (CR) from potentially toxic elements exposure to house dusts from Istanbul-Turkey

Element	DI inhalation				HQ inhalation		CR inhalation	
	Child		Adult		Child	Adult	Child	Adult
	Non-carcinogens	Carcinogens	Non-carcinogens	Carcinogens				
Water								
Al	3.72E−08	3.19E−09	8.73E−08	7.49E−09	x	x	x	x
Cr	1.27E−09	1.09E−10	2.99E−09	2.56E−10	4.24E−06	9.97E−06	1.64E−10	3.84E−10
Cu	3.92E−09	3.36E−10	9.2E−09	7.88E−10	9.79E−08	2.3E−07		
Ni	1.61E−09	1.38E−10	3.77E−09	3.23E−10	8.03E−08	1.89E−07	1.16E−10	2.72E−10
Zn	1.57E−08	1.34E−09	3.68E−08	3.15E−09	5.22E−08	1.23E−07	x	x
Mn	2.37E−09	2.03E−10	5.56E−09	4.77E−10	1.69E−08	3.97E−08	x	x
Total	6.2E−08	5.31E−09	1.46E−07	1.25E−08	4.49E−06	1.05E−05	2.79E−10	6.56E−10
ALF								
Al	1.09E−07	9.3E−09	2.55E−07	2.19E−08	x	x	x	x
Cr	2.13E−09	1.83E−10	5E−09	4.29E−10	7.1E−06	1.67E−05	2.74E−10	6.43E−10
Cu	9.86E−09	8.45E−10	2.32E−08	1.99E−09	2.47E−07	5.79E−07	x	x
Ni	2.96E−09	2.54E−10	6.95E−09	5.95E−10	1.48E−07	3.47E−07	2.13E−10	5E−10
Zn	1.28E−07	1.1E−08	3.01E−07	2.58E−08	4.28E−07	1E−06	x	x
Mn	1.56E−08	1.33E−09	3.66E−08	3.13E−09	1.11E−07	2.61E−07	x	x
Total	2.67E−07	2.29E−08	6.28E−07	5.38E−08	8.04E−06	1.89E−05	4.87E−10	1.14E−09
PSF								
Al	2.92E−08	2.5E−09	6.85E−08	5.87E−09	x	x	x	x
Cr	1.73E−09	1.49E−10	4.07E−09	3.49E−10	5.78E−06	1.36E−05	2.23E−10	5.24E−10
Cu	6.04E−09	5.18E−10	1.42E−08	1.22E−09	1.51E−07	3.55E−07	x	x
Ni	2.86E−09	2.45E−10	6.71E−09	5.75E−10	1.43E−07	3.35E−07	2.06E−10	4.83E−10
Zn	8.36E−08	7.17E−09	1.96E−07	1.68E−08	2.79E−07	6.54E−07	x	x
Mn	1.1E−08	9.39E−10	2.57E−08	2.2E−09	7.82E−08	1.84E−07	x	x
Total	1.34E−07	1.15E−08	3.16E−07	2.7E−08	6.43E−06	1.51E−05	4.29E−10	1.01E−09
Gamble's solution								
Al	1.39E−08	1.19E−09	3.26E−08	2.79E−09	x	x	x	x
Cr	1.45E−09	1.24E−10	3.39E−09	2.91E−10	4.82E−06	1.13E−05	1.86E−10	4.36E−10
Cu	4.50E−09	3.86E−10	1.06E−08	9.06E−10	1.13E−07	2.64E−07	x	x
Ni	1.31E−09	1.12E−10	3.08E−09	2.64E−10	6.56E−08	1.54E−07	9.44E−11	2.22E−10

Table 3 (continued)

Element	DI inhalation				HQ inhalation			CR inhalation	
	Child		Adult		Child	Adult	Child	Adult	
	Non-carcinogens	Carcinogens	Non-carcinogens	Carcinogens					
Zn	1.19E−08	1.02E−09	2.79E−08	2.39E−09	3.96E−08	9.30E−08	x	x	
Mn	2.79E−09	2.39E−10	6.56E−09	5.62E−10	2.00E−08	4.69E−08	x	x	
Total	3.58E−08	3.07E−09	8.40E−08	7.20E−09	5.06E−06	1.19E−05	2.8E−10	6.58E−10	

ALF and PSF can be originated by the acidic condition of these medium. The published studies indicated that the survival of the bacteria can be related to various factors including temperature, pH, chemical composition of the environment (Singh et al., 2022). Previous studies also showed that pathogenic bacteria concentration were declined when culturing at pH 6 compared to pH 7 and 8 (Chan et al., 2019). Various studies have been globally conducted to assess microbial content in indoors including hospitals, schools, offices, industrial areas; however, these studies have limited in residential areas compared to other indoor areas and outdoors (Guo et al., 2020; Kumar et al., 2021). For instance, Sidra et al. (2015) observed the bacteria concentration at 275–14,469 CFU/m^3 in living rooms of Lahore, Pakistan; Kumar et al. (2021) were counted bacteria level at 738 ± 443.59–1654 ± 876.87 CFU/m^3 residential houses in Delhi. Hameed et al. (2024) was also studied microorganisms and ionic composition in the settled dusts collected from museum with a similar experimental manner, and they found that bacteria 2.7×10^4–8.02×10^6 CFU/g in restoration lab, hunting hall, Thorne, residence, reception hall, and outdoor areas of the museum. Higher bacteria counts and ionic compound levels were detected in indoor areas compared to outdoors, and they indicated that microbial concentrations changed by ion nature and its concentration.

3.3 Impact of the inhalable bio-fractions of household-dusts on pathogen behaviors

The bacterial activity results showed that bio-fractions of household-dusts have a significant role on the activity of gram-negative *E. coli* and *P. aeruginosa,* and gram-positive *S. aureus* and *S. epidermidis* (Fig. 2). The activity of *E. coli* and *P. aeruginosa* with the impact of household-dusts declined according to the following sequence: PSF > Gamble's solution > water > ALF, and the activity order of *S. aureus* and *S. epidermidis* is Gamble's solution > PSF > water > ALF. Different responses in simulated bio-fluids referred to importance of physiological conditions on the microorganisms. For example, the activity of gram-negative *E. coli* and *P. aeruginosa* was higher in PSF compared to other bio-fractions (e.g., water, ALF, and Gambles' solution). The higher activities in the PSF and Gamble's

