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Potential toxic metals in household dusts extracted in simulated body fluids and their interaction with culturable pathogens responses

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Research Article

Keywords: trace elements, indoor, bioaccesibility, homedust, Istanbul, bacteria

Posted Date: October 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2168443/v1

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Abstract

In the last decade, a great deal of research has focused on the determination of potential toxic metals in indoors due to an important source of the toxicity and health risks. The previous studies have commonly focused determination of total concentration of metals and identification the microorganisms in dust. On the other hand, determination bio-relevant forms (e.g., inhalable) of metals instead of total contents, examination the behavior of microorganism under these bio-relevant conditions and revealing the interaction between metals and pathogens is vital and necessary for realistic approach. However, previous studies have been ignored these topics in this field. Therefore, the present study aimed to (i) investigate metals in household dusts extracted in simulated body fluids by inhalation, (ii) examine the culturable pathogen responses in the presence of household dusts extracted in simulated body fluids, and (iii) assess their relations and risks using the model approaches by inhalation. Here, 25 household-dusts were extracted in four simulated body fluids to determine bio-relevant forms of metals (specifically using inhalation fluids). Moreover, four clinically important 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 metals and pathogens responses were evaluated, and model and derived approaches were used for risk assessments of metals and pathogens. The higher daily intake metal values obtained in artificial lysosomal fluid fraction of household dust that mimicking the inflammatory condition compared to other body fluids. The highest risk of the pathogens originated by biofilm formation.

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 [1, 2], 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 to outdoor air [2, 3]. 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 potential toxic metals, and indoor contaminants [1, 2, 4, 5]. Moreover, microorganism (e.g., pathogens, allergens, bio-based toxins) in indoor dust are more likely to introduce human lungs, triggering serious health conditions [6] (Wu et al. 2021). Therefore, household-dust has significant potential impact on the human health and indoor air quality.

Moreover, various studies evaluated the potential toxic metals in household-dusts using the model approach by the United States Environmental Protection Agency (USEPA) to estimate human health risk that is caused by contaminants, including hazard identification, exposure assessment, dose-response assessment, and risk characterization [2] (Tan et al. 2016). These studies have shown that metals can have potential adverse effect on health. On the other hand, impact of metals on human health are not fully understood because of their behaviors in the body fluids. Therefore, determination of potential toxic metals needs extra attention in indoor settled dust under realistic conditions and the associated health risk assessment [3](Gohain and Deka 2020). The available studies mostly determined the total concentration of the tested metals in the household-dusts. However, humans cannot take the total amount of metals since metals can be dissolved partly with biological fluids [7, 8]. Furthermore, recent studies indicated that the total metal content might not be a reliable parameter for assessing the exposure risks of metals in air or dust [9–11]. Therefore, risk assessment based on total concentrations of metals cannot reflect the potential health risks of metals in household-dust on human, and assessing the bio-relevant concentration of metals, instead of with their total concentration, can be more realistic approach to find their potential risks.

To understand the bio-relevant forms of toxic substances, various approaches (e.g., *in vivo* and *in vitro)* can apply. 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 alternative way to measure the bio-relevant forms of potentially toxic substances due to being the simple, rapid, reproducible, and economic [7, 12, 13]. Moreover, varied human health (biological) conditions have been applied to understand the immunologic response of organic, inorganics 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., metals, that are released from dust after meeting biological fluids and their comparison using various *in vitro* (simulated biological) solution [12, 14]. Various extraction techniques have been used to assess the bio-relevant forms of metals during inhalation, ingestion, or dermal contact [13, 15]. 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 metal analysis. Moreover, water-soluble forms of metals often show the greatest cellular uptake and toxicity for all exposure ways [12]. Specifically in inhalation, other extraction techniques can be used for the metal 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 [12, 16, 17].

Exposure to such potentially toxic metals and pathogens occurs via inhalation, ingestion, or dermal contact of indoor dust and, eventually, they enter the human body [2, 3, 18]. Epidemiological, animal toxicological and *in vitro* (simulated biological) studies have indicated that the inhaled particles play major role in human health due to their chemical composition, as well as the physical presence, and once they inhaled, the fraction of metal contaminants were readily released into the lung fluid that appeared to be accessible for and toxic to cells [19, 20]. Characteristics of pathogens or microbial communities in indoor dust are affected by the chemical composition of indoor dusts and their availability in the media.

Furthermore, previous studies have indicated that the indoor dust exposure affects the growth, biofilm formation, oxidative stress, and virulence of microorganisms [6, 21–27]. Unfortunately, these studies conducted under controlled conditions, and little is known about the physiological impact of dust exposure on the microorganisms. Also, few examine the behavior of specific microorganisms and their interactions with chemical components.

