Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.2, No.7, 2012

Microbiological Examination and Antimicrobial Susceptibility of Microorganisms Isolated From Salt Mining Site in Ebonyi State

*Anyim C.[1], Aneke C.J.[2], Orji J.O. [1], Nworie O.[1], and Egbule U.C.C. [1]
[1]Department of Applied Microbiology, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria.
[2]Department of Biochemistry, Enugu State University of Science and Technology, P.M.B. 1660, Enugu, Nigeria.
*Corresponding author Email: anyim5live@gmail.com. Tel.: +2347036347015

ABSTRACT
The microbial examination and antimicrobial susceptibility profile of microorganism isolated from salt mining site in Ebonyi state was evaluated in the present study using standard microbiological technique. A total of 300 samples were randomly collected in three sample groups (A, B and C) of 100 each. Isolation, identification and characterization of organisms present on the soil samples were determined by culturing, Gram-staining and biochemical techniques. The result showed that the following organisms were isolated with their frequency as follow: *Bacillus* species (37.3%) and *Staphylococcus* species (23.5%) had the highest frequency in whole sample group A and B, while *Klebsiella* species (15.7%), *Pseudomonas* species (13.7%) and *Erwinia* species (9.8%), had the least. *Rhizopus* species (42.0%) and *Aspergillus* species (26.0%) where the highest fungi isolated, followed by *Penicillum* species (20.0%), while *Mucor* species (4.0%), and *Fusarium* species (8.0%) recorded the least. Sample group C showed high microbial population of all the microbial isolates when compared to sample group A and B. Disc diffusion method was used to determine the susceptibility of isolated bacteria to various antibiotics (ofloxacin, pefloxacin, ciprorex, augumentin, gentamycin, ciproflox, septrin, ampicillin), while agar well diffusion method was used to determine the susceptibility of isolated fungi to some antifungal drugs (metronidazole, ketoconazole, itraconazole, fluconazole). The antibacterial activity of the antibiotics used showed that ciproflox has the best inhibitory effect on all the bacteria isolates, followed by augmentin, while septrin and gentamycin showed no inhibitory effect on all the test bacteria. Ketoconazole showed the highest inhibitory effect on the fungal isolates, followed by itraconazole, while metronidazole and fluconazole showed the least inhibitory effect on the entire test fungal isolates. Hence multiple drug resistance of most isolates to appropriate drugs of choice are of great public health concern and calls for periodic monitoring of antibiograms to detect possible changing patterns. Microbes isolated in the salt mining site can also be used as a source of gene(s) that can increase salt tolerance in different crop species through genetic engineering.

Keywords: Microorganisms, antibacterial, antifungal, resistance, salt mining site, Ebonyi State.

INTRODUCTION

Salt, also known as table salt, or rock salt, is a crystalline mineral that is composed primarily of sodium chloride (NaCl), a chemical compound belonging to the larger class of ionic salts. It is essential for animal life in small quantities, but is harmful to animals and plants in excess. Salt is one of the oldest, most ubiquitous food seasonings and salting is an important method of food preservation. The taste of salt (saltiness) is one of the basic human tastes (Caldwell *et al*., 2000). Salt is obtained from two sources: rock salt and brine. Rock salt is simply crystallized salt, also known as halite. It is the result of the evaporation of ancient oceans millions of years ago. Brine is water containing a high concentration of salt (McCarron *et al*., 2009). Large deposits of rock salt are found in Nigeria; rock salt is available in Benue State, while salt springs found at Awe (Plateau State), Uburu (Imo State) and in Okposi Okwu and Uburu (Ebonyi State) (Okaji, 2009 and ION, 2010). Ebonyi is the second *largest* small-scale *salt producing* areas in *Nigeria*. The small-scale *salt* production in Okposi and Uburu has continued for about 400 years ago (Okaji, 2009).

A salt mine is a mining operation involved in the extraction of rock salt or halite from evaporite deposits (Weller and Dumitroaia, 2005). Prior to the advent of the internal combustion engine and earth moving equipment, mining salt was one of the most expensive and dangerous of operations. While salt is now plentiful, before the Industrial Revolution salt was difficult to come by, and salt mining was often done by slave or prison labour (Taylor *et al*., 2011).

