Received: 3 March 2017 | Revised: 19 July 2017 | Accepted: 21 September 2017
DOI: 10.1002/jobm.201700143

RESEARCH PAPER

Journal of Basic Microbiology

Metal transformation as a strategy for bacterial detoxification of heavy metals

Ashraf M. M. Essa[1,2] | **Mohamed A. Al Abboud**[2] | **Sayeed I. Khatib**[2]

[1] Faculty of Science, Botany Department, Fayoum University, Fayoum, Egypt
[2] Faculty of Science, Biology Department, Jazan University, Jazan, Saudi Arabia

Correspondence
Ashraf M. M. Essa, Faculty of Science, Botany Department, Fayoum University, Fayoum, Egypt.
Email: ashraf.essa@yahoo.com

Microorganisms can modify the chemical and physical characters of metals leading to an alteration in their speciation, mobility, and toxicity. Aqueous heavy metals solutions (Hg, Cd, Pb, Ag, Cu, and Zn) were treated with the volatile metabolic products (VMPs) of *Escherichia coli* Z3 for 24 h using aerobic bioreactor. The effect of the metals treated with VMPs in comparison to the untreated metals on the growth of *E. coli* S1 and *Staphylococcus aureus* S2 (local isolates) was examined. Moreover, the toxic properties of the treated and untreated metals were monitored using minimum inhibitory concentration assay. A marked reduction of the treated metals toxicity was recorded in comparison to the untreated metals. Scanning electron microscopy and energy dispersive X-ray analysis revealed the formation of metal particles in the treated metal solutions. In addition to heavy metals at variable ratios, these particles consisted of carbon, oxygen, sulfur, nitrogen elements. The inhibition of metal toxicity was attributed to the existence of ammonia, hydrogen sulfide, and carbon dioxide in the VMPs of *E. coli* Z3 culture that might responsible for the transformation of soluble metal ions into metal complexes. This study clarified the capability of *E. coli* Z3 for indirect detoxification of heavy metals via the immobilization of metal ions into biologically unavailable species.

KEYWORDS
chelators, detoxification, *Escherichia coli*, extracellular, heavy metals

1 | INTRODUCTION

Heavy metals refer to metals or metalloids with relatively high densities, atomic weights, or atomic numbers. They are known for their toxic impact on most of living organisms even at low levels. Heavy metals are persistent environmental pollutants because they cannot be degraded or destroyed. The environmental pollution with heavy metals induces adverse impacts on terrestrial and aquatic ecosystems. Heavy metals pollution originates from natural sources such as rocks and metalliferous minerals or from anthropogenic activities such as agriculture, metallurgy, energy production, mining, sewage sludge, and waste disposal [1]. Some heavy metals such as antimony, silver, aluminum, gold, lead, and mercury are non-essential and highly toxic pollutants. Others such as manganese, copper, iron, magnesium, arsenic, cadmium, and zinc are essential for living cells at low levels as they act as cofactors for some essential enzymes but they exert toxicity when are found at elevated concentrations. Heavy metals are highly toxic to most living organisms. They have been reported to destroy cellular organelles, alter enzyme specificity, and disrupt cell membranes in addition to the damage of DNA that leads to cell cycle modulation, carcinogenesis, or apoptosis [2–4]. Some metal cations such as Hg^{2+}, Cd^{2+}, and Ag^{+} tend to bind—SH groups resulting in an inhibition of the essential enzymes [5] while other heavy metal can induce oxidative stress [6].

 © 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Heavy metals interact with microorganisms in different ways based on metal species, type of microorganisms and environmental conditions [7]. At the same time, cell structure and metabolic activity of microbial cells influence on the solubility, bioavailability, and toxicity of metals [7]. A large number of microbial species can survive in direct contact with heavy metals because they possess various resistance strategies against these metals. The resistance mechanisms include membrane transport system involved in the cellular accumulation of specific metals [8], metals sequestration via specific binding components [9], active efflux systems encoded by specific genes [10], and enzymatic conversion of toxic metal ions into less toxic forms [11]. Generally, metal resistance genes including those for Hg^{2+}, Ni^{2+}, Ag^{+}, Cd^{2+}, Cu^{2+}, CrO_4^{2-}, and Zn^{2+} are located on plasmids. Besides, some resistance genes are found on bacterial chromosomes such as Hg^{2+} resistance genes, Cd^{2+} efflux genes in *Bacillus subtilis* and arsenic efflux genes in *Escherichia coli* [12,13].

The indirect metal-microbe interaction could represent a promising approach of heavy metal homeostasis in the microbial environment. It assists microorganisms to tolerate high concentrations of heavy metals through producing wide varieties of compounds that are involved in holding the metals away from the cells. The liberation of extracellular non-specific metabolites such as phosphates, sulfides, and carbonates that are formed during microbial metabolic reactions, participate in the diminution of heavy metals toxicity. These compounds are engaged in the precipitation of metals in the microbial habitat [14].

Bioprecipitation by sulfides and phosphates has been intensively investigated because of the low solubility of many of their metal compounds. The release of phosphate via hydrolysis of organic phosphate substrates has been shown as a successful technique for the removal of metals out of solutions as metal phosphates [15]. It is well known that sulfate-reducing bacteria can produce and accumulate large quantities of sulfide that transfer across the cell membrane into the culture medium resulting in the transformation of soluble metal ions into metal sulfides [16].

Some of the extracellular compounds are produced during the microbial metabolism in the form of volatile metabolites such as alcohols, aldehydes, amines, esters, ketones, and mercaptans [17]. The types and quantities of the microbial vapors are significantly influenced by microbial species and growth conditions [18]. Even though the physiological function of the microbial volatiles still not clear, it was proven that they could be contributed to the transformation of some heavy metals into insoluble metal complexes [19,20]. The aim of the current study was to evaluate the toxicity of heavy metals treated with bacterial VMPs against bacterial strains in order to emphasize the role of these vapors in diminishing the harmful influence of heavy metals.

2 | MATERIALS AND METHODS

2.1 | Bacterial isolates and culture conditions

Three bacterial isolates were used in the current study including *Escherichia coli* Z3 (isolated from sludge sample, Egypt [21]) and two bacterial isolates S1 and S2 that were kindly obtained from Faculty of Pharmacy, Jazan University, Saudi Arabia. Cultures were routinely grown aerobically on nutrient broth medium using shaking incubator at 37 °C for 24 h. Proper aseptic techniques and standard laboratory operation procedures were followed during the study with these microorganisms.

2.2 | Identification of the bacterial isolates

The bacterial isolates (S1 and S2) were subjected to 16S rDNA gene sequencing where the genomic DNA was extracted using standard bacterial procedures [22]. Two universal primers were used for the amplification of 16S rDNA gene; (F1) AGA GTT TGA TCC TGG CTC AG and (R1) AAG GAG GTG ATC CAG CCG CA [23]. PCR mixture was prepared according to Essa et al. [24]. PCR has carried out 35 cycles under the following program; denaturation for 40 s at 94 °C, annealing for 1 min at 55 °C, and extension for 2 min at 72 °C. PCR product was analyzed by electrophoresis ($15\ V\ cm^{-1}$, 60 min) on 0.7% horizontal agarose gel. After purification, the products of PCR were subjected to sequencing at GATC Biotech, Constance, Germany. Then the DNA sequences were aligned at NCBI DataBase (www.ncbi.nlm.nlh.gov).

2.3 | Treatment of heavy metal solutions with VMPs of *E. coli* Z3

Analytical grade salts of $CdCl_2$, $CuSO_4$, $HgCl_2$, $AgNO_3$, $Pb(NO_3)_2$, $ZnSO_4$ (Merck) were used to prepare 0.1 M stock solutions. Metal solutions were sterilized using 0.45 µm pore-size polysulfone sterile filters (Whatman). A bioreactor was constructed according to Essa et al. [20] where the growth chamber contained one liter of *E. coli* Z3 culture at mid-log phase (O.D ≈ 0.6) that was reserved under aerobic conditions via pushing sterilized air. At the same time, the bacterial VMPs were passed through one hundred milliliters of the different metal solutions for 24 h exposure time. With the intention of avoiding the contamination of metal solution with bacteria, VMPs were pushed through a 0.2 µm filter (Millipore).

