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Full Length Research Paper

Physico-chemical characteristics of borehole water quality in Gassol Taraba State, Nigeria

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Many people in Africa depend on water from borehole, but purity of the drinking water from this source remains questionable. In a bid to ascertain the health risk local people are exposed to, this study analyses the physico-chemical characteristics of borehole water in Gassol Local Government Area (LGA), Nigeria. For this purpose, water samples were collected from the 12 administrative wards in the LGA. Two samples were collected from each ward, one in the rainy season (March) and another in the dry season (November), a total of 24 water samples in all. The water samples were analyzed for 18 different physical and chemical parameters to ascertain their comparability with the guideline levels recommended by the Standard Organization of Nigeria (SON) and World Health Organization (WHO). Results show that most parameters were within the guideline values in both seasons except for turbidity, pH, fluoride (F^-), chlorine (Cl^+), iron (Fe^{2+}), ammonia (NH_4^+) and manganese (Mn_2^+). Overall, all of the wards had at least one instance in which a parameter falls outside recommended guideline. A further analysis using the mean value test approach to assess level of contamination relative to guideline values showed that the upper bound value (US_{95}) of turbidity, iron, pH and chlorine are greater than their guideline values. This indicates that these are the parameters for which the most urgent action is needed. The high concentration of iron and turbidity outside the prescribed limits in the rainy season suggests that water managers need pay more attention to borehole water quality in the rainy season. There is need for further research across the region to better understand the quality and the contaminants (natural and anthropogenic) of borehole water so as to be able to proffer appropriate remediation strategy.

Key words: Groundwater, guideline value, mean value test, standard organisation of Nigeria (SON), World Health Organisation (WHO).

INTRODUCTION

Water is the most important nutrient essential to the survival of all humanity because it is involved in every bodily function, and makes up about 75% of total body weight (Mack and Nadel, 2011; Offei-Ansah, 2012; Shryer, 2007). The lack of this essential mineral can lead to serious implications such as hypertension, high cholesterol, and heart disease. Recent studies have also linked the lack of water to headaches, arthritis, and

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heartburn (Batmanghelidj and Page, 2012). Therefore, it is recommended that one should drink at least 64 ounces per day (Bellisle et al., 2010). However, despite the need to ensure sufficient water quantity, one of the biggest development challenge is ensuring sufficient water quality (Gundry et al., 2003).

Providing safe drinking water is one of the most complex challenges facing African rural communities. The continent has the highest number of people lacking access to safe, drinkable water. According to World Health Organization (2008), more than 3.4 million people die each year from water sanitation and hygiene-related causes and majority of these are in Africa. The impact of the consumption of unsafe drinking water in Africa has been likened to “death of children at a rate equivalent of a jumbo jet crashing every 4 h” (The United Nations Children's Fund, 2010). In a bid to stem the tide, programs such as the Millennium Development Goals (MDG) which aims at improving the quality of water are widely adopted (World Health Organization, 2006). Emphasis has also been placed on diversifying water sources from reliance on surface water to include rain water and groundwater.

Traditionally many societies have depended on surface water; however with increasing challenges of contaminated surface water resulting in diseases such as bilharzia, sleeping sickness, river blindness and guinea worm, many societies have adopted digging of boreholes (Carpenter et al., 1998; Chigor et al., 2012). Digging of borehole is encouraged by local, national and international organisations as alternative to polluted surface drinking water sources. A lot of funds is been allocated into building boreholes even though sometimes the purity of the drinking water from the boreholes is questionable (Ncube and Schutte, 2005). The quality of borehole water depends upon several factors including local geology, hydrology and geochemical characteristics of the aquifers (Bhattacharya et al., 1997). Apart from these factors, the activities of microorganisms, temperature and pressure are also responsible for the chemical characteristic of groundwater (Fournier and Truesdell, 1973).. Therefore, borehole water often contains dissolved mineral ions whose type and concentration can affect their quality . If certain mineral constituent are present in excessive amounts, some type of treatment may be necessary before the water can be used for the intended purpose.

Water should be free from any physical, chemical or bacteriological contaminant. But unfortunately water is not always found pure. It is for such reason that drinking water quality standard is set up to ensure the safety of drinking water supplies and the protection of public health. This is even more important now because the chemical quality of drinking water during recent years has deteriorated considerably due to the presence of toxic elements, which even in trace amounts can cause serious health hazards (Ikem et al., 2002). Therefore, there is need to ensure that the water people drink and use for household activities is reliable and safe. If not, adequate remedial measures can be put in place. It is the knowledge of the composition and properties of water that is significant for the evaluation of its potential use and management. Knowing the water's physical, chemical and biological characteristics allows experts to determine whether it is suitable for drinking and other domestic uses. On a global scale, World Health Organisation (WHO) produces international norms on water quality and human health in the form of guidelines that are used as the basis for regulation and standard setting, in developing and developed countries world-wide. Various countries have also enforced drinking water standards for the maximum permissible levels of different constituents. In United States, guidance to ensure that drinking water standards are in place to protect human health is set by United States Environmental Protection Agency (USEPA) while the Standards Organisation of Nigeria (SON) has this responsibility in Nigeria.

Nigeria is one of many African countries facing problems of accessibility to clean drinking water. Although it is reported that 27 million new Nigerians have gained access to clean drinking water since 1990, only 47% of the population can access safe water (The United Nations Children's Fund, 2007). The biggest population facing water shortages in Nigeria come in rural Northern Eastern region where over 70% of the population cannot access clean water (Voices, 2013). In a bid to stem the tide individuals, public and private entities have dug boreholes without any effort to ascertain their safety. The Nigerian government even launched a National Borehole Programme to supply water through a motorized system of boreholes to rural communities (Onugba and Sara). As far as North Eastern Nigeria is concerned, borehole water is popular in a region which is entirely within savannah zone. Due to this increased consumption of borehole water in the region, there has been a growing concern about the quality of water from this source. It is against this background that the physical properties and chemical contents in borehole water in Gassol Local Government Area of Taraba State are investigated with the aim of assessing the portability of borehole water and generating information that can serve as a guide in monitoring water contamination in the region. The data generated from this study will be used to create a baseline database of borehole drinking water quality in the region.

MATERIALS AND METHODS

Study area

Gassol Local Government Area (LGA) is one of the 16 LGA’s in Taraba State, Nigeria (Figure 1). It covers a total land area of about 5,500 km^2 and extends between latitude 8°38′00″ north of the equator and 10°46′00″ east of the Greenwich meridian (Taraba State Government, 2015). The area is generally underlined by sedimentary rocks which are very good aquifers (reservoir) for water. The River Taraba which takes its source from the Mambilla plateau in the South is a source of water for domestic uses, fishing and also for irrigation farming during the dry season. The

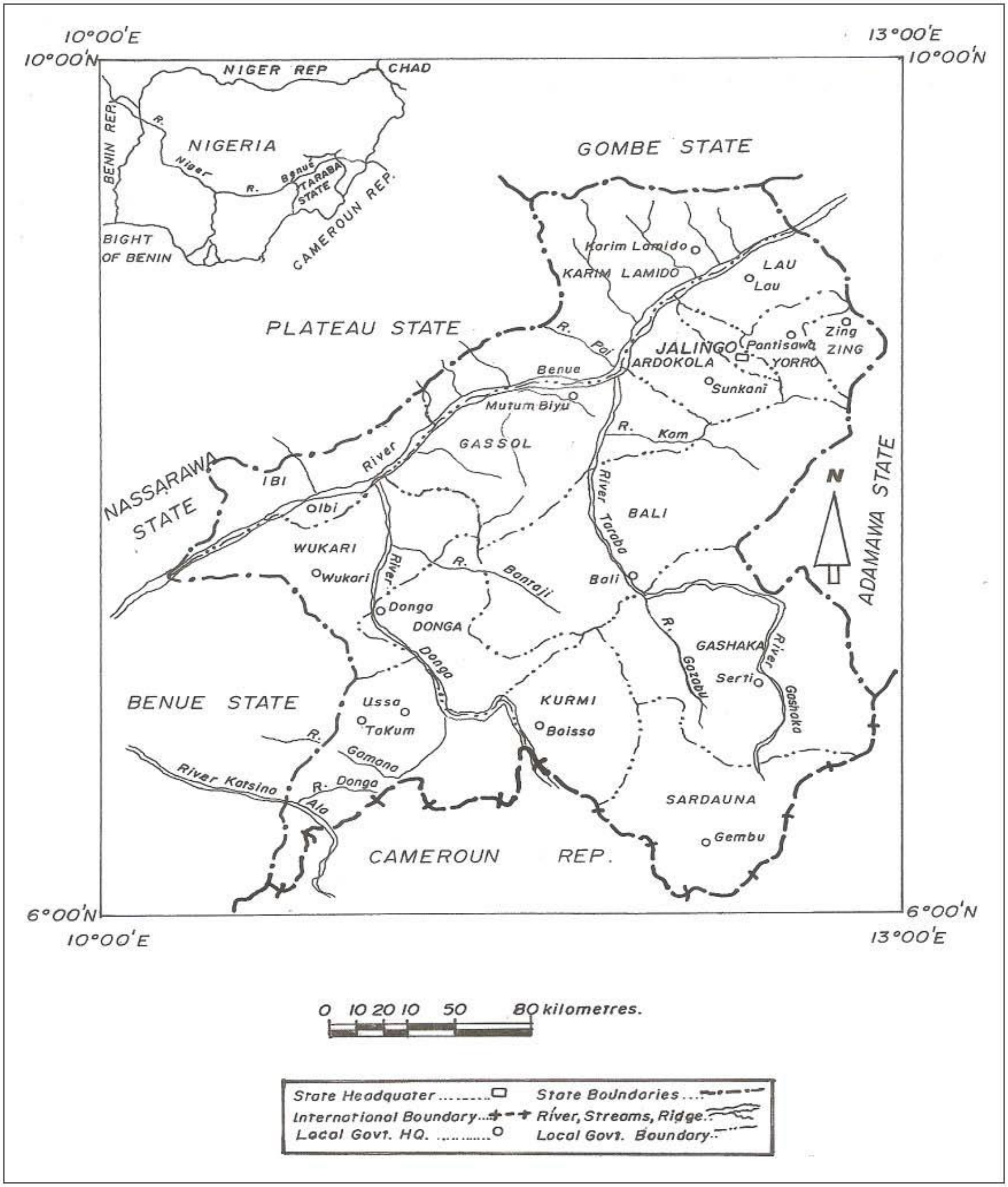

Figure 1. Map of Taraba State showing Local Government Areas (Including Gassol Local Government Area).

temperature regime is warm to hot throughout the year with a slight cool period between November and February. Temperature ranges between 23 to 40°C. There is a gradual increase in temperature from January to April, which also increases the demand of water for domestic uses in the area.

Gassol is an important economic centre because of its cattle market which is well linked to other part of Nigeria. The population of the local government is 245,086 (National Population Commission, 2006) in twelve (12) administrative wards, namely, Sansani, Sendirde, Wuryo, Sabon Gida, Namnai, Yarima, Gassol, Shira, Tutare, Gunduma, Mutum Biyu "A" andMutum Biyu "B".

Water sample collection and analysis

Water samples were collected from randomly selected boreholes. A borehole is selected from each of the 12 administrative wards within Gassol Local Government Area. Water samples were collected from each borehole twice. The first set of samples was collected in May corresponding with the rainy season and the second set in November corresponding with the dry season. In total, 24 water samples were collected for the study. The borehole water samples were collected in prewashed (with detergent, diluted HNO_3 and doubly de-ionized distilled water, respectively) polyethylene bottles. The determinations of the physical and chemical properties of the water samples were performed on the same day of sample were taken. This was done at the United Nations Children's Fund (UNICEF) assisted Rural Water Supply and Environmental Sanitation Agency. Analytical water test tablets (photometer grade) reagents for specific test were used for the preparation of all solutions. Water samples from the boreholes were analysed using a Palintest Photometer 5000, following the procedures set out in the instruction booklet (Palintest, 1980). Each sample was analyzed for 18 parameters. These were turbidity, conductivity, temperature, pH, total dissolved solids (TDS), nitrate (NO^3), fluoride (F^-), chlorine ($C1^-$), iron (Fe^{2+}), ammonia (PO_4^{3-}), hardness ($CaCO_3$), sulphate (SO_4^{2-}), manganese (Mn^{2+}), copper (Cu), magnesium (Mg^{2+}), calcium (Ca^{2+}), total alkalinity and total salinity.

The resultant levels of the parameters were compared with the World Health Organization (WHO) (World Health Organization, 2011) and the Standard Organization of Nigeria (SON) (Standards Organisation of Nigeria, 2007) guideline values to ascertain their compliance with the prescribed recommended limits. These guideline values set maximum allowable limits in drinking water. While the WHO provides a general global guideline the SON is specific to Nigeria. However, while the WHO standards is constantly been updated, the SON standard has not been updated for almost a decade.

Mean value test

The mean value test is a statistical method used to guide decision-making in many regulatory contexts such as in assessment of contaminated land, soil and water quality. This approach which is defined in Appendix A of Contaminated Land Report 7 by Department for Environment Food and Rural Affairs and The Environment Agency (Department for Environment Food and Rural Affairs and The Environment Agency, 2002), assess contaminated sites relative to guideline values. This is based on the estimation of the 95% Upper Confidence Limit of the mean concentration of a contaminant (95%UCL, also referred to as US_{95}) and its use as the appropriate value to be compared with the relevant guideline value or site-specific assessment criterion. This 95%UCL is meant to provide a reasonably conservative estimate of whether the measured concentration is acceptable, considering the uncertainty and variability associated with site investigations.

The necessary calculation involves five steps (Dean, 2007) as follows:

(i) Calculate the arithmetic sample mean, X.
(ii) Calculate the (unbiased) sample standard deviation, s.
(iii) Select an appropriate t value e.g. 95th percentile confidence limit, t. The tabulated “t value” can be obtained from four figure mathematical table.
(iv) Calculate the upper 95th percentile bound of sample as:

$US_{95} = X + (ts / \sqrt{n})$

(v) Compare the upper bound value, (US_{95}) with the guideline value (G).

RESULTS

The results of the borehole water analysis for samples collected in the rainy and dry seasons are presented in Tables 1 and 2. The concentration of each parameter varies from one sample point to the other. This is then compared to the SON and WHO acceptable values to determine and compare the suitability and effect of continual consumption of such water.

Water quality evaluation: parameters within guideline levels

The results showed that all of the boreholes tested were well within the limits prescribed by SON and WHO both in the wet and dry seasons for electrical conductivity (EC), temperature, total dissolved solids (TDS), nitrate (NO_3^-), total hardness ($CaCO_3$), sulphate (SO_4^{2-}), Copper (Cu), magnesium (Mg^+) and Calcium (Ca^{2+}). Conductivity ranged between 392 Ω/cm in Shira to 818 Ω/cm in Gassol. Temperature ranged between 25.0°C in Gunduma and Mutum Biyu "A" in the rainy season to 38.0°C in Tutare and Gunduma in the dry season. Total dissolved solids ranged between 180 ppm in Shira and 428 ppm in Gassol. Nitrate ranged from 0.17 mg/L in Sendirde to 32 mg/L in Shira. Total hardness varied from 22 mg/L in Tutare and Mutum Biyu “B” in the dry season to 75 mg/L in Gunduma in the rainy season. Sulphate varied between 2.4 mg/L in Sendirde to 6.7 mg/L in Shira. Copper ranged between 0.01 mg/L in Sansani, Sendirde, Shira, Mutum Biyu "A", and Mutum Biyu "B" to 0.5 mg/L in Gassol. Magnesium was highest at 1.02 mg/L in Tutare, and Gunduma and lowest at 0.06 mg/L in Sabon Gida. Calcium varied between 2.1 mg/L in Gunduma to 11.5 mg/L in Wuryo. At these levels, these parameters do not pose any health impact and are within the SON and WHO guideline values. As such it will be sufficient to conclude that these parameters are unlikely to be sources of water contamination in Gassol LGA of Taraba State, North Eastern Nigeria. On the other hand, there were incidences in which Turbidity, pH, fluoride, chlorine, iron and manganese are outside guideline values (Appendixes 1 to 7).

Water quality evaluation: parameters outside guideline levels

Turbidity

Turbidity is a physical parameter, which is a measure of the cloudiness of water. It is caused by particles suspended or dissolved in water that scatter light making

Table 1. Chemical and Physical concentration of water samples from wards of Gassol Local Government Area in Rainy Season.

Parameters	Sansani	Sendirde	Wuryo	Sabon Gida	Namnai	Yarima	Gassol	Shira	Tutare	Gunduma	Mutum Biyu "A"	Mutum Biyu "B"	SON	WHO
Turbidity (NTU)	**45.00**	**6.12**	**55.00**	**60.00**	**25.00**	**26.00**	**16.00**	**20.00**	**15.00**	**35.00**	**25.00**	**20.00**	**45.00**	**6.12**
Conductivity (Ω/cm)	778.00	651.00	742.00	627.00	667.00	657.00	818.00	392.00	415.00	720.00	518.00	620.00	778.00	651.00
Temperature (°C)	27.90	27.90	27.90	27.90	27.90	27.90	27.90	27.90	26.00	25.00	25.00	26.00	27.90	27.90
pH	6.97	6.78	6.60	7.04	**6.40**	7.11	7.88	7.10	6.50	6.51	6.70	**6.20**	6.97	6.78
TDS (PPM)	375.00	328.00	371.00	317.00	329.00	330.00	428.00	180.00	210.00	410.00	250.00	310.00	375.00	328.00
Nitrate (NO_3^-) (mg/L)	0.91	0.17	0.28	0.29	0.21	0.79	0.41	32.00	25.00	15.00	16.00	15.000	0.91	0.17
Fluoride (F^-) (mg/L)	1.30	0.79	1.02	0.77	0.34	0.03	1.50	1.48	**2.50**	0.20	0.80	0.50	1.30	0.79
Chlorine (Cl^-) (mg/L)	2.60	3.20	2.80	3.30	3.30	3.50	2.20	2.90	**35.00**	**20.00**	**16.00**	**25.00**	2.60	3.20
Iron (Fe^{2+}) (mg/L)	**0.80**	**0.45**	**0.75**	**0.63**	**0.62**	**0.67**	**0.45**	0.30	0.20	0.01	0.20	0.20	**0.80**	**0.45**
Ammonia (PO_4^{3-}) (mg/L)	0.19	0.03	0.03	0.09	0.06	0.04	0.07	0.10	0.50	0.20	0.50	**0.70**	0.19	0.03
Hardness ($CaCO_3$) (mg/L)	59.00	41.00	37.00	59.10	60.10	65.00	49.10	60.10	72.00	75.00	65.00	62.00	59.00	41.00
Sulphate (SO_4^{2-}) (mg/L)	2.80	2.40	3.50	6.20	6.00	4.50	3.50	6.70	3.20	3.40	5.30	6.20	2.80	2.40
Manganese (Mn2+)(mg/L)	0.01	0.18	0.13	0.14	0.03	0.07	0.11	0.18	0.02	0.20	**0.30**	0.20	0.01	0.18
Copper (Cu) mg/L	0.01	0.01	0.21	0.19	0.25	0.02	0.50	0.01	0.02	0.02	0.01	0.01	1	2
Magnesium (Mg^{2+}) (mg/L)	0.13	0.09	0.11	0.06	0.53	0.62	0.15	0.25	1.02	1.02	1.00	1.00		100
Calcium (Ca^{2+}) (mg/L)	4.70	9.40	11.5	6.50	4.90	6.80	9.60	2.50	3.20	2.10	2.20	2.20		150
Total Alkalinity (mg/L))	40.00	37.00	42.00	54.00	47.50	50.20	40.10	49.10	29.00	15.00	0.02	2.30		
Total salinity (mg/L)	41.00	45.00	33.00	14.00	31.50	6.80	10.50	7.20	3.40	15.00	19.00	19.00		

Bold values indicate incidences where parameters are outside guideline values.

the water appear cloudy or murky. The particulate matters can include sediment - especially clay and silt, fine organic and inorganic matter, soluble coloured organic compounds, algae, and other microscopic organisms (Nemade et al., 2009). Turbidity generally has no direct health effects; however, it can interfere with disinfection and provide a medium for microbial growth (Akoto and Adiyiah, 2007). This may indicate the presence of disease causing organisms such as bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhoea, and associated headaches (Payment et al., 2003).