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

Materials And Methods

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 [28–30]. There are no social-economic differences between sampling areas, and these areas are both highly populated residential areas and having high industrial organizations. Dust samples were obtained from vacuum cleaner bags in regular use of participating volunteers for the purpose of cleaning homes. The information of 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 coarse (80–300 µm), stored in freezer until the analysis [1].

Simulated Bio-relevant Fluids and Analytical Methods for Extractions

The sieved and homogenized dust samples were divided to 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 of 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 [12].

Determination of Bio-relevant Forms of Metals

All the extracting solutions were stored at 4°C before instrumental analysis with Inductively Coupled Plasma Optical Emision Spectrometer (ICP-OES, Spectro Blue- Spectro, Kleve-Germany). For ensuring the quality and accuracy in the sample preperation 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 standart deviation reference material (SRM 2783) analysis was used and > 95% recoveries were obtained [28]. In any cases, we also determined the total concentrations of the metals in all household dust samples using acid digestion method.

Activity, Biofilm Formation, Main Biochemical and Oxidative Stress Responses of Pathogens

Escherichia coli (E. coli) (ATCC 35218), *Staphylococcus aureus (S. aureus)* (ATCC 25923), *Pseudomonas aeruginosa (P. aeruginosa) Staphylococcus epidermidis (S. epidermidis)* (ATCC 35984), and *Bacillus subtilis (B. subtilis)* (ATCC 11774) were selected as a pathogen model approach and used during the experiments. The bacteria were cultivated and incubated at 37 ^oC for 24 hours. Tryptic Soy Broth (TSB) medium was prepared and autoclaved at 121 ^oC for 15 min. Simulated bio-relevant-suspended household-dusts were diluted into TSB medium. Saturated cultures of *E. coli, S. aureus, P. aeruginosa, B. subtilis* grown in TSB medium including simulated bio-relevant-suspended household-dusts at 37 ^0C for 24 hours. For controls, only TSB and cultures in TSB medium was applied [21]. 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 [31]. For the biofilm formation, the crystal violet method was applied using microtiter methods [21, 32].

Model Approaches for Risk Assessments

To evaluate the risks of the metals extracted with bio-relevant solutions, pollution indices and health assessments were applied. For the realistic approach, we used bio-relevant concentrations of the determined metals for the calculation of pollution indices and health assessments.

To assess the anthropogenic impact of the bio-relevant forms of the metals, we used modified form of the geo-accumulation index (Igeo) [20, 33–36]. Igeo evaluates the pollution level of elements in household-dust by comparing the present concentration with pre-industrial levels [20, 33–36]. The Igeo was calculated using Eq. (1) and was classified according to the Table 1:

Table 1
Classification for metals and pathogens

Risk characterisation			Class	
For metals	Geoaccumulation Index	Igeo	Igeo ≤ 0	practically uncontaminated
			0 ≤ Igeo ≤ 1	uncontaminated to moderately contaminated;
			1 ≤ Igeo ≤ 2	moderately contaminated
			2 ≤ Igeo ≤ 3	moderately to heavily contaminated
			3 ≤ Igeo ≤ 4	heavily contaminated
			4 ≤ Igeo ≤ 5	heavily to extremely contaminated
			5 < Igeo	extremely contaminated
	Hazard Quotients	HQ	HQ ≤ 1	unlikely the adverse health effect
			HQ > 1	potential non-carcinogenic health effect
	Hazard Index	HI	HI ≤ 1	unlikely the adverse health effect
			HI > 1	potential non-carcinogenic health effect
	Cancer Risk	CR	CR < 1 < $x10^{-6}$	no significant health effect
			1$x10^{-6}$<CR < 1$x10^{-4}$	acceptable or tolarable cancer risks
			CR > 1$x10^{-4}$	carcinogenic risks
For pathogens	Pathogen Risk	PR	OD ≤ ODc	no risk
			ODc < OD ≤ 2×ODc	weak risk
			2×ODc < OD ≤ 4×ODc	moderate risk;
			4×ODc < OD	strong risk
	Contamination Degree of Pathogen	$CD_{pathogen}$	$CD_{pathogen}$<1	low contamination
			1 ≤ $CD_{pathogen}$<3	moderate contamination
			3 ≤ $CD_{pathogen}$<6	considerable contamination
			$CD_{pathogen}$≥6	very high contamination

$$Igeo = log2(Csample/1.5xCbackground)$$

1

where C_{sample} and $C_{background}$ are the bio-relevant concentrations of the potentially toxic metals in household-dust sample and background, respectively. The 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 [20, 37]. In this study, the daily exposure of potential toxic metals (daily intake (DI)) for inhalation pathway was estimated using a modified version and bio-relevant concentration of metals in Eq. 2 [37].