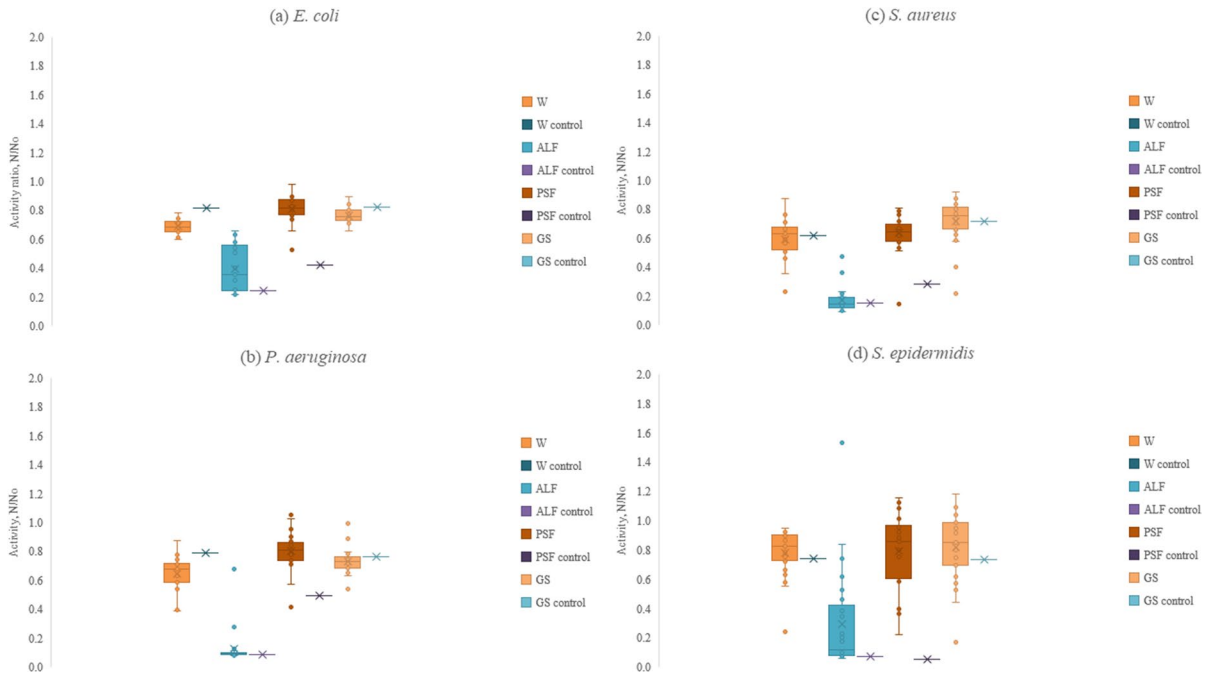

Fig. 2 Box and whisker chart view of the pathogen cultivation in house dust collected in Istanbul-Turkey using different simulated bio-relevant fluids. **a** *E. coli*, **b** *P. aeruginosa*, **c** *S. aureus*, **d** *S. epidermidis.* (W: water, ALF: artificial lysosomal fluid, PSF: phagolysosome simulant fluid, GS: Gamble's solution, N/No meaning N: culture with household dust extracted in bio-relevant fluids, No: culture under controlled condition-no household dust and bio-relevant condition)

solution indicated that the tested pathogens can be easily activated under these lung conditions (PSF and Gamble's solution). These lung conditions are important for the cell responses since PSF reflects the intraphagolysosomal dissolution at acidic pH and it is believed to be a necessary step in the cellular immune response, and Gamble's solution used to simulate the neutral pH extracellular environment of the lung (Innes et al., 2021; Stefaniak et al., 2005; Zupančič et al., 2021). These higher bacterial activities are also critical role on human health and indoor air quality since the tested bacteria strains were determined in many indoor and outdoor air/dust samples. However, bacterial responses due to exposure to air-related samples (particulate matter and dusts) have been limitedly examined in the literature. Some studies in this field examined the bacterial responses through standard reference materials. For instance, Stapleton et al. (2020) examined the mechanisms of respiratory infections through bacterial growth and biofilm formation *S. aureus* and *P. aeruginosa* with the exposure of standard reference material (NIST Standard Reference Material 2584 IAP) and indoor air particulate matter from USA houses. The results indicated that both air particles enhanced bacterial growth and biofilm formation. In another study, the impact of indoor SRM 2585- and outdoor BCR 723-dust standard reference materials exposure on the bacteria (*E. coli, Enterococcus faecalis,* and *P. aeruginosa*) was assessed using bacterial growth, oxidative stress resistance, and biofilm production under nutrient-poor and nutrient-rich conditions (Suraju et al., 2015). The results showed that growth of all three bacterial increased in nutrient-poor conditions, but slowed in nutrient-rich conditions. Similarly, Bado et al. (2017) investigated the effect of house dust (SRM 2585) and road dust (BCR 723) standard reference material exposure on the growth, protein, oxidative and biofilm formation of *K. pneumoniae, E. coli,* and *P. aeruginosa.* The results indicated that house and road dust exposure had influenced the bacterial growth, enhanced oxidative stress resistance, and biofilm formation owing to chemical composition. Deng et al. (2018) examined the growth of *S. epidermidis* and *E. coli* with exposure to natural dust samples from indoor and outdoor living area, traffic

section, industrial factory, and mine. The findings showed that bacterial growth was promoted by dust types with a rising in the concentration of Fe, Ca, Ni, Si, and Al in the culture. Unfortunately, the available studies mostly conducted under controlled conditions, without impact of physiological conditions or real samples. In our previous study, we investigated *S. aureus* and *E. coli* responses including viability, oxidative stress, biofilm formation with the impact of physiological conditions of indoor PM2.5 collected from university laboratory air (Saygin et al., 2023). The bacteria were affected by indoor PM2.5 and physiological conditions due to different in pHs.

Furthermore, the activity levels of pathogens were varied in the presence of bio-fractions of household-dusts and the sequence of the activities was *S. aureus* > *S. epidermidis* > *P. aeruginosa* > *E. coli*, *E. coli* > *S. epidermidis* > *S. aureus* > *P. aeruginosa*, *P. aeruginosa* > *S. aureus* > *E. coli* > *S. epidermidis* and *S. aureus* > *P. aeruginosa* > *S. epidermidis* > *E. coli* in water, ALF, PSF, and Gamble's solution fractions of household-dusts, respectively. The difference between the bacteria levels to the anthropogenic contaminants can be commonly explained by the bacterial cell-wall properties and the dissolution level of substances with the bio-fluids.

To understand the background on the activity responses, the main biochemical (e.g., protein and carbohydrate) and oxidative (antioxidant and LPO) indicators were examined (Fig. 3 and 4). The results showed that protein, carbohydrate, antioxidant and LPO activities declined with the presence of bio-fractions of household-dusts compared to the controls, except carbohydrate levels of gram-negative *P. aeruginosa* and *E. coli*. For gram-negative bacterium, protein, carbohydrate, and antioxidant levels had similar trend with the bio-fractions of household-dusts. The protein, carbohydrate, and antioxidant responses declined following order: PSF > Gamble's solution > water > ALF, ALF > PSF > water > Gamble's solution and Gambles' solution > PSF > water > ALF for, respectively. The response of the LPO activity decreased the following order for *E. coli* and *P. aeruginosa*: water > Gamble's solution > PSF > ALF and PSF > Gamble's solution > water > ALF, respectively. The Pearson correlation analysis also indicated that the activity of *E. coli* with the fractions in water, PSF and Gamble's solution were positively correlated with protein and antioxidant, whereas it was positively correlated with protein, antioxidant and LPO in ALF fraction of household-dusts (Supplementary Table 4). The activity of *P. aeruginosa* had positive correlation with protein and antioxidant in water and PSF fraction of household-dusts; however, the activity of *P. aeruginosa* had positive linked with antioxidant in ALF fraction, and protein in Gamble's solution fraction (Supplementary Table 4). The biochemical responses of the gram-positive *S. aureus* and *S. epidermidis* were similar in protein, carbohydrate and antioxidant levels and the protein, carbohydrate, and antioxidant responses in bio-fractions of household-dusts were decreased following sequences: Gambles' solution > PSF > water > ALF, ALF > Gamble's solution > PSF > water, and Gamble's solution > PSF > water > ALF, respectively. However, the LPO activity of *S. aureus* and *S. epidermidis* was changed as Gamble's solution > PSF > water > ALF and PSF > water > Gamble's solution > ALF, respectively. The Pearson correlation results also indicated that *S. aureus* activity had significant positive and negative correlations with protein and carbohydrate in water fractions, whereas it has positive correlations between protein and antioxidant in ALF fraction, protein, antioxidant and LPO in PSF fractions, and protein and antioxidant in Gamble's fraction (Supplementary Table 4). The activity of *S. epidermidis* was significantly changed with the impact of its protein and antioxidant activities in water and ALF fractions of household-dusts, whereas, the activity showed significant positive dependence to the protein, antioxidant and LPO activity in PSF fraction. In addition to positive correlation to the protein, antioxidant and LPO activity, the activity of *S. epidermidis* had significant negative correlation to the carbohydrate response. These results indicated the various bio-relevant conditions influenced the main metabolism pathways and oxidative indicators of the tested pathogens with different ways. The higher protein and carbohydrate levels indicated the promotion of bacterial growth since they are related to main biochemical metabolism (Saygin et al., 2023). The reduced antioxidant can be originated the oxidative species (de Paula Ribeiro et al. 2020). LPO has been used as a useful parameter of bacterial metabolism, and higher levels indicate the presence of anthropogenic contaminants (Hassanshahian et al., 2010).

