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Insights into the interaction between mineral formation and heavy metals immobilization, mediated by *Virgibacillus* exopolymeric substances

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ABSTRACT

Heavy metal pollution poses significant risks to both the environment and human health due to their toxicity, long residence times, and their ability to bioaccumulate and bio magnify across the food chain. To address this issue, microbial biomineralization has emerged as a promising approach to the bio-removal of heavy metals through immobilization. This process is facilitated by extracellular polymeric substances (EPS), which also play a crucial role in mediating mineral formation. In this study, the interactions between several selected heavy metals (Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}), EPS, and mineral formation were investigated using two mineral-forming *Virgibacillus* strains isolated from the Qatari sabkhas, which are known to be suitable sites for the formation of biominerals. An additional non-mineral-forming *Virgibacillus* strain isolated from the Dukhan oil waste dumpsite was also investigated. Cd^{2+} and Zn^{2+} were to inhibit mineral formation, likely due to competition with Ca^{2+} and Mg^{2+} ions during biomineralization. However, exposure to Ni^{2+} or Cu^{2+} resulted in changes in the FTIR spectra of the EPS, suggesting the presence of specific functional group bindings within the EPS matrix. The EPS produced by each strain was also directly associated with their efficiency (%) at removing heavy metals. Notably, the EPS from *Virgibacillus halodenitrificans* Z4D1, the non-mineral-forming strain, exhibited the highest heavy metal removal efficiency of 31.7 % for Zn^{2+}. These findings reveal that EPS do not only affect the biomineralization process but also that the functional groups in EPS have a direct effect on the immobilization of several heavy metals. Conditions that are not suitable for mineral formation may instead be appropriate for the removal of specific heavy metals.

1. Introduction

Heavy metal pollution poses a significant global threat to the environment and human health due to the high toxicity of heavy metal ions, their ability to bioaccumulate and bio-magnify in organisms, and their long residence times (Ali et al., 2019). The protection of ecosystems necessitates the removal of these metal pollutants from contaminated areas (Kumar et al., 2021). However,

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conventional physicochemical methods of metal removal, such as precipitation, electroplating, and ion exchange, are costly, inefficient at low concentrations, and can generate hazardous byproducts (Nur-E-Alam et al., 2020). There is, therefore, a growing emphasis on the development of eco-friendly and cost-effective alternatives for tackling heavy metal contamination (Elbasiouny et al., 2021; Gaur et al., 2021). In recent years, the bio-removal of heavy metals using bacterial biomass has received a considerable amount of interest from researchers (Gupta et al., 2021; Pham et al., 2022; Priya et al., 2022; Priyadarshanee and Das, 2021). Microbial biomineralization—a complex process involving the formation of biominerals—has been identified as a promising approach. In particular, the precipitation of calcium carbonate minerals has received a significant amount of attention due to its broad technological applications. Heavy metals that have ionic radii that are similar to calcium, such as Cu^{2+}, Cd^{2+}, and Zn^{2+}, can be incorporated into the $CaCO_3$ crystal lattice through the substitution of calcium ions (Kim et al., 2021), preventing the movement of these heavy metals in contaminated soils. Consequently, the use of microbial-induced carbonate precipitation (MICP) has been proposed for various applications beyond soil bioremediation, such as oil recovery, CO_2 capture and storage, the self-healing of concrete structures, and soil stabilization (Choi et al., 2020; Qian et al., 2022; Sohail et al., 2022).

The harsh climatic conditions of the Arabian Gulf region contribute to the intense weathering of pollutants, altering their structure, bioavailability, toxicity, and interaction with microbial cells (Al Disi et al., 2022a; Attar et al., 2017). Microorganisms must adapt to these harsh conditions, consequently enhancing their potential for pollutant biodegradation and bioavailability by synthesizing specific organic molecules to cope with the increased recalcitrance of the altered pollutant structures (Al Disi et al., 2017b; Fouad et al., 2023). Extracellular polymeric substances (EPS) are metabolic products produced by microbial cells; these compounds are composed of various organic substances, including polysaccharides, proteins, extracellular DNA, and lipids. (Angelin and Kavitha, 2020; Costa et al., 2018). EPS matrices are used for a variety of functions, including the formation of a protective layer around cells, shielding them against harsh external conditions. EPS also contains several organic compounds that facilitate the inclusion of Mg in carbonate minerals, providing insights into their role in the biomineralization process (Al Disi et al., 2017a). The use of microbial biofilms for the absorption of heavy metals through the secretion of EPS has been used in several treatment processes (Wu et al., 2021). Previous research has demonstrated that certain functional groups in EPS, such as carboxyl, hydroxyl, phosphorus-containing, and amino groups, provide adsorption sites for heavy metals and other organic toxic contaminants (Gupta and Diwan, 2017; Liu et al., 2023; Pagliaccia et al., 2022). Consequently, the use of EPS in heavy metal ion absorption applications has become increasingly popular.

The toxic nature of heavy metals stems from their ability to disrupt essential cellular processes by binding to enzyme functional groups, inhibiting key biochemical pathways, and generating reactive oxygen species (ROS) that damage cellular structures. Numerous studies have investigated the mechanisms behind bacterial responses to heavy metal stress, highlighting the importance of metal transporters, detoxification systems, and metal-binding proteins in mitigating their harmful effects (Li et al., 2022; Mathivanan et al., 2021). Understanding the impact of heavy metals on bacterial growth is crucial, not only for environmental and ecological considerations but also for the development of strategies to combat metal pollution and enhance bioremediation processes.

