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Editorial Board

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Microbiological quality of selected street foods from Antananarivo on 2016-2017: public health implications

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ABSTRACT

People in a big city as Antananarivo, capital of Madagascar, have leads to take street foods for their daily nutritional needs. This food habits may be a risk for consumers due to contaminations from street environment and bad practices related to hygiene. This study aimed to examine the quality and safety of street vended foods in Antananarivo, on January 2016 to December 2017.Six hundred and sixty two samples including 126samples of melting salads, 70 beef skewers, 54 chicken skewers, and typical Malagasy foods as : mofoanana (67 samples), mofogasy (64 samples), ramanonaka (64), makasaoka (66), mofoakondro (62) and kobandravina(89);were randomly collected from the streetvendors in Antananarivo marketsto evaluate their bacteriological quality.International Methods (ISO) was adopted for to find the load of Total Aerobic Bacteria andEnterobateriaceae,*Escherichia coli* and to search pathogen bacteria as *Salmonella, Campylobacter jejuni, Escherichia coli* O157H7 and *Bacillus cereus* in these foods.The results revealed that the mean values ofthe Total Aerobic Bacteria count was $0.1x10^6$- $4.8x10^6$cfu/g. *Enterobacteriaceae*count range from $0.4x10^2$ to $1.9x10^2$cfu/g. *Escherichia coli* count range from $0.04x10^2$cfu/g. to 0.19 $x10^2$cfu/g.*Salmonella*was only present in melting salads, beef skewers and chicken skewers samples. *Bacillus cereus* count range from $0,1x10^2$ to $1,5x10^2$cfu/g. *Campylobacter jejuni*was only present in samples of ramanonaka and kobandravina. Two strains of presumptive *Eschercichia coli* O157 H7 (βglucuronidase -) were isolated. PCR method was used to confirm the identity of these two isolates. A high contamination above 10^6 cfu/g food and the presence of potential pathogens bacteria could be hazardous. Systematic inspections and training of food vendors on food hygiene and application of hazard analysis critical control point (HACCP) has been recognised as measures to guarantee improvement of the quality of street foods.

Keywords: *streetfoods, Escherichia coli,E. coli O157H7, food borne diseases, Antananarivo, Madagascar, Campylobacter, Bacillus cereus.*

I. INTRODUCTION

Street-vended foods are ready-to-eat foods prepared and sold by vendors on streets or public places for fast consumption (14).

The street food is a growing sector in many developing countries. They provide a source of inexpensive and nutritional meals, although providing a source of income for the vendors (29).

Anyway, street vended food products may represent a risk due to inadequate personnel hygiene of vendors, the bad condition at which it produced, in as using raw materials of bad quality. Such contamination may render the product of inferior quality or unfit for human consumption (31*).*
It has been shown that Street-vended foods have been implicated in outbreaks of foodborne illnesses all around the world. In Madagascar, there was three food bornediseases due to *Salmonella typhi*reported on 2015-2016 and*Escherichia coli* was identified as responsible of so many infections and toxi-infections on 2017 (31, 33).

Escherichia coli, Salmonella and *Campylobacter jejuni*have recognized as a serious bacteria pathogen and has been associated with several out breaks of disease. In Madagascar, there is no available data about these bacteria and street foods. Therefore, this study was led to evaluate the microbial quality of street vended foods in Antananarivo, capital city of Madagascar on January 2016 to December 2017.

II. MATERIAL AND METHODS

Collection of samples

Six hundred and sixty two samples including 126 samples of melting salads, 70 beef skewers, 54 chicken skewers, and typical Malagasy foods as :Mofoanana (67 samples), Mofogasy (64 samples), Ramanonaka (64), Makasaoka (66), Mofoakondro (62) and Kobandravina (89);were randomly collected from the streetvendors in Antananarivo markets. Samples were sent to the laboratory within two hours after collection in a cold-box containing ice-blocks. Characteristics and nature of each food are presented in Table 1.

Table I: Description of foods analysed

Food	Nature	Description
Melting salads	Solid Mixed : cooked and fresh	Salted food, Melting salads composed by spaghetty, vegetables, Minced meat
Beef skewers	Solid Smoked	Beefsmokedpiece
Mofoanana	Solid Cooked	Typically Malagasy foods composed by vegetable, Brede fritter
Makasoka	Solid Cooked	Typically Malagasy foods : a kind of French toast
Mofogasy	Solid Cooked	Typically Malagasy foods:Sweet fried food made of rice flour.
Ramanonaka	Solid Cooked	Typically Malagasy foods : salted fried food made of rice flour
Mofoakondro	Solid Cooked	Sweet banana fritter
Chicken skewers	Solid Smoked	Chickensmokedpiece
Kobandravina	Solid Cooked	Typically Malagasy foods : a kind of pudding made of peanuts, flour, sugar,grinned and cooked inbanana leaves

III. Sample preparations and analysis

Serial dilution

Twenty-five grams (25 g) of each sample was mixed carefully with 225 ml of buffered peptone water. This mixture was homogenized and shacked to obtain a uniform mixture. One ml of the homogenized

food sample was aseptically transferred into a test tube containing 9 ml sterile distilled water. Five dilutions of the homogenates were prepared in conformity with the recommendation of the norm ISO 6887 (6).

Enumeration of Total Aerobic Bacteria

Plate Count Agar (PCA) (Oxoid Ltd, United Kingdom) was used for Total Aerobic Bacteria and was done in conformity with the recommendation of the norm ISO 4833 (7).

Enumeration of *Bacillus cereus*

The recommendation of the norm ISO 7932 was used. 1 ml of the dilution of each food sample was plated onto polymyxin-pyruvate-egg yolk mannitol-bromothymol blue agar plates (Oxoid), which were air dried and incubated at 37°C for 24 to 48 h. Blue colonies with blue zones were subjected to appropriate biochemical tests (9,18).

Detection of *Salmonella* spp.

*Salmonella spp*was detected with the recommendation of the norm ISO 6579. Twenty-five grams (25 g) of each sample was mixed with 225 ml of buffered peptone water and incubated at 37^{o}C for 16 h. One ml of this culture was pipetted into 10 ml of Rappaport-VasilliadisSoya broth (RVS). These were incubated at 41^{o}C for 24 h. The culture was streaked intoHektoen Agar. The agar plate were incubated at 37^{o}C for 24 h. The plate were examined for typical green blue colonies of *Salmonella* (8, 16, 19).

Detection of *Escherichia coli β*glucuronidase +

1 ml of the dilution of each food sample was plated onto Eosin Methylen Blue Agar Medium and incubated at 44°C for 24h to 48 h. Black green metallic colonies were subjected to appropriate biochemical tests according to the norm ISO16649(11,14).

Detection of *Escherichia coli* O157:H7

This strain was determined using sorbitol MacConkey agar (Oxoid) plates. *Escherichia coli O 157 H7* doesn't use sorbitol and gives characteristics colonies on this medium. Then, strains suspects belonging to *E. coli* O157H7 must be identified by PCR, using Kit BAX (Qualicon, Inc. - USA) for screening *Escherichia coli* O157H7 with a detection rate around 96,5 % (12, , 14, 16).

Detection of *Campylobacter jejuni*

25 g of the food sample was mixed with 100 ml Preston broth (Oxoid) and homogenized for 2 min. The enrichment broth was incubated at 42°C for 24 to 48 h. The broth culture was streaked onto Skirrow's agar plates (Oxoid), which were then incubated at 42°C. Colonies were Gram stained and tested for oxidase reaction. Suspect colonies were subjected to appropriate biochemical tests, done in conformity with the recommendation of the norm ISO 10272: 2006 (10).

IV. RESULTS

As shown in Table 2, melting salads, beef skewers, chicken skewers and kobandravina were found to be contaminated. A high level of Total Aerobic Bacteria TAB (>10^{6}ufc/g),*Enterobacteriaceae*(>10^{2}/g) and *Escherichia coli* β glucuronidase + is noted.

The values ofthe Total Aerobic Bacteria count was 0.1x10^{6}- 4.8x10^{6}cfu/g. *Enterobacteriaceae*count range from 0.4x10^{2} to 1.9x10^{2}cfu/g and *Escherichia coli* count range from 0.04x10^{2}cfu/g. to 0.19 x10^{2}cfu/g.

Pathogen bacteria as *Salmonella* was only present in melting salads, beef skewers, chicken skewerssamples. *Bacillus cereus* count range from 0,1x10^{2} to 1,5x10^{2}cfu/g. *Campylobacter jejuni*was only present in samples of beef and chicken skewers. Two strains of*Eschercichia coli* O157 H7 (βglucuronidase -) were isolated and identified by PCR reaction from beef skewers.

Table 2: Microbiological assessment of street foods samples collected in Antananarivo market on 2015-2016.

Number	Samples	TAB. 10^6/g	Ent. 10^2/g	E.C.BG+ 10^2/g	E.C.BG- /g	SLM /g	CAMP /g	BC 10^2/g
126	Melting salads	4,854	1,927	1,259	A	12,49	A	1,445
70	Beef skewers	3,327	1,72	1,19	2	20,93	0,188	A
67	Mofoanana	0,424	0,785	0,04	A	A	A	0,572
66	Makasoka	0,182	0,489	0,09	A	A	A	0,574
64	Mofogasy	0,517	0,933	0,198	A	A	A	0,299
64	Ramanonaka	0,459	0,474	0,147	A	A	A	0,172
62	Mofoakondro	0,311	0,555	0,067	A	A	A	0,374
54	Chicken skewers	3,998	1,961	1,338	A	2,06	0,942	A
89	Kobandravina	1,585	1,199	1,011	A	A	A	1,553

TAB : Total Aerobic Bacteria
Ent :Enterobacteriaceae
E.C.BG + : *Eschercichia coli βglucuronidase* +
E.C.BG - : *Eschercichia coli βglucuronidase* -
SLM :*Salmonella spp*
CAMP :*Campylobacter jejuni*
BC :*Bacillus cereus*
A: Absent

V. DISCUSSION

The result of these different analysis carried out on street foods samplesrevealed that all samples collected were contaminated by microorganisms.This is due to the inadequate personnel hygiene of vendors, the bad condition at which it produced, and using raw materials of poor quality or the fact that they were exposed in an open air because there are several microorganisms (beneficial or pathogen) that we can find in environment (25, 34, 35).

If they are beneficial microorganisms, it is even profitable for the food because it allows to protect them, improve their tastes, their qualities (21). However, for harmful microorganisms, this could have impacts on the food (preservation) and on the consumers.

A high level Total Aerobic Bacteria TAB (>10^6/g) was reported on melting salads, beef skewers, chicken skewers and kobandravina. Its shows a general contamination. The TAB amount allowed to appreciate the general hygiene of the product (from raw material to storage and selling conditions) (31, 35).

Bacillus cereus is only present in highly concentration in melting salads and kobandravina. Their microbial load are superior to the bacteriological criteria. It could be due to the characteristic of this bacteria to metabolize starch while these foods are made of starch.

Enterobacteriaceae is a bacillus Gram negative family. A huge species as *Escherichia coli, Salmonella, Shigella, Klebsiella …*belong to this family.Due to table 2, melting salads, beef skewers, chicken skewers and kobandravinahave a high level of *Enterobacteriaceae*. It shows a faecal contamination of the product from the vendor, form the raw material or during the preparation.However, there are extremely pathogen species in this familyandassociated with many

cases of food borne diseases (31). However, they could easily be resistant on many antibiotics, which could train a treatment failure (30, 31,32).

Salmonella species belongs to the family of *Enterobacteriaceae*. It is only present in melting salads, beef and chicken skewers. It confirms study led on 2016, which showed especially the implication of melting salads and skewers for several cases of Typhoid fever in Madagascar (29, 31).

Escherichia coli is a bacteria that normally lives in the intestines of people and animals. There are many different types of *E. coli*. Most E. coli are found naturally in intestines and play an important role in helping our bodies digest food. However, a few types of *E. coli* can cause diarrhoea and other illnesses when swallowed.

A previous study led in the Urban Commune of Antananarivo, Health Ministry and WHO shows that this species is the first responsible for foodborne disease in Antananarivo on 2016. Indeed, 14 TIAC cases had been noticed in the capital city (29).In fact, melting salads, beef skewers, chicken skewers and kobandravinaare the most contaminated food by this germ. Their consumption could train illness as diarrhoea, dysentery. In 2015, *Tsirinirindravo and al* found that melting salads was the first food associated with foodborne illness in Antananarivo. However, it is very appreciated by consumers (29, 31).

Two species extremely pathogens were found in these street foods:*Campylobacter jejuni*and *Escherichia coli* O157 H7.

E. coli O157:H7 is a toxin producing bacteria that causes intestinal disease in people which lasts about one week. Diarrhoea with blood is typical. Haemolytic uremic syndrome (HUS) is a severe complication of *E. coli* O 157 H7 infection (24).

This germ is only present in beef skewers.Most of the times, it is present in meat or hamburger. *Escherichia coli* O157H7 is noted to be responsible of more than 20000 foodborne diseases per year in the United States (24, 33).

*Campylobacter jejuni*was only present on the two skewers. Infection with *C. jejuni* usually results in enteritis, which is characterised by abdominal pain, diarrhoea, fever, and malaise. Diarrhoea itself can vary in severity from loose to bloody stools (20, 23, 34).

VI. Conclusion

The study aims to determine the microbial quality of pre-cut ready-to-eat vegetable salads sold by food vendors in the Antananarivo markets on 2015-2016.

The most contaminated food are melting salads, beef skewers, chicken skewers and kobandravina. The typical Malagasy food as Mofogasy, Ramanonaka, Menakely are the healthiest, referring to their sanitary hygienic quality.

