ISOLATION AND IDENTIFICATION OF IRON OXIDIZING BACTERIA FROM DIFFERENT SOURCES OF WATER WITHIN KANO METROPOLITANT, NIGERIA

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Abstracts: ***Iron oxidizing bacteria were isolated on winogradsky medium from samples of tap water, iron reservoir, wells, borehole and scratched rusted water-borne pipe collected in Gwale Local Government, Ungogo Local Government and Kano Munincipal, Kano State, Nigeria. The bacterial viable count was ranged from*** 1.4×10^4 ***to*** 3.0×10^4 ***in Gwale while ungogo ranged from*** 5.0×10^3 ***to*** 2.5×10^4***. Also for Kano Munincipal ranged from*** 7.6×10^3 ***to*** 3.2×10^4***. The bacterial isolates were all Gram negative bacilli observed microscopically. The apropriate biochemical test such as indole, methyl red (MR), citrate utilization, vogues proskauer (VP), catalase, oxidase and sugar fermentation (glucose, lactose and sucrose) were done. And the biochemical characterization were comfirmed the presence of Galionella ferruginea, Pseudomonas aeruginosa, Escherichia coli, klebseilla Pneumonia and Thiobacillus thioxidans. Therefore Ungogo show the highest percentages occurrence of the identified bacteria with 41.7%.***

Key words: Isolation, Iron, oxidizing bacteria.

INTRODUCTION:

Microganism plays an important part in the formation of ochereous deposit of bog iron ore that has significant effect on the economic value as well as health problem. These organism have been studied widely by various investigators, the work however has been done from the standard point of biology and geologic (Ehrenberg, 1836). For a number of years, many scientist engaged in study of isolation and identification of iron deposit bacteria which paid attention to the mode origin of these deposit (Weber et al, 2006). Microbiologically influenced corrosion is a reoccuring problem that has attacked nearly all common engineering metals and alloys including carbon, steel, wire steal, stainless steels, Aluminium, copper and high-nickel at varying rate (valde et al, 2008). This iron is found in most water in numerous forms, in true solution, as colloid, in suspension or as complex with other mineral or organic substance, it can impart a bitter taste when present in large amount, making the water unplatable (Yarzabal et al, 2006).

Justification:

Iron oxidizing bacteria have great silence impact to the economical value due to highly destruction of water-borne pipe ways, In Berlin 1971, the whole water distribution system were replaced bacause it was clogged with high deposit of iron bacteria into water system in addition to the health problem as a result of changes occur in water appearance to brownish due to some biochemical activities occurred in their cells. This study was carried out for the purpose to have the actual occurences of the iron bacteria in that particular area of study thereby providing the control or ways to terminate their existing.

Aim and Objectives:

The main aim of the study was to isolated and identified the iron oxidizing bacteria from different water sources.

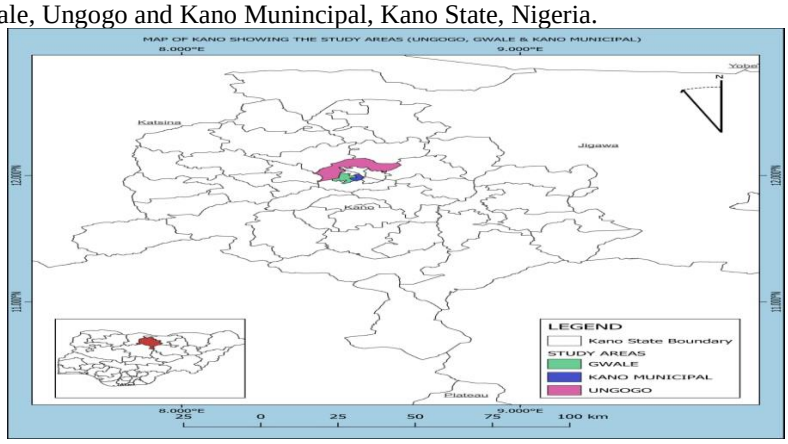
Objectives:

i. Enumeration of aerobic mesophilic bacterial count using standard plate method
ii. Isolation and identification of iron oxidizing bacteria.