Biodiversity is an attribute of an area and specifically refers to the varieties within and among living organisms, assemblage of the living organisms, biotic communities and biotic processes, whether naturally occurring or modified by humans (DeLong, 1996). Extreme environments such as acidic, thermophilic, hypersaline, and arid regions, are important 'hot spots' of microbial 'megadiversity'. These are habitats of microorganisms which have the genetic and physiological capacity to survive and grow under these harsh or extreme conditions through which they have evolved while shaping the environment as we know it today (Olsen *et al*., 1994 and Woese, 1987).

Many types of bacterial species have been isolated from various salt environments. Including the Gram-negative halophilic like species of the genera *Vibrio, Alteromonas, Acinetobacter, Marinomonas* and *Pseudomonas* (Prado *et al*., 1991). Similarly species of the genera *Marinococcus, Sporosarcina, Salinococcus* and *Bacillus* have been recovered from saline soils and salterns (Farrow *et al*., 1992). Previously, a limited study of

water samples collected from Khewra salt mine has indicated the presence of bacteria belonging to *Halomonas magadiensis* and *Virgibacillus halodenitrificans* (Ghauri *et al*., 2006).

Antibiotic resistance is a concern for the management of diseases in humans, animals and plants (Nwosu, 2001).

The present study is the first report of its kind that deals with culturable microbial biodiversity of salt mine site in Ebonyi State and their susceptibility pattern to antimicrobial agents.

MATERIALS and Methods

Study Location

This study was conducted within the Uburu salt mining site, in Okposi Local Government Area of Ebonyi state. The study area is located between Latitude 05^0 55´N and 06^0 00´N, and longitude 07^0 30´E and 07^0 35´E, rainfall pattern is bimodal (April-July), September-November with a short spell sometimes in August. The annual rainfall is between 1000mm-1500mm.The vegetation of the area is predominantly derived Savannah. The mean annual temperature is about 24^{o}C and the relative humidity is between 60-80% (Ofomata, 1975; Ezeh and Chukwu, 2011).

Collection of Soil Sample

The soil samples were randomly collected from various locations in Uburu salt mining site, in Okposi Local Government Area of Ebonyi state. This was done by using sterile hand trowel to dig about 10cm by 10cm depth of the soil. The soil samples were randomly collected in groups A, B and C within the Uburu salt mining site. Group A was collected from Uburu salt lake, Group B around lakeshore, while group C was collected 250 km away from Uburu salt mining site. The soil samples were transported in sterile beaker to the laboratory within 1 hour of collection.

Microbiological Analysis of the Soil Sample

The microbiological analysis of the soil samples were carried out (Oyeleke and Manga, 2008a). The microorganisms were identified and characterized using standard biochemical tests (Cheesbrough, 2006).

Antimicrobial Susceptibility Testing

The antibiotic sensitivity test of the bacterial isolates was performed by disc diffusion technique using commercially available discs on nutrient agar plates (Iroha *et al.,* 2009).

The susceptibility of the fungal isolates on the commonly used antifungal drugs was determined using agar well diffusion method according to Ochie and Kokhalker (2008). The zones of inhibition were determined by measuring the diameter in millimeter of zone to which the antifungal drugs inhibited the growth of the organisms.

RESULT

The various results for the various tests done are shown below.

Table 1: Characteristics of the bacteria isolates

Morphological characterization								**Sugar Fermentation Test**			**Suspected Organisms**
Colour	**Consistency/Texture**	**Gram staining**	**Catalase Test**	**Oxidase Test**	**Indole Test**	**Voges Proskauer**	**Motility Test**	**Glucose**	**Lactose**	**Fructose**	
Grayish	Small round colony	+	-	+	-	-	-	+	-	-	*Bacillus* species
Light yellow	Slightly raised	-	+	+	-	-	-	+	-	-	*Pseudomonas* species
Dirty white	Raised rough surface	-	+	-	+	-	+	+	-	+	*Erwinia* species
Creamy	Raised/ smooth edge	+	+	-	-	-	-	+	-	-	*Staphylococcus* species
Creamy	Flat surface	-	+	-	-	+	-	+	+	+	*Klebsiella* species
Creamy	Raised/ smooth edge	+	+	-	-	-	-	+	-	-	*Staphylococcus* species

The bacterial isolates were then characterized as shown in Table 1 above. Six bacteria (*Bacillus* species, *Pseudomonas* species, *Erwinia* species, *Staphylococcus* species, *Klebsiella* species and *Staphylococcus* species) were isolated in this study and suspected to be found in the salt mining site.