This study aims to investigate the effect of several heavy metals on mineral formation and EPS production by endogenous bacterial strains isolated from Qatari soils and sabkha environments. Cd^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} were selected due to their abundance in sites polluted by oil. In addition, we aim to explore the potential for the bio-removal of heavy metals through the processes of biomineralization and EPS secretion. Hence, the main contribution of this work is the establishment of the relationship between the growth of strains originating in sabkha environments, their tolerance to heavy metals, their potential to form minerals, as well as the role of their EPS in the removal of heavy metals. This study would provide a better understanding of the role of bacteria in the alleviation of heavy metal pollution, particularly due to biomineralization processes.

2. Material and methods

2.1. Bacterial strains and culture media

Three *Virgibacillus* bacterial strains were used in this study. Two of the three strains, *Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2, are mineral-forming strains previously isolated from the sediments of Dohat Faishakh Sabkha in Qatar (Al Disi et al., 2017a), while the remaining strain, *Virgibacillus halodenitrificans* Z4D1, is a non-mineral-forming bacterium isolated from the Dukhan oil wastes dumpsite (Al-Kaabi et al., 2018). An artificial growth medium named MD1 was used to grow the bacterial strains selected in this study to investigate their potential for mineral formation as described by Al Disi (2017a). MD1 is composed of (w/v): 1 % yeast extract, 0.5 % peptone, and 3.5 % sodium chloride, supplemented with a solution containing a molar ratio of 6:1 Mg^{2+}: Ca^{2+}. The solid MD1 medium was prepared by the addition of 16 g/l agar.

2.2. Tolerance of the bacterial strains to heavy metals

An MD1 medium was employed to assess the toxicity of individual heavy metals by supplementing it with escalating concentrations (ranging from 0 to 5 mM) of the following heavy metal salts: copper chloride ($CuCl_2$), nickel chloride ($NiCl_2$), zinc chloride ($ZnCl_2$), and cadmium chloride ($CdCl_2$). Heavy metal stock solutions were prepared using anhydrous, powder, 99.99 % trace metals basis, Sigma Aldrich. The culture media were inoculated with the strains of interest, starting with an initial OD_{600} of 0.15. The minimum inhibitory concentration (MIC) was determined after an incubation period of 72 h by identifying the concentration at which growth reduction exceeded 90 % (Al Disi et al., 2022a; Asadpoor et al., 2021). The experiments were conducted in triplicate at each concentration to ensure accuracy and reliability.

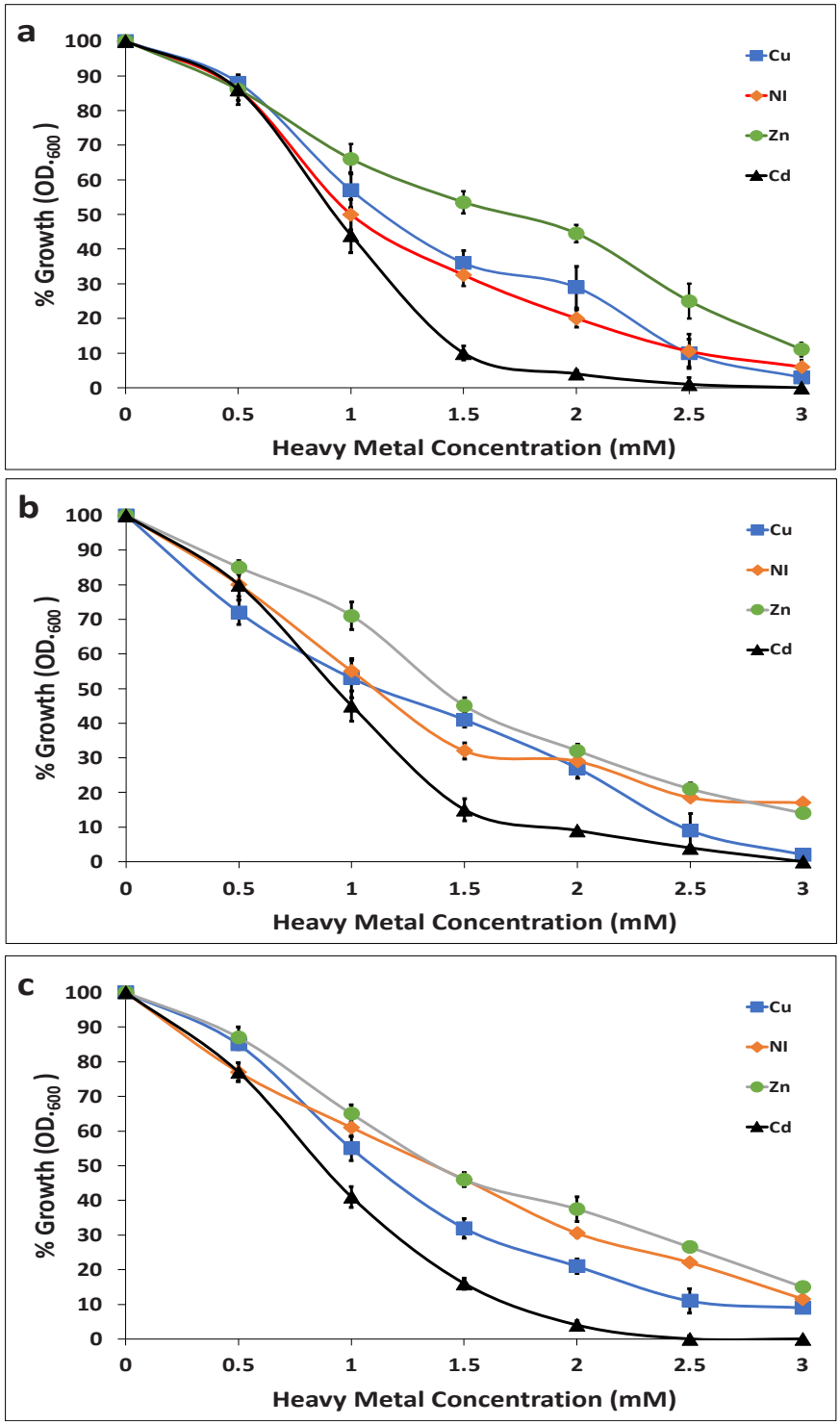

Fig. 1. Growth of a) *Virgibacillus martsimiure* DF1, b) *Virgibacillus* sp. DF2, and c) *Virgibacillus halodenitrificans* Z4D1. Each strain was grown in media supplemented with escalating heavy metal concentrations. The measurements presented here were collected after 3 days of incubation. 100 % represents the growth of the control, which was measured in the absence of heavy metals.