In this study, all the boreholes had turbidity values outside the SON and WHO guideline value of 5 NTU. In the rainy season, turbidity value ranged between 6.12 NTU in Sendirde to 60 NTU in Sabon Gida. The concentration was generally better in the dry season with only Sansani (45 NTU) and Sendirde (6.1 NTU) having turbidity levels above the guideline value. On the average of both seasons, all the boreholes have turbidity level above permissible level. The source of turbidity in Gassol is most likely due to those generated as water moves through the loose soils of the area into the ground water supply. The high concentrations of turbidity in the rainy season when there is high likelihood of mud and silt been washed into underground water will suggest the need to constantly measure this parameter especially in the rainy season.

pH

pH is a measure of hydrogen ions (H^+) and negative hydroxide ions (OH^-) in water. It indicates whether the water is acidic or alkaline (World Health Organization, 2006). In pure water, the concentration of positive hydrogen ions is in equilibrium with the concentration of negative hydroxide ions, and the pH measures exactly 7 on

Table 2. Chemical and Physical concentration of water samples from wards of Gassol Local Government Area in Dry Season.

Parameters	Sansani	Sendirde	Wuryo	Sabon Gida	Namnai	Yarima	Gassol	Shira	Tutare	Gunduma	Mutum Biyu "A"	Mutum Biyu "B"	SON	WHO
Turbidity (NTU)	**45.00**	**6.10**	5.00	4.60	2.50	2.60	1.60	2.10	1.50	3.50	2.20	2.10	5	5
Conductivity (Ω/cm)	778.00	651.00	742.00	627.00	667.00	657.00	818.00	392.00	415.00	720.00	518.00	620.00	1000	2500
Temperature (°C)	30.90	37.00	27.90	35.90	30.90	30.90	37.00	32.00	38.00	38.00	35.00	36.00	23 – 40	23 - 40
pH	6.97	6.78	6.60	7.04	**6.40**	7.11	7.88	7.10	6.50	6.51	6.70	**6.20**	6.5 - 8.5	6.5 - 8.5
TDS (PPM)	375.00	328.00	371.00	317.00	329.00	330.00	428.00	180.00	210.00	410.00	250.00	310.00	500	1000
Nitrate (NO_3^-) (mg/L)	0.91	0.17	0.28	0.29	0.21	0.79	0.41	32.00	25.00	15.00	16.00	15.00	50	50
Fluoride (F^-) (mg/L)	1.30	0.79	1.02	0.77	0.34	0.03	1.50	1.48	**2.50**	0.20	0.80	0.50	1.5	1.5
Chlorine (Cl^-) (mg/L)	2.60	3.20	2.80	3.30	3.30	3.50	2.20	2.90	**35.00**	**20.00**	**16.00**	**25.00**		5
Iron (Fe^{2+}) (mg/L)	0.10	0.10	0.20	0.20	0.02	0.02	0.20	0.10	0.10	0.01	0.20	0.30	0.3	0.3
Ammonia (PO_4^{3-}) (mg/L)	0.19	0.03	0.03	0.09	0.06	0.04	0.07	0.10	0.50	0.20	0.50	**0.70**		0.5
Hardness ($CaCO_3$) (mg/L)	45.00	41.00	37.00	59.10	50.00	45.00	39.00	30.00	22.00	35.00	25.00	22.00	150	500
Sulphate (SO_4^{2-}) (mg/L)	2.80	2.40	3.50	6.20	6.00	4.50	3.50	6.70	3.20	3.40	5.30	6.20	100	250
Manganese (Mn2+) (mg/L)	0.01	0.18	0.13	0.14	0.03	0.07	0.11	0.18	0.02	0.20	**0.30**	0.20	0.2	0.4
Copper (Cu) (mg/L)	0.01	0.01	0.21	0.19	0.25	0.02	0.50	0.01	0.02	0.02	0.01	0.01	1	2
Magnesium (Mg^{2+}) (mg/L)	0.13	0.09	0.11	0.06	0.53	0.62	0.15	0.25	1.02	1.02	1.00	1.00		100
Calcium (Ca^{2+}) (mg/L)	4.70	9.40	11.50	6.50	4.90	6.80	9.60	2.50	3.20	2.10	2.20	2.20		150
Total Alkalinity (mg/L)	40.00	37.00	42.00	54.00	47.50	50.20	40.10	49.10	29.00	15.00	0.02	2.30		
Total salinity (mg/L)	41.00	45.00	33.00	14.00	31.50	6.80	10.50	7.20	3.40	15.00	19.00	19.00		

Bold values indicate incidences where parameters are outside guideline values.

a pH scale ranging from 1 - 14. The SON and WHO set a pH guideline value of between 6.5 and 8.5 as generally considered satisfactory for drinking water.

The pH of borehole water of our study area was generally within the guideline value except in Mutum Biyu “B” where pH value was 6.2 and Namnai where pH was 6.4. The highest pH value of 7.88 was recorded in Gassol. pH is generally considered to have no direct impact on humans. However, long-term intake of acidic water can invariably lead to mineral deficiencies (Fairweather-Tait and Hurrell, 1996). Because virtually all groundwater comes from precipitation that soaks into the soil and passes down to the aquifer, high pH if widespread could also be an indication of acidic rain in the area.

Fluoride

The concentration of fluoride in Tutare ward both in the rainy and dry season when concentration was up to 2.5 mg/L deviate from the 1.5 mg/L suggested as guideline value by SON and WHO. In the study area, fluoride concentrations ranged between 0.03 mg/L in Yarima to 2.5 mg/L in Tutare.

High concentration of fluoride contaminant in ground waters tend to be found in association with crystalline rocks containing fluorine-rich minerals, especially granites and volcanic rocks, shallow aquifers in arid areas experiencing strong evaporation, sedimentary aquifers undergoing ion exchange and inputs of geothermal water. Fluoride has long been found to have a beneficial effect on dental health as such it is an additive in toothpastes and food. However, when present in drinking water at concentrations much above the guideline value of 1.5 mg/L, long term use can result in development of dental fluorosis or at its worst, crippling skeletal fluorosis. Although, the incidence of Fluoride concentration outside guideline value in our study is only restricted to one ward, it is important for water managers to constantly monitor this parameter as other studies in the region have also revealed high incidences of water samples showing high F concentrations (Waziri et al., 2012).

Chlorine

The use of chlorine in drinking water as a disinfectant has played a critical role in the prevention of waterborne diseases. According to the (World Health Organization, 1993), the adoption of drinking water chlorination has been one of the most significant advances in public health protection. However, when concentration of chlorine in water is above the guideline value of 5 mg/L, it could result in irritation of the oesophagus, a burning sensation in the mouth and throat, and spontaneous vomiting. It has also been suggested that episodes of dermatitis and asthma can be triggered by exposure to chlorinated water (Eun et al., 1984; Watson and Kibler, 1933). In this study, there are four wards in which chlorine concentration was outside the guideline values of 5 mg/L. These are Tutare (22 mg/L), Gunduma (35 mg/L), Mutum Biyu "A" (25 mg/L) and Mutum Biyu "B" (22 mg/L).

Iron

Similar to turbidity, the concentration of iron was generally found to be within guideline values of 0.3 mg/L in the dry season. However the concentration of iron in seven wards was well outside the guideline value in the rainy season. These wards are Sansani (0.8 mg/L), Sendirde (0.45 mg/L), Wuryo (0.75 mg/L), Sabon Gida (0.63 mg/L), Namnai (0.62 mg/L), Yarima (0.67 mg/L) and Gassol (0.45 mg/L). Iron concentration range between 0.01 and 0.8 mg/L in the rainy season; and 0.01 and 0.3 mg/L in the dry season. The mean concentration in the rainy season is 0.42 and 0.15 mg/L in the dry season.

It has been suggested that high rainfall is essential in increasing iron concentration in boreholes (Abubakar and Adekola, 2012). Rainwater as it infiltrates the soil and underlying geologic formations dissolves iron, causing it to seep into aquifers that serve as sources of ground water for borehole. Therefore it is not surprising that iron concentration is highest in the rainy season.

Ammonia

Concentration of ammonia in water samples from our study ranges from 0.03 to 0.7 mg/L with an average value of 0.21 mg/L both in the dry and rainy seasons. The value of 0.7 mg/L which is the only one above the 0.5 mg/L guideline value was recorded in Mutum Biyu “B”.

Ammonia can occur naturally in ground water, while in the environment, ammonia originates from metabolic, agricultural activities especially from the intensive rearing of farm animals. Ammonia in water is an indicator of possible bacterial, sewage and animal waste pollution.

Manganese

Manganese occurs naturally in groundwater sources and in soils. However, human activities such as automobile emission are also responsible for manganese concentrations in the environment (Loranger et al., 1996).

In this study, manganese is well within WHO standard but the level in Mutum Biyu A (0.3 mg/L) is outside permissible level for manganese under the SON standard of 0.2 mg/L, but within the WHO standard of 0.4 mg/L.

Water quality across the wards

All the wards in Gassol LGA has at least one incidence of water contamination. This is not helped by the fact that all the boreholes had levels of turbidity outside guideline values in the rainy season. The result show that water samples from Shira ward is of the best quality only falling outside the guideline values which was for turbidity in the rainy season. Mutum Biyu “B” appear to have the worst water quality having four parameters (turbidity, pH, Chlorine and Ammonia) falling outside guideline values in the rainy season alone. Water quality improved in the dry season with five wards (Wuryo, Sabon Gida, Yarima, Gassol and Shira); free from any incidence of water contamination.

Mean value test

The mean value test was conducted using data from each season and then the average value over the two season. The essence of the mean value test as earlier pointed out is to assess the level of water contamination relative to guideline values.

The test to evaluate human health risk of water contamination in Gassol reveals that there is significant difference in mean concentration of contamination indicator and their guideline for turbidity, iron and chlorine (Table 3). The upper bound value (US_{95}) of these parameters are above the guideline value (G) suggested by SON and WHO. Thus, it can be concluded that action is needed to control these contaminants in the area based on the mean value test.

In this circumstance, it is suggested that there should be further sampling to gain a more representative picture of the site. However, precaution will suggest that remedial action is encouraged. This implies that overall; borehole water in Gassol is within guideline values for the majority of the parameters except for these four.

Table 3. Mean Value Test of water samples.

Parameters	Upper bound value (US_{95})			Guideline value	
	Rainy Season	Dry Season	Average	SON	WHO
Turbidity (NTU)	**37.60**	**12.89**	**23.94**	5	
Conductivity (Ω/cm)	702.92	702.92	702.92	1000	2500
Temperature (°C)	27.73	35.90	31.38	23 - 40	
pH	7.05	7.05	7.05	6.5 - 8.5	
TDS (PPM)	358.86	358.86	358.86	500	1000
Nitrate (NO_3^-) (mg/L)	14.73	14.73	14.73	50	50
Fluoride (F^-) (mg/L)	1.29	1.29	1.29	1.5	
Chlorine (Cl^-) (mg/L)	**15.79**	**15.79**	**15.79**		5
Iron (Fe^{2+}) (mg/L)	**0.57**	0.18	**0.35**	0.3	
Ammonia (PO_4^{3-}) (mg/L)	0.33	0.33	0.33		0.5
Hardness ($CaCO_3$) (mg/L)	64.58	43.46	51.69	150	500
Sulphate (SO_4^{2-}) (mg/L)	5.27	5.27	5.27	100	250
Manganese (Mn2+) (mg/L)	0.18	0.18	0.18	0.2	0.4
Copper (Cu) (mg/L)	0.19	0.19	0.19	1	2
Magnesium (Mg^{2+}) (mg/L)	0.71	0.71	0.71		100
Calcium (Ca^{2+}) (mg/L)	7.17	7.17	7.17		150
Total Alkalinity (mg/L)	43.44	43.44	43.44		
Total salinity (mg/L)	27.67	27.67	27.67		

Bold values indicate incidences where parameters are outside guideline values.

However, this analysis is made with great caution, as it is not possible to aggregate boreholes from different wards that are distinct even if it is possible, the presence of a contaminant is enough concern.

DISCUSSION

This study assessed some physical and chemical contamination indictors in borehole water in Gassol Local Government Area of Taraba State, Nigeria. The study reveals that borehole water in the area is not of the best quality as far as the WHO and SON guidelines are concerned. especially considering the fact three para-meters, namely turbidity, chlorine and iron has upper bound value (US_{95}) that are above guideline values. This study like similar studies carried out in the North Eastern region of Nigeria, showed that there are incidences of contamination of borehole water. For instance, Abubakar and Adekola (2012) found borehole water from Yola-Jimeta metropolis to have levels of chloride (Cl^-), iron (Fe^{2+}), nitrate (NO_3^-), pH, sodium (Na^+) and total hardness ($CaCO_3$) which are the main sources of borehole water contamination in the study area. In that study the upper bound value (US_{95}) of pH was found to be above the guideline value. However, this was not the case in the current study.

The presence of these contaminants at levels above guideline values in borehole water poses serious health effect to the population. This underscores the need for water managers to promote efficient water treatment/ management techniques. One approach that might come handy and prove to be easily accessible, low cost and environmentally friendly is the use of natural supplement such as Moringa oleifera seeds as natural absorbent and antimicrobial agent for purification of ground water for drinking purpose. A recent study by (Mangale Sapana et al., 2012) showed that Moringa oleifera seed powder has the potential to be used as treatment for turbidity, TDS, hardness, chlorides, alkalinity and acidity. This is recommended for eco-friendly, nontoxic, simplified water treatment where rural and peri-urban people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water. We therefore, advocate for water agencies to partner with local communities and researchers to ascertain the sustainability of this method.

North Eastern Nigeria is the poorest region in the country where majority lack access to qualitative water for consumption. The region is also the worst hit in terms of access to quality water. The poor water supply in the region has been blamed for causing typhoid fever, cholera and bilharzias especially where water source are not appropriately or sufficiently treated (Alexander, 2010, Uzomah and Scholz, 2002).

It is also noteworthy to point out that while the WHO guideline is been constantly updated, the SON guideline has never been updated since the first version in 2007. Although the SON report stated that “the standard shall be reviewed every three years” (Standards Organisation of Nigeria, 2007), yet this has not happened since the first edition. The lacklustre attitude to updating water guidelines is reminiscent of the poor funding and focus on this sector in Nigeria. There is a need for more attention by local and national government on delivering qualitative water to the populace. It is expected that the Nigerian Standard for Drinking Water Quality will speed up the process of upgrading non-protected water systems and improving the management of all drinking water systems in the country.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Abubakar B, Adekola O (2012). Assessment of borehole water quality in Yola-Jimeta Metropolis, Nigeria. Int. J. Water Resour. Environ. Eng 4(287-293.

Akoto O, Adiyiah J (2007). Chemical analysis of drinking water from some communities in the Brong Ahafo region. Int. J. Environ. Sci. Technol. 4(211-214.

Alexander P (2010). Evaluation of ground water quality of Mubi town in Adamawa State, Nigeria. Afr. J. biotechnol. 7(11).

Batmanghelidj F, Page MJ (2012). Your body's many cries for water, Tantor Media, Incorporated.

Bellisle F, Thornton SN, Hebel P, Denizeau M, Tahiri M (2010). A study of fluid intake from beverages in a sample of healthy French children, adolescents and adults. Eur. J. Clin. Nutr. 64(4):350-355.

Bhattacharya P, Chatterjee, D, Jacks, G (1997). Occurrence of Arsenic-contaminatedGroundwater in Alluvial Aquifers from Delta Plains, Eastern India: Options for Safe Drinking Water Supply. International Journal of Water Resources Development 13(1): 79-92.

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological applications 8(3):559-568.

Chigor VN, Umoh VJ, Okuofu CA, Ameh JB, Igbinosa EO, Okoh AI (2012). Water quality assessment: surface water sources used for drinking and irrigation in Zaria, Nigeria are a public health hazard. Environmental monitoring and assessment 184(5):3389-3400.

Dean JR (2007). Bioavailability, bioaccessibility and mobility of environmental contaminants, John Wiley & Sons.

Department for Environment Food and Rural Affairs, The Environment Agency (2002). Assessment of Risks to Human Health from LandContamination: An Overview of the Development of Soil Guideline Values and Related Research. Bristol, United Kingdom, Environment Agency.

Eun HC, Lee AY, Lee YS (1984). Sodium hypochlorite dermatitis. Contact dermatitis 11(1): 45-45.

Fairweather-Tait S, Hurrell RF (1996). Bioavailability of minerals and trace elements. Nutrition Research Reviews 9(01): 295-324.

Fournier R, Truesdell A (1973). An empirical Na・K・Ca geothermometer for natural waters. Geochimica et Cosmochimica Acta 37(5):1255-1275.

Gundry S, Conroy R, Wright J (2003). A systematic review of the health outcomes related to household water quality in developing countries. J. Water Health. 2(1-13.

Ikem A, Odueyungbo S, Egiebor NO, Nyavor K (2002). Chemical quality of bottled waters from three cities in eastern Alabama. Science of the total environment 285(1): 165-175.

Loranger S, Tétrault M, Kennedy G, Zayed J (1996). Manganese and other trace elements in urban snow near an expressway. Environ. Pollut.n 92(2):203-211.

Mack GW, Nadel ER (2011). Body fluid balance during heat stress in humans. Comprehensive Physiology.

Mangale SM, Chonde SG, Raut P (2012). Use of Moringa oleifera (drumstick) seed as natural absorbent and an antimicrobial agent for ground water treatment. Res. J. Recent Sci. 2277:2502.

National Population Commission (2006). Population and Housing Census of the Federal Republic of Nigeria 2006 Census: Priority Tables, Abuja, Nigeria.

Ncube EJ, Schutte CF (2005). The occurrence of fluoride in South African groundwater: A water quality and health problem. Water SA 31(1):35-40.

Nemade PD, Kadam AM, Shankar HS (2009). Removal of iron, arsenic and coliform bacteria from water by novel constructed soil filter system. Ecological Engineering 35(8): 1152-1157.

Offei-Ansah C (2012). Food habits and preferences as a factor in the choice of meals by students in the University of Cape Coast. Nutrition and health 21(3):151-172.

Onugba A, Sara SG. (2003, September). Rural water supply and handpump development in Nigeria. In Pre-Print of the 29th WEDC Conference Abuja, Nigeria.

Palintest (1980). The Palintest System-Test Instructions for Photometer 5000 Pool Test Kit. Newcastle upon Tyne, Palintest Ltd.

Payment, P, Waite, M, Dufour, A (2003). Assessing microbial safety of drinking water: 47.

Ramsey MH, Argyraki A (1997). Estimation of measurement uncertainty from field sampling: implications for the classification of contaminated land. Science of the total environment 198(3):243-257.

Shryer D (2007). Body Fuel: A Guide to Good Nutrition, Marshall Cavendish.

Standards Organisation of Nigeria (2007). Nigerian Standard for Drinking Water Quality, Lagos, Nigeria.