Another important pathogen activity is biofilm production. The analysis showed that the exposure to

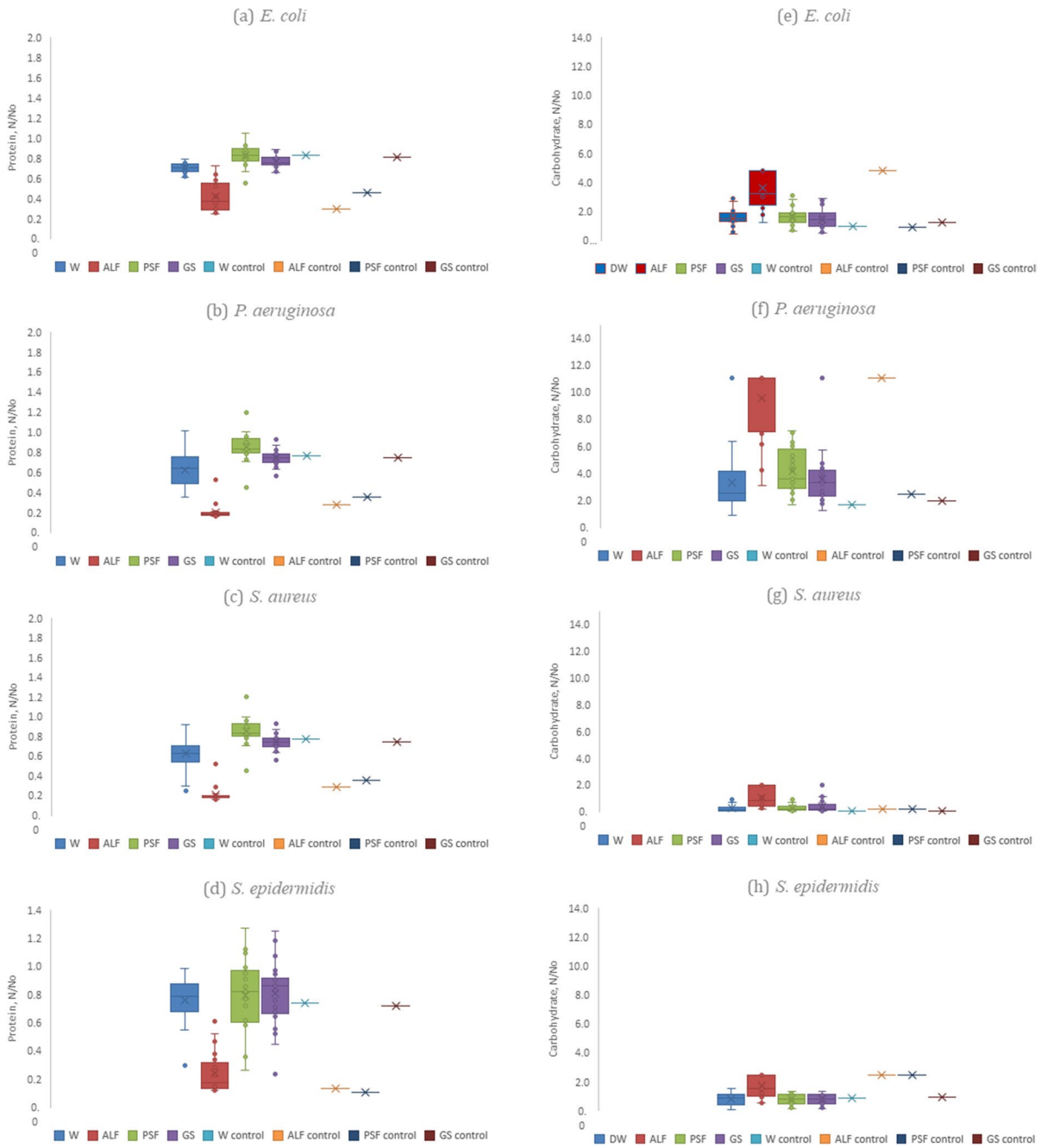

Fig. 3 Box and whisker chart view of the metabolism indicators of pathogens in house dust collected in Istanbul-Turkey using different simulated bio-relevant fluids: protein activity of **a** *E. coli*, **b** *P. aeruginosa*, **c** *S. aureus*, **d** *S. epidermidis* and carbohydrate activity of **e** *E. coli*, **f** *P. aeruginosa*, **g** *S. aureus*, **h** *S. epidermidis* (W: water, ALF: artificial lysosomal fluid, PSF: phagolysosome simulant fluid, GS: Gamble's solution, N/No meaning N: culture with household dust extracted in bio-relevant condition, No: culture under controlled condition-no household dust and bio-relevant condition)

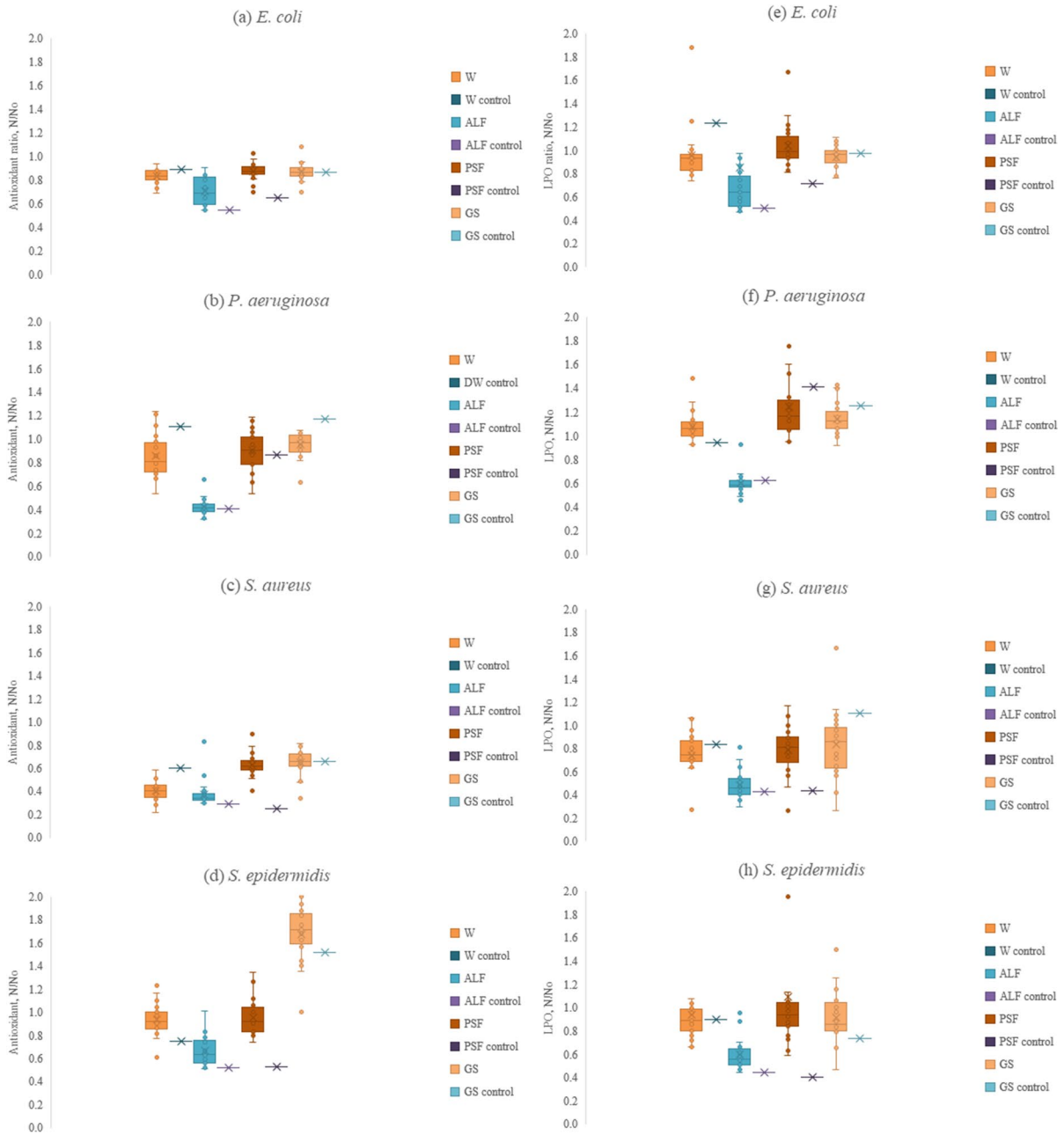

Fig. 4 Box and whisker chart view of the oxidative indicators of pathogens in house dust collected in Istanbul-Turkey using different simulated bio-relevant fluids: antioxidant activity of **a** *E. coli*, **b** *P. aeruginosa*, **c** *S. aureus*, **d** *S. epidermidis* and LPO activity of **e** *E. coli*, **f** *P. aeruginosa*, **g** *S. aureus*, **h** *S. epidermidis* (W: water, ALF: artificial lysosomal fluid, PSF: phagolysosome simulant fluid, GS: Gamble's solution, N/No meaning N: culture with household dust extracted in bio-relevant condition, No: culture under controlled condition-no household dust and bio-relevant condition)