Table 2: Morphological and microscopic feature of fungi isolates

Morphological Characteristics	**Microscopic Examination**	**Suspected Organisms**
White filamentous growth that turns to black sporulation	Long septate hyphae with swollen conidiophore bearing phialide at its apex	*Aspergillus* species
Long hyphael growth that sporulates to black within 48 hours	Long non-septate hyphae with mycelium bearing terminal sporongiosphores on columella	*Rhizopus* species
White wooly growth that turns darker as it sporulates	Non-septate hyphae with straight sporangiophore spherical spores	*Mucor* species
Pink, fluffy and spreading colonies with creamy surface around its edges	Septate hyphae with sickle chlamydospores at the hyphae	*Fusarium* species
Powdery yellow raised rough surface colonies	Septate and branch condioshore with brush like conidial head	*Penicillium* species

The fungal isolates were then characterized as shown in Table 2 above. Five fungi (*Aspergillus* species, *Rhizopus* species, *Mucor* species, *Fusarium* species and *Penicillium* species) were isolated in this study and suspected to be found in the salt mining site.

Table 3: Distribution and percentage frequency of bacterial isolates from group A and B

Bacterial Isolates	**(100 Sample) Group A (%)**	**(100 Sample) Group B (%)**	**Total**
Pseudomonas species	2 (14.3%)	5 (13.5%)	7 (13.7%)
Bacillus species	5 (35.7%)	14 (37.8%)	19 (37.3%)
Erwinia species	1 (7.1%)	4 (10.8%)	5 (9.8%)
Klebsiella species	2 (14.3%)	6 (16.3%)	8 (15.7%)
Staphylococcus species	4 (28.6%)	8 (21.6%)	12 (23.5%)
Total	**14**	**37**	**51**

Table 4: Distribution and percentage frequency of bacterial isolates from group C

Bacterial Isolates	**(100 Sample) Group C (%)**
Pseudomonas species	15 (16.7%)
Bacillus species	25 (27.8%)
Erwinia species	9 (10.0%)
Klebsiella species	13 (14.4%)
Staphylococcus species	28 (31.1%)
Total	**90**

Table 5: Distribution and percentage frequency of fungal isolates from group A and B

Fungal Isolates	(100 Sample) Group A (%)	(100 Sample) Group B (%)	Total
Aspergillus species	8 (42.1%)	5 (16.1%)	13 (26.0%)
Rhizopus species	9 (47.4%)	12 (38.7%)	21 (42.0%)
Mucor species	-	2 (6.5%)	2 (4.0%)
Fusarium species	-	4 (12.9%)	4 (8.0%)
Penicillum species	2 (10.5%)	8 (25.8%)	10 (20.0%)
Total	**19**	**31**	**50**

Table 6: Distribution and percentage frequency of fungal isolates from group C

Fungal Isolates	(100 Sample) Group C (%)
Aspergillus species	11 (19.3%)
Rhizopus species	16 (28.1%)
Mucor species	7 (12.3%)
Fusarium species	9 (15.7%)
Penicillium species	14 (24.6%)
Total	**57**