$$DI_{inh} = C \times \frac{InhR \times EF \times ED}{PEF \times BW \times AT} \quad (2)$$

DI (mg/kg.day): dose contacted (daily intake) through inhalation (DI_{inh}). C (mg/kg) is the content of the metals in each bio-relevant solution. Input assumptions indicated in Supplementary Table 2.

According to USEPA's guideline on human health risk assessment of potential toxic metals (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) [38, 39].

$$HQ_{inh} = \frac{DI_{inh}}{RfDI_{inh}}$$

3

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

$$CR = DIxSF$$

4

where DI is inhalation (DI_{inh}), and SF cancer slope factor for each potentially toxic metals extracted in bio-relevant solution indicated in Supplementary Table 2. HQ and CR classifications were given in Table 1.

To assess the pathogen risk respecting the simulated bio-relevant conditions, two models were derived and proposed. First risk model approach was derived from biofilm formation classification by Christensen et al. [40] classification and called Risk of Pathogen (PR). The risk assessment 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).

Second model approach was derived from Contamination Factor of metals (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):

$$CDpathogen = ODsample/ODcontrol$$

5

For this purpose, $OD_{control}$ is included 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.

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 metal, pathogen, and biochemical parameters.

Results And Discussion

Characterizing Inhalable Bio-relevant Forms of Metals in Household Dusts

As indicated in Table 2, Al, Cr, Cu, Mn, Ni and Zn were determined as potentially toxic metals in bio-relevant 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 metals was greater in the ALF conditions, which mimics to inflammatory conditions, compared to other simulated bio-relevant fractions of household-dusts. This can be explained that ALF is reflected inside the lysosome of macrophages (phagolysosomes) having a more acidic condition, therefore, metal dissolution is generally higher in this condition compared to the neutral lung fluid [13]. Although PSF has same pH with ALF, which is 4.5 and reflects the alveolar macrophage fluid and simulated phagolysosomal fluid, the dissolution of the determined elements was not higher in this condition compared to ALF. This result can be explained by simpler composition of the PSF limiting the dissolution compared to ALF. Moreover, the solubility in ALF also depends on the chemical state of metal. For example, metal oxides, carbonates, chlorides are known to be easily soluble in this media. This suggested these metals in household-dust can be form of oxides, carbonates, and chlorides rather than sulfite, phosphate, and silico [42] (Innes et al. 2021). However, there is no study to compare the findings, and previous studies mostly conducted total amount of metals or using one physiological modelling. In the meantime, quality control of our study applied by triplicate for each of samples and relative standard deviations between replicates were very low, thus the results can be accurately compared.

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

Metals	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

The results also indicated that the average metal 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 metal (e. g., Al) was the predominant metal found in all bio-relevant fractions of household-dusts while toxic metals such as Cu, Cr, and Ni were lower levels. Moreover, the higher contents of Zn and Mn can be originated by the road and windblown dust, biomass burning and traffic activities [43, 44]. These results correlated the study of Hu et al. [7], who reported the *in vitro* inhalation/ingestion solubility of the airborne particle-bound elements in room air conditioners filters. On the other hand, there are no national or international standard limit values for indoor dust samples. Instead, soil reference values which is the standard of Chinese National Soil Quality Standards-National Environmental Protection Agency 1995 are employed to assess level of metals in dusts [45, 46]. The concentration of Cu, Ni and Zn in the household dust samples using various bio-relevant conditions 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 to 3.71 ± 2.51 µg/g for the tested simulated biological solutions (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 metals in soil and plants. Geneva, Switzerland, 1996). Cr concentration in all simulated bio-relevant conditions was greater than this permissible limit. Thus, the potential human health effect from determined metals in bio-relevant solutions cannot be ignored in the household-dust samples.

