Air Quality, Atmosphere & Health (2026) 19:189
https://doi.org/10.1007/s11869-026-02077-3

Could foods from the Mediterranean diet act as a DNA-protective tool against the mutagenic and genotoxic effects of urban fine air particulate matter ($PM_{0.5}$)? An exploratory investigation on bacteria and human cell lines

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Received: 15 May 2026 / Accepted: 4 August 2026

Abstract
Fine particulate matter ($PM_{0.5}$) is an emerging public health concern due to its ability to penetrate deeply into tissues and induce oxidative stress–mediated damage, contributing to the development of chronic diseases. Despite increasing exposure in urban environments, its biological effects and potential mitigation strategies remain insufficiently explored. This study investigated whether selected foods typical of the Mediterranean diet (yoghurt, apples, lemons, peaches, kiwis, broccoli, onions, and tomatoes) can reduce $PM_{0.5}$-induced DNA damage. The protective effects of these foods were evaluated using the Ames test on *Salmonella typhimurium* TA98 and the comet assay on human cell lines derived from lung (A549), liver (HepG2), and colorectal (HT-29) tissues. Exposure to $PM_{0.5}$ organic extracts induced significant mutagenic and genotoxic effects. However, several foods demonstrated notable protective capacity. In bacterial assays, yoghurt, apple, onion, and tomato reduced mutagenicity by over 50%. In human cells, yoghurt and tomato (including tomato purée) showed the strongest protective effects, reducing DNA damage by up to 90% in A549 cells. Additional reductions were observed with peach, apple, and onion across different cell lines. These findings highlight the relevance of investigating $PM_{0.5}$ toxicity given its implications for public health, and suggest that components of the Mediterranean diet, particularly fermented foods and vegetables, may play a role in mitigating DNA damage induced by fine particulate matter. Further studies are warranted to elucidate the underlying mechanisms and confirm the potential protective effects in vivo.

Keywords Vegetables; fermented food · Antimutagenicity · Antigenotoxicity · Urban particulate matter · Fine particulate matter; public health

Introduction

The state of the environment exerts a significant influence on human health and well-being. Among the environmental contaminants, air pollution represents a global concern. The World Health Organisation (WHO) has identified it as the primary environmental health risk and the second most significant risk factor for non-communicable diseases (NCDs) after tobacco. The associated NCDs are heart disease, stroke, lung cancer and chronic obstructive pulmonary disease (WHO Regional Office for Europe 2019). The consequences of these diseases include the loss of healthy life years and preventable deaths. It is estimated that in 2019, outdoor air pollution was a contributing factor in 4.2 million premature deaths on a global scale (WHO 2024). Of the various pollutants, particulate matter (PM) is the most concerning due to its impact on human health (Quezada-Maldonado et al. 2021). The PM is comprised of respirable particles of varying aerodynamic diameters (from 10 µm and above) to which toxic and genotoxic compounds may be absorbed. It is evident that the most significant health effects are caused by the finest fractions of PM, indicated as $PM_{2.5}$, $PM_{0.5}$, $PM_{0.1}$, (fractions containing particles measuring $\leq$2.5, 0.5 and 0.1 µm, respectively). These particles play an important role in the pathogenesis of chronic diseases,

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Published online: 18 August 2026

as they are retained in the alveolar regions of the lungs and diffuse into the bloodstream. Consequently, they have the potential to induce inflammation, oxidative stress and blood coagulation (Quezada-Maldonado et al. 2021; Thangavel et al. 2022; Santibáñez-Andrade et al. 2023; Vilas-Boas et al. 2024). This is particularly relevant given that the vast majority of the global population is exposed to concentrations of fine PM above the health-based guideline level set by the WHO (EEA 2024; WHO 2024). In the European Union, despite a decline in exposure levels in recent years, most of the urban population continues to be exposed to levels of air pollutants that are damaging to health. In Italy, recent evidence suggests that there is still a risk of cause-specific mortality due to long-term exposure to air pollution (Nobile et al. 2024). One of the areas most affected by the issue in Italy is the province of Brescia, where in 2025 the mean annual concentration of PM_{10} was 26 μg/m^3 and that of $PM_{2.5}$ was 17 μg/m^3. In addition, 28 days exceeded the daily threshold for PM_{10} (which is established at 50 μg/m^3 according to Decree n.155 of 2010(Ministerial Decree n. 155 2010)) (Legambiente 2026).

The mechanisms that trigger the adverse health outcomes of the finest fraction of PM (above 2.5 μm), are different, strictly related to each other, and include oxidative stress, DNA damage, chromosome instability, systemic inflammation and changes in DNA methylation (Kazensky et al. 2024; Vilas-Boas et al. 2024; Macrì et al. 2025). Moreover, mounting evidence suggests that urban PM extract is carcinogenic (IARC 2015), mutagenic and genotoxic in different cell types, comprising bacteria, plant and mammalian cells as demonstrated by several studies (Ceretti et al. 2015; Bonetta et al. 2019; Zani et al. 2021; Santibáñez-Andrade et al. 2023), mainly caused through mechanisms related to the oxidative potential of the PM. In light of the fact that the complete elimination of atmospheric particulate matter has been determined to be an unrealistic objective, it is imperative to explore effective methodologies through which to mitigate the adverse impacts of this phenomenon. As outlined by Vilas-Boas and colleagues (Vilas-Boas et al. 2024), the use of antioxidant approaches to counteract PM-induced oxidative stress is a promising strategy, which has been primarily evaluated through the application of defined molecules, some of which are derived from plants.

Recognised by the United Nations Educational, Scientific and Cultural Organization (UNESCO) more than a decade ago as part of the Intangible Cultural Heritage of Humanity (UNESCO 2013), the Mediterranean diet (MD) is a scientific concept that reflects, among many other things, the traditional dietary patterns of the Mediterranean region (Martínez-González et al. 2017). Adherence to the MD has been recognised as having a positive impact on markers of DNA damage, DNA repair and telomere length (Del Bo' et al. 2019).

