Characterizing bioaerosols in PM$_{2.5}$ in a semi-arid region experiencing desert dust events

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Introduction: Fine particulate matter (PM$_{2.5}$) pollution is a global public health concern, particularly in arid and semi-arid regions that experience dust events. Exposure to PM$_{2.5}$ has been associated with various short- and long-term health effects, which can be attributed to the composition of the fine particles. This study aimed to assess the concentration of bacteria and fungi in PM$_{2.5}$ in the air of Isfahan, Iran which experiences air pollution.

Methods: A total of thirty-nine PM$_{2.5}$ samples were collected over 1 year. Bacterial and fungal concentrations associated with PM$_{2.5}$ were measured using both culture-based method and real-time polymerase chain reaction (PCR).

Results: The average concentration of PM$_{2.5}$ was found to be 56 $\mu$g m$^{-3}$, with many samples exceeding the recommended limit of 15 $\mu$g m$^{-3}$ set by the World Health Organization for PM$_{2.5}$ in ambient air. The mean culturable bacterial and fungal concentrations were 39 and 4 CFU m$^{-3}$, respectively which showed a significant difference compared to the results obtained through real-time PCR. No significant correlation was found between the levels of bacterial and fungal cells and PM$_{2.5}$ concentrations. The dominant phyla found in PM$_{2.5}$ were Firmicutes and Actinobacteria, while Proteobacteria as Gram-negative bacteria were rarely detected.

Discussion: The findings suggest that the climate conditions in Isfahan, characterized by its semi-arid nature with low humidity content, influences the activity and viability of microbial populations, resulting in low concentrations of bioaerosols in PM$_{2.5}$. Furthermore, it seems that desert dust events do not contribute to increasing the potential health risks associated with bacterial and fungal bioaerosols in PM$_{2.5}$.

KEYWORDS
air pollution, fine particles, bioaerosols, real-time PCR, dust events, health effect

1 Introduction

Air pollution is becoming a growing concern in certain regions of the world. The expansion of cities, urbanization, and industrial development, coupled with increased traffic, are the main factors contributing to higher levels of atmospheric pollution. This rapid decline in global air quality is having a detrimental effect on human health, the economy, and ecosystems (Shammi et al., 2021; World Health Organization, 2022; Taghizadeh et al., 2023). Air pollution caused by...
Exposure to PM2.5 pollution has significant detrimental effects on human health, leading to increased rate of illness, premature death, and the development of various diseases among affected populations. There is mounting evidence suggesting that fine particulate matter in the air can contribute to the onset and exacerbation of respiratory conditions, including chronic obstructive pulmonary disease (COPD), acute respiratory infections, cardiovascular diseases, type 2 diabetes, and even cancer (Shammi et al., 2021; World Health Organization, 2022; He et al., 2023). The World Health Organization (WHO) estimates the crude death rate and the age-standardized death rate per 100,000 population for individuals aged 25 and above, attributed to mobile sources and industrial activities. However, Middle East dust events, particularly during autumn with strong wind, also contribute to the high PM (Hosseini et al., 2020; Taghizadeh et al., 2023). A study on health burden attributable to fine particulate matter in Iran found that Tehran had the highest number of attributable deaths to ambient PM2.5, followed by Mashhad and Isfahan. The crude death rate and the age-standardized death rate per 100,000 population for individuals aged 25 and above, resulting from ground-monitored PM2.5 in Isfahan, were estimated to be 102 and 122, respectively (Faridi et al., 2022).