Furthermore, the 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 metals, nutients, etc. [37, 47]. Therefore, the correlations were investigated (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 metals in PSF representing the vehicular traffic-related emissions and paints in these conditions [48]. These results showed that the occurance of these potentially toxic metals had no strong relationship with adsorption and transporting the toxic metals 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 characterise the major metal components related the biochemical reactions, Ca, Mg, Na, K, Fe and P were determined in these bio-relevant fractions of household-dusts (Table 2). Similar with the potentially toxic metals, the levels of the major metal components were greater in the ALF fraction of household-dusts due to the acidic and complex environment of the solution compared to other tested bio-relevant solution (water, PSF, and Gamble's solution) and form of these major metals (e.g., oxide, chloride, carbonate) [42]. The highest concentration was determined at Fe in household-dusts extracted in water, ALF, and Gamble's solution compared to other major metals. Since Fe is an abundant element in the earth crust and it has been transported via wind. It can be also associated with soil or street dust, and this impurity can be originated by coal burnt in outdoor environment [43]. This result indicated that street dust might be dominant source for the household-dusts, especially in these bio-relevant fractions. The order of the major metals 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 higher contents of Ca, Na, Mg, and K clearly indicate the impact of soils, paints, and cements on household-dusts and their bio-relevent contents. Moreover, use of insecticides and chemicals for the general cleaning of the house might contribute to K levels [41].

Overall, the results based on the major and potential toxic metal contents in the bio-relevant forms pointed possibility of the anthropogenic sources rather than natural sources in the household dusts.

Impact of the Inhalable Bio-relevant Forms of Household-dusts on Pathogen Behaviors

The household-dust samples, which were extracted with various inhalable bio-relevant fluids, was observed to have a significant impact on the activity of gram-negative *E. coli* and *P. aeruginosa*, and gram-positive *S. aureus* and *S. epidermidis* (Fig. 1). The results indicated that the activity declined in the presence

of bio-relevant fractions of household-dusts in compared to controlled conditions. However, the difference in simulated bio-relevant 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 fractions of household-dusts compared to other bio-relevant fractions (e.g., water, ALF, and Gambles' solution). 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, whereas the activity of *S. aureus* and *S. epidermidis* was higher in the Gamble's solution fraction of household-dusts rather than other bio-relevant fractions, and the order was Gamble's solution > PSF > water > ALF. The higher activities in the fraction of PSF and Gamble's 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 [16, 42].

Furthermore, the activity levels of pathogens were varied in the presence of bio-relevant 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 response to the anthropogenic contaminants have commonly explained by the bacterial cell-wall properties, however, in our case, the cell wall explanation is not valid. Since the household-dusts include many metals, and the dissolution level of substances with the bio-relevant fluids can influence the bacterial activity rather that cell-wall properties.

To understand the background on the activity responses, the main biochemical (e.g., protein and carbohydrate) and oxidative (antioxidant and LPO) indicators were examined (Figs. 2 and 3). The results showed that protein, carbohydrate, antioxidant and LPO activities declined with the presence of bio-relevant fractions of household-dusts compared to the controlled conditions, except carbohydrate levels of gram-negative *P. aeruginosa* and *E. coli*. For the response of gram-negative bacterium, protein, carbohydrate, and antioxidant levels had similar behavior with the bio-relevant 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 response 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). For *P. aeruginosa*, the activity had positive correlation with protein and antioxidant in water and PSF fraction of household-dusts, however, the activity 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 for protein, carbohydrate and antioxidant levels and the response in bio-relevant 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 for protein, carbohydrate, and antioxidant, respectively. However, the LPO activity of *S. aureus* and *S. epidermidis* were different in various bio-relevant fractions of household-dusts and their response was changed as Gamble's solution > PSF > water > ALF and PSF > water > Gamble's solution > ALF for *S. aureus* and *S. epidermidis*, respectively. The Pearson correlation results also indicated that *S. aureus* activity had significant positive correlation with protein and significant negative correlation with carbohydrate in water fractions, whereas it has positive correlation with protein and antioxidant in ALF fraction, positive correlation with protein, antioxidant and LPO in PSF fractions, and positive correlation with protein and antioxidant, and negative correlation with carbohydrate 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 and oxidative indicators of the tested pathogens with different ways.

Another important pathogen activity is the biofilm production. The analysis showed that the exposure of bio-relevant fractions of household-dust influenced the biofilm formation of tested pathogens compared to controlled conditions (Fig. 4). The more biofilms formed with gram-negative *P. aeruginosa* and *E. coli* compared to gram-positive *S. aureus* and *S. epidermidis*. For gram-negative *P. aeruginosa* and *E. coli*, the more biofilms formed in PSF and Gamble's solution fractions of household-dust and rather than water and ALF fractions, and 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* were varied in different bio-relevant fractions of household-dusts. The biofilm formation order is ALF = PSF > water > Gamble's solution and Gamble's solution > PSF > ALF > water for *S. aureus* and *S. epidermidis*, respectively. These results observed that the importance of biofilm formation in the bio-relevant fractions of household dusts and needs extra effort.