Taraba State Government (2015). Local Government Area, Taraba Government House, Jalingo, Nigeria.

The United Nations Children's Fund (2007). Photo essay: Nigeria's water and sanitation challenge.

Uzomah VC, Scholz M (2002). Water Availability Assessment and Corresponding Public Health Implications for a Rural Area in Nigeria. Water and Environment Journal 16(4): 296-299.

Voices (2013). Almost Half Of Nigeria's Population Can't Access Clean Water, Abuja, Nigeria.

Watson, SH, Kibler, CS (1933). Drinking water as a cause of asthma. J. Allergies. 5(197-198.

Waziri M, Musa U, Hati SS (2012). Assessment of Fluoride Concentrations in Surface Waters and Groundwater Sources in Northeastern Nigeria. cancer 5(6.

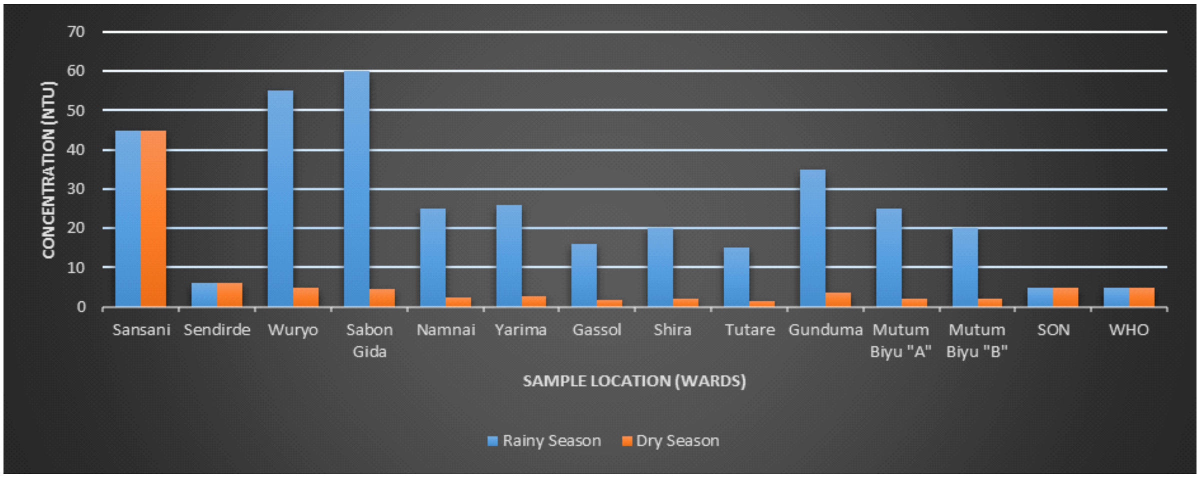
World Health Organization (1993). Guidelines for Drinking-Water Quality. Geneva, Switzerland, World Health Organization.

Unicef. (2010). Diarrhoea: why children are still dying and what can be done. http://www. unicef. org/media/files/Final_Diarrhoea_Report_October_2009_final. pdf. Chicago

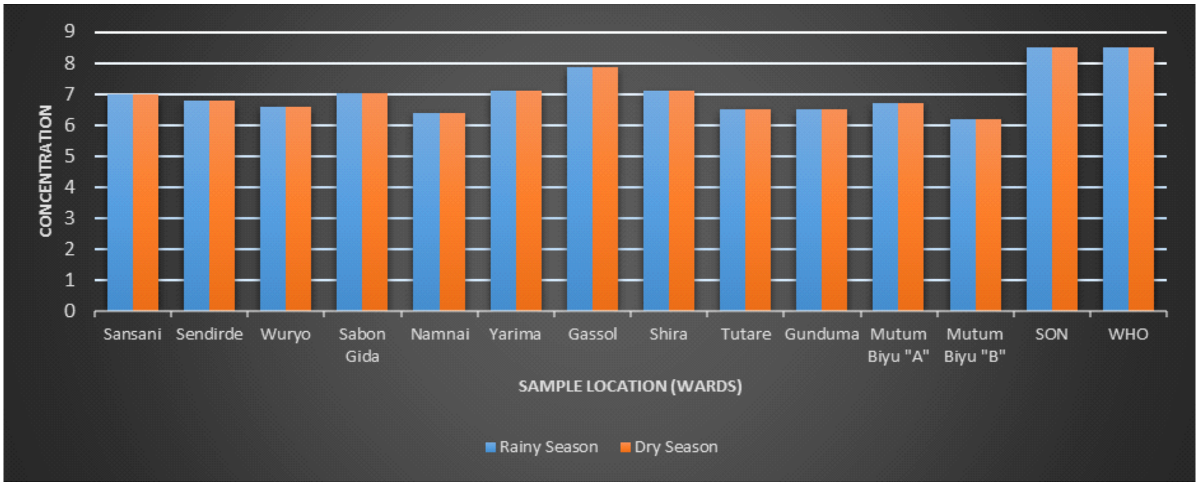
WHO (2006). Meeting the MDG drinking water and sanitation target: the urban and rural challenge of the decade. Geneva, Switzerland, World Health Organization.

WHO (2008). Safer Water, Better Health: Costs, benefits, and sustainability of interventions to protect and promote health. Geneva, Switzerland, World Health Organization.

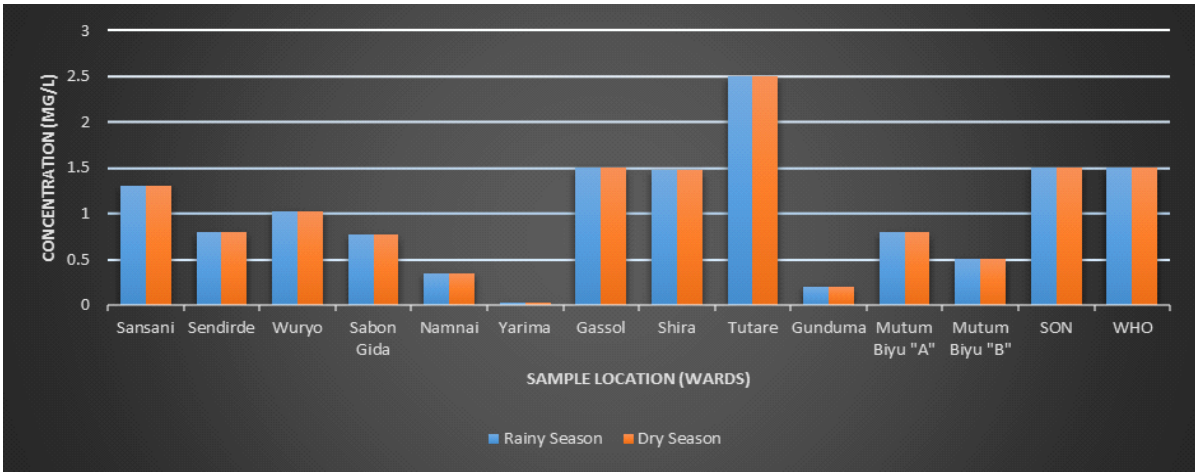
WHO (2011). Guidelines for Drinking-water Quality. Geneva, Switzerland, World Health Organisation.



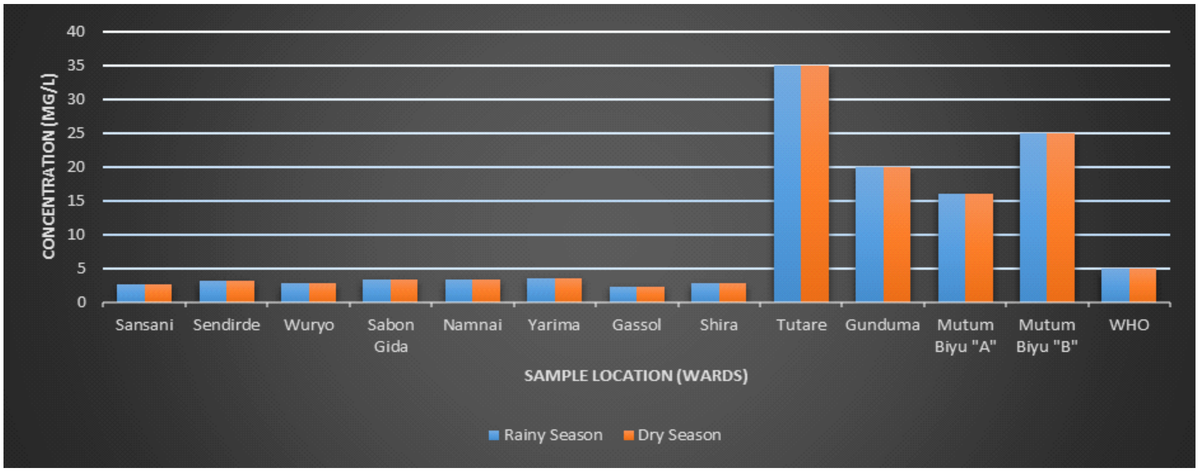
Appendix 1. Graph of analytical values of Turbidity (NTU) in the various wards with SON and WHO guideline values.



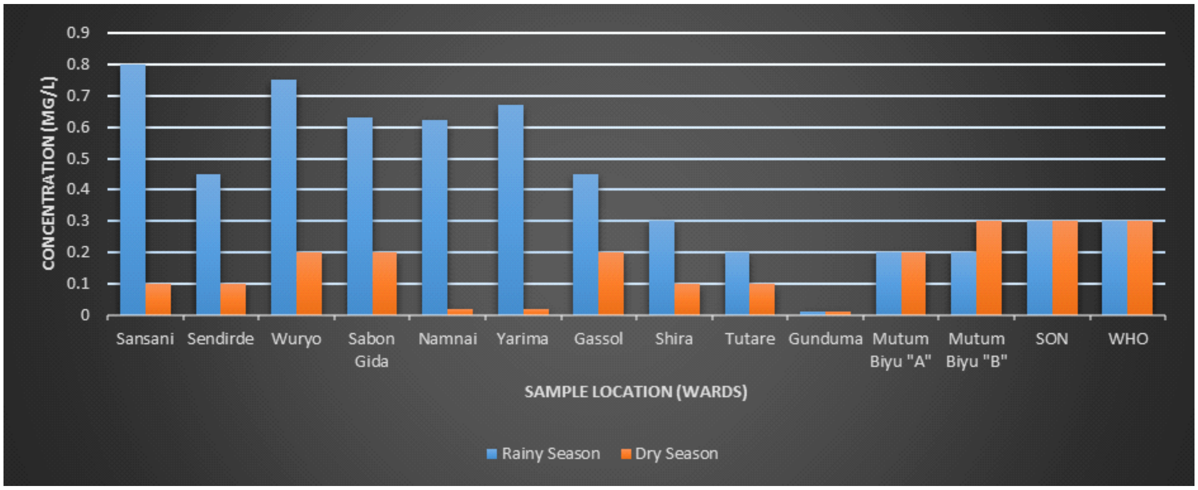
Appendix 2. Graph of analytical values of pH in the various wards with SON and WHO guideline values.



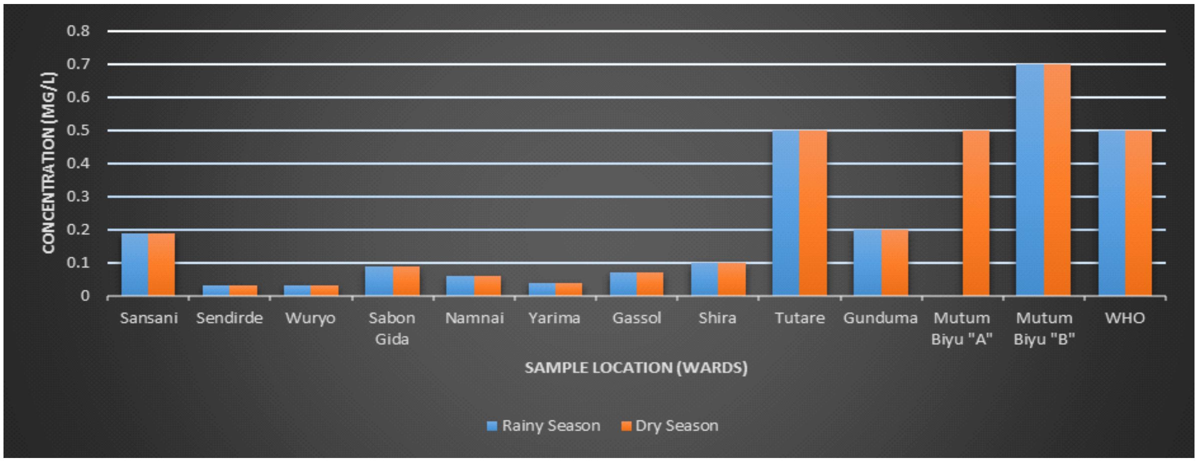
Appendix 3. Graph of analytical values of Fluoride (F-) (mg/L) in the various wards with SON and WHO guideline values



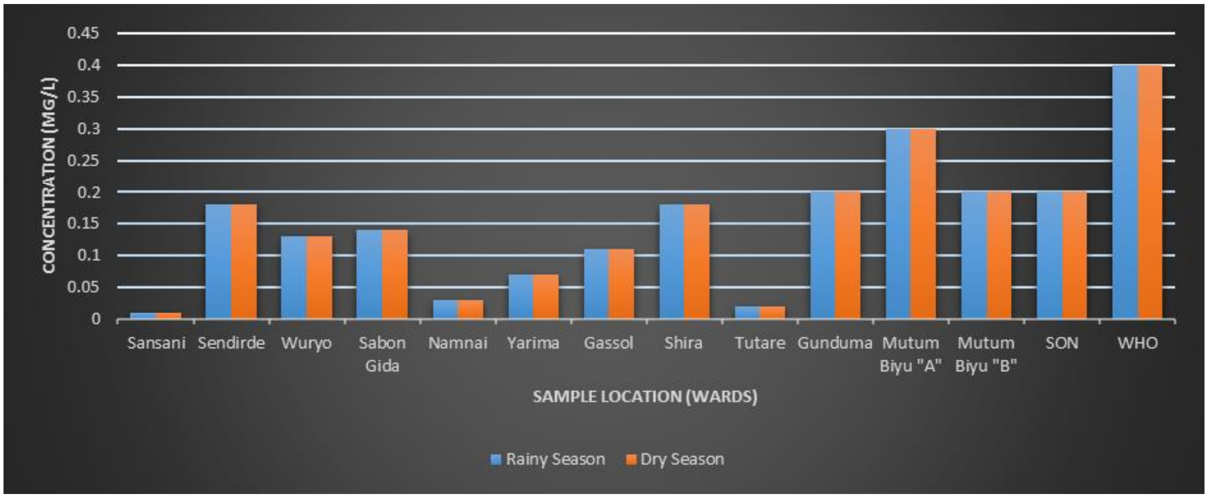
Appendix 4. Graph of analytical values of Chlorine (Cl-) mg/L in the various wards with SON and WHO guideline values.



Appendix 5. Graph of analytical values of Iron (Fe2+) mg/L in the various wards with SON and WHO guideline values.



Appendix 6. Graph of analytical values of Ammonia (PO43-) mg/L in the various wards with SON and WHO guideline values.



Appendix 7. Graph of analytical values of Manganese (Mn2+) mg/L in the various wards with SON and WHO guideline values.

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Full Length Research Paper

The effects of arbuscular mycorrhizal fungi and phosphorus levels on dry matter production and root traits in cucumber (*Cucumis sativus* L.)

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To evaluate the effect of arbuscular mycorrhizal fungi and phosphorus levels on root traits of cucumber plants, a factorial experiment was carried out based on a randomized completely design pot culture. Four phosphorus fertilization treatments, including 2, 5, 10 and 15 mg P kg^{-1} soil possessed phosphorus fertilization levels as the first factor. At the second factor arranged *Glomus mosseae*, *Glomus intraradices* of mycorrhiza species and non-inoculum as a control with three replications were conducted in the greenhouse of agricultural research center of west Azarbaijan province Urmia, in 2013. Results show that above-ground dry matter of inoculated cucumber at both species with 155.00 and 160.83 mg/plant had the highest values. Both species had more root fresh and dry weight, root length and root volume than control. Colonization of *G. mosseae* and *G. intraradices*, with 53.20 and 44.59% had the highest values at the 2 mg P kg^{-1} soil. *G. mosseae* and *G. intraradices* had the highest leaf phosphorus with 486.06 and 477.60 mg/100 g of leaf dry weight at the 15 mg P kg^{-1} soil, respectively. Leaf phosphorus (r = 0.62), root dry weight (r = 0.79**), root length (r=0.44**), root volume (r = 0.82**) and fresh root weight (r = 0.74**) had positive correlation coefficients with above-ground dry matter. Although application of phosphorus increased above-ground dry matter and root traits, but our study clearly demonstrates that mycorrhizal fungi play an important role in the enhancement of growth of cucumber plants under very low phosphorus conditions.**

Key words: Colonization, cucumber, dry matter, insoluble phosphorus, mycorrhiza.

INTRODUCTION

Phosphorus is critical for plant growth, and is a component of the nucleic acid structure of plants and bio-membranes. Therefore, it is important in cell division and tissue development. Phosphorus is also involved in the

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Table 1. Some soil physico-chemical characteristics.

Saturation (%)	Electrical conductivity (ds m^{-1})	pH	Organic carbon (%)	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})	Soil texture
29	1.3	7.4	0.20	2.0	85	Sandy loamy

energy metabolism of cells and is required for the biosynthesis of primary and secondary metabolites in plants. Consequently, plants have evolved a range of strategies to increase phosphorus uptake and mobility (Marschner, 1996), the most common among which is arbuscular mycorrhiza (AM) symbiosis. In AM fungi symbiosis with plant roots, the enhanced uptake of phosphorus is attributed to the fungal partner, and the increase in phosphorus uptake by the colonized roots in turn leads to increased plant growth (Burleigh et al., 2002).

AM fungi are one of the most widespread mycorrhizal associations between soil micro-organisms and higher plants. The function of all mycorrhizal systems depends on the ability of the fungal symbiont to absorb inorganic and organic nutrients available in soil (Marschner and Dell, 1994). The AM fungi infect the roots of a susceptible plant, forming a mutually beneficial, symbiotic relationship. In exchange for carbohydrates from the host plant, AM fungi benefit the plant primarily through increased uptake of soil nutrients, such as phosphorus, zinc and copper (Miller et al., 1986). This enhanced mineral uptake is facilitated by external hyphae of the fungi, which exploit a greater volume of soil than roots, thus accessing nutrients (especially phosphorus) not normally accessible to the plant's root system (Hayman, 1983). Colonization of *Cucumis sativus* by AM fungi affects flowering, fruit production, photosynthesis rates, and disease resistance (Trimble and Knowles, 1995; Valentine et al., 2001; Hao et al., 2005; Kiers et al., 2010).

AM fungi are important due to their great capability to increase plant growth and yield under certain conditions. The major reason for this increase is the ability of plants in association with AM to take some nutrients such as phosphorus efficiently (Podila and Douds, 2001). AM fungi, as obligate symbionts, also depend for their growth and activity on the supply of carbon compounds from the photosynthetic partner (Ocampo and Azcon, 1985; Jennings, 1995). AM symbiosis can cause an important carbohydrate gain in the host plant and up to 20% of total photo-assimilate substances can be transferred to the fungal partner (Graham, 2000). Inoculation with AM fungi, in some vegetables, may improve growth performance (Temperini et al., 2009). The benefits of AM fungi inoculation depend on the genotypic host-fungus combinations and also the type of the inoculums used (Rouphael et al., 2010). The aim of this study was to evaluate the effect of AM fungi species, *Glomus mosseae and Glomus intraradices*, on dry matter production and root traits of cucumber plants under different levels of phosphorus.