bio-fractions of household-dust influenced the biofilm formation of tested pathogens compared to controlled conditions (Fig. 5). The biofilms formation promoted with gram-negative *P. aeruginosa* and *E. coli* compared to gram-positive *S. aureus* and *S. epidermidis.* For gram-negative *P. aeruginosa* and *E.*

Table 4 Proposed possible risk assessments of household dusts by pathogens (PR: pathogen risk, CD: Contamination degree of pathogen, colours in PR; green: no risk, yellow: weak risk, orange: moderate risk, and red: strong risk, colours in CD; green: low contamination degree, purple: moderate contamination risk, orange: considerable contamination risk, red: very high contamination risk)

Pathogen	Risk assessment	Activity				Biofilm			
		Simulated bio-relevant fluids fluids				Simulated bio-relevant fluids			
		Water	ALF	PSF	Gamble's	Water	ALF	PSF	Gamble's
E. coli	PR	no risk	weak risk	weak risk	no risk	moderate risk	moderate risk	moderate risk	moderate risk
	CD	low contamination degree	moderate contamination degree	moderate contamination degree	low contamination degree	moderate contamination degree	moderate contamination degree	moderate contamination degree	moderate contamination degree
P. aeruginosa	PR	no risk	weak risk	weak risk	no risk	strong risk	moderate risk	strong risk	moderate risk
	CD	low contamination degree	moderate contamination degree	moderate contamination degree	low contamination degree	considerable contamination degree	moderate contamination degree	considerable contamination degree	moderate contamination degree
S. aureus	PR	no risk	weak risk	moderate	weak risk	no risk	weak risk	no risk	weak risk
	CD	low contamination degree	moderate contamination degree	moderate contamination degree	moderate contamination degree	low contamination degree	moderate contamination degree	low contamination degree	moderate contamination degree
S. epidermidis	PR	weak risk	moderate risk	strong risk	weak risk	weak risk	moderate risk	strong risk	weak risk
	CD	moderate contamination degree	considerable contamination degree	very high contamination degree	moderate contamination degree	moderate contamination degree	considerable contamination degree	considerable contamination degree	moderate contamination degree

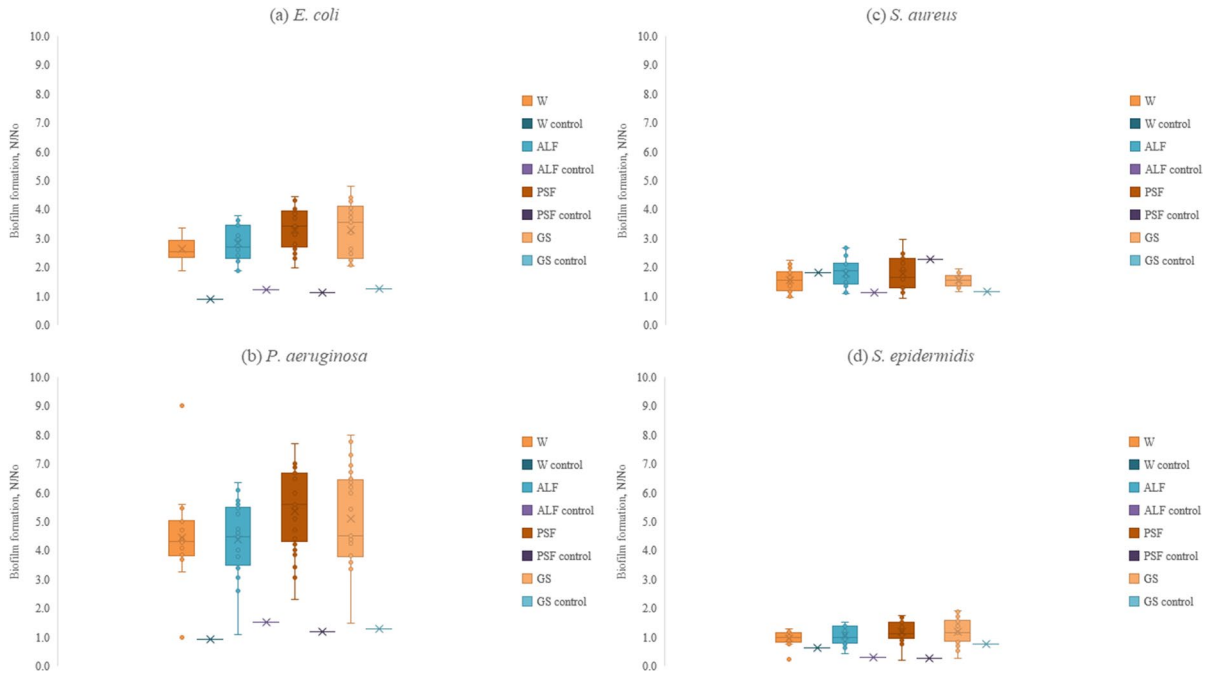

Fig. 5 Box and whisker chart view of the biofilm formation of pathogen in house dust collected in Istanbul-Turkey using different simulated bio-relevant fluids. **a** *E. coli*, **b** *P. aeruginosa*, **c** *S. aureus*, **d** *S. epidermidis* (W: water, ALF: artificial lysosomal fluid, PSF: phagolysosome simulant fluid, GS: Gamble's solution, N/No meaning N: culture with household dust extracted in bio-relevant condition, No: culture under controlled condition-no household dust and bio-relevant condition)

coli, the more biofilms formed in PSF and Gamble's solution fractions compared to water and ALF fractions of household-dust. The formation decreased following order: PSF > Gamble's solution > ALF > water and PSF > Gamble's solution > water > ALF for *E. coli* and *P. aeruginosa.* On the other hand, the biofilm response of *S. aureus* and *S. epidermidis* varied in different bio-fractions of household-dusts. The biofilm formation order of *S. aureus* and *S. epidermidis* is ALF = PSF > water > Gamble's solution and Gamble's solution > PSF > ALF > water, respectively. These results observed that the importance of biofilm formation in the bio-fractions of household dusts. Some available studies respecting dust samples also showed the enhanced biofilm formation due to chemical characteristics. For instance, biofilm formation of *K. pneumoniae, E. coli,* and *P. aeruginosa* was promoted by the exposure of dusts from standard reference materials due to sample ecosystem (Bado et al., 2017). In addition, Suraju et al. (2015) found that dust exposure increased biofilm formation of *E. coli*, *Enterococcus faecalis*, and *P. aeruginosa* owing to specific dust components. The available studies also indicated that more chemical characterization of dusts and various physiological conditions are need to examine for realistic impact of dusts on biofilm formation of microorganisms.