Melting salad, chicken skewers, beef skewers and kobandravina constitute a health risk to consumers, in terms of microbial quality. The contamination could come from unhygienic food preparation, process, environmental conditions, raw materials and improper food handling. Therefor a research should be carried out to determine the antimicrobial susceptibility of the bacteria identified, Street vendors must be trained about hygiene, Good Practice hygiene GPH. The HACCP system have to be build up for street vendors.

REFERENCES

[1.] ACHYUT A., KAREN K. Effect of storage time and temperature on the viability of *E. coli O157:H7, Salmonella spp., Listeria innocua, Staphylococcus aureus, and Clostridium*sporogenes vegetative cells and spores in vacuum-packed canned pasteurized milk cheese. Journal of Microbiology. July 2018, Volume 56, Issue 7, pp 450–459

[2.] BIBEK R., Fundamental Food Microbiology 2nd ed. The CRC Press Ltd Washington, DC 2001, 56-90).

[3.] CHUKWUEMEKA, NNENNE I.I. and CHRISTIAN U.I., Bacteriological quality of foods and water sold by vendors and in restaurants in Nsuka, Enugu State, Nigeria: A comparative study of three Microbiological methods, Journal of Heath Population and Nutrition, 29(6), 2011, 560-566.

[4.] EUN-JIN L., CHOI, J., GROISMAN, E.A. 2014. Control of a *Salmonella* virulence operon by proline-charged tRNApro. Proc. Natl. Acad. Sci. USA 111, 3140–3145.

[5.] ISLAM M., DOYLE M.P., PHATAK S. C., MILLNER P., JIANG X. "Survival of *Escherichia coli* 0157:H7 in Soil and on Carrots and Onions Grown in Fields Treated with Contaminated Manure Composts or Irrigation Water." Food Microbiol. 2005. 22: 63-70.

[6.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique. ISO 6887-1:1999.

[7.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour le dénombrement des micro-organismes. Technique de comptage des colonies à 30 degrés. ISO 4833:2003.

[8.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour la recherche des *Salmonella spp*. ISO 6579:2002.

[9.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Bacillus cereus* présomptifs. Technique par comptage des colonies à 30 degrés C. ISO 7932:2004.

[10.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour la recherche et le dénombrement de *Campylobacterspp*. ISO 10272:2006

[11.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour le dénombrement des *Escherichia coli* bêta-glucuronidase positive. ISO 16649 :2001.

[12.] INTERNATIONAL STANDARD ORGANISATION. Microbiologie des aliments. Méthode horizontale pour la recherche des *Escherichia coli* O157. ISO 16654:2001.

[13.] FOOD AND AGRICULTURAL ORGANIZATION. 1997 .Agriculture food and Nutrition for Africa. A resource book for teachers of Agriculture, Rome. 12 (3):25-29.

[14.] FOOD AND AGRICULTURE ORGANIZATION FAO.1992. *Escherichia coli* and other coliforms. Manual of food quality control. Rev.1- Microbiological Analysis, Food and Agriculture Organization of the United Nation, Rome, Italy, Chap. 3PP, 13-26.

[15.] FOOD AND DRUG ADMINISTRATION "FDA" 2001. *Staphylococcus aureus*. Bacteriological analytical manual .8th Ed. Chapter12. Gaithersburg, p.562.

[16.] FRANZ E., BRUGGEN A. H. 2008. Ecology of *E. coli* 0157:H7 and *Salmonella enterica* in the Primary Vegetable Production Chain." Crit. Rev. Microbiol. 34 (3-4): 143-161.

[17.] GILBERT R., DE LOUVOIS J., DONOVAN T., LITTLE C., NYE K., RIBEIRO C. D., RICHARDS J., ROBERTS D., BOLTON F. B. 2000. Guidelines for the microbiological quality of ready-to-eat foods sampled at point of sale. Commun. Dis. Public Health 3:163–167.

[18.] GRANUM P. E., ANDERSSON A., GAYTHER V., GIFFEL TE M., LARSEN H., LUND T. O., O'SULLIVAN K. 1996. Evidence for a further enterotoxin complex produced by *Bacillus cereus*. FEMS Microbiol. Lett. 141:145–149

[19.] JONGHYUN B., EUNNA C., EUN-JIN L. A rule governing the FtsH-mediated proteolysis of the MgtC virulence protein from *Salmonella entericaserovarTyphimurium*. Journal of Microbiology, August 2018, Volume 56, Issue 8, pp 565–570

[20.] LARPENT J-P., LARPENT-GOURGAUD M. Mémento technique de microbiologie, 3éd. Londres, New York, Paris. Lavoisier, 1997 : 1039 p.

[21.] MANANJARA P., TSIRINIRINDRAVO H.L., RAHERIMANDIMBY M., RANDRIANIERENANA A. Etude des levures endogènes d'*Evodiabilahe*, rutacée endémique de Madagascar. Int. J. Biol. Chem. Sci. 10 (4): 1694- 1701. August 2016.

[22.] NICHOLS S.L., LITTLE C.L., MITHANI V., LOUVOIS J., The Microbiological quality of cooked rice from restaurants and take-away premisis in the United Kingdom, Journal of Food protection,62, 1999, 877-882.

[23.] PRESCOTT L.M., KLEIN D.A. (2008). Pathogenic Organisms. Microbiology, 7th ed McGrew hill, New York. pp 340, ISN 978-0-07-110231-5.

[24.] RANGEL J. M., SPARLING P. H., CROWE C., GRIFFIN P. M., SWERDLOW, D. L. 2005. Epidemiology of *Escherichia coli* O157: H7 Outbreaks in the United States from 1982 to 2002. Emerg. Infect. Dis.11 (4): 603-609.

[25.] RASOLONIAINA L., RANDRIAMAMPIONONA S., ANDRIAMANANTENA H., RAZAFIARIMANANA V., RASOAMAMPIONONA R., RAKOTOARISOA N., TSIRINIRINDRAVO H.L., ANDRIANJAKA A., DUPONNOIS R., ANDRIANARISOA B. « Effets des produits volcaniques sur les cultures du riz et maïs et dynamique de la microflore tellurique sur la culture de maïs » : GDRI-BDDM et les questions de biodiversité 3 au 5 novembre 2010, Lyon-France.

[26.] TAMBEKARAND D.H., MUNDHADA R.H. Bacteriological quality of salad vegetables sold in Amravati City (India), Journal of Biological Sciences, vol.6, no.1, pp.28–30, 2006.

[27.] TINKER I. (1997).Street Foods, Urban Food and Employment in developing Countries. Oxford. University Press, New York, 1997. pp 124-126

[28.] TOMOYASU, T., YAMANAKA, K., MURATA, K., SUZAKI, T., BOULOC, P., KATO, A., NIKI, H., HIRAGA, S., OGURA, T. 1993. Topology and subcellular localization of FtsH protein in *Escherichia coli*. J. Bacteriol. 175, 1352–1357.

[29.] TSIRINIRINDRAVO H. L., RANDRIANIERENANA L. A., ANDRIANARISOA B., RAHERIMANDIMBY M., RANDRIANANTOANDRO H. H., RAZAFINJATOVO D. N., DE PERCIN G., PIERLUIGI B. *Aspects épidémio-cliniques des toxi-infections alimentaires collectives (TIAC) dans la région Analamanga, cas de janvier à juin 2015. Communication lors des Journées QualiReg 2016. 5eme rencontre de l'Agroalimentaire en Océan Indien « La qualité et l'innovation au service du développement des filières agroalimentaire de l'Océan Indien. Saint Pierre, La Réunion.*

[30.] TSIRINIRINDRAVO H.L., ANDRIANARISOA B. Société Française de Microbiologie VIIIe Congrès National Marseille 2010, carrefour des microbiologies du Nord et du Sud Palais des Congrès, parc Chanot, Marseille SFM MARSEILLE 2010 - ABSTRACT n°039 Programme : ACTIVITES ANTIBACTERIENNES DE L'EXTRAIT ISSU DE MESANTHEMUM RUTENBERGIANUM (ERIOCAULACEES). 2, 3 et 4 juin 2010.

[31.] TSIRINIRINDRAVO H. L., RANDRIANIERENANA L. A., ANDRIANARISOA B., RAHERIMANDIMBY M., RANDRIANANTOANDRO H. H., RAZAFINJATOVO D. N., DE PERCIN G., PIERLUIGI B.Espèces de *Salmonella*multiresistantes véhiculées par les aliments de rue à Antananarivo. *Communication lors des Journées QualiReg 2016. 5eme rencontre de l'Agroalimentaire en Océan Indien « La qualité et l'innovation au service du développement des filières agroalimentaire de l'Océan Indien. Saint Pierre, La Réunion.*

[32.] TSIRINIRINDRAVO H.L., ANDRIANARISOA B. Activités antibactériennes de l'extrait des feuilles de *Dalechampiaclematidifolia* (Euphorbiaceae). Int. J. Biol. Chem. Sci. 3(5): 1198-1202, October 2009.

[33.] WATARU H., NORIYUKI N. High prevalence of blaCTX-M-14 among genetically diverse *Escherichia coli* recovered from retail raw chicken meat portions in Japan. Journal of Microbiology. August 2018, Volume 56, Issue 8, pp 530–535

[34.] WORLD HEALTH ORGANIZATION, Food borne diseases; a focus for health education, 53rd world health assembly, Geneva, 2000.

[35.] WORLD HEALTH ORGANISATION (1984). The Role of Food Safety in Health and Development. Technical Report Series 705. Expert Committee on Food Safety, Geneva: WHO: 1-79.

Bioremediation of soils polluted by petroleum hydrocarbons by *Pseudomonas putida*

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ABSTRACT

In order to clean up soils contaminated with hydrocarbons, the bioremediation activity of *Pseudomonas putida* was studied. *Pseudomonas putida* is a bacterium that can withstand the harshest environmental conditions. It is able to metabolize a wide range of petroleum hydrocarbons which is used as a source of carbon and energy. Given the potential of this microorganism, an experiment wasconducted on this strain.

For the isolation of this microorganism, a sample ofsoil from the Vakinankaratra region in the urban commune of Antsirabe II, Madagascar was microbiologically analysed. The bacterial identification was based on a study of the morphological, physicochemical and sequential analysis of the 16S rDNA gene.

The isolated strain was then inoculated into soil polluted by diesel engine oil from the garage at Universityof Saint Joseph Antsirabe, Madagascar. The kinetics ofbacterial growth showed that the biomass increased from 4.10^7 to 3.10^{13} CFU/g at the end of the experimentation. A growth rate of $0,32h^{-1}$ and a generation time of 2,16 hours were noted. The quantification of the residual hydrocarbons according to the EPA method (Environmental Protection Agency) 3540C have made it possible to deduce the capacity of degradation of the bacterial strain which is 0,2 mg of hydrocarbon per gram of soil per day. After 3 months of biological treatment, the concentration of the residual petroleum hydrocarbons had been reduced by 30%(from 18000 mg/kg to 5000 mg/kg). Thus, the ability of *Pseudomonas putida* to decontaminate polluted soils with hydrocarbons has been observed.

***Key words**: petroleum hydrocarbons, bioremediation, Pseudomonas putida, soil, oil.*

I. INTRODUCTION

Petroleum hydrocarbons are potentially toxic organic compounds. Their presence in the soil has negative impacts on both environmental quality and human health (Robert, 1996). Several technics have been proposed to reduce the amountof petroleum hydrocarbons in the soil. Among these technics is bioremediationwhichis to the most ecological and the cheapest.Itconsists to use living organisms or microorganisms to clean up contaminated sites (Ballerini and Vandecasteele, 1999). Bacteria are the most used in bioremediation, however, depolluting activities have been seenwith algae (*Selenastrumcapricornutum*) and fungi (*Aspergillusniger*). Moreover, Madagascar has a broader microbial diversity. There are pathogens microorganisms as *Salmonella, Escherichia coli, ….*And there are beneficial microorganisms, it is even profitable for food, and environments because it allows to protect them, improve their qualities (Tsirinirindravo and *al, 2016 ;*Mananjara and *al, 2016*).

This is why a study was conducted on the bioremediation the soils polluted with hydrocarbons by *Pseudomonas putida* in order to develop an efficient bioremediation system. *P. putida* is an ubiquitous bacterium, mostly found in therhizosphere zone of*Pinusradiata* (Mukerji*et al.,* 2006). The bacterium is classified as a chemo-organotroph because of its capability to metabolize a wide range of carbon compounds including petroleum hydrocarbons. Different manipulations were carried out at the microbiological and physico-chemical laboratories of the University of Saint Joseph Antsirabe, Madagascar.

II. MATERIALS AND METHODS

Biological material

The sampling of the soils to analyze was taken from the soil inVakinankaratra region in the urban commune of Antsirabe II, Madagascar. *Pseudomonas putida* is a ubiquitous bacterium found in large quantity in the root zone of *Pinusradiata* (Bowen *et al.,* 1976). The samples were taken from three different rhizosphere zones of *Pinusradiata* at 15 cm deep.

Bacterial isolation

To make the soil suspension, 10 g of the sample of soil sample are mixed with 90 ml of Buffered Peptone Water. 1 ml of this suspension is plated oncetrimide agar plates and then incubated at 30°C for 48 hours. The fluorescent bacteria colonies at 254 nm are purified in view of being identified (Maksimova*et al.,* 1994).

Bacterial identification

The purified bacterial colonies are identified by a succession of biochemical tests (Garrity*et al*., 2002) and 16S rDNA sequence analysis.

Phenotypic study

The identification of the pure strains is firstly based on a macroscopic observation to determine cultural characteristics. A microscopic observation ata fresh state and after Gram staining made it possible to characterize the bacterial morphology. The respiratory type as well as the respiratory enzymes are both determined. The biochemical characterization is carried out through various tests, namely: test on medium HAJNA-KLIGLER, test on medium SIMMONS CITRATE, test on medium MANNITOL-MOBILITY-NITRATE, test on medium LYSINE-IRON, gelatintest (Nelson, 2002) and the carbon auxanogram (Latour*et al.,* 1997). In addition, an analysis of the 16S rDNA sequence of the presumtived strainof*P. putida* is necessary in order to confirm the strain identity.