Among foods belonging to MD, fermented foods as yoghurts, vegetables and fruit rich in antioxidants (such as phenols, indoles, carotenoids) were studied for their cardiovascular-protective (Delarue 2022) and anticancer effects, as well as for antimutagenic activities (Caldini et al. 2005; Garcia-Gonzalez et al. 2021; Haddad et al. 2021). However, a substantial proportion of the available literature on this topic has focused on a single functional element of the foodstuff under consideration, such as a bacterial strain or a single extracted compound.

Despite the noteworthy findings of these studies, an alternative approach for exploring the protective activity of foods is proposed. This alternative approach involves the use of foods in a slightly manipulated form in order to assess their antimutagenic and antigenotoxic potential. This approach is intriguing due to the high degree to which it resembles real-life food use and the way it contributes to maintaining the complexity and synergism of the mixture.

Several methods can be employed to evaluate the mutagenicity and genotoxicity of compounds or mixtures, as well as their potential protective activity.

The Ames test is the most widely employed and recognised method for detecting mutagenicity (Large et al. 2023). The Ames test constitutes a standardised method (OECD 2020) based on the reverse mutation of *Salmonella typhimurium*. Of the many existing strains, TA98 and TA100 are the most commonly used for assessing environmental matrices (Claxton et al. 2004; Ohe et al. 2004), because of their ability to detected mutagens with different mechanism of action (frameshift mutation and base-pair substitution, respectively). The Ames test has been used to describe how high levels of antioxidants in vegetables can inhibit the effects of pesticides (Isidori et al. 2009), to evaluate the anti-mutagenicity effects of large panels of vegetable and fruit juices on the mutagenicity induced by potent food cooking-borne mutagens, as quinoline, an heterocyclic aromatic amines subgroup (Edenharder et al. 1994) or by N-nitrosamines (Ikken et al. 1999).

Among the methodologies that may be employed to assess genotoxicity, the single-cell gel electrophoresis (SCGE) or comet assay is a well-recognised and extensively utilised method. The comet assay is a sensitive short-term test that detects primary DNA damage in single eukaryotic cells and is commonly used on animal and plant cells to evaluate a wide range of substances and mixtures (Alias et al. 2023, 2024; Collins et al. 2023). This test has been recognised as a powerful tool in compound screening and has been included in toxicology batteries by regulatory authorities (EMA 2012; OECD 2016; Baird et al. 2018). Its use to assess how diet may play a protective role against DNA

instability caused by exogenous and endogenous factors is wide recognised (Wasson et al. 2008; Azqueta et al. 2020; Milić et al. 2021; Mišík et al. 2023).

A preliminary study on the antimutagenic activity of foods belonging to the Mediterranean diet has been conducted recently. The study concluded that the strongest inhibitory effect against the activity of mutagens 2-nitrofluorene, sodium azide, 2-aminofluorene was achieved through the treatment of yoghurt, followed by lemon, kiwi, peach and broccoli (Alias et al. 2025).

The aim of this study was to explore the potential application of foods associated with the Mediterranean diet in mitigating $PM_{0.5}$-induced genomic damage (i.e., point mutations and DNA breakages). The assessment was conducted by using the Ames test and the comet assay to expose *Salmonella typhimurium* and three human cell lines, respectively, to $PM_{0.5}$ organic extract in the presence of a panel of slightly-processed, commercially relevant foods, including yoghurt, apple, lemon, peach, kiwi, broccoli, onion, and tomato.

Material and methods

Mediterranean diet-associated food sample preparation

A panel of fresh, possibly organic, foodstuffs was purchased from the local market. Apple (*Malus domestica*, Golden delicious cultivar), broccoli (*Brassica oleracea*), kiwi (*Actinidia chinensis*), lemon (*Citrus limon*), onion (*Allium cepa*), peach (*Prunus persica*), and tomato (*Lycopersicon esculentum*) were washed, weighed, cut, and pressed to extract the juice using a juice extractor (MJ-L500 Slow Juicer, Panasonic). The juices obtained were centrifuged twice at 3500 rcf (30 min, 4 °C) and filtered (pore size 0.45 µm). Moreover, a commercially available yoghurt containing probiotics (*Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium lactis* strain DN-173010, as indicated by the product label) and a commercially available tomato purée (consisting in fresh tomato cooked, blended and pasteurized) were used. Yoghurt and tomato purée were weighed and centrifuged twice at 3500 rcf (30 min, 4 °C). The pH of the supernatants obtained was measured and the juices were stored in the dark at 4 °C until use or at −20 °C for longer storage.

Particulate matter sampling

The PM was collected in an area of high traffic density of Brescia, Italy (45°33′44,0"N 10°13′58,1"E) by using a high-volume air sampler with a flow rate of 1.13 m^3/min (Air Flow HVS PM10, AMS Analitica S.r.l., Pesaro, Italy). The sampler was situated at ground level throughout the winter period, enabling the collection on pre-weighed fiberglass filters of $PM_{0.5}$ (particle diameter≤0.5 µm). The duration of the PM collection was eight-ten hours per day during the working day of the sampling campaign, for a total of 29 days (18.771,40 m^3). After exposure, the filters were dried, weighed and stored at room temperature until the extraction procedure.

Organic extraction of fibreglass filters

The exposed filters were extracted by sonication using a mixture of hexane, acetone and methanol in a 1:1:1 ratio for two consecutive 30-min cycles (Christensen et al. 2005). The extract was then subjected to vacuum drying in a rotary evaporator and subsequently resuspended in dimethyl sulfoxide (DMSO) at the concentration of 2 m^3_{eq}/µL.