Given that the components and effects of PM2.5 can vary by location, it is crucial to have a correct knowledge about the physiochemical and biological compositions of fine particles to determine the role of PM pollution in potential health outcomes. While the impact of PM2.5 pollution and its physiochemical compositions on human health have been extensively studied (Faridi et al., 2022; He et al., 2023; Tavangar et al., 2023), very few studies have characterized the bioaerosols in PM2.5 and the effect of dust events on this important fraction of fine particles (Alghamdi et al., 2014; Du et al., 2018a; Aziza et al., 2018; Du et al., 2018b; Lu et al., 2018; Xu et al., 2019). Some studies have focused on investigating airborne bacteria and fungi in indoor and outdoor environments, as well as the influence of PM2.5 concentration and dust events on the concentration and type of bioaerosols (Jónez et al., 2011; Mirhosseini et al., 2015; Fang et al., 2016; Goudarzi et al., 2016; Harbizadeh et al., 2019; Hosseini et al., 2020). For instance, Hosseini et al. (2020) reported an average bacterial concentration of 76 CFU m⁻³ and a concentration of fungi of 46 CFU m⁻³ in the ambient air of Isfahan. Because of the health significance of microbial composition of fine particles and the need for relevant researches in this field especially in regions impact from dust events, this study was performed to investigate the microbial characteristics of the PM2.5 in an urban area that frequently experiences PM pollution. This study is the first of its kind in a semi-arid region with dry weather, providing insights into the abundance and types of bioaerosols present in fine particles.

2 Methods

2.1 Sampling location

This study was conducted in Isfahan, Iran, from October 2018 to November 2019. Isfahan with a population of 1.6 million, is a large city in central part of Iran with a semi-arid climate which frequently experiences the PM2.5 pollution. Sampling was performed in the city center, on the roof of a two-story building (32.65226 N, 51.67320 E). This location was selected because there were no multi-story
buildings around it and it was located in a suitable distance from green spaces and other sources of aerosol generation. Figure 1 displays the sampling location.

2.2 Sampling

PM$_{2.5}$ was sampled via a PQ200 with a flow rate of 24 m$^3$ day$^{-1}$ for 24 h (from 9 a.m. to 9 a.m. of the next day). A quartz filter with the diameter of 47 mm was employed for sampling. Before the sampling, the filter was placed at 500°C for 1 h to eliminate its organic substances. Overall, 39 air samples were collected over a year (approximately one sample in 10 days). By measuring the filter’s primary weight and post-sampling secondary weight with precise scale, the PM$_{2.5}$ concentration was calculated and reported as µg m$^{-3}$. Meteorological parameters including relative humidity, temperature, UV index and wind speed during sampling period was obtained from the data of Meteorology Organization of Isfahan.

2.3 Detection of bioaerosols in PM$_{2.5}$

2.3.1 Culture-based method

To evaluate the presence of bacteria and fungi in PM$_{2.5}$, the filter was carefully placed into a sterile 15 mL centrifuge tube containing 7 mL of phosphate buffer solution with 0.02% Tween 20. The tube was then subjected to ultrasonic bath treatment for 5 min, followed by shaking for 30 min to separate the bioaerosols from the filter’s surface. To ensure complete separation, the filter washing process was repeated once more. Next, 250 µL aliquots of the resulting suspension were spread onto duplicate plates of tryptic soy agar (TSA) culture medium supplemented with nystatin for bacterial isolation, and malt extract agar (MEA) culture medium supplemented with chloramphenicol for fungal isolation. The TSA plates were incubated at 30°C for 2–3 days, while the MEA plates were incubated at room temperature for 3–5 days (Nikaeen et al., 2009). The colonies growing on the culture media were enumerated and reported as colony-forming units per cubic meter (CFU m$^{-3}$). Bacterial and fungal colonies were stained and characterized based on the colony and cell morphology.

