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Heavy Metal and Sulfate Tolerance Bacteria Isolated from the Mine Drainage and Sediment of Igun, Osun State, Nigeria

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Abstract

Background*: Mine Drainages (MD) are polluted water associated with mining activities which may contain heavy metals and other chemicals which impact biotic and abiotic factors of such environment. Igun area is known for abandoned mine site with MD. Therefore, the objectives of this study are to determine the physicochemical and microbiological properties of an abandoned mine water and sediment at Igun, and evaluate the heavy metals and sulfate ion tolerance of the indigenous bacteria.*

Materials and Methods*: The Igun MD was divided into grids (4x4 m^2) and from each grid, water and sediment samples were purposively collected in February and October of 2014 to 2016. The chemical analyses of the samples were carried out using APHA methods and compared with the NESREA's recommendation. Microbial analyses of the samples were done using cultural, phenotypic and genomic characteristics to determine the Aerobic Bacteria (AB) and Sulfate Reducing Bacteria (SRB) within the Igun MD. The isolated bacteria were screened for heavy metal and sulfate tolerance using standard methods. Data were analysed using descriptive statistics.*

Results*:The chemical parameters of the mine water samples, except Chromium (2.00mg/L), were low in both February and October samples. Sediment samples in February contained (mg/ kg) Sulphate (37175.58±0.17), Cu (42.50±0.03), Zn (44.97±0.21), Fe (28900.06±0.14), Mn (910.21±0.05), Cr (2280.00±0.17), Co (15.88±0.17), Ni (22.50±0.23) and Au (10.70±0.06), while the October sediment had 78.47±0.12, 21.00±0.04, 51.00±0.21, 22001.00±0.21, 265.50±0.06, 23.00±0.11, 0.50±0.06, 8.00±0.17 and 10.50±0.03, respectively. A total of sixty-nine (69) AB genera present in the water samples were Bacillus (47), Pseudomonas (12), Alcaligenes (3), Staphylococcus (5) and Streptococcus (2). The SRB genera Desulfovibro (2), Desulfotomaculum (4), Desulfobulbus (85), Desulfococcus (3) and Desulfofaba (14) occurred in the sediment samples. Rummeliibacillus stabekisii WED1 (RsWED1) and Desulfobulbus propionicus DS3 recorded the highest tolerance to metal inhibition for Cu^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+} and Fe^{2+} at 250, 300, 250, 450, 200, 450ppm and 100, 450, 650, 150, 100, 650ppm, respectively. The RsWED1 and SRB isolates tolerated up to 120g/l sulfate concentration.*

Conclusion*: The Igun mine drainage contained both aerobic and sulphate-reducing bacteria. Rummeliibacillus stabekisii WED1 (RsWED1) recorded highest metal tolerance to selected metals and sulphate ion.*

Key Word: *Abandoned mine, physicochemical properties, Indigenous bacteria, Rummeliibacillus stabekisii, Desulfofaba gelida.*

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I. Introduction

Environmental pollution became a subject of serious international concern as soon as industry began to evolve. During the global industrial revolution in eighteenth and nineteenth centuries, environmental pollution became a major problem where episodes of high levels of pollutants from various industrial sectors were reported to be associated with increase in diverse chronic health problems such as respiratory and heart diseases and death[1]. Although the quant ity and toxic effects of the pollutants released by various industrial sectors such as iron and steel, textiles and leather, mining and mineral processing, pulp and paper varies, mining andmineral processing facilities produce higher quantity

of hazardous and toxic waste than any other industrial sector[2]. Exposure to such pollutants is a significant genesis of health risks worldwide[3].

The major environmental pollutant of concern with mine drainage are dissolved metals such as Chromium (Cr), Zinc (Zn), Cadmium (Cd), etc., and sulphate ion which are harmful to both ecosystem functions and human health. Heavy metals are widely distributed in almost all types of soils[4], sediment[5], and water bodies[6]. Some heavy metals are essential micronutrients for diverse cellular functions and components of biological macromolecules[7], but can be toxic when accumulated to a certain concentration[4].

Environmental compartments are subject to anthropogenic pressures[8]. As a vital component of aquatic ecosystems, water and sediment microbes play a significant role in the material and energy cycle. However, microbial communities are highly sensitive to environmental changes[9,10]. The excessive heavy metals found in the sediment and water may impose selection pressures on the indigenous microbes[5,6] and even change the diversity of the microbial communities[11,12]. To cope with these situations, an effective strategy for microorganisms is to evolve a system based on biochemical and genetically encoded mechanisms[13]. This has been found in many bacteria strains isolated from different heavy metal polluted scenarios[14,15,16]. Bacteria can thus be used for the remediation of heavy metal polluted areas[13,17]. Nevertheless, present efforts of heavy metal resistance are mainly centered on single isolated strains[15,16].

High sulfate ion concentration is a general characteristic of any mine drainage from sulfide mineral sources.

Sulfate ion concentration has also been reported to be advantageous to some groups of bacteria (sulfate reducing bacteria) as its serves as electron acceptor to produce sulphide in what is called dissimilatory sulphate reduction. Likewise, many bacteria reduce small amounts of sulphates in order to synthesize sulfur-containing cell components; this is known as assimilatory sulphate reduction[18]. Nevertheless, high concentration of sulphate ion in water has been frequently reported to cause various environmental and human health disorder such as odors, metal and concrete corrosion, diarrhea and gastrointestinal problems[19]. However, its damaging mechanisms on microbial cell have been scarcely reported except for its major contribution to electrical conductivity of water. Changes in electrical conductivity can result in variations in osmotic pressure on microbes. High sulfate ion concentration will therefore lead to increased salinity which can retard microbial growth rate and therefore alter the structure of microbial community as a result of the salinity tolerance variations among different microorganisms[20].

A better understanding of the distribution of resistance in heavy metal and sulphate ion contaminated area, especially the long-term polluted sites, is critical to optimize any future remediation schemes.

Igun Gold Mine is one of the ancient mines in Ilesa area Gold field, existing before 1941 when the Nigeria Mining Company started organized mining in the area and the peak production was reached in 1942 at Ilesha goldfield. However, the mining and processing methods employed were crude, primitive, wasteful, and not economical. These left substantial amounts of the gold mineral in the earth since no prospecting was used to support the mining and part of the gold values discarded with the tailings. After the exhaustion of the 'proven' area, everything came to an abrupt end and the mine sites left abandoned around 1995[21].

The objectives of this study are to determine the physicochemical and microbiological properties of an abandoned mine water and sediment at Igun, and evaluate the heavy metals and sulfate ion tolerance of the indigenous bacteria.

II. Materials and Methods

Site Description

The study site is an abandoned mine site located at about 18km south of Ilesa in Atakunmosa West Local Government Area. It has a compass direction of latitude N 7^{o} 31' 25" and longitude E 4^{o}, 40' 22". The size of the open pit is about 180metres by 102metres and the average depth is between 4.5metres and 5.3metres. The area is within one of the six (6) classes of the Basement Complex rock that is from slightly migmatised to non-migmatised, meta-sedimentary and meta-igneous rock or simply called the schist belt[22]. It is one of the eleven (11) schist belts that form part of the major

petrologic units of the Ife-Ilesa schist belt reported by Ademeso *et al.*[22]. The area was reported to have two contrasting lithologies separated by Ifewara fault zone. The west of the fault was reportedly occupied by the amphibole schist, amphibolites, talc- tremolite and pellitic rocks. The eastern part was said to have quartzite, quartz schist and amphibole schist. Gold deposit also occurs in the eastern area where Igun is also located[22].

During the course of this research, the site is almost surrounded with forest. The water is being used for bathing and gold washing. The bottom of the drainage is covered with mud and leaf litters while the South- South- Western side is rocky. The water is being used domestically for washing clothes, fishery and swimming but some community members said it do generate skin irritation sometimes.

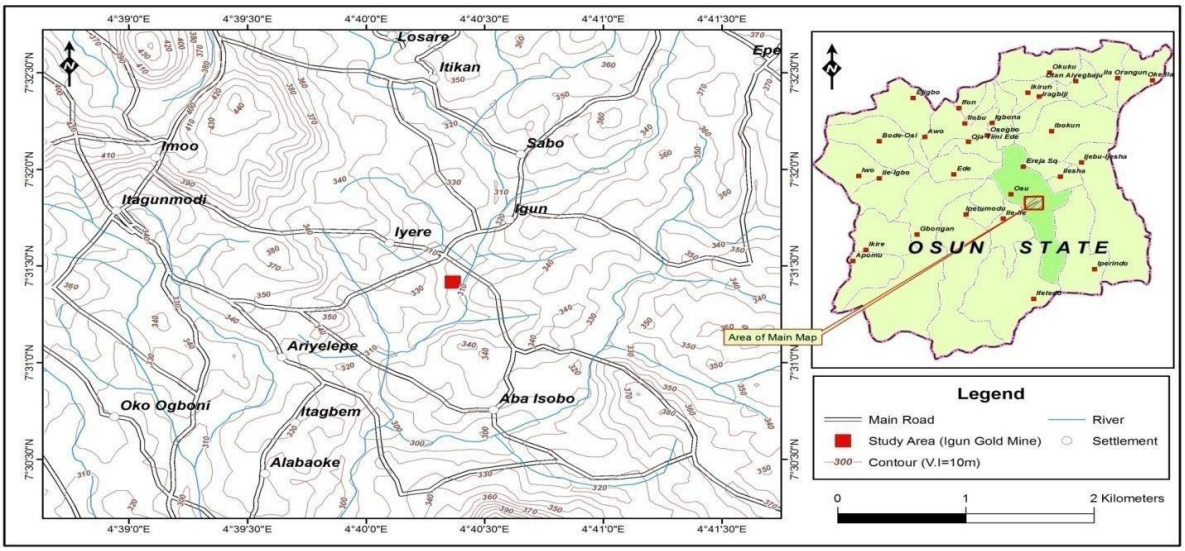


Figure 1: Map of the study area

Water and Sediment Sample collection from Abandoned Igun Gold Mine Site

The abandoned Igun goldmine site was divided into grids of 4m x 4m square, water and sediment samples were purposively collected during dry (February) and raining (October) season between 2014 and 2016. The water samples were collected in 2-lltre polythene bottles previously washed with detergents and disinfected with Hypochlorite solution. The metal composition of the water samples was preserved by the addition of 2 mL concentrated Nitric acid (HNO_3). A van Veen Grab Sampler was used to collect the sediment sample into a stainless basin according to the method of Cavanagh *et al.*[23]. The samples were transported to the Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan in ice chests for the isolation of bacteria immediately after sample collection. Samples were kept under refrigeration condition at $4^{o}C$ prior to other analysis. The samples for the physicochemical parameters were delivered same day to the laboratory for analysis.

