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Ecotoxicological profiling of PM generated by urban heating and transport

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Abbreviations

DCM	Dichloromethane
DMSO	Dimethylsulphoxide
EC	Elemental carbon
EC_{50}	Effective concentration, causes 50% of ecological effect
HH	Household
HMW-PAH	High molecular weight polycyclic aromatic hydrocarbon
LMW-PAH	Low molecular weight polycyclic aromatic hydrocarbon
LOD	Limit of detection
OC	Organic carbon
OSB	Oriented strand board
PAH	Polycyclic aromatic hydrocarbon
PE	Polyethylene
PM	Particulate matter
PM_{10}	Particulate matter with aerodynamic diameter less than 10 µm
$PM_{2.5}$	Particulate matter with aerodynamic diameter less than 2.5 µm
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PVC	Polyvinyl chloride
TC	Total carbon
TU	Toxic unit
VFBIA	*Vibrio fischeri* bioluminescence inhibition assay

Abstract

Airborne particulate matter (PM) is a solid sample. In order to examine the aggregate toxicity of PM samples, most protocols test the liquid phase. According to the reported methods available in the literature, different solvents (either water or organic solutions) are used to prepare an extract.

In the different sample preparation procedures, extraction with organic solvents affect toxicity in extracts, resulting in false outputs. However, the possible option is a whole-aerosol test. This test is based on the kinetic version of the *Vibrio fischeri* bioluminescence inhibition bioassay and mimick an environmentally realistic exposure route. In the whole-aerosol test, use of organic solvents can be avoided. In addition, the test bacteria are in contact with contaminated PM.

The author intended to compare the different extraction procedures with the direct contact test based on the *V. fischeri* bioluminescence inhibition bioassay. Results demonstrated that the sensitivity of the direct contact test is equal to different elutriates. Considering the outcome of the chemical and *Vibrio* test results, the "whole-aerosol" test might be interchangeable with results gained for extracts, taking into consideration the availability of laboratory equipment and/or protocols.

The further goal of the author was to characterise seasonal differences in ecotoxicity of the rural PM samples using different test organisms, including *Vibrio bioassay*. Considering the analytical measurement and *Vibrio bioassay* results, our study proved that *Vibrio bioassay* is a good screening tool for evaluating the ecotoxicity of PM samples.

PM_{10} samples originating from the controlled combustion of illegal wastes (PS, PE, PP, PVC, PU, OSB and Rag) were examined to give initial data on ecotoxicity profile during his research. In the *Vibrio* test, except OSB sample, all samples were categorized as toxic according to the toxic units. Considering the analytical measurement results, the behavior of plastic samples was distinguished based on our findings. The PVC sample contained a high amount of Cd, whereas PP, PS and PU were characterized by high PAH content.

Subsequently, *Vibrio* test and analytical measurements were utilized to investigate the PM samples released from the colored and colorless PET wastes under open combustion experimental setup. Based on chemical composition and *Vibrio bioassay* results, a significant relationship was found both PET waste samples.

Ultimately, *Vibrio* test and analytical measurements were used to assess the Mongolian indoor PM samples through the frame of a Stipendium Hungaricum Programm and use of the possibility to cooperate with the University of Pannonia (Hungary). Based on analytical measurement and Vibrio test outputs, indoor PM in each non-smoking household appears and elevated risk to inhabitants.

The results of the dissertation reveal that the whole-aerosol had excellent discriminative power in different environments.

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1 Introduction

Particulate matter (PM) with an aerodynamic diameter of less than 10 µm (PM_{10}) and 2.5 µm ($PM_{2.5}$) is now identified as one of the most dangerous pollutants on human health by the EU Ambient Air Quality Directive (2008/50/CE).

In the environment, aerosol is a system of solid or liquid particulate matter suspended by a mixture of gases, covering a wide spectrum of small particulate matter including sea salt, mineral dust, drops of sulfuric acid, pollen, bacteria, and viruses. PM is generated by natural and anthropogenic activities. Natural sources (i.e. soil dust, ocean aerosol) are prevailing, and they are approximately 3 to 7 times higher than PM from anthropogenic activities (https://www.eea.europa.eu/themes/air/air-pollution-sources, accessed 08 September 2023). However, the latter are more important PM pollution sources for human health, namely fuel combustion (coal, wood, liquid fuels), including traffic-related emissions, biomass burning and illegal waste burning. The study of Karagulian *et al*. (2015) estimated that globally, the percentage of domestic fuel burning attributed to 20% and 15% of urban ambient $PM_{2.5}$ and PM_{10} , respectively. Addition to domestic fuel burning and traffic-related emissions, illegal waste burning in households have appeared in both developed (Wiedinmyer et al., 2014) and less developed (Lemieux *et al.*, 2000) regions of the world, resulting in a general worsening of air quality in urban areas (Kanellopoulos *et al.*, 2021) and increased PM emissions (Krecl *et al.*, 2021)

In addition, PM has a great impact on several atmospheric episodes, Earth's climate and on the biosphere (https://climate.nasa.gov/news/3027/getting-to-the-heart-of-the-particulate-matter/, accessed 10 August 2023). The climate forcing by aerosols can be realized in direct (scattering and absorbing solar radiation) and indirect radiation forcing (participating in cloud formation processes) (Ghan *et al.*, 2012).

During the combustion process, particulate matter (PM) releases carcinogenic compounds, such as polycyclic aromatic hydrocarbons and transit metals. These compounds may play a major factor concerning toxicity and ecotoxicity of atmospheric PM as they have a high affinity to the very fine and ultrafine aerosol fractions (Sánchez-Piñero *et al.*, 2021). Indeed, toxicological effects of atmospheric PM generated from combustion have been widely studied (e.g., Johnston *et al.*, 2019; Nagy and Szabó, 2019). They are associated with human health effects such as respiratory and cardiovascular mortality and morbidity (Wan Mahiyuddin *et al.*, 2023). However, less information is available on the non-human biota.

Analytical measurements are widely used to examine the effect of pollutants discharged into the environment due to human activities. However, analytical measurements are not sufficient to estimate the environmental impacts of PM due to their long analysis time, costly measurements, and limited information on the concentration of the pollutants. Therefore, it is most important to use simple and reproducible bioassays.

In ecotoxicology testing, so-called single-species bioassays are widely used. The assay based on bioluminescent inhibition of the marine bacterium *Vibrio fischeri* is one of the most frequently applied one due to its numerous advantages. For instance; freeze-dried and liquid-dried kits can be purchased containing *V. fischeri* test organisms to facilitate laboratory work with no need to prepare stock cultures. This test is automatized, as such, researcher errors can be minimized as well.

In addition, in most cases, this bioassay can be successfully employed as the initial screening method to assess the aggregate toxicity of different environmental media, since it is considered a reliable, sensitive, cost-effective, and easy-to-use method. An additional pro of this bioassay is that it requires a rather small amount of the sample. For instance, in case of particulate matter samples which are relatively complicated and costly to collect, a low amount of sample can be collected on a filter and employed for the *V.fischeri* bioluminescence inhibition bioassay. This test has already been proven to be a distinguished tool for screening the potential eco-toxicity of particulate matter (Lin *et al.*, 2002; Roig *et al.*, 2013).

Most frequently, aqueous extract is applied to assess the toxicity of different samples, including particulate matter from urban environments (Roig, Sierra, *et al.*, 2013; Wang *et al.*, 2016). Literature regarding the use of organic solvents (e.g., DMSO, methanol, DCM, and hexane) to prepare an extract for assessing the solid PM samples is also available (Vouitsis *et al.*, 2009; Chang *et al.*, 2013; Verma *et al.*, 2013; Aammi, Karaca and Petek, 2017). However, these solvents are not the best options for extraction as concentration of the potentially toxic compounds in the extract would be strongly depending on the solvent applied, resulting in different research outputs (Verma *et al.*, 2013; Corrêa *et al.*, 2017).

In order to avoid these problems, a novel sample preparation method (so-called "Direct contact" test) was presented by Kováts *et al.* (2012). This method protocol is performed based on the kinetic version of the *Vibrio fischeri* bioluminescence inhibition bioassay, which is available as an ISO standard (ISO 21338:2010: Water quality – Kinetic determination of the inhibitory effects of sediment, other solids and colored samples on the light emission of *V.fischeri* /kinetic luminescent bacteria test/) (Lappalainen *et al.*, 1999, 2001). In the kinetic test, luminescent intensity of turbid and colored samples is assessed in kinetic mode. After bacterial suspension is injected into the extracted sample, luminescence intensity increases to a maximum peak within 30 seconds. That is why the system is called Flash (after this referred to as Flash system). Direct contact test implies that test organisms are in direct contact with suspensions containing PM without further procedures, mimicking naturalistic exposure route.

The reliable test was adapted and used for evaluating the different types of PM samples. Among these, are urban PM, diesel PM, biomass burning emission, PM emission from cooking activities and biodiesel emissions, as well as indoor PM. (Kováts *et al.*, 2012, 2013; Turóczi *et al.*, 2012; Ács *et al.*, 2013; Pintér *et al.*, 2017; Eck-Varanka *et al.*, 2018; C. A. Alves *et al.*, 2021; Vicente *et al.*, 2021).

2 Objectives of the research:

One of the main objectives of the work was to carry out a systematic evaluation of the whole aerosol assay, by comparing its results to outcomes of parallel tests using the same samples but applying different solvents. In this way, I intended to support the proper sensitivity of the test and to establish its further use.

A major part of the thesis consists of different applications in environments where air quality is of crucial importance. One major issue addressed is the illegal burning of domestic waste. The problem is widespread not only in less developed but in developed countries as well, including Hungary.

Illegal burning of domestic waste both in household appliances or open air is a well-known contributor of PM generation and emission. Also, a wide range of studies have addressed its potential effects on human health problems. However, up to know, no systematic ecotoxicological profiling has been done on such emissions. Taking into consideration the gap in available information, my goal was to assess the ecotoxicity of PM generated during the controlled burning of specific waste samples, including a wide range of plastics. Plastics are posing a crucial role as they are significant components of household waste.

Another important field chosen was indoor air quality. Indoor air quality has been more and more in focus of air pollution studies as people spend a considerable ratio of their time indoors. In fact, indoor air quality has been shown to have a serious effect on human health.

Using coal for winter heating is an important factor influencing both indoor air and human health. Mongolia is a typical example, as coal is widely used. Mongolia is a central-Asian landlocked country, spread over 1.564.116 square kilometers and scarcely populated (3.3 million). However, due to its low economic and social indicators it is still considered a developing country. The available literature regarding air pollution in Mongolia is very limited and based on foreign funding and cooperation and it is strictly focused on the capital city (Ulaanbaatar). Besides, performing a whole campaign for air quality sampling is time-demanding and resource-intense, so an initial ecotoxicological screening may show which are the main emission sources and the most relevant topics to target.

As such, my studies were especially targeted to perform an initial screening on the ecotoxicity of indoor samples collected in Ulanbaatar. Typical housing types were chosen since a large percentage of the population still lives in this traditional way. Thus, this study intends to be representative of nearly 60% of the total population living in peripheral area of the capital city without proper heating systems. The study is the first one to use ecotoxicological methods in Mongolian indoor environments.

3 Literature review

3.1 Air pollution

Air pollution is a serious issue that impacts all living organisms and the environment. It consists of harmful compounds, which contaminate both the indoor or ambient environments by any physical, chemical, or biological agent that might change the natural characteristics of the atmosphere. Sources of air pollution are both natural and anthropogenic activities, such as transportation, industrial facilities, household devices, and wildfire (WHO, 2022). The industrial revolution has been the major cause for inducing emissions of toxic pollutants in the atmosphere over the last two centuries. When polluted air mixes with low-altitude clouds, it can generate a type of air pollution, known as smog. There are two types of smog: smog from fossil fuel combustion, mostly during cold period, and photochemical smog which is formed from industrial and motor vehicle emissions during warm period.

Back in history, in 1952, a great episode of smog happened in London. Emissions from fossil fuel were trapped in atmosphere for few days due to the combination of cold weather and an anticyclone, resulting in the death of app. 4000 people and breathing problems in many people (https://www.metoffice.gov.uk/weather/learn-about/weather/case-studies/great-smog, accessed 10 September 2023). Some decades later, in January 1985, another huge smog episode occurred for 5 days in West Germany. 24000 deaths were registered. However, the first major photochemical smog event was observed during the middle of World War II, later this type of smog was generated by photochemical reaction of emission from motor vehicles and power plants in Los Angeles, China, India, and Sydney (Kumar et al., 2017).

Nowadays, air pollution is still the most serious concern in developed and developing countries and more than 90% of the global population breathes unhealthy air that exceeds WHO guideline limits. According to the WHO guideline report, air pollution is responsible for an estimated 7 million deaths each year, and most of them occur in low- and middle income countries (WHO, 2022).

Air pollution can cause a range of diseases such as respiratory diseases, heart failures, chronic obstruction of pulmonary disease (COPD), stroke, lung cancer and other diseases

(WHO, 2022). The number of deaths dramatically increased due to ambient air pollution in all regions of the world. The World Health Organization (WHO) estimated that air pollution had been associated with 4.2 million premature deaths globally in 2019 (WHO, 2022).

Air pollution includes as principal toxic pollutants gases (carbon dioxide, sulfur dioxide, nitrous oxide, methane, carbon monoxide, ammonia, and chlorofluorocarbons) and particulate matter (both organic and inorganic). Of these, carbon dioxide, nitrous oxide, chlorofluorocarbons and methane are greenhouse gases. Across the world, emissions from household devices and transportation are the primary sources of PM and globally, domestic fuel burning emerged as the major contributor in Africa (34%), and the Rest of the Americas (25%), Southern China (21%) and India (19%) (Karagulian *et al.*, 2015). Considering emissions of pollutants in EU, by 2019 all main of pollutants continued to decline. For instance, emissions of both PM_{10} and $PM_{2.5}$ from residential, road transport and agricultural sources fell by 27% and 29% respectively between 2005 and 2019 (https://www.eea.europa.eu/publications/air-quality-in-europe-2021/sources-and-emissions-of-air, accessed 20 Oct 2023).

However, it remains a concerning issue across the world. In 2020, over 50% of European cities exceeded the WHO recommendations for annual $PM_{2.5,}$ and this problem was particularly apparent in the winter period due to the combustion of coal and biomass for household heating. 18% of European cities are chronically polluted, For example, in urban areas in Poland and Bosnia-Herzegovina the average fine PM is above 55 $\mu g\ m^{-3}$ during the heating season (WIAQ, 2020). However, governmental air monitoring stations and reporting in European cities are relatively ubiquitous, which are common in big cities. Whereas in rural areas, governmental monitoring stations are sparse (WIAQ, 2020).

According to the National Oceanic and Atmospheric Administration (NOAA), the global average concentration of carbon dioxide in the atmosphere has been growing for last five decades due to deforestation, burning of fossil fuel, and cement production. The new high record of the carbon dioxide (417 ppm) was recorded in 2022 (https://www.climate.gov/news-features/understanding-climate/climate-change atmospheric-carbon-dioxide, accessed 18 Oct 2023). Ambient levels of CO in developed countries are mostly below limit values, due to use of emission control methodologies, including catalytic converters in automobiles. However, high levels are observed from biomass burning and wildfire (EEA, 2019).

Since the industrial revolution era, methane level has been increased and contributes to global warming. In methane and ammonia emissions, the main source is agriculture (55% and 94%, respectively) in EU countries (https://www.eea.europa.eu/publications/air-quality-in-europe-2021/sources-and-emissions-of-air, accessed 20 Oct 2023). A study by Zhenqi Luo *et al.* (2022) estimated that global ammonia emission in the atmosphere has increased in different regions in the world as follows: India (13% per decades), tropical Africa (33% per decades), South America (18% per decades). According to the NOAA, average methane level in the atmosphere was 1,895.7 ppb in 2021 globally.

Road transport is a principal source of nitrogen oxides, being responsible for 39% of emissions. In EU, emissions of this pollutant has fallen by 36% since 2005 (https://www.eea.europa.eu/publications/air-quality-in-europe-2021/sources-and-emissions-of-air, accessed 20 Oct 2023). Considering sulfur dioxide, energy supply sector is main source. Sulfur dioxide is a deleterious gas that attributes to acid rain, haze, and both human and animal respiratory diseases. Interestingly, average concentration of sulfur dioxide has been dropped in atmosphere (https://www.epa.gov/air-trends/sulfur-dioxide-trends).

By 2019, in EU, emissions sources of heavy metals to atmosphere were manufacturing and extractive industries. Of heavy metals, nickel (61%) and arsenic (54%) have surprisingly dropped since 2005 due to tight regulations based on a result of EU legislation such as Large Combustion Plant Directive 2001/80/EC and Industrial Emissions Directive 2010/75/EU.

3.2 Sources of atmospheric PM

Based on the episodes of PM formation, primary and secondary PM can be distinguished. Primary particulates are directly emitted into the atmosphere from natural sources (volcanic eruption, soil dust, sea salt, biogenic PM and wildfire e.g.) (Fu *et al.*, 2004; Stohl *et al.*, 2011; Vicente *et al.*, 2012; Bian *et al.*, 2019; Chen *et al.*, 2022) and anthropogenic sources (combustion PM) (Gelencsér *et al.*, 2007) (***Fig 1***). The precursors of gases (NO_X, SO_X, NH_3, and Volatile organic compound -VOC) emitted into the atmosphere from fossil fuel combustion are most important for secondary particulate matter emissions (Fowler *et al.*, 2013; Hamid *et al.*, 2019; Isson *et al.*, 2020). Secondary PM are detailed in Section 3.1.4.

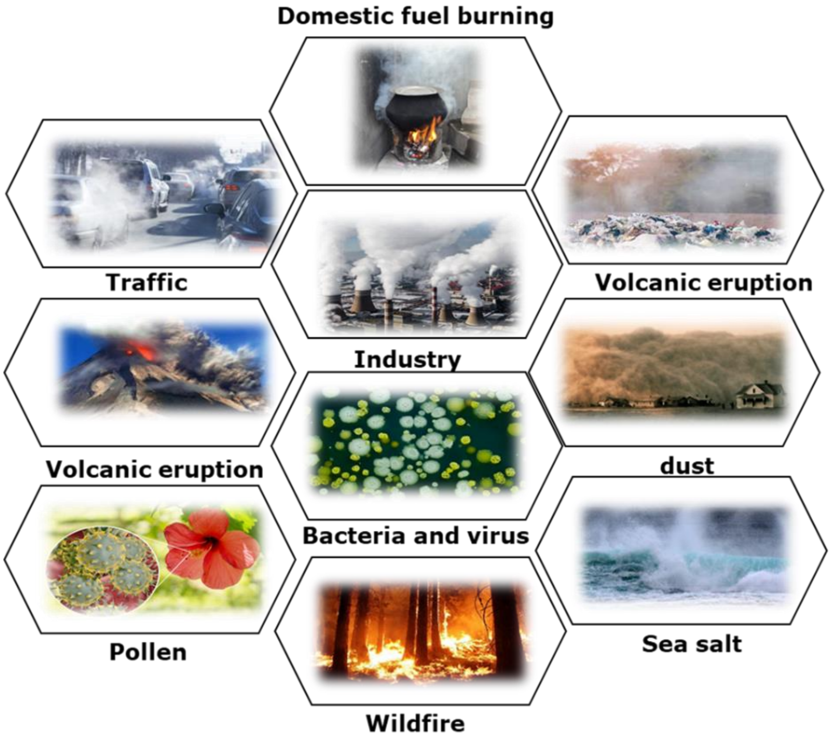

Fig 1 *Particulate matter emission sources*

3.3 Primary PMs

There are two main natural sources of atmospheric PM: ocean and soil dust from dry continental regions.

Soil dust: Soil-derived PM is mainly formed by wind forces or Aeolian erosion in the dry or semi-arid and mining areas as desert or mineral dust. For instance; Saharan dust storm alone accounted to 40-66% of the total dust, which can be transported as long as 4000 km from their origin (Kutiel et al., 2003). Large PM with a diameter greater than 40µm are mainly deposited near the source regions, while the relatively small PM have a lifetime of several days to few weeks and can be transported to thousand kilometers from their origin. Soil PMs are having key role in tropospheric chemical reactions as they interact by heterogeneous chemical reaction with sulfuric acid and nitric acid, influencing gas phase composition and particle acidity (https://nap.nationalacademies.org/read/12743/chapter/5, accessed 10 Oct 2023). For the size distribution, more than 90% of the mass classified as coarse PM. The composition of soil dust is highly varied in different regions, however, aluminum (Al), silicon (Si), calcium (Ca) and iron (Fe) are basically one of the key indicators of soil derived-material (Zhang *et al.*, 2003).

In addition to Sahara dust, in Europe, mainly Italy, Spain and France are most important local dust reservoirs. Annual amount of PM_{10} emitted to atmosphere through the wind from territory has been estimated app. in the range of 0.66 – 0.88 Tg yr^{-1} (Borrelli et al., 2015).

Sea salt: in theory, when water droplets evaporate, salt is left in the atmosphere creating marine aerosol PM (MAPM). Naturally, inorganic (e.g. sodium, chloride and magnesium sulphate) and organic compounds occur in marine atmospheric PM and sea salt is a highly soluble species as well as it plays important roles in tropospheric chemistry and cloud formation (Bian *et al.*, 2019). Emission of sea salt PM is app. 19800 Tg yr^{-1} and the fresh and aged sea salt PM perform key roles in affecting global climate (Schulz et al., 2004). Sea salt PM under 10 µm in diameter are dominant aerosols in marine surface air and can be transported for hundreds of kilometers and even can make a significant contribution to the land-based PM levels, depending on high-speed wind. Sea salt can react with acidic gases such as sulphuric and nitric acids to form sodium sulphate and sodium nitrate and liberate chloride to the gas phase. In urban PM, this phenomenon was demonstrated (Fu *et al.*, 2004).

Biogenic PM: Biogenic particles are omnipresent in the Earth's atmosphere as they impact atmospheric chemistry, biosphere, climate and adverse health effects on living organisms. There are two biogenic PM types based on their formation: primary and secondary ones. Primary biogenic PMs (PBPMs) are directly released into the atmosphere from the biosphere without change in their chemical composition, which consists of different particles such as bacteria, viruses, pollen, spores, algae, fungi, debris of leaves, protozoa and fragment of insects, etc., (e.g., Després *et al.*, 2012). The size of the primary biological PMs (PBAP) are in the range from several nanometers to few hundred micrometers in aerodynamic diameters.

Secondary biogenic particles are formed by gas-to-particle conversion of gaseous organic matter emitted from the biosphere. The biogenic volatile organic compounds (BVOCs), mainly isoprene, and terpenes are released by vegetation, especially deciduous trees (Hamid *et al.*, 2019). In Europe, fungal spores and plant debris are the most important contributors to PM_{10} mass and PM_{10} emission which has been estimated at 0.12 Tg yr^{-1} (Winiwarter *et al.*, 2009).

Volcanic eruption: Primary PM can be released during volcanic eruption. They can also influence climate. For instance: due to volcanic eruption of Eyjafjallajokull, Iceland during April-May, 2010, significant economic and social damage occurred in Europe (Stohl *et al.*, 2011). According to their size, more than 50% of total PM belongs to the coarse category. The volcanic particles can travel to distances of thousands of kilometers (e. g., Stohl *et al.*, 2011).

Wildfire: Wild-land fires, referred as wildfire, are one of the natural emission sources to form PM by burning forest, grassland and other vegetation due to hot temperature, volcanic eruption and lightning as well as human activities. However, in 50% of wildfires, original cause is unclear (WHO, 2022). Wildfires frequently occur in Siberia, California, Australia and in forested Mediterranean countries including Spain, Greece, Italy and Portugal and so. Emissions are predominated by fine PM, which represent app. 92 % of the PM (Vicente *et al.*, 2017). In regions of Europe, the Fire Inventory from NCAR version 1.0 (FINNv1) estimated that coarse and fine PM annual average emission from open combustion were 0.39 Tg yr^{-1} and 0.22 Tg yr^{-1} (Wiedinmyer *et al.*, 2011).

Road transport: Road transport is one of the main contributors to the ambient level of particulate matter in developed and developing countries. Globally, the contribution of road transport to urban PM is around 25% for both PM_{10} and $PM_{2.5}$ (Karagulian *et al.*, 2015). However, there can be significant size-fraction variations regionally, but traffic appears as the main contributor to ambient $PM_{2.5}$ and PM_{10} as data from different regions show : India (37% and 34%), Southwestern Europe (35% and 23%), and Western Europe (25% and 28%), Republic of Korea (23% and 21%), Middle East (12% and 15%), United States of America (24% and 30%), rest of Americas (30% and 38%) and Central and Eastern Europe (19% and 8%) (Karagulian *et al.*, 2015).

Based on the formation processes, PM emission from traffic can be grouped as follows: exhaust emission (PM are formed in internal engine combustion and then released into atmosphere) and non-exhaust emission (tyre wear, brake wear, road surface wear and road dust resuspension). The non-exhaust emission from road traffic can be formed through the mechanical abrasion, grinding, crushing and corrosion processes, while road dust resuspension can generate through the road surface owing to vehicle-generated turbulence (e.g., Harrison *et al.*, 2012).

PM emission rate from diesel vehicles is obviously higher than that of gasoline vehicles (Gertler, 2005). It should be noted that PM emission from diesel powered vehicles differ: Heavy Duty Diesel Vehicles (HDDVs) are higher emitters in comparison to other different diesel categories. However, the PM mass emission rate from diesel vehicles equipped with diesel particle filters (DPF) was on average app. 25 times lower than that of the conventional diesel vehicles (Geller *et al.*, 2006). It is important to note that due to tight regulations, such as the technological updates achieved in the automotive industry towards net-zero emission technologies in European countries, the total percentage of particulate exhaust emissions from traffic dramatically dropped between 2000 and 2020 (Rienda *et al.*, 2023).