Model Approaches for Risk Assessments and Correlation Analysis

To distinguish the anthropogenic inputs in simulated bio-relevant fractions of household-dust, the Igeo was calculated and evaluated (Supplementary Fig. 2). The Igeo values with bio-relevant fractions show that Igeo values was found to be smaller than 1 which indicated the classification of practically uncontaminated (Baysal and Akman 2018). The order of the average Igeo values of the potentially toxic metals in water, ALF and PSF fractions is similar, and the order is Cu > Ni > Cr > Mn > Al > Zn, whereas in Gamble's solution fraction is Cu > Cr > Ni > Mn > Al > Zn. Besides, the Igeo values of each metal 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 metals are greater in bio-relevant fluids having acidic conditions (ALF and PSF) compared to other tested bio-relevant 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 [7, 44]. Moreover, higher concentration of metals in lung fluids may also prove the importance of these metals on human.

Daily intake (DI) of the potentially toxic metals 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 good agreement with the previous studies determined using total metal concentration [48, 50]. Moreover, the DI results indicated that the higher DI values obtained in ALF fractions mimicking the inflammatory condition for all determined metals.

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

Water								
Metal	Dlinhalation				HQinhalation		CRinhalation	
	child		adult		child	adult	child	adult
	non-carcinogens	carcinogens	non-carcinogens	carcinogens				
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								
Metal	Dlinhalation				HQinhalation		CRinhalation	
	child		adult		child	adult	child	adult
	non-carcinogens	carcinogens	non-carcinogens	carcinogens				
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								
Metal	Dlinhalation				HQinhalation		CRinhalation	
	child		adult		child	adult	child	adult
	non-carcinogens	carcinogens	non-carcinogens	carcinogens				
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								
Metal	Dlinhalation				HQinhalation		CRinhalation	
	child		adult		child	adult	child	adult
	non-carcinogens	carcinogens	non-carcinogens	carcinogens				
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
Zn	1.19E-08	1.02E-09	2.79E-08	2.39E-09	3.96E-08	9.30E-08	x	x

Water								
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

Tablo 4. Proposed possible risk assessments of household dusts by pathogens (PR: pathogen risk, CD: Contamination degree of pathogen).

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

The non-carcinogenic risks from determined metals 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 metals. These results were coherent with the studies of other research that they examined the metals in various environmental samples [7, 48, 50]. Moreover, the HQ values for the determined metals 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-relevant fractions that shows selectivity of metals under inflammatory conditions and importance of the conditions. This result also linked with the higher content of metals 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 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-relevant 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 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* didn't 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-relevant 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-relevant 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 the biofilm formation.

The Pearson correlation analysis applied to understand the interaction between metals and pathogens and the results presented in Supplementary Table 5–10. These results indicated that all tested biological parameters of the pathogens were correlated one or more than major and potentially toxic metals, and this linking mainly positive. 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 metals. 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. Contrarily with the activity of *S. epidermidis* in PSF fraction, the biofilm formation of *P. aeruginosa* and *S. epidermidis* in PSF fraction had negative correlations, specifically with Cr, Ni and Mg.

Conclusion

Taken together, these finding suggests that household-dust, depending on the bio-relevant fractions, have influence on the metal dissolution and culturable pathogen responses. The study indicated that the concentrations of metals, their DI and HQ values in ALF fraction of household-dusts were higher compared to other bio-relevant 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-relevant environment, as well as *P. aeruginosa* and *S. epidermidis*. The correlation analysis also observed that pathogen responses were correlated with the tested metals. Therefore, solubility of the major metals and potentially toxic metals in different bio-relevant fluids is important to understand the impact of metals on human health. In the meantime, dissolution of household-dusts in the bio-relevant fluids are also influenced tha pathogen responses by the impact of metals.

In conclusion, to more accurate modelling and the risk assessments of household dusts, both chemical and biological indicators can examine under bio-relevant conditions. Moreover, biological responses can correlate with metals under realistic conditions, and vice versa.

Declarations

Compliance with Ethical Standards

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.

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.

Authors' contributions:

All authors contributed to the study conception and design. Investigation, Resources, Methodology, Writing- Original draft preparation was performed byAsli Baysal. Conceptualization, Investigation, Methodology, Writing- Original draft preparation was written by Hasan Saygin. Methodology was performed by Sevilay Zora. All authors read and approved the final manuscript.