III. Molecular study

Extraction of genomic DNA

The "universal" extraction procedure using SDS and proteinase K has been adopted with some modifications (Daniel *et al.,* 1995). In order to realizethe extraction of the genomic DNA, the strain to be analyzed is firstly cultivated in nutrient broth for 24 hours at 30 °C. Two ml of the bacterial suspension are poured in sterilized Eppendorf tubes and centrifuged at 12000 rpm for 10 minutes. In the obtained pellet are poured 467 µl of the TE buffer solution, 3 µl of 20 mg / ml of proteinase K and 30 µl of 10% SDS. After incubation at 37 °C for 12 hours, the released proteins are precipitated with 400 µl of phenol / chloroform (50:50). The DNA suspended in the aqueous phase is then collected by centrifugation at 12000 rpm for 10 minutes. To ensure that the DNA samples are free of contaminants, 4µl of RNase (100mg/ml) are added then incubated for 2 minutes at room temperature. Finally, the DNA is precipitated by addition of sodium acetate and isopropanol, then collected by centrifugation at 13000 rpm for 10 min and dissolved in TE buffer. The DNA solutions are finally stored at -20 °C for amplification (Maloy, 1990).

Qualitative analysis of DNA on agarose gel

To prepare the agarose gel, 30ml of TBE buffer are mixed with 0,3g of agarose. The obtained mixture is boiled until complete dissolution of the agarose. After having put in place the electrophoresis cell

and the comb, the gel is poured ina manner as to obtain a thickness of 3 to 5 mm. After the solidification of the latter, the comb is removed and the gel is immersedin TBEbuffer as migration buffer. The first well is loaded with a size marker (FERMENTAS, Lambda DNA / HindIII Marker). In the other well are poured: 8 µl of DNA sample, 2 µl of loading buffer (BIO LABS Gel Loading Dye). The manipulation is carried out in a horizontal electrophoresis apparatus (BIO RAD, Mini-Sub Cell GT) at 100 V for 15 minutes. After the migration of the DNA fragments, the gel is stained with ethidium bromide.

Amplification and sequencing of the 16S rDNA region

The target gene is amplified by conventional PCR in a programmable thermocycler(PTC-100). The chosenprimer pair limit the rDNA sequence to be replicated.The latter has been described by Weisburg in 1991:

27F (forward): (5 'AGAGTTTGATCMTGGCTCAG 3')

1492R (reverse): (5 'TACGGYTACCTTGTTACGACTT 3')

The PCR reaction is carried out in 25 µl reaction volume containing 5 µl of genomic DNA, 1 µl of each primer used, 0,125 µl of Taq polymerase, 2,5µl of PCR buffer, 2 µl of MgCl2, 0,5µl. OfdNTP. The thermocycler is programmed as follows: an initial DNA denaturation phase at 94 °C for 5 minutes and then 35 cycles of 1 minute at 94 ° C for DNA denaturation, 1 minute at 60 ° C for hybridization of primers and 1,5 minutes for elongation phase. The reaction is finished by increasing the temperature to 72 ° C for 10 minutes. The amplicons obtained are analyzed by agarose gel electrophoresis.

For sequencing the gene of interest, the PCR products are electrophoresed on an automated Applied Biosystems 3730xl DNA sequencing apparatus, using 50 cm capillary arrays and a POP-7 polymer. The results obtained are analyzed on PE-Biosystems version 3.7.

Phylogenetic analysis of sequences

The sequences are compared with those found in GenBank database the National Center for Biotechnology Information (NCBI) using the BLAST program. Genetic affiliation is evaluated using the Phylip 3.69 software. The results obtained are then represented in the form of a phylogenetic tree.

Biological test

The biological test sits on 3 months. It is realized in a sealed glass container of about 4500 $cm^3$to avoid contamination from outside. The container is covered by perforated aluminum foil to ensure aeration (Tarayre 2012, Bidaud 1998). The strain identified as *Pseudomonas putida* constitutes the inoculum in the experimental device. The setting of the device is done in three steps:

The preparation of the preculture consists in inoculating 3 handles of the strain in 100 ml of nutritive broth. The preparation is incubated at 30 ° C for 24 hours.

Then, 500 g of soil (dry weight) are autoclaved at 121 ° C at 1.5 bar for 2 hours. The soil is then dried at 103 ° C for 24 h and placed in a desiccator until it is cooled. 1.8% of diesel engine oil is added to the soil (El, 1998). Then, 150g of *Pinus* sawdust representing 30% of the weight of the soil is used as texturizer (Bidaud, 1998). Finally, 0.4% of yeast beer autoclaved and grinded which useas a source of nitrogen are also added in the soil (Tarayre, 2012).

The last step consists to inoculate 100ml of preculture in the previously prepared soil. The device is placed in the shade at room temperature (23 ° C). Every seven days, the soil is stirred and 20 ml of distilled water are poured on.

Monitoring bacterial growth

The evolution of the bacterial population is followed by microbiological analysis. To do this, 10 g of the soil sample are mixed with 90 ml of EPT solution in order to make the soil suspension. A succession of dilutions till 10^{-3} is done from the main suspension. Then, 1 ml of each dilution is plated oncetrimide agar plates. After incubation for 24 hours at 30°C, the bacterial count was done according to the ISO7218 standard.

Determination of Residual Hydrocarbons (Method 3540C)

The residual hydrocarbon concentration in the soil is also evaluated during the process. The 3540C method was adopted with some modifications. To do this, the soil is prepared in advance before the extraction of the hydrocarbon. 10 g of soil to be analysed are dried at 103 ° C for 24 h then placed in a desiccator until cooling. 5 g of dried soil are placed in an extraction cartridge then immersed with 150 ml of hexane. The extraction lasts 16 hours and the evaporation of the product is collected in a flask of 250 ml capacity containing pumice stones. The flask is then placed in a rotary evaporator to evaporate the hexane. The solvent and water residues in the flask are removed at 103 °C for 15 minutes, followed by a cooling in a desiccator. Finally, the volume of the extracted product is determined. In addition, the concentration of residual hydrocarbon present in the soil expressed in mg / kg is specified by the following formula (Center of Expertise in Environmental Analysis of Quebec, 2016):

$$C = \frac{AXV}{Q}$$

Thus :

- C : concentration of hydrocarbon in theanalysed sample (mg/kg)
- A : concentration of hydrocarbon in the injected extract (ng/µl)
- V : final volume of the analysed extract (ml)
- Q : dry weight of the analysedsample (g)

IV. RESULTS

Bacterial phenotypic identification

The results of the cultural, morphological, physiological and biochemical studies permit to characterize the isolated bacterial strains.

Six strains were isolated. They are Gram negative, aerobic, mobile, oxidase positive. Fluorescent pigment production at 254 nm was observed on all strains (fig.1). Two strains produce floral notes. The biochemical identification of the strains confirmed the identity of the 6 isolated strains. Of which, 2 were identified as *Pseudomonasputida*, 2 *Pseudomonas aeruginosa*, 1 *Pseudomonas fluorescens* and 1 *Pseudomonas chlororaphis*. Table 1 gives the biochemical characteristics of each isolated bacterial strain.

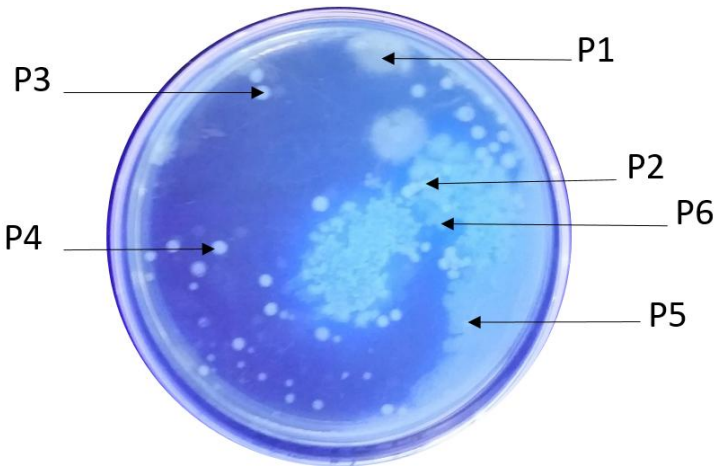

Figure 1 : Bacterial colonies observed at 254 nm after incubation on cetrimide agar

Table 1: Biochemical Characteristics of Isolated Bacterial Strains

Strains	HAJNA-KLIGLER				MANNITOL-MOTILITY		LYSINE-IRON		SIMMONS CITRATE	NITRATE REDUCTASE	GELATIN TEST	CARBON AUXANOGRAM		
	Glu	Lac	H_2S	CO_2	MANNITOL	MOTILITY	LDC	LDA				SACCHAROSE	XYLOSE	TREHALOSE
Pseudomonas aeruginosa	-	-	-	-	-	+	-	+	+	+	+	+	+	-
Pseudomonas chlororaphis	-	-	-	-	-	+	-	+	+	-	-	+	+	+
Pseudomonas fluorescens	-	-	-	+	-	+	-	+	+	-	+	+	+	-
Pseudomonas putida	-	-	-	-	-	+	-	+	+	-	-	+	+	-

LDC : Lysine DeCarboxylase LDA : Lysine DeAminase Source : author, 2017

Molecular identification

Agarose gel electrophoresis of the extracted DNA fragments revealed unique and intact bands. The extract can be used for the amplification step of the 16S rDNA region. Electrophoresis is shown in Figure 2.

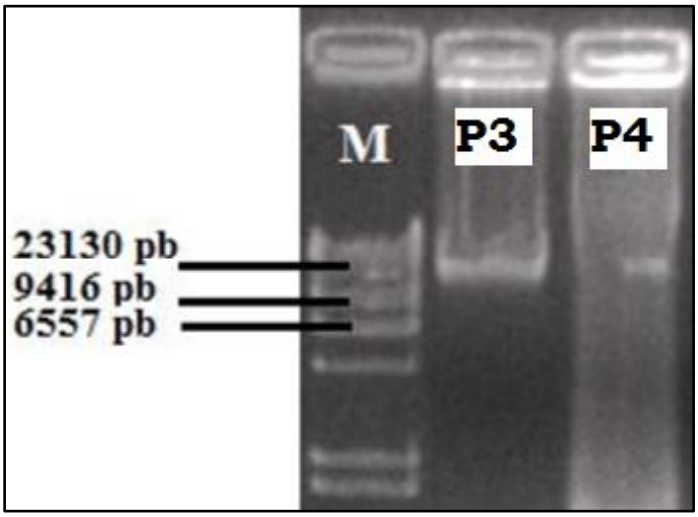

Figure 2 : Agarose gel electrophoresis of the genomic DNA of strains presumed to be *Pseudomonas putida* P3 and P4 (the first band represents the size marker)

Electrophoresis of the amplified 16S rDNA fragments showed that the size of the fragments was around 1500bp. Figure 3 shows Electrophoresis of amplified DNA fragments.

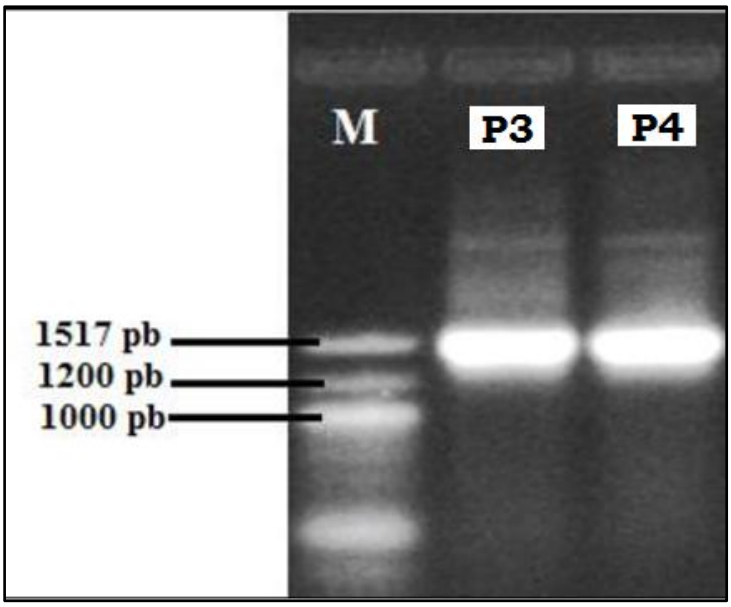

Figure 3 : Agarose gel electrophoresis of the amplified 16S rDNA fragments of the presumptive strain *Pseudomonas putida* P3 and P4 (the first band represents the size marker)

The phylogenetic tree

The assessment in BLAST of the 16S rDNA sequences of the P3 and P4 strains showed respectively 97.8% and 98% sequence similarity with *Pseudomonas putida*. This similarity is represented by a phylogenetic tree in Figures 4 and 5.