PM_{10} emissions form paved roads in Europe, especially in Mediterranean countries, are one of major challenges for sustainable transport (Rienda *et al.*, 2023). In the study by Karanasiou *et al.* (2011) it was stressed that contribution of non-exhaust emissions to PM_{10} in urban areas was higher, especially countries with arid climates, dry seasons and low humidity.

Residential combustion: Globally, residential biomass burning for cooking and heating is one of the largest sources of particulate matter. Currently, more than 35% of the total population on earth consumes solid fossil fuel (wood, charcoal, agriculture waste, and animal dung) as residential energy supply (Bonjour et al., 2013). Despite the widespread availability of electricity and natural gas in households, the consumption of solid fuel (primary source: wood) for household heating (Karagulian *et al.*, 2015) still remains a common practice in other countries, even European countries.

A study by Karagulian *et al.* (2015) estimated that residential fuel burning (wood, gas, and coal) in Central and Eastern Europe contributed up to 32% of $PM_{2.5}$ emissions, and reaching up to 45% of PM_{10} while in Asian countries, including China, the contribution of the domestic fuel burning was 36 % of $PM_{2.5}$ emission and 26% of PM_{10} emissions, respectively.

As of 2017, annual limit values of the PM concentrations ($PM_{2.5}$ and PM_{10}) in large European cities were higher than EU limit values and WHO AQG guidelines, while these values in the rural areas exceeded the standard values as well (EEA, 2019). Several studies reported that biomass (mainly wood) burning from residential sector in Europe is one of the main sources, emitting primary PM during the winter period (Gelencsér *et al.*, 2007; Mărmureanu *et al.*, 2020). Caseiro *et al.* (2009) estimated that contribution of wood burning to PM in rural areas and smaller cities was as high as 70% in December, January, and February.

Likewise, a wide variation in the combustion of fuels in residential sectors in developing countries was found; for example, in Mongolia, solid fuel combustion for heating individual residences in the peripheral area contributed to 60% of the fine PM fraction (World Bank, 2013). The mean $PM_{2.5}$ concentration was 171 $\mu g\ m^{-3}$ during the cold season, with a maximum daily value (24h) up 766 $\mu g\ m^{-3}$ in Ulaanbaatar capital (Wang *et al.*, 2018). Raw coal was banned to burn in many parts of China, however, it is widely employed (Zhihan Luo *et al.*, 2022). Similarly, coal in various forms is still utilized in households in India, Nepal, Bangladesh, and Mongolia (WHO, 2014).

In addition to ambient PM pollution, indoor PM levels should gain more attention as people spend as much as 87% of their time in an enclosed environment, ignoring this threat to human health to which they are exposed (Klepeis et al, 2001). Ahn *et al.* (2019) measured fine PM concentration of a yurt in Ulaanbaatar, Mongolia during the wintertime. Indoor $PM_{2.5}$ concentration in the yurt was extremely high, average peak magnitude was found 407.7 µg m^{-3} with indoor smoking. Another study measured as high as 609 µg m^{-3} 24-h mean $PM_{2.5}$ concentration in kitchens which used solid fuel sampling (Balakrishnan *et al.*, 2013).

Also, high level of outdoor air pollution can penetrate to indoor environments (Sonomdagva et al., 2017; So *et al.*, 2019). A significant correlation was found between outdoor PM and indoor PM concentrations in several studies (Rogula-Kozłowska *et al.*, 2018; Y. J. Hu *et al.*, 2018; Bai *et al.*, 2020).

Illegal waste burning: Today, illegal waste burning is the focus of scientific interest due to its negative impacts on the air quality worldwide. Across the world illegal waste burning is prohibited. Nonetheless, according to available publications, the practice of illegal burning practice is also common in several European countries (Eades *et al.*, 2020; Hoffer *et al.*, 2020; Cieślik et al., 2021). This problem can appear in both developed (Wiedinmyer et al., 2014) and less developed (Lemieux *et al.*, 2000) regions of the world, resulting in a general worsening of air quality in urban areas (Kanellopoulos *et al.*, 2021) and increased PM emissions (Krecl *et al.*, 2021).

To get rid of waste and to reduce fuel cost, energy or fuel poverty might be one of the main causes of the otherwise illegal practice of burning household waste such as plastic, treated wood (furniture, OSB, hardboard, painted wood, and plywood), clothes, tyres, and used oil. The variation percentage in countries as follows; in Hungary (2-10%) (Hoffer *et al.*, 2020), Poland (30%) (Cieślik et al., 2021), Mexico (24%) (Ferronato et al., 2019), finally, England (24%) (Eades *et al.*, 2020). Wiedinmyer et al. (2014) reported that in China, PM_{10} emission from open domestic waste burning (backyard and household, etc.,) is equivalent to 22% of total man-made PM_{10} emission in China. Among these different types of waste, plastic is the most important part of household waste. Of the 250 million tons of municipal solid waste (MSW) generated, 12.4% or 31 million tons was composed of plastic based on EPA estimates (Massaro et al., 2014).

Industrial emission: Globally, 18% of industrial PM_{10} emission was attributed to power generation and industrial emissions from human activities have been found as the highest contributors to PM_{10} in different countries as follows: Japan (34%), Middle East and Southern Asia (27%), Turkey (30%), and Brazil (19%) (Karagulian *et al.*, 2015). In European countries, emissions from the power plants and manufacturing industries represent the second-largest sources of primary PM, especially in central Europe, PM_{10} emissions from industrial activities were 17% (Karagulian *et al.*, 2015).

3.4 Secondary PMs

Secondary PM is not emitted directly into the atmosphere, however, they are created through precursors of gases (NO_X, SO_X, NH_3 and Volatile organic compound -VOC) during the nucleation and condensation process in the atmosphere (Fowler *et al.*, 2013; Hamid *et al.*, 2019; Isson *et al.*, 2020). Basically, secondary aerosols are divided into secondary inorganic aerosol (SIA) and secondary organic aerosol (SOA) based on their formation. Mostly Nitrate (e.g., NO^-_3) and sulphate (e.g., SO_2) components are the main precursors, which are released from mainly anthropogenic activities (e.g., vehicle emission, biomass burning and agricultural activities) as well as from natural sources (e.g., volcanic eruption and sea plankton). For example; ammonium sulphate ($(NH_4)_2SO_4$) and ammonium nitrate (NH_4NO_3) in the troposphere are created mainly from photochemical reactions of main precursors (Bauer *et al.*, 2007), while SOA in the troposphere is formed from both oxidation of biogenic (e.g., isoprene and terpene) and anthropogenic VOC (Hamid *et al.*, 2019). These compounds in SIA are generated from the transformation of precursor compounds (nitrogen oxide - (NO_X) and sulfur oxide - (SO_X)) in the atmosphere to nitric and sulfur acid. They are neutralized by ammonium (NH_4^+) in the atmosphere. Ammonia is precursor compound for atmospheric ammonium. Ammonia is mainly emitted from agricultural sector, including uric acid decomposition in animal husbandry (Allen *et al.*, 2019). In addition, black carbon, mineral dust, and sea salt can be found in SIA. SIA has a significant impact on human health and climate effects (global warming and climate change) due to their physo-chemical characteristics. It is well known that the physio-chemical and optical properties of SOA and SIA are closely associated with homogenous and heterogonous-phase chemical reactions (Allen *et al.*, 2019). They lead to several environmental destructions, including ozone layer depletion, smoke-fog-related accidents, and acid rain formation as well as air quality deterioration, etc., (Allen *et al.*, 2019). In the climate effects of PM, there are two types; direct and indirect effects. They absorb solar radiation and are capable of scattering. Black carbon of PM in the atmosphere absorbs solar radiation, resulting in atmospheric warming. Whereas, PM with a diameter from 0.1 to 2 µm can scatter sunlight, and scattered radiation can reflect into space, resulting in atmospheric cooling. (Allen *et al.*, 2019). In snow and ice, they have a high albedo, meaning that ice and snow reflect most of the incoming radiation. When black carbon (soot) deposits on them, it leads to a decline in surface albedo, subsequently, making them melt faster (Allen *et al.*, 2019). In indirect effects on climate, PM can affect the solar radiation balance by formation of cloud droplets. Cloud droplets in troposphere are generated by condensation of the water vapor onto aerosol PM (cloud condensation nuclei - CCN) when the relative humidity is greater that saturation level (Allen *et al.*, 2019). Sulfate is an indicator of detrimental constituents from coal and oil combustion. In the acute epidemiology study, good correlation was found between diseases (respiratory and cardiovascular problem) and secondary sulfate (Allen *et al.*, 2019). Several researchers hypothesized that sulfate emissions (precursor gases of secondary PM) will continue to increase in Asian countries (e.g., China and India), however, decrease in Europe. Whereas, changes in NOx emissions in China might be increased. In the VOC emissions into the atmosphere, it is mainly released from the biosphere (e.g., deciduous trees), and its future trend is unclear as far (Allen *et al.*, 2019).

3.5 Chemical composition of PM

Generally, the chemical composition of PM is widely varied. Primary PM consists of a complex mixture of substances, including elemental and organic carbon, inorganic ions (e.g., sulfate, nitrates, ammonium, sodium, potassium, calcium, magnesium, and chloride), as well as particle-bound water, crustal material, and metals (e.g., cadmium, lead, nickel, vanadium, copper, zinc, silicon, sulfur, antinomy, and chromium) and polycyclic aromatic hydrocarbons (PAHs) (Valavanidis *et al.*, 2006; Gelencsér *et al.*, 2007; Cheng *et al.*, 2011; Nagy and Szabó, 2019).

PAHs are generated by incomplete combustion of fossil fuels, in urban and rural environments the major sources are domestic heating, transportation, and illegal waste burning (Valavanidis *et al.*, 2006; Gelencsér *et al.*, 2007; Hubai et al., 2022). PAHs released from open-air combustion amounts to 61% of the total man-made air pollution (Wiedinmyer et al., 2014). Recently, air chemistry researchers at the University of Pannonia demonstrated that burning of 12 common domestic waste types emits up to 800 times more PAHs than the dry burning firewood per mass basis (Hoffer *et al.*, 2020). In another study, 47 organic compounds of 8 different classes and bisphenol A in PM_{10} samples were collected in Aspropirgos, an urban and industrial region nearby Athens where uncontrolled combustion activity was monitored during a one-year study. Concentration of PAHs increased in the cold season as these this toxic compound highlighted significant attribution of uncontrolled burning of plastic waste to air quality (Kanellopoulos *et al.*, 2021). It is well documented that PAHs were more abundant (80%) in the fine PM samples compared to coarse PM-bound PAHs (Valavanidis *et al.*, 2006). In EU, the Euro 4 emissions limits on PM for diesel vehicles is 0.025g/km, whereas Euro 5 and 6 emissions limits on PM for diesel cars are 0.005g/km, respectively (https://www.whatcar.com/advice/owning/euro-1-to-euro-6-how-clean-is-my-car/n1190, accessed 10 Oct 2023). Basically, diesel engine equipped with diesel particle filter (DPF) can filter up to 99% of particulate matters. Several study reported that some Euro4, all Euro 5 and 6 standard vehicles equipped with diesel particle filters (DPF) show considerably lower amount PAH emission than gasoline ones (Geller *et al.*, 2006).

In comparison to the older diesel vehicles, DPF-diesel vehicles can reduce 70-95% emissions of metals and trace elements. These trace metals, however, seriously impact air quality. Valavanidis *et al.* (2006) detected Fe, Pb, Zn, Cu, Cr, V, Ni and Cd in PM samples collected from heavily polluted urban areas. In another study, trace elements (Fe, Ti, Ba, Cr, Co, Cu, Zr, and Mn) in PM samples from roadside in urban area were found (Alves *et al.*, 2021).

The major source of metals in traffic-related PM is particulate debris, which is formed by brakes on the road. Metals such as copper (Cu), antimony (Sb), lead (Pb), cadmium (Cd) and Zinc (Zn) were found in tire and brake PM samples (Hjortenkrans et al., 2006). In addition to traffic, metal contents in PM samples collected from open burning of domestic municipal wastes in different countries have been investigated (Park et al., 2013; Kumar *et al.*, 2015). Metals such as As, Cd, Sb, Sn in open-waste burning PM was abundantly observed, higher concentration of metals such as Zn, Cr, Pb, Ni, Cu, and Cd in waste plastic incineration sample were found (Park et al., 2013; Kumar *et al.*, 2015).

3.6 Classification and formation of PM

In terms of source of origin, PM is generally derived in a complex mixture of various tiny particles and gases through the combination of chemical, physical and biological processes in a wide variety of natural and anthropogenic sources (Fu *et al.*, 2004; Stohl *et al.*, 2011; Vicente *et al.*, 2012; Fowler *et al.*, 2013; Bian *et al.*, 2019; Hamid *et al.*, 2019; Isson *et al.*, 2020; Chen *et al.*, 2022). The size of PM in the atmosphere is one of the most important criteria to describe the character of aerosol, influencing both their lifetime and physico-chemical properties. Size of the PM is usually expressed as aerodynamic diameter. Aerodynamic diameter (AD) is a measure of the PM ability to move through air. And also, AD is defined as the diameter of a unit-density sphere with same settling velocity as the PM in question (https://learn.kaiterra.com/en/air-academy/particulate-matter-pm, accessed 21 October 2023). The size of atmospheric PM varies over a widely range, from few nanometer to around 100 micrometers (Harrison et al., 2000).

According to current knowledge, PM is divided into categories as coarse, fine, and ultrafine particles (UFPs) with an aerodynamic diameter less than 10 µm (PM_{10}), < 2.5 µm ($PM_{2.5}$), and <0.1 µm ($PM_{0.1}$) (***Fig 2***). These groups of PM differ significantly in their chemical and physical properties, and formation dynamics (Harrison et al., 2000). The size of the PM cannot be indicated by their source and the nature of their formation.

PM is generated through the complex process that involves the emission of gases (e.g., nitrous oxide, and sulfur oxide) and primary fine PM into atmosphere. These gases are transformed in air to form secondary PM. Whereas, PM can grow up to a size of 1 µm through the condensation, and coagulation processes, and two or more PM combine to form larger PM.

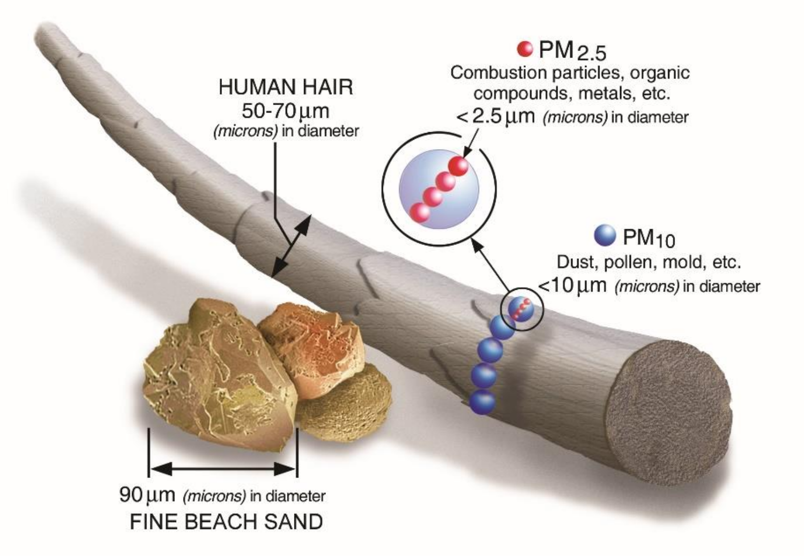

Fig 2 *Size comparisons for PM particles (https://www.sciencelearn.org.nz)*

Distribution of atmospheric PM is basically described by their surface, volume and number. The different groups of PM can be distinguished based on their PM distribution: Aitken or nucleation mode (0.01 – 0.1 µm), accumulation mode (0.1 – 1 µm) and coarse mode (>1 µm). The PM in Aitken or nucleation range are produced by homogeneous and heterogeneous nucleation processes, and collectively named ultrafine mode or ultra PM. Due to rapid coagulation, the lifetime of ultrafine PM is short, from some minutes to hours (Harrison et al., 2000).

PM in Aitken or nucleation and the accumulation mode can create fine PM. Fine PM in atmosphere can accumulate as removal mechanisms are least efficient. As a result, their lifetime in atmosphere is 7 – 10 days. Whereas for coarse mode, their lifetime in atmosphere is short because of their sedimentation (Chen *et al.*, 2022) (***Fig 3)***.

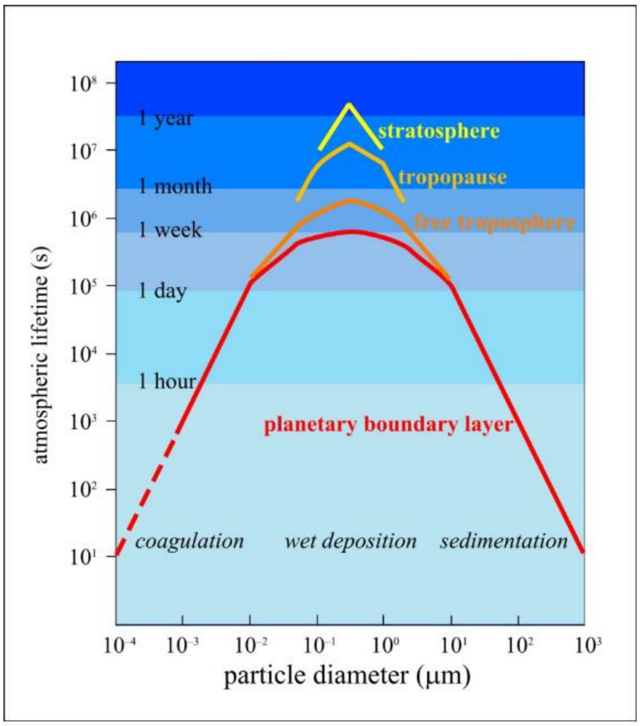

Fig 3 *Atmospheric lifetime of different size particles at different levels of the atmosphere (https://www.mkrgeo-blog.com/).*

3.7 Impacts of PM on health

Worldwide, around the one-third of the population use solid biomass fuel burning (mainly wood, coal, and agriculture waste) for household heating and cooking (Bonjour *et al.*, 2013). Today, emission of $PM_{2.5}$ from solid fuel combustion is the world's greatest environmental health hazard, and the highest premature death observed across the world was associated with the highest concentration of PM (WIAQ, 2020). Adverse health effects are directly increase with decreasing PM size (Camatini et al., 2016; Sopian *et al.*, 2021). Recently, (Vohra *et al.*, 2021) reported that globally, more than 10 million premature deaths were attributed to $PM_{2.5}$ emitted from the combustion of coal, petrol and diesel. The smaller the more dangerous: fine PM is posing the highest hazard for both humans and animals as they can deposit into the lungs and further they can get into blood circulation (https://www.epa.gov/pm-pollution/particulate-matter-pm-basics, accessed 24 October 2023).

Considering PM exposure to the age of a person, children and the elderly are more sensitive to PM than others. Long-term exposure to PM can trigger various respiratory diseases such as asthma, respiratory tract inflammation, and even pneumonia cancer, while short-term exposure to PM can trigger health effects, including eye, nose, lung irritation, coughing, sneezing, nose discharging, and abnormal breathing (e.g., Boing *et al.*, 2022).

Naturally, coarse PM also penetrate into the respiratory system, and are mainly deposited in upper respiratory system. However, they can be removed through cough and sneezing from the upper respiratory system including nose cilia, and mucous membranes of the nasopharynx and throat. According to the available reports suggested that coarse PM can more contribute to asthma and chronic observation pulmonary disease (COPD) (https://www.epa.gov/sciencematters/links-between-air-pollution-and-childhood-asthma, accessed 24 Oct 2023), whereas breathable PM ($PM_{2.5}$) is easily deposited in lung parts (mainly in the alveoli) and then damages the respiratory system by reducing airway epithelial defense and altering the immune response. Fine and ultrafine PM can reach the bloodstream, and then they can penetrate into cells through the cell barrier, resulting in cytotoxicity, genotoxicity, and neurotoxicity (Camatini et al., 2016; Sopian *et al.*, 2021) (***Fig 4***).

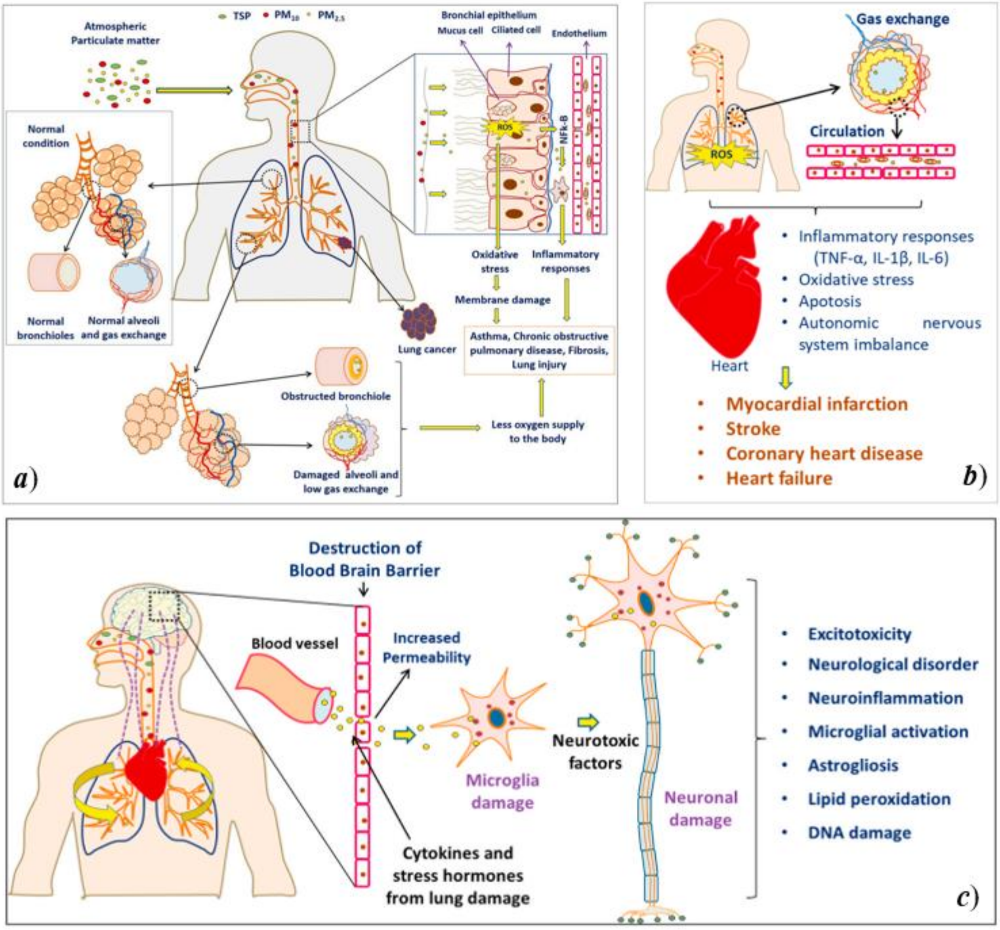

***Fig 4** Health effects of PM in different organ systems; a) respiratory system b) cardiovascular system c) nervous system (Zhu et al., 2021)*

In the EU, the number of the premature deaths attributable to $PM_{2.5}$ dropped by more than 40% since 2005 (https://www.eea.europa.eu/en/analysis/indicators/health-impacts-of-exposure-to.1, accessed 25 October 2023). On the other hand, in Asia (especially Eastern Asia), death rates were much higher than in other continents. In China and India, 3.9 million and 2.5 million deaths attributable to $PM_{2.5}$ generated from the combustion of fossil fuels (such as coal) were reported in 2018 https://www.statista.com/statistics/1203032/fossil-fuel-pollution-deaths-worldwide-by-region/, accessed October 2023).

According to the U.S. Environmental Protection Agency (U.S. EPA), PM bound-species generated from incomplete combustion of solid fuel consist of 16 PAHs compound and metals as priority pollutants. Considering carcinogenic PAHs, the International Agency for Research on Cancer (IARC) classified some PAHs based on the degree of evidence for the carcinogenicity as follows: carcinogenic to humans (group 1), probably carcinogenic to humans (group 2A) and possibly carcinogenic to humans (group 2B) (IARC, 2010). Of the PAH species, the benzo(a)pyrene and dibenzo(a.h)anthracene belong to group 1 and 2A, while benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, and indeno1.2.3CD-pyrene to group 2B. From classification of IARC, As, Cd, and Pb are grouped as carcinogenic, while other metals such as Cu, Zn, Fe and Ni are classified as non-carcinogenic elements based on available literature (Tan *et al.*, 2016).

These harmful compounds play a key role in increasing the production of reactive oxygen species (ROS) from redox reaction in mitochondria, resulting in damaged cells (Camatini et al., 2016; Sopian *et al.*, 2021). A study by Daher *et al.* (2012) reported that coarse and fine particles induced redox activity, suggesting that the chemical compounds in $PM_{2.5}$, such as Copper (Cu), Chromium (Cr), polycyclic aromatic hydrocarbon (PAHs), were strongly and moderately associated with ROS formation. Another study, Vreeland *et al.* (2016) investigated the characteristics and redox activity of $PM_{2.5}$ associated with roadside trash burning in Bangalore, India. The result showed that the highest emission rate of PAHs in trash burning sample (plastic, green waste, and paper) was found, and concluded that exposure to redox-activate PM can be extremely high.