Out of 300 samples analyzed from the salt mining site, a total of 14, 37 and 90 bacteria isolates were obtained from sample group A, B and C respectively as shown in Table 3 and 4, while 19, 31 and 57 fungal isolates were from sample group A, B and C respectively as shown in Table 5 and 6. Out of which 14.3%, 35.7%, 7.1%, 14.3% and 28.6% were *Bacillus* species, *Erwinia* species, *Klebsiella* species and *Staphylococcus* species respectively from sample group A; 13.5%, 37.8%, 10.8, 16.3 and 21.6% were *Bacillus* species, *Erwinia* species, *Klebsiella* species and *Staphylococcus* species respectively from sample group B; and 16.7%, 27.8%, 10.0%, 14.4% and 31.1% were *Bacillus* species, *Erwinia* species, *Klebsiella* species and *Staphylococcus* species respectively from sample group C. 42.1% and 47.4% were *Aspergillus* species and *Rhizopus* species respectively from sample group A; 16.1%, 38.7%, 6.5%, 12.9% and 25.8% were *Aspergillus* species, *Rhizopus* species, *Mucor* species, *Fusarium* species and *Penicillium* species respectively from sample group B; and 19.3%, 28.1%, 12.3%, 15.7% and 24.6% were *Aspergillus* species, *Rhizopus* species, *Mucor* species, *Fusarium* species and *Penicillium* species respectively from sample group C.

Table 7: Antibiotic sensitivity of bacteria isolates

Bacterial Isolate	CPX	CEP	PN	SXT	AU	CN	OFX	PEF
Bacillus species	25	-	-	-	15	-	11	17
Pseudomonas species	21	14	-	-	22	-	17	-
Erwinia species	-	-	19	-	-	-	-	14
Klebsiella species	22	18	-	-	16	-	-	-
Staphylococcus species	10	18	-	-	13	-	-	-

Key: OFX = Ofloxacin, PEF = Pefloxacin, CEP = Ciprorex, AU = Augumentin, CN = Gentamycin, CPX = Ciproflox, SXT = Septrin, PN = Ampicillin, - = Nil

The result of the susceptibility pattern of the commercially used antibiotic disc against the bacterial isolates is shown in Table 7 above. The antibacterial activity of the antibiotics used showed that ciproflox has the best inhibitory effect on all the organism tested with inhibition zone diameter (mm) of 25 mm, 21mm, 22 mm, 18 mm and 10mm against *Bacillus* species, *Pseudomonas* species, *Klebsiella* species and *Staphylococcus* species respectively, but showed no inhibitory effect on *Erwinia* species. Subsequently, augmentin showed a reasonable inhibitory effect on *Pseudomonas* species, *Klebsiella* species, *Bacillus* species and *Staphylococcus* species with inhibition zone diameter of 22 mm, 16 mm, 15 mm and 13 mm respectively. Ciprorex showed inhibitory effect on *Klebsiella* species, *Staphylococcus* species and *Pseudomonas* species with inhibition zone diameter of 18 mm, 18 mm and 14 mm respectively. Ampicillin and pefloxacin were able to show inhibitory effect on *Klebsiella* species, while septrin and gentamycin showed no inhibitory effect on all the test organisms.

Table 8: Antifungal susceptibility of the antifungal drugs

Fungal Isolates	Metronidazole (mm)					Ketoconazole (mm)					Itraconazole (mm)					Fluconazole (mm)				
	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1	2	4	6	8	1
Aspergillus species	-	-	-	-	-	-	-	-	12	17	-	-	6	10	13	-	-	-	-	-
Rhizopus species	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	8
Mucor species	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium species	-	-	-	-	-	-	6	10	10	-	-	-	-	-	-	-	-	-	-	-
Penicillium species	-	-	-	5	8	-	-	-	-	-	-	4	7	12	-	-	-	-	-	-

Key: - = Nil

The result of the susceptibility pattern of the antifungal drugs against the fungal isolates is shown in Table 8 above. Ketoconazole showed inhibitory effect on *Aspergillus* species and *Fusarium* species, and no inhibitory effect on other test organisms. In the same vein, itraconazole showed inhibitory effect only *Aspergillus* species and *Penicillum* species, with no inhibitory effect on other test organism. Metronidazole and fluconazole showed the least inhibitory effect on the entire test organism.