Materials and Methods:

Study Area and Description:

The sample site include Gwale, Ungogo and Kano Munincipal, Kano State, Nigeria.



The study area is located between Latitude 11°58'N to 12°05'N of the Equator, and Longitude 08°32'E to 09°15'E of the meridian. According to National Population Commission census (NPC, 2006) the study area has an estimated population of about 2,962,550 by 2022.

The climate of Kano Metropolis is the tropical wet and dry type, coded as AW in the Koppens climatic classification. The mean annual rainfall in a normal year is about 800mm. However, the climate of the area is characterized by long dry season and short rainy season which a times fluctuates in terms of onset and cessation. Temperature in the area is generally high throughout the year. There are seasonal changes, indicating a gradual increase from January to April where maximum value reaches as high as 43º C (Ibrahim, 2011). The mean annual temperature is about 26°C, April and May is the highest temperature and the lowest in December, and evapotranspiration is generally high throughout the year (Olofin, 1987).

Pre-Cambrian rock of the basement complex which comprises of gneisses, amphibolites, marbles and the older granites which underlie large part of Nigeria including of the Kano Metropolitant (Olofin, 1987). Aquifers of the basement complex area of the area are weathered and fractured rocks in which groundwater exist under watertable conditions. Water table lies at a depth generally less than 7 metres, and the maximum depth of boreholes rarely exceeds 25 metres (Muhammed, 1984).

Obtaining Sample and Handling:
Five different water samples were collected from each sample site (well, bore-hole, iron reservoir, tap and rusted water-borne pipe) using sterile bijou bottle. The samples were transported to the microbiology research laboratory in accordance with the procedure described by Emerson and Floyd (2005).

Media Preparation:
Winogradsky media is one of the selective media suitable for the growth of hetetropic iron oxidizing bacteria and described it's composition below (Greenberg et al, 1992).

Organic matter (glucose or sucrose)	$10g$	
Agar		$20g$
caco_3		trace
*Concentration of salt solution	$5ml$	
Distilled H_2O		$1l$

***Compositions of the concentrated salt solution:**

KH_2PO_4	$0.25g$	
$MgSO_4.H_2O$		$0.125g$
$Nacl$		$0.125g$
$FeSO_4.7H_2O$		$0.005g$
$MnSO_4.4H_2O$		$0.005g$
$Na_2MoO_4.H_2O$	$0.005g$	
Tap Water		$5ml$

The PH of the medium was adjusted to 7.2 and was sterilized at $15ibs$ for 15 minutes. The glucose was autoclave separately at $10ibs$ for 10 minutes and then added to the medium aseptically as described by (Zawarin, 2006).

Aerobic Mesophilic Bacterial Connt:
The aerobic mesophilic bacterial counts were done in accordance with the method outline by mahbubar et al (2010). In this method, the water sample is serially diluted (10-fold) with sterile saline water and $0.1ml$ of each diluted water samples from the test tube were mixed with molten winogradsky medium using pour plate technique, and was incubated at 37℃ for 24 $hours$ under aerobic condition. Isolated colonies were counted and the titres expressed in cfu/ml.

Gram Staining Principle:
Grams staining was carned out as described by cheese brough (2003).

Biochemical Test:
The biochemical test such as indole, methyl red (MR), vogues proskeur (VP), citrate utilization, Respiratory test (oxidase and catalase), coagulase, urease and sugar fermentation (glucose, lactose and sucrose) were performed to identified the presence of iron oxidizing bacteria (Hedrich et al, 2011).

Result and Discussion:
From the mean value of aerobic mesophilic bacterial count. Well water in Gwale have the highest aerobic mesophilic count (MBC) with 1.8×10^4 while bore-hole and iron reservoir have 2.6×10^4 and 1.5×10^4 respectively in both Gwale and Kano Munincipal. Tap water was found to be highest in Ungogo with 2.5×10^4. For the rusted water born pipe, Kano Munincipal had the highest count of 3.2×10^4 as shown in table 1.