3.8 Legal regulation for PM

In the European Union (EU), the most important air quality directives are 2008/50/EC directive on ambient air quality and Cleaner Air for Europe (It was amended with 2015/1480/EC) and 2001/107/EC Directive on heavy metals and polycyclic aromatic hydrocarbons (PAHs) in ambient air. In the EU air quality directive, limit values for PM_{10} are 40 µg m^{-3} (yearly) and 50 µg m^{-3} (daily) while limit values for $PM_{2.5}$ are 25 µg m^{-3} (yearly). The limit value for PM10 is 50 µg m^{-3} (daily). In directive on PAH and heavy metals, the value of BaP is 1 ng m^{-3} (annual), whereas values for heavy metals such as Pb, As, Cd, and Ni are 0.5 µg m^{-3}, 6 ng m^{-3}, 5 ng m^{-3}, and 20 ng m^{-3}, respectively (https://www.eea.europa.eu/themes/air/air-quality-concentrations/air-quality-standards, accessed 26 October 2023)..

In WHO AQG guideline, limit values for PM_{10} are 15 µg m^{-3} (yearly) and 45 µg m^{-3} (daily), while limit values for $PM_{2.5}$ are 5 µg m^{-3} (yearly) and 15 µg m^{-3} (daily) (https://iris.who.int/handle/10665/345334, accessed 26 October 2023). To bring air quality standards closer to the recommendation of the WHO, the European Commission has proposed to revise the Ambient Air Quality Directive on 26 October 2022 as part of the European Green Deal. For instance, the value for $PM_{2.5}$ (yearly) will be decreased by more than half (https://environment.ec.europa.eu/topics/air/air-quality/revision-ambient-air-quality-directives_en, accessed 26 October 2023).

Based on a result of EU legislation such as Large Combustion Plant Directive 2001/80/EC and Industrial Emissions Directive 2010/75/EU, emissions from huge sources have been successfully reduced last years, whereas in contrast, emission from residential solid fuel and biomass combustion appliance has increased in Europe due to lack of regulations (EEA, 2019).

It is important to note that the trend of changes in annual exposure to $PM_{2.5}$ in Europe was reducing (Alikhani et al., 2021). However, as of 2018, in some EU member states including France, Italy, Spain, Hungary, the Czech Republic, United Kingdom, and Germany, the PM_{10} annual limit value was exceeded (EEA, 2019).

In Hungary, Government Decision 1330/2011. (X.12.) contains regulation for decreasing PM emissions and it describes an Action Plan aimed to fulfill proper air quality according to level values of pollutants defined by Ministry of Rural Development Regulation no. 4/2011. (I. 14.). If PM_{10} concentration exceeded and reached 100 µg m^{-3} (threshold value) for two days and no improvement is expected based on the meteorological forecast, a smog alert is declared. A study by Karagulian *et al.* (2015) shows that considerable biomass burning, industry and traffic emissions in Central Europe play a key role in emissions. According to the current regulation, in case of smog alert, traffic is limited, while use of vehicles of certain environmental categories is banned. In addition, illegal waste burning in EU is strongly prohibited. In accordance with that, in Government Decision 1330/2011. (X.12.), one of the major goal is source identification in the Action Plan implemented.

In Asian countries (East Asia), coarse PM emission from fugitive dust is a most concerning issue. In addition to fugitive dust, PM emissions from traffic and domestic fuel burning in Asian countries (East and Southern Asia) are the main sources (Karagulian *et al.*, 2015). In Mongolia, the main source of air pollution is raw coal combustion (Battsengel *et al.*, 2021). Generally, air pollution is still a severe problem in cold periods, reaching values higher than recommendation of the World Health organization (WHO) and National Air Quality Standard MNS 4585:2007 of Mongolia ($PM_{2.5}$ – 50 µg m^{-3} for daily) (Batmunkh *et al.*, 2013). The stove replacement program of households in peripheral area in Ulaanbaatar was implemented to decrease air pollution in 2010. As a result of these huge efforts, in more than 90% of households the stoves were replaced and average concentration PM notably reduced from over 250µg m^{-3} in 2008-2009 to 80µg m^{-3} in 2014 -2015 (Hartman, 2016).

In addition, the Asian Development Bank (ADB) implemented two policy-based loans ($290 million in 2018 and 2019) to improve air quality in the capital city. The program’s first phase was instrumental in supporting the ban on raw coal burning and establishing technical standards for vehicle emissions (Government Decision 1903/2011. (I.124.) - one of the major goal focus on measuring vehicle emissions). The second phase was supported by a policy-based program and embedded the strategies developed under first phase into Mongolian legal and regulation.

The consumption of raw coal was banned for household consumption in the capital city of Mongolia, starting on 15 May 2019 based on Government decisions 1908/2019. (VI.17.) (Byambajav et al., 2021). In the average ambient $PM_{2.5}$ in the winter period of 2019 -2020, percentage of fine PM was dramatically reduced compared to same period in 2016-2017 (app. 51%). However, situation in Mongolia has not improved appreciably since then (WIAQ, 2020). The government of Mongolia’s efforts to reduce air pollution does not provide solution to air pollution from that targeted sectors. Thus, air pollution is challenging Mongolians right to live in a safe and healthy environment, as stipulated in article 16.2 of the Constitution of Mongolia.

4 Ecotoxicology

Ecotoxicology is the study of the effect of toxic compounds on biological organisms, from bacteria to higher taxa, especially at the community, population, ecosystem, and biosphere levels. Ecotoxicology is a multidisciplinary field, which couples or combines toxicology, and ecology (e.g., Kováts *et al.*, 2021; Hubai et al., 2022).

Especially in the case of highly complex media (air samples), chemical analyses are often supported by bioassays (Alves *et al.*, 2021; Vicente *et al.*, 2021; Rienda *et al.*, 2023). These assays evaluate the effect of interaction among toxic substances such as additive, antagonistic, or synergistic effects (Abbas *et al.*, 2018). In ecotoxicology screening, there are a variety of test organisms used as references or monitoring tool to evaluate toxicity in PM samples (Kováts *et al.*, 2012, 2017; Verma *et al.*, 2013; Silva *et al.*, 2015). Among these, the bioassay based on the bioluminescence inhibition of the marine bacterium *V. fischeri* has been widely applied and recommended tool for the initial screening of atmospheric PM (e.g., Kováts *et al.*, 2012; Roig *et al.*, 2013). The test is rapid (requires a short exposure of 15 or 30 minute), cost effective, therefore it is especially useful when samples with unknown toxic potential are to be evaluated (Menz et al., 2013). Test results have proven predictive for acute toxicity in other organisms (Weltens et al., 2014).

The principle of this bioassay based on bioluminescence inhibition of the marine bacteria *V.fischeri* is to measure the inhibition of the light emittance of luminescence bacteria (bioluminescence enzyme system, which contains NAD(P)H flavin mononucleotide (FMN) oxidoreductase and luciferase) in the presence of toxic environment. (Kováts and Horváth, 2016). As bioluminescence is linked with respiration process, it gives a good indication on the metabolic activity of luminescence bacteria (Fort, 1992). In the toxic environment, the bacterial luciferase could be inhibited, and then a quick reduction in light emission of the luminescent bacteria takes place. EC_{50} (the concentration that causes a 50% effect) is a measure of concentration of PM samples, which induces a biological response halfway between the baseline and maximum after exposure time, and also widely used value to characterize toxicity of the sample.

4.1 The *Vibrio fischeri* bacterium

Vibrio fischeri is a gram-negative, rod-shaped, flagellated, motile, non-pathogenic, and heterotrophic bacterium. In the sub-tropic and temperature marine environments, they are ubiquitously distributed as free-living organisms. They can be found in symbiosis with short-tailed squid (*Euprymna scolopes*) in nature and are responsible for the bioluminescence of the organ. They are fed a sugar and amino acid solution by the squid, and in return, they produce light in the special light organ of the host.

The species has been renamed and reclassified in the past. Initially, this species of bacteria was discovered in 1888 by Bernhard Fischer, a German bacteriologist, who named *Einheimischer Leuchtbaccilus*. Subsequently, in 1889, the name of luminous bacterium was changed to *Photobacterium fischeri* by Martinus Willem Beijerinck. Furthermore, Karl Bernhard Lehmann and Rudolf Otto Neumann classified it as *Vibrio fischeri* in 1896 (https://en.wikipedia.org/wiki/Photobacterium, accessed 20 September 2023). The species was re-classified as *Aliivibrio fischeri* based on phylogenic and phenotypic differences (Urbanczyk *et al.*, 2007). However, this nomenclature is still not used in the standards. Thus, the term of *Vibrio fischeri* has been used in our works.

4.2 The light emittance of the bacteria

V.fischeri employs a luciferin-luciferase bioluminescent system to generate light emitted during normal metabolism.

$$\text{Luciferin} + O_2 \xrightarrow{\textit{Luciferase}} \text{LIGHT} + H_2O$$

Considering genetic background and biochemistry, luminescence in bacteria and its regulation mechanism have been completely described (Meighen, 1991). Light emission of luminescent bacteria principally relies on the bioluminescent enzyme system (luciferin-luciferase) which consists of a NAD(P)H:FMN oxidoreductase and a luciferase. Firstly, FMN reduces to form $FMNH_2$ through the reaction catalyzed via NAD(P)H:FMN oxidoreductase enzyme in the presence of a reduced NAD(P)H and a H^+. Subsequently, bacterial luciferase can bind $FMNH_2$, catalyzes the oxidation of reduced $FMNH_2$ to produce oxidized FMN in presence of luciferin (a long-chain aliphatic aldehyde - RCHO) and molecular oxygen, resulting in the blue-green light emission. The spectral band of bioluminescent bacteria is between 420 nm and 660 nm (Meighen, 1991). The flavin reductase of bioluminescence bacteria is perfectly unique as they play important role in catalyzing a flavin redox reaction with flavins.

The regulation and production of induced bioluminescence of *V. fischeri* is genetically regulated by lux operon system which consists of two distinctly transcribed units: the *luxICDABEG* operon and *luxR* gene. The luxA and luxB genes are encoded α (40kDa) and β (37 kDa) subunits of luciferase enzyme. They catalyse the reaction of reduced $FMNH_2$, long chained aldehyde and oxygen to produce FMN, aliphatic acid, water and light. Aliphatic acid reductase complex is endcoded via luxC, luxD and luxE genes that recycles the acid to aldehyde while luxG participates in the metabolism of FMN (Nijvipakul *et al.*, 2008; Miyashiro and Ruby, 2012).

4.3 Quorum sensing

Luminescence in *V. fischeri* is controlled by a bacterial cell population density-responsive regulatory mechanism called quorum sensing. Quorum sensing is the

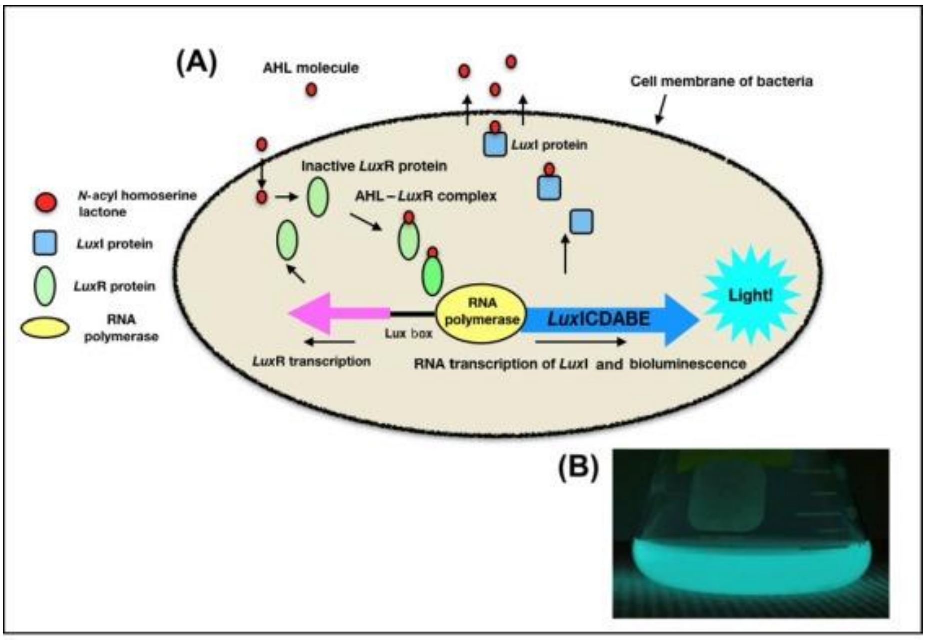

Fig 5 *Quorum sensing in Vibrio fischeri (Prescott et al., 2020)*

regulation of gene expression in response to fluctuations in cell-population density as the mechanism of quorum sensing involves two genes: luxI and luxR. They encode an auto-inducer synthase (known as called luxI) and an auto- inducer-dependent activator of lux operon (called luxR). The luxI synthesizes/or can produce the auto-inducer which is chemically named acylated homoserine lactone (AHL) that rapidly diffuses out of bacterial, and it accumulated in the bacterial cell up to threshold concentration. As a result, high bacterial cell density is needed for the transcription of lux operon and then subsequent production of light (***Fig 5***) (Dunlap, 1999; Lupp *et al.*, 2003; Verma and Miyashiro, 2013). In the ocean, cell density is app. 10^2 cells/ml, this low concentration of cells cannot produce light, as luminescence is not activated.

4.4 *Vibrio fischeri* bioluminescence inhibition test protocol

In 1978, the first version of the bioassay from Beckman Instrument, Inc. USA was marketed as the Microtox® test (including the freeze-dried marine bacteria, salt solution, photometer and methods). Microtox® test based on the bioluminescence inhibition of the marine bacterium *Vibrio fischeri* NRRL-B-1177 is an acute toxicity bioassay and it was used to assess the toxicity of liquid samples (Bulich and Isenberg, 1981). In 1990, Brouwer and his research team developed another version for solid phase assessment (e.g., toxicity of sediments). After that it has become one of the most popular tests in environmental monitoring and screening activities all over the world (Brouwer, 1990). Later on, Brouwer et al (1990) proposed a protocol that allows contact between solid phase and bacteria.

Microtox® test has been modified several times, and subsequently, a modified version of the test by Azur Environmental in 1998 was proposed as a standard for testing solid samples. It is called the Microtox® solid-phase test (MSPT) (https://www.modernwater.com/assets/Technical%20Support/Toxicity/Manuals/ACUTE%20User's%20Manual.pdf).

In the study by Doe *et al.* (2005), MSPT was performed for measuring the toxicity of whole sediment samples, suggesting that interference might occurred by factors connected to the filtrate: i) color (light absorption) ii) turbidity (light scatter) coming from fine particles, which are not removed by filtration. In another study, Ringwood *et al.* (1997) reported that lower light readings occurred due to loss of bacteria in the liquid-phase due filtration procedure. After exposure period, solid particles in the extraction are separated by filtration and then toxicity of water fraction is measured. In addition, during the testing procedure filtration, another confounding factor might be bacteria absorption on the particle surface and physical interference on luminescence from particles (Volpi Ghirardini *et al.*, 2009).

For testing the toxicity of solid samples through direct contact between *V.fischeri* bacteria and contaminated particles, Lappalainen *et al.* (1999, 2001) presented a novel protocol, which can circumvent these problems. In this work the bioluminescence intensity is assessed in kinetic mode. In 2010, this protocol was standardized (ISO 21338:2010: Water quality – Kinetic determination of the inhibitory effects of sediment, other solids and colored samples on the light emission of *V.fischeri* /kinetic luminescent bacteria test/) (ISO 21338, 2010). The kinetic measurement can be used for solid and colored samples in suspension as it can eliminate false toxicity reading that light intensity is calculated by comparing initial and final reading, independently from the control.

The bacterial suspension is injected into the samples, subsequently, peak maximum of luminescence intensity increases within 30 sec (hence, the system is named Flash) (Lappalainen *et al.*, 2001). After 30 min of exposure time, light intensity is read again. During the first 30s, this kinetic reading of bioluminescence can be used for preliminary assessment of the samples (Mortimer *et al.*, 2008) and can show some indication of the behavior of the samples as follows:

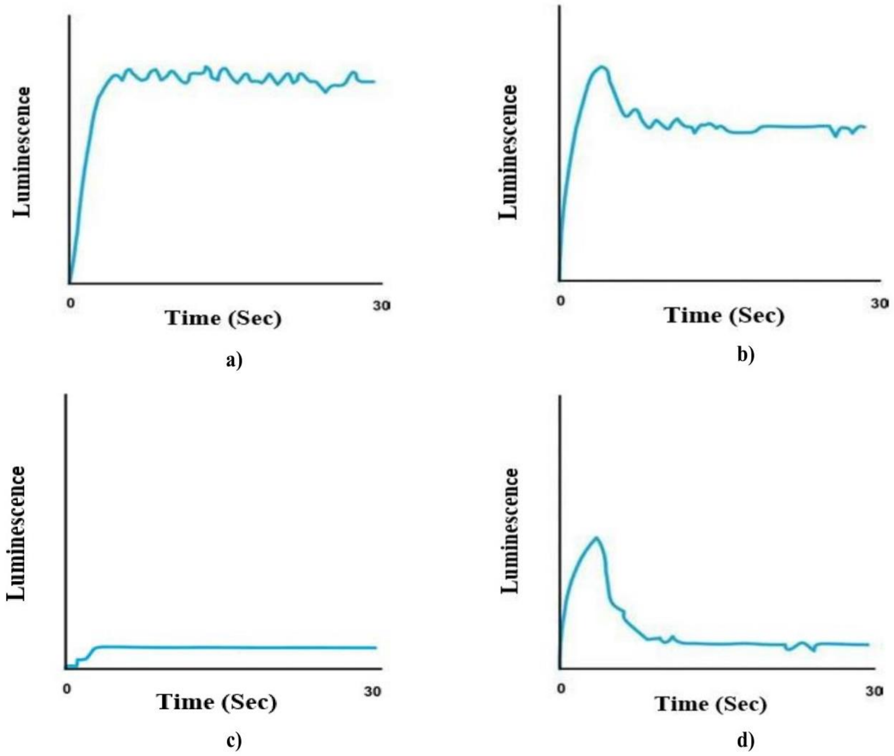

Fig 6 *Luminescence emittance in the a) control, b) toxic, c) colored or turbidity d) colored toxic sample.*

In the control, bioluminescence intensity remains at nearly constant level (***Fig 6a***). In a toxic sample, when luminescence is inhibited, the bioluminescence intensity starts to decrease immediately within 30s (***Fig 6b***). For colored and turbidity samples, the inhibition of bioluminescence intensity can be distinguished from the toxic effect that signal obtained during measurement, because of initial light emission is lower than control during the first 30s, but remains nearly constant thereafter (***Fig 6c***). Whereas, for the colored with toxic sample, the signal obtained in the first 30 sec also shows a characteristic pattern, with an inherently lower light emission compared to the control, which decreases after the initial flash showing the toxic effect (***Fig 6d***).

Nowadays, for ecotoxicological monitoring, a wide variety of different systems are commercially available based on V. *fischeri* bioluminescence inhibition including Ascent luminometer^{TM} (Aboatox Co., Finland), Microtox® (Azur Environmental), ToxAlert® (Merk), LUMIStox® (Dr. Bruno Lange), and ToxScreen (Checklight Ltd., Qiyrat Tiv'on, Israel) (Kováts *et al.*, 2012; Aammi et al. 2017; Corrêa *et al.*, 2017). International and several national standards are available including the European standard (EN ISO 11348 - 2007), the American standard (ASTM D5660- 1995), the French standard (DIN 38412-1990), and the Chinese standard (GB/T 15441- 1995) (Urbanczyk *et al.*, 2007; ISO 21338: 2010; Ye *et al.*, 2011).

The procedures specified in the ISO standard can be distinguished based on the type of bacteria used: 1) ISO 11348-1, Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (luminescent test bacteria) – Part 1: method using freshly prepared bacteria, 2) ISO 11348-2, Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (luminescent test bacteria) – Part 2: method using liquid-dried bacteria, 3) ISO 11348-3:2007, Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (luminescent test bacteria) – Part 3: method using freeze-dried bacteria (ISO 21338: 2010). In Hungary, liquid-dried and freeze-dried bacteria are used for bacterial preparation (Kováts *et al.*, 2012).

Comparing air sample to other ones, air the most complicated to collect, in term of budget and logistic requirements. A small amount of PM sample is deposited on a filter. As such, the size of test organisms is even more restricted; in most cases, bacterial bioassays are used (e.g., Pintér *et al.*, 2017). Back to history, the first attempt utilizing bioluminescence test bacteria was used to assess the toxicity of air sample (Serat et al.,1967). Afterward, in 1998, MSPT based on luminescence bacteria was used to estimate air pollutant ecotoxicity of urban dust collected in London and Hong Kong (Wang *et al.,* 1998). Nowadays this bioassay on air PM samples is widely used (Isidori *et al.*, 2003; Evagelopoulos, 2009; Roig, Schuhmacher, *et al.*, 2013).

In addition, it is important to point out that *V. fischeri* is a marine species, which needs saline environment as mimicking artificial seawater during the sample preparation procedure. To overcome this problem, utilization of freshwater luminescent bacterium was employed. Ye *et al.* (2011) used freshwater luminescent bacterium (*Vibrio qinghaiensis sp. Nov*.) in order to evaluate the acute toxicity assessment of explosive wastewater samples, suggesting that freshwater luminescent bacterium (*Vibrio qinghaiensis sp. Nov*) was highly sensitive. However, this test organism is not currently commercially available in kit form.

4.5 PM sample extraction protocol

According to the conventional protocol (ISO 11348-3:2007), solvents or water are used to prepare an extract. The PM sample is a solid one. Therefore, in order to prepare an extract, different solvents (water and organic solvents: methanol, dichloromethane, dimethyl-sulphoxide, and hexane) need to be applied (Wang *et al.*, 1998; Vouitsis *et al.*, 2009; Kováts *et al.*, 2012; Verma *et al.*, 2012; Chang *et al.*, 2013; Aammi et al., 2017).

In several studies, distilled water was used to make a suspension for determining the toxicity of air samples (Wang *et al.*, 1998; Silva *et al.*, 2015; Wang *et al.*, 2016). For instance; to assess the toxicity assessment of ash samples collected after a wildfire, an aqueous extract of ash was prepared with distilled water (Silva *et al.* 2015). In the study of (Wang *et al.*, 2016), atmospheric PM was collected from three different regions (China) to assess the ecotoxicity of $PM_{2.5}$. The samples were extracted by de-ionized water. In another similar study, in order to assess the ecotoxicity of size-fractioned biomass ash samples, de-ionized water was used as well (Barbosa *et al.*, 2013).

Apart from aqueous extract, different organic solvents have been employed (Vouitsis *et al.*, 2009; Verma *et al.*, 2012; Chang *et al.*, 2013; Aammi et al., 2017). Kahru *et al.* (1996) studied the toxicity of pesticides using luminescent bacteria (*Photobacterium phosphoreum*), suggested that less toxic organic solvents (ethylene glycol, DMSO, methanol, acetone and acetonitrile) are more efficient to dissolve toxic organic substances.

To perform the ecotoxicological evaluation of air pollutants emitted in an industrial settlement of Milazzo (Italy), Semipermeable Membrane Devices (SPMDs) were used to collect organic compounds due to characteristics of the membrane, in which organic contaminants are able to diffuse. SPMDs were rinsed to remove surface fouled residue and then was solubilized with 20 ml of a mixture of acetone and dimethylsulphoxide (DMSO) (1:1 v/v) for 24h. The sample was used after acetone was evaporated by gentle nitrogen stream (Triolo *et al.*, 2008). Similarly, SPMDs were used to collect gaseous pollutants in the urban air of Caserta, South Italy. Cyclohexane was used to dissolve organic contaminants after SPMDs were rinsed with tap water to remove surface fouled residue and then dried with clean paper (Isidori *et al.*, 2003). Lin and Chao (2002) utilized a similar sample preparation method to evaluate acute toxicity of both the organic extract from the PM-associated soluble organic fraction (SOF) and semi-volatile organic component (XOC) of diesel exhaust emission using Microtox bioassay.

In order to apply the Microtox assay, PTFE-coated fibre filter was used to collect PM of light duty vehicles. The filter was Soxhlet extracted in 300 ml Dichloromethane (DCM), extraction took 24h. Subsequently, rotary evaporation was used to reduce the volume to 1ml (Vouitsis *et al.*, 2009). Teflon PTFE filter was used to collect PM samples in the industrialized area in the north-western part of Greece (Kozani) and then hexane and acetone (1:1) mixture was used due to negligible toxicity on *V. fischeri* bioassay (Evagelopoulos et al., 2009). In addition, a Microtox system as bio-indicator was used in the study of Chang *et al.* (2013) to evaluate the toxicity of fly ash samples from a cooling tower after incineration of plastic liquid waste (PSW) and organic liquid waste (OLW), samples were extracted in n-Hexane or dichloromethane/n-Hexane mixtures. Another study used DMSO and ultra-pure water for extraction of $PM_{2.5-10}$ samples collected from several locations (different urban and industrial characteristics) of a megacity (Istanbul) for acute toxicity testing (*V. fischeri* bioassay) (Aammi et al., 2017).

