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Microbiological and chemical evaluation of acid mine drainage from mining sites in Southwestern, Nigeria

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Abstract

Microbial content of acid mine drainage effluents contaminated streams from some geographical areas was evaluated. Water was obtained from acid mine drainage sites in Ekiti and Osun state where twelve (12) samples were obtained from different locations. Culture plating method was used to analyze the samples for bacteria, and Atomic Absorption Spectrophotometer (AAS) was used to determine heavy metals such as Cd, Co, Pb, Cr, Zn, Cu and Mn. The results of biochemical and morphological characterization of the isolates revealed three probable bacterial from the samples which are *Bacillus subtilis*, *Pseudomonas* spp. and *Staphylococcus aureus*; *B. subtilis* being the bacteria with the highest percentage frequency of occurrence. Heavy metals analysis of the mine drains shows that the concentration of Cd, Co, Pb, Cr and Zn exceeded permissible limit set by WHO except Cu and Mn. The results of this study established the presence of bacterial and heavy metals in acid mine drainage sites, which is an indication that the acid mine effluents are contaminated. It is therefore essential for proper dissemination of information concerning the dangers these microbes and heavy metals could pose to human.

Keywords: Acid mine drainage; Bacteria; Heavy metal

1. Introduction

Acid mine drainage is the outflow of acidic water from metal mines or coal mines. It occurs naturally within some environments as part of the rock weathering process. It is often referred to as an extreme environment since its chemical nature does not allow colonization by a diversity of acid-intolerant microorganisms [1]. Areas where the earth has been disturbed (e.g. construction sites, subdivisions, and transportation corridors) may create acid mine drainage [2]. In many localities, the liquid that drains from coal stocks, coal handling facilities, coal washeries, and coal waste tips can be highly acidic, and in such cases it is treated as acid mine drainage [2]. This liquid often contains toxic metals, such as copper or iron. These, combined with reduced pH, have a detrimental impact on the streams aquatic environments [3].

Despite the extreme acidity, heat, and high concentrations of sulfate and toxic metals, a diverse range of microorganisms populate acid mine drainage environments. These organisms can form a chemoautotrophically-based biosphere in the subsurface, ultimately sustained by electron donors derived from sulfide minerals, CO_2, O_2, and N_2 derived from air, and phosphate liberated by water–rock interaction. Microbial activity increases the rate of AMD formation and may be responsible for the bulk of AMD generated [4].

Microbe–mineral interactions are of importance because AMD is a very widespread environmental problem. The organisms can be used in ore processing and are a source of novel biomolecules (especially enzymes) for industrial processes. DNA-based studies of organisms populating mining environments have provided insights into the diversity of acidophilic, metal-tolerant species [5].

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Scientists have recently begun to explore acid mine drainage and mine reclamation sites for unique soil bacteria capable of producing new pharmaceutical leads [6]. Soil microbes have long been a source for effective drugs [7] and new research, such as that conducted at the Center for Pharmaceutical Research and Innovation, suggests these extreme environments to be an untapped source for new discovery[6]. Acid mine drainage is an extreme environment. However, observations of the microbiota indicate that a variety of microorganisms can exist in such an environment. To eliminate acid mine drainage, and also to improve commercial bioleaching, it is imperative to identify the species of bacteria present in such environments.

Colonies of bacteria and archaea greatly accelerate the decomposition of metal ions, although the reactions also occur in an abiotic environment. These microbes, called extremophiles for their ability to survive in harsh conditions, occur naturally in the rock, but limited water and oxygen supplies usually keep their numbers low. Special extremophiles known as Acidophiles especially favor the low pH levels of abandoned mines. In particular, *Acidithiobacillus ferrooxidans* is a key contributor to pyrite oxidation [8].

It is essential to define the microbial population in order to analyze an ecological system. Currently, the primary method of determining which organisms comprise a microbial community involves determining the sequence of 16S ribosomal RNA genes (16S rRNA) from environmental samples. This approach provides information about species richness as well as the evolutionary relationships between lineages. Microbial species composition can then be correlated with environmental data to determine how communities are shaped by geochemical factors [5]. .

In order to eliminate acid mine drainage and to improve bioleaching, it is imperative to isolate and identify the species of autotrophic and heterotrophic bacteria present in such environments. Isolation of microorganisms from the environment requires a good knowledge of the target environment and its bacterial composition.