Salmonella/microsome mutagenicity (Ames) test

The *Salmonella*/microsome assay (Maron and Ames 1983; Mortelmans and Zeiger 2000) was performed using *S. typhimurium* strain TA98, applying the preincubation method (Thompson et al. 2005).

Ames test on PM0.5 organic extract

The mutagenicity of $PM_{0.5}$ extract was evaluated on *S. typhimurium* TA98 strain both without and with S9 mix at doses 25, 50, 75 m^3_{eq}/plate. Briefly, bacteria were mixed with the treatment in presence of PBS or S9 mix for 30 min with shaking (180 rpm) at 37 °C before the addition of molten top agar. The solution was poured onto a minimal glucose plate (1.5% agar, Vogel-Bonner medium E, 2% glucose) and incubated at 37 °C for 72 h. At the end of the incubation period, in accordance with the guidelines delineated in Mortelmans and Zeiger (Mortelmans and Zeiger 2000), signs of toxicity (thinning or absence of background lawn, presence of pinpoint non-revertant colonies) were carefully sought. The dimethyl sulfoxide (DMSO) was used as negative control. The specific positive controls were as follows: 2-nitrofluorene for the TA98-S9 (10 µg/plate), and 2-aminofluorene for TA98 + S9 (20 µg/plate). The mean number of revertants per plate (± standard deviation) was calculated and used to express the mutagenicity ratio (MR), which represents the fold-increase over the negative control value. This was calculated by dividing the mean number of revertant colonies per plate for each sample by the mean number of revertant colonies per plate in the negative control, which represents the spontaneous mutation rate.

Foods antimutagenicity assessment using Ames test

The antimutagenicity of foods was evaluated on *S. typhimurium* TA98 strain both without and with S9 mix in presence of $PM_{0.5}$ at doses 25, 50, 75 m^3_{eq}/plate. The $PM_{0.5}$ doses were chosen based on previous experimental data on the mutagenicity of winter-collected urban PM (Ceretti et al. 2015; Bonetta et al. 2019; Feretti et al. 2019). The food samples were all used at dose of 250 µL/plate, with the exception of lemon and onion, which were used at dose of 63 µL/plate, as determined in a preliminary test described in Alias et al. (Alias et al. 2025). The test was performed in accordance with the procedure described in Sect. "Ames test on PM0.5 organic extract".

The level of mutagenicity inhibition was calculated following the formula described by Tavan and colleagues (Tavan et al. 2002): $M.I.\ \% = [1 - (B/A)] \times 100$, where A was the mutagenic activity of the PM, and B represented the mutagenic activity in the presence of PM and the food. The mean of spontaneous revertants was subtracted from A and B mean revertants values. In the case in which the B term was negative, as a result of a number of revertants in the presence of PM and food lower than that observed in the negative control (spontaneous revertants), the calculated value exceeded 100. In that case, M.I.% values were cut off at 100.0%.

The inhibition capacity of each food in each experimental condition was synthetized using a scoring system of arbitrary values (AV) ranging from 4 to 0, according to the following rules:

- AV 4: inhibition at all doses of mutagen > 50%
- AV 3: inhibition at all doses of mutagen > 25%
- AV 2: inhibition at two doses of mutagen > 25%
- AV 1: inhibition at one dose of mutagen > 25%
- AV 0: inhibition < 25%

Finally, a synthetic index was calculated in order to represent the antimutagenicity capacity of each food. The index, termed "α-Muta Score %", represented the ratio between the sum of the AV assigned to each food and the maximum obtained in each experimental condition (namely, $AV_{max} = 8$) expressed as a percentage. A value of "α-Muta Score %" equal to or greater than 50% was associated to a good performance of the food, in terms of antimutagenicity activity.

Human cell cultures

Lung carcinoma A549 and hepatocellular carcinoma HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (*VWR, Milan, Italy*). Colorectal adenocarcinoma HT-29 cells were cultured in Roswell Park Memorial Institute Medium (RPMI-1640) (*Corning*). Both media were supplemented with 10% foetal bovine serum (FBS; *Sigma-Aldrich, Milan, Italy*), 2 mM L-Glutamine (*Lonza*) and antibiotics (100 U of penicillin G and 100 µg/mL of streptomycin sulphate, *Lonza*). All cell cultures were maintained at 37 °C under a humidified 5% CO_2 atmosphere. Cultures media were refreshed every two or three days during sub-culturing.

Food samples cytotoxicity assessment

To determine the highest sublethal dose of each of the nine foods of the panel for the subsequent genotoxicity assessment, A549, HepG2 and HT-29 cells were exposed to increasing doses of the food samples. Cell viability was then determined using the crystal violet dye method, according to Feoktistova et al. (Feoktistova et al. 2016). Briefly, cells were seeded in 24-well plates at a density of 5×10^4 cells/well and incubated in complete medium for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. Cells were exposed to increasing doses of each food sample (31, 63, 125, 250, 500 µL/mL) or not treated (negative control) for 24 h at 37 °C. At the end, the treatment was removed and the cells were washed twice with tap water. Then, the cells were stained with a 0.5% crystal violet solution for 20 min at room temperature. The plates were then thoroughly washed with tap water and left to air-dry overnight. The wells were then filled with methanol to dissolve the dye for 20 min at room temperature. Finally, the optical density (OD) of each well was measured at 570 nm using a plate reader (EnSight™, Perkin Elmer). The average OD of untreated cells was set to 100%. Cell survival was determined by comparing the OD of the treated cells with that of the untreated cells. The highest non-toxic dose was defined as the dose that produced a survival rate of at least 70%.

PM0.5 extract genotoxicity assessment using the single-cell electrophoresis (comet) test

A549, HepG2 and HT-29 cells were processed in the comet assay under alkaline conditions to determine the genotoxic dose of $PM_{0.5}$ extract. The experimental protocol is outlined below in accordance with the MIRCA recommendations (Møller et al. 2020). The cells were seeded in 24-well plates at a density of 5×10^4 cells/well and incubated in complete medium for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. Cells were exposed to two doses of $PM_{0.5}$ extract (6 and 12 m^3_{eq}) for 24 h at 37 °C. Negative and positive controls were performed using DMSO and methyl methanesulfonate (MMS, 200 µM for 3 h), respectively.