However, concentration of dissolved toxic compound in the extract might be higher due to their solvent uses (Corrêa *et al.*, 2017). The comparison of water and methanol extract was studied to evaluate the toxicity of an ambient $PM_{2.5}$ samples collected on Quartz filters from Atlanta using freshwater rotifers (*Brachionus calyciflorus*). It was found that methanol extract of the PM sample was extremely toxic (8±6 times) to the rotifer compared to the aqueous extract (Verma et al., 2013). A similar study was carried out on diesel exhaust particulate matter (DPM) extracted with a mixture of three organic solvents (dichloromethane, n-hexane, and acetone). Extraction of DPM was tested on different aquatic bioassay, including luminescent bacteria (*V. fischeri*), algae (*Scenedesmus subspicatus*), fish (*Danio rerio*), and daphnids (*Daphnia magna*). Solvent extracts proved extremely ecotoxic (Corrêa *et al.*, 2017).

4.6 ‘Whole-aerosol’ testing protocol

Preparing an extract from a PM sample collected on a filter rises several questions. First, such organic solvents are used in many cases which extract the whole toxic fraction of the sample, both biologically available and biologically unavailable. In this way, actual ecological risk can be seriously overestimated. Second, these solvents are not part of a natural exposure pathway.

In case of other solid samples (soils or sediments) so called whole sediment biotests are used. In these assays test organisms are in direct contact with the toxic medium. Kováts et al. (2012) developed a ‘Whole-aerosol test’ test protocol based on the kinetic *Vibrio* test. Similarly to the whole sediment tests, test bacteria are in direct contact with the toxic particles. The kinetic standard uses a suspension, therefore filter spots are grounded in an agate mortar. After that high-purity water (MiliQ) is used to make a suspension for the test bacteria (Kováts *et al.*, 2012). In the suspension, test bacteria are in fact direct contact with PM without further procedures, mimicking naturalistic exposure route. Naturally, clean filters were also checked when the protocol was evaluated and proved to be non-toxic (inert).

This reliable and new sample preparation method was used to assess the ecotoxicity of different PM samples collected on a quartz filter in different environments, such as diesel particles, urban PM, roadside PM and indoor PM sample (Kováts *et al.*, 2012, 2013; Turóczi *et al.*, 2012; Ács *et al.*, 2013; Pintér *et al.*, 2017; Alves *et al.*, 2021; Mentes *et al.*, 2022; Rienda *et al.*, 2023). Turóczi *et al.* (2012) used the whole aerosol test to assess the overall ecotoxicity of various $PM_{2.5}$/PM_{10}. The results showed that direct emission samples significantly more ecotoxic. Another study, this sample preparation method was used to characterise the ecotoxicity of diesel-powered cars. A significant correlation was found between toxicity and polycyclic aromatic hydrocarbons (Ács *et al.*, 2013). Recently, in the study of Rienda *et al.* (2023) resuspended PM10 road dust was analysed in different types of parking lots, using this method. The result showed that all samples were toxic.

In addition, a direct contact test was also used to assess indoor air quality during cooking different dishes of Latin cuisine. Sampling during preparation of the dishes was carried out in a 15.5 m^2 equipped modern kitchen in the city of Aveiro, Portugal (Alves *et al.*, 2021). Another study, ecotoxicity measurements of indoor PM generated from Portuguese combustion appliances used the direct contact test as well. *V. fischeri* assay proved that indoor particles released from the fireplace were most toxic and sum of PM-bound PAHs was significantly associated with *Vibrio* ecotoxicity ($r= -0.811$, $p < 0.05$) (Vicente *et al.*, 2021).

5 Materials and methods

5.1 PM sampling

5.1.1 Diesel exhaust sampling

PM_{10} aerosol sample was collected from the exhaust of a diesel-powered jeep (Euro 4 environmental standard, age: 13 years, odometer: 256.887 km). Sampling was done at a flow rate of 32 $m^3\ h^{-1}$ for 10 minutes at idling in a closed premise about 1 meter from the tailpipes using the high-volume sampler (KÁLMÁN system KS-303). The quartz filter (Ø15cm) was used to collect PM_{10} sample.

5.1.2 Seasonal PM sampling

K-Puszta (Hungary, 46°58′ N, 19°33′ E) was selected to collect four-season aerosol samples (***Fig 7***). The sampling site is operated for the measurement of background pollution in Hungary.

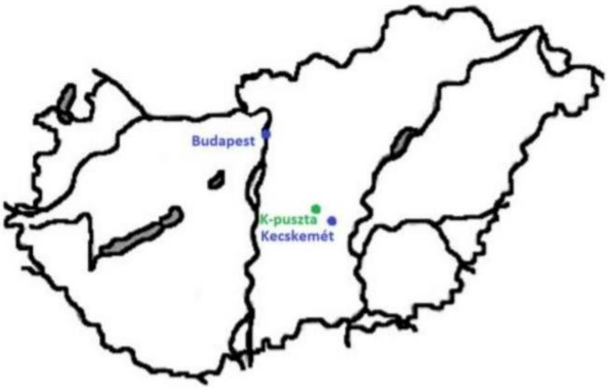

Fig 7 *Location of K-puszta sampling site*

It serves as an air monitoring station by EMEP (European Monitoring and Evaluation Programme) and GAW (Global Atmospheric Watch) and is a part of Hungarian Metrological Service, and WMO (World Meteorological Organization), and also is one of the 20 European supersites within the EU project EUSAAR (European Super-sites for Atmospheric Aerosol Research) Maenhaut *et al.* (2017). Four-season $PM_{2.5}$ samples were collected on a quartz filter using a DIGITEL DH-80, between the 18th of December 2014 and the 22nd of March 2015.

5.1.3 Domestic waste PM_{10} sampling

PM_{10} samples originating from the controlled combustion of Polystyrene (PS), Polypropylene (PP), Polyethylene (PE), Polyurethane (PU), Polyvinyl chloride (PVC), rag (RAG), and Oriented Strand Board (OSB) were collected on quartz filter according to the procedure of experiment conditions of the combustion test (Hoffer *et al.*, 2020). Model waste samples were prepared as follows: The PS waste samples were hard cups or pots of dairy products (yoghurt and pudding), without aluminum foil caps, and pieces of expanded PS insulation board (80 mm × ∼ 100 mm × ∼ 100 mm). PVC: vinyl flooring and hard plastic water pipes. The PP waste sample specimens were a mixture of plastic cups and pots of dairy products (sour cream and pudding), without paper labels and aluminum foil, and quartered plastic trays of meat; PU: packing sponge (average size 120 mm × 100 mm ×15 mm); OSB: slices of OSB material of different brands (average size 120 mm × 100 mm × 20 mm); PE: a mixture of high and low density polyethylene (HDPE and LDPE) and Rag: a mixture of polyester and polyamide fabrics from different clothes (average~ 70 g) (***Fig 8***). The blank samples was also sampled for each type of waste combustion. Before combustion test with analytical balance, all samples were weighted, and then they were burned in commercially available cast-iron stove (Servant S114, 5kW).

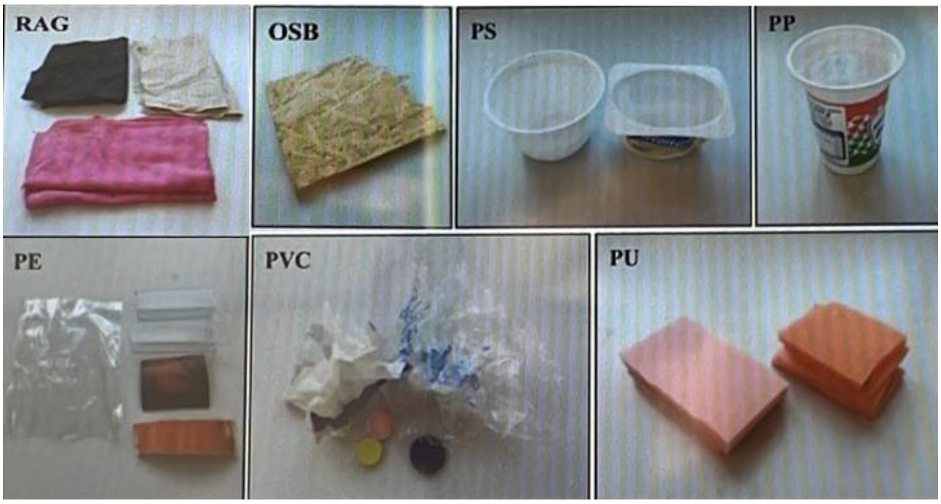

Fig 8 *Domestic waste specimens prepared for combustion test*

The smoldering charcoal was used to heat the cast-iron stove for 1 hour and then 1-10 aliquots of solid waste were burned depending on the waste types. During the experiment, the temperature of the flue gas was measured. PM_{10} samples were collected on quartz filer (Ø15cm) using KALMAN high-volume aerosol sampler (flow rate 32m3 h-1). More detailed description is shown in study of (Hoffer *et al.*, 2020).

5.1.4 Waste PET PM_{10} sampling

Controlled combustion of PET was performed as described above (Hoffer *et al.*, 2020). In this case, two different PET waste were used; one was composed of colored PET bottles and the other of colorless ones. Burning and sampling were repeated for both colored and colorless bottles. In each cycle 1 and 2 liter volume beverage plastic PET bottles without caps and labels were burned. Randomly, all waste PET bottles were selected from most commonly consumed commercial types.

5.1.5 Indoor $PM_{2.5}$ sampling

10 non-smoker households with poor ventilation were selected in Khan-Uul district, Ulaanbaatar (hereinafter named HH-1 to HH -10) that can be regarded as average regarding ambient air quality during the cold season ***(Fig 9)***. The criteria of selected households for our study were as follows: 60% of Mongolians live in the ger area (Guttikunda et al., 2013), app. half of the dwellings in the ger area are made of brick or clay, typically constructed by the owner of each house. We wanted to represent both types, as well as different size of households and different ages of the dwellings. On the other hand, similarities were: location relatively close to the main road; heating type (usage of conventional stoves and coal in each household); only non-smokers lived in the selected families.

Fine indoor particular matter ($PM_{2.5}$) samples from households were collected with an AirChek XR5000 (SKC Ltd) sampler on a Teflon filter at a flow rate of 1.5 – 2.0 L/min for 24 hours, instrument was positioned at 1 to 1.5 meters above ground level to simulate breathing zone between 27 January to 09 February 2018. All households are situated in peripheral area named *Ger khoroolol* which is still not connected to the central heating system. Indoor $PM_{2.5}$ samples were kept in labeled plastic cassettes in cooler box with dry ice, all samples were transported to the laboratory and stored in the freezer at –20^0C at the Department of Public Health, Mongolian National University of Medical

Sciences before being transported to the Centre for Natural Science, University of Pannonia, Hungary.

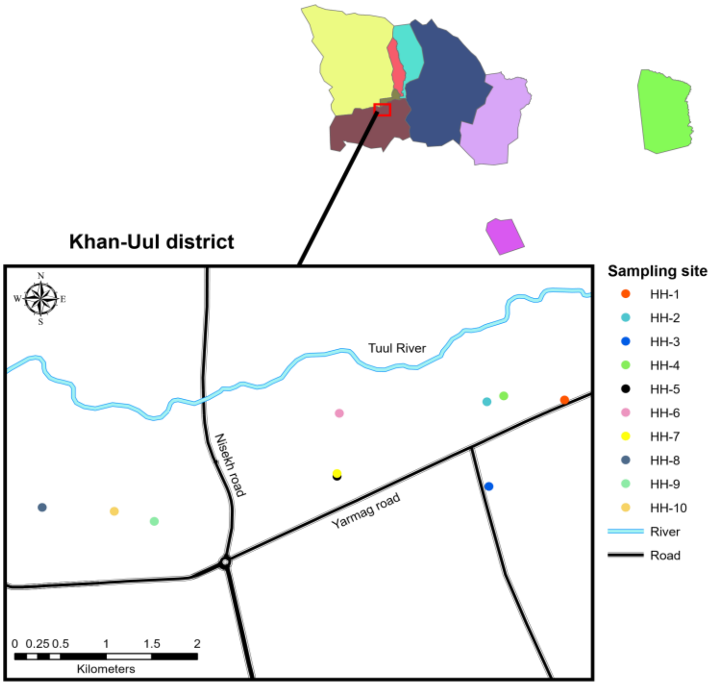

Fig 9 *Map showing the sampling area within Khan-Uul district, Ulaanbaatar, Mongolia*

5.2 Sample preparation

5.2.1 Direct contact test

Direct contact test was conducted according to a novel sample preparation protocol developed by Kováts *et al.* (2012). In case of the diesel emission sample, one-sixth of the filter was ground in an agate mortar while in case of other samples spots of 25 mm with diameter were cut with a special puncher and then grounded in an agate mortar. Samples were transferred into pre-cleaned 4 ml vials with a PTFE-coated spatula. Suspensions were prepared with 2 ml high-purity MiliQ water. Extracted samples were stored in the freezer prior to measurement.

5.2.2 Extraction with Methanol (MeOH)

Extraction was performed according to Verma et al. (2012). One-sixth of the sample filter was cut into pieces and extracted with methanol in a sonication bath for 15 min. The extracted sample was filtered through PTFE 0.45µm pore size filter to remove insoluble materials and any filter fibers. The methanol extract was evaporated to near dryness (1 ml) using a nitrogen stream at 40^0C. The extracted sample was diluted prior to measurement. The 250 µl of the original extracted sample was diluted with dimethyl-sulphoxide (14.75 ml - DMSO). Finally, the sample was stored in a freezer until measurement.

5.2.3 Extraction with Dichloromethane (DCM)

Extraction was performed according to Vouitsis *et al.* (2009). One-sixth of the filter sample was extracted with 300ml dichloromethane (DCM) using the Soxhlet apparatus for 24 hours followed by nitrogen stream evaporation (40^0C) and then was reduced the volume to 1ml. The 1 ml of concentrated extract was changed with 1ml dimethyl-sulphoxide (DMSO). The diluted extraction was stored in a freezer until analysis. About 250 µl of the original extracted sample was added to 14.75 ml with dimethyl-sulphoxide (DMSO).

5.2.4 Extraction with Dimethyl-sulphoxide (DMSO)

DMSO extracts were used (1) to compare the efficiency of different PM extraction protocols and (2) to assess environmental risk of Mongolian (Ulaanbataar) indoor samples. In order to compare different extracts' ecotoxicity, the extract was prepared adopting the study of Aammi et al. (2017). One-sixth of the sample was cut into pieces and placed in a 15 ml plastic tube. The filter was extracted with 10 ml solvent of 2% dimethyl-sulphoxide (DMSO) in a sonication bath (15 min) and then centrifuged to isolate soluble materials from the insoluble ones at 2500 rpm for 10 min. To remove the insoluble materials, the supernatant was filtered using a membrane filter (PTFE 0.45 pore size filter). Extracted sample was kept in the freezer until measurement. Before testing, 3.75 ml of the initial extracted sample was mixed up 5 ml with dimethyl-sulphoxide (DMSO).

In case of the Mongolian indoor samples, the halves of Teflon filters were used for bioluminescence inhibition assay according to the modified protocol of Aammi et al. (2017). Briefly, each half was weighted gravimetrically with a Sartorius microbalance (10µg sensitivity) and then put into a 15 ml centrifuge (plastic) tube, added the 10 ml of

2% DMSO to each sample at 15 minutes with sonication bath. All samples were centrifuged (2500 rpm) for 10 min. To separate supernatants of each sample, Polytetrafluoroethylene (PTFE) 0.45 µm pore size membrane filter was used to remove insoluble components and then transferred into new 15 ml plastic tubes. All samples were kept in the freezer until use.

5.2.5 Exraction with Hexane

According to the sample preparation of Chang et al. (2013), one-sixth of the sample filter was Soxhlet extracted in 300 ml Hexane as followed by evaporated to reduce volume of 1 ml using nitrogen stream at 40^0C. Prior to measurement, 250µl of the original extracted sample was filled up until a volume of 15 ml with dimethyl-sulphoxide (DMSO).

5.2.6 Extraction with de-ionized water

Aqueous extracts were used (1) to compare the efficiency of different PM extraction protocols (2) to assess seasonal pattern in rural $PM_{2.5}$ ecotoxicity and (3) to evaluate PM_{10} from illegal domestic waste burning under controlled combustion. In order to compare different extracts ecotoxicity, the aqueous extract was prepared adopting the study of Wang *et al.* (1998). The filter was cut into pieces and then placed in a beaker with 20 ml de-ionized water (with a resistivity of 418 MΏ cm) in a sonication three times. The extract was kept in a freezer until analysis. About 1.25 ml of the original extracted sample was diluted with 13.75 ml de-ionized water before measurement.

5.3 Analytical measurements

Polycyclic aromatic hydrocarbon (PAH) content in extracts were examined by gas chromatographic mass spectrometry (Agilent 6890GC 5973E MSD GC-MS according to MSZ (Hungarian Standard) 1484-6:2003). The limit of detection (LOD) was 0.001µ/L.

For quality assurance/quality control: (QA/QC) internal standard (p- Terphenyl-d14, 2-fluorobiphenyl from Restek Corporation, Bellefonte, Pennsylvania US) and surrogate standard ((Naphtalene-d8, Acenaphthene-d10, Phenanthrene-d10, Chryzene-d12 Benzo(a)pyrene-d12, and Perylene-d12, from Restek Corporation, Bellefonte, Pennsylvania US) were used for quantification and quantifying of sample and for procedural recovery. Before the analysis standards were freshly prepared and diluted with GC grade solvents (Sigma-Aldrich, St. Louis, Missouri USA). Recoveries for the compounds ranging between 73.5% and 119.4%, this achieved good by the regulatory requirements of the USA-EPA and EU. In our measurements the recoveries were 96-104% for 2-fluorobiphenyl and 108-114% for p-Terphenyl-d14. The recoveries of surrogate standards were acceptable for the standards (Naphtalene-d8, Acenaphthene-d10 82-102%, Phenanthrene-d10 92-109%, Chrysene-d12 95-107%, perylene-D12 82-91%), which were good for making results reliable.

Analytical determinations were carried out by courtesy of the Laboratory of the ELGOSCAR-2000 Environmental Technology and Water Management Ltd. accredited by the National Accreditation Authority (complies with criteria of Standard MSZ EN ISO/IEC 17025:2018), registration number NAH-1-1278/2015.

For heavy metal concentration the extracts were examined by ICP-OES Thermo iCAP 6300, according to EPA 6010C:2007 inductively coupled plasma-atomic emission spectrometry (ICP-AES). Heavy metals were extracted from samples by acid digestion before the measurement. The limit of detection were Hg (0.2 µg/L), Cd, Sb (0.5 µg/L), Au, As, Co, Sn, Pb, Cr, Ti, Se (1 µg/L), Mo, Ni (2 µg/L), Fe, Mn, Cu, Zn (5 µg/L), and Al, B, Ba, Sr (10 µg/L), respectively. The linearity of calibration curve was examined.

5.4 Ecotoxicity testing: *Vibrio fischeri* bioluminescence inhibition test

The principle of this method is to measure the luminescence intensity of the bacterial suspension, and after the sample solution is added to the bacterial suspension, luminescence intensity after a suitable incubation time is measured again. Ecotoxicity testing protocol is shown in ***Fig 10***.

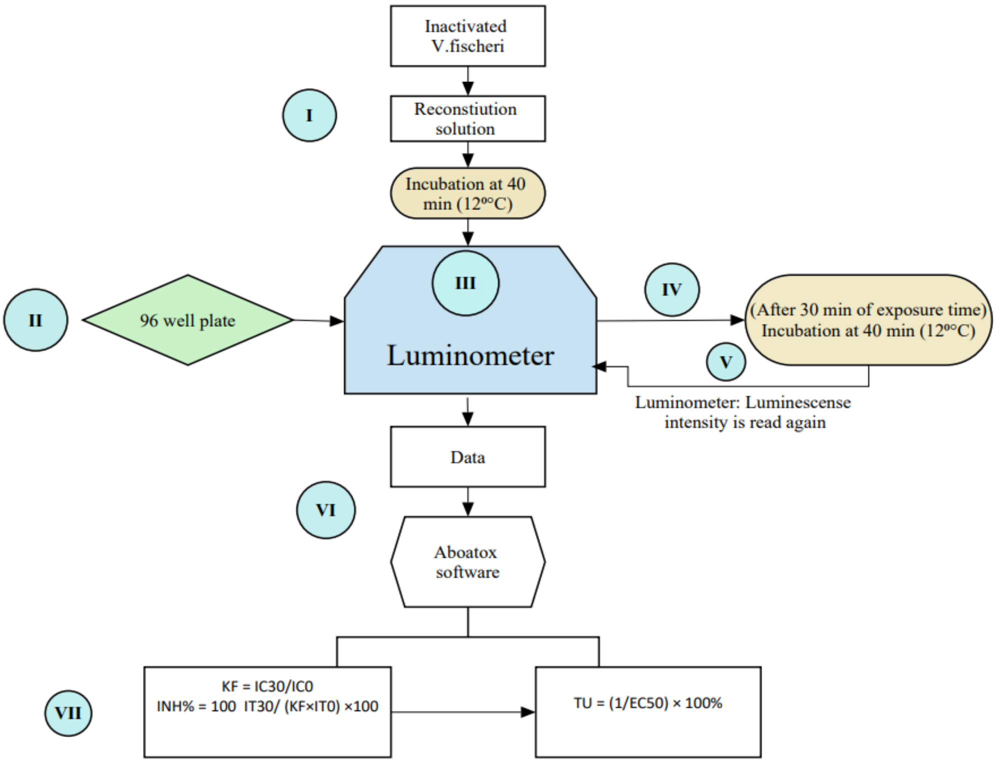

Fig 10 *Scheme of ecotoxicity testing protocol*

This bioluminescence inhibition test is being carried out according to ISO 21338:2010: water quality – kinetic determination of the inhibitory effects of sediment, other solids and color samples on the light emission of marine bacterium *Vibrio fischeri*. Prior to bioluminescent inhibition assay, freeze-dried inactivated luminous *Vibrio fischeri* (NRRL-B-11177) were thawed in cold water with beaker for 2 min after removal from freezer to avoid heat shock and then rehydrated with reconstitution solution (glucose/sodium chloride, buffered to PH 7.0 in a PE bottle). The suspension was incubated at 15^{0}C for 40 min. 2% of saline standard solution was used. The saline solution was prepared originally from LUMIStox standard (LCK480/482-7.5% NaCl).

A 96 micro well plate was used. The micro plate has 8 rows, 12 columns and the volume of the well is 350 µl. 4 samples can be measured simultaneously at 11 dilutions in two replicates (parallel measurement) (***Fig 11***). Luminescence measurements were performed with fully automated Thermo Scientific Luminoskan Ascent® that is a micro plate luminometer under the control of Ascent Software (Aboatox Co., Finland.

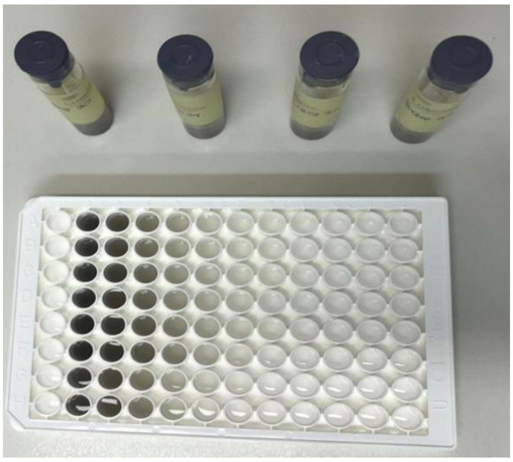

Fig 11 *The samples were filled on the white micro-plate*

The dispenser of the instrument was rinsed with high purity MiliQ water and saline water and subsequently rinsed with bacterial suspension three times before measurement. The microplate with samples was placed in the plate carrier of the apparatus while bacterial suspension was placed in the leakage tray of the apparatus. The samples were injected into the bacterial suspension, bioluminescence intensity was continuously recorded for the first 30 sec. After 30 min of exposure, luminescence intensity was read again.

To calculate the ecotoxicity results, the light output of the unstressed bacteria (the first 30 sec) was used as a reference. EC_{50} or EC_{20} (concentration causing 50% or 20% of bioluminescence inhibition) values were calculated from light inhibition percentage (%) using the Ascent Software provided by the Aboatox Co., Finland.

For samples, the light inhibition (INH %) was estimated based on the following equations:

$$KF = \frac{IC30}{IC0} \qquad INH\% = 100 - \frac{IT30}{KF\ x\ IT0}\ x\ 100$$

Where KF is a correction factor, IC_0 and IC_{30} are luminescence intensities of control at the beginning and after 30 min while IT_0 and IT_{30} are luminescence intensities of the sample at the beginning and after 30 min contact time. From the inhibition data of each concentration the software estimated Gamma using the formula below:

$$\text{Gamma} = \frac{INH\%}{100-INH\%}$$

and the inhibition data which belongs to the Gamma = 1 values gives the EC_{50}.

Afterwards, EC_{50} values were used to calculate toxic units (TU), based on the following equation (Chang *et al.*, 2013):.

$$TU = (1/EC50) \times 100\%$$

The categories of the toxic unit (TU) were classified as follows: non-toxic (<1), toxic (1-10), very toxic (10-100), extremely toxic (>100), respectively.

5.5 Statistical analysis

Principle component analysis (PCA) was used to analyze potential differences in the different waste types. Statistical analyses were performed using the RStudio (RStudio Desktop April 1, 1106) program, ggfortify package (https://CRAN.R-project.org/package=ggfortify). Statistical analysis was carried out using RStudio (RStudio Desktop 1.4.1106) program and R 4.0.0 program (http://cran.r-project.org/src/base/R-4/R-4.0.0.tar.gz) Rcmdr package. Statistical significance was defined as $p \leq 0.05$. For cluster analysis, ward method was used and Euclidean distance was calculated.