Among the bacterial lines of descent are divisions within the proteobacteria, nitrospira, firmicutes, and acidobacteria. The most extensively studied group (but possibly the least relevant under AMD-generating conditions) are the γ-proteobacteria, specifically *Acidithiobacillus* spp. (formerly *T. ferrooxidans*, *Thiobacillus caldus*) and *Thiobacillus* spp. Two β-proteobacterial groups have been detected to date. Among this group are *Thiomonas* sp. and subsequently an isolate designated NO-16 from a Norwegian mine Johnson *et al*., 2001)[10]. The aim of this study is to microbiologically evaluate bacterial strains from acid mine drainage effluents. Specific objectives are; to isolate and identify bacterial strains from acid mine drainage effluents, and to enumerate water samples for the presence of heavy metals

2. Material and methods

2.1. Collection of samples

Environmental water samples were collected from acid mine effluents from some locations in Ekiti and Osun states into sterile bottle, and then transported to the laboratory for further analysis.

2.2. Media Preparation

Nutrient agar and MacConkey agar powder were weighed, dissolved and sterilized according to the manufacturer's instructions. The sterilized molten agar was cooled to 47℃ and about 20 ml was poured into sterile disposable petri dishes. They were allowed to set and kept for subsequent uses.

2.3. Isolation of bacterial

A volume of 100 mL of environmental water was filtered through a Nalgene 150 mL analytical filter unit. After serial dilution in 10 mL of distilled water, 0.2 mL volume of each samples were plated onto prepared solid media. The nutrient agar and MacConkey agar plates were incubated at 37℃ for 18 – 24 hours, while PDA plates were incubated at room temperature (25℃) for 72 hours. Cultures were again plated, and single colonies selected to ensure purity.

2.4. Identification of bacterial isolates

The bacterial isolates were tentatively identified by means of morphological characteristics, cellular and biochemical tests. Morphological characteristics were observed for each bacterial colony after 24 hours of growth. The colony of each isolate on the nutrient agar media were observed for identification of shape, appearance and colour, colony size, margin and emulsification. The biochemical tests carried out include; catalase test, indole test, methyl red, voges proskauer, citrate and oxidase. The isolates were identified using Bergey's Manual of Determinative Bacteriology[10].

2.5. Determination of Heavy Metals

This was done with the use of the Atomic Absorption Spectrophotometer (AAS) by taking 25 mL of the water samples with one drop of nitric acid. For the determination of iron (Fe), copper (Cu), cadmium (Cd), manganese (Mn) and lead (Pb). The analytical method used for the metal concentration was spectrometry and equipment used are AAS Buck Scientific Model 210 VGB, using the calibration and sample analysis. For each element, the instrument was auto-zeroed using the blank (distilled water) after which the standard was aspirated into the flame from the lowest to the highest concentration. The corresponding absorbance was obtained by instrument and the graph of absorbance was plotted against concentration. The samples were analyzed with concentration of metals present being displayed in parts per million (ppm) after extrapolation from the standard curve.

3. Results

The percentage frequency of occurrence of bacterial isolates in the acid mine effluents is shown in figure 1. *Bacillus subtilis* was observed to have higher percentage of occurrence; while *Pseudomonas* spp. and *Staphylococcus aureus* had the lowest percentage of occurrence.

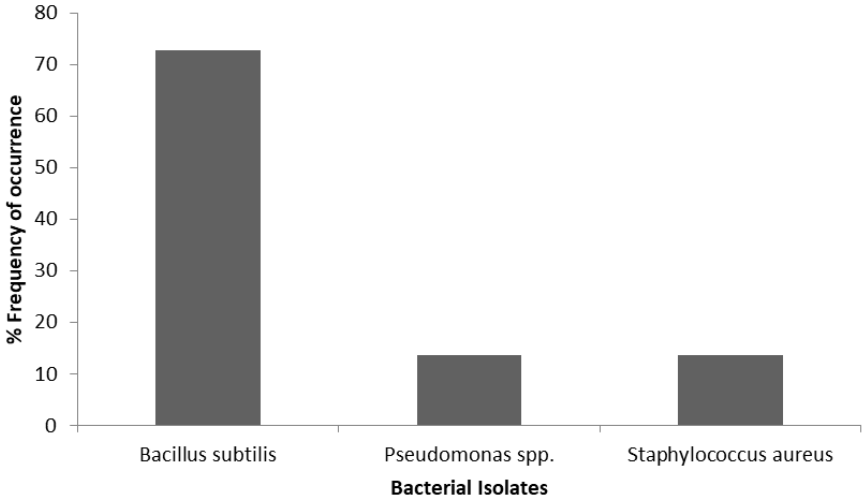

Figure 1 Percentage frequency of occurrence of bacterial isolates in acid mine effluents

The biochemical tests for identification of the bacteria strains were analyzed in Table 1. The morphological characteristics showed the bacteria strains isolated from the study area to be gram negative and gram positive bacteria. There was production of acid during fermentation of glucose, lactose and sucrose by the isolates. Almost all isolates showed positive reaction to glucose and sucrose, while few isolates showed negative reactions. Isolates from location 1, 3, 4 and 7 show positive and negative reactions to lactose; the rest of the isolates showed neither positive nor negative reactions to lactose. The probable identified isolates are *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Table 1 Biochemical tests for identification of isolates.