2.3.2 Non-culture based method

The concentration of total bacteria and fungi (culturable and non-culturable cells) associated with PM$_{2.5}$ was determined using quantitative real-time PCR. DNA was extracted directly from the washed filter suspension using the freeze-thaw method followed by the application of QIAamp DNA Mini Kit (QIAGEN) as per the manufacturer’s instructions. Universal primers for the 16S rRNA and 18S rRNA (ITS) genes (Table 1) were used to amplify the DNA of bacteria and fungi, respectively, with real-time PCR. The concentration of bacterial and fungal cells was calculated by comparing the CT values of the amplified samples with the standard curve. Standard curve was plotted using the serial dilution of E. coli and Aspergillus flavus DNA (10$^5$–10$^6$ genome equivalents (GE)) as the bacterial and fungal indicators (An et al., 2006; Hospodsky et al., 2010). Briefly, the DNA of cultured Escherichia coli and A. flavus was extracted, the concentration of DNA was determined by Qubit fluorometer and the number of cells
TABLE 1 List of primers.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'→3')</th>
<th>Amplified fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eubac 7F</td>
<td>AGAGTTGTATCCGACTTACAGTTAGYTAACCTTGGTACGACTT</td>
<td>~1420</td>
</tr>
<tr>
<td>JTS1</td>
<td>TCGTATGGAACCTGGG</td>
<td>290</td>
</tr>
<tr>
<td>JTS2</td>
<td>GCCTGCGTCTCCTCTAGATGC</td>
<td></td>
</tr>
<tr>
<td>16S F</td>
<td>GAAGATATGACGGATCTAAC</td>
<td>139</td>
</tr>
<tr>
<td>16S R</td>
<td>ATTTCACACCTGACTGACTAT</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2 Concentration of PM$_{2.5}$ and bioaerosols in PM$_{2.5}$ and characteristics of meteorological parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM$_{2.5}$ (μg m$^{-3}$)</td>
<td>56 (38)</td>
<td>12</td>
<td>137</td>
</tr>
<tr>
<td>Total bacteria (CFU m$^{-3}$)</td>
<td>39 (63)</td>
<td>0</td>
<td>213</td>
</tr>
<tr>
<td>Total fungi (CFU m$^{-3}$)</td>
<td>4 (5)</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>UV index</td>
<td>5 (2)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Wind speed (m s$^{-1}$)</td>
<td>2.87 (1.29)</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21 (10.5)</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>26 (19)</td>
<td>6</td>
<td>80</td>
</tr>
</tbody>
</table>

3 Results and discussion

Fine particles, known as PM$_{2.5}$, can have detrimental effects on human health. During the sampling period, concentration of PM$_{2.5}$ ranged from of 12–137 μg m$^{-3}$, with the annual mean of 56 μg m$^{-3}$ (table 2). In the studied region, it was found that only 5% (2 out of 39) of air samples met the WHO guideline limit of 15 μg m$^{-3}$ for fine particle concentration. Additionally, it was observed that PM$_{2.5}$ concentrations exceeded 100 μg m$^{-3}$ on 9 days, further emphasizing the significance of air pollution caused by fine particles in the area. Looking at the data of air quality index (AQI) shows the condition of “Unhealthy for sensitive groups” for 4 months in 2019 due to relatively high levels of PM$_{2.5}$ (IAQI, 2023). In recent years, desert dust events have emerged as one of the major contributors to very high concentrations (over 100 μg m$^{-3}$) of the fine particles in Isfahan. The increased aridity in the Middle East, including Iran, as a result of climatic change and drought has led to more frequent dust events, exacerbating air pollution. Isfahan, being a semi-arid region with low humidity and relatively high temperatures (as shown in table 2) is affected by dust events in the Middle-East region. Alghamdi et al. (2014) reported a mean PM$_{2.5}$ concentration of 60 μg m$^{-3}$ in Saudi Arabia. However, in a study conducted in Ahvaz, Iran, a city heavily impacted by intense Middle East dust (MED) storms, the mean PM$_{2.5}$ concentration during the sampling period was reported as 85.5 μg m$^{-3}$ on regular days and 475 μg m$^{-3}$ on dusty days (Goudarzi et al., 2019). As depicted in Figure 2, high concentrations of PM$_{2.5}$ were observed during autumn followed by winter. Overall, high PM$_{2.5}$ concentrations (exceeding 100 μg m$^{-3}$) were observed during summer (two samples), autumn (5 samples) and winter (4 samples) due to dust events in summer and autumn and thermal inversion in winter. Except for dusty days, high traffic density and industrial activities are the main sources of fine particles in Isfahan throughout the year.