Determination of Physical and Chemical Parameters of Water and Sediment Samples

The physicochemical parameters of the water and sediment samples were determined in duplicates using the standard and classical titrimetric method of APHA[24].

Aerobic Bacterial Isolation from Water and Sediment Sample.

Water and Sediment sample enrichment was done with addition of sterile Peptone water in 1:1 ratio overnight. The samples (fresh and enriched sample) were serially diluted using distilled water as the diluent and 1 mL aliquot of the selected dilutions was plated out on Nutrient agar (Lab M, IDG diagnostics UK); using the standard pour plate technique in duplicates. Incubation of the plates was done at room temperature for 24-48 hours. The plates were observed for growth after the incubation period.

Sulfate-Reducing Bacterial Isolation from Sediment Sample

Sulfate reducing bacterial isolation from the enriched sediment sample and purification was done by agar deep dilution method using Postgate C molten agar medium[25]. The agar tube were set, overlaid with 1ml of freshly prepared Postgate C molten agar medium and then incubated at 28±2^{o}C for 3 to 5 days. The tubes were observed for growth after the incubation period.

Bacterial Isolate Purification

Morphologically distinct colonies of aerobic bacteria were picked and subcultured into fresh Nutrient agar plates by repeated streaking to obtain pure cultures. For the sulfate reducing bacteria, distinct SRB colonies were separated by breaking the tube at a convenient point and colonies were withdrawn with a fine Pasteur pipette. Each colony was break up in 0.5ml sterile saline. The suspensions were observed under microscope using

x40 objective. Pure suspensions were used as inoculum into tubes of freshly prepared Postgate C medium broth. The pure cultures of aerobic bacteria obtained were stored on Nutrient agar slants at 4^{o}C and Peptone-Glycerol broth (containing 15% glycerol) at -10^{o}C while the pure cultures of sulfate reducing bacterial obtained were stored in Postgate C medium broth at 4^{o}C for further studies.

Morphological, Biochemical and Sugar Fermentation Test

The bacterial isolates were subjected to morphological, biochemical and sugar fermentation tests to determine their probable identity. The tests were Gram staining, Spore staining, Motility test, Indole test, Catalase test, Citrate test, Motility test, Methyl red test, Voges-proskaur test, Starch hydrolyses test, Casein hydrolysis test, Gelatin hydrolysis test, Oxidase test, Hydrogen sulfide production test, Urease test, Ammonia production test, Nitrate Reduction test and fermentation of different sugars using the method of Olutiola *et al.*[26].

Determination of Electron donor and Acceptor Requirement for Growth of Sulfate- Reducing Bacterial Isolates

Utilization of electron donors and acceptors for growth by selected sulfate-reducing bacterial isolates was tested using a medium designated as the 'defined medium' generally used for physiological characterization of sulfate-reducing bacteria[27]. Ascorbic acid (0.01%) and thioglycolic acid (0.01%) were added to the 'defined medium' and overlaid with paraffin oil to ensure anaerobic conditions in the medium. The pH was adjusted to 7.0-7.2 with 1 M NaOH. The medium was inoculated with 10% (v/v) of 1.0 McFarland standard (approximately 3.0 x 10^{8} cells) of the suspension of each selected SRB of 24 hour old. Incubation was done at 28±2 ^{o}C for 14 days. Subsequent culture transfer to a freshly prepared medium containing the same composition was done twice to ascertain electron donor/acceptor utilization. SRB growth was determined by the appearance of black precipitate of iron sulfide (FeS) after the incubation period. The electron donors tested were acetate, formate, propionate, butyrate, Iso-butyrate, valerate, Iso-valerate and lactate each at final concentration of 20 mM[27]. Sodium salts of sulfate (20 mM), sulfite (2 mM), thiosulfate (20 mM), nitrate (20 mM) or elemental sulfur (0.1%)[28] was added as possible electron acceptor however with a sulfate-free 'defined medium' that contained chloride at the same concentration as that of sulfate in the defined medium; sodium lactate (20 mM) served as an electron donor.

Desulfoviridin Production by Sulfate-Reducing Bacterial Isolates

The presence of desulfoviridin pigment in the morphologically distinct selected SRB isolates was tested by scooping the concentrated broth culture of each selected isolate with a cotton-tipped swab and 1 drop of 2 N NaOH was directly added onto the swab. The reaction was immediately observed in a dark box under UV light at 630 nm. A red fluorescence was taken as indicating the presence of desulfoviridin pigment[29].

Screening of bacteria Isolates for Heavy Metal Resistance

The heavy metals tested against the isolates obtained from the mine drainage water and sediment samples were copper (Cu), chromium (Cr), Cobalt (Co), Nichel (Ni), Zinc (Zn), Iron (Fe) and Manganese (Mn). Soluble salts of the following heavy metals e.g $CuCl_2$, Cr-KSO_4, $CoSO_4$, $NiCl_2$, $ZnSO_4$, $FeSO_4$ and $MnSO_4$ were used for the preparation of the metal solution. Stock solutions of the

metals (1000ppm) were prepared using the method of Cervantes *et al.*[30]. Selected bacterial isolates were screened on metal-supplimented medium to check for their resistance to increasing concentration of the seven selected heavy metals. Agar dilution method of Cervantes *et al.*[30] was used for the heterotrophic aerobic bacterial isolated. Sterilized solutions of prepared heavy metals were incorporated in to sterilized, molten nutrient agar, mixed gently and then poured in to sterile plates. Looful of 18-24 hours old culture of each selected bacterial isolate was streaked on the metal- supplemented medium and incubated at 28±2^{O}C. The plates were observed for growth till 48 hours and recorded. The observation of no visible growth on the metal-supplemented medium after 72 hours was regarded as 'no growth'. The concentrations of each heavy metal were increased from the initial concentration of 50ppm with a 50ppm at a time. The bacteria growing on each concentration was transferred to the next concentration until it failed to grow. The metal concentration at which bacterial failed to show any visible growth on the medium was taken as its Minimum Inhibitory Concentration (MIC) for its growth.

For the selected sulfate reducing bacteria, broth dilution method of Kieu[31] was used. Solutions of prepared heavy metals were incorporated in to Postgate C broth medium in test-tubes, mixed gently, corked, sterilized and allowed to cool. A 10% (v/v) of 1.0 McFarland standard (approximately 3.0 x 10^8 cells) broth culture of each selected sulfate reducing bacterial isolates (24 hours old) were inoculated into the metal-supplementaed medium and incubated at 28±2^{O}C. The tubes were observed for growth till 14 days and recorded. The concentrations of each heavy metal were increased from the initial concentration of 50ppm with a 50ppm increment at a time. The bacteria growing on each concentration was transferred to the next concentration until it failed to grow. The metal concentration at which bacterial failed to growth by showing black precipitate of iron sulfide (FeS) in the medium was taken as the Minimum Inhibitory Concentration (MIC) for its growth. The formation of black precipitate of iron sulfide (FeS) after the incubation period was regarded as 'bacterial growth'.

Screening of bacteria Isolates for Sulfate Tolerance

The broth dilution method of Al-Zuhair *et al.*[19] was used to screen the selected aerobic bacterial isolates on sodium sulfate-supplemented medium to check for their tolerance to increasing concentration of sulfate ion. For the heterotrophic aerobic bacteria, different quantities of sodium sulfate salt were incorporated in to peptone water solution to form solutions of different initial concentrations of Na_2SO_4, between 50 and 120g/l. The solutions were stirred for total dissolution and dispensed in test-tubes, sterilized and allowed to cool. Loopful of 18-24hrs old culture of the isolates were inoculated in to the sodium sulfate-supplemented medium and incubated at 28±2^{O}C. Bacterial growth was monitored at 24 hours intervals by measuring their Optical Density (OD) at a wavelength of 620 nm using photoelectric colorimeter (model T11D, Techmel and Techmel, USA).

For the selected sulfate reducing bacteria, the same concentrations and preparation of sodium sulfate used for the aerobic bacterial isolates were followed, however, in Postgate C medium (pH 7.0±2 with 1 M NaOH). A 10% (v/v) of 1.0 McFarland standard (approximately 3.0 x 10^8 cells) broth culture of each sulfate reducing bacterial isolates (24 hours old) were inoculated in to the sodium sulfate-supplemented medium and incubated at 28±2^{O}C. The tubes were observed for growth after 14 days and record was made. The formation of black precipitate of iron sulfide (FeS) after the incubation period was regarded as 'bacterial growth'.

Molecular Identification of Heavy Metal Resistant and Sulfate Tolerant Bacterial Isolates.

Bacterial isolates which were able to show resistance to high concentration of the selected heavy metals and tolerate high concentration of sulphate ion, and for the sulfate reducing bacteria, were able to utilize the tested electron donors were selected for molecular (16S rRNA) identification. Genomic DNA was extracted from 5 mL aliquots of overnight Nutrient broth and Postgate C medium culture of each selected bacterial isolate using a ZR 96 Fungi/Bacterial DNA Kit^{TM} (Zymo Research Corporation, USA). The supernatant (5 µL) containing soluble DNA was used for the PCR reactions.

Amplification of the 16S rRNA gene fragments was carried out with the universal primer pair: 27F, (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R, (5' CGGTTACCTTGTTACGACTT 3'). PCR was performed using Dream Taq^{TM} DNA polymerase (Thermo Scientific, USA) according to the manufacturers' instructions and the protocols adopted included an initial denaturation at

95^{O}C for 2 minutes; 35 cycles of 95^{O}C for 15 seconds, annealing at 53^{O}C for 25 seconds and elongation at 72^{O}C for 25 seconds; with a final extension step of 72^{O}C for 5 minutes. Amplicons were resolved on 1.5% agarose gels under UV lights for 45 minutes and the size of the amplicons were verified using a 1Kb DNA ladder (Thermo Scientific, USA). PCR products of the expected size were excised and purified using theZymoclean^{TM} Gel DNA Recovery Kit (Zymo Research Corporation, USA). The PCR products were sequenced on the ABI PRISM^{TM} 3500xl Genetic Analyser. Purified sequencing products (Zymo Research, ZR-96DNA Sequencing Clean-up Kit^{TM}) were analysed using CLC Main Workbench 7. The 16S rRNA sequences were used in BLAST Searches to identify similar sequences using the nucleotide collection of the National Centre for Biotechnology Information (NCBI).

III. Results

Physicochemical Characteristics of Water and Sediment Samples

The physicochemical characteristics of the water and sediment samples obtained in the dry (February, between 2014 and 2016) and raining (October, between 2014 and 2016) season are shown in Table 1. All the physicochemical parameters of the water samples were within the National Environmental Regulations (2009) standard for industrial effluent.