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Figures

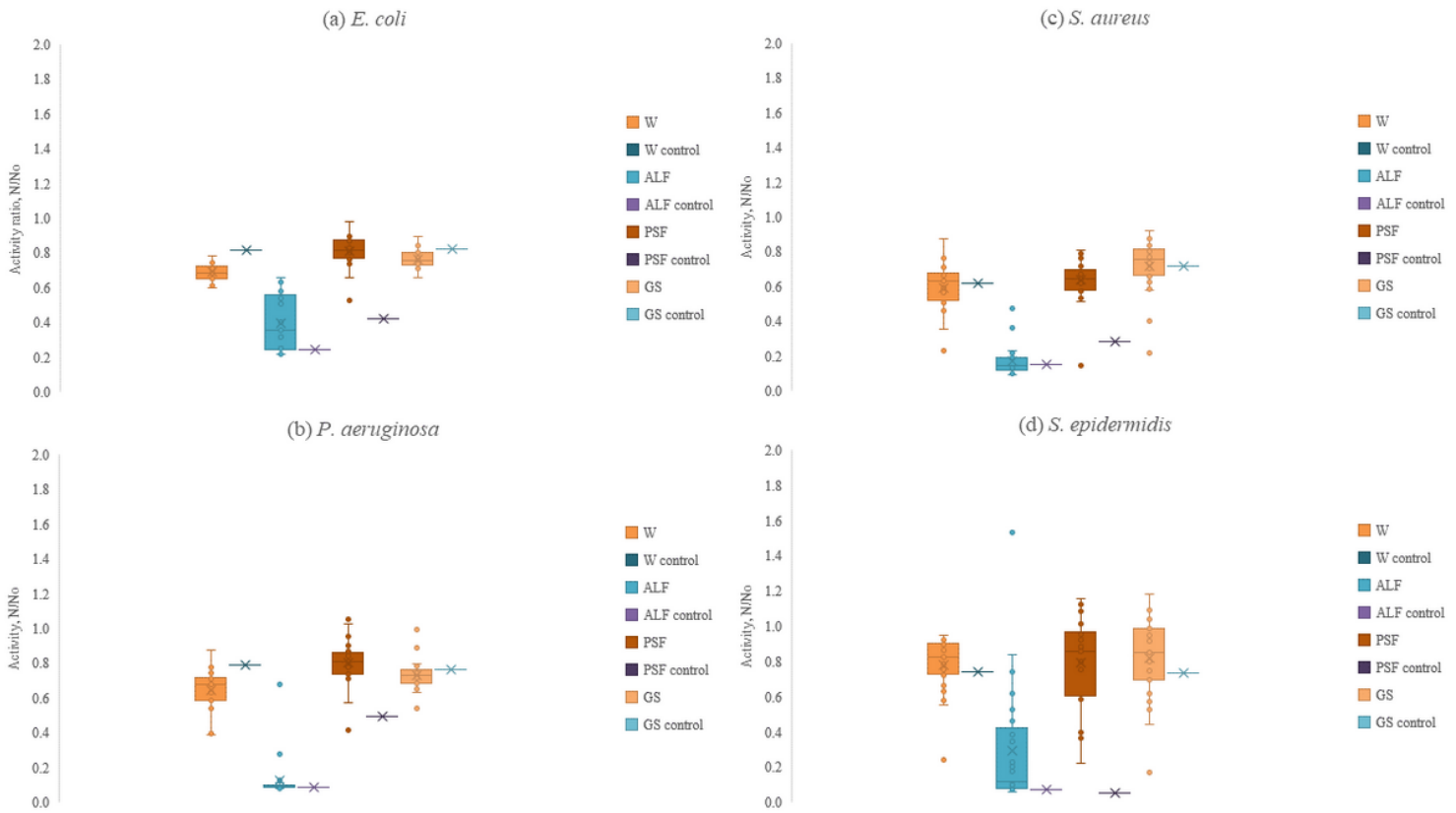

Figure 1

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)