Biological particles, when inhaled alongside fine particles, have been linked to the development of various diseases (Shammi et al., 2021; He et al., 2023). Table 2 also presents the mean concentrations of bacterial and fungal aerosols associated with fine particles. The mean concentration of bacteria in PM$_{2.5}$ was found to be 39 CFU m$^{-3}$, while the mean concentration of fungi was 3 CFU m$^{-3}$. A study conducted on ambient bioaerosols in Isfahan also reported a mean concentration of 76 CFU m$^{-3}$ for bacteria and 46 CFU m$^{-3}$ for fungi (Hosseini et al., 2020). Bragoszewska et al. (2018) reported a range of 50–300 CFU m$^{-3}$ for bacterial aerosols in the Netherlands, which is near to our values. However, a study by Li et al. (2017) in China reported higher mean concentrations of...
bacteria (565 CFU m\(^{-3}\)) and fungi (399 CFU m\(^{-3}\)). Similarly, Gao et al. (2015) found mean concentrations of airborne bacteria and fungi in Beijing, China to be 1110 and 948 CFU m\(^{-3}\), respectively. Furthermore, a study conducted in Ahvaz, Iran, which has an arid climate but relatively high humidity (45%–60%), reported a concentration of airborne bacteria ranging from 70 to 200 CFU m\(^{-3}\) (Haribzadeh et al., 2019). The variation in bioaerosol concentrations across different regions could be attributed to variations in sampling methods, growth media, geographical locations and, most importantly, the climatic and environmental conditions. Low humidity, high temperature, and intensity of UV radiation are major weather conditions that can impact the viability and consequently concentrations of bioaerosols in the ambient air (Kim et al., 2018a). The observed low concentrations of fungi in our study may be attributed to the low humidity content of the air in Isfahan (Table 2). Fungi concentrations are known to be particularly influenced by low humidity levels. This finding is consistent with the results reported by Mirhossein et al. (2015), who observed lower concentrations of fungi in indoor environments in a semi-arid region compared to other studies. Chen et al. (2012) investigated the concentration and size distribution of airborne microorganisms in Guangzhou, China, during the summer and concluded that concentration of bacteria was considerably higher than that of fungi, which aligns with our findings. The results of Alghamdi et al. (2014) also showed a higher concentration of bacteria-associated PM\(_{2.5}\) (45–591 CFU m\(^{-3}\)) than fungi (4–28 CFU m\(^{-3}\)). As suggested by Li et al. (2017), fungal spores are greatly influenced by nutrient availability, plant growth and climatic conditions, and their low concentration can be attributed to a decrease in nutrient sources in arid climates. On the other hand, fungi generally have larger size (2.1–3.3 µm) compared to bacteria (1.1–1.2 µm) which may contribute to their association with particles larger than 2.5 µm (Kim et al., 2018a). It is also worth noting that larger bioaerosols have higher precipitation velocities, which can explain their lower counts compared to bacteria (Fang et al., 2016; Du et al., 2018b). As mentioned earlier, the climate conditions of arid and semi-arid regions, including solar radiation, low humidity and high temperature, play crucial roles in the survival as well as growth of airborne microorganisms. Since, Isfahan experiences a semi-arid climate with dry weather, our results showed a significant difference between the concentrations of bioaerosols detected by the conventional culture method and quantitative real-time PCR (\(p < 0.05\)) (Figure 3). The results show that many microbial species in the semi-arid climate of Isfahan are non-viable or in the viable but non-culturable (VBNC) state and therefore, do not detect via conventional culture methods. This finding indicates that climate conditions of arid regions may lead to VBNC state of bacteria especially Gram-negative ones. This result is supported by the high diversity of identified bacteria in studies which have used next-generation sequences for analysis of bioaerosols in ambient air (Jeon et al., 2011; Li et al., 2017; Lu et al., 2018).
sequencing analysis of microbial communities in PM$_{2.5}$ in Beijing, China showed that Proteobacteria, with an abundance of 33%, were the most frequently detected bacteria (Li et al., 2017). However, when culture methods were used to detect airborne bacteria in locations with different ecological and environmental conditions, it was found that 70%–90% of isolates were generally Gram-positive Bacilli and Cocci as well as Actinomycetes (Alghamdi et al., 2014; Goudarzi et al., 2016; Shammi et al., 2021). An exception to this trend was reported by Fang et al. (2016), who detected relatively high numbers of Pseudomonas species in culturable airborne bacteria of Hangzhou, China. It is important to note that the analysis of biological composition of PM or bioaerosol samples using high-throughput sequencing revealed a different scenario compared to cultivation-based methods, since in several cases there was a predominance of Gram-negative bacteria, mainly belonging to Alpha-, Beta- and Gamma-proteobacteria and Bacteroidetes (Du et al., 2018a; Du et al., 2018b; Lu et al., 2018; Xu et al., 2019). This difference in bacterial community composition could support the hypothesis of low resistance of Gram-negative bacteria in the air and their presence in the VBNC form, which cannot be detected by culture methods. On the other hand, the higher frequency detection of Gram-positive bacteria could be attributed to their greater resistance in arid-conditions. Gram-negative bacteria, with their thin phospholipid layer, have less stability against adverse conditions such as high temperature, low humidity and solar radiation (Kim et al., 2018b). Du et al. (2018a) analyzed the concentration of bacterial and fungal species in the air of Beijing, China, and reported a higher prevalence of bacterial species in winter. Mirhosseini et al. (2015) also reported a higher frequency of detection of Gram-positive bacteria due to their greater persistence against high temperature and dryness. Bacterial spores can survive under unfavorable environmental conditions that are intolerable for the vegetative form of bacteria (Kim et al., 2018a). Since members of Bacillus are spore formers, capable of surviving under adverse conditions such as high temperature and desiccation, therefore an increase in the frequency of Gram-positive Bacilli detection in the summer was observed (Figure 4).