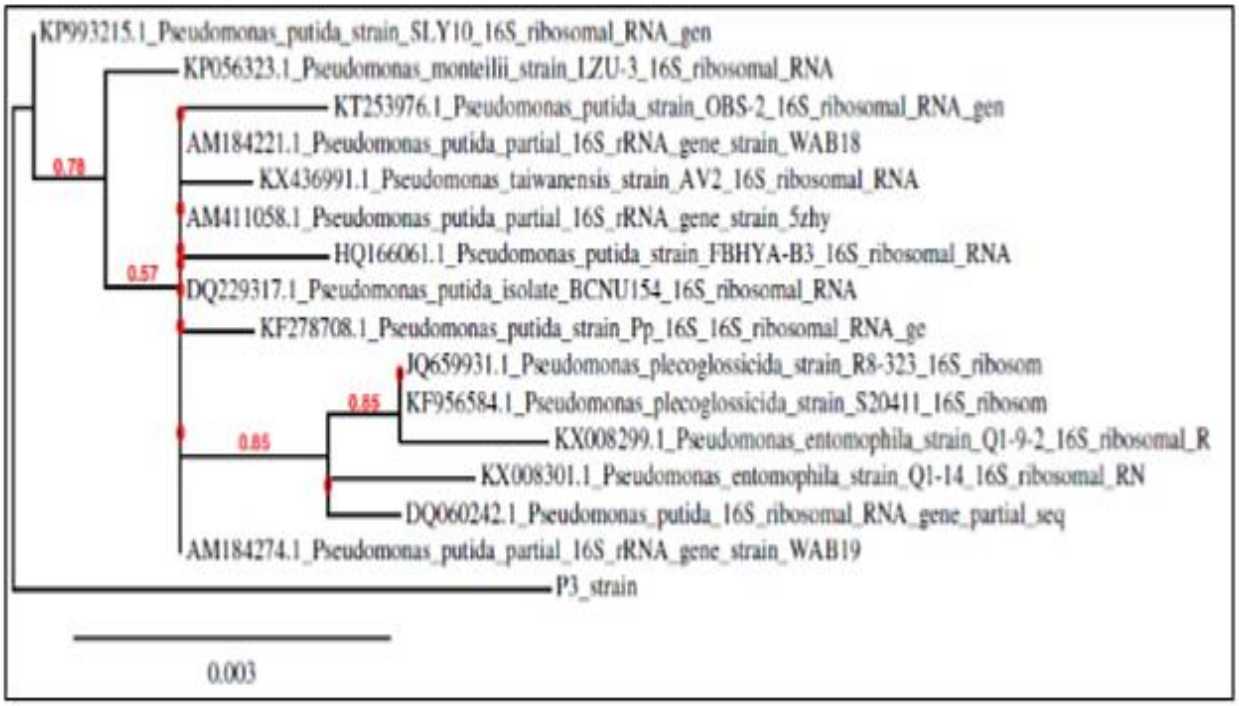

Figure 4: Phylogenetic tree based on the 16S rDNA sequences of Pseudomonasputida P3 strain and related species of BLAST database

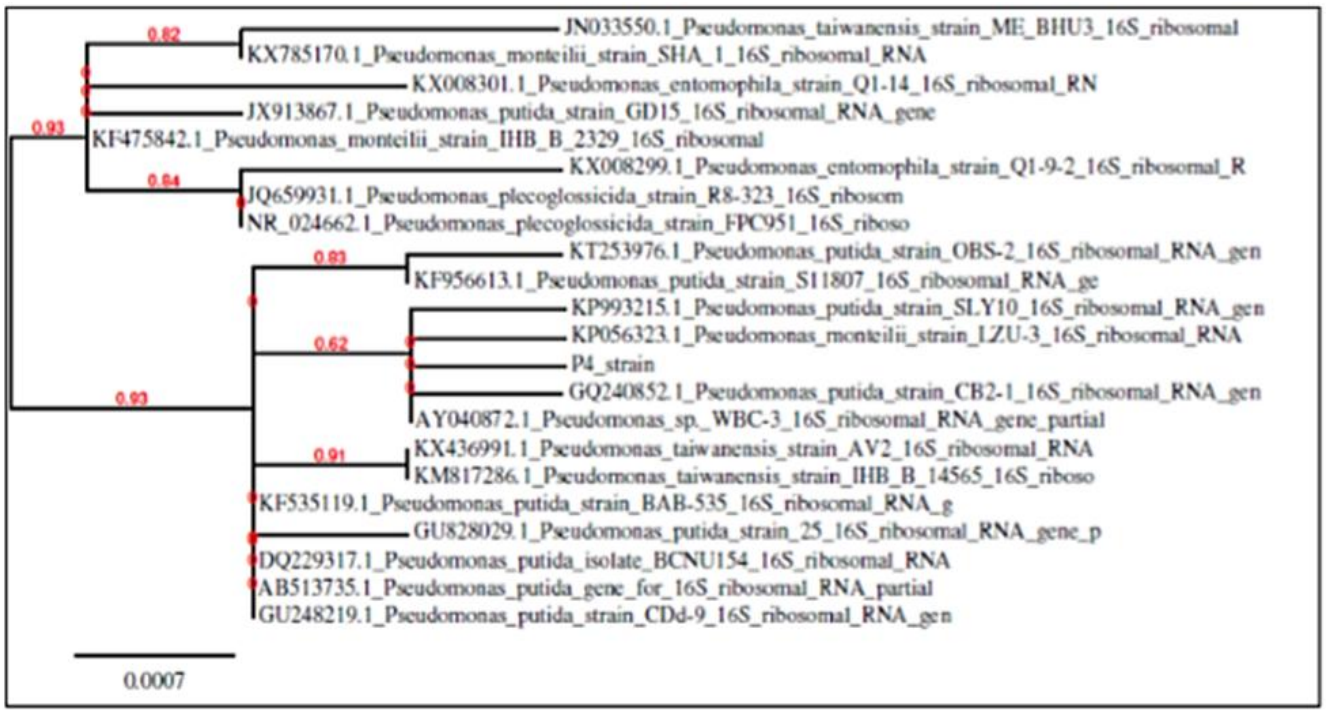

Figure 5: Phylogenetic tree based on 16S rDNA sequences of Pseudomonas*putida* P4 strain and related species of BLAST database

Abundance of microorganisms in the rhizosphere zone of Pinusradiata

Fluorescents *Pseudomonas* are the most common bacterial species found in the rhizosphere zones (Garcia *et al*., 2001). *P. aeruginosa, P. putida, P. fluorescens, P. clhororaphis*belong to this kind of bacteria andthey are called PGPR (Plant Growth Promoting Rhizobacteria). Figure 6 shows the abundance of *Pseudomonas* species in the root zone of *Pinusradiata*.

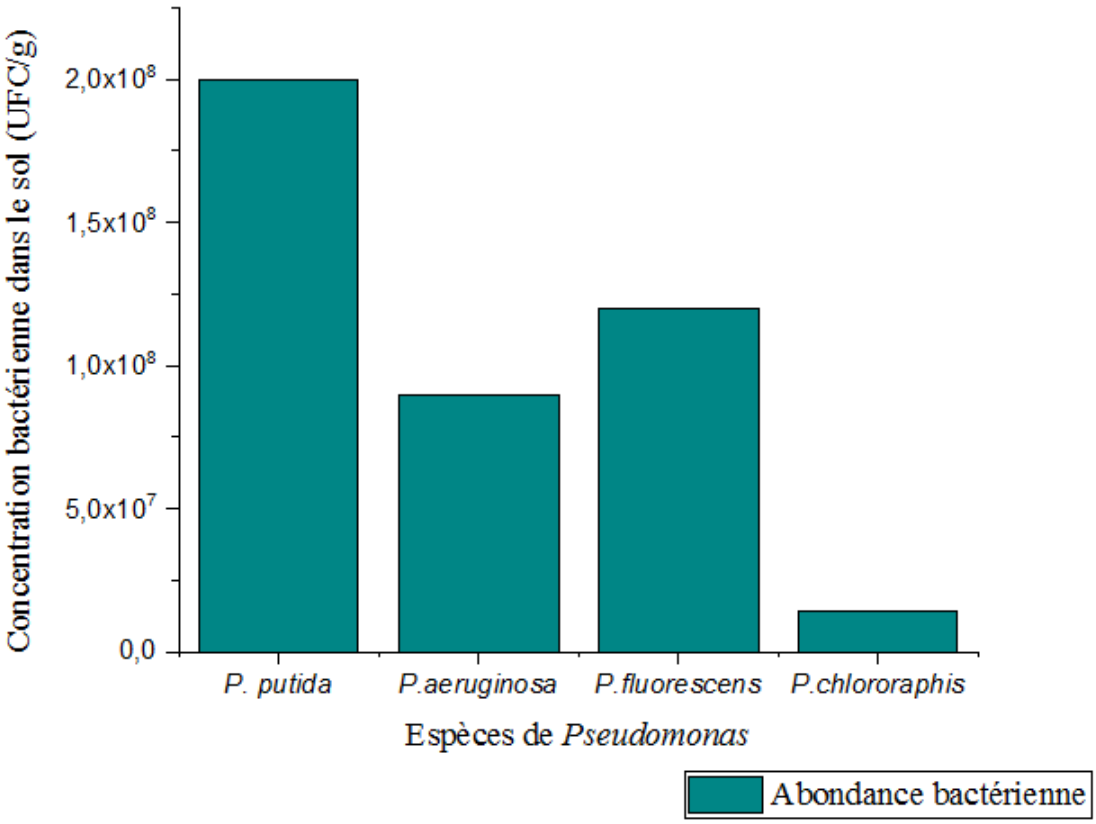

Figure 6: Bacterial distribution of *Pseudomonas* species in the root zone of *Pinusradiata*

Biological test

The results show a reduction of hydrocarbon concentration from 18000 mg/kg to 5000 mg/kg in 3 months. The degradation rates is around 30%. Figure 7 shows the evolution of the degradation rate of the hydrocarbon by *Pseudomonas putida*.

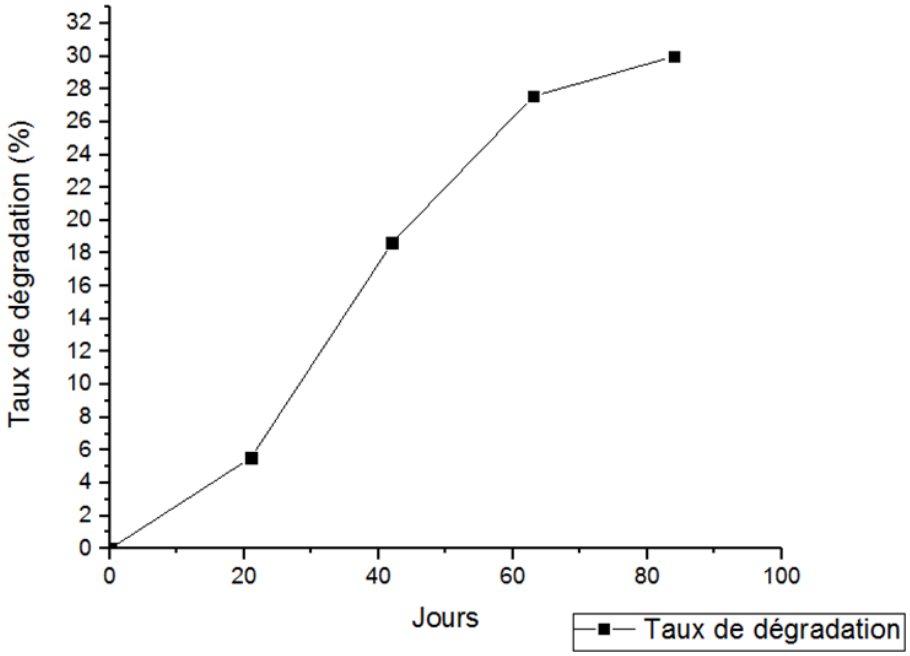

Figure 7: Degradation rate of hydrocarbon on 3 months

The bacterial population increases considerably from 4.10^7 CFU/g to 3.10^{13} CFU/g and the generation time is 2,16h. During the first weeks, bacteria acclimatizes in the new culture medium. It follows an acceleration phase and exponential phase of growth. The bacterial growth is inversely proportional to the degradation of the hydrocarbon (Figure 8).

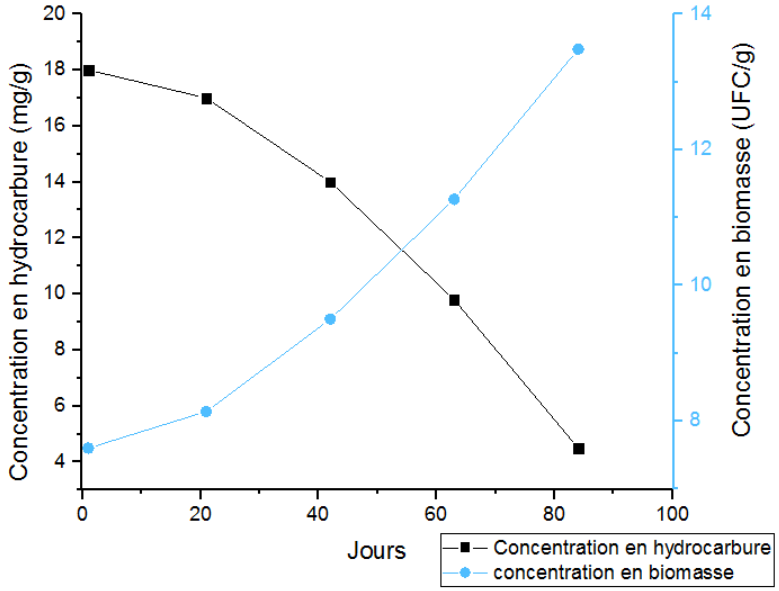

Figure 8: Evolution of the biomass and the hydrocarbon concentrations during the bioremediation of polluted soil using *Pseudomonas putida.*

At the end of the experiment, a production of $1,45.10^{12}$ CFU/g of soil per day was noted. This increased growth correspond to a degradation capacity of $2,52.10^{-4}$g of hydrocarbon per gram of soil per day.

V. DISCUSSION

The bacterial isolation of a soil sample from the region of Vakinankaratra made it possible to deduce the presence of 4 different strains of *Pseudomonas* in the rhizosphere zone of *Pinusradiata*. These strains are*Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas chlororaphis*and *Peudomonasputida*.