Table1: Mean Value of Aerobic Mesophilic Bacterial Count in Gwale, Ungogo and Munincipal.

Sources of Water	Gwale AMBC (cfu/ml)	Ungogo AMBC (cfu/ml)	Munincipal AMBC (cfu/ml)
Well	1.8×10^4	5.0×10^3	7.6×10^3
Borehole	2.6×10^4	8.3×10^3	2.6×10^4
Iron reservoir	1.5×10^4	No growth	1.5×10^4
Tap	1.4×10^4	2.5×10^4	2.2×10^4
Rusted water-borne pipe	3.0×10^4	1.9×10^4	3.2×10^4

AMBC= Aerobic Mesophilic Bacterial Comt. $cfu/ml =$ Colony forming units per millilitre.

From the percentage occurance of bacteria from different sample site, Ungogo have the highest percentage of 41.7% followed by Gwale with 33% and 25% observed in Kano municipal presented in table 2.

Table 2: Bacterial Isolates from Different Sources of Water.

Sources of H_2O	No. of Screened Sample	No. of Positive Isolates	Organism
Well	3	6	*E.coli,K Pneumoniae P. aeruginosa*
Borehole	3	6	*E.coli,K Pneumoniae P. aeruginosa*
Iron reservoir	3	2	*E.coli, G.ferruginea*
Tap	3	4	*E.coli, K. Pneumoniae*
RWBP	3	6	*E.coli, G.ferruginea T.Thiooxidans*

RWBP= Rusted Water-borne Pipe

Three samples were screened from each sources of water in which well, borehole and rusted water borne pipe has the highest of six isolates where iron reservoir has the lowest two positive isolates as shown in table 3.

Table 3: Percentage Occurences of bacteria from sample sites

S/n	Sample Site	No.of Screened Sample	No. of Positive	% Occurences
1	Ungogo	5	10	41.7%
2	Gwale	5	8	33.3%
3	Municipal	5	6	25.0%
	Total	15	24	100%

Discussion:

From the result obtained, Gwale Local Government having the highest bacterial count of 1.8×10^4 in well water while Ungogo had lowest count of 5.0×10^3. Therefore, much iron precipitated in Gwale may necessarily be presented in well which lead to the well degradation gradually. In Kano Municipal and Gwale, iron reservoir and borehole had the same highest bacterial count of 1.5×10^4 and 2.6×10^4 respectively. This information indicated that there must be tendency of high clogged by the iron precipitate in the water distribution system present in the both two site (Mugg, 1972). Particularly Kano Municipal that shown the overall highest count of 1.9×10^4 in rusted water-borne pipe.

Galionella ferruginea were isolated in scratched rusted water-borne pipe and well water, Galionella is the major genera of the members of iron oxidizing bacteria that catalyzed the oxidation of iron in well and coupled with massive corrosion of water-borne pipe system (Ehrenbera, 1836). *Pseudomanas aeruginosa* and *Klebsiella Pneumoniae* were also isolated from well and borehole, *Pseudomanas aeruginosa* is said to be a pioneer colonizer in the process of biofilms in well (San et al, 2014). E.coli were isolated in the entire sample and is said to have the highest number of appearance due to its availability to be found everywhere as a sources of contamination not only as catalyzed the oxidation of iron. While *Thiobacillis thiooxidans* was isolated in only rusted water-borne pipe samples with lowest number of appearance as a result of its high acidity characteristics, the last table concluded that Ungogo has the highest percentage occurance of iron bacteria with 41.7%.

Conclusion:
Conclusively, the presence of iron bacteria revealed that there is subtantial body information available concerning bacterial corrosion in both sample site of study.

Recommendation:
Further isolation will enhance the chance to get wide variety of bacterial species, ways should be employ to create strong barrier between organism and iron water-borne pipe against economic lost which include physical and chemical method.

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