Heavy Metal Composition of the Water and Sediment Samples.

The heavy metal concentration of the water and sediment samples obtained in the dry (February, between 2014 and
2016) and raining (October, between 2014 and 2016) season are shown in Table 2. The chemical compositions of the mine water samples, except Chromium (2.00mg/L), were low in both February and October samples.

Aerobic Bacteria and Sulfate-Reducing Bacteria Isolated.

The numbers of the different aerobic bacterial isolate species from the samples are shown in Table 4 while the sulfate- reducing bacterial isolates obtained from the samples are shown in Table 5. The photomicrographs of the sulfate- reducing bacterial isolates are shown in Plate 1. The total aerobic bacterial isolates obtained was sixty-nine (69) belonging to *Bacillus* strains, *Pseudomonas* strains, *Alcaligenes* strains, *Staphylococcus* strain and *Streptococcus* strain. The most frequently isolated aerobic bacterial species obtained is *Bacillus* sp. (47) while the least (2) is *Streptococcus* strain. The total sulphate reducing bacterial isolates obtained was one hundred and eight (108) belonging to *Desulfobulbus propionicus, Desulfobulbus elongates, Desulfotomaculum* sp., *Desulfococcus* sp., *Desulfococcus multivorans, Desulfofaba* sp. *and Desulfovibrio* sp. *Desulfobulbus propionicus* (74) was the most frequently isolated species of the sulfate-reducing bacteria while *Desulfococcus multivorans* (1) was the least.

Table 1: Mean Physicochemical Properties of the Water and Sediment Samples between 2014 and 2016

Property	Dry Season	Raining Season				NER Standard (2009) for Industrial Effluent
		Water Sample	Sediment Sample	Water Sample	Sediment Sample	
Temperature (^{O}C)		28.00±0.02^{c}	23.00±0.04 ^{c}	26.00±0.01 ^{c}	22.00±0.11^{c}	<40^{O}C
pH		6.20±0.03 ^{b}	5.45±0.07 ^{a}	6.60±0.04 ^{b}	6.70±0.12 ^{b}	6-9
Conductivity (EC) (µS/cm)		89.00±0.11 ^{e}	150.00±0.17 ^{e}	39.00±0.12 ^{d}	119.50±0.23 ^{e}	
Total Dissolved		56.00±0.12 ^{d}	99.00±0.05 ^{d}	28.00±0.14 ^{c}	69.00±0.17 ^{d}	2,000
					8.92±0.03 ^{b}	
Acidity		0.29±0.01 ^{a}	26.00±0.14 ^{c}	0.06±0.03 ^{a}	29.64±0.05 ^{c}	
Alkalinity Dissolved Oxygen		7.96±0.02 ^{b}	ND	8.19±0.02 ^{b}	ND	
Organic Carbon(%)		0.60±0.14 ^{a}	2.55±0.17 ^{a}	0.29±0.11 ^{a}	2.00±0.12 ^{a}	
Sulphate		0.80±0.04 ^{a}	37175.58±0.17 ^{g}	4.61±0.23 ^{b}	78.47±0.12 ^{d}	500mg/l
Calcium (Ca)		5.79±0.01 ^{b}	40.12±0.11 ^{cd}	2.10±0.06 ^{a}	66.50±0.21 ^{d}	200mg/l
Magnesium (Mg)		8.50±0.14 ^{b}	2949.76±0.12 ^{f}	11.65±0.21 ^{bc}	1507.00±0.23 ^{e}	200mg/l

ND=Not detected.

Concentrations are in mg/l (water sample) and mg/kg (sediment sample) except Temp, pH, EC and Org. Carbon)

Values are in mean ± standard deviation. Means with different superscripts are significantly different from one another while the means with similar superscripts are no significantly different at p=0.05.

NB: ND signifies Not detected and were regarded as zero hence it was ranked as ”a ”

Table 2: Mean Metal Concentration of the Water and Sediment Samples between 2014 and 2016

Metal	Dry Season		Raining Season		NER Standard (2009) for
	Water Sample	Sediment Sample	Water Sample	Sediment Sample	Industrial Effluent (mg/l)
Copper (Cu)	0.03±0.00 ^{a}	42.50±0.03 ^{c}	ND	21.00±0.04 ^{c}	<1
Zinc (Zn)	ND	44.97±0.21 ^{c}	0.21±0.17 ^{a}	51.00±0.21 ^{d}	<1
Iron (Fe)	1.10±0.12 ^{b}	28900.06±0.14 ^{f}	8.28±0.11 ^{b}	22001.0±0.21 ^{f}	20
Manganese (Mn)	ND	910.21±0.05 ^{d}	0.43±0.02 ^{a}	265.50±0.06 ^{e}	5
Chro miu m (Cr)	2.00±0.12 ^{c}	2280.00±0.17 ^{e}	0.13±0.05 ^{a}	23.00±0.11 ^{c}	<1
Cobalt (Co)	ND	15.88±0.17 ^{b}	ND	0.50±0.06 ^{a}	
Nickel (Ni)	0.18±0.01 ^{a}	22.50±0.23 ^{bc}	ND	8.00±0.17 ^{b}	<1
Gold(Au)	ND	10.70±0.06^{a}	ND	10.50±0.03^{b}	

ND=Not detected.
Concentrations are in mg/l (water sample) and mg/kg (sediment sample) except Temp, pH, EC and Org. Carbon)

Values are in mean ± standard deviation. Means with different superscripts are significantly different from one another while the means with similar superscripts are no significantly different at p=0.05.
NB: ND signifies Not detected and were regarded as zero hence it was ranked as ”a ”

Table 3: Results of Biochemical Characterization and Carbohydrate Utilization Tests for the Sulfate-Reducing Bacterial Isolates

						Electron Donor								Electron Acceptor				Probable Identity
Isolate Code	Shape	Gram Reaction	Motility	Spore Formation	Desulfoviridin	Formate	Acetate	Propionate	Butyrate	Iso-butyrate	Valerate	Iso-valerate	Lactate	Sulfate	Thiosulfate	Sulfite	Sulfur	Nitrate	
DS1	Vibrio	-	Motile	-	+	+	-	-	+	+	-	-	+	+	+	+	-	-	*Desulfovibro* sp.
DS2/ RS1	Rod	+	Motile	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	*Desulfotom aculum* sp.
DS3/ RS2	Ova	-	Non-motile	-	-	-	-	+	-	-	-	-	+	+	+	+	-	+	*Desulfobulbus propionicus*
DS4/ RS3	Rod	-	Motile	-	-	-	-	+	-	-	-	-	+	+	+	+	-	+	*Desulfobulbus elongatus*
RS4	Sphere	-	Non-motile	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	*Desulfococcus multivorans*
DS5	Globe Shape	-	Non-motile	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	*Desulfococcus* sp.
DS6	Slightly Curved Rod	-	Motile	-	-	+	-	+	+	+	-	-	+	+	+	+	-	-	*Desulfofaba* sp.

KEY: D=Dry Season; R=Raining Season; S=Sulfate-reducing Bacteria; +=Present/Growth; - =Absent/No growth

Table 4: Aerobic Bacterial Isolates from the Water and Sediment Sample

Isolate	Water		Sediment		
	Dry Season	Raining Season	Dry Season	Raining Season	Total
Bacillus sp.	11	14	15	7	47
Pseudomonas sp.	4	1	5	2	12
Alcaligenes sp.	1	-	2	-	3
Staphylococcus sp.	1	2	2	-	5
Streptococcus sp.	-	1	-	1	2
Total	17	18	24	10	69

Table 5: Sulfate-Reducing Bacterial Isolates from the Water and Sediment Samples

Isolate			Water Sample		Sediment Sample		Total
Code	Genera	Species	Dry Season	Raining Season	Dry Season	Raining Season	
DS3/RS2	*Desulfobulbus*	*Desulfobulbus propionicus*	ND	ND	42	32	74
DS4/RS3	*Desulfobulbus*	*Desulfobulbus elongatus*	ND	ND	6	5	11
DS2/RS1	*Desulfotomaculum*	*Desulfotomaculum* sp.	ND	ND	2	2	4
DS5	*Desulfococcus*	*Desulfococcus* sp.	ND	ND	2	ND	2
RS4	*Desulfococcus*	*Desulfococcus multivorans*	ND	ND	ND	1	1
DS6	*Desulfofaba*	*Desulfofaba* sp.	ND	ND	14	ND	14
DS1	*Desulfovibrio*	*Desulfovibrio* sp	ND	ND	2	ND	2
		Total	-	-	68	40	108