3.4 Model approaches for risk assessments and correlation analysis

To distinguish the anthropogenic inputs in simulated bio-fractions of household-dust, the Igeo was calculated and evaluated (Supplementary Fig. 2). The Igeo values with bio-fractions show that Igeo values was found to be smaller than 1 which indicated the classification of practically uncontaminated (Baysal & Akman, 2018; Baysal and Saygin, 2022). The order of the average Igeo values of the potentially toxic elements in water, ALF and PSF fractions is similar, and the order is Cu > Ni > Cr > Mn > Al > Zn, and in Gamble's solution fraction is Cu > Cr > Ni > Mn > Al > Zn. Besides, the Igeo values of each element by dissolution media declined following order: ALF > water > PSF > Gamble's

solution, ALF > PSF > Gamble's solution > water, ALF > PSF > Gamble's solution > water, PSF > ALF > water > Gamble's solution, PSF > Gamble's solution > water > ALF, ALF > PSF > water > Gamble's solution for Al, Cr, Cu, Ni, Zn, and Mn, respectively. These results showed that risks of the potentially toxic elements are greater in bio-relevant fluids having acidic conditions (ALF and PSF) compared to other tested bio-fractions. Although scarcely study examined the different in vitro conditions, recent studies indicated the ALF solubility of Zn, Cr, Cu, Ni had higher than Gamble's solution (Hu et al., 2018; Ogundele et al., 2018). Moreover, higher concentration of elements in lung fluids may also prove the importance of these elements on humans.

Daily intake (DI) of the potentially toxic elements through inhalation on exposure to household-dust was assessed and evaluated the potential human health risks (Table 3). DIs of children were higher than adults for inhalation exposure way. These results were in good agreement with the previous studies determined using total element concentration (Baltas et al., 2020; Ogundele et al., 2018). Moreover, the DI results indicated that the higher DI values obtained in ALF fractions mimicking the inflammatory condition for all determined elements.

The non-carcinogenic risks from determined elements in the household-dusts extracted in bio-relevant fluids via inhalation were also calculated (Table 3). The HQ values for non-carcinogenic risks of adults were lower than children for the determined elements. These results were coherent with the studies of other research that examined the elements in various environmental samples (Baltas et al., 2020; Ogundele et al., 2018). Moreover, the HQ values for the determined elements via inhalation were all lower than safe level indicating the non-carcinogenic risks. Similarly, with daily intake, the non-carcinogenic risks of household-dusts are higher in ALF fraction compared to other bio-fractions that show selectivity of elements under inflammatory conditions and importance of the conditions. This result is also linked with the higher content of elements in the ALF.

The inhalation carcinogenic risks (CR) were evaluated through Cr and Ni. As shown in Table 3, the CR are greater for children than adults. Furthermore, carcinogenic risks were found to be greater in ALF fraction of household-dusts compared to other fractions.

As shown in Table 4, the risk assessment of pathogens is evaluated through two derived models. These models (PR and $CD_{pathogen}$) showed that higher risks by the activity were found for *S. epidermidis* in ALF and PSF fractions household-dusts compared to other pathogens and bio-fractions. The lower PR and $CD_{pathogen}$ was measured in the household-dusts extracting in water compared to ALF, PSF and Gamble's solution. Moreover, the PR of *E. coli* activity was no risk with the fraction of water and Gamble's solution, and it was weak risk in ALF and PSF fractions. The PR results of *P. aeruginosa* were similar to the PR of *E. coli* activity. This result indicated that lower pH of the medium of household-dusts has potential to increase the PR through the activity of gram-negative pathogens. Furthermore, their $CD_{pathogen}$ was similar response for both gram-negatives (*P. aeruginosa* and *E. coli*), and they showed low contamination degree under water and Gamble's solution and moderate contamination degree in ALF and PSF. This result indicated that the ALF and PSF fractions of household-dusts have potentially more risks for *P. aeruginosa* and *E. coli* activity compared to fractions in water and Gamble's solution. Contrarily to gram-negatives, gram-positive *S. aureus* and *S. epidermidis* did not show similar response by their risk assessments upon the activity, except for the PR and $CD_{pathogen}$ of the *S. aureus* and *S. epidermidis* activity under Gamble's solution. The risks evaluations indicated that the PR of *S. aureus* activity increased following order: water < ALF = Gamble's solution < PSF. The $CD_{pathogen}$ of the *S. aureus* activity increased following sequence: water < ALF = PSF = Gamble's solution. Besides, the PR and $CD_{pathogen}$ of *S. epidermidis* activity increased following order: water = Gamble's solution < ALF < PSF. This result also showed that pH of the environment may influence the risks evaluation of these pathogens and acidic conditions have more risks compared to neutral conditions for the activity of gram-positives.

The biofilm risk assessments of the tested pathogens in the presence of various bio-fractions of household-dusts were also evaluated according to the derived approaches and the results is present in Table 4. The PR were the highest level that is strong risk with the extraction of household-dusts in water and PSF for *P. aeruginosa,* and in PSF for *S. epidermidis.* The PR in the water, ALF, PSF, and Gamble's solution fractions of household-dust decreased

following order: *P. aeruginosa* > *E. coli* > *S. epidermidis* > *S. aureus, P. aeruginosa* = *E. coli* = *S. epidermidis* > *S. aureus, P. aeruginosa* = *S. epidermidis* > *E. coli* > *S. aureus,* and *P. aeruginosa* = *E. coli* > *S. epidermidis* = *S. aureus*, respectively. This result indicated that the risk of the biofilm formation is greater for *P. aeruginosa* and *S. epidermidis* mainly in water and PSF fractions of household-dusts. The PR results also showed that the biofilm production has greater risks rather than the bacterial activity when their classifications were compared for all pathogens, except *S. aureus*. The $CD_{pathogen}$ of *E. coli* was same level for all simulated bio-fractions and it is moderate contamination degree. The $CD_{pathogen}$ of *P. aeruginosa* was water = PSF (considerable contamination degree) > ALF = Gamble's solution (moderate contamination degree). This response has adverse trend for *S. aureus* and it is water = PSF (low contamination degree) < ALF = Gamble's solution (moderate contamination degree). The $CD_{pathogen}$ of *S. epidermidis* was considerable contamination degree in ALF and PSF fractions, and moderate contamination degree in fractions of water and Gamble's solution. The order of $CD_{pathogen}$ by pathogens is *S. aureus* < *E. coli* = *S. epidermidis* < *P. aeruginosa, S. aureus* = *E. coli* = *P. aeruginosa* < *S. epidermidis, S. aureus* < *E. coli* < *P. aeruginosa* = *S. epidermidis,* and *S. aureus* = *E. coli* = *P. aeruginosa* = *S. epidermidis* in fractions of water, ALF, PSF and Gamble's solution, respectively. These results of $CD_{pathogen}$ indicated that the highest contamination risks originated by *P. aeruginosa* and *S. epidermidis* in PSF fractions of household-dust. This result suggested that acidic and intraphagolysosomal conditions may support the risk of biofilm formation.

The Pearson correlation analysis applied to understand the interaction between elements and pathogens and the results presented in Supplementary Table 5–10. These results indicated that all tested biological parameters of the pathogens were correlated to one or more than major and potentially toxic elements. For example, the activity of *S. epidermidis* in PSF fraction which obtained the highest risk was positively correlated with Al, Fe and Ni. The activity has also significant linking with its protein, antioxidant and LPO indicators, and these responses have correlations with elements. The protein response in PSF fraction positively correlated with Al, Fe, Ni, Zn, and K, and negatively correlated with P, antioxidant and LPO indicators of *S. epidermidis* in PSF fraction had positive correlation with Al. The biofilm formation of *E. coli* had significant negative and positive correlations with Cu in water and Ca in PSF, respectively. Except Fe in water and PSF, the biofilm formation of *P. aeruginosa* was significant negative linking with Cr in PSF, Ni in PSF, Ca in ALF and Gamble's solution, K in Gamble's solution, Mg in all bio-fluids, and Na in Gamble's solution. The biofilm production of *S. aureus* had significant negative correlation with Cr and Ni in water and PSF, respectively. Moreover, a significant positive link was found between Zn and P levels in ALF and Gamble's solution and biofilm formation of *S. aureus.* In addition, significant negative correlations were found between the biofilm formation of *S. epidermidis* and Cr in water and PSF, Ni in PSF, Ca in ALF, Na in Gamble's solution, and Mg in all bio-fluids.