Pearson correlation test was used to determine the relationship between indoor and outdoor PM samples variables (defined as $p \leq 0.05$). Microsoft Excel was used to calculate concentration and percentage contribution of different molecular weight PAHs for as well. In addition, Software Origin Pro 8 (© OriginLab Corporation, USA) was used to estimate PAH source apportionment based on their ratios and toxic units (TU).

5.6 PAH Source identification

The PAHs isomer ratio is a widely used technique to apportion original emission sources (Yunker *et al.*, 2002). The diagnostic ratio approach for identifying PAHs source requires comparison of ratios of constantly established PAHs pairs.

Diagnostic ratio of PAHs, such as ratios of fluoranthene to fluoranthene plus pyrene Flt/(Flt+Pyr), Benzo[a]anthracene plus chrysene BaA/(BaA+Cry), can be applied (Yunker *et al.*, 2002). The ratio of Flt/(Flt+Pyr) below 0.4 likely implies petroleum input, ratio between 0.4 and 0.5 petroleum combustion and ratio above 0.5 grass/wood and coal combustion. BaA/(BaA+Cry) ratio below 0.2 indicates petroleum, from 0.2 and 0.35 either petroleum or combustion as mixed source and ratio above 0.35 indicates fossil fuel and vegetation combustion. Ind/(Ind+BghiP) ratio >0.5 likely implies combustion of grass, wood and coal, while ratio between 0.2 and 0.5 petroleum combustion and <0.2 petroleum (Yunker *et al.*, 2002).

6 Results and Discussion

6.1 Validation of the direct contact test

In different sample preparation procedures, extraction with organic solvents influenced toxicity in extracts and ecotoxicity results, in addition, it does not reflect a realistic environment exposure route. Therefore, the main goal of present study was to compare toxicity results of different sample preparation to direct contact test (Kováts *et al.*, 2021).

6.1.1 PAH concentration

The concentrations of PAHs in samples extracted by different organic solvents are given in ***Table 1***. The lowest total PAH concentration was found in water extract, whereas in the other extract samples, such as DCM, hexane, and DMSO, highest PAH content were detected. In the methanol extract sample, total PAH content was 0.568 µg/mg, whereas in the DMSO extract, highest PAH content was 2.151 µg/mg.

Table 1 *Chemical characteristics of extracts*

Sample	Water	Methanol	DMSO	Hexane	DCM
Naphthalene (µg/mg)	7.340	5.430	7.478	5.004	5.728
2-methil-naphthalene (µg/mg)	0.190	0.142	0.291	0.130	0.147
1-metil-naphthalene (µg/mg)	0.090	0.063	0.166	0.058	0.066
Acenaphthylene (µg/mg)	0.000	0.008	0.012	0.006	0.004
Fluorene (µg/mg)	0.010	0.008	0.013	0.011	0.008
Phenanthrene (µg/mg)	0.080	0.182	0.290	0.249	0.271
Anthracene (µg/mg)	0.014	0.016	0.034	0.026	0.033
Fluoranthene (µg/mg)	0.020	0.122	0.431	0.255	0.305
Pyrene (µg/mg)	0.000	0.165	0.523	0.366	0.450
Benz (a) anthracene (µg/mg)	0.000	0.017	0.120	0.075	0.082
Chrysene (µg/mg)	0.000	0.009	0.110	0.041	0.075
Benz (b) fluoranthene (µg/mg)	0.000	0.006	0.115	0.069	0.048
Benz (5) fluoranthene (µg/mg)	0.000	0.020	0.055	0.043	0.031
Benz (e) pyrene (µg/mg)	0.000	0.004	0.068	0.044	0.029
Benz (a) pyrene (µg/mg)	0.000	0.006	0.042	0.026	0.031
Dibenz (a. h) anthracene (µg/mg)	0.000	0.000	0.001	0.000	0.000
Indeno (1.2.3-cd) pyrene (µg/mg)	0.000	0.000	0.007	0.004	0.002
Benz (g.h.i) perylene (µg/mg)	0.000	0.000	0.001	0.001	0.001
Total PAHs	**0.174**	**0.568**	**2.151**	**1.219**	**1.372**
Total PAHs + naphthalene	7.794	6.203	10.086	6.411	7.313

Bold values are total PAH concentration without naphthalene

Considering the profile of PAH isomers, light molecular weight (LMW) PAHs are abundant in water extract. The low amounts of fluoranthene and phenanthrene content in water extract were 0.020 µg/mg and 0.080 µg/mg, respectively. Higher molecular weight (HMW) PAH contents were determined in methanol, DMSO, hexane and DCM extracts. HMW PAHs (5- to-6-ring) are dominant in diesel emission, high molecular weight PAHs are causing the most adverse health effects such as carcinogenicity etc., (e.g., Islam *et al.*, 2019). In ecotoxicology screening, most often chemical measurements are coupled with EC_{50} values. The PAHs content in extracts can cause the variances in ecotoxicity results. The PAH concentration without naphthalene is in concordance with the outcomes of toxicological examination.

6.1.2 Ecotoxicity assessment

In the *Vibrio* test, comparative values of samples extracted with different organic solvents and direct contact are illustrated in ***Table 2*** expressed as EC_{50}. Highest toxicities were observed in hexane and DCM extract. EC_{50} value in DMSO extract was 9.92%. In the water extract, lower toxicity was determined. Whereas, EC_{50} value in direct contact was relatively high, similarity to methanol extract.

Table 2 *Ecotoxicity of different extracts vs. the direct contact test*

Sample	Methanol	Hexane	DCM	DMSO	Direct contact	Water
EC_{50}	12.53	6.79	6.39	9.92	11.97	78.31

The *Vibrio* bioassay was employed by Baran *et al.* (2019) to assess the impact of the bioavailable contamination fraction of sediments samples collected from the silted part of the Roznów reservoirs (Southern Poland). Toxicity results were calculated as percent effect (PE) and assessed by following criteria: non-toxic ≤20%; slightly toxic ≤50%; toxic ≥50%; and highly toxic samples (percentage effect = 100%). Based on this classification, extracts using organic solvents showed highly toxic results, with maximum inhibition in concentrated sample of 100%. On the contrary, water extract gave non-toxic result as maximum inhibition in concentrated sample was 14.5%.

Fig 12 gives the 30 sec kinetic curves of *Vibrio fischeri* bioluminescence inhibition bioassay on samples prepared with different solvents. According to 30 sec kinetic diagram results, samples extracted by Methanol, Hexane, DCM, and DMSO were found highly toxic. However, the water extract was obviously less toxic then other samples prepared with different organic solvents. The kinetic *Vibrio fischeri luminescence inhibition* test is widely used to assess the toxicity of solid and colored environment samples including sediment, particulate matter, etc., and preliminary assessment of the toxicity can be performed based on their kinetic mode (Mortimer *et al.*, 2008; Jarque *et al.*, 2016). *V. fischeri* 30-sec kinetic bioassay was used to assess the toxicity associated to sediments, which samples were collected from five localities with different contamination inputs in the South-eastern part of the Czech Republic. Results suggested

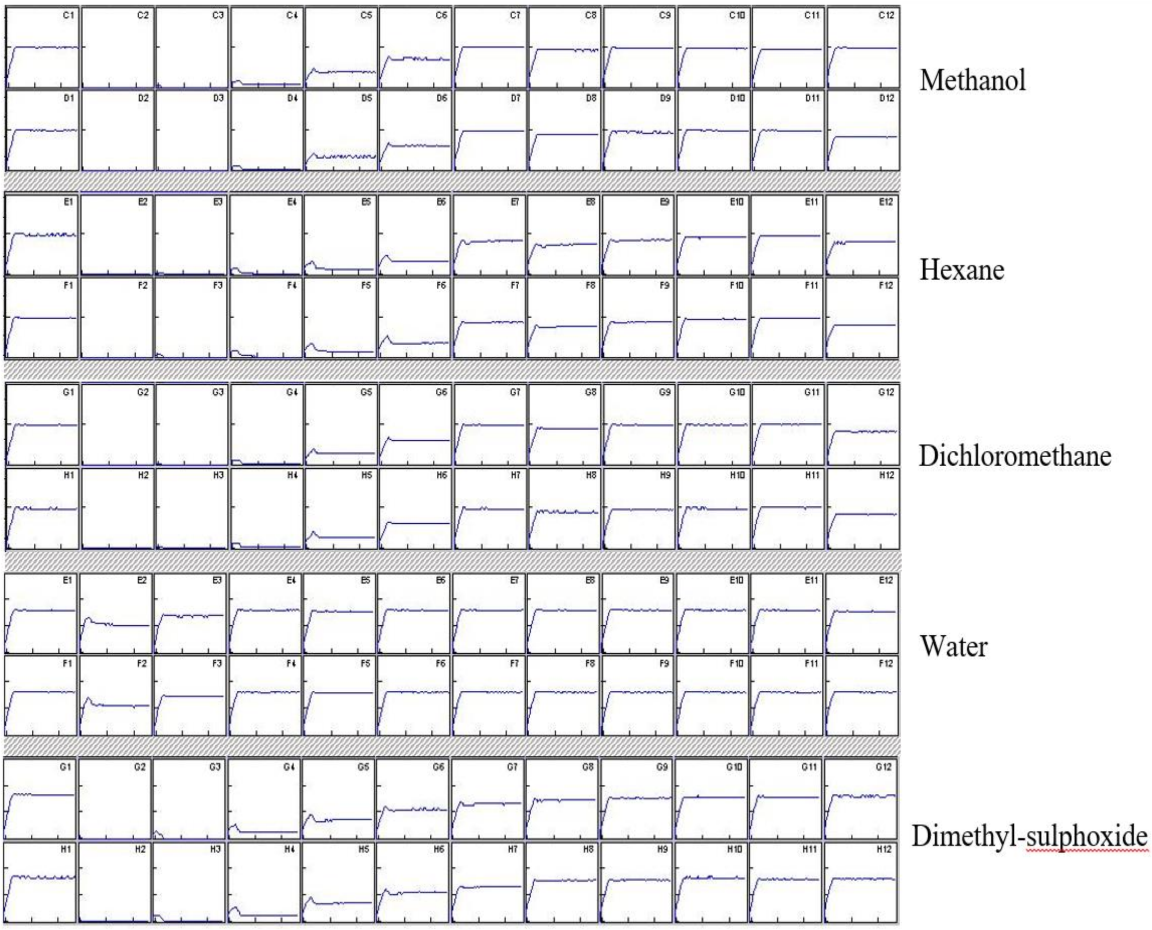

Fig 12 Kinetic diagram of the samples. Well1: control. Well2: highest, Well12: lowest concentrations. Light output is recorded in the first 30 s interval. In the control, light output remains even but in the actual samples light emittance is reduced, showing concentration-dependent pattern. Each sample is analyzed using two replicates (indicated by two rows in the diagram)

the method as a good screening tool for evaluating toxicity (Jarque *et al.*, 2016). In another study, the kinetic *V. fischeri* test was used for the initial and real-time screening of the toxicity of the sample of nanoparticles (NPs) samples. The 30 sec kinetic diagram of bioassay in these samples clearly showed toxicity (Mortimer *et al.*, 2008).

The dithiothreitol (DTT) assay was used to examine the ROS-generating (or oxidative) potential of the $PM_{2.5}$ samples collected from urban site near central Atlanta. In the methanol extract, DTT assay response was significantly higher (Verma *et al.*, 2012). In another study, bioassays were employed to compare the efficiency of bioremediation processes in heavily petroleum contaminated soil samples collected in two biopiles at the Czechowice oil refinery, Poland. The ecotoxicological test was performed in DMSO/H2O and DCM/DMSO soil extracts. Higher toxicity in all samples was found (Płaza *et al.*, 2005). In the study of Selivanovskaya *et al.* (2010), a comparison was performed between direct contact biotest with traditional elutriate test for evaluation of wastes hazards. The result showed the lowest inhibition dilution (LID_{10}) obtained in aqueous elutriate test was 6.4 times lower in comparison to that obtained in contact bioassay. The bioavailability of particulate matter plays a crucial role, therefore, when potential impacts are to be assessed, the bioaccessibility of pollutants is the determining factor (e.g. Turner, 2011). Regulatory frameworks for the assessment and remediation of contaminant soils based on a risk-based approach have been moved towards due to considering contaminant bioavailability (Kim *et al.*, 2015). Many reports demonstrated that the influence of bioavailability particulate-bound compounds strongly affects ecotoxic environmental impacts (e.g., Varshney et al., 2016; Jan *et al.*, 2018; Sah *et al.*, 2019). In order to determine bioavailable fraction of toxic compounds and exposure pathway, it should be noted that direct contact bioassay seems the most suitable screening tool. In the study of Tositti *et al.* (2018), bioluminescence inhibition test in a direct contact version was applied to assess the ecotoxicity of PM_{10} samples collected from a coastal location in N-W Italy. Cluster analysis on chemical composition and percentage of inhibition ratio (IR%) data revealed that a statistically significant correlation was detected between adverse effects on living cell and the specific chemical species in PM_{10} samples.

Unfortunately, studies on comparing the toxicity of different extracts and the whole medium using the same test organism are restricted. In order to evaluate the risk assessment of solid media including sediment, soil, compost and so, most of the studies apply a whole battery of bioassays representing the screening tool (Juvonen *et al.*, 2000). Biotests on liquid and solid phases of contaminated soils from industrial sites in the North of France were compared using ecoscores for evaluating ecotoxicology. Results of solid phase bioassay showed a higher sensitivity (Lors *et al.*, 2011). In the study of Tuikka *et al.* (2011), a sediment contact battery tests representing three different trophic level including the *Vibrio* test was utilized to assess the sediment toxicity. The result demonstrated that flash test with *Vibrio* test was efficient for discriminating the contaminated sediments.

Vibrio fischeri bioassay has been utilized to assess the different phases of the same sediment/ soil samples in some studies. Microtox Solid and liquid phase assay were employed to assess the overall sensitivity for the initial ecotoxicological screening of estuarine sediments. In the solid phase, greatest toxicity in all sediments was detected, while lower toxicity of samples was found in liquid phase test. For elutriate samples, there was no toxicity, whereas only pore water sample from the East wall site was toxic (Davoren *et al.*, 2005). In Poland, luminescent bacteria were used for the assessment of ecotoxicity. Microtox® screening test was employed for testing soil elutriates, while Microtox® Solid phase test was applied for testing solid samples. Relatively low toxicity was found in soil elutriates, whereas in the SPT- Microtox®, high reduction in bioluminescence appeared, EC_{50}s ranged from 4.8 to 36.4% and all samples were toxic (Klimkowicz-Pawlas et al., 2019). In an another study, Acheson *et al.* (2004) compared the solid phase and saline extract Microtox® assay for soil contaminated with polycyclic aromatic hydrocarbons (PAHs). Solid phase assay was more sensitive than saline extract assay, suggesting that solid phase Microtox® assay was appropriate for examining the toxicity of soil contaminated with PAHs.

Gonzalez-Merchan *et al.* (2014) used solid and liquid phase Microtox® assay to characterize sediments from stormwater retention basin in the region of Lyon (France), suggesting the SPT- Microtox® test in all samples showed higher ecotoxicity. A similar result was observed in the study of Paulovits *et al.* (2012) who used the three different phase (sediment, elutriate and pore water) BioTox® Flash assay (kinetic bioluminescence bioassay) to characterize the toxic nature of the sediments in the 2nd reservoir of the Kis-Balaton Water Protection System (KBWPS). Direct contact test in all sampling site showed higher ecotoxicity as followed by elutriate samples, whereas no pore water toxicity was found.

Direct contact and water extract tests using microbial test organism (*V. fischeri and A. agilis*) were used to evaluate soil toxicity. Soil samples were collected from contaminated sites. Direct contact test (*V. fischeri and A. agilis*) in all sample sites were more sensitive and detected high toxicity (Leitgib et al., 2007).

Among organic solvents, DMC is widely used to prepare an extract from different media such as sediment (Masood *et al.*, 2018). In order to assess the genotoxic and mutagenic potential of organic material extract in PM emission of a typical automotive diesel engine loaded with different alternative and/or renewable fuels, extract was prepared using DCM solvent (Soriano *et al.*, 2020). PM-bound PAHs samples from urban industrial area in South Africa were extracted with DCM solvent to determine their concentration and carcinogenic and mutagenic health risk of $PM_{2.5}$ bound PAHs (Morakinyo et al., 2020) as well. In the study of Jing *et al.* (2019), luminescent bacteria were used to evaluate a method for $PM_{2.5}$ biotoxicity assay. $PM_{2.5}$ samples were collected from campus of Fudan University (Shanghai, China) where this site was influenced by commercial, industrial and residential activities. A residual toxicity of the $PM_{2.5}$ samples, extracted by eight common extraction solvents, was measured and found that bioluminescence intensity of samples extracted with methanol, 1/1 N-hexane/dichloromethane, N-hexane, and dichloromethane (DCM) were not affected. Results indicated that rate for light inhibition of dimethyl-sulphoxide (DMSO) was 1%, which concluded that extraction solvent would affect results of toxicity test when being used in PM extraction process. From findings of this study, considering both residual toxicity and the polarity of extraction solvent, N-hexane/dichloromethane, methanol, and ultrapure water seem available solvents to prepare an extraction of PM samples for luminescence bacteria testing.

6.2 Seasonal $PM_{2.5}$

In our study, *Vibrio* bioassay was used to characterize seasonal differences in rural $PM_{2.5}$ (Kováts *et al.*, 2020).

6.2.1 PAH content

In this study, PAHs content in all four season extracts was measured. The highest ∑PAHs content was detected in autumn, followed by winter and spring. Whereas lowest total PAH content was found in summer extract (***Fig 13***).

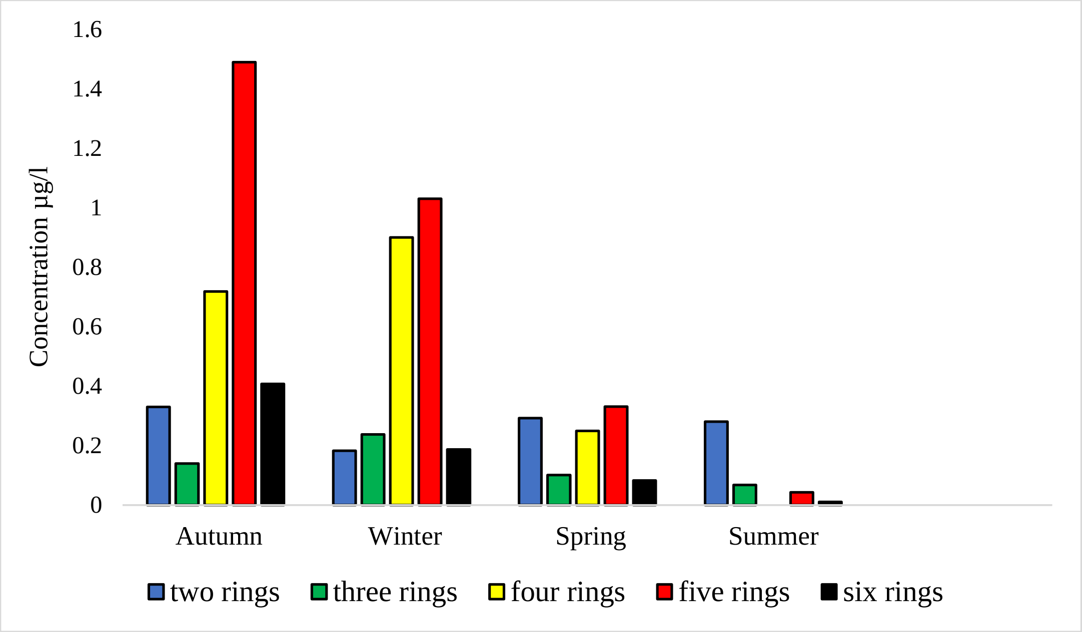

Fig 13 *Distribution of PAHs in the seasonally collected aerosol samples. PAHs are categorized into groups according to the number of rings*

HMW PAHs (4, 5 and 6-ring PAHs) were predominant in cold season samples compared to warm season ones. Mostly, the concentration of 5-ring PAHs in cold season was much higher than warm season. Surprisingly, 4-ring PAHs were not found in the summer extract sample.

Considering the individual PAHs characteristics, benzo[k]fluoranthene (BkF), indenol 1.2.3CD-Pyrene (IDP) and Benzo[g.i.h]perylene (BghiP) are excellent markers, which are associated with vehicle exhaust (Pant *et al.*, 2017) while Fluorene (Flu), Pyrene

(Pyr), Benzanthracene (BaA), Chrysene (Cry) and DbaA are considered as good markers of incineration source (Eiguren-Fernandez *et al.*, 2004). In our study, the highest concentration of BghiP in the autumn extract sample was observed, so it indicated traffic exhaust. Whereas concentration of Pyr, Cry and BaA in cold season samples were significantly higher compared to those in warm seasons, concentrations of Pyr in winter and autumn were 0.285 μg/l and 0.207 μg/l, values in spring and summer sample were 0.079 μg/l and 0.015 μg/l respectively. The concentration of Cry in the autumn and winter samples were 0.203 µg/l and 0.200 µg/l whereas in the spring and summer samples these values were 0.052 µg/l and 0.011 µg/l. The concentration of BaA in heating season samples was found to be higher (3 to 8 times) than those in non-heating season. Characteristics of individual PAHs in the heating season indicated biomass combustion (Gelencsér *et al.*, 2007). A similar study in Romania observed biomass combustion primary emission as main source in wintertime (Mărmureanu *et al.*, 2020), where highest amount of PAHs could be detected (Valavanidis *et al.*, 2006).

Considering the distribution of PAHs in vapor and particulate phase, HMW PAHs with 5-6 rings are abundantly observed in particle phase (Lu et al., 2008). Ambient temperature and lower photochemical activity are most important factor to increase the level of the PAHs in cold period (Eiguren-Fernandez *et al.*, 2004). For instance: mean total PAHs concentration at the urban site was 66.9 ng m^{-3} during April (average air temperature was 17.0°C) and 203 ng m^{-3} during January and February (average air temperature was 4.9°C) (Morville et al., 2011). The 17 PAHs of the atmospheric gas and particle-phase were measured in four seasons between 2002 and 2004 in the east of France, seasonal variation of PAHs showed higher values in the cold season. Significant differences in concentrations between rural, suburban and urban areas were observed and the prevalence of HMW PAHs was generally characteristic during the cold season as well (Morville et al., 2011). Similar seasonal variation trends have been reported (Li *et al.*, 2006; Esen et al., 2008). To evaluate the air quality of Győr (Hungary), 18 individual PAHs in urban PM_{10} samples were measured in the period of 2008 to 2012. Higher concentration of total PAHs was found in cold period and prevalence of HMW PAHs appeared in the heating season (Szabó et al., 2015).

6.2.2 PAH source identification

In this study, seasonal PM extracted samples are shown in ***Fig 14*** to identify their source emission based on these two ratios. Flt/(Flt+Pyr) ratios in winter, spring and summer samples were higher than 0.5 while Flt/(Flt+Pyr) ratio in autumn sample was between 0.4 and 0.5, its value was 0.48. BaA/(BaA+Cry) ratios in autumn, spring and summer samples were above 0.35, indicating combustion input, while ratio in winter sample was near to transit point of two kinds of combustion sources, indicating mixed sources.

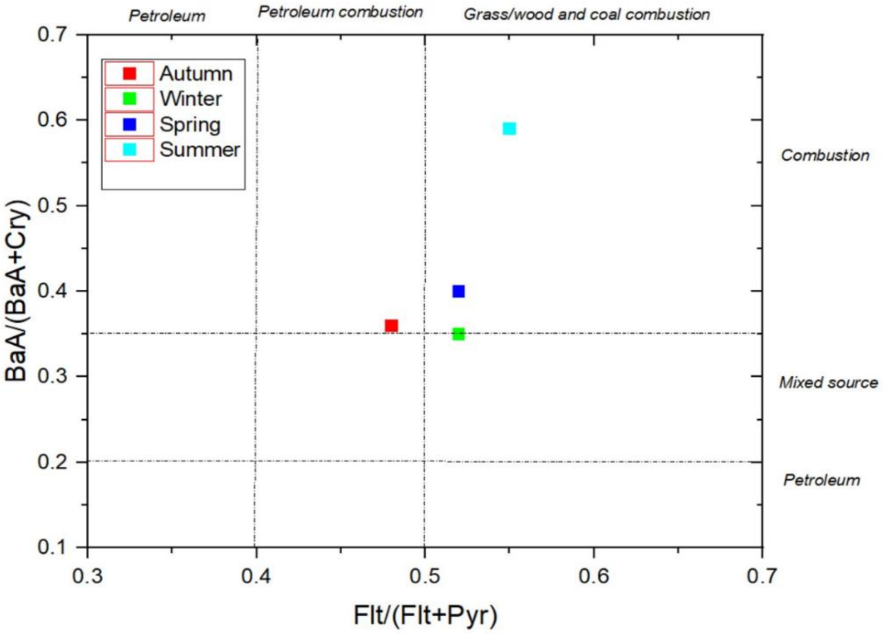

Fig 14 *Cross plot of BaA/(BaA+Cry) against Flt/(Flt+Pyr)*

These two ratio indicated that biomass burning and petroleum combustion are most important source emission of spring, autumn and winter samples in rural area, Hungary. Gelencsér *et al.* (2007) estimated that the main source of $PM_{2.5}$ was biomass burning in winter period at 6 sites along a west to east transect in Europe. A similar study discussed source identification of summer and the winter aerosol using data collected in Magurele, Romania between 2012 and 2013, suggesting the primary source of organic aerosol was biomass burning for the winter season (Mărmureanu *et al.*, 2020).