Based on the phenotypic characters, it was noted that all these strains belong to the genus *Pseudomonas* (Public Health England, 2015). The comparison of the identification results obtained with those of Bossis*al* in 2000 makes it possible to identify the strains P3 and P4 as *Pseudomonas putida*. However, phenotypic studies are not sufficient to confirm the identity of the strains. Thus, a molecular characterization has been carried out. The comparison in NCBI of the 16S rDNA of strains P3 and P4 respectively displays a similarity of 97,8% and 98% with *Pseudomonas putida*. Results of the obtained 16S rDNA sequences show that the P3 and P4 encoded strains belong to the species *Pseudomonas putida*.

About the experiment carried out on*Pseudomonas putida*, a bioremediation activity was observed. Indeed, the decrease in hydrocarbon concentration means that the strain is able to use petroleum hydrocarbon as the unique carbon and energy source (Jirasripongpun, 2002). This variation of the hydrocarbon concentration corresponds to a degradation capacity of 0,25 mg of hydrocarbon per day, which is not insignificant. The work carried out by Vinothini and its collaborators in 2015 on *Pseudomonas putida* showed a degradation rate of 98,8%, compared to the values obtained during the experiment, the latter is not yet optimized. Especially since during the first weeks of experimentation, the rate of degradation was not considerable (5,5%), a preculture in a medium containing hydrocarbon is therefore required.

There are several parameters to consider for effective bioremediation. On the one hand, the use of a consortium shows to be more efficient because of the diversity of catabolic enzymes that each microorganism used (Ghazali*et al*., 2004). Physicochemical characters also affect bioremediation. Specifically, the addition of texturizer such as sawdust promotes the supply of oxygen as an electron acceptor during metabolic reactions (Bidaud, 1996). The addition of bio-surfactant, temperature, pH, and moisture also contribute to the increased ability of the bacteria to degrade (Thwaites*et al*., 2007).

VI. CONCLUSION

This experiment shows the presence in soil of a strain able to clean up soil contaminated by petroleum hydrocarbons. The results obtained reflect the ability of *Pseudomonas putida* to decrease to 30% the hydrocarbons concentration in 3 months. Despite the complexity of the chemical composition of the engine oil, the strain is found to be able to degrade this pollutant and to resist its toxic activity. The effectiveness of bioremediation depends not only on the biological agent used, but also on the physicochemical characteristics of the soil to be treated. Nitrogen content, moisture and aeration of the soil significantly affect this bacterial activity. The biological system used, the bioaugmentation and biostimulation has been appropriate.

However, several factors such as oxygen supply, pH, and humidity must be optimized to promote the bioremediation activity of the strain.

REFRENCES

[1.] **Ballerini D., Vandecasteele J. P.** La restauration par voie microbiologique des sols contaminés par les polluants organiques. Biotechnologie : Edition Tech et Doc. 1999 ; 865p.

[2.] **Bidaud C.**Biodegradation des hydrocarbures aromatiques polycycliques. Thèse de doctorat : Génie des Procédés.: Institut National Polytechnique de Grenoble et de L'Ecole Nationale Supérieure des Mines de Saint-Etienne, 1998 ; 250p.

[3.] **Bossis E., Lemanceau P., Latour X., Gardan L.** The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida* : current status and need for revision. Agronomie. EDP Sciences, 2000 :20(1) ; 51-68.

[4.] **Bowen G.D., Rovira A.D.** Microbial colonization of plant roots. AnnualReview of Phytopathology. 1976 ; 14: 121-144.

[5.] **Centre d'expertise en analyse environnementale du Québec**. Détermination des HAPs : dosage par chromatographie en phase gazeuse couplée à un spectromètre de masse, 2016 ; 5: 21p.

[6.] **Daniel G., Inge P., Michael R., Martin A.** A simple « universal » DNA extraction procedure using SDS and proteinase K is compatible with direct PCR amplification. ResearchGate. PCR methods and applications. 1995 ; 4: 368-370.

[7.] **El Amine F.** Biodégradation et gestion des huiles de coupes usées. Mémoire : Sciences de l'environnement : Université de Québec, 1998 ; 155p.

[8.] **Garcia S., I. E., Hynes, R. K., Nelson, L.**Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol. 2001 ; 47: 404-411.

[9.] **Garrity,G.M., M. Winters, D.B. Searles.** Taxonomic outline of the prokaryotes Bergeys manual of systematic bacteriology. 2th edition. 2002, 1-350.

[10.] **Ghazali F. M., Rahman R. N. Z. A., Salleh A. B., Basri M.** Biodegradation of hydrocarbons in soil by microbial consortium. Biodeterior. Biodegradation. 2004, 54 : 61–67.

[11.] **Jirasripongpun K.** The characterization of oil-degrading microorganisms from lubricating oil contaminated (scale) soil, 2002, 35(4): 296-300.

[12.] **Latour X., Lemanceau P.** Métabolisme carboné et énergétique des *Pseudomonas*spp fluorescents saprophytes à oxydase positive. Agronomie. EDP Sciences. 1997, 17 (9-10) : 427-443.

[13.] **Maksimova NP., Blazhevich OV., Lysak VV., FomichevluK.**Characterization of the fluorescent pigment pyoverdine Pm, produced by Pseudomonas putida bacteria. Mikrobiologia, 1994, 63(6) : 1038-44.

[14.] **Maloy, S.R., Raj, H.D.** "Proposal of *Cyclobacteriummarinus* for a marine bacterium previously assigned to the genus Flectobacillus." Int. J. Syst. Bacteriol. (1990) 40 : 337-347.

[15.] **Mananjara P., Tsirinirindravo H.L., Raherimandimbym.,Randrianierenana a.** Etude des levuresendogènesd'*Evodiabilahe*, rutacéeendémique de Madagascar. Int. J. Biol. Chem. Sci. 10 (4): 1694- 1701. August 2016.

[16.] **Mukerji K. G., Manoharachary C.; Jagjit S.** Microbial activity in the rhizosphere Sol biologie. New York : Springer. 2006, 7 : 349.

[17.] **Nelson K.E., Weinel C., Paulsen I.T., Dodson R.J., Hilbert H., Martins dos Santos V.A.** Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. Environ Microbiol. 2002, 4: 799–808

[18.] **Public Health England**. Identification of *Pseudomonas* species and other non-Glucose Fermenters. Bacteriology-Identification. Microbiology Services Standards Unit. 2015, 3: 1-41.

[19.] **Robert M.** Le sol. Interface dans l'environnement. Ressources pour le développement. Paris. 1996 ; 146p.

[20.] **Tarayre C.**Bioremédiation des sols pollués aux hydrocarbures. Amélioration de la bio-augmentation et biostimulation. Allemagne : Edition universitaires européennes. 2012 ; 101p.

[21.] **Thwaites J.M., Farrell R.L., Duncan S.D., Lamar R.T., White R.B.** Microbiology of extreme environments. New-York : In C. Edwards. 2007, 147-177.

[22.] **Tsirinirindravo H. L., Randrianierenana l. A., Andrianarisoa B., Raherimandimby M., Randrianantoandro H. H., Razafinjatovo D. N., de Percin g., Pierluigi b**. Espèces de *Salmonella*multiresistantes véhiculées par les aliments de rue à Antananarivo. Communication lors des Journées QualiReg 2016. 5eme rencontre de l'Agroalimentaire en Océan Indien. Saint Pierre, La Réunion.

[23.] **Vinothini C., Sudhakar S., Ravikumar R.**Biodegradatiion of pétroleum and crude oil by *Pseudomonasputida* and *Bacillus cereus*. *International Journal Of Current Microbiology an Applied Sciences*, 2015, 4(1) : 318-329.

[24.] **Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J**. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*, (1991) 173 : 697–703.

The Soil Problems in Constructions of Airport

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ABSTRACT

Time, in the globalized world, is one of the most important factors about the economy, science and health. Mankind has made various efforts to use time efficiently for many years. In these studies transport came to the fore and it has become indispensable. In the light of today's technological conditions, air transport is developing at an increasing rate. Every day many aircrafts are produced, which have different speeds, weight and volume, for serve to transport. Therefore to make structures for easy and safe transport need a stable soil. Particularly suitable areas for the airport grounds in cities today, not being physically proper that construction of the airport made on soil with low bearing capacity, swelling potential of an expansive soil, settlement of soil etc, areas. In this study, soil problems encountered in the construction of airports will be explained and a summary of studies on the solution of these problems will be presented.

Keywords—*component; formatting; style; styling; insert (key words)*

I. INTRODUCTION

Soil commonly used throughout the history of mankind, and perhaps the most complex building material. Most of the deformations that occur in civil engineering works cause of the ground movements. The roads, dams, nuclear power stations and the airports are built on the soil so before the building design and the construction, first have to know about settlement and bearing capacity for safety. Today, swelling of soil and collapsing are common soil issues. Clay layers has a low permeability. Clay layers have very high degree of swelling. While the dry state or very low water consistency is view of a solid structure, the percentage of water increases, it is large changes in volume and texture in soil. Between grains of the ground to deteriorate over time and after this strong bond structure leads to deterioration of the natural structure. These features cause sometimes permanent cracking and exceeding the permissible settlements. Swelling soils, weak soil and longer settlement time of soft clay lead to very serious disturbances and complete failure of structures on the especially roads, dams and most importantly on the filling airports. to problematic soils, usually it is used additives in geotechnical practice. This additive materials are lime, Portland cement, fly ash, lime-cement-fly ash admixture, emulsified asphalt, cement kiln dust, Geofiber reinforcement, salt, and non-traditional polymer stabilizers.

Lime is popular additives used to improve fine grained soils. Construction of Denver International Airport was used lime stabilization method. Lime treatment advantages, a decrease in the liquid limit and an increase in the plastic limit results in a significant reduction in plasticity index and it gives the ability to process the problematic soils, due chemical reaction between soil and lime a reduction in water content occurs, lime addition increases the optimum water content but decreases the maximum

dry density and immediate increase in strength and modulus results. The effect of lime on soil is shown immediate and long term stabilization. Increased workability of soil is the result of immediate modification and increased strength and durability is considered long term stabilization that takes place during and after curing. The immediate increase in strength results from flocculation-agglomeration reaction and leads to better workability, whereas long term strength gain is due to pozzolanic reactions. Generally, It can be observed that as lime content increases, swell pressure decreases significantly. This enables its use in reducing the swelling potential of expansive soils (Mallela et al. 2004, Thompson 1966).

Another method of soil improvement is cement stabilization. Cement treatment causes chemical reaction similar to lime. It is observed soils and clayey materials with low plasticity index are better suited to be stabilized with cement (Currin et al. 1976; Engineering manual 1110-3-137 1984). Whit cement stabilization, reduction in plasticity index and swell potential, and increase in strength, modulus of elasticity and resistance against the effects of moisture and freze thaw can be achieved by cement stabilization. The addition of cement was found to increase optimum water content but decrease the maximum dry density (Tabatabi 1997). In addition stabilization method is Fly ash and Lime-Cement-Fly ash stabilization. Fly ash contains silica, alumina, and different oxides and alkalis in its composition, and is considered as a pozzolanic material (Das 1990). Fly ash can improve the engineering properties of soil. Coarse grained soils can be stabilized by a combination of lime and fly ash. it is used to produce a hardened cementitious material with improved compressive strength when mixed with lime and fly ash (Muhunthan and Sariosseiri 2008).

II. Soil Problems In Airport

The lack of availability of higher quality materials and the added costs for these materials are replaced with materials of better quality. Especially, it is used more than soils for construction of higway and airport. These material high water content and low workability of these soils pose difficulties for construction projects. Frequently, additives such as lime, cement, fly ash and cement kiln dust are used to improve their engineering properties. The choice and effectiveness of an additive depends on the type of soil and its field conditions.

Construction of Kansai International Airport was used vertical sand drains. It is considered that of the airport construction experienced some problems which, could be characterized as a geotechnical failure. In construction, firstly, the top 20 m of the seabed (Holocene clays) were treated within the design island area by installing vertical sand drains to accelerate compaction under the backfill (Figure 1). Next, the perimeter seawalls were built (Figure 2). Later, the land reclamation took place, in which the granular fill, taken from a number of excavations in the Osaka area, was placed within the seawalls up to a depth of about 3 m below the water level using bottom-dump barges. Last was accomplished by means of four large barges, anchored inside the seawalls, which transferred the fill brought by the smaller barges from across the bay, to bring the island to the required 4 m above the water level. This height is to guarantee that the airport will not be swamped by high tides brought by typhoons that hit the coast of Japan every September. Thanks to the vertical drains, the top 20 m of the Holocene clay reached almost 90% of its final 6 m settlement during the construction (Handy, 2002). These settlements were being compensated by an additional 6 m thick layer of fill and additional height of the seawalls (Puzrin et al 2010).

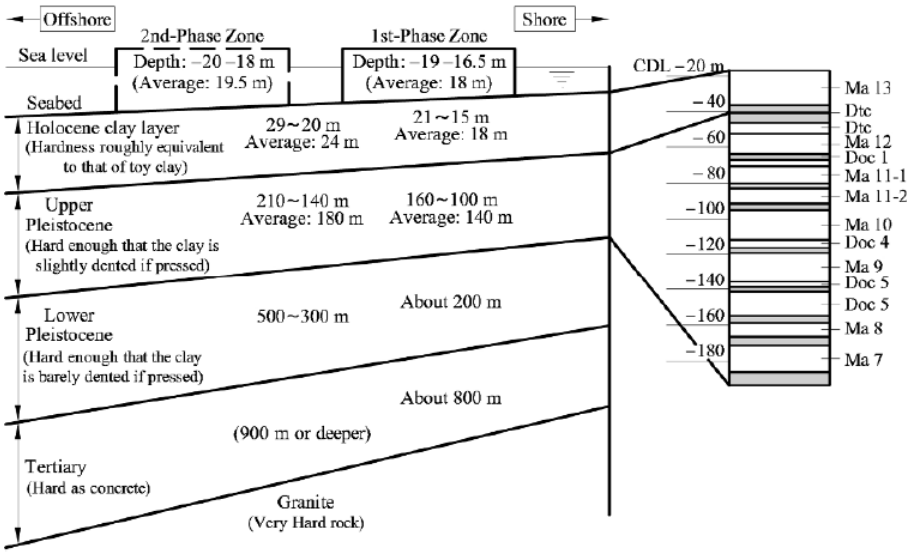

Figure 1. The soil profile of the seabed (Puzrin et al 2010)

In Kentuck Airport was used cement modified soils. During the design it was discovered that the soils to support the one to three foot taxiway embankment were weak and therefore would create issues with stability. To improve the stability of the weak in-situ soils was used porland cement to construction of the embankment. In Figure 3 is shown mixer incorporating portland cement (Smith 2009).