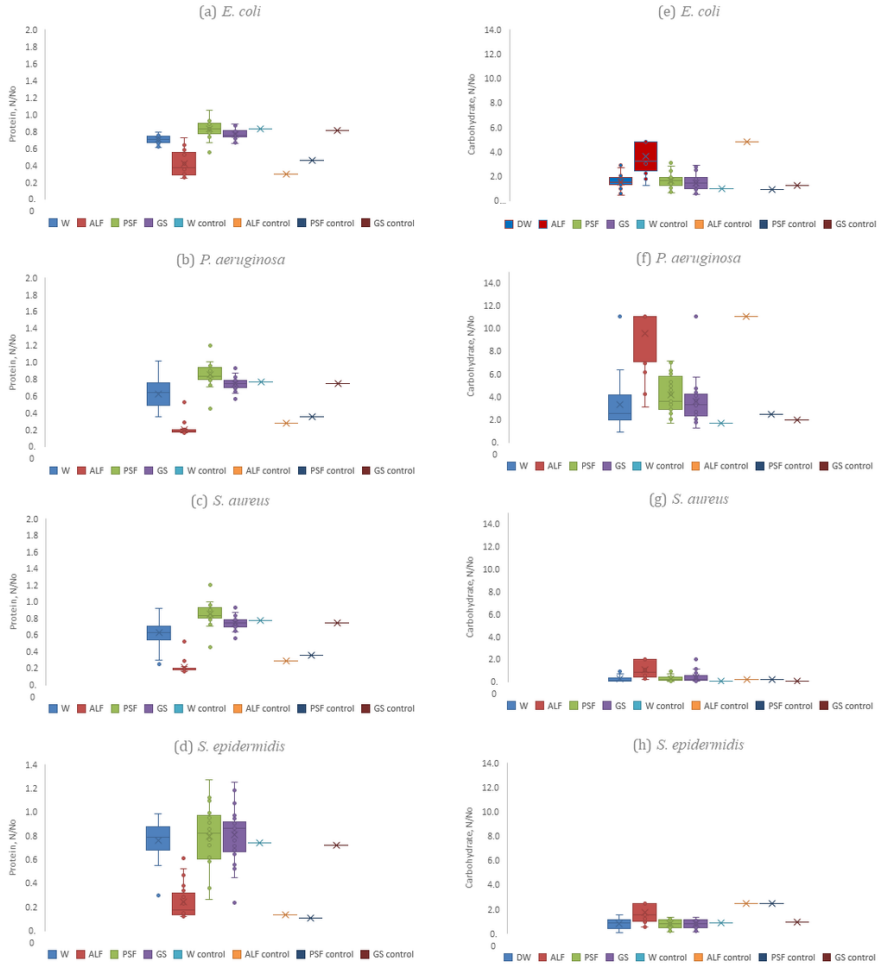

Figure 2

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)

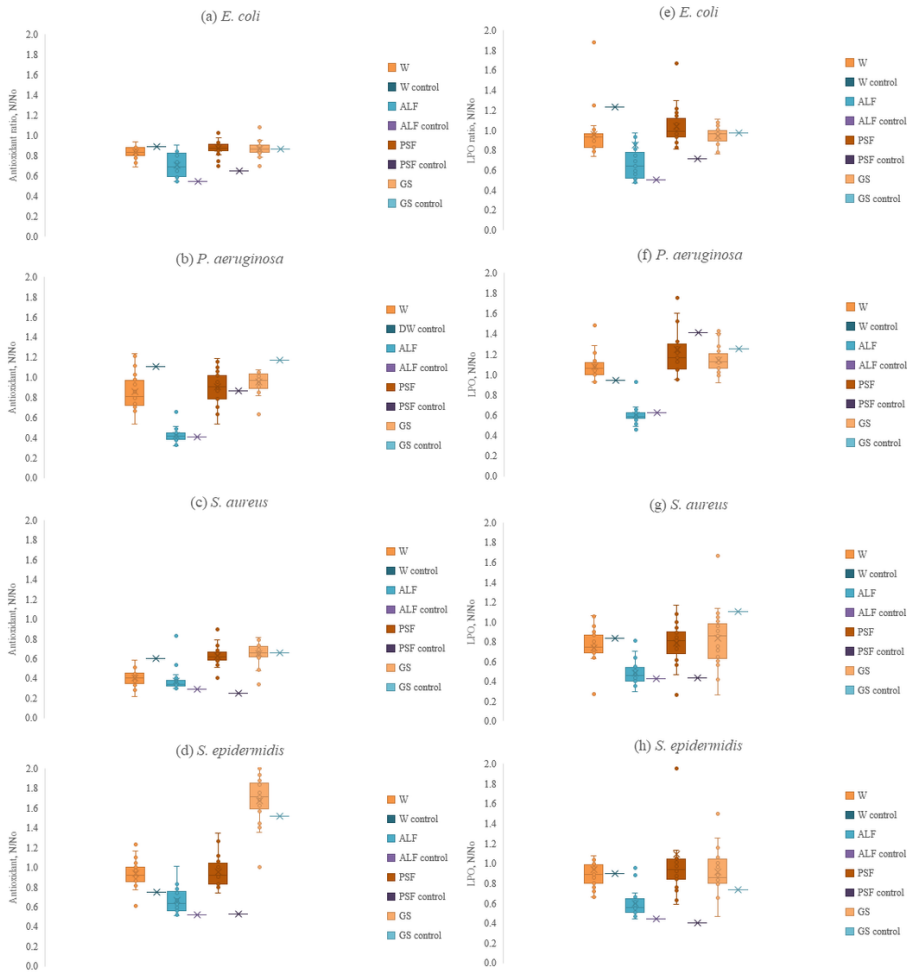

Figure 3

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)