2.4 | Toxicity assay using standard agar well diffusion method

Since the bacterial strain *E. coli* Z3 demonstrated a clear resistance against some of the tested metals such as mercury, lead, and silver (data not shown), the toxicity of the treated

and untreated heavy metals was measured against A Gram-negative model (*E. coli* S1) and A Gram-positive model (*Staphylococcus aureus* S2) using the modified agar well diffusion method [25]. Nutrient agar plates were inoculated with 100 µl of the tested bacterial strains (10^8 cell ml^{-1}). Once the agar was solidified, it was punched with 8 mm diameter wells and filled with 25 µl of low and high concentrations of metal solutions as follows: 0.1 and 0.2 mM for mercury and silver; 1.0 and 2.0 mM for cadmium; 2.0 and 4.0 mM for lead, copper, and zinc. Experiments were carried out in triplicate and the diameters of the zones of inhibition were measured by millimeter after 24 h incubation at 30 °C. Streptomycin (100 µg ml^{-1}) was used as a positive control.

2.5 | Toxicity assay using minimum inhibitory concentration (MIC)

Mueller–Hinton (MH, Merck) agar plates were supplemented with different heavy metals. Analysis of metal resistance was performed by mid-log phase cultures of *E. coli* S1 and *S. aureus* S2. Cells were streaked on MH agar plates containing serial concentrations of metals as follow: 0.1–2.0 mM for mercury and silver; 1.0–20.0 mM for cadmium, lead, zinc, and copper. Growth was recorded after 2 days of incubation at 37 °C. The lowest concentration of metal that completely prevented bacterial growth was termed the minimal inhibitory concentration.

2.6 | Effect of treated and untreated heavy metals on bacterial growth

The toxic effect of treated and untreated metal solutions against the bacterial strains *E. coli* S1 and *S. aureus* S2 was monitored. Nine milliliters of nutrient broth containing various doses of treated and untreated metals were inoculated with 1 ml of mid-log phased cultures of the fresh culture of each bacterial strain (O.D ≈ 0.6). The metal concentrations were 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 mM for mercury and silver, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 mM for cadmium and 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 mM for lead, copper, and zinc. After incubation at 37 °C for 24 h, the bacterial growth was monitored by measuring the optical density at 600 nm using a UV-VIS spectrophotometer (UNICAM, 8625). A culture grown in the absence of metal served as the control. At the same time, the protein content of bacteria exposed to treated and untreated metals was estimated using the bicinchoninic acid (BCA) assay kit (Sigma–Aldrich BCA-1, B9643), with bovine serum albumin as a standard. The developed purple color was measured spectrophotometrically at 562 nm [26].

2.7 | SEM and EDX analysis of metals precipitates

Each treated metal solution collected from the bioreactor was centrifuged at 100,000 rpm for 30 min. The supernatant was filtered (0.2 µm filters) and stored for analysis. The metal precipitates were washed with 20 ml de-ionized water followed by centrifugation as before (three times). Each metal precipitate was collected and dried at 30 °C. Then metal precipitates were examined with a JEOL JSM 5900 scanning electron microscope with the composition determined by EDX using an Oxford Link ISIS System according to Essa and Khallaf [27]. The elements of the samples assigned concentration values based on the known compositions of standards that were analyzed under the same conditions as described by Tylko et al. [28]. The analysis was carried out by determining the intensity of a characteristic X-ray peak of a chosen element on a standard that has no interfering peaks.

2.8 | Heavy metals analysis

The purified supernatants were subjected to heavy metal analysis (Cd^{2+}, Cu^{2+}, Hg^{2+}, Ag^{+}, Pb^{2+}, and Zn^{2+}) using a Flameless Atomic Absorption Spectrophotometer (AAS, Perkin Elmer 2380). The removal percentage was calculated for each metal in comparison to the VMPs unexposed metals.

2.9 | Levels of biogenic sulfides, ammonia, and CO_2 in the bioreactor

The sulfide content of a water sample exposed to the VMPs of *E. coli* Z3 for 24 h was assayed using the protocol developed by Siegel [29]. One hundred microliters of sample were transferred into 1.5 ml microcentrifuge tubes. Then 100 µl N,N-dimethyl-p-phenylenediamine sulfate (0.02 M) in 7.2 N HCl and 100 µl of $FeCl_3$ (0.3 M) in 1.2 N HCl were added. After incubation in the dark for 20 min, tubes were subjected to centrifugation at 10,000 rpm for 10 min and the optical density of the supernatant was measured spectrophotometrically at 670 nm. Sulfide concentration was determined by comparing results to standard curve developed with Na_2S standards. At the same time, the concentration of ammonia in a water sample exposed to the bacterial VMPs for 24 h was measured using modifying Nessler method according to Jeong et al. [30]. The absorbance of the samples was measured at 400 nm and compared to standard curve developed with NH_4Cl. Regarding biogenic CO_2, trapping experiment was carried out by exposing 10 ml 0.1 M $Ba(OH)_2$ to the bacterial VMPs. After 24 h, the dry weight of $BaCO_3$ was determined by filtering the solution through Whatman No. 1 filter paper and drying at 50 °C for 4 days.

2.10 | Statistical analysis

The data presented in the current study are the mean value of at least three replicates. Standard errors were calculated for all the values using MS Excel 2007.

TABLE 1 Effect of treated and untreated heavy metals against *E. coli* S1 and *S. aureus* S2 using standard agar well diffusion method

	Escherichia coli S1				*Staphylococcus aureus* S2			
	Low conc.		High conc.		Low conc.		High conc.	
Heavy metals	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Streptomycin	29 ± 1				24 ± 2			
$HgCl_2$	22 ± 1	14 ± 2	30 ± 1	15 ± 1	20 ± 1	12 ± 2	28 ± 1	16 ± 2
$AgNO_3$	15 ± 2	10 ± 1	27 ± 2	13 ± 1	13 ± 2	ND*	19 ± 2	ND*
$CdCl_2$	15 ± 1	12 ± 1	25 ± 1	12 ± 2	21 ± 2	12 ± 1	24 ± 1	15 ± 2
$Pb(NO_3)_2$	18 ± 2	12 ± 2	26 ± 1	15 ± 2	24 ± 1	15 ± 2	32 ± 2	18 ± 1
$ZnSO_4$	10 ± 2	ND*	11 ± 2	ND*	11 ± 2	10 ± 1	18 ± 1	10 ± 1
$CuSO_4$	17 ± 1	ND*	18 ± 1	ND*	12 ± 1	ND*	15 ± 2	ND*

Experiments were carried out in triplicate and diameters of the zones of inhibition were measured by millimeter. Each heavy metal was used at two concentrations; 0.1 and 0.2 mM for mercury and silver, 1.0 and 2.0 mM for cadmium; 2.0 and 4.0 mM for lead, copper, and zinc. Data are the means of three replications ± standard errors.
*ND, not detected.

3 | RESULTS

3.1 | Identification of bacterial strains

Based on the morphological characteristics and Gram stain, the bacterial isolates were classified as Gram-negative bacilli (S1) and Gram-positive cocci (S2). The molecular identification of the bacterial isolates was performed using 16S rDNA gene sequencing. The obtained DNA sequences (1500 bp) were aligned at NCBI DataBase. The isolate S1 was identified as *E. coli* S1 with 99% identity to the accession number BA000007 while S2 was identified as *S. aureus* S2 with 99% identity to the accession number LOSC01000004.