Sample locations		Gram rxn	Catalase	Coagulase	Citrate	Indole	Motility	Methyl red	Oxidase	Spore staining	Voges-Proskauer	Fermentation			Probable isolates
												Glucose	Lactose	Sucrose	
1	1	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
	2	- rod	+	-	+	-	+	-	+	-	-	-	-	-	*Pseudomonas*
2	1	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
	2	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
3	1	+ cocci	+	-	+	-	-	+	-	-	+	+	+	+	*Staph aureus*
	2	+ rod	+	-	+	-	+	-		+		+		+	*Bacillus subtilis*
4	1	+ cocci	+	-	+	-	-	+	-	-	+	+	+	+	*Staph aureus*
	2	- rod	+	-	+	-	+	-	+	-	-	-	-	-	*Pseudomonas*
5	1	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
	2	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
	3	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
6		+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
7	1	+ cocci	+	-	+	-	-	+	-	-	+	+	+	+	*Bacillus subtilis*

	2	- rod	+	-	+	-	+	-	+	-	-	-	-	-	*Staph aureus*
8		+ rod	+	-	+	-	+	-		+	+	+		+	*Pseudomonas*
9		+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
10	1	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
	2	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
11	1	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
	2	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
12	1	+ rod	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*
	2	+ cocci	+	-	+	-	+	-		+	+	+		+	*Bacillus subtilis*

+ = Positive; – = Negative

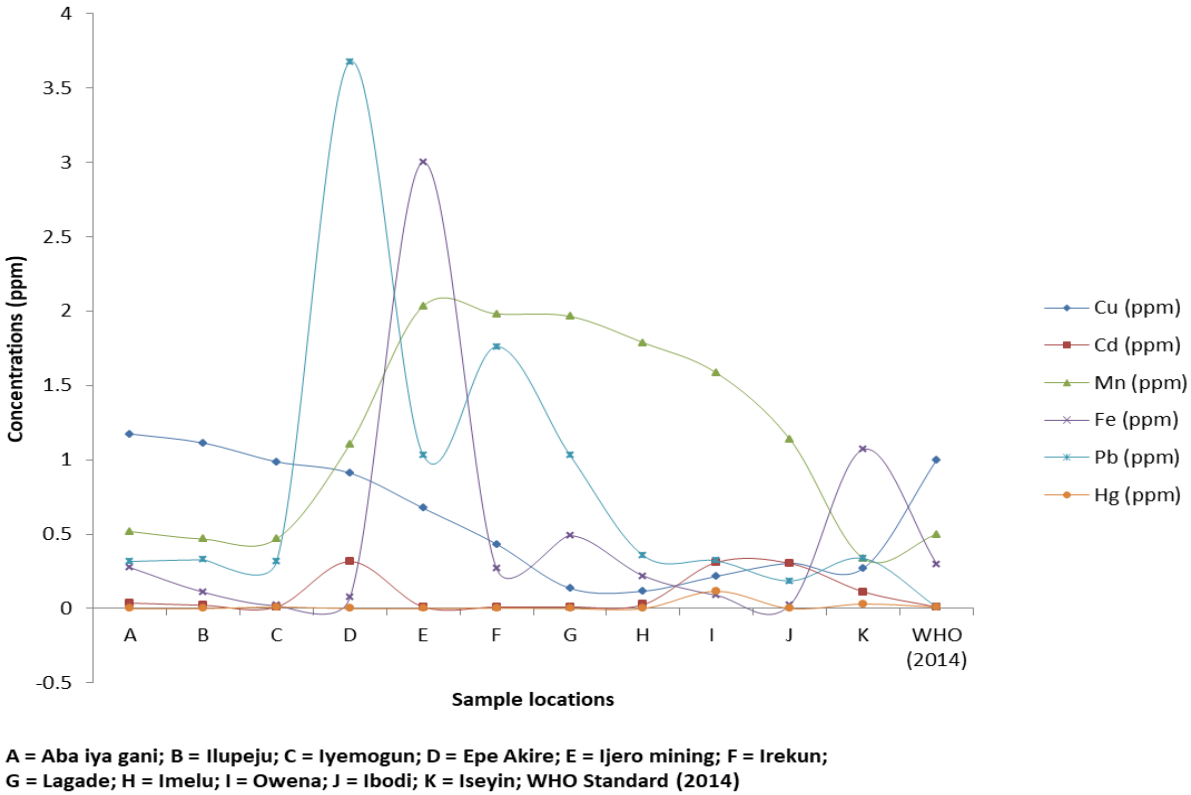

Figure 2 Concentration of heavy metals in acid mine water effluents from different locations

Figure 2 presents the concentrations of heavy metals in acid mine effluents from different locations. The heavy metals analyzed were iron (Fe), copper (Cu), cadmium (Cd), manganese (Mn), lead (Pb) and mercury (Hg).