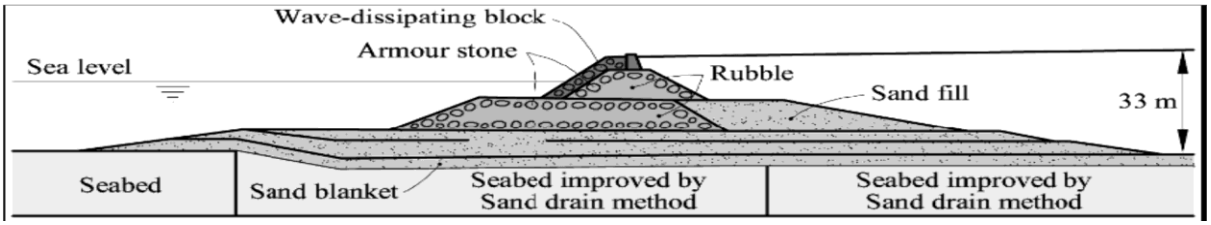

Figure 2. The cross-section of a seawall (Puzrin et al 2010)

Figure 3. The cross-section of a seawall (Puzrin et al 2010)

The subgrade soils of the Washington Dulles International Airport were significantly weak, therefore it was used the cement stabilized to support base (Figure 4). To address the issue of poor subgrade support conditions, the following options were considered undercut the poor subgrades and replace them with good quality borrow materials with a minimum CBR value of 20, crush the demolished concrete pavement structure and use it as crushed recycled concrete base to improve the subgrade support conditions, and addition of small amounts of lime, fly ash and / or ordinary Portland cement to the top 12 inches of the existing subgrades (Syed, 2007). When lime stabilized subgrades, require 7-days or more of curing to develop the desired strength. Similar concerns were also expressed with the use of lime-fly ash as the stabilizer. So ordinary Portland cement was evaluated as the candidate stabilizer in view of the high early strength provided by cement stabilized subgrade soils (Skokie, 1995).

Construction of the Suvarnabhumi Airport was used preloading with PVD for ground improvement on soft clay layer (Bangkok Clay). Most of geotechnical studies focused on the strength and deformation characteristics of the soils and used them for ground improvement design. The effectiveness of ground improvement using preloading with PVD is presented through the deformation and settlement of a series of test embankments and the change of soilproperties as well. The initial swamp areas consisted mainly of unconsolidated sedimentary beds with succession of soft peat layers. The thickness of these unconsolidated sediments is known to vary from a few meters to up to meters in some areas in the Botany Basin. Despite surcharging the fill in the course of the airport developments over the years, it is considered that consolidation settlement will be continued throughout the life of the airport (Chin et al 2008). In Figure 5 is given comparison of settlements in the PVD and non-PVD areas. In addition, construction of the Houston International Airport was used lime-cement-fly ash stabilization (Little et al 2000) and construction of Denver International Airport was used lime stabilization method.

Figure 4. Mixing soil-cement for subgrade stabilization

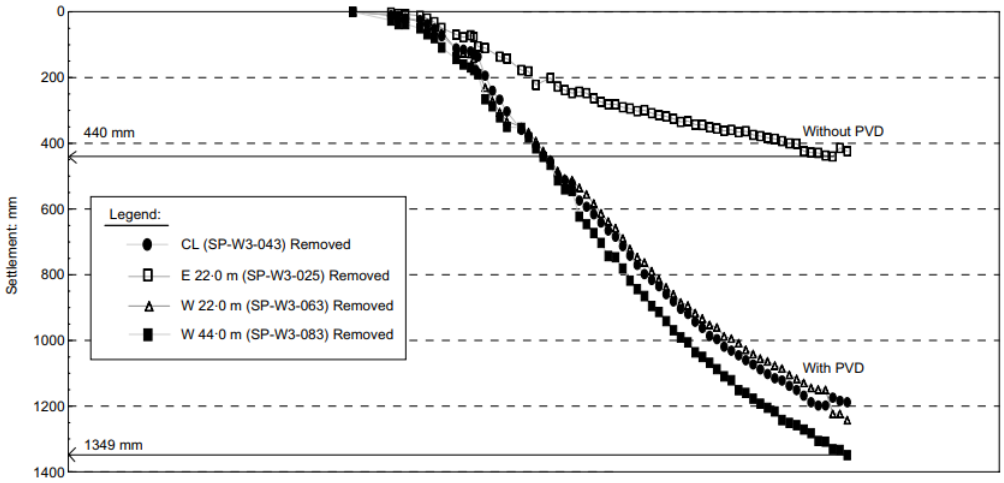

Figure 5. Comparison of settlements in the PVD and non-PVD areas (Moh and Lin, 2003).

III. Conclusion

Some geotechnical area is on the issue of the use of soils (silts, soft clay) for fills ofhigway and airport. The lack of availability of higher quality materials and it is added lime, cement, fly ash, soilswereinadequatetosupporttheconstructionoftheairport,itwouldchoiseproperimprovementmethod.Itis wantedthismethodistobetechnicallyviable,cost-effectiveandtimelytomeettheprojectdeadlines.Theadditionofthesematerialswasfoundtoimprovethedrying rate,workabilityandcompactioncharacteristicsofthesoils.Significantimprovementinunconfinedcompressiv estrength,modulusofelasticity,settlementofproblemsoilsareattainedby thesematerials.

References

[1] Chin, C. T, Chang, J. F., Hu, I.C. and Chen, J.R. (2008), Geotechnical site characterization for Suvarnabhumi Airport, Taylor & Francis Group, London, UK.

[2] Currin, D. D., Allen, J. J., and Little, D. N. (1976). " Validation of soil stabilization index system with manual development." Report No. FJSRL-TR-0006, Frank J. Seisler Research Laboratory, United States Air Force Academy, Colorado.

[3] Das, B. M. (1990), Principle of foundation engineering, PWS-KENT publishing company, Boston

[4] Engineering manual 1110-3-137. (1984), Soil stabilization for pavements mobilization construction, Department of the Army, Corps of engineers office of the chief of engineers.

[5] Little, D. N., Males, E. H., Prusinski, J. R., and Stewart, B. (2000), "Cementitious stabilization, Transportation in the New Millennium: State of the Art and Future Directions", Perspectives from Transportation Research Board Standing Committees.

[6] Mallela, J., Quintus, H. V., and Smith, K. (2004), Consideration of lime-stabilized layers in mechanistic-empirical pavement design, The National Lime Association.

[7] Moh, Z.C. and Lin, P.C. (2003), "From Cobra Swamp to International Airport: Ground Improvement at Suvarnabhumi International Airport" Ground Improvement, 7(2), 87–102.

[8] Muhunthan, B and Sariosseiri, F. (2008), "Interpretation of Geotechnical Properties of Cement Treated Soils", Research Report FHWA Contract DTFH61-05-C-00008.

[9] Puzrin, A. M., Alonso, E. E., Pinyol, N. M. (2010), Geomechanics of Failures, DOI 10.1007/978-90- 481-3531-8_2.

[10] Skokie, I. L. (1992), Portland Cement Association, Soil-Cement Laboratory Handbook, Portland Cement, Association.

[11] Smith, D. (2009), Cement-Modified Soil Solves: Kentucky Airport Problem, Pavement, PG, PCA Southeast Region.

[12] Syed, I. M. and Brown, T.L. (2007), "Innovation in Cement Stabilization of Airfield Subgrades", The 2007 FAA Worldwide Airport Technology Transfer Conference, Atlantic City, New Jersey, USA.

[13] Tabatabi, A. M. (1997),Pavement [Roosazi Rah], University's publication center, Tehran, Iran.

[14] Thompson, M. R. (1966), Shear strength and elastic properties of lime-soil mixtures, Highway Research Record, Washington, D.C., 139, 1-14.

Predictive Regression Models of Water Quality Parameters for river Amba in Nasarawa State, Nigeria

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ABSTRACT

The challenges of river water quality management are so enormous, due to the unpredictive modes of contamination. Monitoring different sources of pollutant load contribution to the river basin is also quite tasking, resulting to laborious and expensive process which sometimes lead to analytical errors. This study deals with the assessment of the physico– chemicaland bacteriological parameters of water samples from River Amba during the period of August 2017 to January 2018 and developing regression models. Water quality Parameters such as Temperature, Turbidity (NTU), Suspended solids (mg/l), Colour, Total solids, Total dissolved solids, Electrical conductivity (µs/cm), pH, Hardness, Chemical Oxygen Demand, Dissolved Oxygen (DO), and Total Coliform were obtained and compared with water quality standards. The results of the water quality analysis of the study in comparison with drinking water quality standard issued byWorld Health Organization(WHO) and National Agency for Food and Drug Administration Control (NAFDAC) revealed that most of the water quality parameters were not adequate to pronounce the water potable. Hence adequate water treatment processes should be employed to make the water fit for consumption and other domestic uses. Statistical analysis was done, in which the systematic correlation and regressionanalysis showed a significant linear relationship between different pairs of water quality parameters. The highest correlation coefficient between different pairs of parameters obtained is (r = 0.999), resulting from the correlation between TS and SS. Multiple regression analysis was also carried out and regression equations were developed. It was observed that the parameters studied had a positive correlation with each other.

KEYWORD: physio-chemical parameters,Regression models, Statistical study, standards, water quality.

I. INTRODUCTION

Water is an essential element that supports life. However, statistics reveal that, from the advent of the new millennium, one billion people lacked access to safe drinking water and 2.4 billion to adequate sanitation[15]. The resultant effect is that people source for various means of water supply of which streams and rivers are part of, especially in rural areas.With the emphasizes placed on the need for the potability of water, water quality monitoring network could serve as an important tool in the management and assessment of surface water quality which could be improved by means of accurate forecasts of surface water variables.The assessment of surface waterquality is not just meant for the suitability of human consumption but also in relation to agricultural, industrial, recreational, commercial uses and its ability to sustain aquatic life[20].Water quality monitoring is therefore a fundamental tool in the management of freshwater resources. To emphasize its significance, World Health Organization (WHO), UnitedNations Environment Programme (UNEP), United Nations Educational, Scientific and CulturalOrganization (UNESCO) and World Meteorological Organization (WMO) launched in 1977, a water monitoring programme to collect detailed information on the quality of global ground and surface water.

There are quite a number of literatures on water quality monitoring and assessment, suggesting all together, the possible increase in pollution loads in streams and rivers which resultantly changes or alters water quality parameters such as heavy metals, nutrients and organic matter, soluble ions, oil and grease, and organic chemicals such as pesticides and poly-nuclear aromatic hydrocarbons (PAHs),[20].Therefore, it is possible to

control water pollution problems through monitoring as well as enforcement of emission standards by industries [4].

This could also be achieved by developing models that can assist in the monitoring and assessment of these water quality parameter.[13] pointed out that the systematic calculation of correlation coefficients between water quality parameter variables and linear or multiple regression analysis provide an indirect means for the rapid monitoring of water quality. Statistical correlation and regression analysis have been found to be a set of dependable tools for correlating different parameters and developing models which becomes a mechanism for prediction or forecasting [12]. This prediction and forecasting forms an integral part of surface water monitoring in the aspects of water resource impact assessment, environmental impact assessment, and pollution monitoring and control.

II. Materials and Methods

The Study Area

River Amba lies within the River Amba Basin. The catchment is situated within the capital territory of Lafia in Nasarawa State, 90 km North of Makurdi, capital of Benue State. The perennial River has a catchment area of 115 square kilometers.Lafia town through which River Amba encompasses is situated on longitude 08°.30′ East and latitude 08°.32′ North. The area is located in the middle climatic belt that is generally very warm and humid with dry and rainy seasons. It has a mean temperature range of 26°C to 30°C, a mean rainfall range of 1120 mm to 1500 mm, relative humidity of 60 – 80 % and falls within the Guinea Savannah kind of vegetation. The Amba River system rises as small tributaries, like the Dutse, BukanKwatu, and Angogo springs, from the Eastern Agyaragu-Obi minor highland areas. The rivulets join the mainstream of the River Amba which flows roughly East-West to the West where joining other tributaries, it empties through the major River Oriye, into the River Mada.