After treatment, cells were detached and resuspended in PBS. An aliquot of suspension (10 µL) was used to

evaluate the cellular survival using the Trypan Blue exclusion technique. The test was carried out only on samples with viability > 70%, according to the International Workshop on Genotoxicity Test Procedures (Tice et al. 2000). The remaining cell suspension was mixed to low melting agar (LMA) to final concentration of 0.6% and distributed onto agarose pre-coated slides. The slides were immersed in lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 8 mM Tris HCl, 10% DMSO, 1% Triton X-100, pH 10) at 4 °C overnight. After that, the slides were immersed in cold buffer solution (1 mM Na_2EDTA, 300 mM NaOH, pH > 13) for 20 min of unwinding, followed by 20 min of electrophoresis (300 mA, 0.8 V/cm) in an ice-bathed electrophoretic cell. After neutralization in distilled water, the agarose microgels were dehydrated by placing the slides in absolute ethanol for 5 min. Immediately before scoring, air-dried slides were stained with 75 µL of GelRed Nucleic Acid Gel Stain (Biotinum) and analysed under a fluorescence microscope (Olympus CX 41RF, Japan) connected to a system for the analysis of images (Komet v5, Kinetic Imaging Ltd) measuring the parameter tail intensity (TI) as the primary comet assay descriptor. Fifty nuclei were analysed for each slide (100 nuclei/experimental condition). The median value of the TI values recorded per slide was used as the measure of the central value of the comet scores. The results per condition were expressed as the mean TI (± SD). The statistical analysis was performed by using Student's *t*-test, where $p < 0.05$ was considered significant.

Foods antigenotoxicity assessment using the single-cell electrophoresis (comet) test

Cells were exposed to the genotoxic dose of $PM_{0.5}$ extract in absence and presence of food sample based on the results of the cytotoxicity test (described in Sect. "Food samples cytotoxicity assessment") following the same procedure described in Sect. "PM0.5 extract genotoxicity assessment using the single-cell electrophoresis (comet) test". The level of DNA damage reduction was calculated adapting the procedure described by Tavan and colleagues (Tavan et al. 2002) to the comet assay data (TI values). Each experiment was conducted in duplicate.

Results

Antimutagenicity of foods in presence of PM0.5 organic extract

The antimutagenic potential of foods against the effect of $PM_{0.5}$ extract was evaluated on the *S. typhimurium* TA98 strain, since the $PM_{0.5}$ extract did not induce mutagenic effects in other frequently used bacteria strains (e.g., TA100 strain), as already observed in previous studies on urban PM collected in Brescia (Bonetta et al. 2019; Feretti et al. 2019). The antimutagenicity activity of foods was evaluated in the presence of $PM_{0.5}$ organic extract without and with metabolic activation (± S9 mix), and expressed in terms of percentage of mutagenicity inhibition (Fig. 1). The values on mean revertants, MR and mutagenicity inhibition are reported in the Supplementary Table 1.

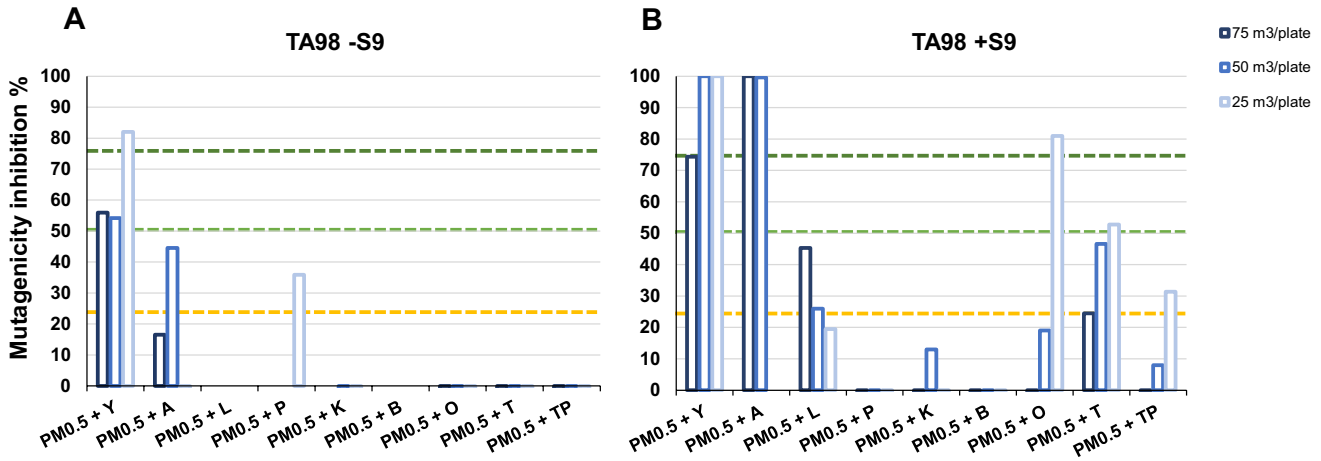

Fig. 1 Results of the Ames test on *S. typhimurium* TA98 of food samples (Y: yoghurt, A: apple; L: lemon; P: peach; K: kiwi; B: broccoli; O: onion; T: tomato; TP: tomato purèe) in presence of $PM_{0.5}$ organic extract without and with metabolic activation (± S9 mix) (panels A and B, respectively), expressed as percentage of mutagenicity inhibition. The yellow, light green and dark green dotted lines highlight the mutagenicity inhibition levels of 25%, 50% and 75%, respectively. Negative controls (mean revertants/plate ± SD): TA98-S9 from 23.0 ± 0.0 to 26.3 ± 8.5; TA98 + S9 from 30.7 ± 8.6 to 33.5 ± 16.3; Positive controls: TA98 ± S9 > 1000