2.3. Evaluation of bacterial growth at a heavy metal concentration of 1 mM

The inoculum of each strain was separately prepared in an MD1 medium via overnight incubation in a shaker set at 30 °C and 200 rpm by inoculating 15 ml of medium with a separate colony from an overnight culture in 1.5 % agar-MD1 plates. The volume of the inoculum was calculated to ensure an initial OD_{600} of 0.15 in the culture. The initial concentration of heavy metals in the culture was 1 mM. The cultures were then incubated at 30 °C in a shaker operating at 200 rpm. The colony-forming units (CFU) method was employed to determine the number of viable bacterial cells in the liquid cultures. This involved calculating the number of distinct colonies formed in 100 µL of serial dilutions spread on LB plates after overnight incubation at 30 °C. The obtained CFU values were then used to construct the growth kinetics curves for each of the studied strains in the presence of individual heavy metals; these were compared to the control group incubations that were performed without the addition of any of the heavy metals. All cultures were prepared in triplicate.

2.4. EPS production and extraction

After a five-day incubation period, the cells were separated from each broth by centrifugation at 15,000 rpm for 15 min at 4 °C. The resulting supernatant underwent two additional rounds of centrifugation and was then precipitated by adding two volumes of chilled 95 % (v/v) ethanol to one volume of supernatant. The resulting precipitates were resuspended in distilled water and subjected to additional dialysis to remove low molecular weight compounds. Dialysis was performed at 4 °C against distilled water for 24 h using a cellulose membrane with a cutoff of 12 kDa. The EPS obtained were freeze-dried and stored at −20 °C for subsequent analysis (Al Disi et al., 2019).

2.5. Impact of heavy metals on the ability of the Virgibacillus strains to form minerals

The mineral-forming strains, *Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2, along with the non-mineral-forming strain, *Virgibacillus halodenitrificans* Z4D1, were cultured on MD1 solid medium plates supplemented with 1 mM of each of the heavy metals of interest. These MD1-HM culture plates were then incubated for 30 d at 30 °C. Periodic observations were made using an optical microscope to monitor the formation of mineral crystals. Immediately following the incubation period, the mineral crystals in each culture were carefully collected using a scalpel and transferred into a 50 ml centrifuge tube. The samples were washed three times in distilled water to remove any salts and impurities. The minerals were left to dry at 40 °C before being subjected to Scanning Electron Microscopy/Energy-Dispersive X-ray Spectroscopy (SEM/EDX) analysis and SEM mapping.

2.6. Scanning electron microscopy/energy-dispersive x-ray spectroscopy

SEM images were collected, and SEM mapping was conducted using a Nova Nano Scanning Electron Microscope, equipped with a Bruker EDX detector. The images were captured at a 5 nm resolution and a 200,000× magnification. EDX analysis and SEM mapping were conducted following the procedures described in "ASTM standard method E1508–12a" (Das et al., 2014). The EDX analysis was conducted with a spot size of 5, an accelerating voltage of 20 kV, and an error rate of 4 %.

2.7. Elemental analysis using inductively coupled plasma-optical emission spectrometry

Elemental analysis was performed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) on an Optima 7300 DV instrument (PerkinElmer). ICP-OES analysis was conducted on the freeze-dried EPS samples in triplicates. The samples were initially acid digested using a combination of the following strong acids: 6 ml of concentrated HCl, 2 ml of concentrated HNO_3, and 2 ml of concentrated HF. The sample digestions were carried out using a Microwave Digestion System MARS 6 (CEM Corporation).

3. Results and discussion

3.1. Tolerance of the bacterial strains to the toxicity of heavy metals

The growth and tolerance of the bacterial isolates towards a range of heavy metals (Cd^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}) were investigated in MD1 culture media. Fig. 1 shows that the bacterial strains investigated in this study exhibited remarkable resilience to heavy metals, being tolerant to heavy metal concentrations as high as 2.5 mM for the heavy metals tested. However, it was evident that Cd^{2+} exhibited the highest level of toxicity among all the strains with an MIC of 1.5 mM.

These results highlight the tolerance of the bacterial isolates to heavy metal exposure, which has potential implications for their environmental and industrial applications. Further research in this area could provide insights into the mechanisms underpinning the observed tolerances and toxicity variations of specific heavy metals to the bacterial isolates. Which would contribute to our understanding of bacterial responses to heavy metal stress. The impact of heavy metals on the growth of the examined strains was assessed by measuring the OD_{600} values after a period of incubation, which is commonly used in similar studies (Al Disi et al., 2022b; Goff et al., 2023; Zhang et al., 2022). Furthermore, relatively tolerant microorganisms should be examined not only in terms of the accumulation of cells within the culture but also in terms of their behavior during growth, especially since heavy metals are known to exert a significant impact on bacterial growth and physiology (Campillo-Cora et al., 2023). Bacteria have been known to be adversely affected

when exposed to high levels of heavy metals, such as experiencing reduced growth rates, alterations in their cellular metabolism, and increased oxidative stress.