DISCUSSION

Microorganisms from samples collected from the various sites of salt mining sites were found highly diverse group of halotolerant and halophilic microorganisms with different morphological characteristics (Table 1 and 2). However, the morphological characteristics alone were not enough to differentiate the bacterial and fungal isolates and could lead to identification problems. The main reason for this is the standardization of a conventional method, when it was applied to halophilic microorganisms because their growth characteristic highly dependent on many factors such as temperature, pH, medium composition and NaCl concentrations. Our results of morphological characteristics are in accordance with Fritze (2002) who recommended that morphological characterization results cannot be directly compared without full background knowledge of the precise conditions used for a particular test. Hence the microorganisms were subjected to various biochemical tests (Table 1 and 2). Several studies have been conducted on the ecology, taxonomy, and phylogeny of halophilic bacteria as well as their biochemical properties (Lichfield and Gillevet, 2002; Aneela *et al*., 2012).

The results of this work showed that *Bacillus* species (37.3%) and *Staphylococcus* species (23.5%) were more predominant bacteria from the salt mining site (sample group A and B), followed by *Klebsiella* species (15.7%) and *Pseudomonas* species (13.7%), while *Erwinia* species recorded the least frequency of 9.8%. These results are in agreement with those reported by Quesada *et al*. (1982), and Rodriguez-Valera (1988), who reported higher frequencies of moderately halotolerant and halophilic bacteria compared to extremely halophilic bacteria in saline environments. Subsequently, *Rhizopus* species (42.0%) and *Aspergillus* species (26.0%) were more predominant fungi from the salt mining site (sample group A and B), followed by *Penicillium* species (20.0%) and *Fusarium* species (8.0%), while *Mucor* species recorded the least frequency of 4.0%. This result is also in line with the work of Shweta *et al*. (2012), who reported the presence of salt tolerance of halophilic fungi from mangroves and solar salterns. And also the work of Mbata (2008) who isolated fungi from hyper saline Dead Sea water.

The frequency occurrence of the bacterial isolates in this work has shown that out of the five bacterial isolates, *Bacillus* species (37.3%) has the highest frequency in salt mining site (sample group A and B), followed by *Staphylococcus* species (23.5%) and *Klebsiella* species (15.7%), while *Pseudomonas* species and *Erwinia* species had the least frequency of 13.7% and 9.8% respectively. This is in agreement with the work of Nasrin *et al.* (2012), who reported the *Bacillus* genus are more prevalent in salt containing environments compared to other microbial groups that may be found under such environments. However bacterial diversity was more evident on the basis of presence/absence of endospores in case of our isolates related to *Bacillus* species. Results of this study are also in accordance with those of Rohban *et al*. (2009), who isolated bacterial strains belonging to the genera *Bacillus* species from Saltan lake of Iran. In the same vein Lucretia *et al*. (2009), also revealed the presence of *Bacillus* species at the highest frequency when compared to other microbial population isolated from Sua pan solar salterns, South Africa. However, the high frequency of *Staphylococcus* species (23.5%) isolated in this work is also similar to the work of Nasrin *et al.* (2012), who showed that *Staphylococcus* species can also be isolated from salt mining site at high frequency. Aneela *et al.* (2012) reported the presence *Staphylococcus* species (4.8%) at a very low frequency from salt mines of Karak, Pakistan, which is contrary to the finding of this work, but also an indication that *Staphylococcus* species can be isolated from salt mining site. *Pseudomonas* species in this work was isolated in low proportion, this is in accordance with the work of Aneela *et al.* (2012), who reported the presence *Pseudomonas* species (4.8%) at a very low frequency from salt mines. Similarly, Dombrowski (1963)

reported the *Pseudomonas* species in Paleozoic salt deposits. Abdulla (2009) reported the presence of *Erwinia* species, which were highly resistance to heavy metals (Cu, Cd, Hg, Mn, Ni and Zn). *Erwinia* species in this work was isolated in the lowest percentage frequency of 9.8%. Hence this is an indication that *Erwinia* species have high tolerance to metals hence can be isolated from halophilic environment which are known to contain some of this heavy metals at a reasonable proportion.