During the year of sampling, black fungi had the highest frequency, while yeasts had the lowest frequency (Figure 6). The higher level of black fungi compared to other genera could be attributed to the presence of pigment, mainly melanin, in their cell membrane structure. Melanin provides protection against environmental stress and enhances their adaptation to the surrounding conditions. Melanin protects organisms from the
damaging effects of UV radiation (Lang-Yona et al., 2012) which is high in the studied region during warm seasons. In autumn, winter and summer, black fungi were the most frequent, while *Penicillium* spp. were the most identified fungi in spring (Figure 6). Meklin et al. (2007) conducted a microbiological investigation on the air of Ohio and found that *Cladosporium*, *Aspergillus* and *Penicillium* had the highest counts. According to Alghamdi et al. (2014), *Aspergillus* and *Penicillium* were identified as the predominant fungal genera present in PM$_{2.5}$ air samples from Jeddah, Saudi Arabia. The authors suggested that the prevalence of airborne *Aspergillus*, *Penicillium*, and *Alternaria* can be attributed to their adaptability to diverse substrates in all regions, regardless of weather conditions. Additionally, these fungi possess a high capability to produce and release a large number of spores into the air.

However, it is important to consider that factors such as climate conditions and geographical differences can influence the concentration and type of fungi isolated from the ambient air.