This study evaluated the effects of heavy metals on the growth of bacterial strains by measuring the potential viability of the cells based on the growth curves established by the CFU counts across five days of incubation, during which the growing cells were exposed to 1 mM of heavy metals (Fig. 2). When cultivated in MD1 liquid media supplemented with Cu^{2+}, the growth of the mineral-forming strains (*Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2) was not inhibited by the presence of heavy metals; indeed, *Virgibacillus martsimiure* DF1 exhibited enhanced growth during the incubation period. A decrease in the viable cell counts was observed on day 5 of

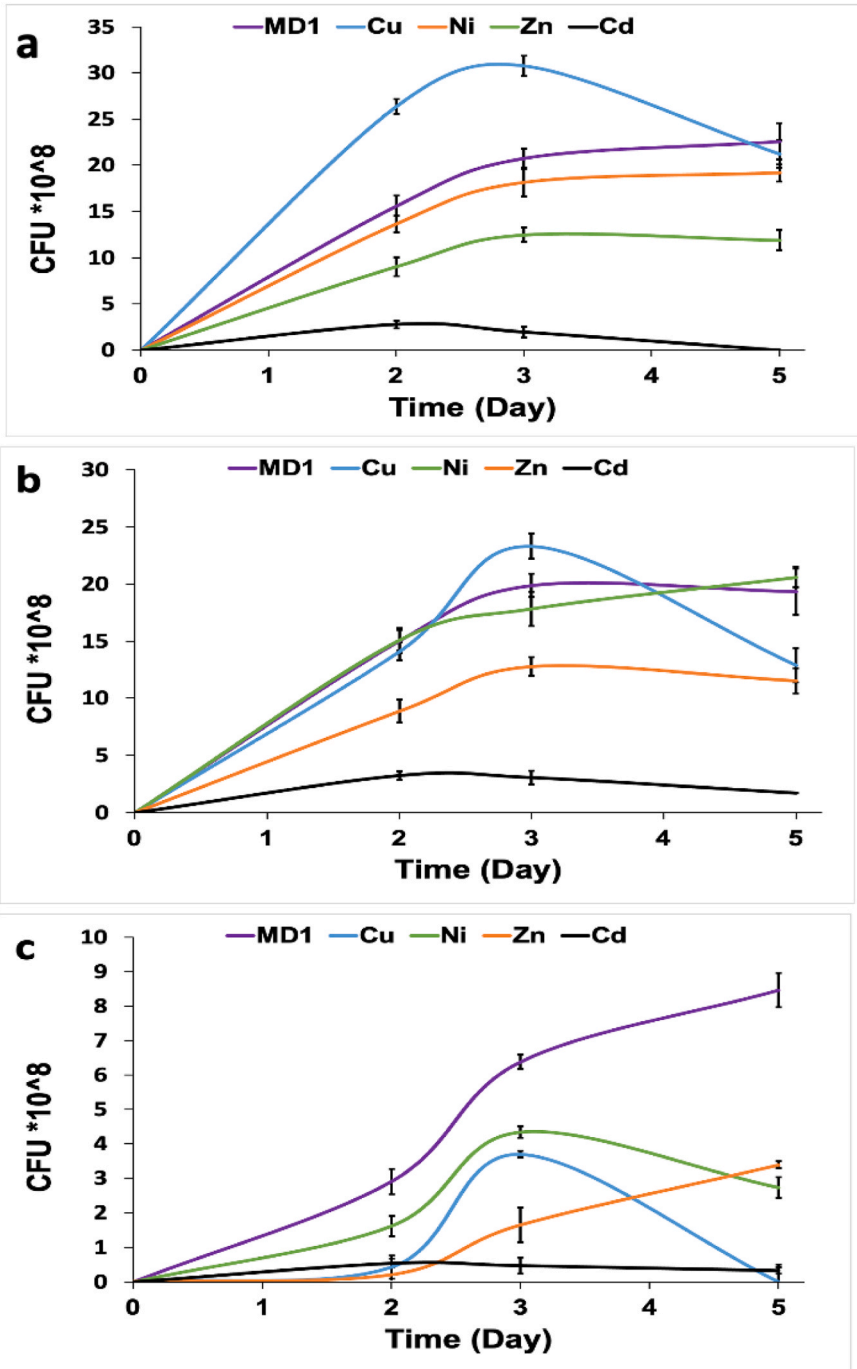

Fig. 2. Growth curves of a) *Virgibacillus martsimiure* DF1, b) *Virgibacillus* sp. DF2, and c) *Virgibacillus halodenitrificans* Z4D1 over an incubation period of five days in the presence of 1 mM of heavy metals.

Table 1
Results of mineral formation on MD1 media as well as MD1 media supplemented with heavy metals.

Mineral Formation					
Bacterial Strain/Media	**MD1**	**MD1-Cu**	**MD1-Ni**	**MD1-Cd**	**MD1-Zn**
Virgibacillus martsimiure DF1	Yes	Yes	Yes	No	No
Virgibacillus sp. DF2	Yes	Yes	Yes	No	No
Virgibacillus halodenitrificans Z4D1	No	No	No	No	No

incubation. However, the presence of copper clearly inhibited the growth of the non-mineral forming strain, *Virgibacillus halodenitrificans* Z4D1. In addition, the inhibitory effect of Ni and Zn on *Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2 was relatively low compared to *Virgibacillus halodenitrificans* Z4D1.