ND=Not detected, D= Dry season, R=Raining season, S=SRB

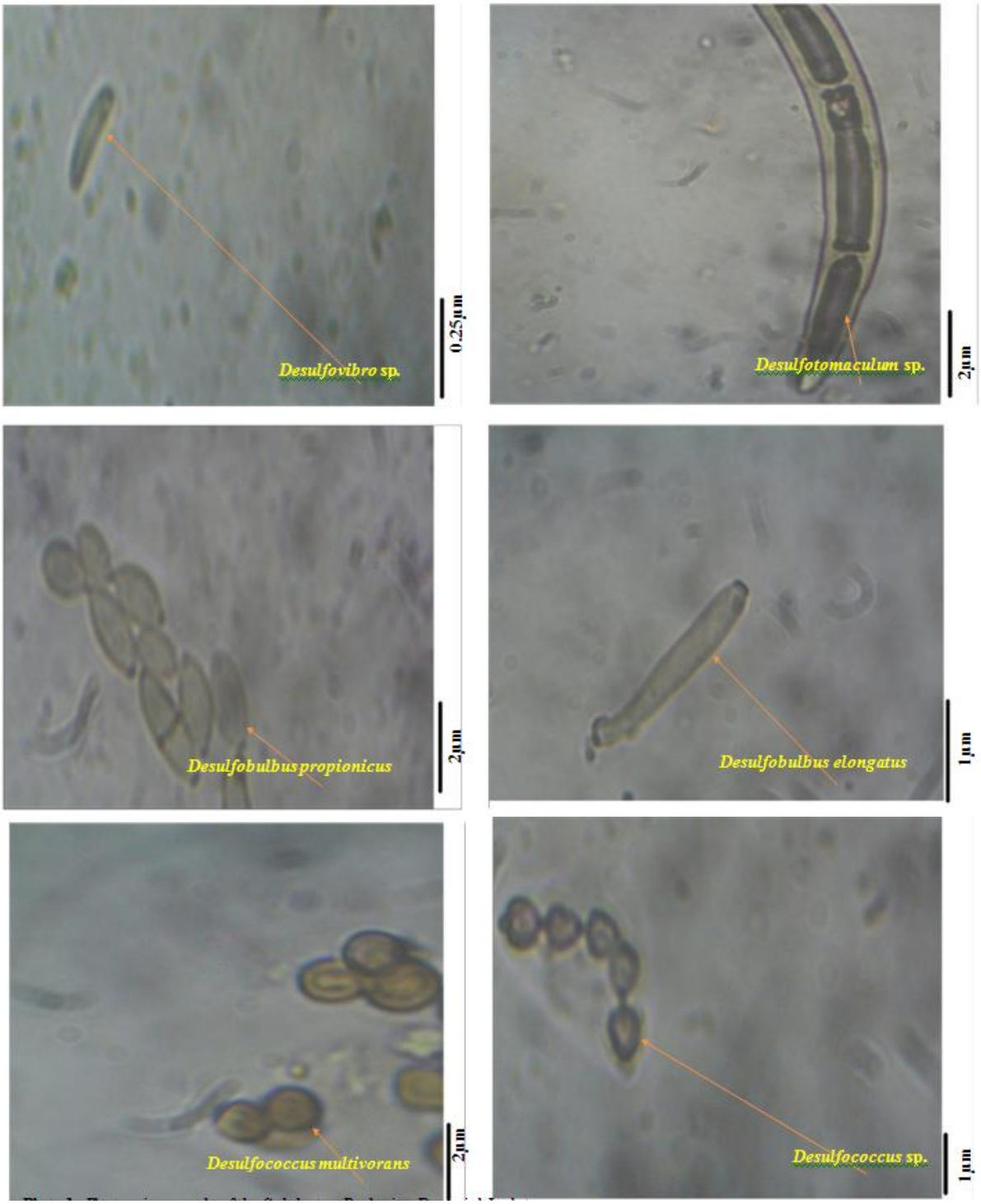


Plate 1: Photomicrograph of the Sulphate - Reducing Bacterial Isolates

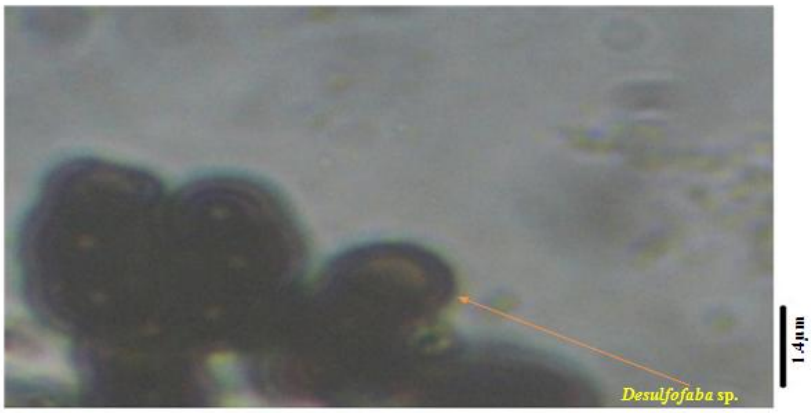


Plate 1(Cont.): Photomicrograph of the Sulphate - Reducing Bacterial Isolates

Minimum Inhibitory Concentration (MIC) of the Heavy Metals on the Isolated Bacteria

The minimum inhibitory concentration (MIC) of the metals on each isolated aerobic bacterium (water and sediment sample) during the dry season and raining season is shown in Table 6a and 6b respectively. All the bacteria showed varied level of resistance to the six metals employed. The MIC value shown by the bacterial isolates during the raining season generally lower compared to the dry season bacterial isolates except for iron. Bacillus sp. (WED1) recorded the highest tolerance to metal inhibition acr o s s the six metals Cu2+, Cr3+, Co2+, Ni2+, Zn2+ and Fe2+ at 250, 300, 250, 450, 200, 450ppm respectively. The MIC of the metals on each isolated sulphate-reducing bacteria during the dry season and raining season is shown in Table 7 . Desulfobulbus propionicus DS3 recorded the highest tolerance to metal inhibition for Cu2+, Cr3+, Co2+, Ni2+, Zn2+ at 100, 450, 650, 150, 100, 650ppm, respectively

Effects of Sulphate Concentration on the Growth of the Isolated Bacteria.

The effect of sulphate concentration on the growth of the selected aerobic bacterial isolates during dry season and raining season is shown in Figure 2D and 2R respectively. The general trend shown by all the aerobic bacterial isolates was that at lower sulfate concentrations, there was no observable effects on the growth of the bacterial isolates while at higher sulfate concentrations the growth of the bacterial isolates were significantly affected. All the dry season aerobic bacterial isolates tolerated sulphate ion concentration to at least 80g/l w hile the raining season isolates tolerated sulphate ion concentration to 40g/l except Bacillus sp. (WER17) that tolerated sulphate ion concentration up to 60g/l . All the sulphate reducing bacteria tolerated the highest (120g/l) sulphate ion concentration tested.

Table 6a: Minimum Inhibitory Concentration (ppm) of the Heavy Metals on the Dry Season (February) Aerobic Bacterial Isolates

Isolate (Water)	Heavy Metals							Isolate (Sediment)	Heavy Metals						
	Cu^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Fe^{2+}	Mn^{2+}		Cu^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Fe^{2+}	Mn^{2+}
Bacillus sp. (WED1)	250	300	250	450	200	450	ND	*Bacillus* sp. (SED1)	150	300	150	400	150	500	ND
Pseudomonas sp.(WED2)	200	300	200	550	200	450	ND	*Pseudomonas* sp. (SED2)	150	300	150	350	100	500	ND
Bacillus sp.(WED3)	200	300	100	450	50	450	ND	*Bacillus* sp. (SED3)	150	300	100	350	100	500	ND
Bacillus sp.(WED4)	200	300	200	550	200	450	ND	*Bacillus* sp. (SED4)	150	300	150	350	100	500	ND
Alcaligenes faecalis (WED5)	200	300	200	500	200	450	ND	*Pseudomonas* sp. (SED5)	150	250	100	250	50	400	ND
Pseudomonas sp.(WED6)	100	300	100	350	50	450	ND	*Bacillus* sp. (SED6)	150	300	100	350	100	500	ND
Bacillus sp. (WED7)	200	300	250	450	200	450	ND	*Pseudomonas* sp. (SED7)	150	300	100	300	100	550	ND
Bacillus sp. (WED8)	150	300	200	400	50	400	ND	*Alcaligenes* sp. (SED8)	150	300	100	200	100	500	ND
Bacillus sp. (WED9)	150	300	200	400	50	450	ND	*Bacillus* sp. (SED9)	150	300	100	300	100	550	ND
Bacillus sp.(WED10)	150	300	200	400	50	450	ND	*Bacillus* sp. (SED10)	150	300	100	200	50	550	ND
Bacillus sp.(WED11)	200	300	200	600	150	400	ND	*Bacillus* sp. (SED11)	150	300	150	350	50	500	ND
Staphylococcus sp(WED12	150	300	100	350	50	400	ND	*Bacillus* sp. (SED12)	150	300	150	300	100	450	ND
Pseudomonas sp.(WED13)	200	300	150	550	150	400	ND	*Staphylococcus* sp. (SED13)	150	250	100	300	50	450	ND
Bacillus sp.(WED14)	200	300	250	500	150	400	ND	*Staphylococcus* sp. (SED14)	200	300	100	300	50	500	ND
Pseudomonas sp.(WED15)	200	300	250	500	200	400	ND	*Bacillus* sp. (SED15)	200	300	100	300	50	500	ND
Bacillus sp.(WED16)	200	300	250	600	200	400	ND	*Pseudomonas* sp. (SED16)	150	300	100	300	50	550	ND
Bacillus sp.(WED17)	150	300	150	350	150	500	ND	*Bacillus* sp. (SED17)	100	250	100	250	50	500	ND
								Bacillus sp. (SED18)	150	300	100	200	50	450	ND
								Bacillus sp. (SED19)	100	250	100	300	50	500	ND
								Bacillus sp. (SED20)	150	300	100	200	50	400	ND
								Bacillus sp. (SED21)	150	300	100	300	100	400	ND
								Pseudomonas sp. (SED22)	150	250	100	200	100	500	ND
								Alcaligenes sp. (SED23)	150	250	100	200	50	400	ND
								Bacillus sp.(SED24)	150	300	100	250	100	400	ND

KEY: ND=Not Determined (> 650ppm concentration tested); W=Water; S=Sediment; E=Aerobic bacteria; D=Dry Season.

Table 6b: Minimum Inhibitory Concentration (ppm) of the Heavy Metals on the Raining Season (October) Aerobic Bacterial Isolates