4 Conclusion

Taken together, these findings suggest that the bio-fractions of household-dust have influenced the element dissolution and culturable pathogen responses including activity, biofilm formation and oxidative stress indicators. The study also indicated that the concentrations of elements, the DI and HQ values in ALF fraction of household-dusts were higher compared to other bio-fractions. It is important since ALF reflects the body immunological response when phagotosysis of inhaled dusts. On the other hand, the pathogen-based risk assessments (PR and $CD_{pathogen}$) suggested that the PSF fraction of household-dusts had greater risk compared to other bio-fractions, as well as *P. aeruginosa* and *S. epidermidis*. The correlation analysis also observed that pathogen responses were correlated with the elements. Therefore, solubility of the major elements and potentially toxic elements in different bio-fluids is important to understand the impact of elements on pathogen responses and human health.

In conclusion, to more accurate modeling and the risk assessments of household dusts, both chemical and biological indicators can examine under bio-relevant conditions.

Author contributions All authors contributed to the study conception and design. Investigation, resources, methodology,

writing—original draft preparation were performed by Asli Baysal. Conceptualization, investigation, methodology, and writing—original draft preparation were written by Hasan Saygin. Methodology was performed by Sevilay Zora. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose. The authors declare no conflict of interest. The authors did not receive support from any organization for the submitted work.

Ethical approval The manuscript does not report on or involve the use of any animal or human data or tissue. The manuscript does not contain data from any individual person.

References

Bado, M., Kwende, S., Shishodia, S., & Rosenzweig, J. A. (2017). Impact of dust exposure on mixed bacterial cultures and during eukaryotic cell co-culture infections. *Applied Microbiology and Biotechnology, 101*(18), 7027–7039. https://doi.org/10.1007/s00253-017-8449-4

Baltas, H., Sirin, M., Gökbayrak, E., & Ozcelik, A. E. (2020). A case study on pollution and a human health risk assessment of heavy elements in agricultural soils around Sinop province. *Turkey Chemosphere, 241*, 125015. https://doi.org/10.1016/j.chemosphere.2019.125015

Barron, V., & Torrent, J. (2013). Iron, manganese and aluminium oxides and oxyhydroxides. *EMU Notes in Mineralogy, 14*(9), 297–336.

Baysal, A., & Akman, S. (2018). Assessment of chromium and nickel levels in surface sea waters and sediments from industrial marine area in Tuzla Aydinli Bay. *Istanbul Turkey Marine Pollution Bulletin, 130*, 293–298.

Baysal, A., Baltaci, H., Ozbek, N., Destanoglu, O., Ustabasi, G. S., & Gumus, G. (2017). Chemical characterization of surface snow in Istanbul (NW Turkey) and their association with atmospheric circulations. *Environmental Monitoring and Assessment., 189*(6), 275. https://doi.org/10.1007/s10661-017-5982-7

Baysal, A., & Saygin, H. (2022). An assessment of ecological and possible human health risks from Cr and Ni in sediments affected by the industrial marine area in Tuzla Aydinli Bay, Istanbul, Turkey between 2016–2020. *Stochastic Environmental Research and Risk Assessment*. https://doi.org/10.1007/s00477-022-02289-w

Boisa, N., Elom, N., Dean, J. R., Deary, M. E., Bird, G., & Entwistle, J. A. (2014). Development and application of an inhalation bioaccessibility method (IBM) for lead in the PM10 size fraction of soil. *Environmental International, 70*, 132–142. https://doi.org/10.1016/j.envint.2014.05.021

Bouillard, L., Michel, O., Dramaix, M., & Devleeschouwer, M. (2005). Bacterial contamination of indoor air, surfaces, and settled dust, and related dust endotoxin concentrations in healthy office buildings. *Annals of Agricultural and Environmental Medicine, 12*(2), 187–192.

Brągoszewska, E., & Biedroń, I. (2018). Indoor air quality and potential health risk ımpacts of exposure to antibiotic resistant bacteria in an office rooms in southern Poland. *International Journal of Environmental Research and Public Health, 15*(11), 2604. https://doi.org/10.3390/ijerph15112604

Brągoszewska, E., Biedroń, I., & Hryb, W. (2020). Microbiological air quality and drug resistance in airborne bacteria ısolated from a waste sorting plant located in Poland-A case study. *Microorganisms, 8*(2), 202. https://doi.org/10.3390/microorganisms8020202

Chan, W. L., Chung, W. T., & Ng, T. W. (2019). Airborne survival of escherichia coli under diferent culture conditions in synthetic wastewater. *International Journal of Environmental Research and Public Health, 16*(23), 4745.

Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., & Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. *Journal of Clinical Microbiology, 22*(6), 996–1006. https://doi.org/10.1128/jcm.22.6.996-1006.1985

Deng, J., Dong, F., Dai, Q., et al. (2018). Interface effect of natural precipitated dust on the normal flora of *Escherichia coli* and *Staphylococcus epidermidis*. *Environmental Science and Pollution Research, 25*, 22340–22347. https://doi.org/10.1007/s11356-017-9666-1

de Paula Ribeiro, J., Quijano, M. F. C., Ferreiro, J. D., Gioda, A., Jiménez-Vélez, B., Monserrat, J. M., & Gioda, C. R. (2020). Aqueous particulate matter (PM2.5) from Brazil alters antioxidant profile responses and causes oxidative stress. *Atmospheric Pollution Research 11*(3), 511–519. 10.1016/j.apr.2019.11.023

Dias da Silva, L. I. D., Yokoyama, L., Maia, L. B., Monteiro, M. I. C., Pontes, F. V. M., Carneiro, M. C., et al. (2015). Evaluation of bioaccessible heavy element fractions in PM10 from the metropolitan region of Rio de Janeiro city, Brazil, using a simulated lung fluid. *Microchemical Journal, 118*, 266–271.

Dimitroulopoulou, S., Dudzińska, M. R., Gunnarsen, L., Hägerhed, L., Maula, H., Singh, R., Toyinbo, O., & Haverinen-Shaughnessy, U. (2023) Indoor air quality guidelines from across the world: An appraisal considering energy saving health productivity and comfort. *Environment International, 178*, 108127. https://doi.org/10.1016/j.envint.2023.108127

Doyi, I., Strezov, V., Isley, C. F., Yazdanparast, T., & Taylor, M. P. (2020). The relevance of particle size distribution and bioaccessibility on human health risk assessment for trace elements measured in indoor dust. *Science of the Total Environment, 733*, 137931. https://doi.org/10.1016/j.scitotenv.2020.137931

Duzgoren-Aydin, N. S., Wong, C. S., Aydin, A., Song, Z., You, M., & Li, X. D. (2006). Heavy element contamination and distribution in the urban environment of Guangzhou, SE China. *Environmental Geochemistry and Health, 28*(4), 375–391. https://doi.org/10.1007/s10653-005-9036-7

Eneji, I. S., Adams, I. U., & Julius, K. A. (2015). Assessment of heavy elements in ındoor settled harmattan dust from the University of Agriculture Makurdi, Nigeria. *Open Journal of Air Pollution, 4*, 198–207. https://doi.org/10.4236/ojap.2015.44017

Gao, P., Guo, H., Zhang, Z., Ou, C., Hang, J., Fan, Q., He, C., Wu, B., Feng, Y., & Xing, B. (2018). Bioaccessibility and exposure assessment of trace elements from urban airborne particulate matter (PM10 and PM2.5) in simulated digestive fluid. *Environmental Pollution, 242*, 1669–1677. https://doi.org/10.1016/j.envpol.2018.07.109

Gohain, M., & Deka, P. (2020). Trace elements in indoor dust from a university campus in Northeast India: Implication for health risk. *Environmental Monitoring and Assessment, 192*(11), 741. https://doi.org/10.1007/s10661-020-08684-6