3.2 | Toxicity of the treated and untreated heavy metals

The toxicity of the treated metals was determined using standard agar well diffusion method. The obtained results (Table 1) showed a clear toxicity reduction of the treated metals in comparison with the untreated metals. In the case of *E. coli* S1, the inhibition zones of the treated at high concentrations were diminished by 50.1% for Hg, 51.8 % for Ag, 52.0% for Cd, and 42.3% for Pb. Simultaneously, the treated copper and zinc demonstrated a complete disappearance of their toxicity at the tested concentrations. Similar results were obtained with *S. aureus* S2 (Table 1). The diameter of the inhibition zone of the treated metals in relation to the untreated ones was reduced by 42.8% for mercury, 37.5% for cadmium, 43.7% for lead, and 44.3% for zinc at the higher concentrations. At the same time, a complete detoxification of treated copper and silver was recorded at the tested metal levels against *S. aureus* S2.

In order to clarify the toxicity of the treated heavy metals in comparison to the untreated metals against the bacterial strains, minimum inhibitory concentration (MIC) assay was conducted (Table 2). Overall, there was a clear reduction in the toxicity of metals exposed to the bacterial VMPs. The MIC values of the treated metals were higher than those of the untreated metals. In case of *E. coli* S1, the recorded MICs of treated metals were 1.0 mM for mercury, 2.0 mM for silver, 14.0 mM for cadmium, 12.0 mM for lead and copper, and 16.0 mM for zinc. Analogous data were collected with Gram-positive *S. aureus* S2 where high MIC values were determined with treated metals. The recorded MICs of the treated metals were 0.8 mM with mercury, 1.6 mM with silver, 10.0 mM with lead, and 12.0 mM with cadmium, copper, and zinc.

3.3 | Impact of treated and untreated heavy metals on bacterial growth

In order to investigate the influence of the treated and untreated metals on the bacterial growth, an experiment was

TABLE 2 Minimum inhibitory concentration (MIC) of the treated and untreated heavy metals against *E. coli* S1 and *S. aureus* S2

	Minimum inhibitory concentration (mM)			
	Escherichia coli S1		*Staphylococcus aureus* S2	
Heavy metals	Untreated	Treated	Untreated	Treated
$HgCl_2$	0.2	1.0	0.2	0.8
$AgNO_3$	0.3	2.0	0.3	1.6
$CdCl_2$	3.0	14.0	2.0	12.0
$Pb(NO_3)_2$	6.0	12.0	4.0	10.0
$ZnSO_4$	6.0	16.0	4.0	12.0
$CuSO_4$	4.0	12.0	3.0	12.0

Data are the means of three replications.

carried out in nutrient broth where the tested strains (*E. coli* S1 and *S. aureus* S2) were exposed to various doses of the treated and untreated metals. The obtained results (Fig. 1) revealed a clear suppressive influence of mercury and silver on the growth of *E. coli* S1 even at low levels. The other metals such as lead, copper, and zinc demonstrated a less inhibitory effect on the bacterial growth especially at low doses while at high doses they exerted a marked discourage impact on the bacterial growth. On the other hand, treated heavy metals clarified a less inhibitory impact against *E. coli* S1 up to 0.3 mM for Ag and Hg, 4.0 mM for Cd, 8.0 mM for Pb, Cu, and Zn. Simultaneously, *S. aureus* S2 demonstrated a compatible behavior against the treated metals. Data in Fig. 2 demonstrated a clear reduction of the

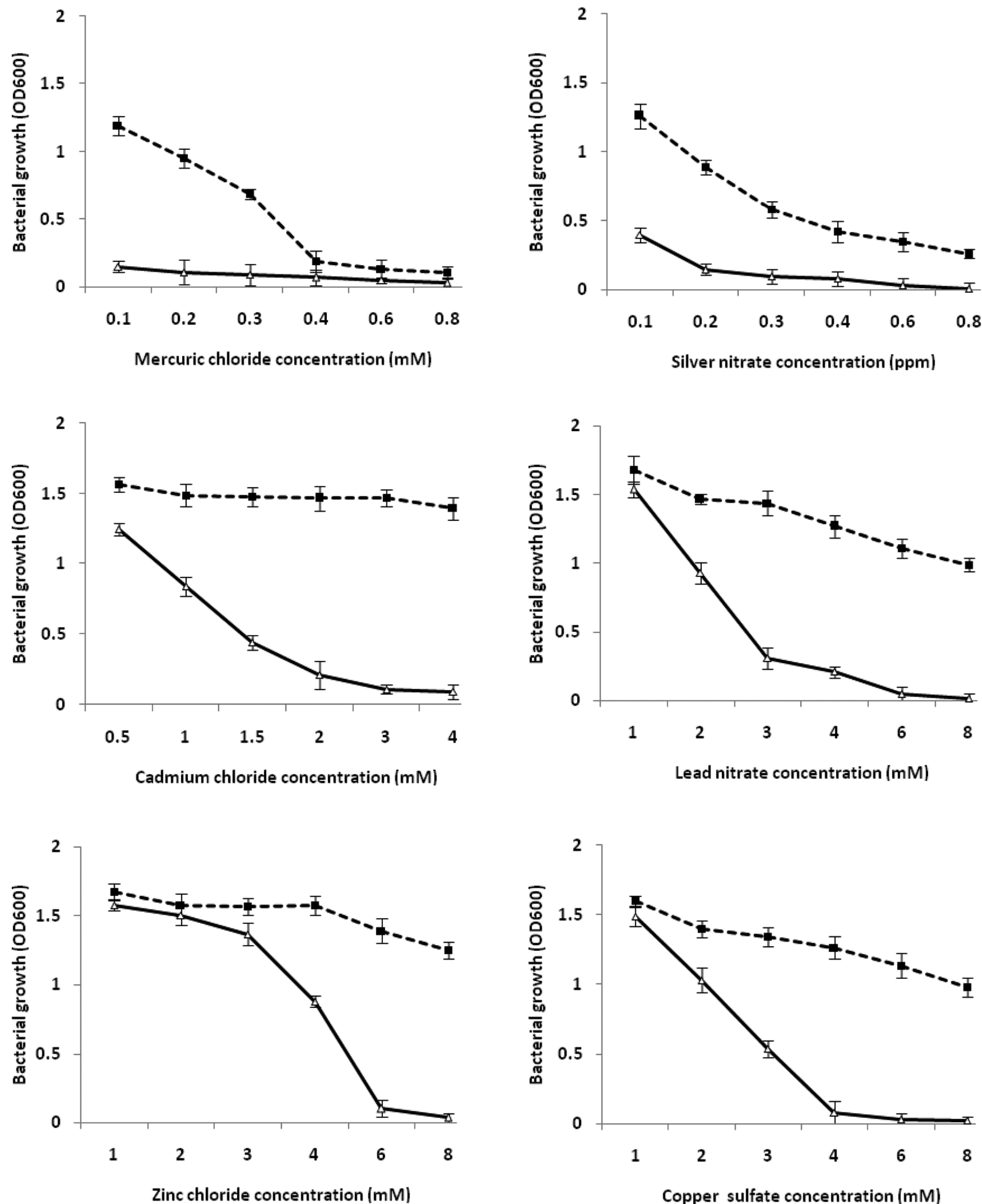

FIGURE 1 Effect of treated and untreated heavy metals on the growth of The experiment was carried out in triplicate and values are means of replicates ± standard error. The optical *E. coli* S1. Bacterial growth was monitored spectrophotometrically by measuring optical density at 600 nm. Density of bacteria grown under the same conditions in absence of heavy metals was 1.53 ± 0.1. Dash line with black squares represents metals treated with bacterial VMPs while solid line with white triangle represents untreated metals

detrimental impact of heavy metal compared to the untreated metals. The treated heavy metals demonstrated a less suppressive effect against *S. aureus* S2 impact up to 0.4 mM for Hg and Ag, 4.0 mM for Cd, 6.0 mM for Pb and Cu, and 8.0 mM for Zn.