Figs. 1 and 2 compare the differences between the three bacterial strains to show that each strain has different tolerances to each of the different heavy metals. Copper plays a crucial role in the growth and development of bacteria, serving as both an essential micronutrient and a potential stressor. Functioning as an essential trace element, copper participates in vital enzymatic reactions and redox processes within bacterial cells. This includes electron transport chains and superoxide dismutase activity, which collectively contribute help maintaining cellular homeostasis and protecting against oxidative stress. The beneficial role of copper in cell growth could explain the enhanced growth of *Virgibacillus martsimiure* DF1. However, copper can also exhibit antimicrobial properties, interfering with cellular processes and causing damage to microbial membranes and DNA (Frei et al., 2023). Not only can the presence of copper in growth media influence bacterial growth rates and cell viability but it can also affect biofilm formation (Gomes et al., 2020). Understanding the intricate balance between the beneficial and harmful effects of copper on bacteria growth is crucial for a variety of fields, including biotechnology, environmental microbiology, and medicine.

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3.2. Investigation of the impact of heavy metals on mineral formation

All the studied bacterial strains were able to grow in the culture media containing 1 mM of each heavy metal (Cu^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}). *Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2 formed minerals in MD1 media, as well as MD1 media supplemented with Cu^{2+} and Ni^{2+}. However, quantitative correlation between the growth rates of individual bacterial cell and the mineral formation

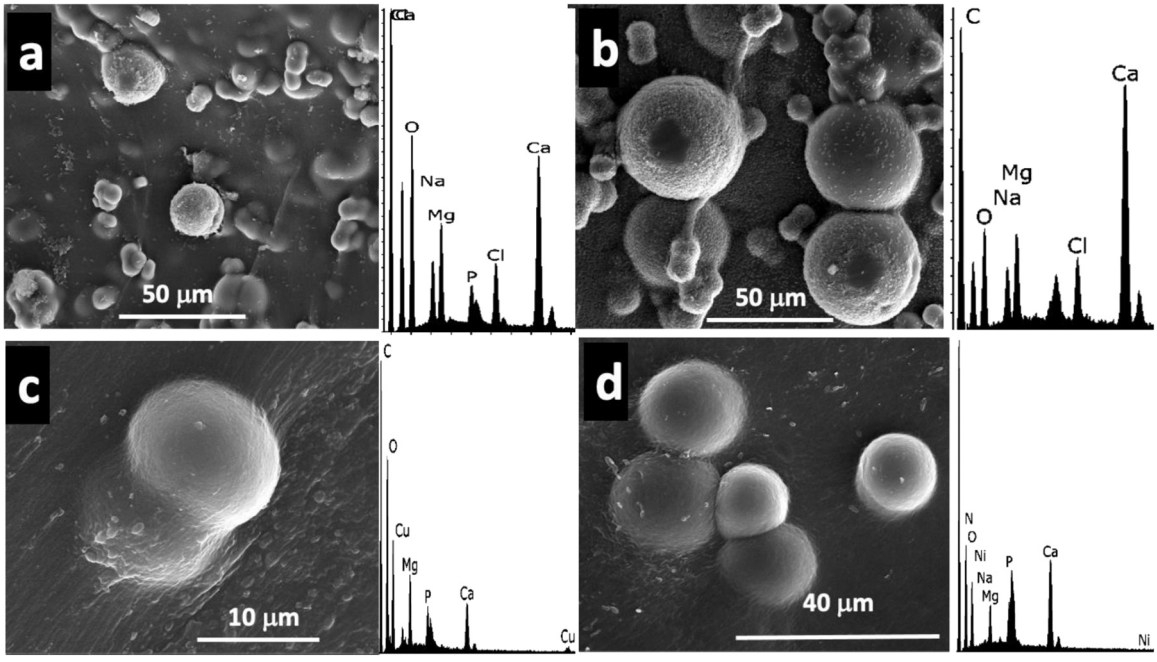

Fig. 3. Representative SEM/EDS analyses of the minerals formed in the pure cultures of a) *Virgibacillus martsimiure* DF1-MD1, b) *Virgibacillus* sp. DF2-MD1, c) *Virgibacillus martsimiure* DF1-MD1-Cu, and d) *Virgibacillus* sp. DF2-MD1-Ni.

processes could not be precisely assessed due to the inability to quantify the formed minerals in the culture. It is crucial to note that heavy metals can exert various influences on bacterial growth and biomineralization (Al Disi et al., 2022a). Notably, no mineral formation was observed in the MD1 culture media supplemented with Zn^{2+} and Cd^{2+} (Table 1). This inhibitory effect of Zn^{2+} and Cd^{2+} on mineral formation may be attributed to the potential competition between these heavy metal ions and Ca^{2+} and Mg^{2+}. Specifically, Cd^{2+} and Zn^{2+} might have occupied the available Ca^{2+} and Mg^{2+} nucleation sites in the EPS matrix (Su et al., 2022).

3.3. Characterization of formed minerals using SEM/EDX and SEM mapping

The SEM/EDX analysis of the recovered minerals showed that *Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2 mediated the formation of high magnesium carbonate minerals (Fig. 3). The minerals formed by the bacterial cells, primarily exhibit a spherical morphology. Although there is a possibility of some cells being entrapped within the formed minerals, our findings indicate that this entrapment has a minor impact on cell growth. The growth of the bacterial cells is not significantly hindered by the mineral formation process. Therefore, the selected calcium concentration aligns with the successful mitigation of heavy metal pollution in our experimental system.

SEM mapping was employed to investigate the spatial associations between heavy metals, minerals, EPS, and bacterial cells. The results revealed that the copper precipitates were either integrated within or deposited onto the surface of Mg-calcite crystals (Fig. 4a). While Ni was found to be incorporated into the Mg-calcite crystals (4b). Furthermore, the heavy metals were observed to be adsorbed onto the EPS produced by the bacterial strains as well as on the surfaces of bacterial cells (Fig. 4c and d). These findings highlight the roles of minerals, EPS, and bacterial cells in sequestering and immobilizing these pollutants, contributing to our understanding of the interactions between bacteria and heavy metals.

