

**HEAVY METAL ION RESISTANCE AND BIOREMEDIATION CAPACITIES OF
BACTERIAL STRAINS ISOLATED FROM AN ANTIMONY MINE**

BY

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Declaration

I declare that the dissertation hereby submitted to the University of Limpopo for the degree Master of Science has not previously been submitted by me for a degree at this or any other University, that this is my own work in design and in execution, and that all materials contained therein have been duly acknowledged.

Signed: _____

Date: _____

Dedication

This work is fully dedicated to Sekhula M. Joseph, Sekhula K. Maria and to the family of my former high school principal Mrs. P.S. Mohale and the husband Mr. L. Mohale for their support.

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List of Abbreviations

• AAS	-	Atomic absorption spectroscopy
• ABC	-	ATP-binding-cassette
• ADP	-	Adenosine diphosphate
• AEM	-	Analytical electron microscope
• <i>ArsB</i>	-	Arsenic resistance gene B
• <i>ArsB</i>	-	Arsenic resistance protein B
• <i>ArsC</i>	-	Arsenic resistance gene C
• <i>ArsC</i>	-	Arsenic resistance protein C
• <i>ArsD</i>	-	Arsenic co-regulator gene
• <i>ArsD</i>	-	Arsenic co-regulator protein D
• <i>ArsRDABC</i>	-	Arsenic operon
• ATP	-	Adenosine triphosphate
• <i>CadA</i>	-	Cadmium resistance gene A
• <i>CadA</i>	-	Cadmium resistance protein A
• <i>CadC</i>	-	Cadmium resistance gene C
• CDF	-	Cation diffusion facilitator
• Cd-MT	-	Metallothionein-bound cadmium
• <i>Cnr</i>	-	Cobalt/nickel resistance
• <i>CorA</i>	-	Magnesium ion transporter
• CPM	-	Cytoplasmic membrane
• Cyto	-	Cytoplasmic space
• <i>Czc</i>	-	Cadmium, zinc and cobalt resistance
• DNA	-	Deoxyribose nucleic acid

• G1pF	-	Glycerol facilitator
• G-SH	-	Reduced glutathione
• GS-SG	-	Oxidized glutathione
• HoxN	-	Integral membrane protein for Ni
• MgtE	-	Magnesium transport protein E
• MH broth	-	Mueller-Hinton broth
• MHA	-	Mueller-Hinton agar
• MHB	-	Mueller-Hinton broth
• MIC	-	Minimal inhibitory concentration
• MIT	-	Metal inorganic transport
• mRNA	-	Messenger RNA
• NADPH	-	Reduced Nicotinamide adenine denucleotide phosphate
• <i>Ncc</i>	-	Nickel, cobalt and cadmium resistance determinant
• OM	-	Outer membrane
• Peri	-	Periplasmic space
• Pit	-	Phosphate inorganic transport
• PstABC	-	Phosphate transport-ATP-binding cassette
• RNA	-	ribose nucleic acid
• RND	-	Resistance, nodulation and cell division
• SEM	-	Scanning electron microscope
• SH	-	Sulfhydryl group
• <i>Smt</i>	-	Metallothionein operon
• <i>SmtA</i>	-	Metallothionein gene A
• <i>SmtB</i>	-	Metallothionein gene B
• TEM	-	Transmission electron microscope
• TMA	-	TRIS-buffered mineral salt agar medium
• XRD	-	X-ray and electron diffraction analysis

- ZiaA - Zinc exporter protein
- ZntA - Zinc transport protein A

List of symbols

- $\mu\text{g/g}$ - Microgram per gram
- μM - Micromolar
- μm - Micro-meter
- As(III) - Arsenite
- As - Arsenic
- As(V) - Arsenic ion
- As^{3+} - Arsenic ion
- AsO_2^- - Arsenite
- AsO_4^{3-} - Arsenate
- $\text{C}_4\text{H}_4\text{KO}_2\text{Sb} \cdot \frac{1}{2}\text{H}_2\text{O}$ - potassium antimony tatrte
- CaCl_2 - Calcium chloride
- Cd - Cadmium
- Cd^{2+} - Cadmium ion
- $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ - Cadmium chloride
- C_{eq} - Equilibrium concentration
- C_i - Initial concentration
- Co - Cobalt
- Co^{2+} - Cobalt ion
- $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ - Cobalt chloride
- Cr^{2+} - Chromium ion
- Cu - Copper
- Cu^{2+} - Copper ion
- $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ - Copper chloride
- $\text{Fe(III)(NH}_4\text{)} \cdot 12\text{H}_2\text{O}$ - Ferric ammonium sulfate dodecahydrate
- Fe^{2+} - Ferrous ion
- g - grams