In the absence of metabolic activation (Fig. 1A), a reduction in the mutagenic effect of all three doses of $PM_{0.5}$ (75, 50, 25 m^3/plate) was observed with the administration of yoghurt (56.0%, 54.2%, 82.1%, respectively). Inhibition effects were induced by apple in the presence of the 75 and 50 m^3/plate of $PM_{0.5}$ (16.5% and 44.6%, respectively) and by peach juice in the presence of the lowest $PM_{0.5}$ dose (35.9%). Conversely, lemon, kiwi, broccoli, onion, tomato and tomato purée did not inhibit the mutagenicity of $PM_{0.5}$. Some signs of toxicity were observed in the presence of $PM_{0.5}$ and three of the food samples. Specifically, all doses of $PM_{0.5}$ in the presence of lemon, the doses of 75 and 50 m^3/plate in the presence of peach, and the kiwi in presence of 75 m^3/plate of $PM_{0.5}$ extract.

In the presence of metabolic activation (Fig. 1B), the observed outcomes were different. An initial observation pertains to the complete absence of toxic effects. With regard to the reduction of the mutagenic effect of $PM_{0.5}$, the inhibition caused by yoghurt was marked against all three doses of $PM_{0.5}$ (75, 50, 25 m^3/plate), with a reduction of mutagenicity of 74.4%, 100.0%, 100.0%, respectively. The apple treatment of the highest doses of $PM_{0.5}$ (75 and 50 m^3/plate) resulted in a significant inhibition of their mutagenicity (100.0% and 93.9%, respectively). Surprisingly, the treatment of the low dose of $PM_{0.5}$ (25 m^3/plate) caused toxicity. The administration of lemon resulted in a reduction of mutagenicity for all doses of $PM_{0.5}$, although to a lesser extent (45.3%, 26.0%, and 19.5%, respectively). The onion did not inhibit the effect of 75 m^3/plate of $PM_{0.5}$, slightly reduced the mutagenicity of 50 m^3/plate of $PM_{0.5}$ (19%), while inhibited the mutagenicity of the lowest dose of PM (25 m^3/plate) of 81.0%. The tomato was found to be effective in reducing the mutagenic activity of $PM_{0.5}$ at all the three doses of 24.5%, 46.6% and 52.8%, respectively. Differently, the tomato purée reached a significant reduction only for the lowest dose of $PM_{0.5}$ (31.4%). Finally, peach, kiwi and broccoli treatments did not induce a considerable reduction (equal or greater than 25%) in the mutagenicity of $PM_{0.5}$ extract.

Consequently, the antimutagenic potency of the tested foods was found to be heterogeneously distributed, as described in Table 1. The yoghurt demonstrated the most significant antimutagenic potential, as indicated by its 100% α-Muta Score. This was followed by apple with 37.5% and by lemon and tomato, which both demonstrated an antimutagenic effect of 25.00%, and by peach, onion and tomato purée, which demonstrated a minimal antimutagenic effect with 12.50% α-Muta Score values. Neither kiwi nor broccoli juices were effective in protecting against the mutagenic effect of $PM_{0.5}$.

Antigenotoxicity of foods in presence of PM0.5 organic extract

To evaluate the protective capacity of foods against the genotoxic damage caused by $PM_{0.5}$ extract on human cells,

Table 1 Determination of the mutagenicity inhibition capacity of foods in the presence of $PM_{0.5}$ organic extract, in terms of percentage of mutagenicity inhibition, arbitrary values and α-Muta Score

	Dose of $PM_{0.5}$ (m^3_{eq}/plate)	Yoghurt		Apple		Lemon		Peach	
		M.I.%	AV	M.I.%	AV	M.I.%	AV	M.I.%	AV
TA98 -S9 + $PM_{0.5}$	75	56.0	4	16.5	1	n.d	0	n.d	1
	50	54.2		44.6		n.d		n.d	
	25	82.1		-		n.d		35.9	
TA98 + S9 + $PM_{0.5}$	75	74.4	4	**100.0**	2	45.3	2	-	0
	50	**100.0**		99.6		25.9		-	
	25	**100.0**		n.d		19.5		-	
AV_{tot_food}		8		3		2		1	
α-Muta Score %		100		37.5		25.0		12.5	

	Kiwi	Broccoli		Onion		Tomato		Tomato purée		
	M.I.%	AV	M.I.%	AV	M.I.%	AV	M.I.%	AV	M.I.%	AV	$AV_{tot\_\pm S9}$
TA98 -S9 + $PM_{0.5}$	n.d	0	-	0	-	0	-	0	-	0	6
	-		-		-		-		-		
	-		-		-		-		-		
TA98 + S9 + $PM_{0.5}$	-	0	-	0	-	1	24.5	2	-	1	12
	12.9		-		19.0		46.6		8.0		
	-		-		81.0		52.8		31.4		
AV_{tot_food}	0	0	1	2	1						
α-Muta Score %	0.0	0.0	12.5	25.0	12.5						

$PM_{0.5}$: particulate matter with aerodynamic diameter of particles ≤ 0.5 μm. The values of M.I.% in bold font are those which were determined to exceed 100%. The complete set of original data can be found in the Supplementary material Table S1

n.d.: not determined due to toxicity; -: inhibition < 0%; AV 0: inhibition < 25%; AV 1: inhibition at one dose of mutagen > 25%; AV 2: inhibition at two doses of mutagen > 25%; AV 3: inhibition at all doses of mutagen > 25%; AV 4: inhibition at all doses of mutagen > 50%

Table 2 Doses of food samples for each cell line that tested in the anti-genotoxicity assessment

Sample	Dose (µL/mL)		
	A549	HepG2	HT-29
Yoghurt	250	125	125
Apple	250	250	125
Peach	125	125	125
Lemon	Tox	Tox	125
Kiwi	Tox	Tox	Tox
Broccoli	Tox	Tox	Tox
Onion	63	63	Tox
Tomato	250	125	125
Tomato purée	250	250	125

it was necessary to determine the highest non-toxic dose of each food. The cytotoxicity of food samples was evaluated using a dye-based assay on three cell lines: lung carcinoma A549, hepatocellular carcinoma HepG2 and colorectal adenocarcinoma HT-29. In each cell line, three out of the nine tested foods resulted cytotoxic (cell viability < 70%). Specifically, lemon, kiwi and broccoli were toxic to A549 and HepG2 cells, while kiwi, broccoli and onion were toxic to HT-29 cells. For all the other foods was possible to determine the highest non-toxic dose. The results are shown in Table 2.