3.4. FTIR analysis of the EPS functional groups

The FTIR spectra of the EPS produced by the bacterial strains investigated in this study under different growth conditions with and without heavy metals are presented in Fig. 5. Notably, consistent FTIR peaks at 1535 cm^{-1} (N-H bending in Amide II), 1419 cm^{-1} (symmetric stretching of C═O in COOH), and 1231–1235 cm^{-1} (asymmetric stretching vibration of P = O) were observed among the mineral-forming strains, *Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2. However, the FTIR spectra of the EPS produced by *Virgibacillus halodenitrificans* Z4D1 did not contain the bands around 1235 cm^{-1} and 875 cm^{-1} which correspond to asymmetric stretching vibration of P = O and Phosphate functional groups respectively (Fig. 5a). A rightward shift in the FTIR peaks corresponding to the C–O vibration of polysaccharides was observed (ranging from 1016 to 1026 cm^{-1}) in the EPS produced in MD1 supplemented with heavy metals. Interestingly, major changes included the appearance of an FTIR peak at 1228 cm^{-1} corresponding to the asymmetric stretching vibration of P = O were observed in FTIR spectra produced by Z4D1in the presence of Zn^{2+}(Fig. 5d). Furthermore, shifts in the FTIR peak at 1010 cm^{-1} corresponding to the C–O vibration of polysaccharides was observed in the EPS produced by *Virgibacillus halodenitrificans* Z4D1 (Fig. 5d). These alterations in the FTIR spectra of the EPS produced by the bacterial strains may be attributed to the potential adsorption of the divalent heavy metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}). This adsorption is likely influenced by the varying affinities of these heavy metals towards the negatively charged functional groups within the EPS matrix (Felz et al., 2020).

3.5. Estimation of removal efficiency of heavy metals by EPS

The ability of EPS to remove heavy metals from liquid cultures was investigated using ICP-OES analysis; the corresponding percentages of heavy metals found in the EPS are presented in Table 2. The results revealed that the EPS removal efficiencies (%) of Cu^{2+} ($0.13 \pm 0.01 - 0.31 \pm 0.02$) and Ni^{2+} ($0.19 \pm 0.01 - 0.48 \pm 0.02$) were significantly lower than that of Zn^{2+} ($19.4 \pm 1.0 - 31.7 \pm 1.6$) and Cd^{2+} ($8.7 \pm 0.4 - 10.6 \pm 1.1$). Notably, the EPS of the non-mineral-forming strain, *Virgibacillus halodenitrificans* Z4D1, exhibited higher removal efficiencies than the EPS of mineral-forming strains. These findings highlight the diverse potential of bacterial strains in heavy metal removal applications and underscore the importance of EPS as a potent tool in heavy metal sequestration.

These findings provide compelling evidence that the presence of Cu^{2+} and Ni^{2+} ions does not hinder the formation of minerals by mineral-forming bacterial trains (*Virgibacillus martsimiure* DF1 and *Virgibacillus* sp. DF2). Indeed, ions of both heavy metals were found to be incorporated into the carbonate minerals precipitated by these organisms. Consequently, EPS produced in the presence of Cu^{2+} and Ni^{2+} exhibited reduced efficacy in removing these heavy metals, reflected in maximum removal efficiencies of approximately 0.31 ± 0.02 for Cu^{2+} and 0.48 ± 0.02 for Ni^{2+}, respectively. In contrast, the presence of Zn^{2+} and Cd^{2+} ions noticeably hindered mineral formation aligning with earlier conclusions presented in Sections 3.2 and 3.3 that suggest potential competition between Zn^{2+} and Cd^{2+} with Mg^{2+} and Ca^{2+}, vital elements for mineralization (Su et al., 2022). Noteworthy is the significantly higher percentage of Zn^{2+} found within the EPS produced by the bacterial strains, implying a greater affinity for binding and sequestering Zn^{2+} ions, possibly as a defense mechanism against their toxic effects (Felz et al., 2020). This observed phenomenon suggests that the mineral-forming strains, under the influence of Zn^{2+} and Cd^{2+}, exhibit a diminished ability to form minerals, as evidenced by the lower removal efficiency. The higher removal efficiency for Zn^{2+} and Cd^{2+} implies that these metal ions are more effectively incorporated or sequestered by the EPS, potentially hindering the availability of essential nucleation sites for Ca^{2+} and Mg^{2+} in the mineralization process. Notably, It Cd^{2+} exhibited high toxicity, inhibiting the bacterial growth and mineral formation underscoring its detrimental impact on bacterial physiology and mineral formation capabilities (Xu et al., 2020).

Furthermore, the EPS matrix, characterized by negatively charged functional groups, serve as sites for both nucleation of mineral