In another study, to assess air quality, PM concentration and PM bound PAHs in

PM_{10} and $PM_{2.5}$ aerosol samples collected at the monitoring station of Győr, were measured. Source apportionment indicated that main source emission of PM bound PAHs was biomass burning and traffic emission, BaP/BghiP ratio demonstrated traffic source in 70% of all the samples (Szabó et al., 2015; Szabó et al., 2019). The study of Angyal *et al.* (2021) highlighted that biomass burning (39%) and traffic emission (31%) in Debrecen were major contributors to $PM_{2.5}$.

6.2.3 Ecotoxicity assessment

In the *V.fischeri* bioluminescence inhibition assay, higher ecotoxic value (EC_{20}s) was found in winter period as followed by autumn and spring time. Whereas no ecotoxic effect was detected in summer period (***Fig 15***). *Vibrio fischeri* bioluminescence inhibition bioassay (Ascent Luminometer system) was used by (Kováts *et al.* (2012) to assess the ecotoxicity of winter and summer PM_{10} samples collected from residential background station at the main observatory of the Hungarian Metrological Service, Budapest (Hungary). As result, the winter sample was more ecotoxic while in summer sample, no toxic effect was observed. Wang *et al.* (2016) used bioluminescent bacteria (*Photobacterium phosphoreum* PPT_3) to assess the ecotoxicity of atmospheric PM in the period from March 2014 to January 2015 in Nanjing (China), and found that heating season samples collected from all locations showed higher ecotoxicity in comparison to others.

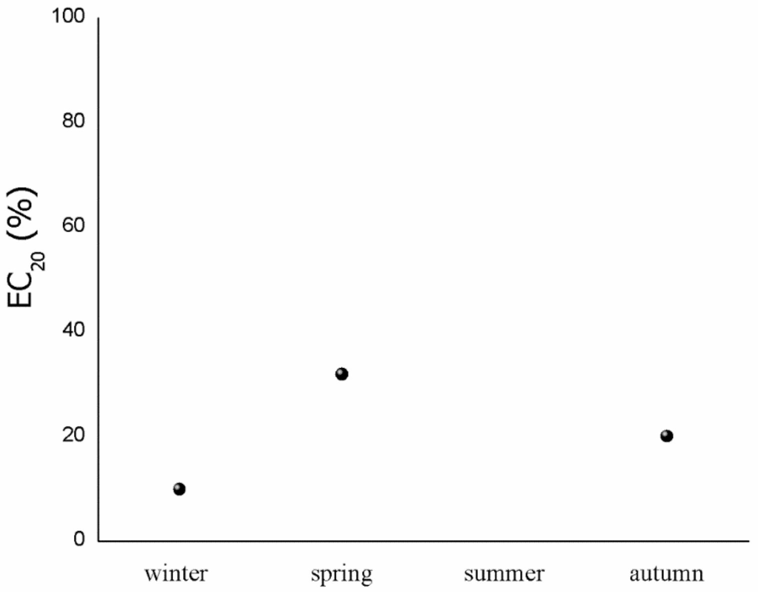

***Fig 15** Ecotoxicity of seasonal PM extract samples*

Aammi et al. (2017) also assessed the ecotoxicity levels of atmospheric $PM_{2.5-10}$ using *V. fischeri* inhibition bioassay at several location of a megacity (Istanbul, Turkey). An extremely high toxic unit (TU) in extracted samples from polluted area was found in the cold season (between January and March). *V. fischeri* inhibition bioassay was employed in the study of Roig *et al.* (2013) in various areas of Catalonia (Spain), and ecotoxicity test in urban and cold season samples showed significantly higher ecotoxicity than samples from rural areas and summer respectively. In addition, positive correlation was found between the concentration of metals and organic toxic substances in atmospheric PM_{10} and the bioassay outcomes.

6.3 Domestic waste PM_{10}

To provide an initial estimation of the ecotoxicity profiling of PM_{10} samples originating from controlled combustion of different waste types, such as polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PU), oriented strand board (OSB) and rag (RAG), present study was performed (Kováts *et al.*, 2022).

6.3.1 Concentration of PAHs and heavy metals

PAH concentrations in each PM_{10} emitted from different types of waste combustions are illustrated in ***Table 3***. Highest total concentration of PAHs was found in PS sample (407µg/l), followed by PU sample (321 µg/l) and PP sample (167 µg/l). Lowest total concentrations in RAG (8.95 µg/l), OSB (13.7 µg/l) and PVC (20 µg/l) samples were detected, respectively. In the PE sample, concentration was 48.7 µg/l.

Table 3 *Summary of PAHs of aqueous extract. Concentration values are given in µ/L. PS: polystyrene, PE: polyethylene, PP: polypropylene, PVC: polyvinyl chloride, PU: polyurethane, OSB: oriented strand board, and RAG: rag. Limit of detection (LOD) was 0.001 µg/L*

	PS	PE	PP	PVC	PU	OSB	RAG
Naphthalene	<0.001	0.339	2.8	2.15	6.68	<0.001	0.144
2-methyl-naphthalene	0.18	0.15	0.966	<0.001	1.66	0.12	0.123
1-methyl-naphthalene	0.31	0.108	0.533	<0.001	1.64	<0.001	0.132
Acenaphthylene	26.9	1.22	7.96	0.49	56.2	0.67	0.101
Acenaphthene	0.58	0.154	0.345	0.067	128	0.09	0.024
Fluorene	38.8	1	7.54	0.565	6.79	0.277	<0.001
Phenanthrene	213	8.91	44.6	3.15	32.8	1.39	0.253
Anthracene	15	0.7	3.22	0.28	10.2	0.33	0.027
Fluoranthene	60.7	15.6	36.3	6.59	28	4.82	2.34
Pyrene	30.2	16.8	51.1	3.5	37.6	4.11	1.66
Benzanthracene	4.61	0.738	2.94	0.57	2.18	0.83	0.375
Chrysene	8.71	1.24	2.69	1.34	2.22	0.24	2.05
Benzo(b)fluoranthene	4.44	0.623	0.676	0.67	2.13	0.32	0.851
Benzo(k)fluoranthene	0.826	0.17	1.24	0.151	0.54	0.04	0.155
Benzo(e)pyrene	0.98	0.282	2.33	0.225	0.88	0.157	0.38
Benzo(a)pyrene	1.04	0.401	0.979	0.19	1.79	0.185	0.238
Dibenzo[a.h]anthracene	0.43	0.145	0.074	0.086	0.54	0.07	0.085
Indeno1.2.3CD-Pyrene	0.44	0.027	0.07	<0.001	<0.001	0.019	<0.001
Benzo(g.h.i)perylene	0.26	0.122	1.04	0.06	0.738	0.07	0.014
Total PAHs	407	48.7	167	20	321	13.7	8.95

Considering individual PAHs in each sample, 3-to 4-rings PAHs in PS, PP, and PU samples were observed as dominant. Of heavy PAHs (5-to 6-ring), values of dibenzo[a.h]anthracene and benzo[g.h.i]perylene were 0.54 µg/l and 0.74 µg/l, respectively.

Heavy metal contents in RAG and PE samples were rather low, toxic metal, concentrations of cadmium (Cd) were below the detection limits (see ***Table 4***). Highest concentrations of Cd were found in PVC sample (22.4 µg/l), followed by PU (5.54 µg/l) and OSB (2.89 µg/l). High concentration of Pb was detected in the PVC sample, with 1.01 µg/l, while in other samples, concentrations of Pb were below the limits of detection. Highest concentration of Zn was observed in PVC sample as well. However, in other samples, concentrations of Zn were relatively low.

Table 4 Summary of metal concentration of aqueous extract. Concentration values are given in µ/L. PS: polystyrene, PE: polyethylene, PP: polypropylene, PVC: polyvinyl chloride, PU: polyurethane, OSB: oriented strand board, and RAG: rag. Limit of detection (LOD) 0.2 - 10 µg/L

	PS	PE	PP	PVC	PU	OSB	RAG
Ag	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000
Al	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
As	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000
B	72.1	54.4	66.8	58.9	94.6	79.5	64.2
Ba	<10.0	<10.0	<10.0	122	<10.0	<10.0	<10.0
Cd	<0.500	<0.500	<0.500	22.4	5.54	2.89	<0.500
Co	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000
Cr	1.43	<1.000	3.73	<1.000	<1.000	<1.000	<1.000
Cu	<5.00	6.72	<5.00	6.26	<5.00	6.33	7.61
Mo	2.42	<2.00	5.63	2.08	2.14	<2.00	<2.00
Ni	<2.00	<2.00	2.02	2.5	<2.00	<2.00	<2.00
Pb	<1.000	<1.000	<1.000	1.01	<1.000	<1.000	<1.000
Se	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000
Sb	<0.500	<0.500	0.557	4.03	<0.500	<0.500	8.4
Sn	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000	<1.000
Zn	12.8	8.23	11.4	78.8	11.9	11.3	6.73
Na	3.84	3.1	3.81	0.684	3.3	4.54	2.92
Hg	<0.200	<0.200	<0.200	<0.200	<0.200	<0.200	0.228

Considering the chemical composition of all extracted samples, PS and PP samples could be identified as PAHs –type samples, whereas PU sample is mixed nature based on their high content of PAHs and metals. In the experimental combustion study of Hoffer et al. (2020) the concentration of PAHs in PM_{10} emission of twelve different types of common solid waste (e.g., plastic beverage packaging and other plastic wastes, textiles waste, fiberboard, furniture, tyres and coloured paper wastes) were measured, extremely high PAH emissions was found in PS, PP, and PU samples.

PVC sample could be somewhat categorized as metal-type based on the high content of Cd and Zn. In our case, low amount of PAHs in the PVC sample was found. However, it should be noted that high amount of PAHs was detected in incineration products of PVC linoleum, especially concentrations of benzo[a]anthracene and chrysene were high (142.5 and 143.13 µg/g) (Gotlib et al., 2001). Valavanidis *et al.* (2008) studied metals and PAHs accumulation in PM emission from controlled combustion of 6 types of plastic. As result showed, high concentrations of Pb and Zn in PVC sample were detected, with 64.2±6.7 µg/g and 13.1±0.5 µg/g respectively. Another experimental study (Park et al., 2013) studied the emission levels of PM_{10} / $PM_{2.5}$, PAHs, and heavy metals from test combustion of individual domestic waste such as paper, wood, and plastics. The most common heavy metal in mixed waste plastics emission samples was Zn, followed by Cr, Pb, Ni, Cu, and Cd. High amount of PAHs in mixed plastic emission sample was observed, the highest concentration of phenanthrene was detected (330.9 µg/g) followed by Fluoranthene (275.7 µg/g), Pyrene (90.5 µg/g), and Indeno (1,2,3-cd)pyrene (62.0 µg/g).

Several studies on the open burning of municipal solid waste, which emits heavy metals that contribute to atmospheric PM, have been demonstrated. For instance; Wang *et al.* (2017) reported that a large amount of 9 toxic heavy metals (Hg, As, Pb, Cd, Cr, Se, Cu, Zn, and Ni) was emitted from open-municipal waste burning in six districts in China, especially large toxic heavy metals emission was mainly observed in densely populated urban areas and large municipal solid waste generation places. In another Indian study, trace metals in open waste burning aerosol samples were investigated in landfill sites, in New Delhi during the cold period. As result showed that the metals such as, As, Cd, Sb, and Sn content in aerosol samples were predominant (Kumar *et al.*, 2015).

PAH concentrations of the OSB and Rag samples were rather low, TU value in Rag was similar to PE sample (1.85), but practically no toxicity was detected in OSB sample. For heavy metal analysis, heavy metal such as, Cd, and Pb were detected in limit of detection (LOD).

6.3.2 Sample characterization

In our study, PCA analysis was used to identify the toxic waste samples. They can be distinguished as PAHs type and heavy metal type based on the ecotoxicity assay result and a chemical composition. The biplot of PCA analysis for different type of waste combustion samples is presented in ***Fig 16***.

Fig 16 illustrates the PC1 component accounted for 44.16% of the total variance and the PC2 component accounted for 19.65% of the total variance. In the PCA analysis, three cluster of samples could be identified based on their similarity. Samples such as PE, RAG, and OSB created one group, while PS, PU and PP samples formed another group. PVC sample was separate.

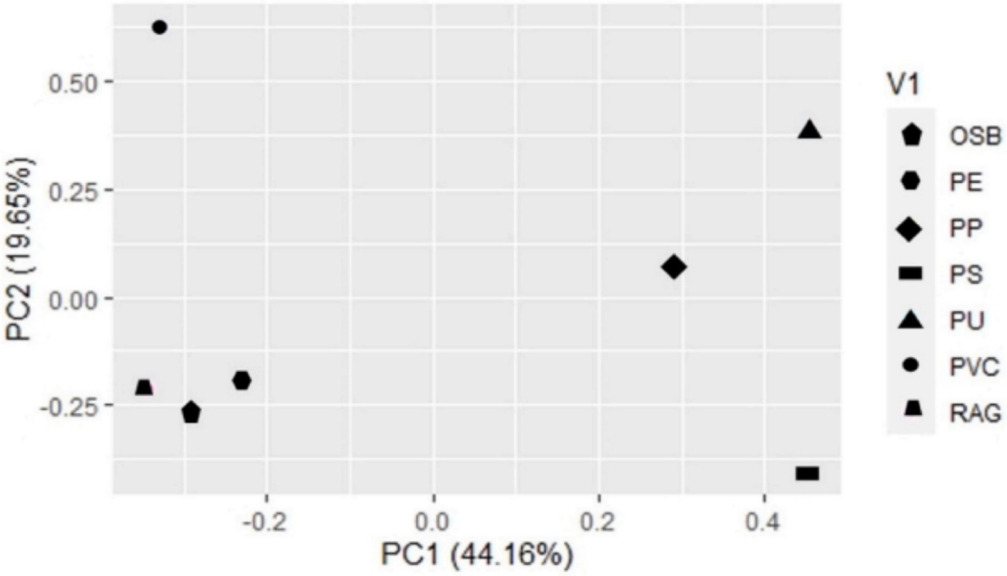

Fig 16 *PCA diagram of the samples based on chemical composition and ecotoxicity results. PP: polypropylene, PS: polystyrene, PU: polyurethane, PE: polyethylene, PVC: polyvinyl chloride, RAG: rag, OSB: oriented strand board.*

6.3.3 Ecotoxicity assessment

In the *V.fischeri* bioluminescence inhibition assay, the toxic units (TU) of each PM_{10} emitted from different types of waste combustions showed clear differences (***Fig 17)***. For all extracted samples except the OSB sample, the categories of the TU were regarded as toxic. However, TU values in extracted waste samples were slightly different, the toxic unit of PU was 4.21, followed by PVC (3.3), PS (2.57) and PP (2.14) respectively. TU values of RAG and PE were very close to each other, 1.85 and 1.87, respectively. Whereas, for the OSB sample, no ecotoxic effect was found. In this case, each blank samples were also measured and found to be non-toxic.

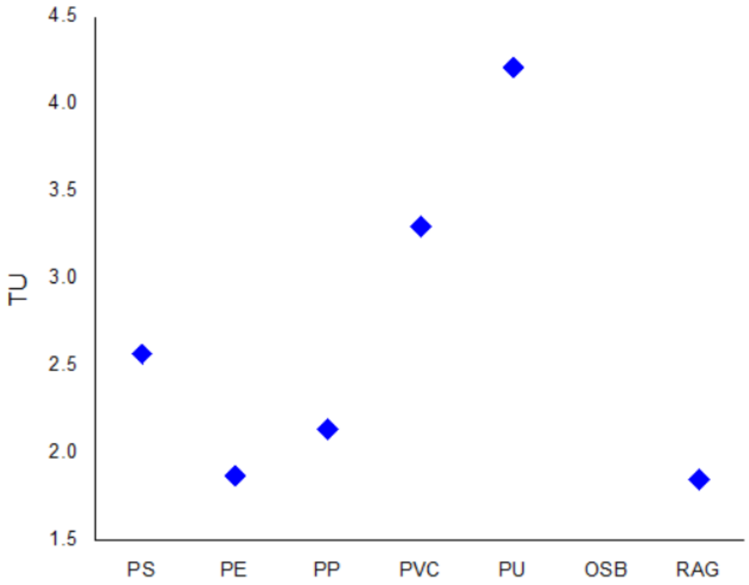

Fig 17 *EC_{50} of PM_{10} emission from different illegal waste burning under the controlled combustion*

Fig 18 shows the kinetic diagram of light output of stressed and control bacteria as recorded during the first 30 sec of the measurement (Lappalainen *et al.*, 1999, 2001) and gives the kinetic curves of OSB, Rag, PE, PVC, PU, PP and PS extracted samples. For OSB sample, kinetic diagram showed far less toxicity than any of the other extracted waste samples.

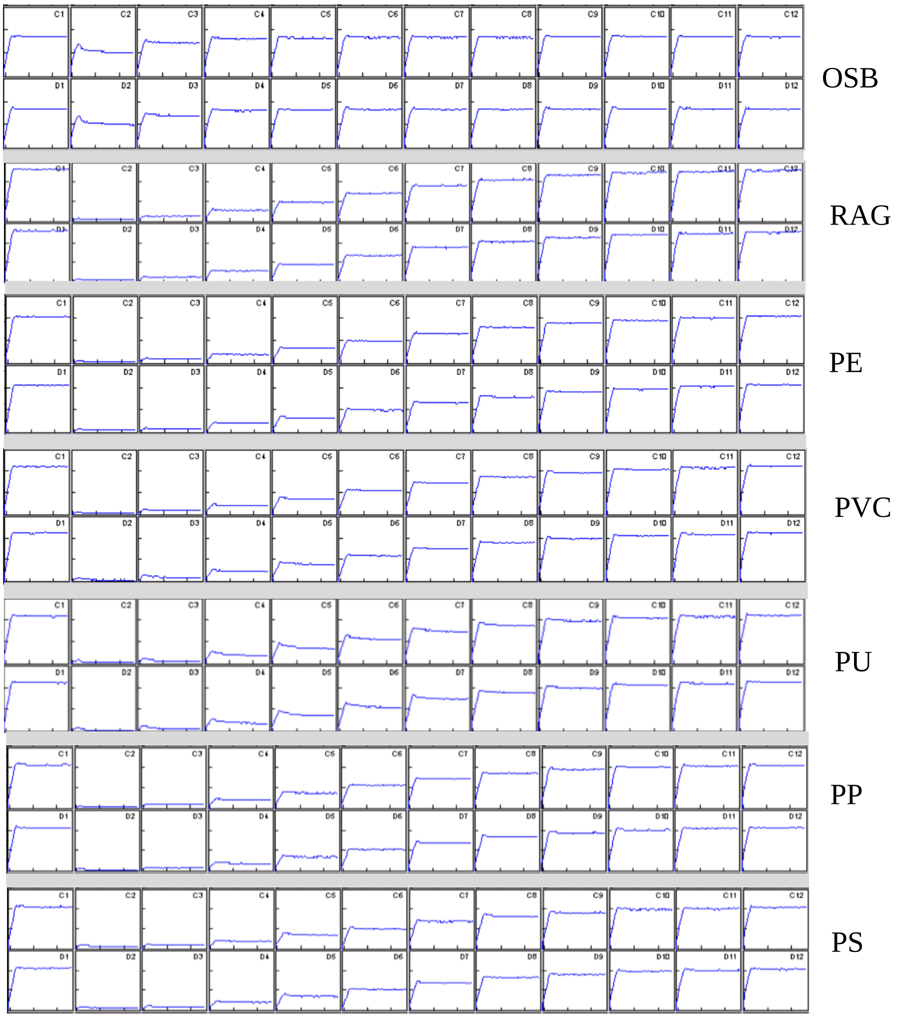

Fig 18 *The kinetic diagram of the different types of waste combustion aerosol PM10 samples. Each samples were measured in two replicates, C1 to D1- controls, C2 to D2 – Highest concentrations, C12 to D12 – lowest concentrations.*

These diagrams give a visual estimation of the expected behavior of test bacteria and can be used for the initial and real-time screening of the toxicity of the sample (e.g. Jarque *et al.*, 2016). In our study, OSB, Rag, PE, PVC, PU, PP, and PS extracted samples were regarded as toxic based on a preliminary assessment of kinetic curves.

6.4 Waste PET PM_{10}

The direct contact test based on *V. fischeri* bioluminescence inhibition bioassay was used to assess the potential environmental impact of the PM_{10} sample emitted from colored and colorless PET wastes under the open-combustion experimental setup (Kováts *et al.*, 2023).

6.4.1 Concentration of heavy metals

The concentration of heavy metals in PM_{10} samples originating from the controlled combustion of colorless and colorful PET waste bottles is shown in ***Table 5***. High concentration of Antimony (Sb) in the PM_{10} samples from both PET waste bottles was detected. Antimony trioxide (ATO) is one of main catalysts, which is commonly used in the manufacture of PET bottles. Antimony content was measured in 12 brands of bottles in the study of Chapa-Martínez *et al.* (2016), it ranged from 73 mg/kg to 111.3 mg/kg. In another similar study, the antimony content examined in ten brands of PET package materials varied between 210 mg/kg and 290 mg/kg (Keresztes *et al.*, 2009).

Table 5 *Metal concentrations in the PM10 samples (mg/kg)*

Metals	Colorless1	Colorless2	Colored1	Colored2
Ag	<0.020	<0.020	<0.020	<0.020
As	0,022	0,032	0,029	0,02
B	1,66	2,35	1,77	2,00
Ba	<0.200	<0.200	0,207	<0.200
Cd	<0.010	<0.010	<0.010	<0.010
Co	<0.020	<0.020	<0.020	<0.020
Cr	0,065	0,082	0,084	0,096
Cu	<0.100	<0.100	<0.100	<0.100
Mo	0,087	0,085	0,101	0,085
Ni	0,273	0,112	0,056	0,054
Pb	0,096	0,091	0,066	0,077
Se	<0.020	<0.020	<0.020	0,022
Sb	14,8	16,9	6,93	14,4
Hg	<0.004	<0.004	<0.004	<0.004
Sn	<0.020	<0.020	<0.020	<0.020

Two main types of removing processes of the antimony (Sb) in ambient air are transfer to different environments including soil or water via wet and dry deposition (Smichowski, 2008). In the soil antimony is more easily absorbed by roots and transported to shoots by plant and can be accumulated (Zhong *et al.*, 2020). There are a number of studies, which can give data about its ecotoxicity on various test organisms including earthworms, mussels and higher plants, etc.

Xu *et al.* (2021) estimated the toxicity of soil antimony to earthworm *Eisenia fetida* (Savingy) before and after aging process. The results showed that the values of net avoidance response, escape and mortality of earthworm in aged treatment generally decreased. In the study of Zhu *et al.* (2022), hydroponic culture experiments were performed to establish the toxicity of different forms of Sb to rice plant. High toxicity and water vapor deficit was found. In another study, effects of six different polymer types as 4 mm microplastic on *Nelumbo nucifera* (Lotus) in water and sediment were investigated. Highest inhibition of germination and growth on two plastic samples was detected, while significantly increased Catalase and Glutathione S-transferase activities were observed.

Chemical composition and ecotoxicity of four types of plastic leachates including PET to aquatic test organism were examined in the study of Capolupo *et al.* (2020). Highest concentration of antimony in leachate was detected and inhibition of normal embryonic Mediterranean mussel (*Mytilus galloprovincialis*) development was significantly induced. Based on evidence reports on human health, antimony occurring in higher concentration than 8.87 mg/m^3 seem to cause respiratory problems (e.g., pneumoconiosis). Cardiovascular disease, dermal problem (antimony spot), reproduction disorders, genotoxic and carcinogenic effects are associated with chronic antimony exposure as well (Sundar and Chakravarty, 2010). Neurotoxicity of antimony in the early life stage of Zebrafish was examined in the study of Xia *et al.*(2021). As result, level of acetylcholinesterase (*AChE*) in Zebrafish embroyes was reduced, along with decreased expression of neurofunctional markers including *gfap, nestin, mbp,* and *shha*.

6.4.2 Concentration of PAHs

The concentrations of PAHs measured in PM$_{10}$ samples originating from the controlled combustion of both PET waste samples are presented in ***Table 6***. Total PAHs content of both PET waste samples varied in the samples and ranged from 290 mg/mg to

425 mg/kg. Higher difference of total PAHs content was observed in the 2 colored samples. Whereas, on the contrast, values of total PAHs in 2 colorless samples were rather close to each other, 378 mg/kg and 425 mg/kg. In the work of Hoffer *et al.* (2020), controlled combustion test were conducted with twelve different types of domestic waste including PET and PAH content in samples was examined. The highest concentration of PAHs was detected when burning PET waste (3.0 µg total PAH in 1mg PM_{10} aerosol).