MATERIALS AND METHODS

A experiment was conducted at greenhouse with a day/night cycle of 16 h at 22°C and 8 h at 19°C (relative humidity: 50 to 70%) in the agricultural research center of west Azarbaijan province, Urmi, Iran. The greenhouse located in longitude 37°, 35', 32'' north, latitude 45°, 3′, 39'' east and 1330m altitude. Some physicochemical properties of soil which is used to growth the cucumber plants were determined (Table 1). Soil was collected from a low P (2 ppm Olsen extractable P) field in the region of Shaharchay River around of Urmia.

A factorial experiment based on a randomized completely design was carried out with three replications. Four phosphorus fertilization treatments in this study, including 2, 5, 10 and 15 mg P kg^{-1} soil (KH_2PO_4) were incorporated into the soil by hand and mycorrihzal treatments, a control with no inoculums and two mycorrhizal fungi inoculums, *G. mosseae* and *G. intraradices* arranged as the first and second factors, respectively.

Seeds of the cucumber cultivar were provided by the Agricultural Research Station of Urmia. The two species of AM fungi used in this study were *G. mosseae* and *G. intraradices*, which were produced on maize (*Zea mays* L.) host plants by Dr. E.M. Goltapeh at Tarbiat Modarres University, Tehran, Iran. The mycorrhizal inoculum was a mixture of sterile sand, mycorrihzal hyphae, spores (25 spores g^{-1} inoculums), and colonized root fragments. Seeds of cucumber were surface sterilized with 0.05% sodium hypo-chloride for 45 min before sowing them. Seeds were sown in sterilized soils in plastic pots (12 ×12 cm) at a depth of 3 cm on 25 June 2013. Thirty grams of the appropriate inoculums was placed into the hole below each seed, and then covered with sterile soil. For non-mycorrhizal control plants were sown with no inoculation. The plants were grown in a greenhouse under natural photoperiods for 6 weeks during which only distilled water was applied. In addition, twice a week, each pot was supplied with 100 ml of a nutrient solution containing 720 mg of $MgSO_4.7H_2O$, 295 mg of Ca $(NO_3)2.4H_2O$, 240 mg of KNO_3, 0.75 mg of $MnCl_2.4H_20$, 0.75 mg of KI, 0.75 mg of $ZnSO_4.7H_2O$, 1.5 mg of H_3BO_3, 0.001 mg of $CuSO_4.5H_20$, 4.3 mg of FeNaEDTA and 0.00017 mg of $Na_2MoO_4.2H_2O$ supplemented without phosphorus (Vosatka and Gryndler, 1999).

The root fresh weights were measured before drying at 72°C for 24 h that leads to the weights of the dry matter for root. Length and volume of roots, root dry weights and above-ground dry matter of seedlings were determined after harvesting. At 6 weeks after

Table 2. Mean squares traits of cucumber affected by mycorrhizal infection and different levels of phosphorus.

S.O.V	df	Mycorrhizae colonization	Total dry weight	Leaf phosphorus	Root fresh weight	Root dry weight	Root length	Root volume
Phosphorus (P)		399.60**	8866.67**	7560.99**	52496.30	1368.52**	26.59**	0.08**
Mycorrhizae (M)	2	5562.57**	6633.11**	5353.48**	233858.33**	2952.78**	28.53**	0.19**
M × P	6	103.63**	680.56	753.39**	10054.63	32.41	38.12*	0.01
Error	24	2.40	1113.89	78.63	21369.44	75.00	12.97	0.02
CV (%)	-	6.32	23.11	2.01	23.20	17.71	24.93	24.55

* Significant at the 5% probability level; ns, not significant; ** Significant at the 1% probability level.

Table 3. Comparison of colonization percentage, Leaf phosphorus and Root length of cucumber affected by mycorrhizal infection and different levels of phosphorus.

Mg phosphorus kg^{-1} soil	Mycorrhizal symbiosis	Mycorrhizae colonization (%)	Leaf phosphorus (mg/100g dry leaf)	Root length (cm)
2	Non-mycorrhizal	0.00^{g}	392.36^{e}	14.33^{bcd}
	G. mosseae	53.20^{a}	440.12^{c}	13.67^{cd}
	G. intraradices	44.59^{b}	416.12^{d}	11.00^{d}
5	Non-mycorrhizal	0.00^{g}	400.46^{e}	11.67^{d}
	G. mosseae	42.78^{b}	434.52^{c}	17.93^{abc}
	G. intraradices	39.58^{c}	427.32^{cd}	11.67^{d}
10	Non-mycorrhizal	0.00^{g}	390.46^{e}	10.67^{d}
	G. mosseae	33.86^{d}	461.05^{b}	12.00^{d}
	G. intraradices	28.52^{e}	459.08^{b}	20.33^{a}
15	Non-mycorrhizal	0.00^{g}	475.92^{ab}	14.00^{bcd}
	G. mosseae	29.34^{e}	486.06^{a}	18.00^{abc}
	G. intraradices	23.97^{f}	477.60^{ab}	18.67^{ab}

Means followed by the same letter(s) in each column are not significant differences.

planting, the percentage of colonization of cucumber roots by AM fungi was determined per experimental unit. Root colonization was measured in fresh roots cleared in 10% KOH for 10 min at 90°C and stained in 0.05% lactic acid–glycerol–Trypan Blue (Phillips and Hayman, 1970). The percentage of root colonization by AM fungi was microscopically determined using the gridline intersection method (Giovannetti and Mosse, 1980). To measure leaf phosphorus, dried leaves were milled, digested, and analyzed as described by Watanabe and Olsen (1965) and Ohnishi et al. (1975). The method described for phosphorus involves drying, homogenization, and combustion (4 h at 500°C) of the leaf sample. The plant ashes (5 mg) are digested in 1 ml of concentrated HCl. The samples are then filtered, and total phosphorus is quantified as PO4– using the ascorbic acid method (Watanabe and Olsen, 1965). The amount of PO4– in solution was determined calorimetrically at 882 nm (Graca et al., 2005).

Analysis variance of data was performed using MSTATC software. The effects of phosphorus, application of mycorrhizae, and the interactions of these two factors were analyzed by ANOVA and the means compared by the Duncan's Multiple Range test ($P \leq 0.05$). Also, correlation coefficients were calculated.

RESULTS AND DISCUSSION

Different levels of phosphorus and mycorrhizae for traits of root fresh weight, root dry weight, root volume, total dry weight and interaction between them for leaf phosphorous accumulation, root length and mycorrhiza colonization had significant differences (Table 2).

Colonization percentage of *G. mosseae* was more than *G. intraradices* and was less reduced with increasing phosphorus levels. Variations of this trait were for *G. mosseae* between 29.34 to 53.20 and *G. intraradices*23.97 to 44.59. Colonization mycorrhiza was reduced due to increasing phosphorus fertilization (Table 3). Both species of mycorrhiza had more root fresh weight,

Table 4. Means comparison of cucumber traits by mycorrhizae species

Mycorrhizal symbiosis	Total dry weight (mg/plant)	Root fresh weight (mg/plant)	Root dry weight (g/plant)	Root Volume (cm^3)
Non-mycorrhizal	117.50^b	470.83^b	30.83^b	0.38^b
G. mosseae	160.83^a	687.50^a	56.67^a	0.60^a
G. intraradices	155.00^{ab}	731.67^a	59.17^a	0.59^a

Means followed by the same letter(s) in each column are not significant differences.

Table 5. Means comparison of cucumber traits by different levels of phosphorus

Mg Phosphorus kg^{-1} soil	Total dry weight (mg/plant)	Root fresh weight (mg/plant)	Root dry weight (mg/plant)	Root volume (cm^3)
2	114.44^b	-	36.11^b	0.42^b
5	131.11^b	-	45.00^b	0.48^{ab}
10	144.44^{ab}	-	48.89^b	0.55^{ab}
15	187.78^a	-	65.56^a	0.64^a

Means followed by the same letter(s) in each column are not significant difference.

root dry weight and root volume with 731.67 mg, 59.17 mg, 0.59 cm^3, respectively than non-inoculated cucumber plants (Table 4). Root dry weight and root volume increased with improved phosphorus fertilization. Phosphorus fertilization treatments of 2 and 15 mg kg^{-1} soil were 36.11 mg and 0.42 cm^3 and 65.56 mg and 0.64 cm^3 values of them, respectively (Table 5). Expanded roots of mychorrhizal plants enhanced root area (Allen et al., 1981). Therefore, water and nutrient uptake in mycorrhizal plants was due to more root expansion than control (Huang et al., 1985).

Analysis of the phosphorus accumulation in leaves of the cucumber plants showed that the highest phosphorus accumulation in leaves (486.06 mg/100 g dry leaf) was obtained from the plants inoculated with *G. mosseae* and phosphorus treatment 15 Mg P kg^{-1} soil (Table 3). The minimum phosphorus accumulation in leaves (392.36 mg/100 g dry leaf) was obtained from the non-mycorrhizal and 2 mg P kg^{-1} soil, followed by the non-mycorrhizal plants 5 and 10 mg P kg^{-1} soil. The phosphorus concentration in the leaves of cucumber plants in each treatment was significantly higher than that in the control. Plants were more responsive to additional phosphorus in the low to medium phosphorus (2 to 15 mg phosphorus kg^{-1} soil) range, while AM infected plants were more responsive in the low phosphorus (2 and 5 mg phosphorus kg^{-1} soil) range, with increasing colonization and acquiring phosphorus (Table 3). The significance of sufficient phosphorus availability during early crop growth has been reported in different crop species (Grant et al., 2005). It has been reported that enhanced early-season phosphorus nutrition in maize increased dry matter at early stages partitioned to the grain at later development stages (Parewa et al., 2010). Likewise, in wheat and barley, phosphorus supply during earlier growth had superior effect on final grain yield than phosphorus supply in later stages (Smith and Smith, 2011).

Plenets et al. (2000) reported a greater difference in dry matter accumulation of maize under phosphorus deficiency during early stages of growth. The above ground dry matter accumulation was observed to be severely reduced (up to 60%) during early stages of maize growth, while there were only slight differences on dry matter accumulation at harvest and grain yield. The effect of early phosphorus deficiency on decline in shoot growth occurs because of slight stimulation of root growth (Mollier and Pellerin, 1999). The initial reduction in growth related to phosphorus deficiency has an ultimate effect on the final crop yield, which is experienced by the crop throughout the remaining of the growing period. Phosphorus is critical for plant growth and makes up about 0.2% of dry mass, but it is one of the most difficult nutrients for plants to acquire. In soil, it may be present in relatively large amounts, but much of it is poorly available because of the very low solubility of phosphates of iron, aluminum, and calcium, leading to soil solution concentrations of 10 mM or less and very low mobility (Ryan et al., 2005). The ability of

Table 6. Correlation coefficients between cucumber traits

Treatment	Mycorrhizae colonization	Total dry weight	Leaf phosphorus	Root dry weight	Root length	Root volume
Total dry weight	0.21					
Leaf phosphorus	0.29	0.62**				
Root dry weight	0.48**	0.79**	0.73**			
Root length	0.11	0.44**	0.55**	0.46**		
Root volume	0.35*	0.82**	0.55**	0.81**	0.53**	
Root fresh weight	0.43**	0.74**	0.45**	0.78**	0.39*	0.80**

* and ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

AM fungi to enhance host-plant uptake of relatively immobile nutrients, in particular phosphorus and Zn (Balakrishnan and Subramanian, 2012), and their requirement for up to 20% of host-plant for establishment and maintenance, is well accepted (Subramanian et al., 2009).

Analysis of the root length of cucumber plants showed that the highest root length (20.33 cm) was obtained from the plants inoculated with *G. intraradices* and phosphorus treatment 10 mg P kg^{-1} soil (Table 3). The minimum root length (11.67 and 10.67 cm) was obtained from the non-mycorrhizal and 5, 10 mg P kg^{-1} soil (Table 3). Marulanda et al. (2007) reported that in lavender inoculated plants with *G. mosseae* and *G. intraradices* improved root growth 35 and 100%, respectively.

Between different levels of phosphorus application, 15 mg P kg^{-1} soil had the most above-ground dry matter with 187.78 mg/plant and the lowest phosphorus application (2, 5 mg P kg^{-1} soil) had above-ground dry matter with 114.44 and 131.11 mg/plant. Both Species with 160.83 and 155.00 mg/plant above-ground dry matters had the highest values than control (Tables 4 and 5). Subramanian et al. (2006) observed that root colonization by the AM fungus significantly increased dry matter yield and ultimate increased the production. Total dry weight differences in mycorrhizal treatments are related to water absorption and mineral nutrients (AL-Karaki et al., 2004; Demir, 2004; Kaya et al., 2003; Pelletier and Dione, 2004; Sanches-blanco et al., 2004).

Correlation coefficients of traits showed that mycorrhizae colonization with root dry weight (r=0.48**), root volume (r=0.35*) and fresh root weight (r=0.43**) had significant differences (Table 6). In addition, leaf phosphorus (r = 0.62**), root dry weight (r = 0.79**), root length (r=0.44*), root volume (r = 0.82**) and root fresh weight (r=0.74*) had significant differences with above-ground dry matter. These observations indicate that plants having a higher leaf phosphorus, root dry weight and root volume produce higher total dry weight.

Conclusions

Inoculated plants with *G. mosseae* and *G. intraradices* showed more Leaf phosphorus, root fresh and dry weight, root length and volume than control. Root related traits such as root fresh and dry weight, root length and root volume increased in more phosphorus application and consequently will lead to increase above-ground dry matter. Relationships between traits showed that with increasing leaf phosphorus, root dry weight and root volume in inoculated mycorrhizal cucumber plants enhanced above-ground dry matter. Furthermore, since the formation of mycorrhizae often leads to increases in root traits and above-ground dry matter, the effect of mycorrhizae on Leaf phosphorus is also probably partly caused by the enhanced phosphorus nutrition. The overall results show that the use of mycorrhizal fungi is an essential element for the production of strong seedlings of cucumber and reduction in consumption of phosphate fertilizers.

Conflict of interest

The author did not declared any conflict of interests.

REFERENCES

Al-Karaki GN, McMichael B, Zak J (2004). Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263-269.

Allen MF, Smith WK, Moore TS, Christensen M (1981). Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal Bouteloua gracilis H.B.K. Lag ex Steud. New Phytol. 88:683-693.

Balakrishnan N, Subramanian KS (2012). Mycorrhizal symbiosis and bioavailability of micronutrients in maize grain. Maydica 57:129-138.

Burleigh SH, Cavagnaro T, Jakobsen I (2002). Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J. Exp. Bot. 53:1593-1601.

Demir S (2004). Influence of arbuscular mycorrhiza on some physiological, growth parameters of pepper. Turkish J. Biol. 28:85-90.

Giovannetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

Graca MAS, Barlocher F, Gessner MO (2005). Methods to study litter decomposition: A practical guide. Springer-Verlag, Dordrecht, the Netherlands. 329 p.

Graham JH (2000). Assessing cost of arbuscular mycorrhizal symbiosis in agroecosystems. In: Podila GK, Douds DD eds. Current Advances in Mycorrhizae Research pp. 127-140. APS Press, St Paul.

Grant C, Bittman S, Montrea M, Plenchette C, Morel C (2005). Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. Canadian J. Plant Sci. 85:3-14.

Hao ZP, Christie P, Qin L, Wang CX, Li XL (2005). Control of fusarium wilt of cucumber seedlings by inoculation with an arbuscular mycorrhical fungus. J. Plant Nut. 28:1961-1974.

Hayman DS (1983). The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Canadian J. Bot. 61:944-963.

Huang RS, Smith WK, Yost RS (1985). Influence of vesicular-arbuscular mycorrhiza on growth, water relations, and leaf orientation in Leucaena leucocephala Lam. De Wit. New Phytol. 99:229-243

Jennings DH (1995). The Physiology of Fungal Nutrition. Cambridge: Cambridge University Press.

Kiers ET, Adler LS, Grman EL, Heijden MGA (2010). Manipulating the jasmonate response: How do methyl jasmonate additions mediate characteristics of aboveground and below-ground mutualisms? Func. Ecol. 24:434-443.

Marulanda A, Porcel R, Barea M, Azcon R (2007). Drought tolerance and antioxidant activities in laventies in lavender plants colonized by native drought-tolerant or drought-sensitive *Glomus* species. Mic. Ecol. 54:543-552.

Marschner H (1996). Mineral Nutrition of Higher Plants. London: Academic Press.

Marschner H, Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159:89-102.

Miller JC, Jr, Rajapakse S, Garber RK (1986). Vesicular-arbuscular mycorrhizae in vegetable crops. Horti. Sci. 2l:974-984.

Mollier A., Pellerin S (1999). Maize root system growth and development as influenced by phosphorus deficiency. J. Exp. Bot. 50:487-497.

Ocampo JA, Azcon R (1985). Relationship between the concentration of sugars in the roots and VA mycorrhizal infection. Plant and Soil 86:95-100.

Parewa HP, Rakshit A, Rao AM, Sarkar NC, Raha P (2010). Evaluation of maize cultivars for phosphorus use efficiency in an Inceptisol. Int. J. Agri. Environ. Biotech. 3:195-198.

Pelletier S, Dionne J (2004). Inoculation rate of Arbuscular Mycorrhizal Fungi *Glomus intraradices* and *Glomus etunicatum* affects establishment of landscape Turf with no irrigation or fertilizer inputs. Crop Sci. 44:335-338.

Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapidassessment of infection. Trans. British Mycol. Soci. 55:158-161.

Plenets D, Mollier A, Pellerin SI (2000). Growth analysis of maize field crops under phosphorus deficiency. II. Radiation use efficiency, biomass accumulation and yield components. Plant and Soil 224:259-272.

Podila GK, Douds DD (2001). Current Advances in Mycorrhizae Research. APS Press, St. Paul.

Temperini O, Rouphael Y, Parrano L, Biagiola E, Colla G, Mariotti R, Rea E, Rivera CM (2009). Nursery inoculation of pepper with arbuscular mycorrhizal fungi: An effective tool to enhance transplant performance. Acta Horti. 807:591-596.

Rakshit A, Bhadoria PBS, Das DK (2002). An overview of mycorrhizal symbioses. J. Interacademicia 6:570-581.

Rouphael Y, Cardarelli M, Mattia ED, Tulli M, Rea E, Colla G (2010). Enhancement of alkalinity tolerance in two cucumber genotypes inoculated with an arbuscular mycorrhizal biofertilizer containing *Glomus intraradices*. Biol. Fertil. Soils 46:499-509.

Ryan MH, Herwaarden AF, Angus JF, Kirkegaard JA (2005). Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonisation by arbuscular mycorrhizal fungi. Plant and Soil 270:275-286.

Sanchez-Blanco MJ, Ferrandez T, Morales MA, Morte A, Alarcon JJ (2004). Variations in water status, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomusdeserticola* under drought conditions. J. Plant Physiol. 161:675-682.

Smith SE, Smith FA (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular toecosystem scales. Ann. Rev. Plant Biol. 62:227-250.

Subramanian KS, Tenshia V, Jayalakshmi K (2009). Biochemical changes and zinc fractions in arbuscular mycorrhizal fungus (*Glomus intraradices*) inoculated and uninoculated soils under differential zinc fertilization. Appl. Soil Ecol. 43:32-39.

Subramanian KS, Santhanakrishnan P, Balasubramanian P (2006). Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci. Horti. 107:245-253.