Following the determination of the highest non-toxic dose, it was also necessary to determine the genotoxic dose of $PM_{0.5}$. An evaluation was conducted by means of a comet test on each cell line in the presence of different doses of $PM_{0.5}$ extract. The dose–response curve demonstrates that the exposure to a dose of $PM_{0.5}$ equivalent to 12 m^3 of air, results in a significant increase in DNA damage compared to the negative control ($p < 0.05$) (Figure S1).

Subsequently, the antigenotoxicity potential of various foodstuffs was assessed in the presence of a $PM_{0.5}$ extract dose, which elicited a significant increase in DNA damage (expressed as tail intensity) in the A549, HepG2 and HT-29 cell lines (TI = 8.0 ± 2.6, TI = 6.0 ± 0.5 and TI = 3.7 ± 1.0, respectively) ($p < 0.01$) (Fig. 2A, B, C).

The A549 cells exhibited resistance to $PM_{0.5}$-induced damage in the presence of the yoghurt, peach, onion, tomato and tomato purée treatments (TI = 0.8 ± 0.4, TI = 2.3 ± 1.0, TI = 2.9 ± 0.9, TI = 2.5 ± 1.3 and TI = 1.7 ± 0.6, respectively), corresponding to a strong DNA damage reduction of 90.3%, 70.8%, 63.3%, 68.1% and 79.0%, respectively (Fig. 2A and D). Despite the preliminary evaluation of the toxicity of foods, data on exposure to $PM_{0.5}$ in presence of apple were excluded due to the low vitality (< 70%) recorded after the

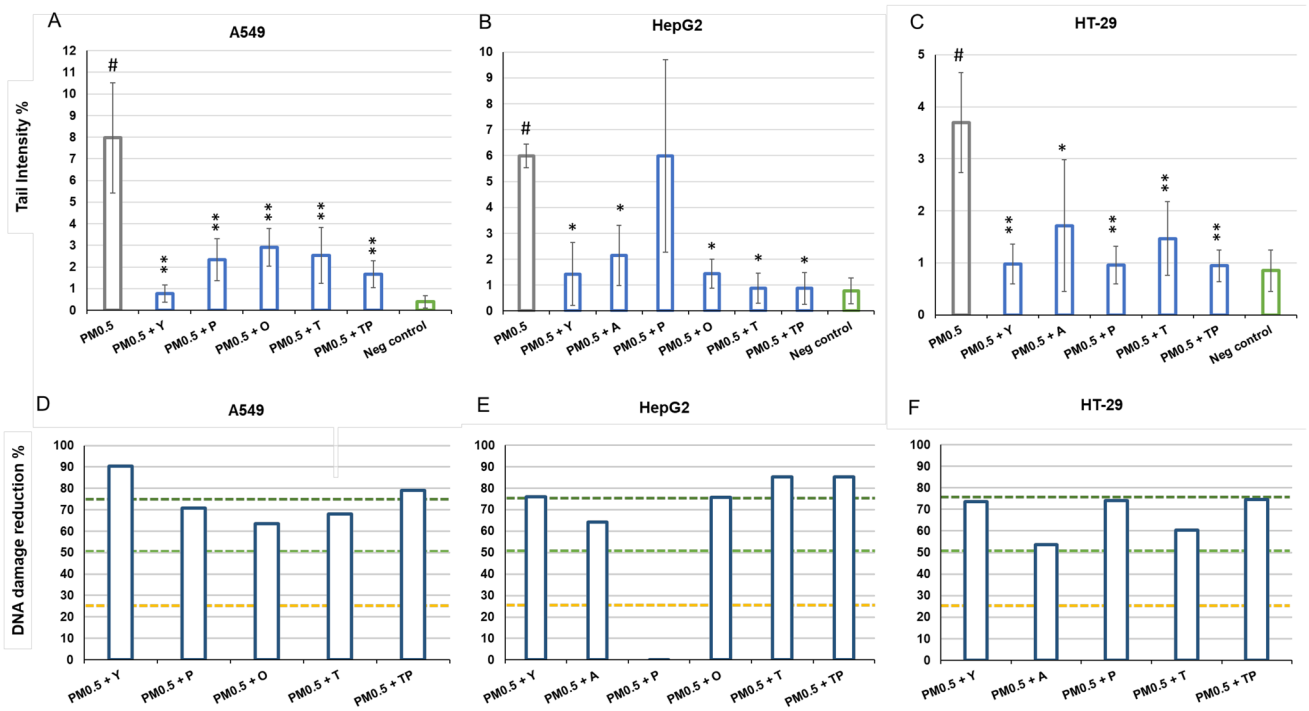

Fig. 2 Results of the comet test on A549, HepG2 and HT-29 cells treated with food samples (Y: yoghurt, A: apple; P: peach; O: onion; T: tomato; TP: tomato purèe) in presence of $PM_{0.5}$ organic extract (dose equivalent to 12 m^3 of air), expressed as tail intensity (mean ± standard deviation; data for each condition were obtained from 50 nuclei/slide in duplicate) (panels A, B and C, respectively) and percentage of DNA damage reduction (panels D, E and F, respectively). Negative controls (TI% mean ± SD): A549 0.4 ± 0.3; HepG2 0.8 ± 0.5; HT-29 0.9 ± 0.4; positive controls (TI% mean ± SD): A549 4.9 ± 0.0; HepG2 5.2 ± 0.6; HT-29 6.3 ± 1.1. #: $p < 0.01$ statistically significant *versus* negative control; *: $p < 0.05$, **: $p < 0.01$ statistically significant *versus* $PM_{0.5}$ according to Student's *t*-test. The yellow, light green and dark green dotted lines highlight the damage reduction levels of 25%, 50% and 75%, respectively

treatments, as recommended by the comet test guidelines (Tice et al. 2000).