The frequency occurrence of the fungal isolates has showed that out of the five fungal isolates, *Rhizopus* species (42.0%) has the highest frequency from the salt mining site (sample group A and B), followed by *Aspergillus* species (26.0%), *Penicillium* species (20.0%) and *Fusarium* species (8.0%), while *Mucor* species had the least frequency of 4.0% (Table 5). *Rhizopus s*pecies has the highest frequency from the salt mining site. This in line with the work of Altaf and Mairza (2003), who isolated *Rhizopus s*pecies at a very high proportion from Khewra salt mine in Pakistan. This is an indication that *Rhizopus s*pecies can be isolated from salt mining sites. The reasonably high percentage frequency of *Aspergillus* species in this work is in accordance with the work of Mbata (2008), who isolated *Aspergillus versicolor* at a very high frequency 44.1% from hypersaline Dead Sea water. *Penicillium* species and *Fusarium* species were also isolated from the salt mining site. This is similar with the work of Asya *et al.* (2009), who reported the presence of *Penicillium* species, *Fusarium* species and *Mucor* species, and other fungi from Dead Sea in Syria-Africa.

The sample group C, which is obtained outside the salt mining site showed more bacterial and fungal isolates when compared to the salt mining site (group A and B). However, microbial population increases as one moves away from the salt mining site and observation that conforms to the work of Dobler *et al*. (2001), who reported that substrate metabolism (utilization) by microbes' increases with distance away from the point of heavy pollution. The type of microorganism present in terrestrial environment is often dependent on the nature of nutrient available and the competency of the microbial population to withstand the pressure (stress imposed on them by the pollutants) (Barkay and Pritchard, 1988). Their detection stems from their ability to survive and function under stress (Kivisaar, 2003).

Antibiotic resistance is a concern for the management of diseases in humans, animals and plants. The intense research efforts to elucidate mechanism of resistance have focused on genes derived from a narrow range of environments. Most of the known resistance determinants have been discovered in clinical and vertinary bacterial isolates, whereas the environmental reservoirs of antibiotic resistance are not well characterized (Nwosu, 2001 and Seveno *et al*., 2002).

Antibiotics used in this study included ofloxacin, pefloxacin, ciprorex, augumentin, gentamycin, ciproflox, septrin and penicillin. *Bacillus* species was fully susceptible ciproflox, pefloxacin, augumentin and resistant to ofloxacin, ciprorex, gentamycin, septrin and ampicillin (Table 7). This result in line with the work of Belma *et al*. (2002), who isolated *Bacillus* species from soil sample and reported their resistance to ampicillin, gentamycin and ciproflox. In the same vain Ikpeme *et al*. (2011) reported the resistance of *Bacillus* species to gentamicin, penicillin and other commonly used antibiotics. *Pseudomonas* species were resistant to pefloxacin, gentamycin, septrin and penicillin, and showed moderate resistance to ofloxacin and ciprorex. *Klebsiella* species were resistant to ofloxacin, pefloxacin, gentamycin, septrin and ampicillin this work. *Staphylococcus* species were resistant to almost all the antibiotics tested. *Erwinia* species showed resistance to all the antibiotics tested in this work. The fact that several bacterial species are known to be resistant to a wide array of antibiotics was confirmed by Aseffa *et al.* (1997).

The result of this work showed that *Rhizopus* species and *Mucor* species were resistant to all the antifungal drugs tested. Ketoconazole showed inhibitory effect on *Aspergillus* species and *Fusarium* species, and no inhibitory effect on other test organisms. In the same vein, itraconazole showed inhibitory effect only *Aspergillus* species and *Penicillium* species, with no inhibitory effect on other test organism. Metronidazole and fluconazole showed the least inhibitory effect on the entire test organisms. This might be attributed to inappropriate prescription of these antifungal drugs by healthcare providers or unnecessary use of antifungal drugs by human.

REFERENCES

Abdulla, D.A. (2009). Plasmids profiles, antibiotic and heavy metal resistance incidence of endophytic bacteria isolated from grapevine (*Vitis vinifera* L.). *African Journal of Biotechnology*. 8 (21), 5873-5882.