Trimble MR, Knowles NR (1995). Influence of vesicular–arbuscular mycorrhizal fungi and phosphorus on growth, carbohydrate partitioning and mineral nutrition of greenhouse cucumber (Cucumus sativus L.) plants during establishment. Canadian J. Plant Sci. 75:239-250.

Valentine AJ, Osborne BA, Mitchell DT (2001). Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. Scien. Horti. 88:177-189.

Vosatka M, Gryndler M (1999).Treatment with culture fractions from *Pseudomonas putida* modifies the development of *Glomus fistulosum* mycorrhiza and the response of potato and maize plants to inoculation. Appl. Soil Ecol. 11:245-251.

Watanabe FS, Olsen SR (1965). Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Soil Sci. Soci. America 29:677-678.

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Full Length Research Paper

Influence of initial glycerol concentration upon bacterial cells adaptability and biodegradation kinetics on a submerged aerated fixed bed reactor using Biocell® (PE05) packing

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The present paper reports an experimental work of the influence of initial substrate concentration on the adaptability of bacterial cells and the biodegradation kinetics of the substrate in a submerged aerobic fixed-film reactor, by studying some physicochemical parameters. The bioreactor used in this study is gotten from the biological aerated filter (BAF), but the used filter media is made of plastic of a large size than that used usually in BAF and with a high specific surface. For this purpose, various synthetic wastewaters were prepared based on a non-toxic substrate, in this case, glycerol, and some salts of nitrogen, phosphorus and some oligo-elements with different initial chemical oxygen demand (COD) of: 330 MgO_2/L (S1), 480 MgO_2/L (S2), 860 MgO_2/L (S3) and 1120 MgO_2/L (S4). Experiments have been carried out at laboratory scale in a cylindrical reactor, made of PVC (height of 1 m and diameter of 0.125 m). The pilot was filled by a Biocell® (PE05) packing fixed between two grids. The analysis of various physicochemical parameters, during biodegradation (COD, dissolved oxygen, pH, turbidity), showed that the performances of the submerged aerobic fixed-film reactor were influenced by the initial substrate concentration. In low concentrations, the adaptability of bacterial cells was easy and relatively quick. Biodegradation kinetics constants reached 0.407, 0.346, 0.341 and 0.232 d^{-1} respectively for synthetic wastewaters S1, S2, S3 and S4. It has been found that the physicochemical parameters could be used for monitoring the adaptability and the biodegradation process of the substrate. Turbidity was revealed as a good indicator for biofilm growth, mainly because of its decrease during the adaptation phase and its increase during the biodegradation phase. In the same time, the pH increased especially during biodegradation phase. In parallel, DO decreased gradually.

Key words: Submerged aerobic fixed-film reactor, adaptability, biodegradation kinetics, packing, synthetic wastewater, chemical oxygen demand (COD).

INTRODUCTION

Submerged aerobic fixed-film reactors are mainly used for carbonaceous, and ammonia removal in the aerobic treatment of urban wastewaters. They are also used in secondary or in tertiary treatment. Today, these

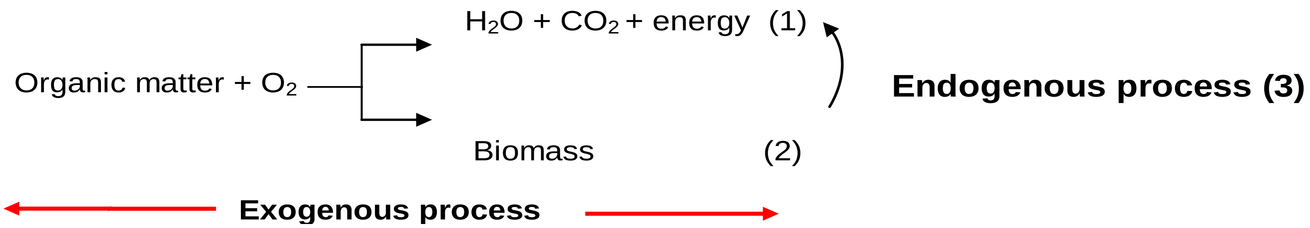

Figure 1. Removal of biological process of organic matter.

bioreactors have several applications in industry (Mendoza-Espinoza et al.,1999; Chaudhary et al., 2003). They combine compactness and high removal efficiencies in a large range of hydraulic and organic load. In secondary treatment, aerobic biological degradation of organic matters in submerged aerobic fixed-film reactor is made in the presence of bacteria according to the reactions in Figure 1.

This degradation involves three reactions in the same time: the first one (Reaction 1) corresponds to an oxidation of organic matter with H_2O and CO_2 as byproducts, it releases also energy, necessary to bacterial cells maintenance and growth; it uses external organic matter (Exogenous process). The second one (Reaction 2) is related to multiplication of bacterial cells using organic matters. The third one (Reaction 3) uses biomass when the dissolved substrate becomes rare (Endogenous process) and bacteria cells use their own reserves. The kinetics of the endogenous process reaction is relatively low as compared to the exogenous processes.

The packing filling the reactor allows the development of biofilm by its colonization over the available surface area, in which bacterial cells adhere to each other. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) allowing the formation of biofilm (Horan, 2003; Harvey et al., 2011).

It is well known that the performances of bioreactors depends on biofilm growth, which could be influenced by several factors, among others: packing characteristics (Mendoza-Espinoza, 1999; Cheremisinoff, 2002; Prenafeta-Boldú et al., 2008), aeration conditions (Kassab et al., 2010; Jin et al., 2012; Albuquerque et al., 2012), substrate concentration and its nature in wastewater (Amrouche et al., 2011; Chen et al., 2012).

The submerged aerobic fixed-film reactor performances could be assessed by the biodegradation yield (Y). It is expressed as follows:

$$Y\ (\%) = \frac{L_0 - L}{L_0} \qquad (1)$$

where: Y, Yield (%) removal of the substrate (in terms of BOD); L_0, Initial organic load of the substrate, expressed in BOD (MgO_2/L); L, final organic load of the substrate at time t, expressed in BOD (MgO_2/L).

The removal performances could also be assessed by calculating biodegradation kinetics constants of organic pollution. In batch reactor, the most significant period in the growth cycle is the exponential growth phase, when the population of biomass is perfectly adapted to the substrate. The first-order model, neglecting endogenous respiration, provides accurate simulations of biodegradation kinetics. It can be written as follows (Cheremisinoff, 2002; Mara, 2003):

$$\frac{dL}{dt} = -k_t \times L \qquad (2)$$

where:
t: time (d); kt: first-order BOD biodegradation kinetics constant expressed as (d^{-1}) depending on the temperature according to the following expression:

$$k_t = k_{t_0} \times \theta^{(T-T_0)} \qquad (3)$$

where: first-order BOD biodegradation kinetics constant at T0, expressed as (d^{-1}); : temperature coefficient, equal to 1.032, this value is in the range of those given by Lesouef et al. (1992) and Queinnec et al. (2006).
T: Temperature of the synthetic wastewater expressed in °C; T_0: Standard temperature at 20°C.

The integration of equation 2, taking into account equation 3, provide the expression of substrate degradation in time:

$$L = L_0 \times 10^{-\left[k_{t_0} \times \theta^{(T-T_0)}.t\right]} \qquad (4)$$

The linearization of this equation allows the determination of the constant using semi-logarithmic coordinates according to Equation 5.

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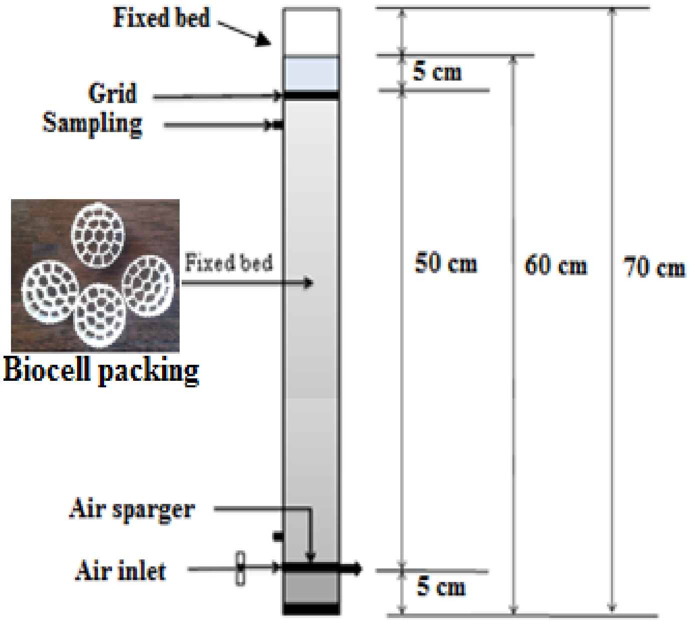

Figure 2. Experimental setup.

$$\log \frac{L}{L_0} = k_{t_0} \theta^{(T-T_0)} \times t \qquad (5)$$

This equation was used in this study. Glycerol substrate used is pure. So the COD will be considered almost equal to the BOD (Mara, 2003).Other physicochemical parameters such as conductivity, pH, dissolved oxygen and turbidity could be linked to the assessment of submerged aerobic fixed-film reactors performances (Mendoza-Espinoza et al., 1999; Akin et al., 2005; Albuquerque et al., 2012) and could give some explanations about the efficiency of this bioreactor against wastewater treatment. This work aimed to study the effect of initial concentration of the synthetic wastewater on the adaptability of bacterial cells on a submerged aerobic fixed-film reactor packing on surface area and the biodegradation kinetics; four tests were made with concentrations: 330 MgO_2/L (S1), 480 MgO_2/L (S2), 860 MgO_2/L (S3) and 1120 MgO_2/L (S4).

MATERIALS AND METHODS

Experimental setup

Experiments were carried out in a reactor operating in batch mode (Figure 2). It was a cylindrical column, with height of 1 m and diameter of 0.125 m, made of opaque PVC. It was filled with 5 L of the synthetic wastewater. The air was introduced at the bottom of the column through an air flow equal to 10 L/h, by a rectangular diffuser (8 x 1 cm).

The bioreactor was filled with Biocell® packing (Figure 2) with excellent physical characteristics (Table 1). It was supported by a grid placed just above the air sparger at the bottom of the column for maintaining the packing fixed. Another grid was used to fix this packing to avoid its flotation by the effect of crossing air bubbles.

Table 1. Physical properties of packing.

Name	Biocell® (PE05)
Color	White
Material	Virgin Polythene
Average particle diameter (mm)	25
Average particle height (mm)	12
Specific surface area (m^2/m^3)	~ 427
Density (kg/m^3)	95
Number of particles (/ m^3)	1.353×10^5
Bed porosity (%)	92.5

Synthetic wastewaters preparation and experiments conduct

Synthetic wastewaters were prepared in COD/N/P ratio of 100/4/1 (EPA, 1997; Mara, 2003) (Table 2), using distilled water. Pure glycerol was the organic substrate. Salts of sodium nitrate (nitrogensource) and potassium phosphate (phosphorus source) and some oligoelements ($FeSO_4$, $MgSO_4$, $MnSO_4$ and $CaSO_4$) were also added to synthetic wastewaters prepared. Doses of oligoelements were low (Mara, 2003; Jin, 2012).

To initiate the adaptation, inoculation by bacterial flora was made in the reactor. This bacterium was extracted from soil (Mara, 2003; Horan, 2003). Table 3 illustrates the physicochemical characteristics of synthetic wastewaters.

Submerged aerobic fixed-film reactor performances monitoring

Chemical oxygen demand (COD) and turbidity were monitored using a photometer type Palintest 7000. pH and dissolved oxygen were measured using a Hach sensor 40d-HQ -Multi parameters, which can also measure the temperatures of water and air. The conductivity was measured by Orion model 125. For measuring the COD concentrations, samples from the bioreactor were filtered through filters having a pore size of 0.45 µm. For the other parameters, measurements were realized directly in samples.

RESULTS AND DISCUSSION

COD and biodegradation kinetics evolution

COD results are presented in Figure 3. According to the figure, the same appearance was shown in COD curves. In fact, it shows three principal phases. The first slow phase correspond to the adaptation of bacteria cells to the substrate and also to the hydraulic batch mode conditions (adaptation phase). The time of this adaptation increased with the initial concentration as notified by some authors (Amrouche et al., 2011; Dey et al., 2010). This time was 1 day for S1 and S2, 2.5 days for S3 and 3.5 days for S4. After this time, the substrate concentration

Table 2. Synthetic wastewaters compositions.

Synthetic wastewaters	S1	S2	S3	S4
	(Mg/L)			
$C_3H_8O_3$	300	400	750	1000
$NaNO_3$	12	16	20	40
KH_2PO_4	3	4	5	10
$ZnSO_4.7H_2O$	0.3	0.4	0.8	1
$FeSO_4.7H_2O$	0.3	0.4	0.8	2
$MnCl_2$	0.3	0.4	0.8	3
$CaSO_4$	0.07	0.1	0.23	0.25
$MgSO_4.7H_2O$	0.03	0.04	0.1	0.1

Table 3. Physicochemical characteristics of synthetic wastewaters.

Parameters of synthetic wastewaters		S1	S2	S3	S4
COD	(MgO_2/L)	330	480	860	1120
Water T°	(°C)	25	25.5	15.1	14.3
Air T°	(°C)	25.3	26.2	14.5	14
Conductivity	(µS/cm)	1185	1320	1584	1955
Turbidity	(NTU)	10	28	28	30
pH	-	7.92	8.2	7.63	8.23
DO	(Mg/L)	-	10.32	10.28	9.35

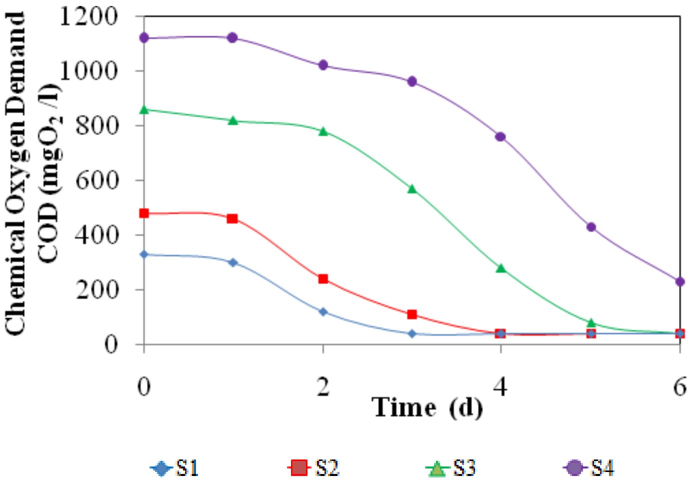

Figure 3. COD evolution versus time.

manifested a slow decrease. The second phase corresponded to the removal of the substrate. This removal depended on initial concentration. In low COD concentration (330 MgO_2/L), the time required was 3 days, moreover, this time was 4, 5 and more than 6 days, respectively for 480, 860 and 1120 MgO_2/L.

The third phase manifested a deceleration of the substrate concentration decrease. The COD tended to stabilize at times, an endogenous respiration has probably triggered after an almost total removal of the substrate (Reaction 3).

The biodegradation kinetics constants could be determined by linearization of the equation 5 and plotting log (L/L_0) against time as shown in Figure 4. Two different slopes of the glycerol biodegradation kinetics for each COD concentration removal have been noted. The first

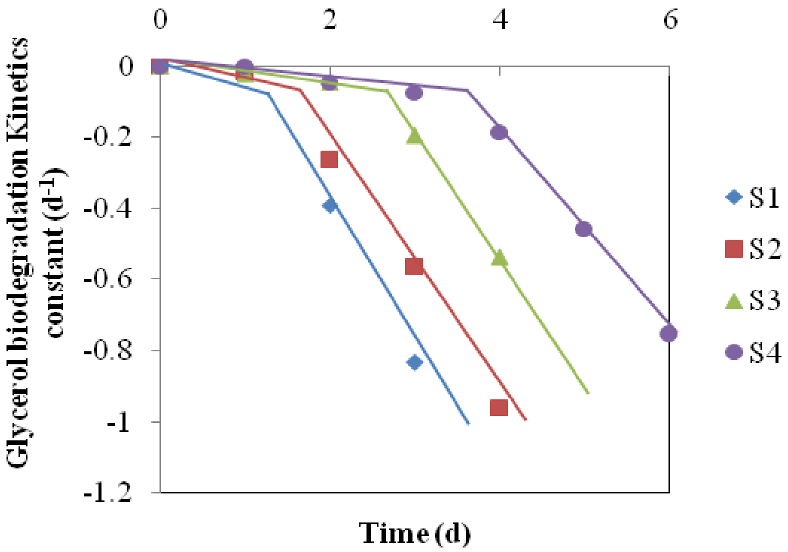

Figure 4. Semi-logarithmic glycerol biodegradation kinetics constants versus time.

Table 4. Biodegradation kinetics constants.

Synthetic wastewater (MgO_2/L)	S1(330)	S2 (480)	S3 (860)	S4 (1120)
Biodegradation kinetics k_t (d^{-1})	0.407	0.346	0.341	0.232

Table 5. Submerged aerobic fixed-film reactor purification yields (%) for S1, S2, S3 and S4.

Synthetic wastewater (MgO_2/L)	S1 (330)	S2 (480)	S3 (860)	S4 (1120)
Purification yield (%)	94.9%	91.7%	91.8%	79.5%

one was slight; it corresponded to the adaptation phase where a little part of substrate was consumed. The second slope was pronounced; it represented the biodegradation phase. The substrate removal was a function of the initial concentration of glycerol. Table 4 shows the values of biodegradation kinetics constants of degradation phase depending on the initial concentration. Biodegradation yields of these tests were illustrated in Table 5.

Both Tables 4 and 5 showed that the adaptability of bacterial cells (quantified by biodegradation kinetics constants and purifications yields) was better at a low initial concentration of glycerol. This adaptability behavior does not depend on the glycerol molecule (not toxic), unlike other tests where it is associated with the toxicity of the substrate when it becomes limited, as found by some authors (Duan, 2011; Marrot, 2006). This result is consistent with the study of Dey et al. (2010). Papadia et al. (2011), also highlighted the influence of the initial concentration of substrate on different biological treatment systems (trickling filter, activated sludge, bioflottation, flow jet aeration) working in continuous hydraulic regime. It has been shown that the increase of the organic load affected adversely the removal rate of the wastewater pollution. Silva et al. (2011) conducted tests to evaluate biodegradation kinetics of winery wastewaters in an aerobic batch, varying substrate concentrations (1, 3, 5 and 7 g/L). The evolution of COD removal efficiency presented a sharp drop of COD concentration at the beginning of the biodegradation curve, better for low concentrations, followed by a gentle drop.

Authors indicated that the adsorption, possibly occurring opposite intermediate adsorption of some compounds before biodegradation, is an important factor that may affect the biodegradation process and lead to exaggerated degradation slopes (Li et al., 2009). Matsuo et al. (2001) indicated that the initial concentration of the substrate influenced the properties of biofilm and its performance of purification. They indicated also that in the presence of heavily loaded waste water, a thick and less dense biofilm is formed; this caused a difficulty in the transfer of nutrient for the development of the bacteria inside the biofilm, unlike weakly charged wastewater.