The HepG2 cells were significantly and effectively protected by five out of the six tested foods, with a reduction in DNA damage greater than 75% induced by yoghurt, onion, tomato and tomato purèe and a mitigation of the DNA damage very near this target triggered by apple (64.1%) (Fig. 2B and E).

The HT-29 cells demonstrated a significantly decreasing in level of damage when exposed to $PM_{0.5}$ in the presence of yoghurt, apple, peach, tomato and tomato purée ($TI = 1.0 \pm 0.4$, $TI = 1.7 \pm 1.3$, $TI = 1.0 \pm 0.4$, $TI = 1.5 \pm 0.7$ and $TI = 1.0 \pm 0.3$, respectively), quantified in a reduction of DNA damage of 73.6%, 53.6%, 74.0%, 60.4% and 74.5%, respectively (Fig. 2C and F). Also in this case, despite the preliminary evaluation of the toxicity of foods, data on exposure to $PM_{0.5}$ in presence of lemon were excluded due to the low vitality (< 70%) recorded after the treatments. The data set pertaining to the comet tests are presented in the Supplementary material (Table S2).

Discussion

The present study examined the potential protective capabilities of a selection of foodstuffs from the Mediterranean diet against the mutagenic and genotoxic effects of the urban fine PM ($PM_{0.5}$) collected in Brescia, a northern Italian town, in *Salmonella typhimurium* TA98 and in human cell lines derived from pulmonary, hepatic, and colorectal tissues (A549, HepG2, and HT-29, respectively).

The most efficacious food capable of mitigating the effects of $PM_{0.5}$ was the yoghurt, which demonstrated protective capabilities in both bacteria and human cell lines with an inhibition of damage always greater than 50%. As has been previously established, the antimutagenic properties of fermented milk have been extensively documented in relation to mutagens, with this efficacy attributed to the lactic acid bacteria (LAB) (Wollowski et al. 1999; Tavan et al. 2002; Caldini et al. 2005) or of a probiotic product in its entirety (Limeiras et al. 2017). The present study, which did not incorporate LAB components in the experimental setting, corroborated earlier observations on yoghurt antimutagenicity, thereby reinforcing the hypothesis that soluble, secretory factors produced by enzymatic conversion and/or the transformed substrates and the consequent formation of bioactive or bioavailable end-products (Marco et al. 2017, 2021) support the antimutagenic activity of chemicals (Alias et al. 2025), also those associated with urban $PM_{0.5}$ exposure.

Among the panel of plant-derived samples, robust protective activity was observed following treatment with apple in both bacteria and human cells (HepG2 and HT-29). The protective potential of apple is attributable to its well-described chemical and nutraceutical characteristics. Indeed, apple possess a range of biologically active compounds, including quercetin, catechin, epicatechin, procyanidin, coumaric acid, chlorogenic acid, and gallic acid, recognized to have antihypertensive, antioxidant and anti-inflammatory properties (Luca et al. 2016; Patocka et al. 2020).

A further protective treatment was that of tomato, which was effective against the $PM_{0.5}$-induced damage in bacteria with metabolic activation and in all the tested human cells, particularly the metabolic competent cell line HepG2. The cells that were equipped with metabolic machinery, both of exogenous and endogenous origin, demonstrated a higher reduction in DNA damage. Indeed, in the presence of the metabolic activation system, the *S. typhimurium* TA98 strain exhibited an inhibition capacity of foods that was twice as significant as in its absence, as demonstrated by the sum of AV for each condition ($AV_{tot\_-S9} = 6$ and $AV_{tot\_+S9} = 12$). In a similar manner, in the HepG2 cells, five out of the six foods that were tested (83%) led to a mitigation in DNA damage of very near or above 75% in comparison to A549 and HT-29 (40% and 60% of foods with a reduction over 75%, respectively). This places the HepG2 cells at the top of a rank of responsive cells within this study. These observations are in line with those made by several studies regarding the efficacy of some fruits and vegetables in counteracting the genotoxicity of xenobiotics, such as the heterocyclic aromatic amines, in metabolically competent animal cells (Edenharder et al. 2002; Platt et al. 2010). Despite the observed differences in responsiveness, it is noteworthy that all the cellular models exhibited susceptibility to the inhibitory activity of the majority of the tested foods, thus confirming their effectiveness as appropriate toxicological models.

Further consideration is warranted with respect to the damage effector, namely the urban $PM_{0.5}$ organic extract. In relation to the experimental setting of the Ames test, it appears that the mitigation of $PM_{0.5}$ activity through the slightly manipulated foods is a more substantial challenge than the counteraction of known mutagens (as outlined by Alias and colleagues (Alias et al. 2025)). Indeed, among the food panel that was tested, only yoghurt was capable of counteracting $PM_{0.5}$ mutagenicity to a significant extent. This is presumably attributable to the markedly complex nature of the mixture (Monarca et al. 1997; Drudi et al. 2024). As is well documented, the organic preparations derived from urban PM samples are characterised by an enrichment in polycyclic aromatic hydrocarbons (PAHs) including known carcinogenic PAHs such as benzo(a)pyrene, nitro-PAHs (Bonetta et al. 2019; Feretti et al. 2019; Zani et al. 2021),

organosulfur and organonitrogen substances (Li et al. 2024) and aromatic amine compounds (Zhang et al. 2025).