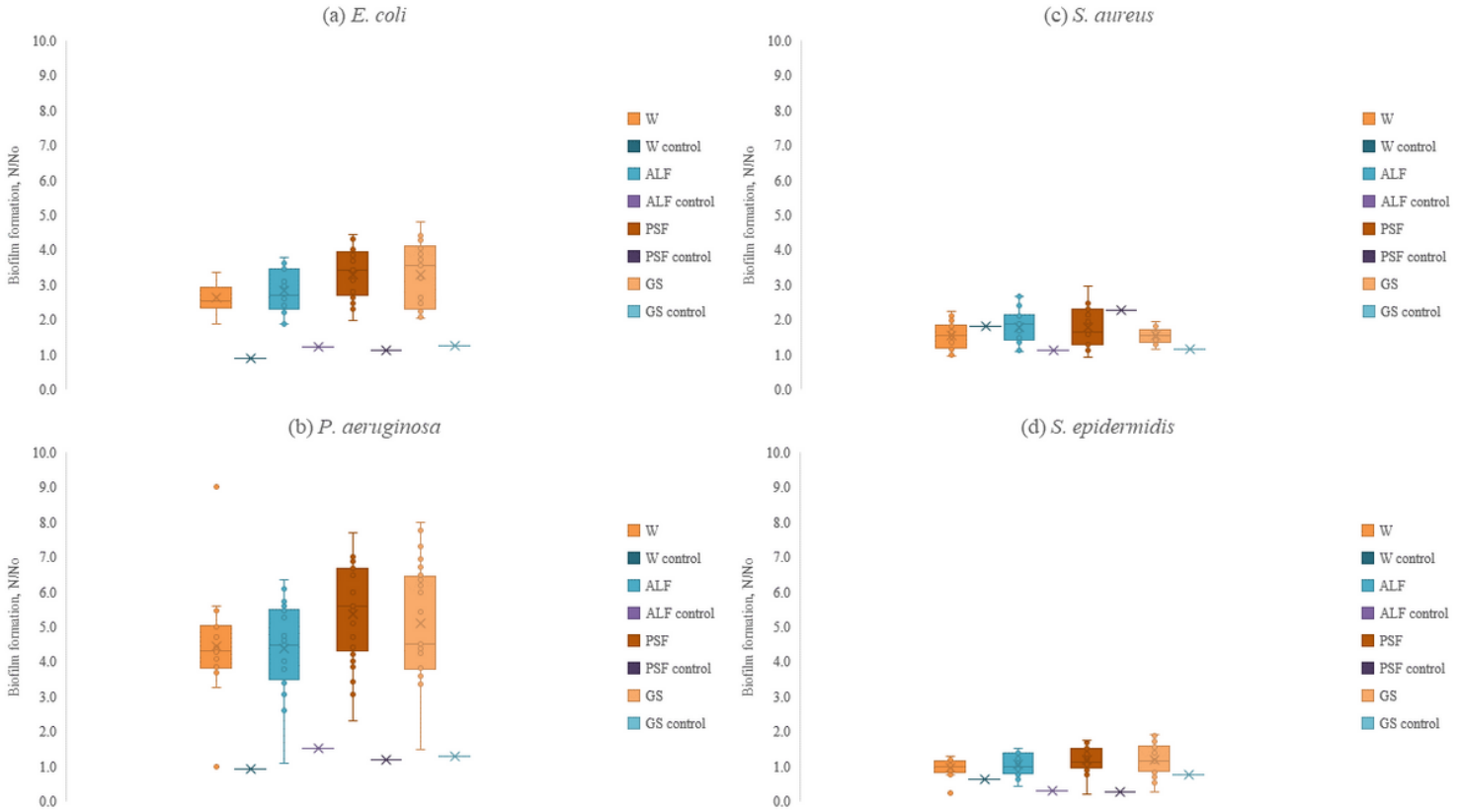

Figure 4

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)

Supplementary Files

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- supplementarymaterials.docx