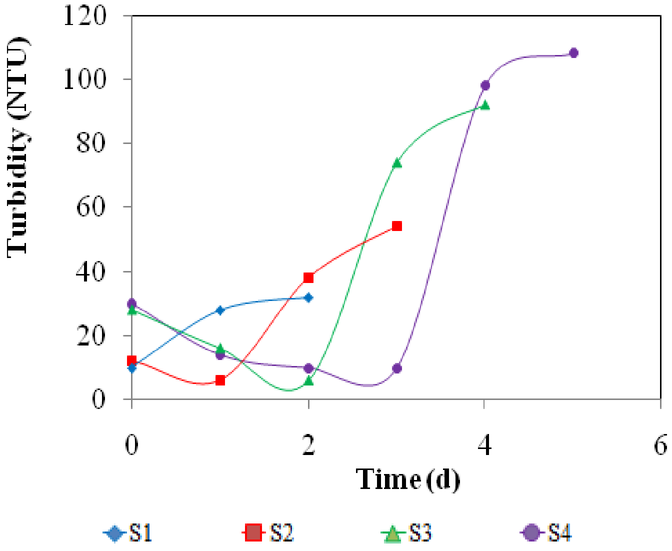

Figure 5. Turbidity evolution versus time.

Turbidity evolution

Turbidity results for the different synthetic solutions are presented in Figure 5. Results illustrated in Figure 4 shows that the turbidity followed the same trend for all initial concentrations tested, except for S1 (330 MgO_2/L).The turbidity decreased at the beginning for S2, S3 and S4, and then, it increased to reach a maximum. But for S1, turbidity increased probably because of the quick adaptation of bacteria as mentioned above, inducing an increasing in biomass formation (Reaction 2).

The different synthetic wastewaters have different initial turbidities, from 10 to 30 NTU. This was due to the quantity of matters, especially from soil, added to each synthetic wastewater, despite the filtration of synthetic wastewaters. On the adaptation phase, turbidity decreased probably by physical interception and the adsorption and flocculation of biofilm due to extracellular polymeric substances (EPS) secreted by bacteria (Zhang and Liu, 2005; Hongyuan et al., 2013).

The increase of turbidity coincided with the beginning of the degradation phase. These two processes occurred at the same time according to the Reactions 1 and 2. This increase may be due to some factors: augmentation of biomass quantity, a part of which can be detached by abrasion of biofilm due to the air flow or by gases (CH4, N2, CO_2, etc.) released by anaerobic/anoxic processes, generally produced when the thickness of biofilm became high. Turbidities reached a maximum, which depended on the initial concentration (Table 6).

Turbidity results confirm clearly those of COD, and allow the same conclusions concerning the behavior of batch bioreactor (Valentis, 1988; Kwok et al., 1998). So, the turbidity was a monitoring parameter which could characterize the stage of the biological process.

pH evolution

pH experimental results are presented in Figure 6. Figure 6 shows, at the beginning, an increase of pH for S1 and S2 tests, a slight decreased for S3 and a constant evolution for S4. After the adaptation phase, a pH increasing trend was observed for all tests. The pH behavior in test S1 could be probably explained by their rapid kinetics. It could be corroborated by COD and turbidity results of S1.

During biodegradation, the pH measured in synthetic wastewaters resulted from an equilibrium between bacterial cells catabolism process (CO_2 formation from Reaction 1) and from the denitrification process (increase of pH), as reported by some researchers (Fabregas, 2004; Akin et al., 2005). It could also be affected by the stripping of CO_2 (Cohen et al., 2004, Morales et al., 2013).

To explain pH behavior, the biofilm detachment caused a degradation of biomass protein. Akin et al. (2005) and Fabregas (2004) showed that this increase could be explained by the denitrification which occurred during anoxic phase in a SBR. The noted decrease of DO as shown above limited the oxygen transfer in the synthetic wastewater and promotes the conditions of the denitrification which occurred in the biological matter. The pH could be used to monitor the biological process. Its evolution depended on turbidity and the aeration of synthetic wastewater.

Dissolved oxygen evolution

DO results are illustrated in Figure 7. It has been clearly oxygen decreased continuously in S2 and S3 tests. Whereas, in S4 test, the observed initial DO was low

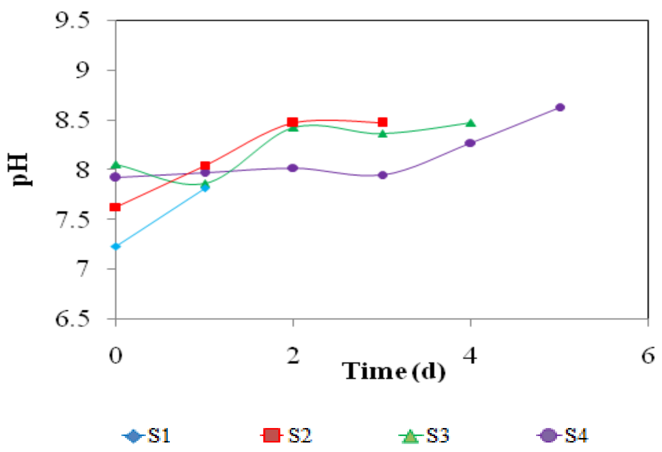

Figure 6. pH evolution versus time.

Table 6. Maximum turbidity versus initial concentration.

Synthetic wastewater (MgO_2/L)	S1 (330)	S2 (480)	S3 (860)	S4 (1120)
Maximum turbidity (NTU)	35	~ 50	~ 90	110

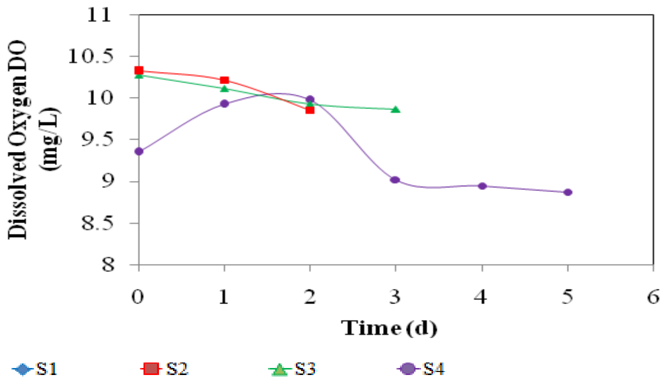

Figure 7. Dissolved oxygen evolution versus time.

able 7. Dissolved oxygen concentration as function of initial concentration of substrate.

Test	Dissolved oxygen concentration
S2 (480 MgO_2/L)	10.32
S3 (860 MgO_2/L)	10.28
S4 (1120 MgO_2/L)	9.35

(Table 7). DO increase before a decrease. This behavior can be explained by the viscosity of S4 synthetic wastewater, due to its high concentration (1120 MgO_2/L), which prevents an efficient oxygen transfer (Stemmet et al., 2008; Kherbeche et al., 2013). But, during the adaptation phase a little part of substrate was consumed, so a DO increase was observed. After that, the DO decreased because of the substrate consumption by bacterial cells during biodegradation phase (Reaction 1).

The oxygen transfer could also be decreased because of the biological suspended matter, as reported by some authors (Rosenberger, 2003; Germain et al., 2007). It controlled both kLa20 and α-factor. Moreover, biological suspended matter accounted for the effects of the viscosity (Garcia-ochoa et al., 2000; Jin et al., 2001; Rosenberger, 2003). On the other hand, the α-factor was affected by the surfactants through EPS secreted by biological matter. Jimenez et al. (2014) showed a strong depression in bubble rise velocity and mass transfer in the presence of surfactants.

When transferring, oxygen must penetrate through the soluble microbial products (SMP) and then diffuse through the flock matrix (EPS) (Jimenez et al., 2014).

However, during biological process, the substrate could be transformed to some bio products which acted as surfactant agents, and affected the oxygen transfer (Garcia-ochoa et al., 2005; Painmanakul, 2005). So, the DO was correlated with the process biodegradation through its diminution during the process.

Conclusions

The main conclusions that could be drawn from this study are:

1. The various stages of the process of biological degradation occurring in a batch reactor are the adaptation, the degradation of the substrate and the endo genous

respiration.

2. The biological kinetics presents two slopes, the first one is slight and corresponds to the adaptation phase, the second one is accentuated, and it corresponds to the biodegradation phase.

3. When the substrate concentration is low, the duration of the adaptation phase is small and the elimination of the substrate is easy.

4. The tests gave excellent results in terms of the abatement rates: 94.2% (S1), 91.7% (S2), 91.8% (S3) and 79.5% (S4) which corroborated with calculated biological kinetics constants (0.407 d^{-1} (S1), 0.346 d^{-1} (S2), 0.341 d^{-1}(S3), and 0.232 d^{-1}(S4)). These results confirmed also the good adaptability at low concentrations of substrate.

5. The turbidity was revealed as a great indicator of bacteria metabolism. Its increase coincided with the beginning of the substrate degradation; several factors were acting in the same time: development of bacteria cells in which, a part was detached by abrasion due to the air flow or by the anaerobic/anoxic process occurring when the thickness of biofilm increased.

6. In the same time, the pH increased when the turbidity increased and dissolved oxygen decreased because of aerobic bacteria metabolism, striping of CO_2 and increasing in viscosity due to some metabolites produced during biological reaction.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Akin B, Ugurlu A (2005). Monitoring and control of biological nutrient removal in a sequencing batch reactor. Process Biochem.40:2873-2878.

Albuquerque A, Makinia J, Pagilla K (2012). Impact of aeration conditions on the removal of low concentrations of nitrogen in a tertiary partially aerated biological filter. Ecol. Eng. 44:44-52.

Chaudhary DSVigneswaran S, Ngo H, Shim WG, Moon H (2003).Biofilter in Water and Wastewater Treatment. Korean J. Chem. Eng., 20:1054-1065.

Chen S, Hu W, Xiao Y, Deng Y, Jia J, Hu M (2012). Degradation of 3-phenoxybenzoic acid by a Bacillus sp. PLoS One.7: e50456.

Cheremisinoff NP (2002). Handbook of Water and Wastewater Treatment Technologies. Elsevier.

Cohen Y, Kirchmann H (2004). Increasing the pH of wastewater to high levels with different gases CO_2 stripping.Water Air Soil Pollut. 159:265-275.

Dey S, Mukherjee S (2010). Performance and kinetic evaluation of phenol biodegradation by mixed microbial culture in a batch reactor. Int. J. water Environ. Eng.. 2:40-49.

Duan Z (2011). Microbial degradation of phenol by activated sludge in a batch reactor. Environ. Prot. Eng. 37:53-63.

EPA (1997).Wastewater Treatment manuals - Primary, secondary and tertiary treatment. Environmental protection agency.

Fabregas TV (2004). SBR Technology for wastewater treatment : suitable operational conditions for a nutrient removal. Thesis.

Garcia-ochoa F, Castro E, Santos V (2000). Oxygen transfer and uptake rates during xanthan gum production. Enzym. Microb. Technol. 27:680-690.

Garcia-ochoa F, Gomez E (2005).Prediction of gas-liquid mass transfer in sparged stirred tank bioreactors. Biotechnol. Bioeng. 92:761-772.

Germain E, Nelles F, Drews A, Pearcec M, Kraumeb M, Reida E (2007). Biomass effects on oxygen transfer in membrane bioreactors. Water Res. 41:1038-1044.

Harvey G, Hasibul H, Dipesh D, Charles M, Hung YT (2011). Biofilm Fixed Film Systems. Water. 3:843-868

Hongyuan L, Wenchao G (2013). Research on biological aerate filter with volcanic filter for pretreatment of micro-polluted source water in lower temperature. Afr. J. Microbiol. Res. 7:4794-4800.

Horan N (2003). Handbook of Water and Wastewater Microbiology. Elsevier.

Jin B, Yu Q, Yan X, Van Leeuwen J (2001). Characterization and imporvement of oxygen transfer in pilot plant external air-lift bioreactor for mycelial biomass production. MicrobBiotechnol. 17:265-272.

Jin Y, Ding D, Feng C, Tong S, Suemura T, Zhang F (2012). Performance of sequencing batch biofilm reactors with different control systems in treating synthetic municipal wastewater.Bioresour. Technol. 104:12-18.

Kassab G, Halalsheh M, Klapwijk A, Fayyad M, Van Lier JB (2010). Sequential anaerobic-aerobic treatment for domestic wastewater - a review. Bioresour. Technol. 1013299. 101:3299-3310.

Kherbeche A, Milnes J, Jimenez M, Dietrich N, Hébrard G, Lekhlif B (2013). Multi-scale analysis of the influence of physicochemical parameters on the hydrodynamic and gas–liquid mass transfer in gas/liquid/solid reactors. Chem. Eng. Sci. 100: 515-528.

Kwok W, Picioreanu C, Ong S, Van Loosdrecht MC, Ng W, Heijnen J (1998). Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. Biotechnol. Bioeng. 58:400-407.

Lesouef A, Payraudeau M, Rogalla F, Kleiber B (1992). Optimizing nitrogen removal reactor configurations by on-site calibration of the IAWPRC activated sludge model. Water Sci. Technol. 25:105-123.

Li WW, Li XD, Zeng KM (2009). Aerobic biodegradation kinetics of tannic acid in activated sludge system. Biochem. Eng. J. 43:142-148.

Mara D (2003).Domestic Wastewater treatment in developing countries.

Marrot B, Barrios-Martinez A, Moulin P, Roche N (2006). Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. Biochem. Eng. J. 30:174-183.

Matsuo T, Hanaki K, Takizawa S, Satoh H (2001). Advances in Water and Wastewater treatment technology - Molecular technology, nutrient removal, sludge reduction and environmental health.

Mendoza-Espinoza L, Stephenson T (1999). A review of biological aerated filters (BAF) for wastewater treatment. Environ Eng Sci. 16:201-216.

Morales N, Boehler MA, Buettner S, Liebi C, Siegrist H (2013). Recovery of N and P from urine by struvite precipitation followed by combined stripping with digester sludge liquid at full scale.Water. 5:1262-1278.

Painmanakul P (2005). Analyse locale du transfert de matière associé à la formation de bulles générées par différents types d'orifices dans différentes phases liquides Newtoniennes : étude expérimentale et modélisation. Thèse de Doctorat.

Papadia S, Rovero G, Fava F, Di Gioia D (2011). Comparison of different pilot scale bioreactors for the treatment of a real wastewater from the textile industry. Int. Biodeterior. Biodegradation. 65:396-403.

Prenafeta-Boldú FX, Illa J, van Groenestijn JW, Flotats X (2008). Influence of synthetic packing materials on the gas dispersion and biodegradation kinetics in fungal air biofilters. Appl. Microbiol. Biotechnol. 79:319-327.

Queinnec I, Ochoa J, VandeWouwer A, Paul E (2006). Development and calibration of a nitrification PDE model based on experimental data issued from biofilter treating drinking water. Biotechnol.Bioeng. 94:209-222.

Silva F, Pirra A, Sousa J, Arroja L, Capela I (2011). Biodegradation Kinetics of winery wastewater from port wine production. Chem. Biochem. Eng. 25:493-499.

Stemmet CP, Bartelds F, Van Der Schaaf J, Kuster BFM, Schouten JC (2008). Influence of liquid viscosity and surface tension on the gas–liquid mass transfer coefficient for solid foam packings in co-current two-phase flow. Chem. Eng. Res. Des. 86:1094-1106.

Valentis G (1988). Epuration par cultures fixées sur support géotextile. Ecole Nationale des ponts et chaussées. Thèse de Doctorat.

Zhang T, Liu X (2005). Effect of bidosicai activated carbon filter on treatment of micro-polluted source water. China Water and Wastewater. 21:39-40.

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Full Length Research Paper

Determination of mechanical characteristics and reaction to fire of "RÔNIER" (*Borassus aethiopum Mart.*) of Togo

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The "rônier" *or Borassus aethiopum Mart* is a wood material which is used as an element for construction and public works in Togo. The goal of this study was to determine its mechanical characteristics and its reaction to fire which are the fundamental parameters of works dimensioning. The analyses and tests of *B. aethiopum* samples enabled to realize that the *B. aethiopum* possesses mechanical features much superior to those of resinous and leafy wood (1.08 times for the pruning to 2.5 times for the axial drive). The resistance to the axial compression is 3.5 times the transversal one. With a strong content in cellulose, the use of the *B. aethiopum* should be avoided at temperature exceeding 180°C. For a temperature of 676°C for 2 min, with a humidity of 8.17%, the sample of *B. aethiopum* lost 78.54% of its weight.

Key words: Togo, *Borassus aethiopum*, mechanical characteristics, reaction to fire.

INTRODUCTION

The Togolese population prefers to use the rônier, currently called "cocker" rather than other woods in the building of houses and lintels (Samah, 1998). The rônier, scientifically called *Borassus aethiopum* is a material that can be found locally. It is the kind of spermaphyte angiosperme of the class of monocotyledons belonging to the family of Arecaceae. It is used in different construction activities, especially in the works of civil engineering. The rônier develops a smooth and grey stipe that, at the adult age, measures 15 to 20 m and provides an aspect of a slightly thickened column from the start and strongly swollen in the middle (Diallo, 1987).

In Asia, it was cultivated for its sugar and other uses, especially the construction (wood, leaves); in this area, though it faces a competition from other material such as bamboo, it derives its prestige from its unrivaled quality of resistance. It is a material that does not rot and stands sea mollusk (Tsyboulsky et al., 1971). In Togo, though data are not available on wooden characteristics from rônier, it is mostly used. Indeed, a test on traditional buildings in Togo, carried out in "cocker", showed that time and bad weather have no impact on their elements and the "cocker"

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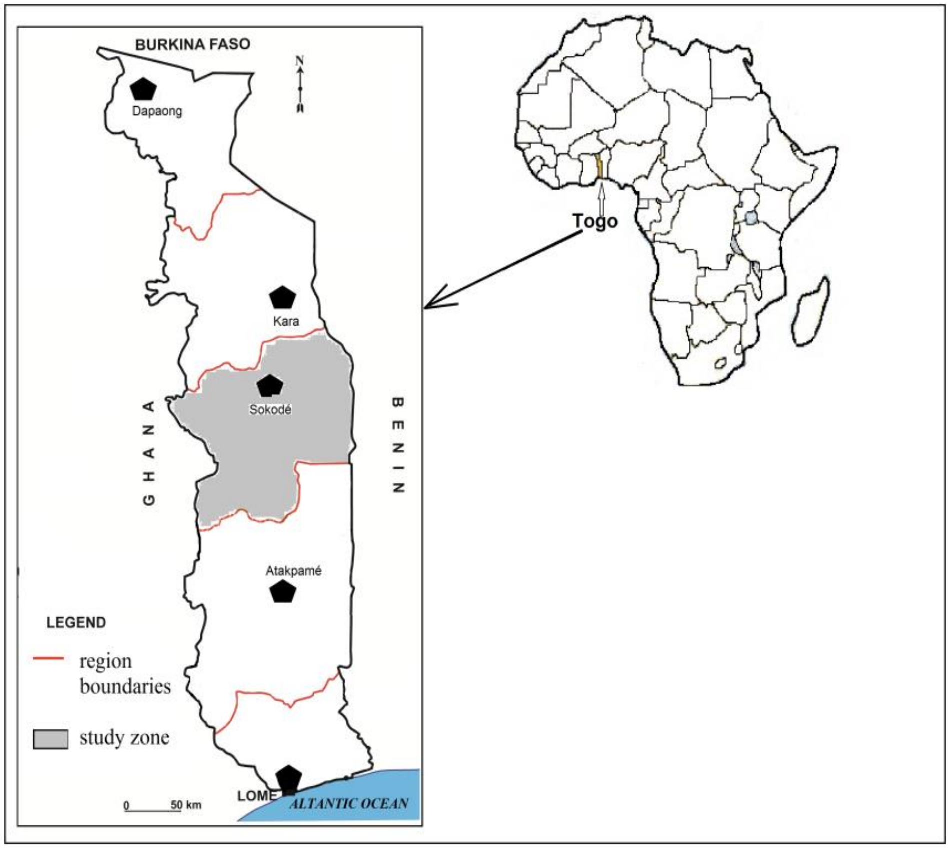

Figure 1. The map of Togo showing the study zone.

left no sign of aging and deterioration by water, humidity, insects and mollusks (Tsyboulsky et al., 1971); but its use is old-fashioned, which can be dangerous for the works if dimensions of parts of works become too weak.