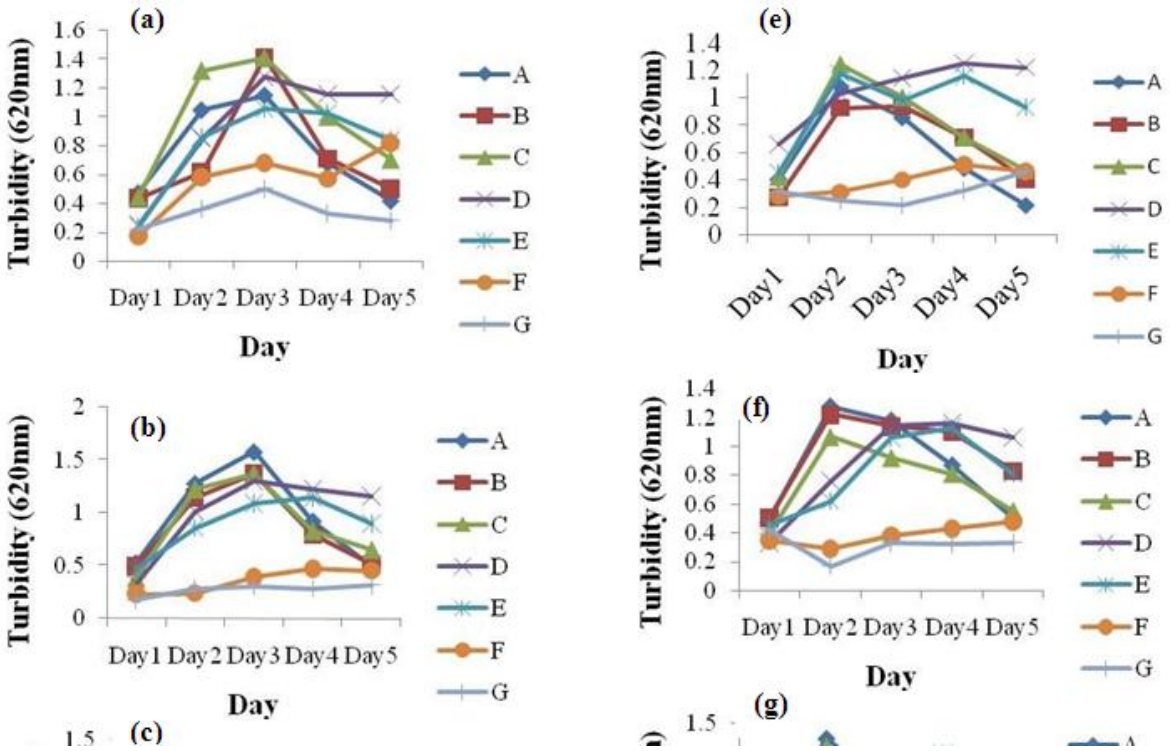
Isolate (Water)	Heavy Metals							Isolate (Sediment)	Heavy Metals						
	Cu^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Fe^{2+}	Mn^{2+}		Cu^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Fe^{2+}	Mn^{2+}
Bacillus sp. (WER1)	200	300	150	200	100	450	ND	*Bacillus* sp. (SER1)	200	300	200	200	100	450	ND
Bacillus sp. (WER2)	200	300	150	150	100	450	ND	*Bacillus* sp. (SER2)	100	300	150	200	50	450	ND
Streptococcus sp.(WER3)	200	350	250	250	250	600	ND	*Pseudomonas* sp. (SER3)	100	250	100	150	100	450	ND
Bacillus sp. (WER4)	150	300	200	200	200	450	ND	*Pseudomonas* sp. (SER4)	200	300	150	200	100	450	ND
Staphylococcus sp. (WER5)	200	300	150	250	250	600	ND	*Bacillus* sp. (SER5)	150	300	150	200	100	450	ND
Bacillus sp. (WER6)	200	300	150	150	100	450	ND	*Bacillus* sp. (SER6)	100	250	150	200	100	450	ND
Bacillus sp. (WER7)	100	300	150	150	50	450	ND	*Bacillus* sp. (SER7)	150	300	150	200	100	450	ND
Bacillus sp. (WER8)	200	300	150	200	150	600	ND	*Bacillus* sp. (SER8)	100	250	150	200	100	450	ND
Pseudomonas sp.(WER9)	200	350	150	200	150	600	ND	*Bacillus* sp. (SER9)	200	250	150	200	100	450	ND
Bacillus sp. (WER10)	150	250	150	150	100	450	ND	*Streptococcus* sp.(SER10)	150	250	150	200	100	450	ND
Bacillus sp. (WER11)	200	300	150	200	150	600	ND								
Bacillus sp. (WER12)	100	250	150	200	100	450	ND								
Bacillus sp. (WER13)	200	300	150	200	50	450	ND								
Bacillus sp. (WER14)	200	250	150	200	50	450	ND								
Bacillus sp. (WER15)	200	350	250	250	200	600	ND								
Staphylococcus sp(WER16	200	300	150	200	100	600	ND								
Bacillus sp. (WER17)	200	300	200	250	200	600	ND								
Bacillus sp. (WER18)	100	300	100	200	100	450	ND								

KEY: ND=Not Determined (> 650ppm concentration tested); W=Water; S=Sediment; E=Aerobic bacteria; R=Raining Season

Table 7: Minimum Inhibitory Concentration (ppm) of the Heavy Metals on the Sulfate-Reducing Bacterial Isolates

Isolate (Dry Season)	Heavy Metals							Isolate (Raining Season)	Heavy Metals						
	Cu^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Fe^{2+}	Mn^{2+}		Cu^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Fe^{2+}	Mn^{2+}
Desulfotomaculum sp. (DS2)	100	350	600	200	50	550	ND	*Desulfotomaculum* sp. (RS1)	100	400	600	200	50	600	ND
Desulfobulbus propionicus (DS3)	100	450	650	150	100	650	ND	*Desulfobulbus propionicus* (RS2)	100	350	500	100	50	550	ND
Desulfobulbus elongatus (DS4)	100	400	500	50	50	650	ND	*Desulfobulbus elongatus* (RS3)	200	300	450	200	100	600	ND
Desulfococcus sp. (DS5)	150	400	600	250	50	550	ND	*Desulfococcus multivorans* (RS4)	100	300	600	50	50	600	ND
Desulfovibro sp. (DS1)	100	400	600	50	50	600	ND								
Desulfotaba sp. (DS6)	50	400	650	200	50	650	ND								

KEY: ND=Not Determined (It is greater than the 650ppm concentration tested); D=Dry Season; R=Raining Season; S=Sulfate- reducing bacteria



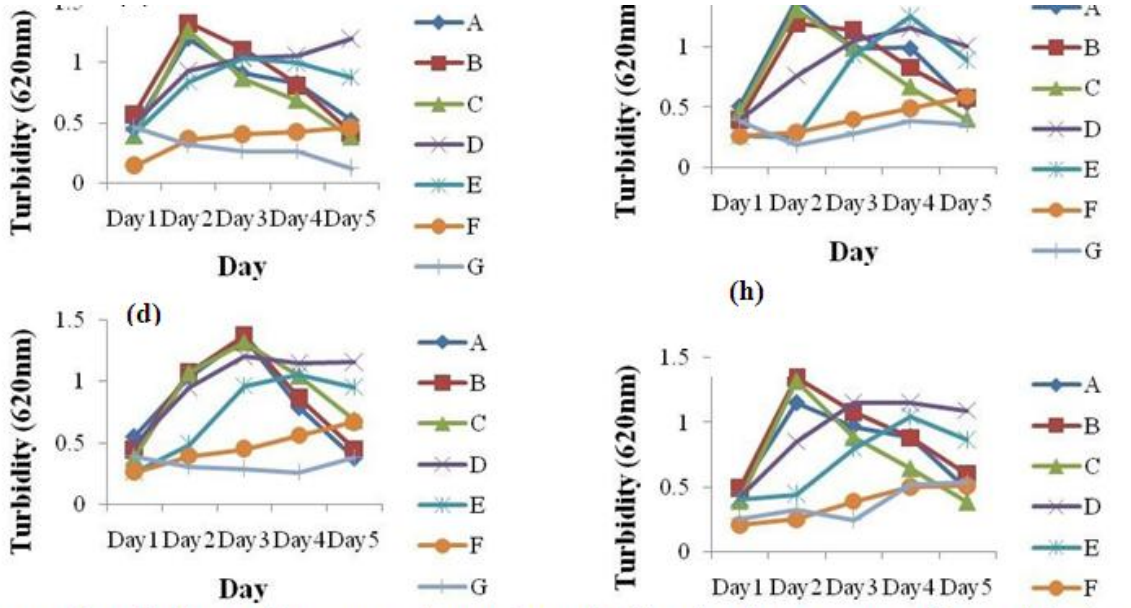
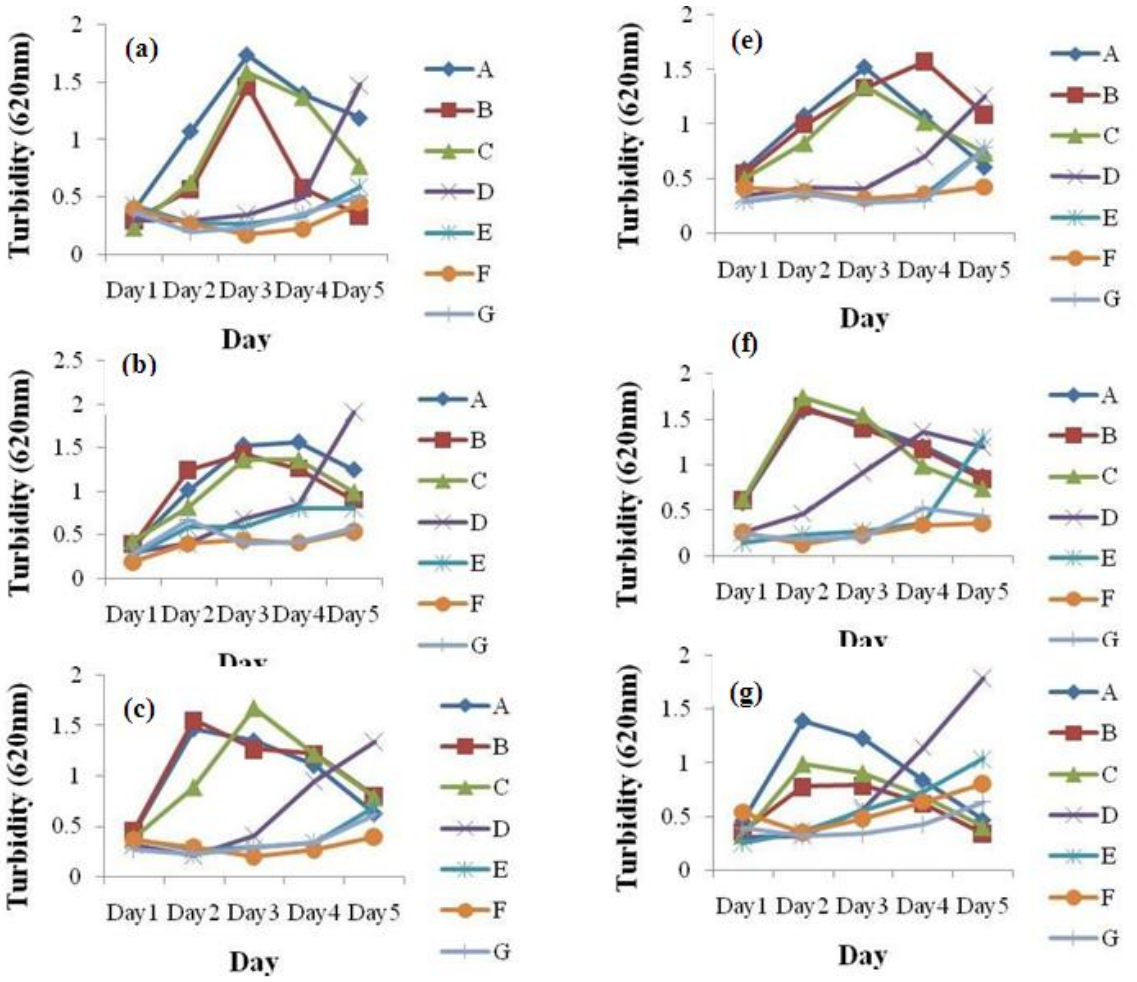


Fig 2D: Effect of Different Sulfate Concentrations on the Growth of the Selected Dry Season Aerobic Bacterial Isolates