Table 6 *PAH concentrations in the PM10 samples (mg/kg)*

	PAHs	Colorless1	Colorless2	Colored1	Colored2
two rings	Naphthalene	<0.01	0.22	0.07	0.06
	2-methyl-naphthalene	<0.01	0.02	0.03	0.03
	1-methyl-naphthalene	<0.01	0.02	0.02	0.02
three rings	Acenaphthylene	0.16	0.21	0.12	0.21
	Acenaphthene	0.02	0.02	0.03	0.01
	Fluorene	0.13	0.16	0.11	0.01
	Phenanthrene	3.07	0.45	2.7	4.02
	Anthracene	0.36	0.56	0.29	0.62
four rings	Fluoranthene	26.6	29.3	16	27.2
	Pyrene	14.2	17.6	11.5	17.3
	Benz(a)anthracene	33.5	41.3	26.1	36
	Chrysene	95.7	92.1	66.2	105
five rings	Benzo(b)fluoranthene	88.2	103	66.9	90.6
	Benzo(k)fluoranthene	14.4	17.3	12.8	15.8
	Benzo(e)pyrene	27.5	32.3	21.2	30.2
	Benzo(a)pyrene	19.4	25.1	18	23.3
	Dibenzo(a.h)anthracene	23.9	29.1	20.6	25.8
six rings	Indeno1.2.3CD-Pyrene	11.8	14.2	8.14	11.2
	Benzo(g.h.i)perylene	19	22	19.3	22.9
	Total PAHs	378	425	290	410

Fig 19 shows the distribution of PAHs, which are categorized into groups based on the number of rings in both PET waste samples. In our study, prevalence of 4-and 5-ring PAHs in all samples was found. Highest concentration of chrysene in 2 colored samples was detected with the values of 66.2 mg/kg and 105.0 mg/kg. The concentrations of chrysene in 2 colorless samples were 92.1 mg/kg and 95.7 mg/kg.

In the 5-ring PAHs, high concentration of benzo(b)fluoranthene in all samples was found and ranged between 66.9 mg/kg and 103 mg/kg. The concentration of benzo(b)fluoranthene in 2 colorless samples ranged from 88.2 mg/kg to 103 mg/kg. The benzo(b)fluoranthene content was also high in 2 colored samples (ranged from 66.9 mg/kg to 90.6 mg/kg). Of 6-ring PAHs, the concentrations of the indeno1.2.3CD-Pyrene and benzo(g.h.i)perylene in both PET waste samples were relatively high as well. The concentration of indeno1.2.3CD-pyrene ranged from 11.8 mg/kg to 14.2 mg/kg in 2 colorless PET samples while in 2 colored samples, concentrations were 8.14 mg/kg and 11.2 mg/kg.

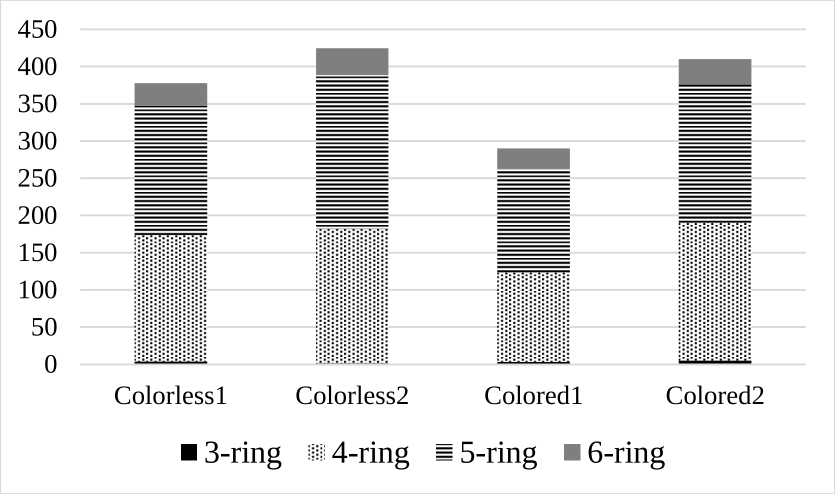

Fig 19 *Concentration of different PAH isomers in the samples*

In the study of Xiu et al. (2014), bioaccumulation and oxidative damages such as lipid peroxidation (LPO), protein carbonyl (PC) and DNA strand breaks were examined in soft parts of juvenile scallops (*Chlamys farreri*) to evaluate effects of the benzo(b)fluoranthene (BbF) and chrysene (Cry). Higher accumulation of chrysene in soft part of scallops was found and LPO levels, PC contents and DNA damage were increased significantly. Another report assessed the effects of chrysene (Cry) on detoxification enzyme activities, bioaccumulation and effect of chrysene on biomolecule damage in gills, muscle and hepatopancreas tissues of the juvenile white shrimp *Litopenaeus vannamei*. Test organisms were treated with chrysene for 21 days at different concentrations. A sharply increased chrysene accumulation was observed. Also, detoxification enzyme activities of glutathione –S-transferase (GST), sulfotransferase

(SULT) and uridinediphosphate glucuronyltransferase (UGT) were induced while higher activity of antioxidant defense system (superoxide dismutase- SOD) in gill and hepatopancreas was found. In addition, lipid peroxidation (LPO), protein carbonyl (PC) and DNA damage appeared (Ren et a., 2015). Guo *et al.* (2017) also proved that mixture of chrysene and benzo(a)pyrene concentration in tissues of the scallop *Chlamys farreri* increased rapidly in a time and dose effect pattern.

Liver parameters were assessed in chrysene-exposed C57BL/6 mice in the study of (Tao *et al.*, 2021). Increase in morphometric parameters of liver (e.g., liver weight and hypotocyte swallowing) appeared while higher amount of the liver enzymes (ALT) in serum was detected.

PM-bound PAHs were measured in $PM_{2.5}$ and PM_{10} samples collected seasonally in an urban region in Zhengzhou (China) between 2014 and 2015. Chrysene and benzo(b)fluoranthene content in air samples during the cold period were detected at high levels (Wang *et al.*, 2017). Human health hazards of PAHs in road dust were investigated in the study of Soltani *et al.* (2015). Cancer risk assessment was estimated by inhalation and dermal contact. An average estimated levels via dermal contact and ingestion pathway in adult and children were found as much as 2.97×10^{-4} and 2.24×10^{-4} in children and 3.10×10^{-4} and 1.75×10^{-4} in adults. In another study, PAHs emission from burning of agricultural waste (crop residue) was measured. Highest mean concentration of benzo(b)fluoranthene was 1.30 µg/mg. This study also demonstrated that the toxicity of PAHs was due to presence of benzo(b)fluoranthene for most samples, while extremely high cancer risk was estimated (10^{-1}) (Sunday *et al.*, 2022).The concentration of benzo(b)fluoranthene in human milk of the healthy, non-smoking, Italian adults living in rural or low traffic zones was detected (0.560±1.39 µg/kg as wet weight) (Bubba *et al.*, 2005). In animal experiment study Kim *et al.* (2011) revealed that sperm quality in the benzo(b)fluoranthene-exposed mice was decreased. Another study also reported that mixture of benzo(b)fluoranthene and benzo(a)pyrene had higher cytotoxicity and facilitated ROS production (Branco *et al.*, 2021).

6.4.3 Ecotoxicity assessment

Fig 20 shows the toxic unit (TU) of PM_{10} samples originating from the controlled combustion of PET wastes. Based on TU classifications, both PET waste samples were regarded as toxic. However, differences in TU values in colorless PET waste samples appeared, they were 4.01 and 5.63. In the colored samples, TU values fell closer to each other, at 3.9 and 4.33.

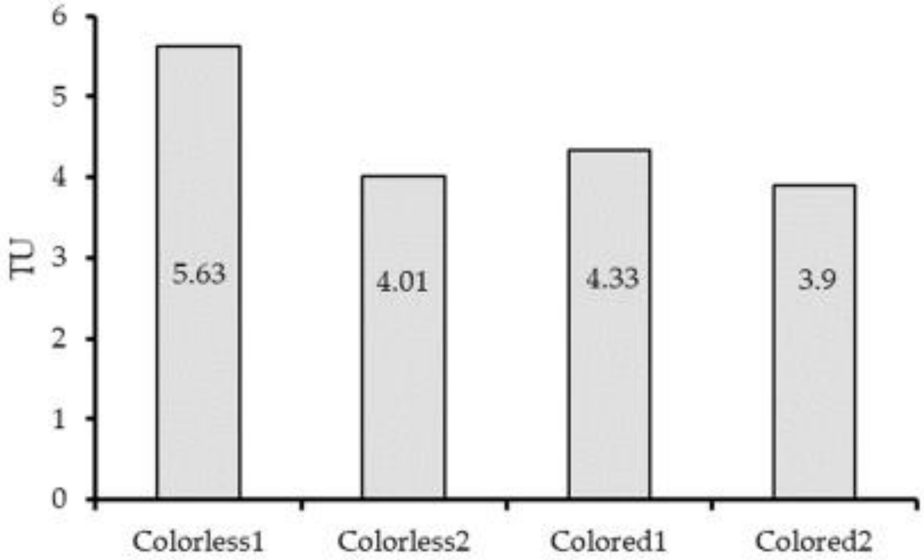

Fig 20 *TU values in the 4 PM samples*

Based on outcomes of ecotoxicology and chemical measurements in this study, particle emissions from illegal PET waste burning indicated high toxicity, which might pose a serious environmental risk. In China, microplastic pollution in atmospheric dust from different environments was studied. Four colors of microplastic in dust were determined and PET sources mainly from fabric fragment emissions (Zhang et al., 2022).

6.5 Indoor $PM_{2.5}$

V.fischeri bioluminescence inhibition bacterium test was used to assess the indoor $PM_{2.5}$ in non-smoking households in Mongolia (Sainnokhoi *et al.*, 2022).

6.5.1 Concentration of indoor $PM_{2.5}$

Concentration of indoor $PM_{2.5}$ in different household ranged between 62.8µg m_3 and 324.8 µg m_3. Highest concentration was detected in HH-6 (324.8 µg m_3) and HH-2 (320 µg m_3) while lowest concentration was found in HH-9 (62.8 µg m_3) (***see table 7***). However, concentration of $PM_{2.5}$ in all household exceeded the standard of the WHO (10 µg m_3) and Mongolian National Ambient Air Quality Standard (50 µg m_3).

6.5.2 PAH concentrations

Table 8 shows the concentrations of 19 individual PAHs in indoor air of ten non-smoking ten households. The highest ∑PAHs content was detected in HH-7 (175.7 ng m^{-3}), followed by HH-2 (137.3 ng m^{-3}), and HH-5 (105.1 ng m^{-3}) while the lowest ∑PAHs content was found in HH-9 (46.2 ng m^{-3}).

In this study, comparison with colder regions appears to be more appropriate as more representative meteorological conditions can be taken into consideration. In the study of Mohammed *et al.* (2016), the distribution pattern of $PM_{2.5}$ bound PAHs in indoor air in Harbin city (China) was examined. The levels of the sum of 16 US EPA priority PAHs were found high during heating months, average value in indoor air was 102 ±75.8 ng m^{-3}. Krugly *et al.* (2014) reported the characterization of PAHs in indoor $PM_{2.5}$ fraction of a primary school, sampling was conducted during the cold season period in the city of Kaunas, Lithuania between 2011 and 2012. The concentration of total PAHs in the $PM_{2.5}$ fraction ranged from 20.3 ng m^{-3} to 131.1 ng m^{-3}. In another study in Hangzhou (China), PAHs pollution in residential air was investigated, total concentration of 8 PAHs ranged from 7.1 ng m^{-3} to 320 ng m^{-3} (Lu et al., 2011). Seasonal variation and sources of PAHs in urban indoor particulate and gas phase samples were investigated in a semiarid region of India, average PAHs level in residential homes was 233 ng m^{-3} (Masih *et al.*, 2012). In another Chinese study, Li *et al.* (2017) reported an average of 39.6 ng m^{-3} PAH content in indoor $PM_{2.5}$ samples collected in cold season from suburban hotels in Jinan (China).

While our results fall into a similar range, in the study of Zhu *et al.* (2009), the highest concentration of total 16 PAHs (36200 ng m^{-3}) was detected in residential air samples collected from Hangzhou, China.

Table 7 *Description of sampling sites*

	Concentration of indoor PM2.5 (μg/m^3)	**Huse type**	**Residency year**	**Sampling place**	**Households area (m^2 /room number)**	**Family members**	**Heater type**	**Ventilation per day**	**Heating time per day**	**Cooking per day**	**Nearby outdoor PM source**	**Tobacco smoke**
HH-1	107.2	Fired clay brick	12	Living room	60/ 3 rooms	5	Stove	-	Coal, processed coal and wood/ 4	3	Next to main road	No
HH-2	320.7	Breeze-block	12	Living room	27/ 2 rooms	7	Stove	1	Coal, processed coal and wood/ 3	1	Main road, unpaved road	No
HH-3	176.5	Fired clay brick	7	Living room	64/ 3 rooms	5	Electric/stove	1	Coal, processed coal and wood/ 6	2	Next to unpaved road	No
HH-4	137.2	Breeze-block	10	Sleeping room	71/ 2 rooms	8	Stove	2	Coal, processed coal and wood/ 2	3	Next to unpaved road	No
HH-5	93.6	Fired clay brick	4.5	Living room	31.15/ 1 room	5	Stove	-	Coal, processed coal and wood/ 3-4	3	Next to mini power plant, main road	No
HH-6	324.8	Fired clay brick	45	Living room	160/ 2 rooms	7	Electric/ stove	-	Coal, processed coal and wood/ -	2	Unpaved road	No
HH-7	214.6	Clay and breeze-block	40	Living room	24/ 1 room	6	Stove	-	Coal, processed coal and wood/ 3	1	Next to mini power plant, main road	No
HH-8	246.2	Fired clay brick	10	Living room	18/ 1 room	6	Stove	-	Coal, processed coal and wood/ 4	-	Unpaved road	No
HH-9	62.8	Breeze-block	26	Living room	104/ 3 rooms	9	Electric/ stove	-	Coal, processed coal and wood/ 3	2	Next to unpaved road	No
HH-10	142.8	Breeze-block	50	Kitchen room	32/ 1 room	3	Electric/ stove	-	Coal, processed coal and wood/ 2	2	Next to unpaved road	No

Table 8 *Concentration (ng m_3) of PAHs in the indoor PM2.5 samples of households, carcinogenic PAHs are given in italic. (ND: not detected)*

	HH-1	**HH-2**	**HH-3**	**HH-4**	**HH-5**	**HH-6**	**HH-7**	**HH-8**	**HH-9**	**HH-10**
Naphthalene	6.2	5.4	7.0	6.1	6.2	5.1	4.7	5	5.8	5.6
2-methyl-naphthalene	5.6	4.5	6.2	5.8	6.2	5.4	4.6	4.7	6.5	5.3
1-methyl-naphthalene	5.5	1.9	2.4	2.5	6.1	2.2	1.9	1.85	2.7	2.3
Acenaphthylene	0.4	0.5	0.3	0.4	ND	0.4	0.6	0.25	0.3	0.7
Acenaphthene	0.2	0.1	0.6	0.3	ND	0.2	0.2	0.15	0.2	0.2
Fluorene	1.1	0.8	1.4	0.9	0.8	0.8	0.8	0.85	0.9	0.8
Phenanthrene	6.7	6.5	7.0	7.9	5.4	5.5	6.1	5.5	4.4	5.3
Anthracene	0.2	0.4	0.2	0.3	7.3	0.3	0.4	0.4	0.2	0.3
Fluoranthene	2.3	5.3	2.4	5.2	6.3	0.3	7.4	3	1.4	2.0
Pyrene	1.6	4.4	1.4	2.4	10.1	2.5	8.1	2.95	1.3	1.7
Benzo (a)anthracene	2.1	9.0	1.1	2.3	6.9	4.3	13.7	3.95	1.5	2.8
Chrysene	1.5	7.1	0.9	5.5	5.9	3.3	9.0	3.25	1.2	2.4
Benzo(b)fluoranthene	11.5	28.8	4.5	12.9	13.1	13.1	37.7	11.4	5.6	11.8
Benzo(k)fluoranthene	3.4	8.9	1.0	2.4	3.8	3.9	12.6	2.85	2.2	3.6
Benzo(e)pyrene	5.3	12.2	1.8	3.4	7.7	5.9	17.5	5.35	2.7	6.6
Benzo(a)pyrene	7.8	20.4	4.1	6.9	9.5	8.3	25.1	5.3	4.8	8.9
Dibenzo[a.h] anthracene	1.1	1.6	0.4	2.1	ND	0.5	1.7	0.75	0.3	0.8
Indeno1.2.3CD-Pyrene	4.4	11.8	2.4	4.0	5.4	4.9	14.4	2.95	2.6	5.1
Benzo(g.h.i)perylene	3.0	7.7	1.7	3.4	4.8	3.0	9.2	2.6	1.6	3.5
Total PAH	70.2	137.3	46.7	74.1	105.1	69.5	175.7	63	46.2	69.7

Fig 21 illustrates the total amount of different molecular weight PAHs in indoor $PM_{2.5}$ air of ten non-smoking households. In HH-7 and HH-2, the concentration of HMW PAHs (5 and 6-ring) was notably higher compared to others. The prevalence of five-ring PAHs was obvious in all household except HH-3. Amongst five-ring PAHs in each sample, benzo[b]fuoranthene (BbF) and benzo[a]pyrene (BaP) were represented as a most significant fraction.

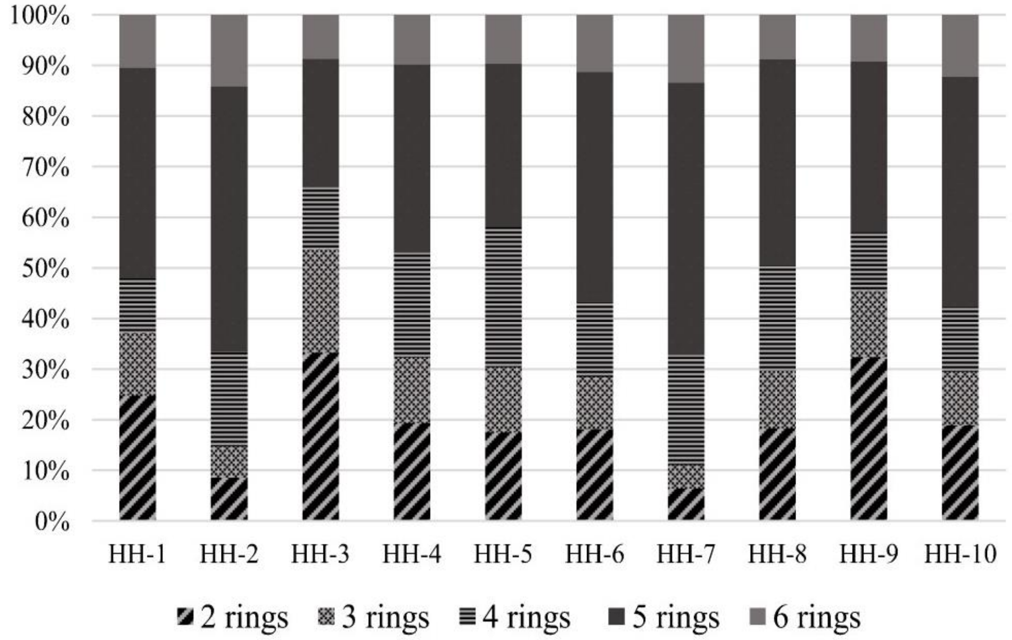

Fig 21 *Total amount of different molecular weight PAHs in households*

As shown in ***Fig 22***, the percentage contribution of different molecular weight PAHs in indoor $PM_{2.5}$ air samples in all household was dominated by 5-ring and 4-ring PAHs. The result showed that 5-ring PAHs represented 54% of total PAHs in HH-7, followed by HH-2 (52%), and HH-6 (46%), HH-10 (46%), HH-1 (42%), HH-8 (41%), HH-9 (34%), HH-5 (32%), and HH-1 (25%).

4-ring PAHs in indoor $PM_{2.5}$ in each sample were abundant as well, in decreasing order: HH-5 (28%; 17.58%), HH-7 (21.71%; 6.42%), HH-8 (20.87%; 18.33%), HH-2 (18.75%; 8.57%). In addition, 6-ring PAHs in indoor $PM_{2.5}$ in each sample showed high contribution, accounted for 8.8% to 14% of total PAHs.

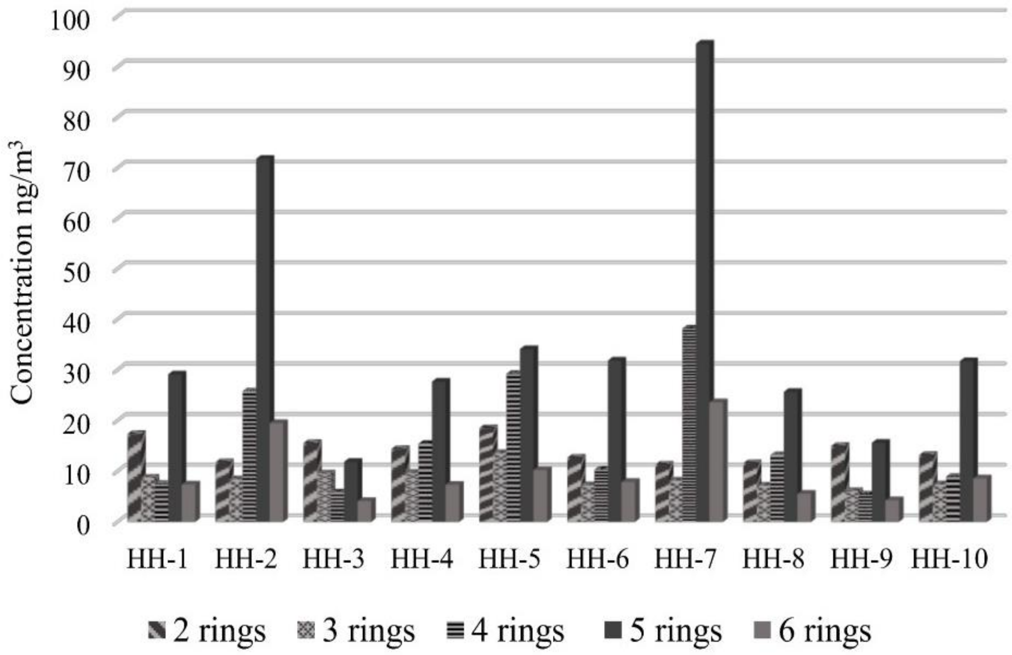

Fig 22 *Percentage contribution of different molecular weight PAHs in the households*

These results show that 5-and 6-ring PAHs were obviously the predominant fractions of total PAHs in indoor $PM_{2.5}$ of ten non-smoking households. Our findings are in concurrence with the research results of other reports (e.g., Wu *et al.*, 2015). On the one hand, Wu measured the concentration of 16 PAHs absorbed by indoor $PM_{2.5}$ air in rural households of Henan Province (China) during the heating period. The ratio of heavy molecular PAHs (4- to 6-rings) to total PAHs in $PM_{2.5}$ in winter ranged from 79 to 94.8%. On the other hand, particulate-bound PAHs in indoor PM from public spaces (shopping centres and train stations) were found to be abundantly sorbent on $PM_{2.5}$ with proportion of 59-97% to total particulate phase (Lu et al., 2008).

6.5.3 Correlation factor between outdoor $PM_{2.5}$ and indoor $PM_{2.5}$

In this study, data of ambient $PM_{2.5}$ concentrations from the National Agency of Meteorology and Environmental Monitoring were used. As far 15 air monitoring stations in Ulaanbaatar city are available (http://agaar.mn/index). There are two air stations (Misheel Expo and Nisekh) that are quite close to these ten households, HH-1, HH-2, HH-3, and HH-4 are located app. 5 – 5.3 km from these air monitoring stations. HH-5, HH-6, and HH-7 are located app. 4.4 km from Nisekh air monitoring station, whereas distance between HH-8, HH-9, and HH-10 and the Nisekh air monitoring station are app. 345 to 500 meter. Model based correlation between mean concentrations of indoor and outdoor $PM_{2.5}$ is illustrated in ***Fig 23*** and moderate relationship was found (R2 = 0.66, p <0.015).

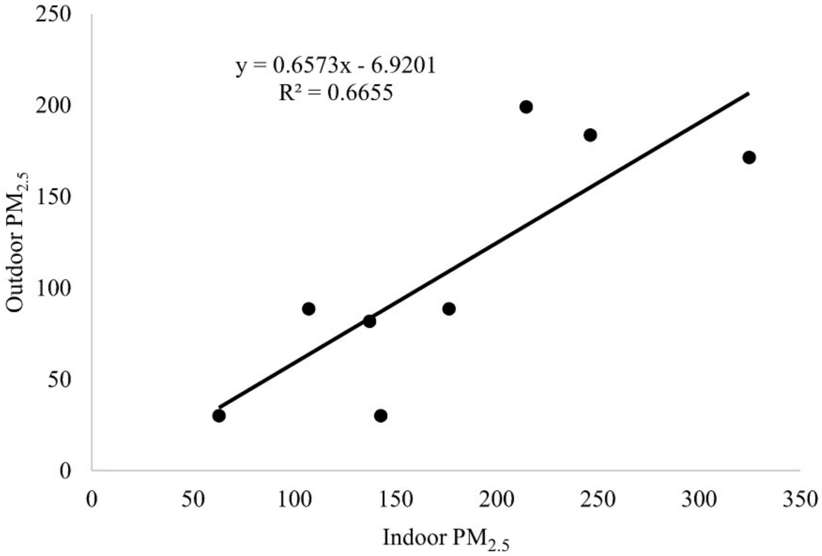

Fig 23 *Correlation between concentrations of indoor and outdoor $PM_{2.5}$ in the winter period*

One-year real-time monitoring of indoor and ambient $PM_{2.5}$ concentration in campus environment, Northeast China, was examined, significant correlation was found between indoor and outdoor $PM_{2.5}$ concentrations (Bai *et al.*, 2020). There is a case study in Guangzhou (China). Field sampling from different districts was carried out in three typical urban setting including school, office and residential areas. A good correlation was found between indoor and outdoor particle number concentration at all three sampling settings (Hu *et al.*, 2018). Byambatseren et al. (2018) concluded that indoor air quality in households located between the Ger area and residential district of Ulaanbaatar city was strongly affected by background $PM_{2.5}$ concentrations. In wintertime, coal combustion in peripheral areas where access to basic infrastructure (e.g., central heating and sewage system) is very limited, these sources represent the main contribution to both indoor and outdoor $PM_{2.5}$ (Enkhbat *et al.*, 2016).