At the same time, the data in Table 3 confirmed the toxicity reduction of the treated metals against *E. coli* S1 where high amounts of proteins were accumulated in the cells exposed to the treated metals in correlation to the untreated metals. The greatest protein content was spotted at 0.2 mM of Hg and Ag, 4.0 mM of Cd, 8.0 mM of Pb, 6.0 mM of Cu, and 8.0 mM of Zn. Regarding the protein content of *S. aureus* S2, the obtained results (Table 4) showed a massive increase in the protein levels in the cells grown in the presence of the

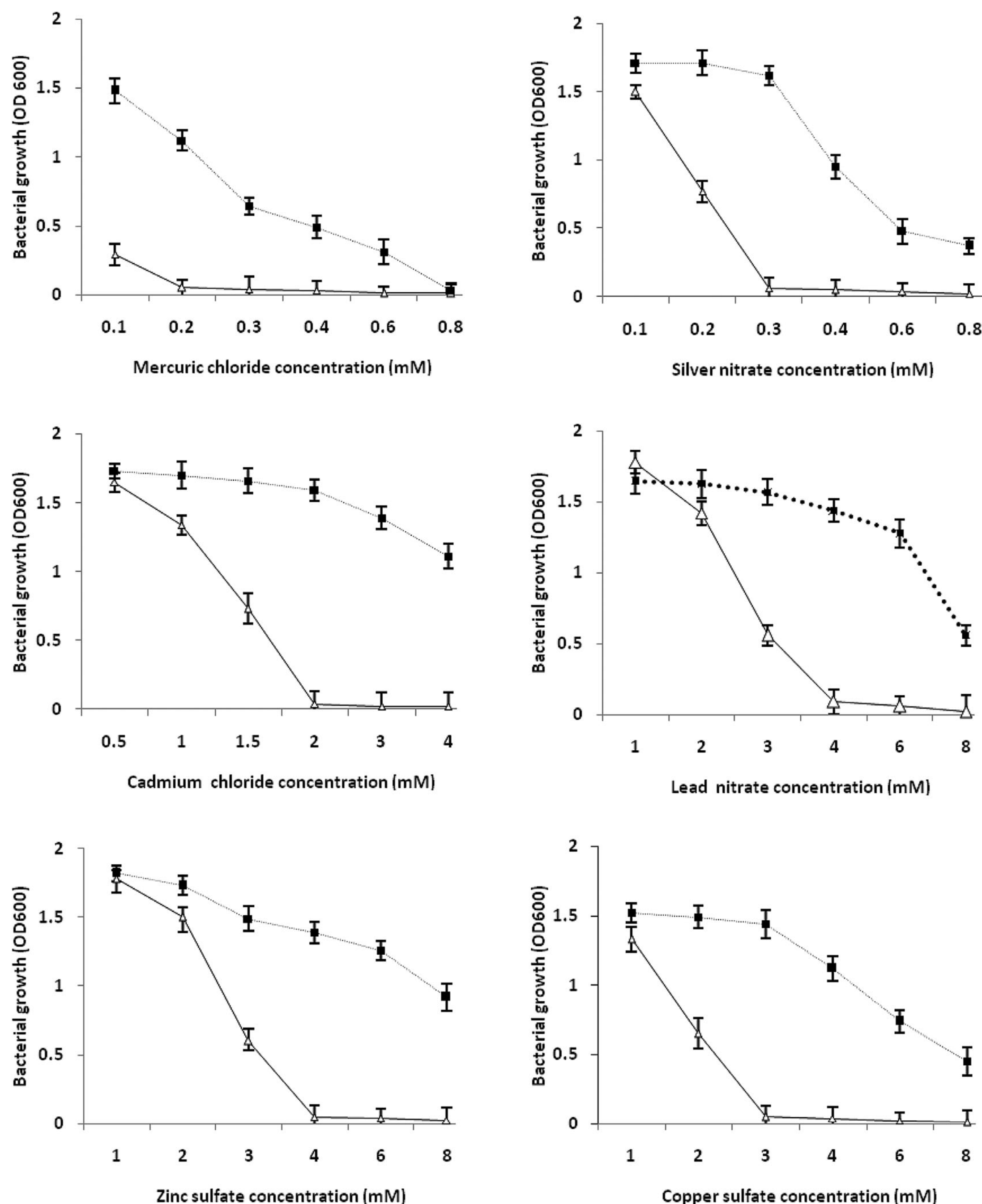

FIGURE 2 Effect of treated and untreated heavy metals on the growth of *S. aureus* S2. Bacterial growth was monitored spectrophotometrically by measuring optical density at 600 nm. The experiment was carried out in triplicate and values are means of replicates ± standard error. The optical density of bacteria grown under the same conditions in absence of heavy metals was 1.67 ± 0.13. Dash line with black squares represents metals treated with bacterial VMPs while solid line with white triangle represents untreated metals

treated metals even at the high metals concentrations. The maximum percentage of protein increase was recorded at 0.3 mM of Hg and Ag, 2.0 mM of Cd, 4.0 mM of Pb, 3.0 mM of Cu, 6.0 mM of Zn.

3.4 | Scanning electron microscope and electron dispersive X-ray analysis of metal precipitates

Scanning electron microscope analysis of the metal precipitates collected from the bioreactor showed the presence of tiny particles (10–50 µm in diameter) with mercury, lead, silver, cadmium, copper, and zinc (Figs. 3 and 4). At the same time, the electron dispersive X-ray system demonstrated the elemental composition of metal precipitates (Table 5). In case of Hg-precipitate, it was comprising of 72.6% mercury, 6.6% sulfur, 11.5% carbon, 3.9% nitrogen, and 5.2% oxygen while Pb-precipitate contained 77.8% lead, 9.2% sulfur, 7.8% carbon, 0.2% nitrogen, and 4.8% oxygen. Similarly, Ag-precipitate consisted of 71.5% silver, 11.6% sulfur, 8.4% carbon, and 8.2% oxygen where Cd-precipitate contained 63.5% cadmium, 18.9% sulfur, 7.1% carbon, 1.6% nitrogen, and 8.6% oxygen. Likewise, Zn-precipitate consisted of Zn (57.8%), S (7.4%), C (7.8%), N (1.0%), and O (25.7%) while Cu-precipitate contained Cu (61.6%), S (9.1%), C (4.0%), N (1.9%), and O (23.1%).

3.5 | Levels of biogenic sulfides, ammonia, and CO_2 in the bioreactor

The obtained results (Fig. 5) showed the presence of various levels of biogenic ammonia and sulfide in the precipitation chamber of the bioreactor. There was an increase of ammonia and sulfide concentration by increasing the exposure time. The maximum ammonia concentration (1.3 mM) was achieved after 24 h while the sulfide level has reached a constant level (111 µM) after 12 h. At the same time, elevated levels of CO_2 (5.8-fold) were detected in the VMPs released from the bacterial culture in comparison to abiotic experiment running under the same conditions (absence of bacterial VMPs).