Guo, K., Qian, H., Zhao, D., et al. (2020). Indoor exposure levels of bacteria and fungi in residences, schools, and offices in China: A systematic review. *Indoor Air, 30*(6), 1147–1165. https://doi.org/10.1111/ina.12734

Hameed, A. A. A., El-Gendy, S., & Saeed, Y. (2024). Characterization and decontamination of deposited dust: A management regime at a museum. *Aerobiologia, 40*, 217–232. https://doi.org/10.1007/s10453-024-09813-1

Hassanshahian, M., Emtiazi, G., Kermanshahi, R. K., & Cappello, S. (2010). Comparison of oil degrading microbial communities in sediments from the Persian Gulf and Caspian Sea. *Soil Sediment Contam, 19*(3), 277–291. https://doi.org/10.1080/15320381003695215

Hu, X., Xu, X., Ding, Z., Chen, Y., & Lian, H. Z. (2018). In vitro inhalation/ingestion bioaccessibility, health risks, and source appointment of airborne particle-bound elements trapped in room air conditioner filters. *Environmental Science and Pollution Research International., 25*(26), 26059–26068. https://doi.org/10.1007/s11356-018-2403-6

Huang, H., Jiang, Y., Xu, X., & Cao, X. (2018). In vitro bioaccessibility and healthassessment of heavy elements in atmospheric particulate matters from three different functional areas of Shanghai China. *Science of Total Environmen, 610*, 546–554. https://doi.org/10.1016/j.scitotenv.2017.08.074

Innes, E., Yiu, H. H. P., McLean, P., Brown, W., & Boyles, M. (2021). Simulated biological fluids—a systematic review of their biological relevance and use in relation to inhalation toxicology of particles and fibres. *Critical Reviews in Toxicology, 51*(3), 217–248. https://doi.org/10.1080/10408444.2021.1903386

Jadoon, W. A., Abdel-Dayem, S., Saqib, Z., Takeda, K., Sakugawa, H., Hussain, M., Shah, G. M., Rehman, W., & Syed, J. H. (2021). Heavy elements in urban dusts from Alexandria and Kafr El-Sheikh, Egypt: Implications for human health. *Environmental Science and Pollution Research International., 28*(2), 2007–2018. https://doi.org/10.1007/s11356-020-08786-1

Jayarathne, A., Egodawatta, P., Ayoko, G. A., & Goonetilleke, A. (2018). Assessment of ecological and human health risks of elements in urban road dust based on geochemical fractionation and potential bioavailability. *Science of the Total Environment, 635*, 1609–1619. https://doi.org/10.1016/j.scitotenv.2018.04.098

Kastury, F., Smith, E., & Juhasz, A. L. (2017). A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of element(loid)s from ambient particulate matter or dust. *Science of Total Environment, 574*, 1054–1074. https://doi.org/10.1016/j.scitotenv.2016.09.056

Kastury, F., Smith, E., Karna, R. R., Scheckel, K. G., & Juhasz, A. L. (2018). Methodological factors influencing inhalation bioaccessibility of element(loid)s in PM2.5 using simulated lung fluid. *Environmental Pollution, 241*, 930–937. https://doi.org/10.1016/j.envpol.2018.05.094

Kumar, P., Singh, A. B., & Singh, R. (2021). Seasonal variation and size distribution in the airborne indoor microbial concentration of residential houses in Delhi and its impact on health. *Aerobiologia (bologna), 37*(4), 719–732. https://doi.org/10.1007/s10453-021-09718-3

Kurt-Karakus, P. B. (2012). Determination of heavy elements in indoor dust from Istanbul, Turkey: Estimation of the health risk. *Environment International, 50*, 47–55. https://doi.org/10.1016/j.envint.2012.09.011

Lagier, J.-C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M., & Raoult, D. (2015). Current and past strategies for bacterial culture in clinical microbiology. *Clinical Microbiology Reviews, 28*, 208–236. https://doi.org/10.1128/CMR.00110-14

Li, L., Qiu, Y., Gustafsson, A., et al. (2019). Characterization of residential household dust from Shanghai by particle size and analysis of organophosphorus flame retardants and elements. *Environmental Sciences Europe, 31*, 94. https://doi.org/10.1186/s12302-019-0279-9

Liu, X., Hurt, R. H., & Kane, A. B. (2010). Biodurability of single-walled carbono nanotubes depends on surface functionalization. *Carbon, 48*, 1961–1969.

Liu, X., Ouyang, W., Shu, Y., Tian, Y., Feng, Y., Zhang, T., & Chen, W. (2019). Incorporating bioaccessibility into health risk assessment of heavy elements in particulate matter originated from different sources of atmospheric

pollution. *Environmental Pollution, 254*(Pt B), 113113. https://doi.org/10.1016/j.envpol.2019.113113

Liu, Y., Ma, J., Yan, H., Ren, Y., Wang, B., Lin, C., & Liu, X. (2016). Bioaccessibility and health risk assessment of arsenic in soil and indoor dust in rural and urban areas of Hubei province China. *Ecotoxicology and Environmental Safety., 126*, 14–22. https://doi.org/10.1016/j.ecoenv.2015.11.037

Morawska, L., & Huang, W. (2022). WHO health guidelines for indoor air quality and national recommendations/standards. *Handbook of Indoor Air Quality*. https://doi.org/10.1007/978-981-10-5155-5_49-1

Mukhtar, A., & Limbeck, A. (2013). Recent developments in assessment of bio-accessible trace element fractions in airborne particulate matter: A review. *Analytica Chimica Acta., 774*, 11–25. https://doi.org/10.1016/j.aca.2013.02.008

Novak Babič, M., Gostinčar, C., & Gunde-Cimerman, N. (2020). Microorganisms populating the water-related indoor biome. *Applied Microbiology and Biotechnology, 104*(15), 6443–6462. https://doi.org/10.1007/s00253-020-10719-4

Ogundele, L. T., Olasinde, R. T., Owoade, O. K., & Olise, F. S. (2018). Composition and source identifcation of chemical species in dust from selected indoor environments in Ile-Ife Nigeria. *Earth Systems and Environment, 2*, 323–330. https://doi.org/10.1007/s41748-018-0052-z

Onat, B., Çalışkan, N. S., Şahin, Ü. A., & Uzun, B. (2020). Assessment of the health risk related to exposure to ultrafine, fine, and total particulates and elements in a element finishing plant. *Environmental Science and Pollution Research International, 27*(4), 4058–4066. https://doi.org/10.1007/s11356-019-06891-4

O'Toole, G. A. (2011). Microtiter dish biofilm formation assay. *Journal of Visualized Experiments, 47*, 2437. https://doi.org/10.3791/2437

Ozbek, N., & Baysal, A. (2016). A new approach for the determination of sulphur in airborne particles by HR-CS ETAAS. *International Journal of Environmental Analytical Chemistry, 96*(6), 505–514. https://doi.org/10.1080/03067319.2016.1180381

Paleologos, K. E., Selim, M. Y., & Mohamed, A. M. O. (2021). Indoor air quality: pollutants, health effects, and regulations. In K. E. Paleologos, M. Y. Selim, & A. M. O. Mohamed (Eds.), *Pollution assessment for sustainable practices in applied sciences and engineering* (pp. 405–489). NY: Butterworth-Heinemann. https://doi.org/10.1016/B978-0-12-809582-9.00008-6

Pelfrêne, A., Cave, M. R., Wragg, J., & Douay, F. (2017). In vitro investigations of human bioaccessibility from reference materials using simulated lung fluids. *International Journal of Environmental Research and Public Health 14*(2), 112. https://doi.org/10.3390/ijerph14020112

Pompilio, A., & Di Bonaventura, G. (2020). Ambient air pollution and respiratory bacterial infections, a troubling association: Epidemiology, underlying mechanisms, and future challenges. *Critical Reviews in Microbiology, 46*(5), 600–630. https://doi.org/10.1080/1040841X.2020.1816894