(a) Bacillus sp. (WED1); (b) Pseudomonas sp. (WED2); (c) Bacillus sp.(WED4); (d) Alcaligenes faecalis (WED5); (e); Bacillus sp. (WED7); (f) Pseudomonas sp. (WED15); (g) Bacillus sp. (WED16); (h) Bacillus sp. (SED1)

KEY: A=0g/l; B=20g/l; C=40g/l; D=60g/l; E=80g/l; F=100g/l; and G=120g/l



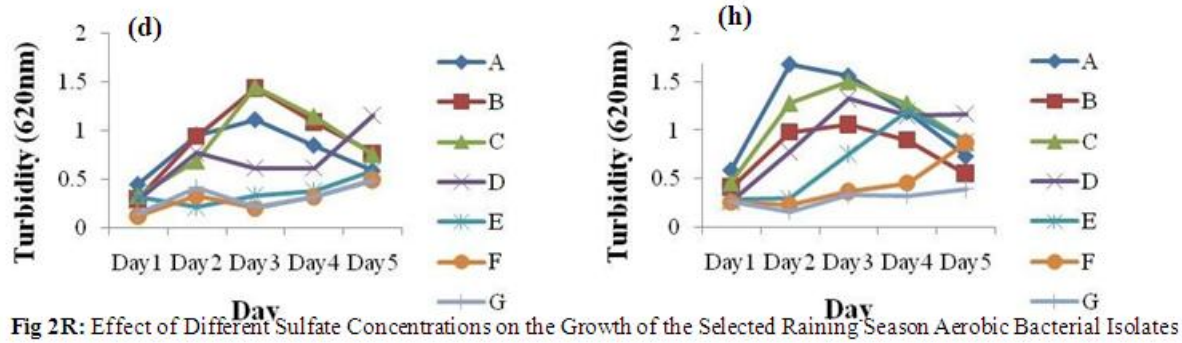


Fig 2R: Effect of Different Sulfate Concentrations on the Growth of the Selected Raining Season Aerobic Bacterial Isolates (a) Streptococcus sp. (WER3); (b) Bacillus sp. (WER4); (c) Staphylococcus sp.(WER5); (d) Bacillus sp.(WER8); (e); Pseudomonas sp.(WER9); (f) Bacillus sp. (WER11); (g) Bacillussp. (WER15); (h) Bacillus sp. (WER17) KEY: A=0g/l; B=20g/l; C=40g/l; D=60g/l; E=80g/l; F=100g/l; and G=120g/l

The Molecular (16S rRNA) Identity of the Heavy Metal Resistant and Sulfate Tolerant Bacteria Isolated and their Phylogenetic Tree

The sequencing results of the heavy metal resistant and sulfate tolerant bacteria strains (Bacillus sp. WED1, Alcaligenes faecalis WED5, Desulfococcus sp. DS5 and Desulfofaba sp. DS6) which were compared with the genes already deposited in the NCBI Genebank showed that the bacteria identity are: Rummeliibacillus stabekisii(WED1), a gram positive rod bacterium belonging to the Firmicute family; Alcaligenes faecalis (WED5), a gram negative rod bacterium belonging to Beta- proteobacteria phylogenetic group; Desulfococcus sp. (DS5), a gram negative cocci and Desulfofaba gelida (DS6), a gram negative rod both belonging to Delta-proteobacteria phylogenetic group respectively. The gene sequences of these isolates had been deposited in the gene bank with the accession number: MN250294.1, MN250293.1, MF629792.1 and MF629791.1 respectively.

The phylogenetic tree (Neighbor-Joining) showing the relationship of each of the bacterial isolates to the ones already isolated and sequence submitted to the NCBI genebank are shown in Figure 3a

– 3d.

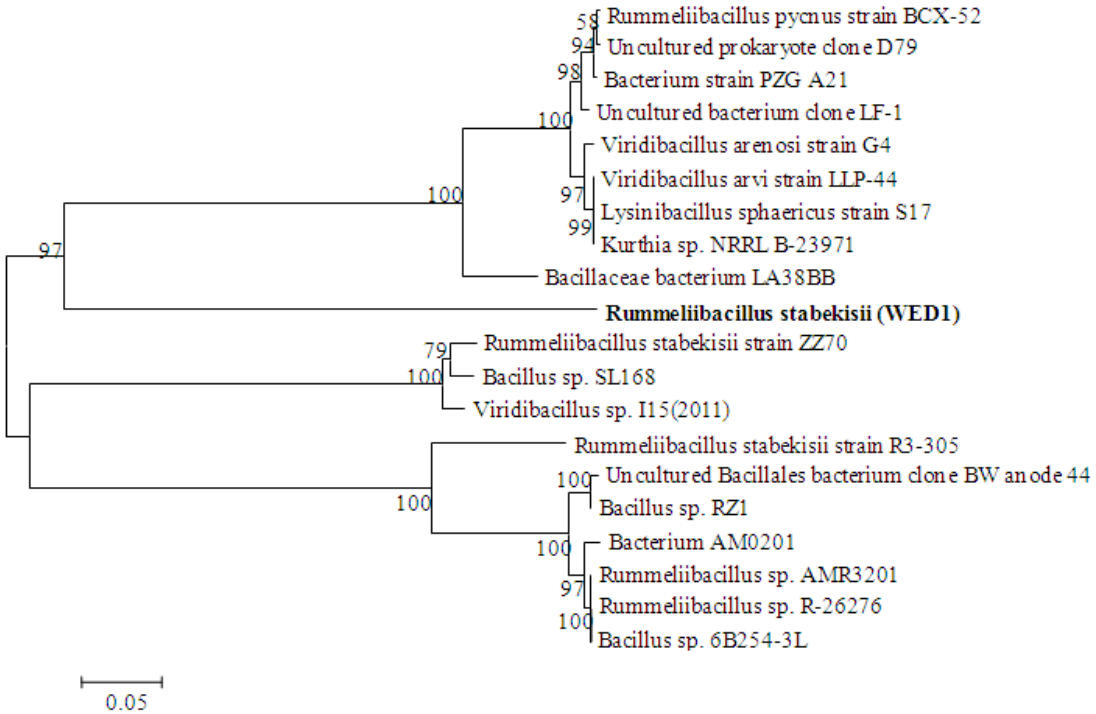


Fig 3a: Neighbor-Joining Phylogenetic Tree of Partial 16S rRNA Sequence of Rummeliibacillus stabekisii (WED1)

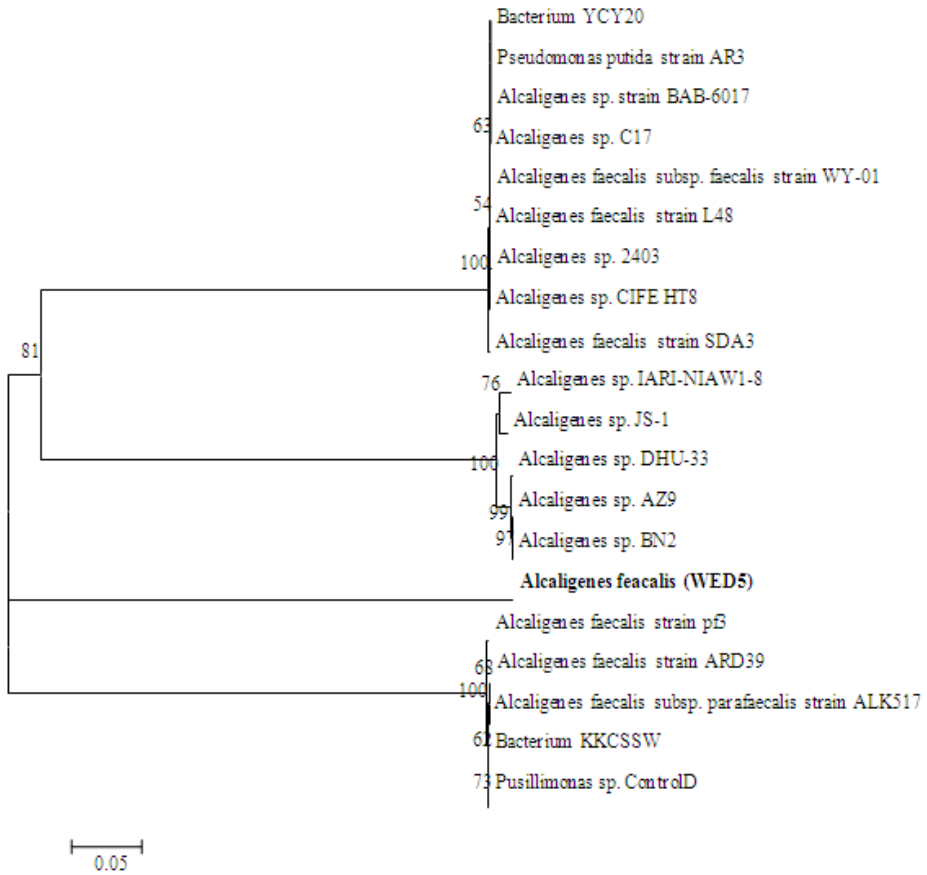


Fig 3b: Neighbor-Joining Phylogenetic Tree of Partial 16S rRNA Sequence of Alcaligenes faecalis (WED5)

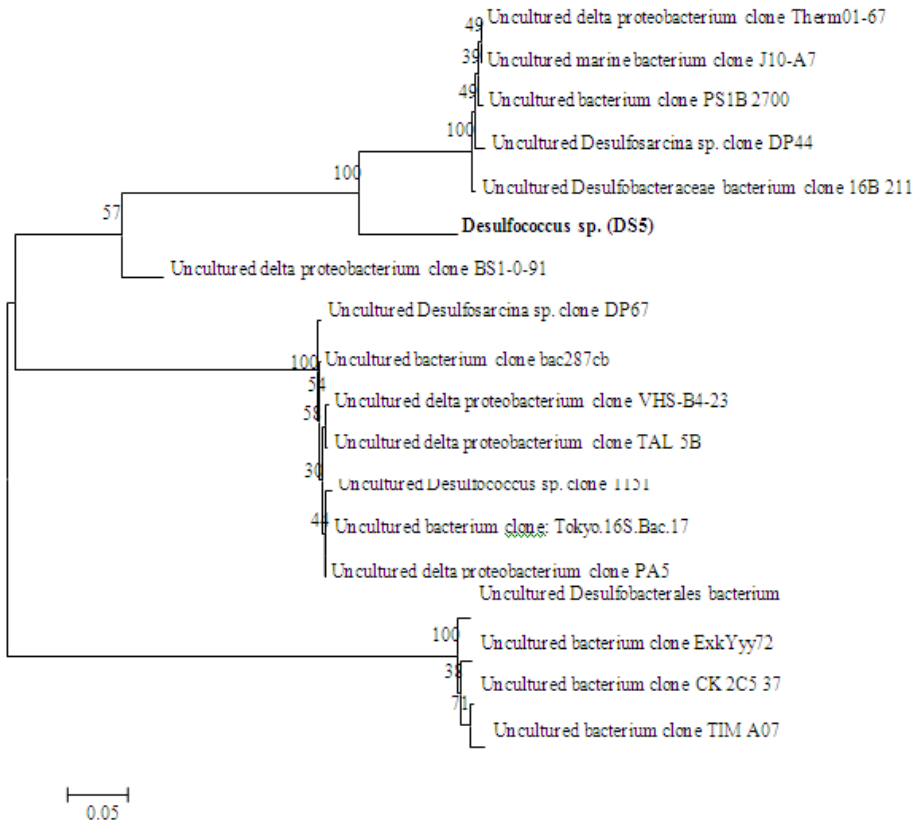


Fig 3c: Neighbor-Joining Phylogenetic Tree of Partial 16S rRNA Sequence of Desulfococcus sp. (DS5)