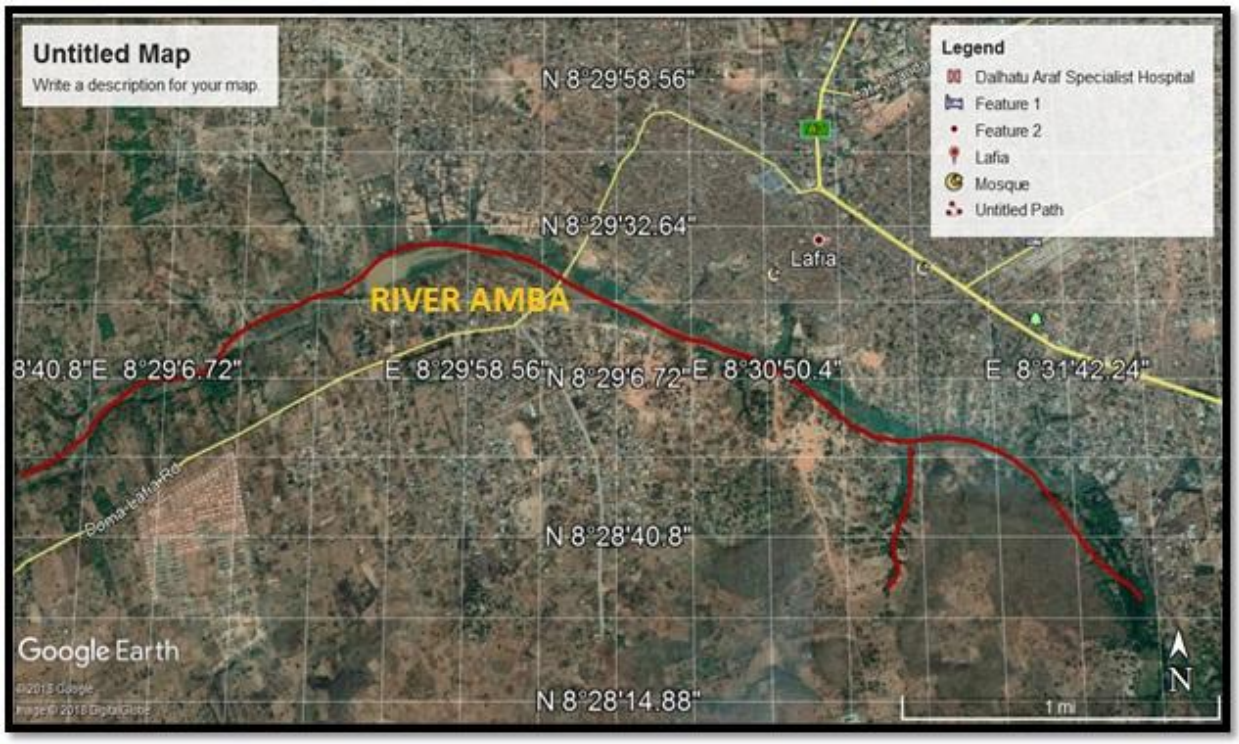

Figure 1: Google Earth Map Show RiverAmba

Sampling

Three sampling stations were chosen based on upstream, middle stream and downstream, and was represented as A, B and C. The stations were chosen at a distance of about ten meters apart to effectively cover the entire

length of the river. Water samples for physico-chemical analysis were collected at subsurface from sampling stations one every sampling day. 250mL reagent bottles and 1000 mL plastic containers were washed, dried and corked, labeled and used for sample collection. Subsurface water samples were collected at a depth of about 10 – 20cm. Dark colored 250 mL glass bottles were used for the Dissolved oxygen (DO) and Biochemical Oxygen Demand (BOD) analysis while 1000mL plastic containers were used to collect water for the general purpose analyses. The sampling bottles and containers were rinsed three times with the river water at each sampling station before the collection of samples. Water samples were collected by lowering the sampling bottles or containers by hand to a depth of about 20cm below the surface level. Each sample container was treated according to the analysis required to be carried out on them. Testing was done once a month, for six months: from August 2017 to January 2018. A total of 135 samples were collected and analyzed monthly.

III. Methodology

Samples were collected and analyzed for 13physico-chemical parameters, such as Temperature (T), Turbidity (NTU), Suspended solids (SS), Colour (C), Total solids (TS), Total dissolved solids (TDS), Electrical conductivity (EC), pH, Hardness (H), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), and Total Coliform (TC).Temperature was measured at the point of sampling by the use of mercury-in-glass thermometer. A battery powered digital pH meter was used to measure the pH of the samples at the points of sampling. The values are reported to the nearest 0.1pH unit. The Colourof each water sample was measured using a Hach potable colorimeter model DR/890. The turbidity of the water samples was measured with the use of HACH 2100P turbidimeter. The readings were read off in nephelometric turbidity units (NTU).A high powered microcomputer conductivity meter JENWAY 40701 model H19032 with a degree of accuracy of 0.01 was used to measure the conductivity of the water samples. The instrument was standardized using potassium chloride solution of conductivities 500$\mu s/cm$ and 1500$\mu s/cm$. The unit is in micro siemens per centimeter. Total solids (TS) of the water samples were estimated by gravimetric methods. To determine (TS), 100mL of the unfiltered portion of the water sample was evaporated in a porcelain dish to dryness and washed. The dish was oven dried at a temperature of 105^0C, cooled in a desiccator to room temperature to a constant weight and the weight recorded. The weight difference between the weight of dish with 100mL of water and weight of dish after evaporation less weight of dish alone was considered as the weight of solids (TS). The level of dissolved oxygen (DO) in each sample was determined using Winkler's method. The BOD was determined using the 5 – day BOD dilution test, applying the iodometric method for the determination of dissolved oxygen (DO). The difference between the initial dissolved oxygen value measured on the first day and the value of dissolved oxygen determined after five days of incubation gave the BOD of the sample**.** The total coliform count of the water sample expressed in cfu/100 mL was determined using membrane filtration technique. The water sample was filtered through the membrane and then transferred to appropriate growth medium and after incubation and inoculation, the growth colonies were counted.

IV. Statistical analysis

Statistical analysis in the form of descriptive statistics, correlation and regression analysis of the physico-chemical and microbial properties of a river basin gives a fairly good amount of information like their average values and possible predictions. Pearson's correlation coefficient and linear regression were performed between pairs of water quality parameters. Significantcorrelation coefficient ($r \geq 0.7$) and linear regression equations are summarized in Table 1 below.

Table 1: Significant correlations coefficient ($r \geq 0.7$) and linear regressions

S/N	Pairs of Parameter	Correlation Coefficient (r)	Regression Equation	Regression Coefficient (R^2)
1	Temp – C	0.9349	C =291.35Temp - 9017.3	0.8741
2	Turb - TDS	0.9193	TDS = 0.015Turb + 40.35	0.8452
3	Turb – SS	0.9995	SS = 0.888Turb - 5.8585	0.999
4	Turb – TS	0.9833	TS = 1.035Turb + 10.759	0.9669
5	C- EC	0.9651	EC = 0.523C + 66.58	0.9314
6	TDS – SS	0.9303	SS = 10.543TDS - 414.54	0.8654
7	TDS – TS	0.9459	TS = 12.795TDS - 489.79	0.8948
8	SS – TS	0.9865	TS = 1.774SS + 17.286	0.9731
9	DO – BOD	0.9366	BOD = -36.238DO + 254.42	0.8772

Table 2 shows the water quality analysis viz., Mean, Standard Deviation (SD), Standard Error (SE) and Coefficient of Variation (CV). The Coefficient of Variation from Table 2 showed turbidity, suspended solids, total solids, and colourto be 53.7%, 56.6%, 50.6%, and 54.9%. While the Coefficient of Variation for pH, Turb, COD, BOD, DO, H, EC, TC were found to be 1.1%, 1%, 9.5%, 32.43%,20.48%, 44.74%, 10.2%, 3.9%, 15.8%, 11.9%, and 33% respectively. The coefficient of variation showed that there was significant variation in the value of some parameters measured for the six months. Though a considerable amount was not high, thus,the variation range is narrow.

Table 2: Descriptive Statistics of Water Quality Parameters of River Amba

Parameters	Maximum	Minimum	Range	Mean	SD	SE	CV (%)
T	210	60	150	131.8	70.8	28.9	53.7
SS	180	47.5	132.5	110.3	62.4	25.5	56.6
TDS	55.9	42.2	13.7	49.8	5.5	2.2	11
TS	227.1	58.6	168.5	147.2	74.5	30.4	50.6
TEMP	32.6	31.5	1.1	32.1	0.59	0.24	1
C	496	81.3	414.7	335.1	184	75.4	54.9
EC	96.6	71.7	24.9	84.1	10	4.1	11.9
pH	6.48	6.27	0.21	6.4	0.07	0.03	1.1
H	70	50	20	62	9.8	4	15.8
S	70	21	49	39.9	17.4	7.1	43.6
COD	152	119	33	136.3	13	5.3	9.5
$DO_{2(1)}$	5.3	4.9	0.4	5.1	0.2	0.1	3.9
DO2(2)	4.1	3.6	0.5	3.9	0.3	0.1	7.6
BOD	79	60	19	69	7.1	2.9	10.2
TC	1113	525	588	771	254.6	104	33

Multiple Regression Results

Multiple linear regression is a kind of modelling technique that allows one to establish a relationship between physico-chemical parameters by fitting a linear equation to the observed data set. In this study, multiple linear regression equations were developed to predict certain parameters based on the physico-chemical parameters analyzed. In determining what models would be appropriate in predicting certain parameters for water quality monitoring, stepwise regression method was applied to select the best possible fitted multiple linear regression model. The factor considered in estimating parameters of importance in the regression model is the p-value which must be less than 0.05 (i.e. P-value < 0.05). Table 3 shows the developed multiple regression models using stepwise method in multiple regression analysis.

One of the most commonly used criterion to evaluate the performance of a model is its coefficient of determination (R^2). This R^2 value also tells us how good the model fits with the data used to develop the models. The range of R^2 value for the multiple regression developed is from 0.7304 to 0.9999. The regression between turbidity, suspended solids, total solids, and temperature had a correlation coefficient of 0.999 (i.e. r = 0.999).

Table 3**:** Multiple Regression Equation using Stepwise method

S/N	Parameter	Regression Equation	Regression Coefficient (R^2)
1	Turb - SS,TS,Temp	Turb = -145.418 + 1.215(SS) -0.100(TS) + 4.919(Temp)	0.9999
2	SS - TS,Temp, Turb	SS = 119.273 + 0.083(TS) - 4.035(Temp) + 0.822(Turb)	0.9999
3	TDS - TS,Temp	TDS = 178.752 + 0.096(TS) - 4.462(Temp)	0.9922
4	TS – SS	TS = 17.236 + 1.177(SS)	0.9731
5	Colour - SS,TDS	Colour = 2497.424 + 6.858(SS) - 58.632(TDS)	0.8883
6	Sulphate – COD	Sulphate = -116.663 + 1.149(COD)	0.7304
7	DO - BOD,TC	DO = 6.764 - 0.027(BOD) + 0.000243(TC)	0.9824

Table 4: Correlation Coefficient Matrix of water quality parameters

	T	*SS*	*TDS*	*TS*	*TEMP*	*C*	*EC*	*pH*	*H*	*S*	*COD*	*DO2(1)*	*BOD*	*TC*
T	1													
SS	0.999493563	1												
TDS	0.919135091	0.930281	1											
TS	0.983253753	0.986443	0.945919	1										
TEMP	0.783114352	0.76691	0.516241	0.760147	1									
C	0.706902748	0.690825	0.407452	0.663636	0.934945	1								
EC	0.722168074	0.70656	0.443396	0.657	0.876003	0.965104	1							
pH	-0.72979493	-0.7206	-0.52929	-0.66816	-0.73751	-0.86501	-0.95706	1						
H	0.503890795	0.505237	0.527419	0.432526	0.123858	-0.02778	-0.01466	0.021393	1					
S	0.825888365	0.838142	0.886164	0.825173	0.403694	0.461555	0.556838	-0.7184	0.322042	1				
COD	0.954715659	0.957391	0.88601	0.972591	0.787209	0.76284	0.755671	-0.77556	0.263428	0.854643	1			
DO2(1)	-0.93719287	-0.94643	-0.94901	-0.96414	-0.62465	-0.59223	-0.58844	0.647348	-0.40049	-0.90917	-0.9657	1		
BOD	0.945282855	0.945812	0.849566	0.941368	0.788227	0.803438	0.827636	-0.86362	0.230022	0.877649	0.986819	-0.93658	1	
TC	0.05106811	0.034531	-0.1869	-0.03388	0.326464	0.558829	0.681432	-0.71225	-0.5092	0.145334	0.1331	0.055649	0.273715	1

* Correlations are significant at the 0.05 level (2 tailed) ** Correlations is significant at the 0.01 level (2 tailed)

V. Results and Discussion

Table 5: Comparison of Water Quality Parameters of River Amba with Water Standards

S/N	Parameters	WHO	NAFDAC	Present study value
1	Turbidity(NTU)	5	5	132
2	Suspended solids(mg/l)	5	-	112
3	Total dissolved solids (mg/l)	1200	500	50
4	Total Solids (mg/l)	-		147

5	Temperature(^{o}C)	25	Ambient	32.1
6	Colour	15	15	327
7	Electrical conductivity (µs/cm)	1250	1000	84
8	pH	6.5-8.5	6.5-8.5	6.4
9	Hardness (mg/l)	100	150	62
10	Sulphate (mg/l)	200	100	40
11	Chemical Oxygen Demand(mg/l)	100Below	-	136
12	Dissolved Oxygen($DO_{2(1)}$)(mg/l)	4below	-	5.1
13	Dissolved Oxygen($DO_{2(5)}$)(mg/l)	-	-	4
14	Biochemical Oxygen demand (BOD)mg/l	50below	-	69
15	Total Coliform per100ml of water	0	10	771

* WHO-World Health Organization, NAFDA- National Agency for Food and Drug Administration Control

Results of the analysis for the six months as reported in Tables 5 shows that the temperature of the water during the study period was in a range of31.2°C to 32.6°C on the average. The maximum value of temperature observed was in the month of October, while the minimum was in the month of January. The pH values varied from 6.27 to 6.48. The maximum and minimum pHvalue was observed in the month of January and September respectively. The electrical conductivity (EC) varied between 71.7µs/cm to 96.6µs/cm. The maximum value was recorded in the month of September and minimum in the month of January. The values of hardness were in a range of 50.0 mg/l to 70.0mg/l. The maximum value was recorded in the month of January and minimum in the month of August. The turbidity values were in a range of 60NTU to 210NTU. The maximum value was in the month of September and minimum in the month January. The SS values recorded were between 180 mg/l to 475 mg/l.The maximum value was recorded in the month of January while the minimum was in the month of September.