Lim *et al.* (2018) measured indoor $PM_{2.5}$ concentration affected by conventional and improved stoves in a ger during heating season. Average 24-h $PM_{2.5}$ concentration detected in ger were significantly higher in the case of improved stoves rather than conventional ones. Accordingly, Barabad *et al.* (2018) studied the emission characteristic of particulate matter on Mongolian bituminous coal in controlled heating experiment, concluding that $PM_{2.5}$ emission would depend on the coal used in households.

6.5.4 PAH source identification

Ratio of Flt/(Flt+Pyr) in all household were above 0.5, which indicated combustion of solid fuel (Yunker *et al.*, 2002). It is important to note that in HH-5, Flt/(Flt+Pyr) ratio was 0.38, which implied petroleum input. The results from our study suggested that coal and wood was the most important source of indoor air $PM_{2.5}$ bound PAHs for all households except HH-5 and HH-7.

(BaA+Cry) ratio was greater than 0.35 for all household except HH-4, which implied combustion input and value in HH-4 was 0.29, suggesting mixed source. The cross plots of BaA/(BaA+Cry) against Flt/(Flt+Pyr) was suggesting that in case of the most of households, the main important source was coal and wood combustion (Yunker *et al.*, 2002) (***Fig 24***). In HH-5 and HH-7, the crossplots of BaA/(BaA+Cry) against Flt/(Flt+Pyr) indicated that petroleum and petroleum combustion might have been the main source. It is most interesting to note that environment of mentioned two households was very distinguishable to each other; one of the most possibility source might be the vicinity of an old mini power plant and petroleum station.

In the cross plots of Ind/(Ind+BghiP) against Flt/(Flt+Pyr) indicated that grass, wood and coal combustion for all household (except HH-5 and HH-7) was the main source (Yunker *et al.*, 2002; Anenberg *et al.*, 2013; Bonjour *et al.*, 2013). Several study have reported that coal combustion could be the main source of air particular matter emission in peripheral area during the heating period in Ulaanbaatar city (Enkhbat *et al.*, 2016; Hill *et al.*, 2017; Barabad *et al.*, 2018). In this study, most of the PAHs identified in indoor air $PM_{2.5}$ in all household implied that coal was the main indoor pollution in households which burned coal. It is most common that in ger district, Ulaanbaatar, coal is widely used as source of fuel for cooking and heating in the winter period (Battsengel *et al.*, 2021).

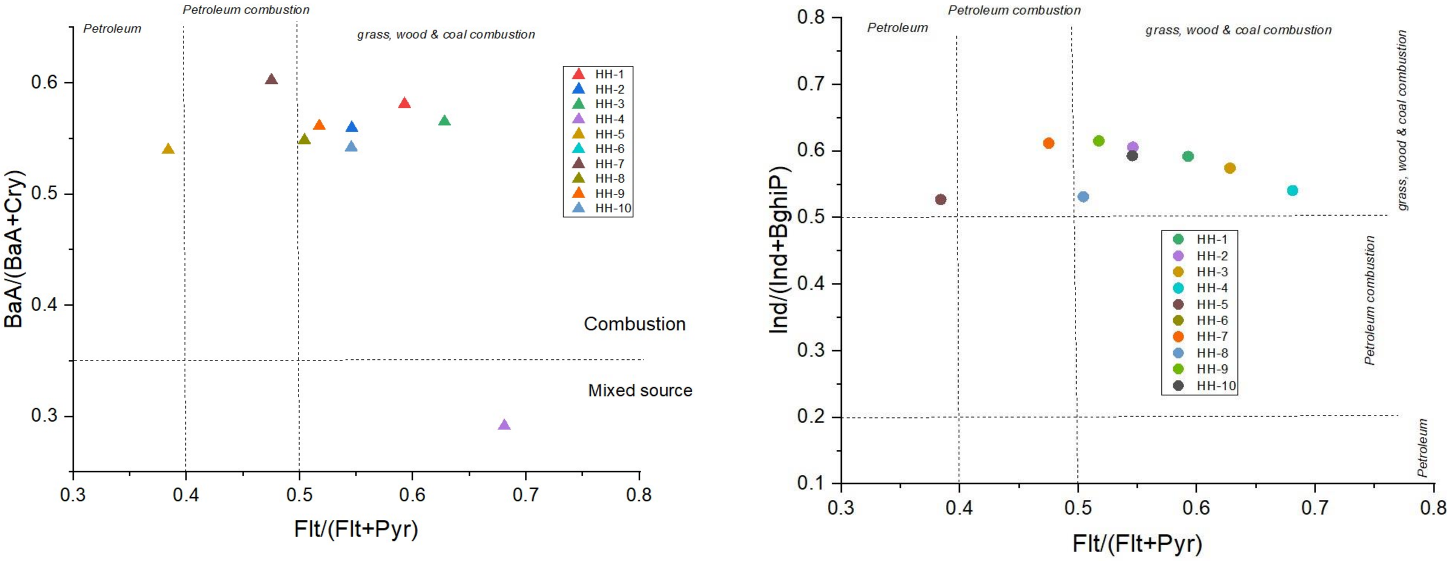

Fig 24 *a) Cross-plot of BaA/(BaA + Cry) ratio vs. Flt/(Flt + Pyr) in ten households. b) Cross-plot of Ind/(Ind + BghiP) ratio vs. Flt/ (Flt + Pyr) in ten household.*

6.5.5 Ecotoxicity assessment

In the *Vibro* ecotoxic assay, all samples were regarded as toxic based on calculated toxic units (TUs) (Chang et al., 2013) (***Fig 25***). TU values of all samples were in a wide range, TUs were rather similar in HH-2 (5.4), HH-6 (5.5) and HH-8 (4.9), whereas low TU was found in HH-10 (1.8). TU value above 4 were in HH-7 (4.0), HH-3 (4.1), HH-4 (4.2), and HH-5 (4.4) while below 4, TU values were 3.6 and 3.7 in HH-9 and HH-1 respectively. PAH content of these samples was examined, however, differences in the ecotoxicity can be partially explained by the PAH concentrations: highest percentage of HMW PAHs (5- to 6-rings) was found in HH-2 (66.5%), TU was also remarkably high in this sample (5.4). The highest amount of HMW PAHs was also detected in HH-6 (57%), TU was 5.5. In HH-5, ratio of HMW PAHs amounted to 42.2%, TU was 5.5 respectively.

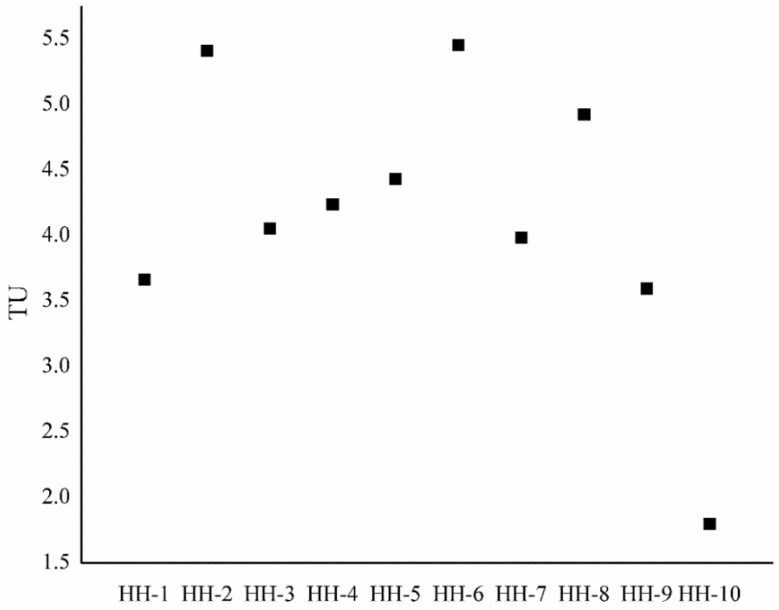

Fig 25 *The toxic unit values of an indoor* $PM_{2.5}$ *from ten household*

Evagelopoulos et al. (2009) studied the toxicity of fine and coarse particles utilizing the *V.fischeri* bioluminescence inhibition bioassay. The particles samples were collected from an urban area of Kozani in Greece between 2005 and 2006. A significant correlation was found between ecotoxicity and PAHs contents in the samples.

Alves *et al.* (2021) evaluated the ecotoxicity of indoor PM_{10} samples collected during cooking activities in modern kitchen utilizing *V. fischeri* bioluminescence inhibition bioassay. The result demonstrated that an excellent correlation was found between HMW PAHs and toxicity values ($r^2 = 0.94$), but there was no good relationship between LMW PAHs and toxic units. Isidori *et al.* (2003) also used *V.fischeri* bioassay to appraise the ecotoxicity of urban air of Caserta, South Italy, at 17 points in two seasons of the year, a correlation was found between chemicals in air and toxicity test results in most of the sites. In another study, the *V.fischeri* test was used to assess air pollutant ecotoxicity in the industrial settlement of Milazzo (Italy) on agriculture, a similar tendency appeared in this study (Triolo *et al.*, 2008).

In this study, an excellent relationship was found between indoor $PM_{2.5}$ levels and TU values (t =2.4803, df = 8, p value = 0.03809; $R^2 = 0.6593202$).

In the study of Wang *et al.* (2021), indoor and outdoor $PM_{2.5}$ samples collected from the coal-fired area in winter of Qian'an City of Tangshan City, Hebei Province (China), suggested that heavy metals such as Cr, Cu, Zn, Ni, Cd, and Pb in indoor and outdoor $PM_{2.5}$ samples were the main contributors to air pollution in coal combustion.

V.fischeri bioluminescence bioassay was applied to assess the ecotoxicity of PM_{10} air samples from Catalino (Spain), good correlation was found between ecotoxicity reading and heavy metals (Roig *et al.*, 2013). In our study, Vibrio ecotoxicity might be attributed to heavy metals as well.

Based on indoor $PM_{2.5}$ concentration, TU, and individual PAHs, the output of cluster analysis of households is presented (***Fig 26***). Three groups could be categorized.

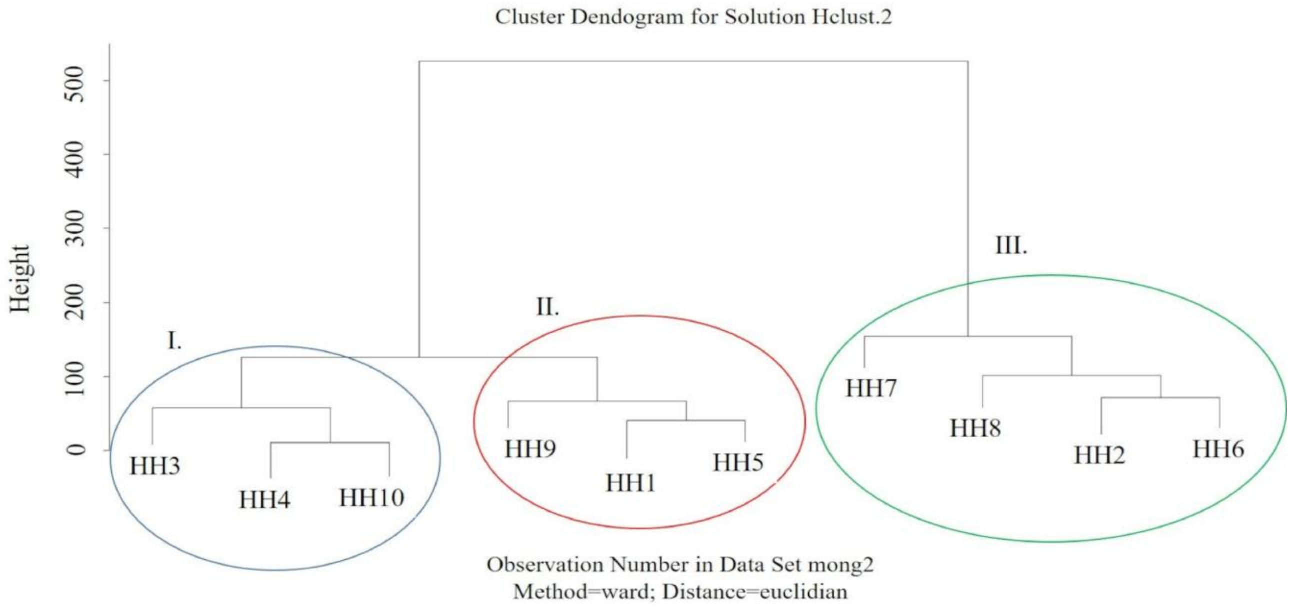

Fig 26 *Dendogram of different households based on the individual PAH concentrations, TU, and indoor* $PM_{2.5}$ *concentrations*

A significant difference was found between group I and III (*t* = - 2.2638, *df* = 21, *p*=0.0343) while no-significant differences were found between group I and II (*t* = - 1.2537, *df* = 21, *p*=0.2237) and group II and group III (*t* = - 0.70335, *df* = 21, *p*=0.4896). Considering the proximity of HH-2, HH-6, and HH-8, these households are similar to each other and having high indoor toxicity. ***Fig 27*** illustrates light emission of kinetic mode of *V. fishceri* on different indoor $PM_{2.5}$ extract samples as continuously recorded during first 30s of measurement. In our study, the kinetic diagram of indoor $PM_{2.5}$ extracted samples of households were higher than typical curves of each control samples, however, these diagram of samples are regarded as far less toxic.

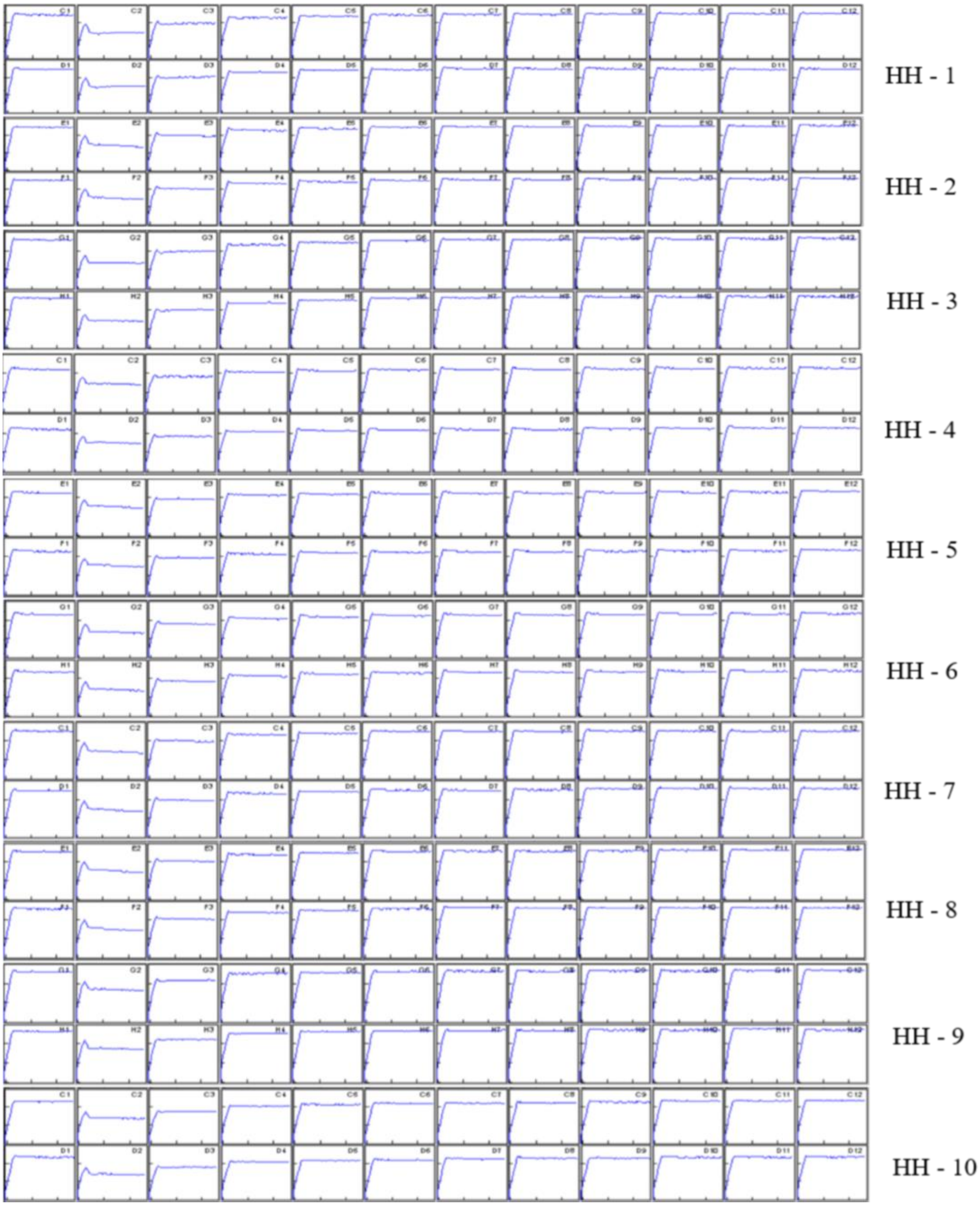

Fig 27 *The kinetic curve of the indoor PM2.5 air samples of the ten households. Each samples were measured in two replicate, C1 to D1- controls, C2 to D2 – Highest concentrations, C12 to D12 – lowest concentrations.*

7 Total summary

Airborne particulate matter samples are labour-intense and expensive to collect, whereas sometimes only low quantities of PM can be collected on a filter due to logistical challenges. Therefore, finding a trustworthy technique that requires the conditions of field collection and laboratory treatment can improve the quality of the scientific work reducing the overall costs.

For assessing the overall ecotoxicity of the sample, *V. fischeri* bioluminescence inhibition bioassay is an excellent screening tool is, due to its many benefits. It is simple, easy to use, cost-effective, rapid, and also low sample quantity is needed. On the other hand, conventional protocol is required using water extract but in scientific reports, different types of organic solvents are used to prepare an extract. This preparation has some drawbacks, which need to be improved for mimicking the naturalistic exposure route. Therefore, a proper evaluation of the nature of the solvent and the samples, as well as of the extraction procedure for direct contact test is needed.

The present study aimed to compare the different sample preparation protocols for PM ecotoxicity and to validate the whole aerosol version of the kinetic *Vibrio fischeri* bioluminescence inhibition bioassay using different samples. They were as follows: Four season PM samples in rural region; PM emission of the different illegal waste burning; PM emission of colored and colorless PET burning; indoor air samples in Mongolian households.

The main outcomes of the study were summarized as follows:

1. **Protocol comparison** – Present study was the initial research work to compare toxicity of different extraction (PM sample extracted by hexane, DCM, DMSO, water and methanol) to a direct contact test when assessing PM pollution. Based on *Vibrio* test and chemical analysis, highest PAH content and lowest EC_{50} value determined in DCM, hexane, and DMSO extracts. Whereas lowest amount of PAH and highest EC_{50} value in water extract were measured. Our result proved that the sensitivity of the direct contact test equals to that of extraction procedures using organic solvents. From these findings, direct contact test might be

interchangeable with results gained for these extract, taking into consideration the availability of laboratory development such as instruments, and protocol.

2. **Seasonal $PM_{2.5}$** – *V.fischeri bioassay* was used to assess the seasonal differences in rural PM. Considering the outcomes of the *Vibrio* test and chemical analysis, highest total PAH content and lowest EC_{20} values were detected in autumn and winter extracts. In the cold season extracts, the prevalence of HMW PAH (4, 5, and 6-ring) were observed. Lower total PAH content and highest EC_{20} values were found in warm season samples. In the summer extract, 4-ring PAHs were not detected. These findings provided vital information that can be used for the assessment of PM samples, and our result demonstrated that the luminescence bacteria test is an excellent screening tool.
3. **Domestic waste PM_{10}** – *Vibrio* test was used to give initial data on ecotoxicity profiling of PM_{10} samples originating from controlled combustion of individual domestic waste types (polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PU), oriented strand board (OSB) and rag (RAG)). In the *Vibrio* test, according to the TU values of PM_{10} emissions of different illegal waste burning, all samples were categorized as toxic except OSB sample. The lowest total PAH content and heavy metals in OSB, PE, and Rag were detected. Whereas, the PU sample showed a mixed nature, and a high amount of Cd and PAH was found. In the PP and PS samples, a highest amount of PAH was detected. Extremely high amount of Cd and Zn were detected in PVC sample.
4. **PET waste PM_{10} –** *Vibrio test* was used to assess the PM_{10} samples originating from the controlled combustion of the waste PET (colorless and colored) samples. TU values in both samples were classified as toxic. In the chemical compositions, the highest total PAH and antimony (Sb) in 2 colored and 2 colorless samples were detected. Considering PAH isomers in all samples, 4 and 5-ring PAH was predominant.
5. **Indoor $PM_{2.5}$** – It was first time to assess the indoor $PM_{2.5}$ samples in Mongolian non-smoking household without connecting central heating using the unique *V.fischeri* bioluminescence inhibition assay. According to the TU values of

indoor PM samples from non-smoking ten households of Mongolia, all samples were classified as toxic. In the chemical measurement, 5-ring PAHs were dominant in all samples, amounting to 25% - 53% of the total PAHs. This study can be also a precedent for guiding future researchers in the design and implementation of sampling programs based on the evidence herein offered.

As a general conclusion, it can be stated that the whole-aerosol test had excellent discriminative power in different environments.

8 New scientific results

- During the comparison of the direct contact test with extracts prepared using diferent solvents (organic ones and water), it was demonstrated that the sensitivity of the direct contact test based on *Vibro fischeri* bioluminescence inhibition bioassay equals the conventional ISO 11348 standard in cases when extracts are prepared with the application of 'aggressive' organic solvents such as methanol, DCM, or hexane.
- Considering the characterisation of seasonally collected rural samples, the *Vibrio fischeri* bioluminescence inhibition bioassay revealed an excellent screening tool, detecting no toxicity in the summer sample and the highest inhibition in the winter sample. Our results are in concordance with those reported in the literature.
- Assessing the toxic potential of PM emission generated under controlled burning of waste types such as polypropylene (PP), polystyrene (PS), polyurethane (PU), polyethylene (PE), polyvinyl chloride (PVC), rag and oriented strand board (OSB), results indicated that all individual domestic waste samples except OSB posed high ecotoxicity. Samples could be characterized by the chemical composition of emitted particles as 'PAH-type' and 'heavy metal-type' samples, both resulting in significant toxicity. Results also proved that illegal burning of domestic waste does pose high environmental risk.
- Toxicological and chemical analyses of PM emission generated during controlled burning of colored and colorless PET bottles proved the highly toxic nature of these emissions. A good correlation between chemical composition and ecotoxicity of the colored and colorless PET waste samples was detected, and also revealed that samples contained a significant amount of antimony, as well as 4- and 5-ring PAHs.
- Our study was the first to investigate the ecotoxicity of indoor samples collected in Ulanbaatar (Mongolia) during heating season. Results proved that all indoor PM samples in non-smoking households posed high toxicity, with 5-to-6-ring PAH of total PAH having been highly predominant in the indoor air of each

household. The study stresses the risk posed by traditional heating systems on human health.

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10 List of publications

Scientific publications related to the dissertation

- **Sainnokhoi TA**., Kováts N., Gelencsér, A., Hubai K., Teke G., Pelden P., Tserenchimed T., Erdenechimeg Z., and Galsuren J. (**2022**: Characteristics of particle - bound polycyclic aromatic hydrocarbons (PAHs) in indoor in the Southwest part of Ulaanbaatar capital, Mongolia. *Environ Monit Assess*, 194:665. **IF: 3.3 Q2.**
- Kováts, N., Hubai, K., **Sainnokhoi, TA**., Hoffer, A., Teke, G. **(2021):** Ecotoxicity testing of airborne particulate matter—comparison of sample preparation techniques for the *Vibrio fischeri* assay. *Environmental Geochemistry and Health*, 43(5) **IF: 4.9, Q1.**
- Kováts, N., Hubai, K., **Sainnokhoi**, **TA**., Eck-Varanka, B., Hoffer, A., Tóth, Á., & Teke, G. **(2022):** Ecotoxic emissions generated by illegal burning of household waste. *Chemosphere*, *298*, 134263. **IF: 8.94, Q1.**
- Kováts, N., Fábián, V., Hubai, K., Diósi, D., **Sainnokhoi, TA**., Békéssy ,Z., and Teke, G. (**2020**)**:** Seasonal Differences in Rural Particulate Matter Ecotoxicity. *Aerosol Science and Engineering*., 4(3), pp. 169–177. **Q3**.
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Scientific publications not related to the dissertation

- Hubai, K., Kovats, N., **Sainnokhoi**, **TA**., and Teke, G. **(2021):** Accumulation pattern of polycyclic aromatic hydrocarbons using Plantago lanceolata L. as passive biomonitor. *Environmental Science and Pollution Research*, 29: 7300–7311**. IF: 5.19, Q1.**
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