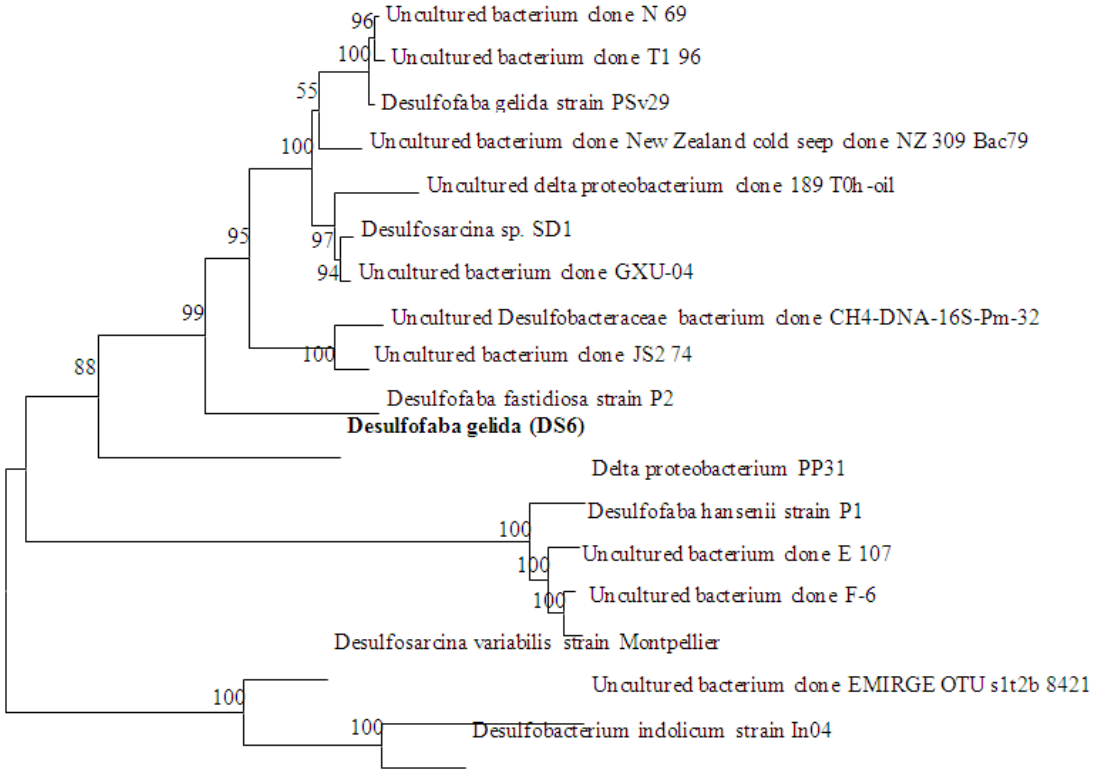


Fig 3d: Neighbor-Joining Phylogenetic Tree of Partial 16S rRNA Sequence of *Desulfofaba gelida* (DS6)

IV. Discussion

The Physicochemical Properties and Heavy Metal Composition of the Abandoned Mine drainage sample

The National Environmental Standards and Regulations Enforcement Agency (NESREA) established in 2007 by an Act came up with the National Environmental Regulations32 for each sector in Nigeria in the year 2009. The aim of these regulations was to come up with a permissible level for components of wastewater discharged from these sectors. The regulations for all categories of industries and that of Mining and Metallurgy sector were chosen for this study. The physicochemical properties and metal composition of the abandoned mine drainage sample employed in this study fall within the permissible limit of NER32 except chromium which had higher concentrations (2.00mg/L) in the dry season water sample than the permissible limit (<1mg/L). This higher concentration of chromium couple with the presence of some other metals at a significant concentration calls for urgent actions to be taken on the proper monitoring of the abandoned mine drainage and effective treatment technique to forestall a potential public and environmental health issues.

Bacteria Composition of the Dry (February) and Raining Season (October) Water and Sediment Samples

Several species of bacteria have earlier been reported to inhabit heavy-metal contaminated environment and the distridution of these bacterial species are greatly dependent on factors such as metal species and concentration, sulphate ion concentration and organic matter among many others33. Because sediment receives deposition of microbes and organic matter from the upper water layer and provides a matrix of complex nutrients and solid surfaces for microbial growth, it microbial biomass and taxon richness are generally greater than those of the corresponding water body34. The monthly survey of the heterotrophic population in water, sediment and fish tissues of the Awash River (Ethiopia) by Wondimu et al.34 revealed the heterotrophic bacterial population (2.3 X 104) in the sediment sample was higher than that of the water sample (0.2 X 104) in February which is in agreement with the report of this present study where an higher number of the aerobic bacterial isolates (24) was recorded in the sediment sample than in the water sample (17) during the dry season. In accordance to the report of this present study where higher number of the aerobic bacterial isolate (24) was obtained in the dry season than in the raining season (10) sediment sample, Unimke et al.35 reported a higher heterotrophic bacterial population count (2.55 × 107 cfu/g) during the dry season than in the raining season (2.46 ×

107 cfu/g) in the heavy metal contaminated sediment of the Imo River estuary of the Niger Delta mangrove ecosystem (Nigeria). The possible reason for the lower percentage distribution of the aerobic bacterial isolates in the raining season sediment sample could have been as a result of the release of toxic heavy metals possibly due to sulfide mineral dissolution which eventually reduced heavy metal concentration in the sediment during the raining season.

The most abundant aerobic bacterial species obtained in this study was Bacillus sp. with eleven (11) species (water sample) and fifteen (15) species (sediment sample) in the dry season, and fourteen (14) species (water sample) and seven (7) (sediment sample) in the raining season. This is in agreement with the work of Zampieri et al.36 who reported that Bacillus sp. was the most frequently isolated from heavy metal contaminated sediments of the Araca Bay and Sao Sebastiao on the Sao Paulo coast of Brazil. They furthermore suggested that Bacillus sp. may be dominant genus in heavy metal-polluted areas. The predominance of Bacillus sp. in the water and sediment sample in this present study could be as a result of its ubiquitous nature and extensive distribution in the environment couple with its ability to withstand extremes or harsh conditions through spore formation37. Staphylococcus sp. was also one of the bacterial isolates reported by Zampieri et al.36 which is in concordance with this study in which Staphylococcus sp. was one of the isolated bacterial species.

The isolation of Pseudomonas sp. from heavy metal contaminated environment as observed in this study has also been earlier reported by Mihdhir et al.38 who isolated Pseudomonas aeruginosa (S7) from the heavy metal- contaminated industrial wastewater ponds found in Makkah city (Saudi Arabia)

All the sulfate-reducing bacteria (SRB) isolated in this study were obtained from the sediment sample. Sulfate reducing bacteria are strict anaerobes that are often found in biotopes where oxic conditions can temporarity exist therefore, they are usually restricted to reduced environment such as sediment of soil 1, 39. Sanchez-Andrea et al.40 and Bomberg et al.39 reported the occurrence of SRB in an alkaline, cold Kotalahti mine drainage (Leppavirta, Finland) and the acidic, metal ladened Tinto River sediment respectively. Nevertheless, SRB have been identified in the water column of the oxygen minimum zone in the Pacific Ocean, where sulfate reduction was found to be active41. The isolation of SRB only from the sediment sample in this study could be as a result of the presence of relatively high dissolved oxygen concentration in the water sample in this present study. Furthermore, the high concentration of sulfate ion and percentage organic carbon (as terminal electron acceptor and donor respectively for SRB) in the sediment could have supported SRB growth in the drainage sediment.

The higher number of SRB isolates (68) obtained in the dry season than in the raining season (40) sediment sample is similar to the work of Li et al.42 who reported a higher SRB population during autumn than spring in the profundal sediments of a freshwater lake, Lake Kizaki (Japan). The reduction in the sediment sulfate concentration and the release of toxic heavy metal concentration in the sediment during the raining season could have brought about the lower number of SRB isolate in the raining season sediment sample in this study.

Desulfobulbus propionicus was the most frequently isolated species of the sulfate- reducing bacteria in this study with fourty-two (42) and thirty-two (32) in number during the dry and raining season respectively. Similarly to this result, Giloteaux et al.43 reported that the predominant dsrAB sequences detected in a three- year studies of SRB community pattern in Carnoule`s acidic mine water (France) were most similar to the family Desulfobulbaceae which was detected in all samples regardless of the sample station or period. Likewise, Li et al.42 reported three SRB genera (Desulfobulbus, Desulfobacterium and Desulfovibro) detected in the profundal sediments of a freshwater lake, Lake Kizaki (Japan) of which Desulfobulbus had the highest relative 16S rRNA abundance (RNA index) almost in all of the 15 months study periods (1.4% relative abundance on average in the 0-

3cm and 3-6cm layers), while Desulfobacterium and Desulfovibro exhibited low relative abundance. Furthermore, the author reported that the RNA index of Desulfobulbus correlated with the rate of sulfate reduction in the sediment and concluded that Desulfobulbus appears to be dominant in the active SRB population in the surface sediment. The predominance of Desulfobulbus propionicus in the abandoned mine drainage sediment in this present study could be probably because it has a competitive advantage for limited resources such as carbon source in the environment.

Minimum Inhibitory Concentration (MIC) of the Heavy Metals on the Isolated Bacteria

Several bacterial species have been reported to show resistance to different heavy metals. The MIC of copper on Bacillus sp. isolated from an oil field soil in Moran, Dibrugarh district, Assam was reported to be 600µg/mL (600ppm) by Saikia et al.44. This MIC value is comparatively higher than the highest MIC value of 250 ppm for copper shown by Bacillus sp. (WED1) in this present study. However, Elsik et al.45 reported a lower MIC value of 2.0mM (128ppm) for Bacillus anthracis PS2010 isolated from heavy metal polluted site in

Egypt. The variation in the sampling sources and the concentration of the copper in the sampling locations might be responsible for these differences.