• h	-	Hour
• Hg	-	Mercury
• Hg(II)	-	Mercuric ion
• HgO	-	Mercuric oxide
• KCl	-	Potassium chloride
• l	-	litre
• M	-	Mass of dry weight
• MgCl	-	Magnesium chloride
• mM	-	Millimolar
• Mn ²⁺	-	Manganese ion
• Na ₂ HAsO ₄ .7H ₂ O	-	Sodium sulphate anhydrous
• Na ₂ HPO ₄	-	Di-sodium-orthohosphate
• Na ₂ SO ₄	-	Sodium sulphate
• NaAsO ₂	-	Sodium arsenite
• NaCl	-	Sodium chloride
• NH ₄ Cl	-	Ammonium chloride
• Ni	-	Nickel
• Ni ²⁺	-	Nickel ion
• NiCl ₂ .6H ₂ O	-	Nickel chloride
• nM	-	Nanomolar
• °C	-	Degrees Celsius
• q	-	Metal uptake
• rpm	-	Revolution per minute
• Sb	-	Antimony
• Sb(V)	-	Antimony ion
• Sb ⁵⁺	-	Antimony ion
• V	-	Volume
• Zn	-	Zinc
• Zn ²⁺	-	Zinc ion
• ZnCl ₂	-	Zinc chloride

Abstract

Six aerobic bacterial strains [GM 10(1), GM 10 (2), GM 14, GM 15, GM 16 and GM 17] were isolated from an antimony mine in South Africa. Heavy-metal resistance and biosorptive capacities of the isolates were studied. Three of the isolates (GM 15, GM 16 and GM 17) showed different degrees of resistance to antimony and arsenic oxyanions in TYG media. The most resistant isolate GM 16 showed 90 % resistance, followed by GM 17 showing 60 % resistance and GM 15 was least resistant showing 58 % resistance to 80 mM arsenate (AsO_4^{3-}). GM 15 also showed 90 % resistance whereas isolates GM 16 and GM 17 showed 80 % and 45 % resistance respectively to 20 mM antimonate (SbO_4^{3-}). Arsenite (AsO_2^-) was the most toxic oxyanion to all the isolates.

Media composition influenced the degrees of resistance of the isolates to some divalent metal ions (Zn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} and Cd^{2+}). Higher resistances were found in MH than in TYG media. All the isolates could tolerate up to 5 mM of the divalent metal ions in MH media, but in TYG media, they could only survive at concentrations below 1 mM. Also, from the toxicity studies, high MICs were observed in MH media than TRIS-buffered mineral salt media. Zn^{2+} was the most tolerated metal by all the isolates while Co^{2+} was toxic to the isolates.

The biosorptive capacities of the isolates were studied in MH medium containing different concentrations of the metal ions, and the residual metal ions were determined using atomic absorption spectroscopy. GM 16 was effective in the removal of Cu^{2+} and Cd^{2+} from the contaminated medium. It was capable of removing 65 % of Cu^{2+} and 48 % of Cd^{2+} when the initial concentrations were 100 mg/l, whereas GM 15 was found to be effective in the biosorption of Ni^{2+} from the aqueous solutions. It was capable of removing 44 % of Ni^{2+} when the initial concentration was 50 mg/l. GM 17 could only remove 20 % of Cu^{2+} or Cd^{2+} . These observations indicated that GM 16 could be used for bioremediation of

Cu^{2+} and Cd^{2+} ions from Cu^{2+} and Cd^{2+} -contaminated aqueous environment, whereas GM 15 could be used for bioremediation of Ni^{2+} .