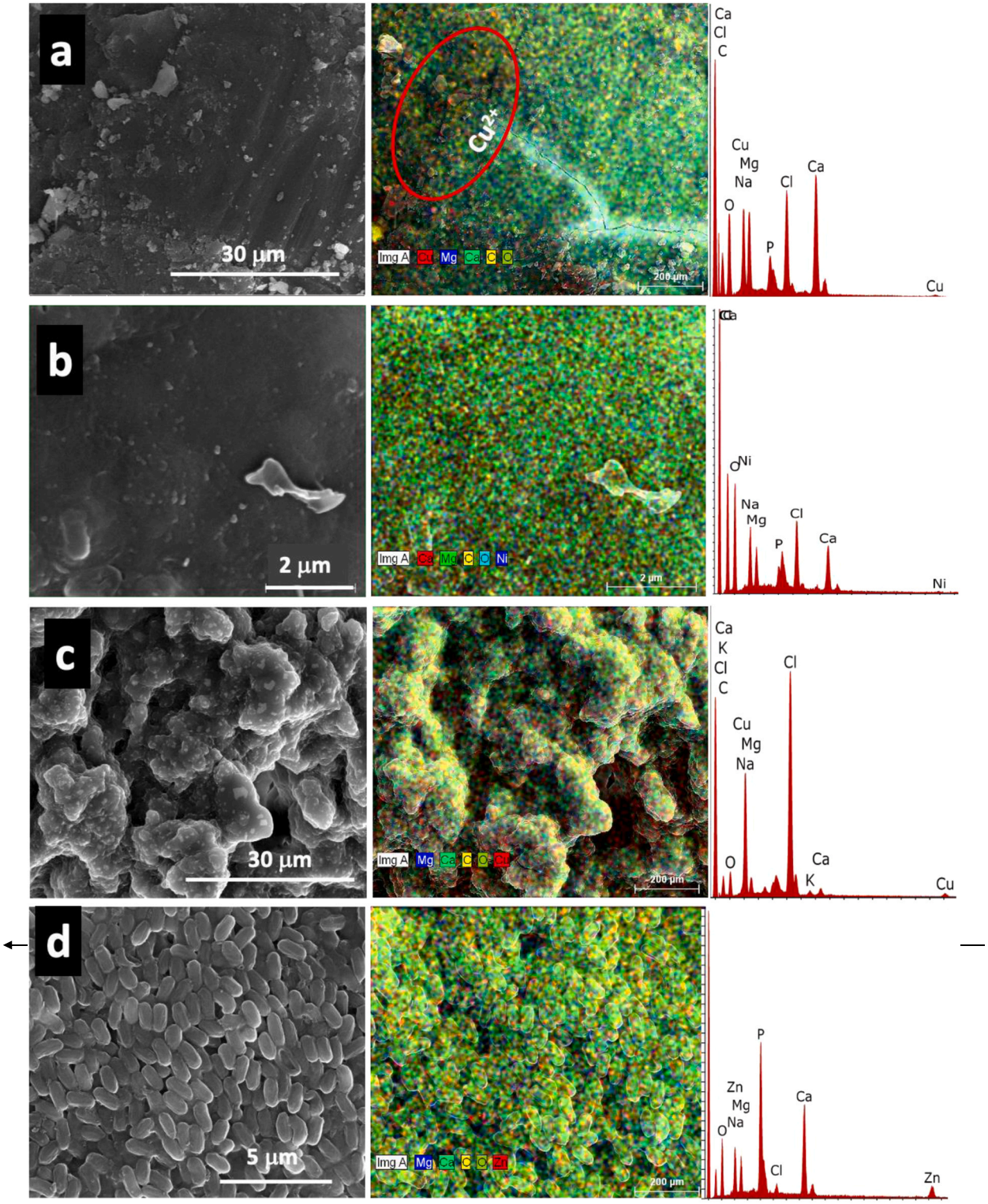

Fig. 4. SEM images and elemental mapping showing a) Cu^{2+} deposited on the surface of an Mg-calcite crystal; b) Ni^{2+} incorporated into the Mg-calcite crystal; c) Cu^{2+} adsorbed on EPS, and d) Zn^{2+} adsorbed onto the bacterial cells of *Virgibacillus martsimiure* DF1.