In the present study, no significant relationship was found between the increase in PM$_{2.5}$ concentrations and bacterial and fungal numbers. Similar to our results, a study conducted in Beijing, China also found no correlation between bacterial and fungal abundance in PM$_{2.5}$ and pollution levels of fine particles (Du et al., 2018b). Similarly, Alghamdi et al. (2014) reported that bacteria associated with PM$_{2.5}$ seemed to be independent of PM
mass concentrations. In contrast to our findings, some studies have identified a positive correlation between the number of bacterial colonies and PM concentrations (Jeon et al., 2011; Goudarzi et al., 2016; Shammi et al., 2021; Zhang et al., 2022). Jeon et al. (2011) reported a significant increase in airborne culturable bacterial concentration and a change in bacterial community structure during Asian dust events, with the mean concentration of culturable bacterial cells associated with PM$_{10}$ on dusty days being seven times higher than that on normal days. Li et al. (2017) also evaluated the concentration and size of bioaerosol particles in the air of Qingdao, China and reported an increase in bioaerosols concentration during dust storms. A study conducted in Ahvaz, Iran also showed an increase in airborne bacterial concentrations during dust event days compared to normal days with concentrations being three times higher (Goudarzi et al., 2016). This difference across different regions could be attributed to variations in sampling methods (bioaerosol alone or bioaerosol in PM), geographical locations and, most importantly, the climatic and environmental conditions. However, according to table 3, high concentrations of PM$_{2.5}$ could influence the predominant bacterial species associated with PM$_{2.5}$. Bacillus spp., known for their high resistance, were more frequently identified on dusty days, while Staphylococcus spp. were not detected (Table 3). It has been reported that Bacillus species were more abundant in dusty days (almost 23.8%) than the non-dust days (Shammi et al., 2021). Certain microorganisms in the air can only survive in clean climatic conditions due to their cell structure. As PM$_{2.5}$ concentration rises, both organic and non-organic compounds such as sulfate, nitrate and ammonia also increase, which can promote the metabolism and growth of some microorganisms (Madhwal et al., 2020; Wei et al., 2020). However, a further increase in particle concentration can result in the presence of chemical and toxic compounds within fine particles, which may inhibit the growth of sensitive microorganisms. Therefore, only microorganisms that can adapt to harsh conditions can survive. Bacilli and Actinomycetes are capable of surviving at high particle concentrations due to their cellular characteristics (Wei et al., 2020).

In the present study, several opportunistic pathogens were also identified. Staphylococcus epidermidis, a coagulase-negative Staphylococci, was identified and is known to cause 75% of infections in this category. Pathogenic S. equorum and Staphylococcus gallinarum species were also identified (Qin et al., 2019). Streptomyces species, typically found in soil flora, have recently been identified as immune system suppressants which cause systemic diseases (Xu et al., 2019). Saccharomonospora spp. can also cause respiratory system infections. Nocardia dassonvillei, an opportunistic pathogen, is known to causes infections in the nose, lung, biliary tract and skin (Lu et al., 2018). However, it is important to note that the concentrations of these pathogenic airborne bacteria were relatively low in the present study, making it difficult to assess their adverse effects on human health. Further research is needed to understand the potential risks associated with the presence of these opportunistic pathogens in the air.

### 4 Conclusion

The concentration of PM$_{2.5}$ in a significant number of ambient air samples in Isfahan exceeded the recommended value by the WHO. However, the concentrations of bacteria and fungi in PM$_{2.5}$ were lower than those reported in similar studies, and there was no correlation with PM$_{2.5}$ concentrations. This could be attributed to the warm and arid climatic conditions in Isfahan, which may contribute to the lower concentration of airborne microorganisms. Additionally, the results of molecular examination revealed much higher concentrations of bacteria and fungi compared to the conventional culture methods, indicating the potential presence of VBNC bioaerosols. Although the molecular identification of isolated bacteria showed the presence of some pathogenic bacteria in the urban air, it appears that air pollution based on the elevated PM$_{2.5}$ concentrations does not

### Table 3 Comparison of bacterial species identified in non-dust and dust days.

<table>
<thead>
<tr>
<th>PM$_{2.5}$ (µg m$^{-3}$)</th>
<th>Cocci</th>
<th>Bacilli</th>
<th>Actinomycetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25</td>
<td>Staphylococcus epidermidis&lt;br&gt;Staphylococcus gallinarum</td>
<td>Bacillus safensis&lt;br&gt;Ornithinimicrobium sp.</td>
<td>Saccharomonospora sp.</td>
</tr>
<tr>
<td>100–150</td>
<td>-</td>
<td>Bacillus paralicheniformis&lt;br&gt;Bacillus megaterium</td>
<td>Kocuria sp.</td>
</tr>
</tbody>
</table>
have health impacts in terms of bacterial and fungal bioaerosols. Further studies are required to determine the chemical properties of these particles and their health impacts.

**Data availability statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

**Author contributions**

MN: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing—original draft, Writing—review and editing. HK: Formal Analysis, Investigation, Writing—original draft, Writing—review and editing. ZS: Data curation, Investigation, Methodology, Writing—review and editing. YH: Methodology, Writing—review and editing.

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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