4 | DISCUSSION

Microorganisms are directly concerned in metal biogeochemistry with a variety of bioprocesses. They have been known with their capacity to change the bioavailability of

TABLE 3 Effect of treated and untreated metals on protein content of *E. coli* S1

Heavy metals	Protein content (µg ml^{-1})					
$HgCl_2$ (mM)	0.1	0.2	0.3	0.4	0.6	0.8
Treated	203 ± 11**	151 ± 12**	106 ± 14**	54 ± 9**	28 ± 13*	26 ± 14*
Untreated	76 ± 16**	28 ± 13**	26 ± 10	22 ± 12**	23 ± 10*	21 ± 11
$AgNO_3$ (mM)	0.1	0.2	0.3	0.4	0.6	0.8
Treated	311 ± 19**	226 ± 13**	129 ± 11**	103 ± 11**	86 ± 16**	58 ± 12**
Untreated	112 ± 11**	57 ± 16**	34 ± 13**	28 ± 10**	25 ± 13*	26 ± 10**
$CdCl_2$ (mM)	0.5	1.0	1.5	2.0	3.0	4.0
Treated	347 ± 12**	328 ± 15*	302 ± 14**	288 ± 13**	275 ± 17**	244 ± 14**
Untreated	329 ± 10*	218 ± 14**	95 ± 13**	56 ± 10**	29 ± 14**	24 ± 11**
$Pb(NO_3)_2$ (mM)	1.0	2.0	3.0	4.0	6.0	8.0
Treated	378 ± 15	360 ± 13*	328 ± 19**	287 ± 13**	206 ± 11**	189 ± 14**
Untreated	366 ± 12*	214 ± 11**	73 ± 11**	45 ± 10*	27 ± 13**	22 ± 12**
$CuSO_4$ (mM)	1.0	2.0	3.0	4.0	6.0	8.0
Treated	365 ± 18*	332 ± 11**	304 ± 16**	287 ± 12*	243 ± 19**	172 ± 14**
Untreated	353 ± 13**	311 ± 15**	67 ± 10**	25 ± 12**	24 ± 10**	21 ± 11**
$ZnCl_2$ (mM)	1.0	2.0	3.0	4.0	6.0	8.0
Treated	377 ± 17*	362 ± 11*	329 ± 12**	298 ± 17**	243 ±13**	186 ± 11**
Untreated	369 ± 16	353 ± 14**	317 ± 11**	181 ± 14**	59 ± 9**	26 ± 15**

The experiment was carried out in triplicate and the total protein content in absence of metals was 361 ± 11 µg ml^{-1}. Statistical significance of differences compared to control (without metals).

*Significant at $p < 0.05$.

*Significant at $p < 0.01$.

TABLE 4 Effect of treated and untreated metals on protein content of *S.* aureus S2

Heavy metals	Protein content (μg ml^{-1})					
$HgCl_2$ (mM)	0.1	0.2	0.3	0.4	0.6	0.8
Treated	282 ± 9**	214 ± 14**	109 ± 11**	86 ± 10**	63 ± 16**	35 ± 12**
Untreated	94 ± 13**	57 ± 16**	28 ± 10**	26 ± 10**	27 ± 13**	25 ± 11**
$AgNO_3$ (mM)	0.1	0.2	0.3	0.4	0.6	0.8
Treated	335 ± 12**	316 ± 13*	294 ± 15**	201 ± 10**	77 ± 12**	76 ± 13**
Untreated	318 ± 15**	126 ± 17**	41 ± 10**	29 ± 11**	25 ± 10**	26 ± 14*
$CdCl_2$ (mM)	0.5	1.0	1.5	2.0	3.0	4.0
Treated	369 ± 15*	354 ± 11*	339 ± 14*	312 ± 12**	251 ± 15**	195 ± 13**
Untreated	348 ± 13	354 ± 16**	143 ± 11**	35 ± 16**	28 ± 15*	22 ± 11**
$Pb(NO_3)_2$ (mM)	1.0	2.0	3.0	4.0	6.0	8.0
Treated	369 ± 12*	346 ± 10**	329 ± 13**	302 ± 12**	277 ± 17*	131 ± 14**
Untreated	381 ± 15*	316 ± 13**	123 ± 15**	37 ± 10**	23 ± 11**	25 ± 12**
$CuSO_4$ (mM)	1.0	2.0	3.0	4.0	6.0	8.0
Treated	361 ± 12*	345 ± 16*	323 ± 11**	248 ± 16**	191 ± 14**	115 ± 12**
Untreated	342 ± 14**	149 ± 12**	36 ± 14**	22 ± 11**	26 ± 10**	28 ± 12*
$ZnCl_2$ (mM)	1.0	2.0	3.0	4.0	6.0	8.0
Treated	374 ± 14	351 ± 10*	328 ± 11**	286 ± 13**	235 ± 10**	176 ± 13*
Untreated	359 ± 11*	347 ± 10**	138 ± 15**	36 ± 12**	28 ± 13*	29 ± 10**

The experiment was carried out in triplicate and the total protein content in absence of metals was 373 ± 14 μg ml^{-1}. Statistical significance of differences compared to control (without metals).
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.

metal in the environment either through solubilization or precipitation. On the whole, the ability of a microorganism to resist the toxic metals is often associated with their ability to immobilize these metals into insoluble species [31]. Immobilization process reduces the free metal species and may enable metals to be transformed in situ [7].

According to the obtained results of the current study, the immense decline of the toxicity of heavy metals treated with VMPs of *E. coli* Z3 was attributed to the transformation of the soluble metal ions into insoluble metal complexes resulting in a marked diminish of free metal ions. Data in Table 6 demonstrated a complete metal removal with Hg and Pb while the percentage of metal removal of the other metals was 88% with Zn, 87% with Cd, 82% with Ag and 71% with Cu after treating them with the VMPs of *E. coli* Z3. In addition, the obtained EDX data showed the existence of sulfur, nitrogen, and oxygen in the collected metal precipitates that could be assigned to the presence of some biogenic chelating molecules such as sulfide, ammonia, and CO_2 in the VMPs of the bacterial culture.

The capability of *E. coli* Z3 strain to produce hydrogen sulfide under aerobic conditions was confirmed by the formation of black color during their growth on Kligler iron agar. At the same time, data in Fig. 5 showed the presence of various levels of sulfides in water sample exposed to bacterial VMPs that were not observed in abiotic experiment running under similar condition (absence of bacterial VMPs). Sulfide plays a major role in the co-precipitation of heavy metals as metal sulfides that are characterized by low solubility products [32]. Previous studies had confirmed the capability of an assortment of microorganisms for the release of volatile sulfide under aerobic conditions [33,34]. The oxic production of sulfide might confer microbial cells with the ability to survive in presence of high levels of metals through precipitating them into metal sulfides [35]. Moreover, several microbial metabolic pathways of sulfide have been proposed where cysteine desulfhydrase pathway was the most evident [36].

The bacterial precipitation of heavy metals as metal sulfides could not be assigned only to the presence of H_2S but also to the occurrence of thiol compounds in the bacterial VMPs. Thiol-compounds are characterized by their affinity to coordinate and complex various metals in their solutions. In our previous research, dimethyldisulfide was identified in *Klebsiella pneumoniae* M426 biogas. This compound played a chief task in the transformation of mercury and other metals into organo-sulfur metal precipitates [37]. Similarly, El-Shanshoury et al. [38] have attributed the transformation of lead into lead sulfide by *Bacillus anthracis* PS2010 to the