Our study consists in searching the characteristics of this rônier material that is complex and used in the constructions in Togo according to the required direction. We sampled woods of rônier that we tested to determine mechanical features and to fire-proof. The following tests were therefore carried out: Resistance to the compression, traction, flexion and pruning; resistance to fire.

MATERIALS AND METHODS

In order to conduct this study the experimental device consisted of: a universal hydraulic press of the kind UPD-10 with a maximum pressure of 10 000 kPa; a ventilation oven (60 to 200°C), volume 393 L, weight 175 kg, and power 6600 watts; an oven (40 to 180°C); volume 25 L, weight 29 kg, and power 500 watts; an electric scales of the kind Sartorius model A2005F1 with maximum mass of 202 g, precision of 0.0001 g; a Roberval's balance with flaw and maximum mass of 25 kg; a metallic tube that is 165 mm long, 50 mm diameter with a burner tip of 7 mm; a manufactured domestic gas as fuel; a chronometer; wood test tube from rônier, cut according to the type of test; metallic mould and laboratory mixer for constructing concrete test tube.

Method

The rônier used for this study was taken from the central region of Togo as is shown in Figure 1. Due to the texture of rônier wood that is very fibrous, heterogeneous and anisotropic, the samples collected were from stems that were taken from the inferior half of the rônier trunk outside the core of the trunk. The following two series of test were carried out: on one hand, a series on samples in the parallel way to fibers of these latter: axial load; on the other hand, a series on samples but in the perpendicular way to the fibers.

Tests of compression were conducted on cubic samples of 25 mm of ridge with the help of universal hydraulic press of the kind UPD-10. The constraints of axial compression (0°) or perpendicular (90°) $f_{c,0\ or\ 90}$ are determined by the following expression:

$$f_{c,0\,ou\,90} = \frac{N_{c,0,90}}{S_{c,0,90}} \qquad (1)$$

With $N_{c,0,90}$ load crushing axially applied (0°) or perpendicular to fibers in N and $S_{c,0,90}$ surface of crushing of the sample in mm^2

Tests of traction on the samples of section 30 x10 mm and of length 750 mm in the parallel way to the fibers are only made in the parallel way (0) to the fibers. The crushing is carried out on universal hydraulic press of the kind UPD-10. The constraints of axial traction are determined by the following expression:

$$f_{t,0} = \frac{N_{t,0}}{S_{t,0}} \qquad (2)$$

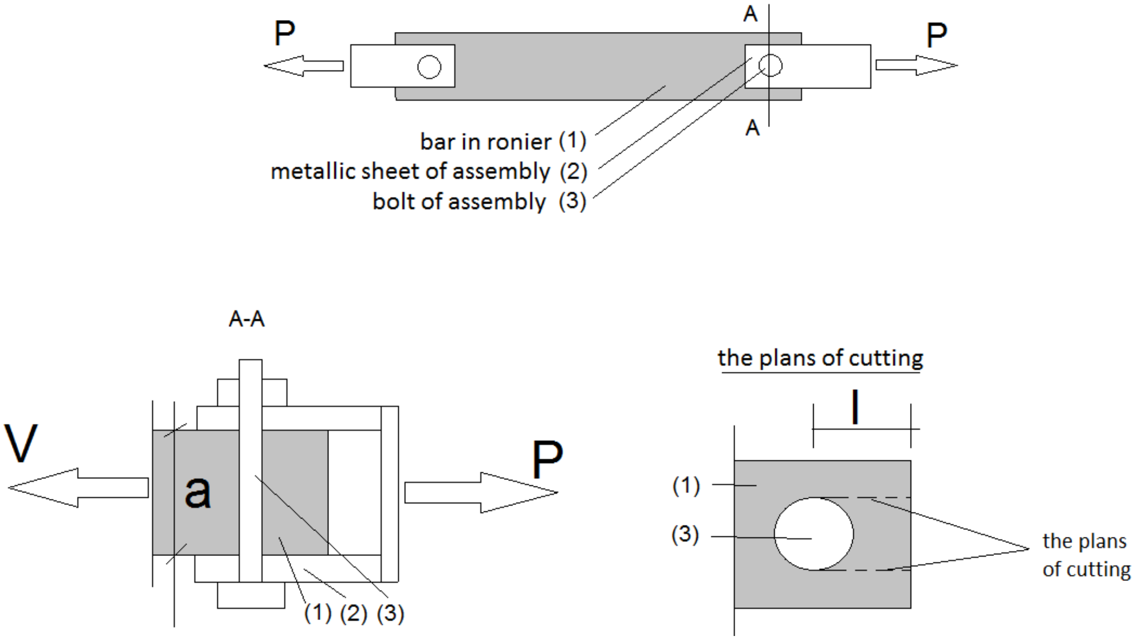

Figure 2. The setting-up device of the cutting test.

Whereby $N_{t,0}$ is load crushing axially applied (0°) to fibers in N and $S_{t,0}$ the surface of crushing of the sample in mm^2.

The universal hydraulic press of the kind UPD-10 enabled to determine the bending strength of the rônier (cocker). The tests are carried out on the sample with dimensions 15 x 30 x 800mm.

The expression of the constraint of bending (f_m) on the extreme fibers (positioned to y=h/2) of the neutral axe of the bar is given by:

$$f_m = \frac{3PL}{2h^2 b} \tag{3}$$

Where, f_m is constraint of bending in MPa, P being the load of crushing applied to half-reach in N, L being the reach of the bar (distance between the two press hold) in mm and h, b the height and the length of the section of the bar in mm.

The test of pruning is made in the axial way. The setting-up device is given in Figure 2.

The expression of the constraint of pruning (f_v) is given as:

$$f_V = \frac{V}{2l.a} \tag{4}$$

With V, the load of pruning in N, a the length of the room and $2l$ the two lengths of the plan of pruning (Figure 2).

The test of the fire-proof is also conducted on the purpose of appreciating the resistance to fire of our material. The sample with a unit of initial humidity of 8.17% is set on fire at a temperature of 676°C for 2 min. Weights before and after the test enabled to determine the burning effect that is observed by appreciating the loss of weight of test tubes.

The appreciation of the resistance to fire of woods is carried out as follows: a material that is far from fire and stops burning is considered as resistant to fire if it loses less than 20% of its weight; a material that is far from fire and burns, but if after 5 min the fire dies out, it is considered as somewhat resistant to fire; a material that is far from fire and burns easily, is considered as non resistant to fire if it loses more than 20% of its weight.

RESULTS AND DISCUSSION

The summary of results on the average values of mechanical tests (traction, compression, flexion and pruning) and the characteristic values of leafy and resinous woods are shown in Figure 2 (Benoit et al., 1995). As for Figures 3 and 4, they give graphic illustration of results.

Figure 3 shows a significant difference between the constraints in axial traction (105 MPa), in axial compression (92.5 MPa) and in perpendicular compression (26 MPa); which confirms the anisotropic aspect of the rônier (Figure 4)

The results also show that the resistances of rônier are very high in the axial direction (axial compression: 92.5 MPa; axial traction: 105 MPa; which confirms the behavior of this material as a fibrous, rigid, and hard resistant material (Table 1). On the other hand the resistance is very weak in the transversal direction (perpendicular compression: 26 MPa); the wood therefore behaves like a plastic deforming material (Benoit et al., 2009). It is also noticed that the resistance to the axial compression is 3.5 times that which is perpendicular to fibers; this ratio is 3.72, according to the works of GBAGUIDI et al. (2010), on the rônier in the Republic of Benin; it is only 2.52 in the highest gap for the case of characteristic constraints of ordinary woods. This very important difference of resistance to the axial compression and to that of the perpendicular compression (3.5 times) is due to the presence of fibers. These fibers would be very rich in cellulose, agent responsible for the resistance of the woods. Indeed, the cellulose is the constituent of the wood that plays the role of support of the mould in pectolignous cement

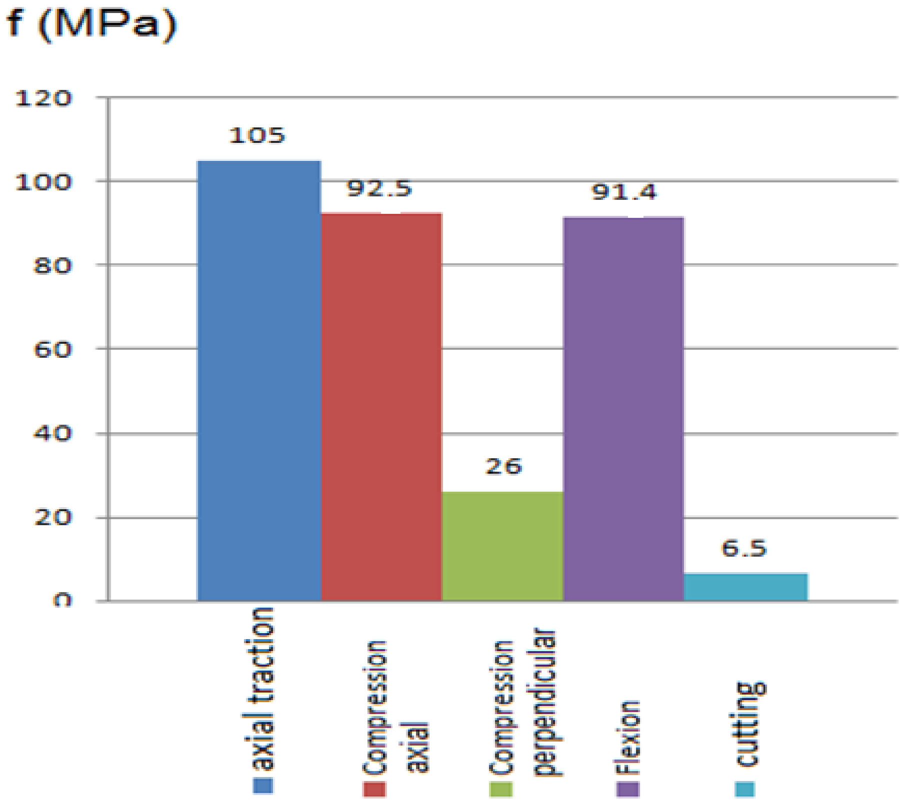

Figure 3. The constraints of breaking up of rônier.

Table 1. Resistances of "rônier" and woods.

Average Values (MPa)	Type of wood	Axial traction (MPa)	Compression (MPa)		Flexion (MPa)	Pruning (MPa)
			Axial	Perpendicular		
Rônier	-	105	92.5	26	91.4	6.5
Resistances 4	Leafy and woods	18 to 42	23 to 34	8 to 13.5	30 to 70	3 to 6
	Resinous woods	8 to 24	2 to 2.9	2 to 2.9	14 to 40	1.7 to 3.8

(Kompella et al., 2002). It is important to note that the cellulose deteriorate at a temperature exceeding 180°C with gas emission (Champetier, 1959).

The constraints of breaking up of the rônier are much higher than those of leafy and resinous woods that are the most resistant (Figure 4); the ratios of resistance of rônier on the wood resistance are: resistance to axial traction: 2.50; resistance to axial compression: 2.75; resistance to perpendicular compression:1.93; resistance to axial traction:1.31; resistance to pruning: 1.08.

According to GBAGUIDI's works (EROCODE 5, 1995), the constraint of breaking up to the traction is 303 MPa, while it is 105 MPa according to the study. This difference would be due to the complex aspect of the rônier wood the features of which are linked to its chemical composition (cellulose, hemicelluloses, lignin, extractics etc.) and the different proportions of component are also based on kinds, climate conditions, the age of the plant etc. (Kollmann et al., 1984)

The fire proof test resulted in a loss of weight of the test tube of 78.54%, which is superior to 20%. This allows concluding that the rônier belongs to the group of ligneous materials that are not resistant to fire. Thus, the rônier will start losing its mechanical characteristics and becomes almost inexistent at the temperature of 676°C. However, the fibres, with their significant resistances, would be resistant to alterations due to insects, mushrooms and microorganisms; which would free them from prior protections (Tsyboulsky et al., 1971)

The very high resistance of the *B. aethiopum* "rônier" gives it the characteristic of a material that is much adapted even better to the construction works of civil

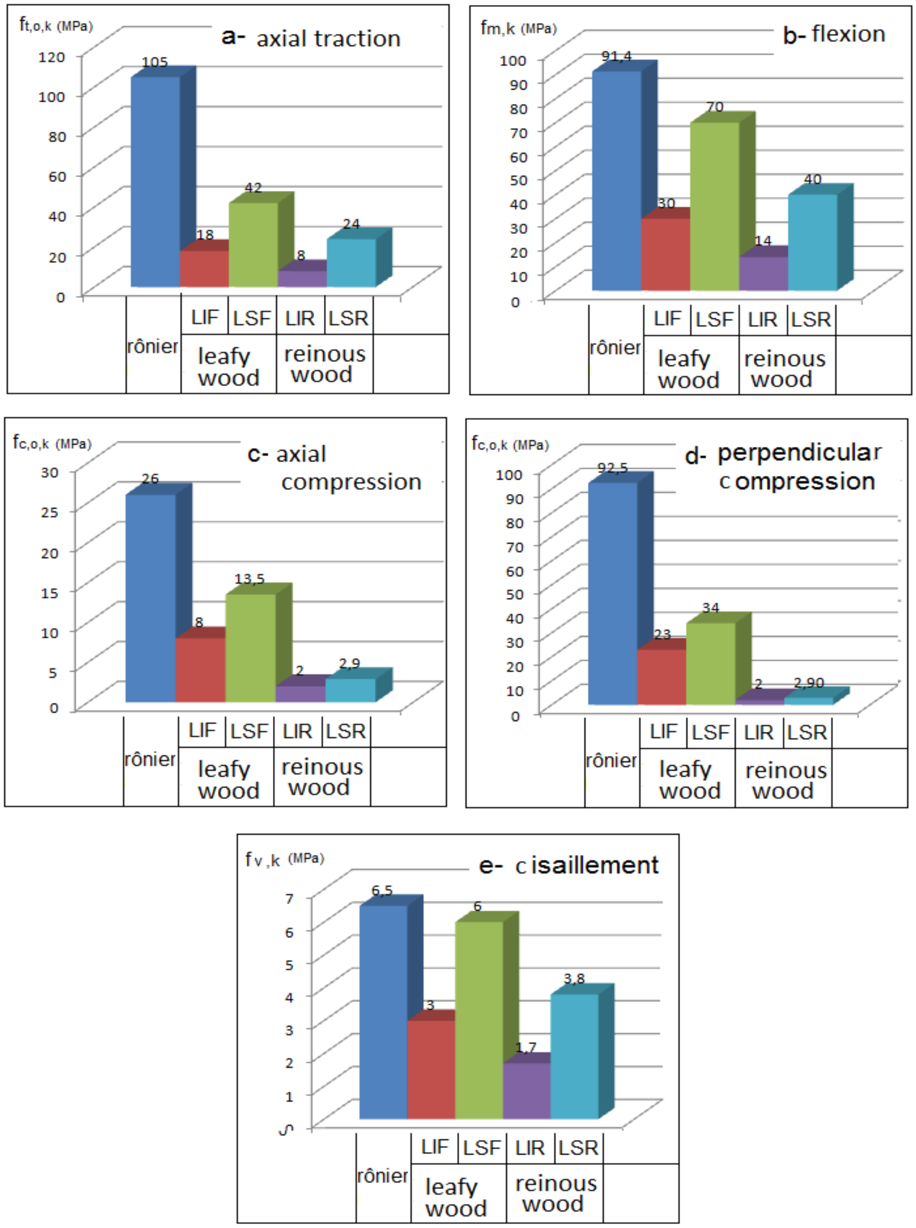

Figure 4. The constraints of the rônier and woods.

engineering for the parts of works in: traction: tie-rods, strained ribs of lattice work truss; compression: posts and compressed ribs of lattice work truss; flexion beams, lintels at the openings in the buildings and trusses with full soul of framework.

Their usage has to be reduced for works that are exposed to temperature higher than 180°C.

Conclusion

This study has identified some mechanical characteristics of *B. Aethiopum* needed for works of civil engineering design studies. The behavior of this material was also tested in the presence of high temperature. It emerged from the analysis that, this material possesses resistance strength of 1.08 times to pruning and 2.5 times to axial drive than to resinous and leafy wood. Its resistance to the axial compression is 3.5 times than that of the transversal one. The use of the material should be avoided at a temperature above 180°C. The use of this material in constructions must be accentuated and encouraged especially for works such as: frames, architraves and columns.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Benoit Y, Legrand B, Tastet V (2009). Calcul des structures bois – guide d'application. 2ème édition. Amendement A1+feu+vent. Edition Eyrolles.
Champetier G. (1959). Les fibres textiles, naturelles, artificielles et synthétique. Collection Armand Collin. Paris. Pp 10-40.
Diallo M (1987). Le comportement du rônier (Borassus Aethiopum) dans les rôneraies paysanes des régions de Fatick et de Thiès, Sénégal. Université de Laval (Québec).
Gbaguidi VS, Gbaguidi-Aisse LG, Gibigaye M, Adjovi E, Sinsin BA, Amadji A (2010). Détermination expérimentale des principales caractéristiques physiques et mécaniques du bois du rônier (borassus aethiopum mart.) d'origine béninoise : Cas des poutres. JRSUL Lomé (Togo). Série E, 12(2):1-9.
Kompella MK, Etlambros J (2002). Micromechanical characterization of cellulose fibers. Polym. Test. 21(5) :523-530.
Kollmann FP, Côté WA Jr (1984). Principles of wood science and technology. Springer Series in Wood Science, I, NY, USA 592p.
Samah OD (1988). Utilisation des matériaux locaux dans la construction des ouvrages du Génie Civil : Le cocker : caractéristiques et utilisation. CCL Cacaveli.

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Perspective

Is climate change human induced?

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Climate is the most vital element of our planet and its liveability is key concern for every habitat. From Silent Spring till present, debate is on whether humankind has impact on nature. Since its establishment in 1988, the Intergovernmental Panel on Climate Change (IPCC) has been playing pivotal role in raising public concerns on human-induced climate change through its various assessment reports. These reports follow exhaustive review process, and are widely accepted. In 2007, IPCC's 4th assessment report- 'Climate Change 2007 – Impacts, Adaptation and Vulnerability' came into question on Himalayan glacier melt. The climate gate in 2009 further strengthened the confusion on credibility of IPCC`s projections. The present study analyses district level temperature and rainfall patterns of Uttarakhand- a Himalayan state, and examines the validity of IPCC's projection. Uttarakhand is a tourism oriented economy. The state is best known for its religious places and natural sites. Rapid urbanisation in mountainous regions is disturbing regional eco-balance, but increasing vehicular pollution in climate-sensitive areas seems to have greater impact on temperature and precipitation patterns. Result shows a noticeable shift in the variability of temperature and rainfall, and a significant warming especially in mountainous districts. However, human activities do not correlate very well with these changes.

Key words: Climate-sensitive sectors, monsoon, climatic variability, polar caps, vehicular pollution.

INTRODUCTION

India is considered highly vulnerable to climate change, not only because of high physical exposure to climate-related disaster, but also because of the dependency of its economy and majority of population on climate-sensitive sectors (for example, agriculture, forests, tourism, animal husbandry and fisheries). More than 40 million hectares of India land (12%) is prone to floods and river erosion; of the 7,516 km long coastline, close to 5,700 km is prone to cyclones and tsunamis; 68% of the cultivable area is vulnerable to drought and hilly areas are at risk of landslides and avalanches (NDMA, 2007). The country has a unique climate system dominated by the monsoon, and the major physiographic features that drive this monsoon are its location in the globe, the Himalayas, the Central Plateau, the Western and Eastern Ghats and the oceans surrounding the region.