Table 2 Heavy Metal (mg/L) Analysis of Mine Drains

Location	Ni	Cu	Mn	Cd	Co	Pb	Cr	Zn
1	0.02	0.01	0.00	0.05	0.23	0.37	3.31	3.85
2	0.05	0.00	0.01	0.12	0.21	0.34	3.25	3.65
3	0.03	0.00	0.00	0.03	0.23	0.39	3.31	3.25
4	0.00	0.00	0.00	0.08	0.27	0.42	3.33	3.22
5	0.03	0.00	0.02	0.07	0.23	0.39	3.35	4.21
6	0.05	0.00	0.00	0.03	0.22	0.44	3.26	3.12
7	0.06	0.00	0.01	0.01	0.23	0.49	3.45	3.00
8	0.03	0.00	0.01	0.04	0.21	0.55	3.32	3.23
9	0.06	0.01	0.03	0.06	0.00	0.63	3.45	2.96
10	0.03	0.00	0.03	0.85	0.18	0.33	4.11	3.72
11	0.98	0.00	0.00	0.06	0.16	0.33	3.24	3.11
12	0.06	0.00	0.01	0.00	0.18	1.32	3.32	4.18
WHO/PL	0.02	0.00	0.02	0.02	0.02	0.01	0.02	3.00

4. Discussion

The results suggest that the heterotrophic bacteria, which can be recovered from an acid mine drainage sites on nutrient agar medium, probably represents transient organisms which are not indigenous to the acid mine sites. The isolated bacterial include *Bacillus subtilis*, *Pseudomonas* spp. and *Staphylococcus aureus*. The low number of organisms recovered from the each location suggests that a small population of transient heterotrophs was tolerant to the acid environment but did not proliferate there. This result correlates with Tuttle *et al*. (2006)[11] who reported low number of bacterial species from acid mine streams.

The population of *Bacillus subtilis* was higher (72.7%) compared to the other bacterial isolated from the study areas. This may be caused by (i) the high of acidic waters which results from high concentrations of oxidized compounds, (ii) a low concentration of organic material, and (iii) the absence of those microorganisms that utilize oxygen while metabolizing organic materials. The anaerobic species isolated from acid mine effluents did not include sulfate-reducing bacteria. Sulfate-reducing organisms were either lacking or present in very small numbers in acid mine water according to Tuttle *et al*. (2006)[11].

The bacilli were generally non-motile, oxidase-negative, aerobic or facultative species. The bacteria formed white- or cream-colored colonies on the medium employed. One aerobic oxidase-positive bacterium was also isolated. The organisms were not thoroughly characterized but examined seemed to be transients, entering from a nonacid environment. Therefore, it can be presumed that the gram-negative bacteria have a greater permeability barrier to hydrogen ions in the environment which gives the gram-negatives survival advantage. The permeability barrier may be related to the higher lipid content of the gram-negative cell envelope as compared to that of the cell wall of gram-positive bacteria.

The result as shown in Table 2 indicate the heavy metals (Ni, Cu, Mn, Cd, Co, Pb, Cr and Zn) assessed. Ni, Co, Pb, Cr and Zn concentration is above the permissible level except in Ni, Cd and Co concentrations in location 4, 7, 12 and 9 respectively. This could be related with the corrosive water effects on household plumbing systems containing lead in pipes, solder, fittings or the service connections to homes. It can also be attributed to anthropogenic activities.

The concentration of Cu and Mn as shown in Table 2 were found relatively in low concentrations in all the water samples.

5. Conclusion

The results of this study established the presence of bacterial in acid mine drainage sites, and this is indication that the acid mine effluents contained an acid-tolerant microflora. And heavy metal analysis revealed the presence of iron (Fe), copper (Cu), cadmium (Cd), manganese (Mn) and lead (Pb); though in few concentration which could not cause any harm to human.

There is need to disseminate information concerning the presence of microbial life in acid mine drainage sites; due to the pathogenicity of some microbes. Further research is recommended on the antibiotic resistance of these bacterial in order to establish their virulence factor.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest in this manuscript.

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