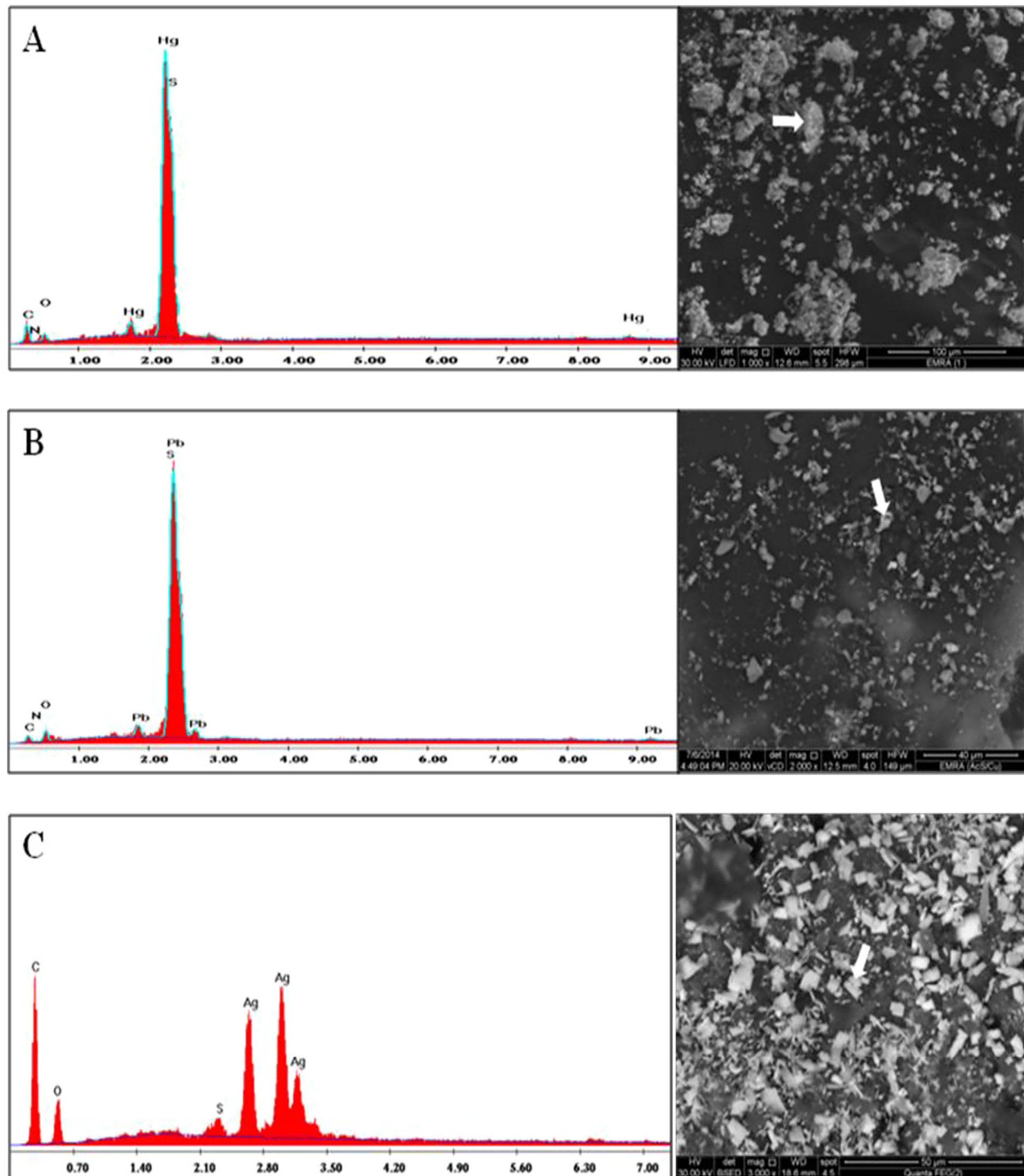

FIGURE 3 Scanning electron microscope (SEM) and energy dispersive X-ray (EDX) analysis of precipitates produced in the bioreactor after 24 h through the interaction of VMPs of *E. coli* Z3 with mercury chloride solution (A), lead nitrate solution (B), and silver nitrate solution(C)

release of dimethyldisulfide from the bacterial cells. Biogenic oxidized volatile sulfur species could be formed through the conversion of methionine, into reduced mercaptans that are transformed rapidly into oxidized volatile sulfur species under aerobic conditions [39].

The obtained results (Fig. 5) clarified the presence of elevated amounts of ammonia in the water sample exposed to bacterial VMPs that were not observed in abiotic experiment running under similar condition. The release of ammonia in addition to some volatile nitrogenous compounds during bacterial growth could be attributed to the enzymatic breakdown of various organic substrates [40]. It is well-known that some metal ions have strong tendency to coordinate with amine groups [41]. Mubarak et al. [42]

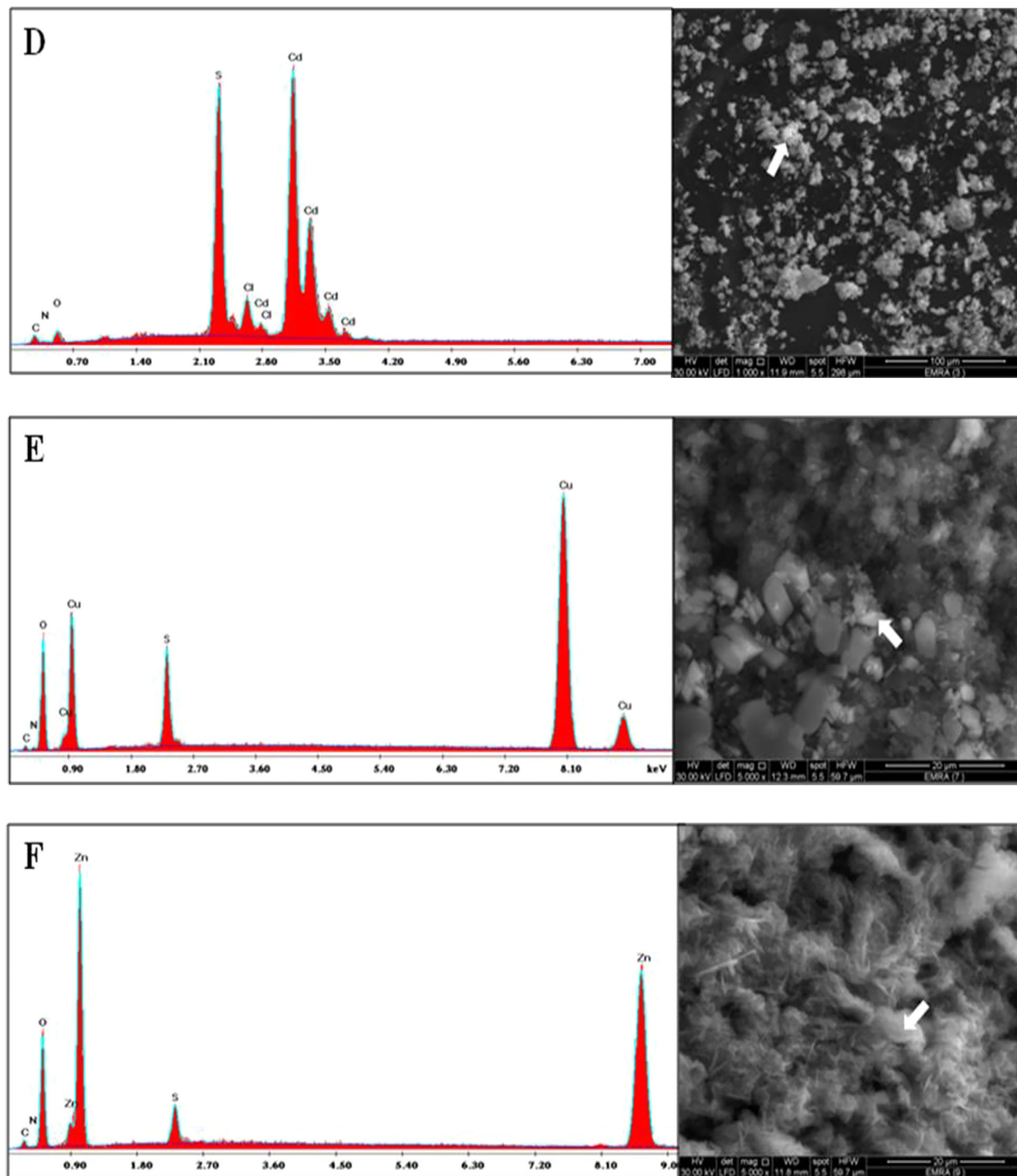

FIGURE 4 Scanning electron microscope (SEM) and energy dispersive X-ray (EDX) analysis of precipitates produced in the bioreactor after 24 h through the interaction of VMPs of *E. coli* Z3 with cadmium chloride solution (D), copper sulfate solution (E), and zinc sulfate solution (F)

have identified the amine groups in nano-silver particles fashioned by some cyanobacterial strains. Similarly, culture VMPs of *K. pneumoniae* with their nitrogenous metabolites were used successfully to precipitate Au(III) ions out of an industrial wastewater [43]. Moreover, the accumulation of ammonia in metal solution could be responsible for the rapid pH change of the precipitation chamber toward alkalinity (pH = 8.5–9.1). Hydrogen ion activity is possibly the key factor affecting metal speciation, solubility, and ultimately bioavailability of metals in aqueous solutions. The shifting pH toward alkalinity might induce the formation of metal hydroxide where the solubility of metal hydroxide minerals decreases with increasing pH value [44]. Additionally, high ammonia concentration in metal solution can