Altaf, H. and Mirza, S.B. (2003). Comparative Study of the Effect of Na^{+}, K^{+} and Ca^{++} Metals and *Rhizopus species* on the Growth of *Acacia nilotica* and *Peganum harmala* Seeds, Khewra Salt Mine, District Jhelum and Muzaffarabad, Azad Kashmir, Pakistan. *Pakistan Journal of Biological Sciences*. 6(15), 1324-1327.

Aneela, R., Iftikhar, A., Muhammad, I. and Muhammad, J. (2012). preliminary isolation and characterization of halotolerant and halophilic bacteria from salt mines of Karak, Pakistan. *Pak. J. Bot*. 44, 365-370.

Aseffa, A., Gedhi, E. and Asmelash. J. (1997). Antibiotic resistance of prevalent *Salmonella* and *Shigella* strains in North West Ethiopia. *East African Medical Journal*. 174, 708-713.

Barkay, T. and Pritchard, H. (1988). Adaptation of aquatic microbial communities to pollutant stress. *Microbiol. Sci.* 5, 165-669.

Belma, A., Necdet, S. and Yavuz, B. (2002). Determination of Some Properties of Bacillus Isolated from Soil. *Turk J. Biol*. 26, 41-48.

Caldwell, J.H., Schaller, K.L., Lasher, R.S., Peles, E. and Levinson, S.R. (2000). Sodium channel Nav1.6 is localized at nodes of Ranvier, dendrites, and synapses. *Proceedings of the National Academy of Sciences*. 97 (10), 5616–5620.

Cheesbrough, M. (2006). Distrcit laboratory practice in tropical countries, part 2. Cambridge University Press, Cambridge, United Kingdom. PP. 137-150.

DeLong, D.C. (1996). Defining biodiversity. *Wildlife Soc. Bull.* 24, 38-749.

Dobler, R., Burri, P., Gruiz, K., Brandl, H. and Bachofen, R. (2001). Variability in microbial population in soil highly polluted with heavy metals in the basis of substrate utilization pattern analysis. *J. soils and Sed.* 1 (3), 151-158.

Dombrowski, H. (1963). Bacteria from paleozoic salt deposits. *Ann. Acad. Sci.* 108: 453-460Dombrowski, H.J. (1966). Geological problems in the question of living bacteria in Paleozoic salt deposits. Northern Ohio Geological Society, USA. PP. 215-219.

Ezeh, H.N. and Chukwu, E. (2011). Small scale mining and heavy metals pollution of agricultural soils: The case of Ishiagu mining district, South Eastern Nigeria. *Journal of Geology and Mining Research*. 3 (4), 87-104.

Farrow, J.A.E., Ash, C., Wallbanks, S. and Collins, M.D. (1992). Phylogenetic analysis of the genera *Planococcus, Marinococcus* and *Sporosarcina* and their relationship to the genus *Bacillus*. *FEMS Microbiol. Lett.* 93, 167-172.

Fritze, D. (2002). *Bacillus* identification traditional approaches. In: Applications and Systematics of Bacillus and Relative*s*,(Eds.): R. Berkeley, M. Heyndrickx, N. Logan and P. DeVos. Oxford: Blackwel. PP. 100-122.

Ghauri, M.A., Khalid, A.M., Grant, S., Grant, W.D. and Heaphy, S. (2006). Phylogenetic analysis of bacterial isolates from man-made high-pH, high-salt environments and identification of gene-cassette-associated ORFs. *Curr. Microbiol.* 52, 487-492.

Ikpeme, E., Nfongeh, J., Enyi-Idoh, K., Eja, M.E. and Etim, L (2011). Antibiotic susceptibility profiles of enteric bacterial isolates from dumpsite utisols and water sources in a rural community in Cross River State, Southern Nigeria. *Nature and Science*. 9(5), 46-50.

Iroha, I.R., Adikwu, M.U., Esimone, C.O., Aibinu, I. and Amadi, E.S. (2009). Extended spectrum Beta- L actamase (EBSL) in *E. coli* isolated from a tertiary hospital in Enugu state, Nigeria Pak. *Journal of Medical Sciences*. 25(2), 279-282.

Kivisaar, M. (2003). Stationary mutagenesis: Mechanisms that accelerate adaptation of microbial population under environmental stress. *Environ. Microbial*. 5 (10), 814-827.