The DO values ranged between 4.9 mg/l to 5.3 mg/l. The maximum value was recorded in the month of January and the minimum was in the month of September. The BOD values were in a range of 60 mg/l to 79mg/l. The maximum value was recorded in the month of December and minimum in the month of January. The COD values were between 119 mg/l to 152 mg/l. The maximum value was observed in the month of Septemberand minimum in the month of January.
The sulphate values were in a range of21 mg/l to 70mg/l. The maximum value was obtained in the month of September and minimum in the month of August. The TDS values ranged between 42.2 mg/l to 55.9 mg/l. The maximum value was recorded in the month of September and minimum was in the month of August. The TS values were between 58.6 mg/l to 227.1 mg/l. The maximum value was observed in the month of Octoberand minimum in the month of January.

Physical Water Quality Parameters

Temperature

Table 5 shows in details the water quality parameters of the study in comparison with WHO and NAFDAC standards for acceptable drinking water. The temperature of potable water according to WHO and NAFDAC is 25^{o}C. The study has on the average for the six months a temperature of 32.1^{o}C. The temperature is higher than the acceptable temperature stipulated by these two bodies. Ascertaining the temperature of a river is very important because the temperature controls the rate of all chemical reactions that take place in the river, and it

also affects the growth of fishes, its reproduction and immunity. Extreme temperatures or temperature fluctuations can be fatal to the fishes in the river. The high temperatures as observed during the study may be due to an increase in atmospheric temperature resulting from the anomalies caused by climate change. However, the observed range of the temperature allows for optimum proliferation of most of the bacterial especially when isolated from the water samples. Bacteria such as enterobateriaceac and mesophiles grow optimally at a temperature ranging from 20^{o}C to 32^{o}C [7].

Turbidity

The value of turbidity obtained during the study is 132NTU which is above the stipulated value of 5NTU by WHO and NAFDAC water standards. This signifies that the water is very turbid. Turbidity in water may be caused by the growth of Phytoplankton [6].One of the major causes of turbidity can be attributed to human activities around the river such as construction, mining, and agriculture which tend to disturb the stability of suspended particles in the water. The human activities commonly found around the banks of river Amba is rice milling, block making and agricultural activities such as sugar cane plantation. This can lead to high levels of sediments entering into the river during precipitation due to storms water runoff[5].

Total Dissolved Solids (TDS)

The quality of potable drinking water is mostly characterized by the level of total dissolved solids in the water. Hence, the need to ascertain the level of TDS in the water. The value of TDS concentration of the water under study is 50mg/l. In comparison with WHO and NAFDAC water quality standards, which stipulates that the value of total dissolved solids should be within a permissible minimum of 500mg/l and a permissible maximum limit of 1200mg/l, the water analyzed was observed to be low in total dissolved solids concentration. The presence of TDS in water may affect its taste. Water containing TDS concentrations below 1200mg/l is usually acceptable to consumers. However, water with an extremely low concentration of TDS may also be unacceptable to consumers because of it's flat, insipid taste and corrosive impact on water supply systems [22]. On the contrary, high (TDS) in water can result in the formation of an increased amount of residue which in turn renders the water unfit for consumption and could result in gastrointestinal irritations [1].

High levels of TDS in water may also be said to be objectionable to consumers owing to the resulting taste and to excessive scaling in water pipes, heaters, boilers, and household appliances [22]. Some dissolved organic matter can also contribute to an increased level of TDS which also indicates that the water is polluted [14]. While water with extremely low concentrations of TDS may also be unacceptable to consumers because of its flat and insipid taste. High TDS might be due to the presence of a large number of organic salts such as carbonate, bicarbonate sodium, potassium and calcium and also some non-volatile substance [17].

Suspended Solids(SS)

The value of suspended solids as presented in Table 5 is 112 mg/l which is above the value stipulated by WHO and NAFDAC standards presented in the same table. SS in water are indications of suspended and solid materials present in the water[16]. Suspended solids in rivers are mostly due to high levels of sediments carried by surface runoff into the water after precipitation. These includes runoff from natural and anthropogenic (human) activities in the watershed [21].

Electrical Conductivity (EC)

The electrical conductivity of water is synonymous to the amount of total dissolved salt (TDS) present in the water. In other words, it could be referred to as a direct function of total dissolved salts [9]. Electrical Conductivity of river Amba is 84.0µs/cm as presented in Table 5 which is not acceptable with the WHO and NAFDAC limits. When electrical conductivity is high, it increases the corrosive nature of water [14]. High electrical conductivity value might be due to the presence of a high amount of dissolved inorganic substances in ionized form. But it is not the case with this study. However, the water from River Amba can be classified as mesotrophic because it falls below the stipulated 1200µs/cm by WHO [8].

Chemical Water Quality Parameters

pH

The pH value of the water was also presented in Table 5. The pH of a water is considered an important water quality parameter because it supplies information regarding the acidity or alkalinity of water. The study has pH value of 6.4 which falls slightly below the range of 6.5 -8.5 prescribed by WHO and NAFDAC. The lower the pH value of a water the higher the corrosive nature of water. Dissolved gases such as carbon (IV) oxide, hydrogen sulphide, and ammonia also affect the pH of water. One of the significant environmental impacts of pH is the effect that it has on the solubility and thus the bioavailability of other substances [11].

Hardness

Ca and Mg salts are the two main causes of water hardness [10], and they have a detrimental effect on humans as it puts the heart at risk. The hardness of water above approximate values of 200mg/l may result in the formation of scales especially if the water is to be transported to consumers through pipes [19]. The result obtained for hardness is 62 mg/l as presented in Table 3 which is below the limit specified by WHO and NAFDAC.
The water from River Amba is considered free from hardness. Water hardness is mostly due to the presence of multivalent metal ions which comes from minerals dissolved in the water. One of the most prevailing impacts of water hardness on fishes and other aquatic life appears to be the effect some ions have on more toxic metals such as Lead, Cadmium, Chromium, and Zinc. Generally, the harder the water, the lower the toxicity of other metals to aquatic life [14].

Sulphate

The amount of sulphate ions obtained from the analysis of the water under study is 40mg/l. WHO and NAFDAC specified the range of 100 – 400mg/l for minimum and maximum tolerance of sulphate content in water. The amount of sulphate content in excess of the stipulated range can result in diarrhea. Hence, the value of the present study shows that the water is free from sulphate problems.

Chemical Oxygen Demand (COD)

The COD amount obtained from the study is 134mg/l which is well above the WHO and NAFDAC acceptable limits. COD is the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water. High COD has an undesirable consequence on aquatic life [2].

Biological Water Quality Parameters

Dissolved Oxygen (DO)

Thedissolved oxygen content of River Amba is 5.1mg/l as presented in Table 5. This is slightly above WHO and NAFDAC standards of 4.0mg/l. DO is one of the most important water quality parameters. If in contact with a water body, it gives direct and indirect information about the reactions in the water such as bacterial activity, photosynthesis, availability of nutrients, stratification etc. Some of the effects of dissolved oxygen is that it corrodes water lines, boilers and heat exchangers, and affects the survival of marine animals at low levels [18]. Variation in dissolved oxygen might be due to temperature, photosynthesis, respiration, aeration, organic water and sediment concentration [3].

Biochemical Oxygen Demand (BOD)

The BOD concentration of the river presented in Table 5 is 69mg/l. As observed, it is higher than the value specified by WHO and NAFDAC standard. High BOD decreases the level of dissolved oxygen in water [17].

Coliform Count

The total coliform count for the sample considered was exceedingly higher than the value specified by WHO and NAFDAC. The value of the coliform count is 771per 100ml of water which is way higher than the value of 0 and 10 specified by WHO and NAFDAC respectively.
The result of the water quality analysis of the study in comparison with drinking water quality standard issued by WHO and NAFDAC revealed that most of the water quality parameters were not adequate to pronounce the water potable. Hence, the people consuming this water are at risk of contracting water-borne and/ or sanitation-

related diseases as highlighted by the microbiological quality of the water they use for drinking and other domestic uses. Proper treatment of this river water is necessary for it to become potable.

VI. CONCLUSION

Water quality parameters like temperature, turbidity, suspended solids, Electrical conductivity, pH, COD, DO, BOD, and coliform count had values above that stipulated by the WHO and NAFDAC. Water quality parameters like TDS, Hardness, sulphate were observed to be within the permissible limit for both WHO and NAFDAC water quality standards.The people consuming this water are at risk of contracting water-borne and/ or sanitation-related diseases as highlighted by the microbiological quality of the water if used for drinking and other domestic uses.The generated water quality information indicates a high concentration of suspended solids. The proposed models are based on the generated data from the study at the river scale and would therefore be directly applicable to the study area. The models developed form a basic tool to support water quality monitoring and land use management in future.

REFERENCES

[1.] Adak, M. G. and Purohit, K. M (2001), *Poll. Res*., 20, 575.

[2.] Boyd, C. E and Lichikoppler, F. (2008). Water quality in fish pond culture research and development. Experimental station Aubm, *International Center for Agriculture*. P.22.

[3.] Budget, U.S and Verma, A.K (2006) Limnological Studies on J.N.U. Lake, New Delhi, India.*Bulletin of Botanical Society*. 32: 16-23.

[4.] Ekiye, E. and Zejiao, L. (2010). Water quality monitoring in Nigeria; Case Study of Nigeria'sindustrial cities. *Journal of American Science*, Vol. 6, No. 4, pp. 22-28. ISSN: 1545-1003.

[5.] EPA (2002). Water, a comprehensive treatise Unilever research laboratory. England. P.335

[6.] Eze, S. O., and Chigbu, G. C (2015) Physical, Chemical and Microbiological parameters of IYI OKAI stream in Abirba, Ohafia Local Government Area, Abai State, Nigeria. *European Journal of Pure and Applied Chemistry*. 2(1): 8-21

[7.] Fransolet, G., Viuers, G, Masschevn, W.J (2006) Influence of temperature on bacterial development in waters. *Ozone science*. 7:205-227.

[8.] Garg, R. K., Rao, R. J., Uchchariya, D., Shukla, G., Saksena, D. N. (2010) Seasonal Variations in Water Quality and Major Threats to Ramsagar Reservoir*. African Journal of Environmental Science and Technology*, Vol 4(2), pp. 61 – 76.

[9.] Harilal, C.C., Hashim A., Arun P.R. and Baji S., (2004), *J Ecology Environ Conservation*, 10(2), 187-192.

[10.] Kateria, J., Nithya A and Mohan S C, (2010). Correlation analysis of drinking water quality in and around Perur block of Coimbatore District, Tamil Nadu, India, *Rasayan Journal 13. Chemistry*, 3(4): 649654.

[11.] Khan, R.M., Jadhar, M.S. and Ustad, I.R. (2012). Physico-chemical analysis of Triveni lake water of Amrovati district in India. Biological science discovery 3 (1): 64-66.

[12.] Kumar, N. and Sinha, O.K. (2010). Drinking Water Quality Management through Correlation Studies among various Physico-chemical Parameters: A Case Study. *International Journal of Environmental Sciences*, 3 (2): 253-259.

[13.] Mohammad, M.H., Ali, A., Seyed, M.R. and Seyed, M.A.H. (2013). Correlation Study and Regression Analysis of Drinking Water Quality in Kashan City, Iran, *Middle-East Journal of Scientific Research,*13 (9): 1238-1244.

[14.] Navneet, K., Sinthan, D. (2010). Drinking water quality management through correlation studies among various physicochemical parameters*. International Journal ofenvironmental sciences* 1 (2): 253-259.

[15.] Octavian Postolache, Pedro Silva Girão and José Miguel Dias Pereira (2012). Water Quality Monitoring and Associated Distributed Measurement Systems: An Overview, Water Quality Kostas Voudouris and DimitraVoutsa, IntechOpen, DOI: 10.5772/32159. Available from: https://www.intechopen.com/books/water-quality-monitoring-and-assessment/water-quality-monitoring-and-associated-distributed-measurement-systems-an-overview

[16.] Oladiji A.T., Adeyemi, O., Abiola, 0.0. (2007) Toxicological evaluation of the surface water of Anilegbe River using rats. *Nigerian Society of Experimental biology.* 16: 94-101.

[17.] Prasanthi, S., Rao, V.S., Shanmukha, K.J. and Prasad K. R. S (2012) Physicochemical analysis of water samples of Najendle area in Gunter District of AndnraIndiaInternational*Journal of chemical Technology Research 4 (2): 691-699.*

[18.] Premlata, V., (2009). Multivariant Analysis if Drinking water Quality parameters of lake pichhola in Udaipur, India. *Biological Forum International Journal.* 1 (2): 97-102.

[19.] Sudhakar. G,andLatha S G,(2013), Seasonal Variation of Ground-Water Analysis from BapatlaMandal, Guntur District, Andhra Pradesh*Int. Jrnl of sci& research*, 10.12.

[20.] Taiwo, A.M., Olujimi, O.O., Bamgbose, O. andArowolo, T.A. (2012). Surface Water Quality Monitoring in Nigeria: Situational Analysis and Future Management Strategy, Water Quality Monitoring and Assessment, Dr. Voudouris (Ed.), ISBN: 978-953-51-0486-5, InTech, Available from: http://www.intechopen.com/books/waterquality-monitoring-and-assessment/surface-water-quality-monitoring-in-nigeria-situational-analysis-and-futuremanagement-strategy

[21.] United states Environmental Protection Agency (USEPA) (2006) volunteer stream monitoring: A methods manual. Washington, Dc. 841-843.

[22.] WHO (1993). Guidelines for Drinking Water Quality. Volume 1; Recommendations 2nd edition, World Health Organization, Geneva.