TABLE 5 Elemental composition of the metal precipitates determined by energy dispersive X-ray analysis (EDX)

	Metals precipitates					
Elemental composition (wt%)	**Hg**	**Pb**	**Cd**	**Zn**	**Cu**	**Ag**
Metal	72.6 ± 2.7	77.8 ± 1.8	63.5 ± 1.3	57.8 ± 0.9	61.6 ± 1.8	71.5 ± 1.7
Carbon	11.5 ± 0.5	7.8 ± 1.1	7.1 ± 0.8	7.8 ± 0.4	4.0 ± 0.9	8.5 ± 0.4
Nitrogen	3.9 ± 0.7	0.2 ± 0.1	1.6 ± 0.7	1.0 ± 0.3	1.9 ± 0.6	0.0
Oxygen	5.2 ± 0.6	4.8 ± 0.7	8.6 ± 0.8	25.7 ± 1.3	23.1 ± 0.8	8.2 ± 0.4
Sulfur	6.6 ± 0.8	9.2 ± 0.5	18.9 ± 1.4	7.4 ± 0.6	9.1 ± 0.7	11.6 ± 0.9

The experiment was carried out in triplicate and values are means ± standard error.

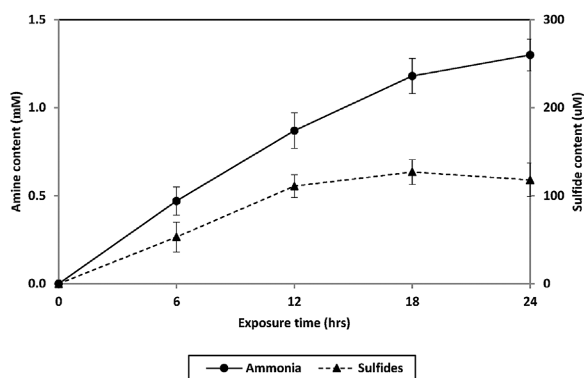

FIGURE 5 Ammonia and sulfide contents in the deionized water sample exposed to *E, coli* Z3 culture off-gas for 24 h in the bioreactor. The experiment was carried out in triplicate and values are means (M) of replicates ± standard error

induce the formation of ammonia-metal complexes with various metals. Some of these complexes are water soluble such as tetraammine-copper(II) where the amminomercury complex is an example for insoluble ammonical-metal complexes [43,45].

Likewise, the current study clarified the presence of elevated levels (5.8-fold) of CO_2 in the VMPs. The biogenic CO_2 that is liberated during microbial catabolism could be found in aqueous solutions in the form of carbonate ions under alkaline conditions. It is known that carbonate ions have an affinity to co-precipitate some cations into metal carbonates [46,47]. The XRD analysis showed the presence of Hg and Pb carbonate (data not shown). Microorganisms are known to induce carbonate precipitation by altering solution chemistry through a wide range of metabolic pathways such as photosynthesis, ammonification, and denitrification [48].

In conclusion, the present study highlighted the transformation of metal ions into metal precipitates as a mechanism through which *E. coli* Z3 can indirectly reduce the toxicity of heavy metals. The bacterial transformation of heavy metals depends on the discharge of various volatile metabolic products including nitrogenous compounds, hydrogen sulfide, organothiol compounds, and CO_2 (Fig. 6). Hydrogen sulfide and volatile organothiols tend to coordinate metal ions into sulfur-based metal precipitates. Simultaneously, the presence of ammonia in the bacterial biogas affects on the metal solutions in two ways. Firstly, it can precipitate some metal ions as nitrogen-based complexes. Secondly, the accumulation of ammonia shifts pH of metal solutions toward alkalinity that participate in the formation of water insoluble metal hydroxides. Moreover, the microbial capability of producing elevated levels of CO_2 in parallel with the increase of pH value favors the deposition of some cations as metal carbonates. More studies would be required to identify the VMPs components of different Gram-negative and Gram-positive bacterial strains and to investigate their role in the heavy metal transformation process. At the moment, another study is running aiming to purify and upload certain

TABLE 6 Residual metal concentration after treating metal solutions with the VMPs of *E. coli* Z3 for 24 h in the bioreactor

Heavy metals	Initial concentration (ppm)	Residual concentration (ppm)	Percent of metal removal
$HgCl_2$	69 ± 9	BDL	100
$AgNO_3$	42 ± 4	7 ± 8	82
$CdCl_2$	70 ± 8	9 ± 5	87
$Pb(NO_3)_2$	47 ± 6	BDL	100
$ZnSO_4$	88 ± 5	11 ± 7	88
$CuSO_4$	63 ± 7	18 ± 6	71

The experiment was carried out in triplicate and values are means ± standard error where BDL indicates that metal concentration is below detection limit.

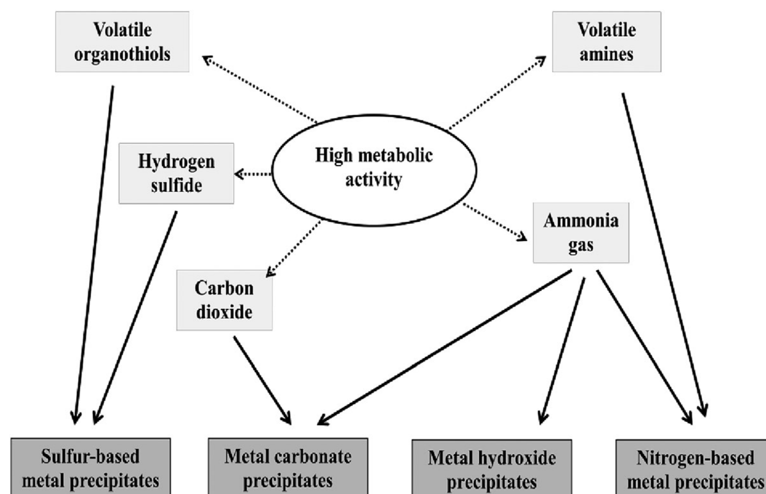

FIGURE 6 Schematic representation for the indirect heavy metal precipitation by *E. coli* Z3

ingredients of the *E. coli* Z3 VMPs onto organic carriers in order to apply these composite structures for the removal of heavy metals out of industrial wastewater.

ACKNOWLEDGMENTS

The authors gratefully acknowledge all the staff of the Biology Department, Faculty of Science, Jazan University, especially Dr. Yahya Masrahi and Dr. Hassien Elnashrei.

CONFLICTS OF INTEREST

No conflict of interest to be declared.

ORCID

Ashraf M. M. Essa http://orcid.org/0000-0001-9050-2685

REFERENCES

[1] Oves M, Khan S, Qari H, Felemban N. Heavy metals: biological importance and detoxification strategies. J Bioremed Biodeg 2016;7:334.

[2] Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 2008;82:493–512.

[3] Patlolla A, Barnes C, Yedjou C, Velma V. Oxidative stress, DNA damage and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. Environ Toxicol 2009;24:66–73.

[4] Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. Molecular, clinical and environmental toxicology. Basel: Springer; 2012. pp 133–64.

[5] Nies DH. Microbial heavy metal resistance. Appl Microbiol Biotechnol 1999;51:730–50.

[6] Kachur AV, Koch CJ, Biaglow JE. Mechanism of copper catalyzed oxidation of glutathione. Free Radic Res 1998;28:259–69.