The highest MIC value for chromium obtained in this study is 350ppm shown by Streptococcus sp. (WER3), Pseudomonas sp. (WER9) and Bacillus sp. (WER15). This MIC value i s however higher than the MIC value of 280µg/mL (280ppm) reported in the work of Singh et al.46 for Bacillus sp. SG-1 from industrial effluent Kanpur U. P., India. A lower MIC value of 200ppm for chromium was also reported for Pseudomonas sp YSY-15 and YSY-17 by the same author. However, some Pseudomonas sp. strains isolated from the Dandaru River in Ibadan (Nigeria) were reported by Adekanbi and Falodun47 to show MIC value of 500ppm for chromium which is higher than the highest MIC value of 350ppm shown by the Pseudomonas strains for chromium in this study.

The variation in the sampling sources and the concentration of the metal in the sampling locations might be responsible for the differences. Tamiru et al.48 reported a higher chromium (Cr^{3+}) MIC value for Streptococcus sp. MB16 (1000ppm) and Streptococcus sp. MB17 (600ppm) isolated from the Rhizosphere soils contaminated with Tennary effluent in Bahir Dar (Ethiopia). Likwise, the two strains of Streptococcus isolated from the soil of an industrial area of Abeokuta (Ogun Stae, Nigeria) were reported by Wani et al.49 to have higher MIC value of 400ppm (Streptococcus sp. PZ2) and 700ppm (Streptococcus sp. PZ4) respectively for chromium. However, Owolabi and Hekeu50 reported that the Streptococcus sp. PC2B isolated from a Paint Company dumpsite, Ota (Ogun State, Nigeria) showed the Maximum Tolerable Concentration (MTC) of 4mM (208ppm) when grown on chromium amended agar media with increasing concentration of 2, 4, 6, 8 and 10mM had Maximum Tolerable Concentration (MTC) of 4mM (208ppm) and therefore the MIC value of 6mM (312ppm) which is lower than the highest MIC value (350ppm) for chromium shown by Streptococcus sp. WER3 in this study. The difference in the MIC value might be as a result of different concentration of chromium in the sample locations.

The highest MIC value for colbat obtained in this study is 250ppm shown by Bacillus sp. (WED1, WED7, WED14, WED16 and WER15), Pseudomonas sp. (WED15), and Streptococcus sp. (WER3). This MIC value is higher than the highest MIC value of 140µg/mL (140ppm) for Cobalt reported by Singh et al.46 for Bacillus sp. SG-1 from industrial effluent Kanpur U. P., India. Singh et al.51 reported that Pseudomonas sp YSY-13, YSY-15, YSY-17 and YSY-19 isolated from nine different rhizospheric soil samples from wheat & pigeon pea field (Allahabad district, Uttar Pradesh) had MIC value of 100, 50, 100 and 100ppm respectively for cobalt.

In a study by Rajbanshi52, the MIC value for nickel reported for Bacillus sp. isolated from Guheswori sewage treatment plant (Napal) was 200µg/mL (200ppm), this value is comparatively lower to the highest MIC value of 600ppm shown by Bacillus sp. WED11 and WED16 in this present study. This difference in the MIC value could be as a result of difference in the geographical location of the sampling sites and the bacteria were obtained from different sampling sources.

The highest MIC value for zinc obtained in this study is 250ppm shown by Streptococcus sp. (WER3) and Staphylococcus sp. (WER5). Zinc resistance by Streptococcus sp. and Staphylococcus spp. isolated from wastewater and polluted soil have been reported by several authors to show various resistance to toxic heavy metals in accordance with this present study, notably among them were Saikia et al.44 and Tamiru et al.48. The MIC value for zinc shown by a Staphylococcus sp. isolated from an oil field soil in Moran, Dibrugarh district, Assam as reported by Saikia et al.44 was 600ppm which is not in agreement with this present study. Likewise, Tamiru et al.48 reported higher MIC value of 1000ppm for Streptococcus sp. MB16 isolated from Rhizosphere Soils Contaminated with Tannery Effluents in Bahir Dar (Ethiopia). Wani et al.49 also reported that the MIC values for Streptococcus sp. PZ2 and

Streptococcus sp. PZ4 isolated from the Industrial Area of Abeokuta, Ogun State (Nigeria) were 700 and 400ppm respectively for zinc. The diiference in sampling sources and concentration of zinc in the sample locations might be responsible for these differences.

In a study by Ka-ot et al.53, two Bacillus sp. strains, Bacillus subtilis subsp. Inaquosorum SK22 and Bacillus cereus SK44, isolated from Rat-Hole Coal Mines of Meghalaya (India) were reported to have MIC values of 1000ppm and 800ppm respectively. These MIC values are greater than the highest MIC value (600ppm) obtained for the Bacillus sp. WER8, WER11, WER15 and WER17, Staphylococcus sp. WER5 and WER16, Streptococcus sp. WER3 and Pseudomonas sp. WER9 in this present study. These differences could be as a result of differences in the sampling sites from which the Bacillus sp. strains are isolated.

The inhibitory effect of heavy metals on the sulfate reducing bacteria has been reported by several authors. A gram negative sulfate reducing bacteria, Desulfovibrio desulfuricans, in the work of Natarajan and Padukone54 was reported to tolerate up to 50ppm cobalt concentration which is comparatively lower to the concentration of the same metal tolerated by the sulfate reducing bacterial strains in

this present study. However, a soil enriched culture of sulfate reducers, Desulfosporosinu auripigmenti and Citrobacter freundii from a for mer uranium- mining site (Ronneburg, Germany) was reported by Sitte et al.55 (2013) to tolerate up to 40mM (2,360ppm) cobalt concentration which is higher than the cobalt concentration tolerated by any of the sulfate reducing bacterial strains in this present study where the highest MIC value of 650ppm was shown by Desulfobulbus propionicus (DS3) and Desulfofaba sp. (DS6). The variation in the tolerance level might be as a result of the different sample sources and metal concentrations from which the SRB strains are obtained

Azabou et al.56 reported that the MTC (Maximum Tolerable Concentration) value for a gram negative rod SRB, Desulfomicrobium sp., isolated from a wastewater treatment plant (Mahres, Tunisia) was 100ppm for nickel. This result is not in accordance with this present study where some of the isolated gram negative sulfate reducing bacteria such as Desulfococcus sp. (DS5), Desulfofaba sp. (DS6) and Desulfobulbus elongatus (RS3) tolerated higher nickel concentrations (between 150-250ppm). The highest MIC value for nickel was 250ppm shown by Desulfococcus sp. (DS5) in this present study. The variations in the metal tolerance might be as a result of the fact that the bacteria were obtained from different sampling sources.

In a study by Azabou et al.57, a mixed culture of SRB enriched from a sludge obtained from a wastewater treatment plant (Mahres, Tunisia) was reported to show MIC value of 200ppm for zinc. Also, a gram negative, mesophilic heavy-metals-tolerant sulfate-reducing bacterium, Desulfomicrobium sp., from an enriched culture of a wastewater treatment plant (Mahres, Tunisia) using phosphogypsum as a sulfate source was reported by Azabou et al.56 to show MTC (Maximum Tolerable Concentration) value of 125ppm for zinc. These reports are not in accordance with the result of this present study where the isolates tolerated a lower concentration of zinc with the highest MIC value of 100ppm shown by the gram negative sulfate- reducing bacteria, Desulfobulbus propionicus (DS3) and Desulfobulbus elongatus (RS3). These differences might be as a result of the zinc concentration from the sampling sources

Effects of Different Sulfate Concentrations on the Growth of the Isolated Bacteria.

Sulfate ion concentration majorly contributes to electical conductivit y of water of which its variation can lead to changes in osmotic pressure on microorganisms. High sulfate ion concentration will therefore lead to high salinity which can reduce microbial growth rate1. Crisler et al.58 reported that the number and growth rate of cultivable aerobic heterotrophic bacteria from Martian soils (Oklahoma, United States) decreases with increase in magnesium sulfate concentration which is in agreement with this present study where the growth of all the selected aerobic bacterial isolates decreases with increased concentration of sulfate ion. Also, similar to this study where Bacillus sp. (WED1) was observed to be the most sulfate tolerant aerobic bacteria which grew at the highest sulfate ion concentration (120g/l) tested, the same authors reported that the increase in the MgSO4 concentration to 2M (240g/l) (the highest concentration tested) in the cultured medium contaning 1% NaCl lead to the growth of only eighteen (18) isolates which were mainly Halomonas and Bacillus spp.

V. Conclusion

In this study, the metal resistance and sulfate tolerance of the indigenous bacterial isolates of the abandoned Igun goldmine drainage were investigated. It was observed that the physicochemical and heavy metal parameters of the abandoned mine drainage from Igun goldmine were within the limit set by the National Environmental Standards and Regulations Enforcement Agency (NESREA) except chromium which was more than twice greater than the NESREA limit.

The mine drainage water and sediment of the Igun abandoned goldmine sampled in this study are habitats of metal-resistant Gram positive and Gram negative aerobic bacteria belonging to the genera *Bacillus, Pseudomonas, Alcaligenes, Staphylococcus, Streptococcus* and metal- resistant Gram positive and Gram negative Sulfate reducing bacteria belonging to the genera *Desulfotomaculum, Desulfobulbus, Desufococcus, Desulfovibro and Desulfofaba*; where *Bacillus* sp. and *Desulfobulbus propionicus* were the most populous bacterial species.

The bacterial species showed varying degree of resistance to metals a n d tolerance to sulfate with *Rummeliibacillus stabekisii* (MN250294.1), *Alcaligenes faecalis* (MN250293.1), *Desulfococcus* sp. (MF629792.1) and *Desulfofaba gelida* (MF629791.1) being most tolerant to metals, sulfate and the *Desulfococcus* sp. (MF629792.1) and *Desulfofaba gelida* (MF629791.1) utilized more than one electron donor tested, .

VI. Recommendation

It is recommended that proper legislation to enforce mining operators to abide with the Federal Environmental Protection Agency regulations guiding the discharge of mine drainage from abandoned mines and mining facilities into receiving water bodies and the environment as a whole. Also, the existing abandoned mine drainages need to be cleaned up by the concern government organization. This is very necessary at this time because of the presence of toxic metal species in the mine drainage generated from abandoned mine as discovered in this study. This will go a long way in preventing the general populace from exposure to dangerous toxic chemicals via the discharge of mine drainage from abandoned mines into water resources and leading to further environmental degradation.

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