Conversely, in the comet assay approach the efficacy in counteracting $PM_{0.5}$ genotoxicity has been observed following the treatment with a variety of foods, including yoghurt, peach, onion, tomato and tomato purée. This finding could be attributable to the oxidative mechanism by which the PM acts as genotoxic. As the alkaline comet test is capable of detecting a variety of damage (e.g., single- and double-strand breaks, alkali-labile sites, DNA crosslinks) (Collins et al. 2023), it is conceivable that a multitude of mechanisms could be triggered by foodstuffs, thus offering protection at the single-cell level on a widespread scale. This hypothesis is further substantiated by the observation that the Ames test on the *S. typhimurium* TA98 strain exclusively identifies one particular mechanism, namely the frameshift point mutation, lowering the chance of mitigation of the PM-induced damage at only the food that trigger this specific mechanism.

A noteworthy observation pertains to the foods that failed to counteract the deleterious effects of $PM_{0.5}$. In this study, kiwi and broccoli, which have been identified as highly active in protecting DNA in numerous other studies (Mišík et al. 2023; García-Yagüe et al. 2026), did not emerge as protective foods for two reasons. On the one hand, neither foodstuff exhibited any significant protective activity in bacteria, likely attributable to the aforementioned high complexity of the $PM_{0.5}$ organic extract mixture and the use of the sole TA98 strain. On the other hand, both exhibited high toxicity in all the cell lines examined. A comprehensive analysis of kiwi and broccoli has revealed a plethora of compounds, predominantly phenolic acids, which have been shown to exhibit significant cytotoxic properties against a range of cancer cell lines, including HepG2, HT-29, A549 and Caco-2 (Dias et al. 2020; Le et al. 2020). Two other foods, namely apple and lemon, were partially excluded from the evaluation of their antigenotoxic potential due to the low cellular vitality recorded after treatment in the presence of $PM_{0.5}$ extract, despite the use of a previously established non-toxic dose of both. One potential explanation for this phenomenon relates to the generation of toxic by-products from interactions between PM organic extract compounds and lemon or apple juice samples. It is evident that the validation of such a hypothesis would require the implementation of rigorous analytical and toxicological methodologies. This exhaustive approach was beyond the scope of the present study, but it could be contemplated for future investigations. Furthermore, in order to achieve a comprehensive evaluation of the potential mitigation effect of foods, it would be advisable to consider the use of lower doses of foods that have demonstrated toxicity (in their own raw state or in the presence of $PM_{0.5}$) and different time of exposure in subsequent studies.

The primary objective of the study was to assess the efficacy of slightly manipulated foods in mitigating a mutagenic/genotoxic insult with a view to recognising a foodstuff (or more foodstuffs) with the potential to offer a significant protective effect. This study focuses to a greater extent on the inherent complexity of the foodstuff than on purified active molecules that might be obtained via specific preparations (e.g., integrators).

It is clear that this study is an in vitro investigation on bacterial and human cells, and is subjected to the inherent limitations associated with experimental methodologies of this nature. The in vitro analysis requires the utilisation of samples that are suitable, in terms of preparations and doses, for the biological models and the experimental conditions. The procedures employed in this particular research study on the protective potential of food resulted in the samples obtained not being representative of typical human consumption patterns or the fate of food in the human body, among all the gastrointestinal digestion process. Despite the fact that a direct correlation with human health remains unattainable, the data collated in such studies establishes a foundation for further in vitro and in vivo research into the antimutagenic/antigenotoxic potential of foods, representing a pioneering endeavour to mitigate the mutagenicity/genotoxicity induced by $PM_{0.5}$ through the use of foods from the Mediterranean diet.

The present study is further limited by the absence of a detailed chemical characterization of both the PM extract and the food samples. However, it is imperative to acknowledge that the principal objective of the study was to evaluate the efficacy of foodstuffs in reducing the mutagenic and genotoxic insult, with the aim of identifying a food (or group of foods) that exhibits the potential to offer a substantial protective effect.

In the course of this encouraging trajectory, further studies will be conducted to investigate the effects of foods belonging to the Mediterranean diet on human cells in models of increasing complexity (e.g., three-dimensional cell culture, long-term exposure), even in different combinations (e.g., digested foods, contemporary presence of different foods), including in-depth chemical characterization of the tested mixture (both PM extracts and food preparations), and the integration of proteomic and metabolomic analysis to develop evidence-based dietary strategies to protect the health of urban populations exposed to high levels of air pollution.

Conclusions

The study provided promising results in the mitigation of genotoxicity induced by urban $PM_{0.5}$ by means of foods belonging to the Mediterranean diet. Furthermore, it represents a significant step forward in the knowledge of food-$PM_{0.5}$ extract interaction and provides a substantial foundation for future research on the inhibitory capacity of foods on $PM_{0.5}$-induced genotoxicity. The findings of this research could be usefully integrated in enhancing public health educational interventions to reduce the burden of air pollution-related diseases in vulnerable urban populations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11869-026-02077-3.

Acknowledgments Carlotta Alias was supported by Fondazione Umberto Veronesi. The authors would like to thank the B+LabNet Environmental Sustainability Laboratory of the Università di Brescia for allowing the use of some of the equipment required for the study.

Authors' Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Carlotta Alias, and Ilaria Zerbini. The first draft of the manuscript was written by Carlotta Alias and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by Università degli Studi di Brescia within the CRUI-CARE Agreement. The research was supported by Fondazione Umberto Veronesi and the Open Access Funding was provided by Università di Brescia within the CRUI-CARE Agreement.

Data Availability The raw data supporting the conclusions of this article will be made available by the authors on request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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