Raisi, L., Aleksandropoulou, V., Lazaridis, M., et al. (2013). Size distribution of viable, cultivable, airborne microbes and their relationship to particulate matter concentrations and meteorological conditions in a Mediterranean site. *Aerobiologia, 29*, 233–248. https://doi.org/10.1007/s10453-012-9276-9

Ren, H., Yu, Y., & An, T. (2020). Bioaccessibilities of element(loid)s and organic contaminants in particulates measured in simulated human lung fluids: A critical review. *Environmental Pollution, 265*(Pt B), 115070. https://doi.org/10.1016/j.envpol.2020.115070

Ruiz-Gil, T., Rilling, J. I., Campos, M., et al. (2023). Airborne bacterial communities associated with particulate matter in Temuco (Chile), one of the most air-polluted cities in South America. *Aerobiologia, 39*, 457–476. https://doi.org/10.1007/s10453-023-09803-9

Saygin, H., & Baysal, A. (2022). Single and combined effects of antibiotics and nanoplastics from surgical masks and plastic bottles on pathogens. *Comparative Biochemistry and Physiology Part c: Toxicology & Pharmacology, 257*, 109340. https://doi.org/10.1016/j.cbpc.2022.109340

Saygin, H., Baysal, A., Onat, B., et al. (2023). Oxidative stress and chemical characteristics of indoor PM2.5: A case study in an underground (-3rd) floor. *Air Quality, Atmosphere and Health, 16*, 1345–1356. https://doi.org/10.1007/s11869-023-01346-9

Saygin, H., Tilkili, B., Kayisoglu, P., & Baysal, A. (2024). Oxidative stress, biofilm-formation and activity responses of *P. aeruginosa* to microplastic-treated sediments: Effect of temperature and sediment type. *Environmental Research, 248*, 118349. https://doi.org/10.1016/j.envres.2024.118349

Settimo, G., Manigrasso, M., Avino, P. (2020). Indoor air quality: A focus on the european legislation and State-of-the-Art research in Italy. *Atmosphere, 11*(4), 370. https://doi.org/10.3390/atmos11040370

Sidra, S., Ali, Z., Sultan, S., Ahmed, S., Colbeck, I., & Nasir, Z. A. (2015). Assessment of airborne microflora in the indoor micro-environments of residential houses of Lahore, Pakistan. *Aerosol Air Quality Research, 15*, 2385–2396. https://doi.org/10.4209/aaqr.2014.12.0338

Singh, N. K., Sanghvi, G., Yadav, M., et al. (2022). Assessment and characterization of bioaerosols from an indoor environment-operated wastewater management facility: Unraveling pathogenicity in research laboratories. *Aerobiologia, 38*, 519–531. https://doi.org/10.1007/s10453-022-09763-6

Stapleton, E. M., Manges, R., Parker, G., Stone, E. A., Peters, T. M., Blount, R. J., Noriega, J., Li, X., Zabner, J., Polgreen, P. M., Chipara, O., Herman, T., & Comellas, A. P. (2020). Indoor particulate matter from smoker homes induces bacterial growth, biofilm formation, and impairs airway antimicrobial activity. *A Pilot Study Frontiers in Public Health, 7*, 418. https://doi.org/10.3389/fpubh.2019.00418

Stefaniak, A. B. (2010). Persistence of tungsten oxide particle/fiber mixtures in artificial human lung fluids. *Particle and Fibre Toxicology, 7*, 38. https://doi.org/10.1186/1743-8977-7-38

Stefaniak, A. B., Guilmette, R. A., Day, G. A., Hoover, M. D., Breysse, P. N., & Scripsick, R. C. (2005). Characterization of phagolysosomal simulant fluid for study of beryllium

aerosol particle dissolution. *Toxicology in Vitro, 19*, 123–134. https://doi.org/10.1016/j.tiv.2004.08.001

Suraju, M. O., Lalinde-Barnes, S., Sanamvenkata, S., Esmaeili, M., Shishodia, S., & Rosenzweig, J. A. (2015). The effects of indoor and outdoor dust exposure on the growth, sensitivity to oxidative-stress, and biofilm production of three opportunistic bacterial pathogens. *Science of the Total Environment, 538*, 949–958. https://doi.org/10.1016/j.scitotenv.2015.08.063

Tan, S. Y., Praveena, S. M., Abidin, E. Z., & Cheema, M. S. (2016). A review of heavy elements in indoor dust and its human health-risk implications. *Reviews on Environmental Health, 31*(4), 447–456. https://doi.org/10.1515/reveh-2016-0026

Thompson, J. R., Argyraki, A., Bashton, M., Bramwell, L., Crown, M., Hursthouse, A. S., Jabeen, K., Marinho Reis, P., Namdeo, A., Nelson, A., Pearce, D. A., Potgieter-Vermaak, S., Rasmussen, P. E., Wragg, J., & Entwistle, J. A. (2021). Bacterial diversity in house dust: Characterization of a core indoor microbiome. *Frontiers in Environmental Science, 9*, 754657. https://doi.org/10.3389/fenvs.2021.754657

Turner, A. (2011). Oral bioaccessibility of trace elements in household dust: A review. *Environmental Geochemistry and Health, 33*(4), 331–341. https://doi.org/10.1007/s10653-011-9386-2

USEPA. (1989). Risk assessment guidance for superfund (volume I) human health evaluation manual, In *Office of Soild Waste and Emergency Response*, pp. 1–89, US Environmental Protection Agency

U.S. Environmental Protection Agency (EPA) (2009) Risk assessment guidance for Superfund: Volume I-human health evaluation manual (Part F, supplemental guidance for inhalation risk assessment). Washington DC: EPA

Wang, Y., Fang, F., Lin, Y., Cai, J., Zhang, C., & Ge, Y. (2020). Pollution and influencing factors of heavy elements from rural kitchen dust in Anhui Province China. *Atmospheric Pollution Research, 11*(7), 1211–1216. https://doi.org/10.1016/j.apr.2020.04.011

White, J. K., Nielsen, J. L., Larsen, C. M., & Madsen, A. M. (2020). Impact of dust on airborne Staphylococcus aureus' viability, culturability, inflammogenicity, and biofilm forming capacity. *International Journal of Hygiene and Environmental Health, 230*, 113608. https://doi.org/10.1016/j.ijheh.2020.113608

Wu, M. W., Dong, W. J., Guan, D. X., Li, S. W., & Ma, L. Q. (2024). Total contents, fractionation and bioaccessibility of nine heavy metals in household dust from 14 cities in China. *Environmental Research, 243*, 117842. https://doi.org/10.1016/j.envres.2023.117842

Wu, Z., Lyu, H., Liang, W., Jing, X., Wang, Y., & Ma, X. (2021). Microbial community in indoor dusts from university dormitories: Characteristics, potential pathogens and influence factors. *Atmospheric Pollution Research, 12*(3), 321–333. https://doi.org/10.1016/j.apr.2020.12.018

Xu, Z., Liang, Y., Lin, S., et al. (2016). Crystal violet and XTT assays on *Staphylococcus aureus* biofilm quantification. *Current Microbiology, 73*(4), 474–482. https://doi.org/10.1007/s00284-016-1081-1

Zhang, X., Zha, T., Guo, X., Meng, G., & Zhou, J. (2018). Spatial distribution of element pollution of soils of Chinese provincial capital cities. *Science of Total Environment, 643*, 1502–1513. https://doi.org/10.1016/j.scitotenv.2018.06.177

Zheng, N., Hou, S., Wang, S., Sun, S., An, Q., Li, P., & Li, X. (2020). Health risk assessment of heavy elements in street dust around a zinc smelting plant in China based on bioavailability and bioaccessibility. *Ecotoxicology and Environmental Safety, 197*, 110617. https://doi.org/10.1016/j.ecoenv.2020.110617

Zupančič, M., Šušteršič, M., Bavec, Š, & Gosar, M. (2021). Oral and inhalation bioaccessibility of potentially toxic elements in household dust from former Hg mining district, Idrija, Slovenia. *Environmental Geochemistry and Health*. https://doi.org/10.1007/s10653-021-00835-z