[7] Gadd GM. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 2010;156:609–43.

[8] Porcheron G, Garenaux A, Proulx J, Sabri M. Iron, copper, zinc, and manganese transport and regulation in pathogenic Enterobacteria: correlations between strains, site of infection and the relative importance of the different metal transport systems for virulence. Front Cell Infect Microbiol 2013;3:90.

[9] Hetzer A, Daughney CJ, Morgan HW. Cadmium ion biosorption by the thermophilic bacteria *Geobacillus stearothermophilus* and *G. thermocatenulatus*. Appl Environ Microbiol 2006;72:4020–7.

[10] Teitzel GM, Parsek MR. Heavy-metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Appl Environ Microbiol 2003;69:2313–20.

[11] Khan S, Hesham AL, Qiao M, Rehman S. Effects of Cd and Pb on soil microbial community structure and activities. Environ Sci Pollut Res 2010;17:288–96.

[12] Kunst F, Ogasawara N, Moszer I, Albertini AM. The complete genome sequence Gram-positive bacterium *Bacillus subtilis*. Nature 1996;390:249–56.

[13] Rosen BP. Biochemistry of arsenic detoxification. FEBS Lett 2002;529:86–92.

[14] Fomina M, Charnock JM, Hillier S, Alvarez R. Role of fungi in the biogeochemical fate of depleted uranium. Curr Biol 2008;18:375–7.

[15] Park JH, Bolan N, Megharaj M, Naidu R. Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. J Hazard Mater 2011;185:829–36.

[16] Zhou Q, Chen Y, Yang M, Li W. Enhanced bioremediation of heavy metal from effluent by sulfate-reducing bacteria with copper-iron bimetallic particles support. Bioresour Technol 2013;136:413–7.

[17] Bunge M, Araghipour N, Mikoviny T, Dunkl J. On-line monitoring of microbial volatile metabolites by Proton Transfer Reaction-Mass Spectrometry. Appl Environ Microbiol 2008;74:2179–86.

[18] Schafer H, Myronova N, Boden R. Microbial degradation of dimethylsulfide and related C1-sulfur compounds: organisms and pathways controlling fluxes of sulfur in the biosphere. J Exp Bot 2010;61:315–34.

[19] Essa AM, Macaskie LE, Brown NL. A new method for mercury removal. Biotechnol Lett 2005;27:1649–55.

[20] Essa AMM, El-Said EM, Ali RM. Biogenic volatile compounds of the activated sludge and application for metal bioremediation. Afr J Biotechnol 2012;11:9993–10001.
[21] Essa AM. The effect of continuous mercury stress on mercury reducing community of some characterized bacterial strains. Afr J Microbiol Res 2012;6:4006–12.
[22] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1989.
[23] Edwards U, Rogall T, Blocker H, Emde M. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 1989;17:7843–53.
[24] Essa AM, Radwan TE, Ibrahim WM, Reyad AM. Biodegradation of the organophosphorus insecticide diazinon by *Pseudomonas aeruginosa* isolated from agricultural drainage ditches. Egypt J Bot 2016;56:353–70.
[25] Perez C, Pauli M, Bazerque P. An antibacterial assay by agar well diffusion method. Acta Bio Med Exp 1990;15:113–5.
[26] Smith PK, Krohn RI, Hermanson GT, Mallia AK. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85.
[27] Essa AM, Khallaf MK. Antimicrobial potentiality of consolidation polymers impregnated with copper nanoparticles. BMC Microbiol 2016;16:144. https://doi.org/10.1186/s12866-016-0766-8
[28] Tylko G, Banach Z, Kilarski W. PROZA and calibration curves for quantitative X-ray microanalysis of biological samples. Microchim Acta 2004;144:271–6.
[29] Siegel LM. A direct microdetermination for sulfide. Anal Biochem 1965;11:126–32.
[30] Jeong H, Park J, Kim H. Determination of NH_4^+ in environmental water with interfering substances using the modified Nessler method. J Chem 2013;2013:1–9. Article ID 359217.
[31] Majumder A, Bhattacharyya K, Bhattacharyya S, Kole SC. Arsenic-tolerant, arsenite-oxidising bacterial strains in the contaminated soils of West Bengal, India. Sci Total Environ 2013;46:1006–14.
[32] Chang JC. Solubility product constants. In: Lide DR, editor. CRC handbook of chemistry and physics. Boca Raton: CRC Press; 1993. pp 8–39.
[33] Essa AM, Macaskie LE, Brown NL. Mechanisms of mercury bioremediation. Biochem Soc Trans 2002;30:672–4.
[34] Lefebvre DD, Kelly D, Budd K. Biotransformation of Hg(II) by cyanobacteria. Appl Environ Microbiol 2007;73:243–9.
[35] Sinha S, Mukherjee SK. *Pseudomonas aeruginosa* KUCD1, a possible candidate for cadmium bioremediation. Braz J Microbiol 2009;40:655–62.
[36] Edwards CD, Beatty JC, Loiselle JB, Vlassov KA. Aerobic transformation of cadmium through metal sulfide biosynthesis in photosynthetic microorganisms. BMC Microbiol 2013;15:161–71.
[37] Essa AM, Creamer NJ, Brown NL, Macaskie LE. A new approach to the remediation of heavy metal liquid wastes via off-gases produced by *Klebsiella pneumoniae* M426. Biotechnol Bioeng 2006;95:574–83.
[38] El-Shanshoury AR, Elsilk SE, Ateya PS, Ebeid EM. Synthesis of lead nanoparticles by *Enterobacter* sp. and avirulent *Bacillus anthracis* PS2010. Ann Microbiol 2012;62:1803–10.
[39] Engels WM, Alting AC, Arntz MT, Gruppen H. Partial purification and characterization of two aminotransferases from *Lactococcus lactis* subsp. *cremoris* B78 involved in the catabolism of methionine and branched-chain amino acids. Int Dairy J 2000;10:443–52.
[40] Purvina S, Bechemin C, Balode M, Verite C. Release of available nitrogen from river-discharged dissolved organic matter by heterotrophic bacteria associated with the cyanobacterium *Microcystis aeruginosa*. Estonian J Ecol 2010;59:184–96.
[41] Essa AM, Mostafa SM. Biomineralization of some heavy metals by cyanobacterial biogas. Egypt J Bot 2011;31:112–21.
[42] Mubarak D, Sasikala M, Gunasekaran M, Thajuddin N. Biosynthesis and characterization of silver nanoparticles using marine cyanobacterium, *Oscillatoria willei* NTDM01. Dig J Nanomater Biostruct 2011;6:385–90.
[43] Macaskie L, Creamer N, Essa A, Brown N. A new approach for the recovery of precious metals from solution and from leachates derived from electronic scrap. Biotechnol Bioeng 2007;96:631–9.
[44] John DA, Leventhal JS. Bioavailability of metals. In: du Bray EA, editor. Preliminary Compilation of Descriptive Geoenvironmental Mineral Deposit Models. US Department of the Interior US. Geological Survey Open-File Report 95-831. Denver, Colorado 1995.
[45] Dougal JC, Gans P, Gill JB, Johnson LH. Complexation of noble transition metals in liquid ammonia. Pure Appl Chem 1988;60:1731–42.
[46] Dupraz C, Reid RP, Braissant O, Decho AW. Processes of carbonate precipitation in modern microbial mats. Earth Sci Rev 2009;96:141–62.
[47] Wei S, Cui H, Jiang Z, Liu H. Biomineralization processes of calcite induced by bacteria isolated from marine sediments. Braz J Microbiol 2015;46:455–64.
[48] Zhu T, Dittrich M. Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review. Front Bioeng Biotechnol 2016;4:4.

How to cite this article: Essa AMM, Al Abboud MA, Khatib SI. Metal transformation as a strategy for bacterial detoxification of heavy metals. *J Basic Microbiol*. 2017;1–13. https://doi.org/10.1002/jobm.201700143