Lichfield, C. D. (2002). Halophiles. *J. Ind. Microbiol. Biotechnol.*, 28, 21–22.

Lucretia, G., Lureshini, N. and Mathabatha, E.S. (2009). Isolation of hydrolase producing bacteria from Sua pan solar salterns and the production of endo-1, 4-bxylanase from a newly isolated haloalkaliphilic *Nesterenkonia* spp. *African Journal of Biotechnology*. 8(20), 5458-5466.

Ofomata, G.E.K. (1975). Land regions. In: Nigeria in maps: Eastern States. Ethiope pub. House. Benin. PP. 33-37.

Olsen, G.J., Woese, C.R. and Overbeek, R.C. (1994). The winds of (evolutionary) change: breathing new life into microbiology. *J. Bacteriol.* 176, 1-6.

Oyeleke, S.B. and Manga, S.B. (2008a). Essentials of laboratory praticals in microbiology. 1st edition. Tobest Publishers, Minna, Nigeria.

Mbata, T.I. (2008). Isolation of fungi in hyper saline Dead Sea water. Sudanese *Journal of Public Health*. 3 (4), 170-172.

McCarron, D.A., Geerling, J. C., Kazaks, A.G. and Stern, J.S. (2009). Can Dietary Investment Opportunities in Nigeria, ION (2010). Salt production in Nigeria: The opportunities. *Market Metroportal Review*. 25, 3.

Nasrin, A., Muhammad, A.G., Aamira, I., Munir, A.A. and Kalsoom, A. (2012) Biodiversity and phylogenetic analysis of culturable bacteria indigenous to Khewra salt mine of Pakistan and their industrial importance. *Brazilian Journal of Microbiology*. 39, 143-150.

Nwosu, V.C. (2001) Antibiotic resistance with particular reference to soil microorganisms. *Res Microbiol* 152: 421–430.

Prado, B., DelMoral, A., Quesada, A., Rios, R., Monteoliva-Sanchez, M., Campos, V. and Ramos-Cormenzana, A. (1991). Numerical taxonomy of moderately gram negative rods isolated from solar de Atacama Chile. *Syst. Appl. Microbiol.*, 14, 275-281.

Quesada, E., Ventosa, A., Rodriguez-Valera, F. and Ramos- Cormenzana, A. (1982). Types and properties of some bacteria isolated from hypersaline soils. *J. Appl. Microbiol*. 53: 155-161.

Rodriguez-Valera, F. (1988). Characteristics and microbial ecology of hypersaline environments. Boca Raton: CRC Press, London. PP. 3-30.

Rohban, R., Amoozegar, M.A. and Ventosa, A. (2009). Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran. *Ind. J .Microbiol. Biotechnol*. 36, 333-340.

Séveno, N.A., Kallifidas, D., Smalla, K., van Elsas, J.D., Collard, J.M., Karagouni, A.D. and Wellington, E.M.H. (2002). Occurrence and reservoirs of antibiotic resistance genes in the environment. *Rev. Med. Microbiol.* 13, 15–27.

Shweta, S.N., Valerie, G. and Sarita, W.N. (2012). Isolation and salt tolerance of halophilic fungi from mangrove. Indian Journal of Geo-Marine Sciences. 41(2), 164-172.

Taylor, R.S., Ashton, K.E., Moxham, T., Hooper, L. and Ebrahim, S. (2011). Reduced dietary salt for the prevention of cardiovascular disease: A meta-analysis of randomized controlled trials (Cochrane Review). *American journal of hypertension.* 24 (8), 843–53.

Okaji, O.O. (2009) Salt production in Uburu and Okposi okwu autonomous community, Ebonyi State. Ebonyi State Business Directory. 11: 1-3.

Weller, O. and Dumitroaia, Gheorghe (2005). The earliest salt production in the world: an early Neolithic exploitation in Poiana Slatinei-Lunca, Romania. *Antiquity.* 79: 306.

Woese, C.R. (1987). Bacterial evolution. *Microbiol. Rev.* 51, 221-271.