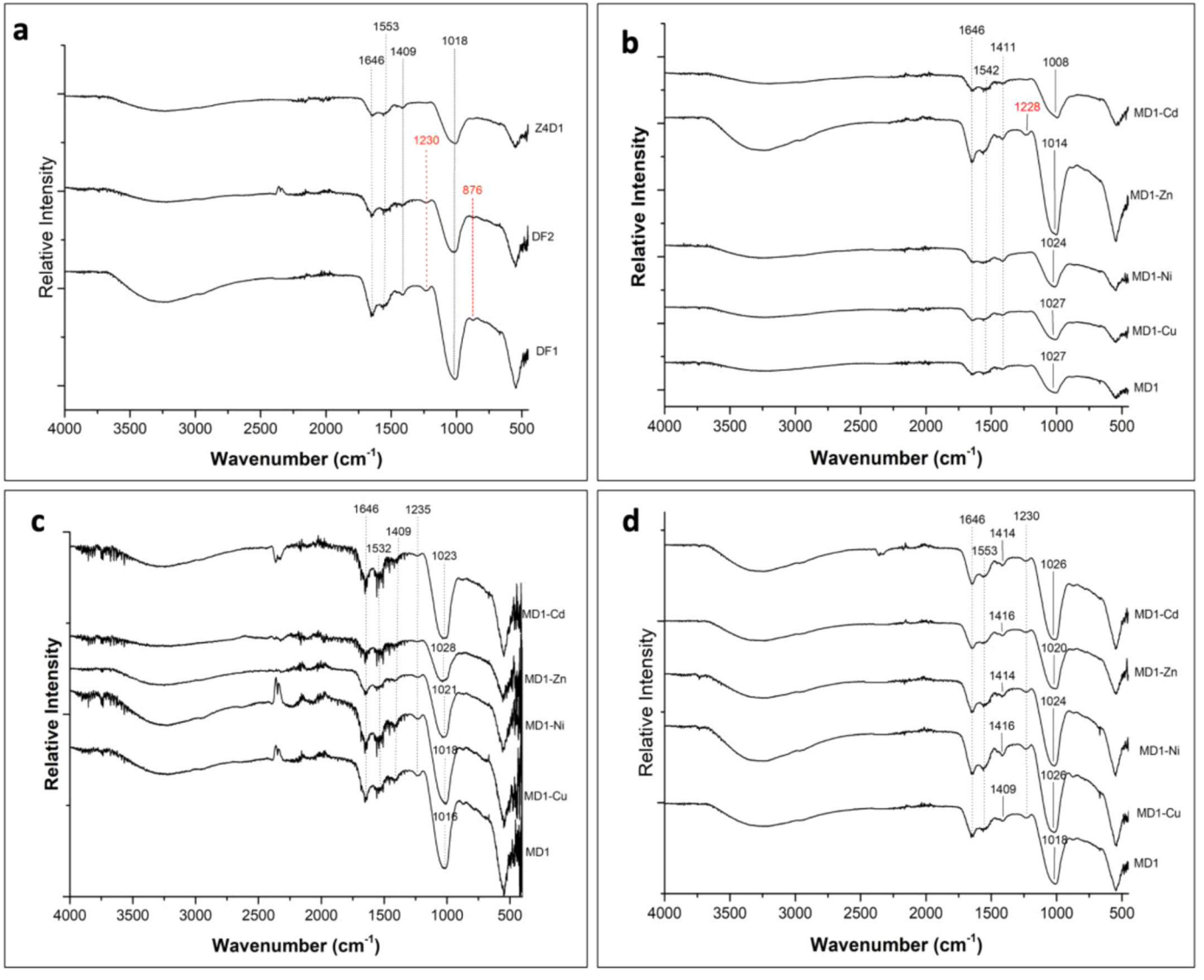

Fig. 5. Illustrative FTIR spectra of the EPS produced by a) all studied strains on MD1, b) the mineral-forming strain *Virgibacillus martsimiure* DF1, c) the mineral-forming strain *Virgibacillus* sp. DF2, and d) the non-mineral-forming strain *Virgibacillus halodenitrificans* Z4D1.

Table 2
Heavy metal removal efficiency (%) by the EPS produced by the studied bacterial strains.

HM Removal efficiency (%)				
Bacterial strain	**Cu^{2+}**	**Ni^{2+}**	**Zn^{2+}**	**Cd^{2+}**
Virgibacillus martsimiure DF1	0.14 ± 0.01	0.32 ± 0.02	24.3 ± 1.2	8.7 ± 0.4
Virgibacillus sp. DF2	0.13 ± 0.01	0.19 ± 0.01	19.4 ± 1.0	9.9 ± 0.5
Virgibacillus halodenitrificans Z4D1	0.31 ± 0.02	0.48 ± 0.02	31.7 ± 1.6	10.6 ± 1.1

formation and adsorption of heavy metals (Robles-Fernández et al., 2022). The preferential affinity or adsorption of EPS functional groups to heavy metals depends on the composition and structure of EPS (Wang et al., 2020). Interestingly, the non-mineral-forming EPS may be more suitable for trapping heavy metals as evidenced by the *Virgibacillus* strain Z4D1 isolated from oil waste. In this case, the absence of biomineralization processes doesn't hinder its ability to effectively remove heavy metals. This could be attributed to the specific characteristics of its EPS, which effectively immobilizes heavy metals through adsorption. Further research is needed to explore the selectivity of this process for specific heavy metals.

Microbial exopolymeric substances (EPS) exhibit significant promise in effective removal of heavy metals from diverse environments (Zeng et al., 2020). EPS feature appropriate functional groups like carboxyl, hydroxyl, and amino groups, facilitating effective binding with heavy metal ions (Wu et al., 2021). They demonstrate biocompatibility, allowing for versatile production under different environmental conditions (Waoo et al., 2023). The selectivity of EPS in metal binding is advantageous for targeting specific contaminants within complex matrices (Huang et al., 2022). The formation of stable complexes between microbial EPS and heavy metals prevents re-release into the environment, by their immobilization (Pande et al., 2022). The versatility of EPS production under a range of environmental conditions makes them adaptable for various applications (Mohd Nadzir et al., 2021). Despite these advantages, challenges such as optimizing EPS production and efficient recovery of EPS-metal complexes persist, prompting ongoing research to enhance the feasibility and efficiency of microbial EPS in heavy metal removal processes.

4. Conclusion

This study aimed to explore the biomineralization capabilities of specific bacterial strains and to investigate their potential heavy metal removal. In general, mineral formation occurred when the culture media lacked heavy metals or when supplemented with Cu^{2+} and Ni^{2+}. The presence of Cd^{2+} and Zn^{2+} were found to hinder mineral formation, likely due to competition with Ca^{2+} and Mg^{2+} ions. The FTIR analysis revealed significant changes and shifts in the peaks of EPS produced by the strains under different growth conditions, highlighting the interactions between the heavy metals and the functional groups present in the EPS. Moreover, the findings highlight the inhibitory effects of Cd^{2+} and Zn^{2+} on mineral formation, as well as provide insights into the underlying mechanisms of these interactions and suggest that the EPS produced by the strains possess the capability to interact with and potentially adsorbed heavy metals. This study thus contributes to the development of strategies for environmental remediation and the restoration of heavy metal-contaminated soils through biomineralization approaches.

In fact, the bacterial strains investigated in this study, along with their produced extracellular polymeric substances (EPS), hold promisee as alternative for the development of remediation of both contaminated soils and heavy metal-contaminated wastewaters. However, it was necessary to investigate the interaction between the two processes; the mineral formation which could incorporate many metals into insoluble forms and the immobilization of heavy metals outside the minerals, both mediated by the EPS of the bacterium *Virgibacillus*. Following this study, further investigations will be performed to consider the two processes separately or to search for appropriate EPS composition which mediate both processes in parallel.

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CRediT authorship contribution statement

Zulfa Al Disi: Conceptualization, Investigation, Methodology, Formal analysis, Visualization, Writing original draft, **Dalal Omar Mohamed:** Investigation, Visualization, Writing original draft, **Mohammad A. Al-Ghouti:** Formal analysis, Review and editing, **Nabil Zouari:** Supervision, Conceptualization, Methodology, Formal analysis, Visualization, Review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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