The Himalayas influence the climate of the Indian subcontinent by sheltering it from the cold air mass of Central Asia. The range also exerts a major influence on monsoon and rainfall patterns. They prevent frigid and dry arctic winds from blowing south into the subcontinent keeping South Asia much warmer when compared with regions located between corresponding latitudes throughout the globe.

Himalayan glaciers cover about three million hectares or 17% of the mountain area. They form the largest body of ice outside the polar caps and are the source of water for the innumerable rivers that flow across the Indo-Gangetic

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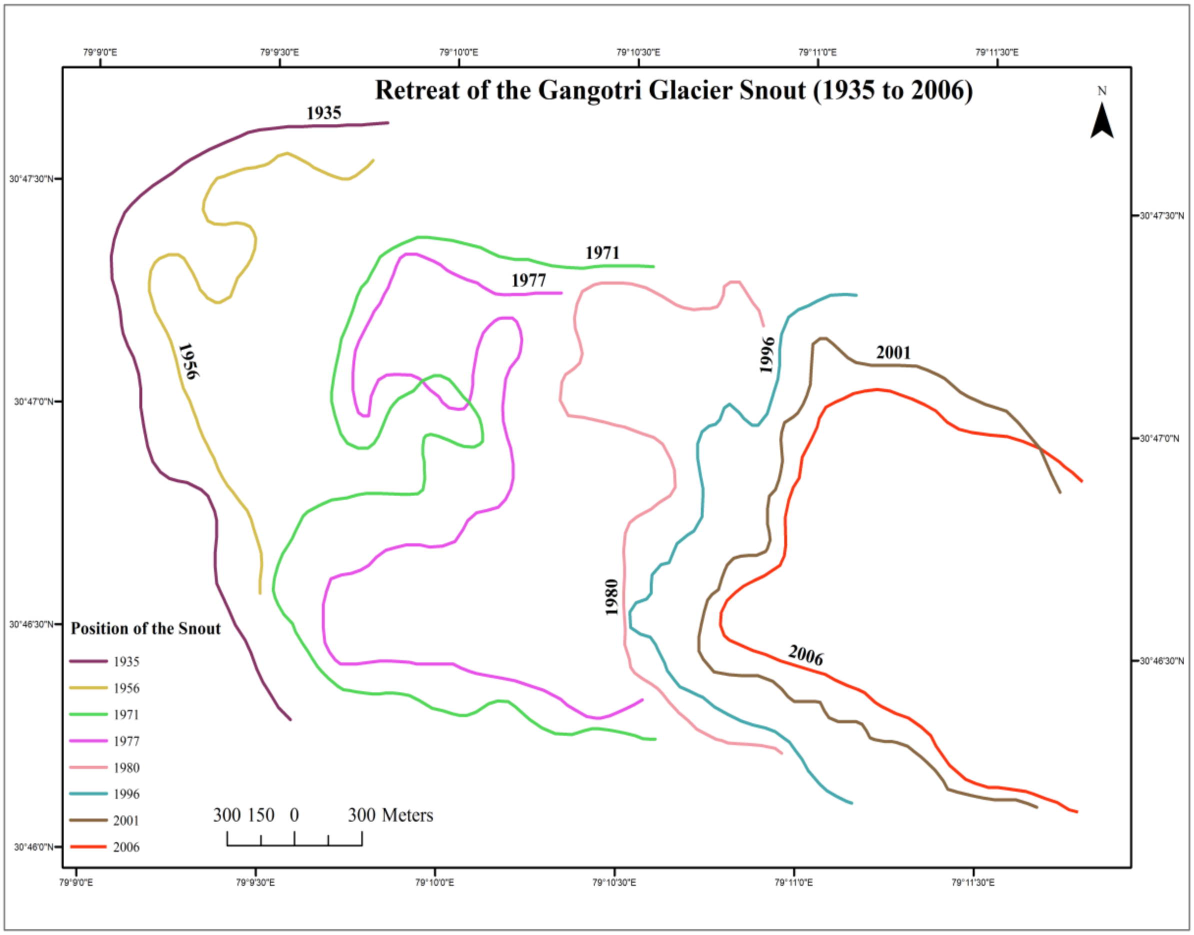

Figure 1. Retreat of the Gangotri glacier snout (1935 to 2006 based on maps made by the Geological Survey of India).

plains. About 15,000 Himalayan glaciers form a unique reservoir which supports perennial rivers such as the Indus, Ganga and Brahmaputra which, in turn, are the lifeline of millions of people in South Asian countries (Pakistan, Nepal, Bhutan, India and Bangladesh). The Gangetic basin alone is home to 500 million people, about 10% of the total human population in the region.

The Himalayan ecosystem is highly vulnerable to the stress caused by increased pressure of population, exploitation of natural resources and other related challenges. Climate change may adversely impact the Himalayan ecosystem through increased temperature, altered precipitation patterns and episodes of drought. According to IPCC`s 4th assessment report "glaciers in the Himalaya are receding faster than in any other part of the world and, if the present rate continues, the likelihood of them disappearing by the year 2035 and perhaps sooner is very high if the Earth keeps warming at the current rate. Its total area will likely shrink from the present 500,000 to 100,000 km^2 by the year 2035" (Cruz et al., 2007 cited in IPCC, 2007).

Syed Iqbal Hasnain, India's well-known Glaciologist, observes that "The Ganga system is about 60 to 70% snow and ice. There are more than 800 glaciers in the Ganga basin. The Gangotri is the big one. It used to cover more than 250 square kilometers, but now it's breaking up in many places. You will see blocks of dead ice that are no longer connected to the main ice body. I'm afraid that if the current trends continue, within 30 or 40 years most of the glaciers will melt out" (Black, 2009).

Contrary to Hasnain`s view, a white paper on the status of Himalayan glaciers and global warming by V.K. Raina, former Deputy Director General of the Geological Survey of India, suggests that "in most cases glaciers have stopped retreating. While the Gangotri glacier stopped receding in the 2007-09 period, glaciers like Pindari in Kumaon continue to record a high annual retreat of almost 10 m annually". He further states that "The glaciers are undergoing natural changes, witnessed periodically" (Raina, 2010). According to assessments made during 1935-2006 by the Geological Survey of India, the Gangotri region has not shown any evidence of major retreat (Figure 1).

A glacier is affected by a range of physical features and a complex interplay of climatic factors. Establishing change in climate of the Himalayan region on the basis of

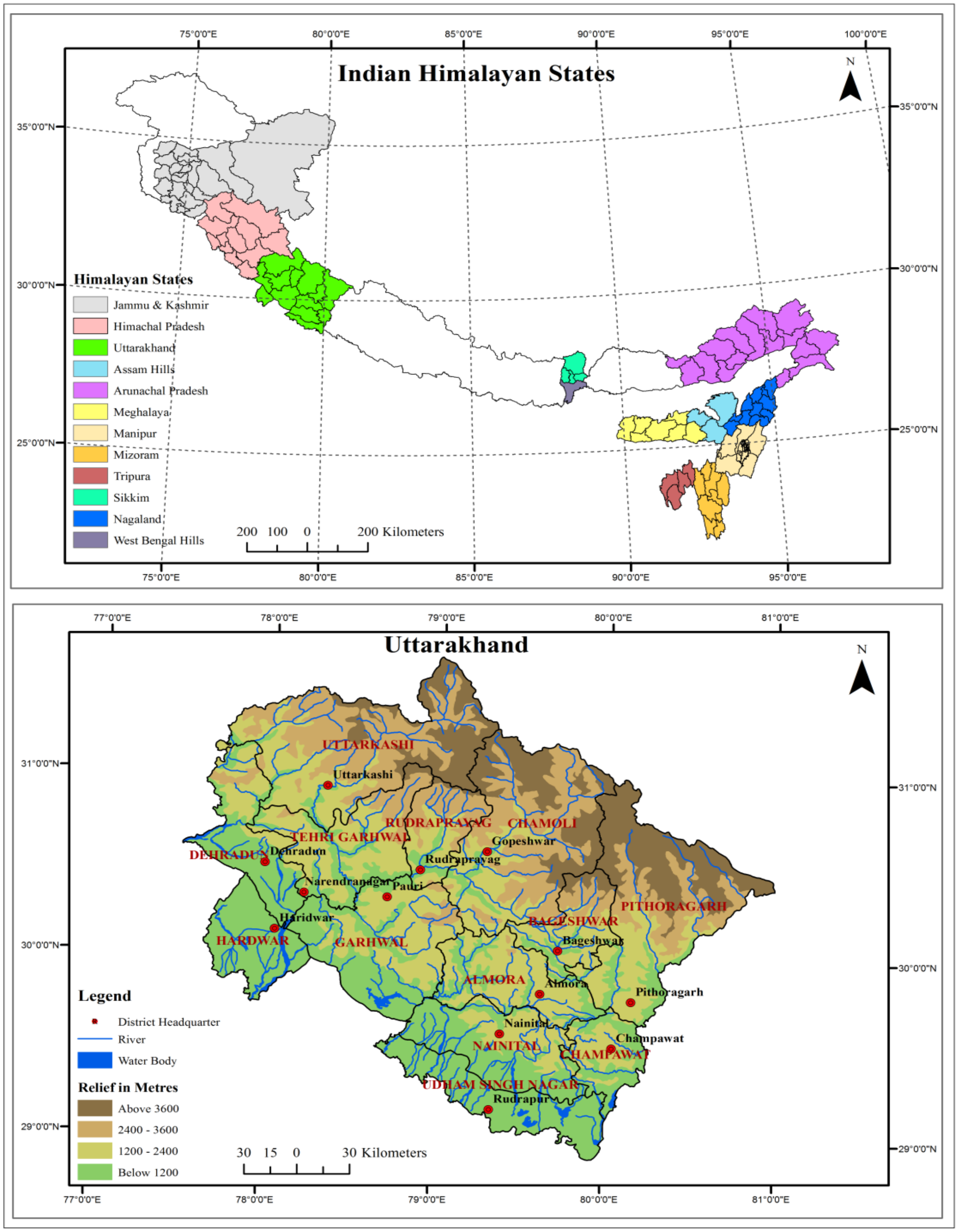

Figure 2. Location map of Uttarakhand.

movement of glaciers, and attributing this change to human activities without analysing the local climate variability, departures and level of human interference does not seems reliable.

The present work is an attempt to identify anthropogenic influence over natural climatic variability of the Himalayan region by considering Uttarakhand as the case of study. The data has been collected from Census of India, India Meteorological Department, Survey of India and Geological Survey of India, and simple correlation and regression techniques have been used for analysis of temperature and rainfall patterns.

Uttarakhand is a part of the Indian Himalayan region (Figure 2). Owing to its immense natural beauty, rich

Table 1. Regression result for temperature and rainfall patterns.

S/N	District	Correlation between temperature and time series (r)	Regression result for temperature series		Correlation between rainfall and time series (r)	Regression result for rainfall series	
			R^2	F		R^2	F
1	Almora	0.266	0.071	7.598**	-0.209	0.044	4.579*
2	Bageshwar	0.299	0.089	9.826**	-0.221	0.049	5.125*
3	Chamoli	0.315	0.099	10.984**	-0.184	0.034	3.515
4	Champawat	0.291	0.085	9.246**	-0.242	0.058	6.211*
5	Dehradun	0.232	0.054	5.694*	-0.035	0.001	0.125
6	Garhwal	0.247	0.061	6.515*	-0.122	0.015	1.503
7	Haridwar	0.221	0.049	5.124*	0.023	0.001	0.051
8	Nainital	0.260	0.068	7.254**	-0.201	0.041	4.229*
9	Pithoragarh	0.342	0.117	13.250**	-0.236	0.056	5.919*
10	Rudraprayag	0.308	0.095	10.514**	-0.153	0.023	2.404
11	Tehri Garhwal	0.263	0.069	7.435**	-0.114	0.013	1.311
12	Udham Singh Nagar	0.260	0.068	7.275**	-0.164	0.027	2.769
13	Uttarkashi	0.308	0.095	10.455**	-0.103	0.011	1.075

**: $p<0.01$, *: $p<0.05$.

biological succession and India's great rivers feeding glaciers- Gangotri, Ponting, Milam, Pindari etc., the region is regarded as Devbhumi- abode of Gods, and Tapobhumi- land of asceticism in Indian scriptures.

The northern region of the state is part of the Great Himalayan Range, covered in snow and glaciers. Two of the Indian subcontinent's most important rivers- the Ganga and the Yamuna- also originate from the glaciers of Uttarakhand. The natural resources of the region provide life supporting, provisioning, regulating and cultural 'eco-system' services to millions of local as well as downstream people. The state lies between the longitudes 77°34′-81°02′E and latitudes 28°43′-31°27′N having a maximum dimension of east-west 310 km and north-south 255 km. It covers an area of 53,484 km^2 with the elevation ranging from 210 to 7817 mt. The state shares border with China (Tibet) in the North and Nepal in the East and inter-state boundaries with Himachal Pradesh in the West, Northwest and Uttar Pradesh in the South. Broadly, the region constitutes of 13 districts falling in two major administrative unit viz., Garhwal (northwest portion) and Kumaon (southeast portion).

The climate of Uttarakhand is temperate, marked by seasonal variations in temperature but also affected by tropical monsoons. January is the coldest month, with daily high temperatures averaging below freezing in the north and near 21°C in the southeast. In the north, July is the hottest month, with temperatures typically rising from 7 to about 21°C daily. In the southeast, May is the warmest month, with daily temperatures normally reaching the highest around 38°C from a low around 27°C. Most of the state's roughly 1,500 mm of annual precipitation is brought by the southwest monsoon, which blows from July through September.

RECENT CLIMATE TREND

Although climate represents a set of factors and determinants showing long term averaged state of the atmosphere over a region but temperature and rainfall are the two most prominent elements among them. The present study examines trends of rainfall and temperature at annual and monthly time scales for the periods of 1911-2012 to understand the climatic variability of the region.

Uttarakhand has two physiographic zones- montane and non-montane. The mountainous regions have recorded more significant warming and declining rainfall trend, while Hardwar, which is almost plain, noticed positive rainfall trend (Table 1). It is evident that the temperature and rainfall departures from centennial average are significantly high in higher altitudes (Figure 3).

Data shows four distinct phases of temperature patterns during the last century- no warming or cooling up to 1950, warming trend during 1950-1080, cooling trend between 1980-2000 and again warming after 2000. On the other hand, the rainfall patterns recorded no significant shift from natural variability at centennial scale (1911-2012) (Figure 4).

Analysis of monthly average temperature data shows very striking results. Colder months- January, February, November and December, have recorded significant warming while the hottest months- June, July, August and September, have shown cooling trend. The study area receives most of its rainfall during the months of July and August but during last century (1911-2011), these months have recorded declining precipitation trend. The months of March and May on the other hand have received more rainfall than normal (Figure 5). Interestingly, the districts of non-montane physiographic

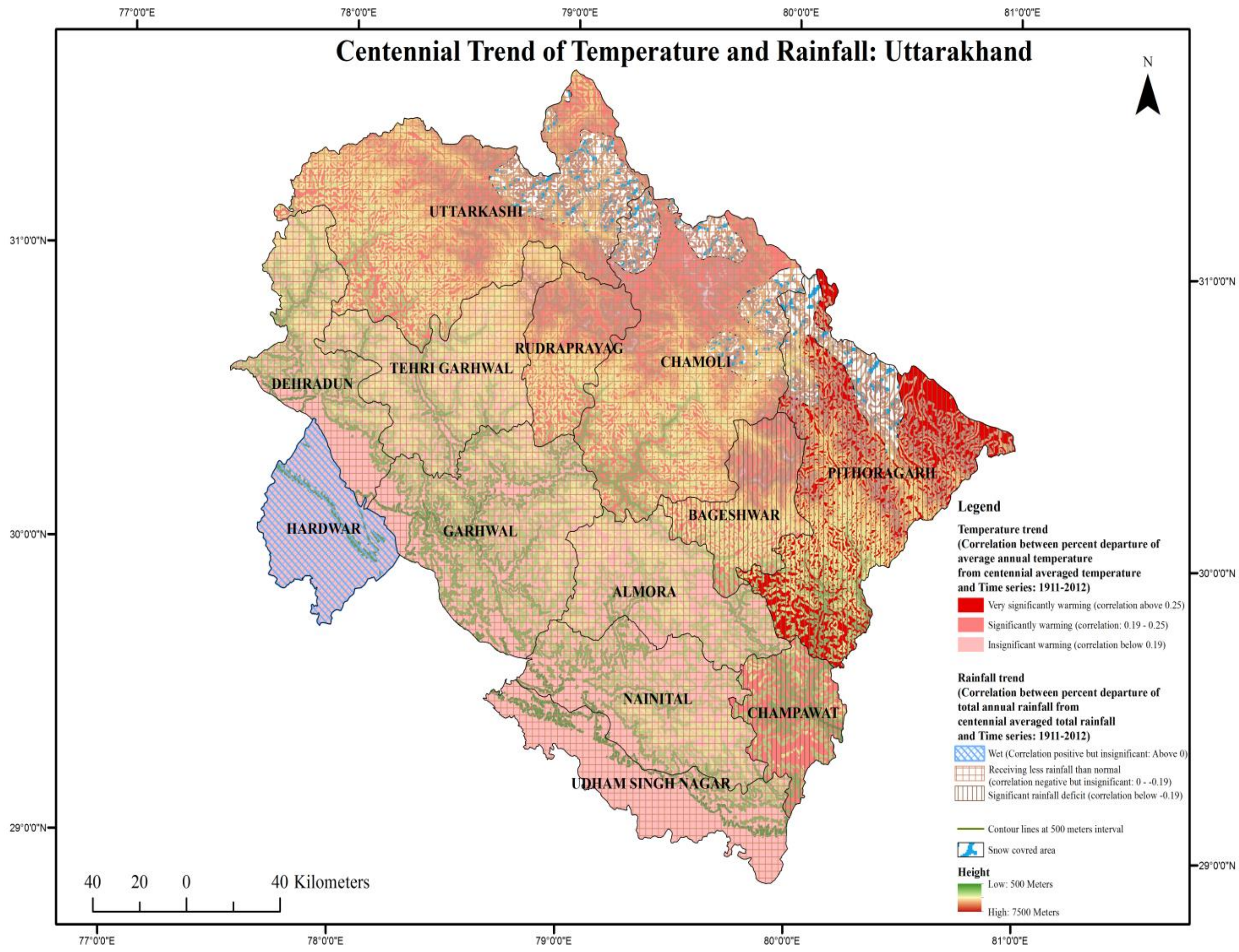

Figure 3. Temperature and rainfall trend of Uttarakhand.

zone have shown lowest departures.

The state has recorded a continuous growth in population during the last century except in 1921-31, however the urban population has grown at faster rate. Most of the population lives in the lower districts where urban share is high (Figure 6).

Hardwar and Dehradun districts record the highest population and percent urbanisation, and have the highest number of census towns (Table 2). These two districts have noticed less warming than others. Contrary to other districts, Hardwar has recorded an increasing rainfall trend although this trend is insignificant. Evidently urbanisation holds no significant association with temperature and rainfall trends.

Heavy forest diversion for basic infrastructures is also being accused for deteriorating local climate's stability. But data suggests that green cover removal is not directly related with the warming. Dehradun and Hardwar which have recorded largest forest diversion are not the warmest districts of the region (Table 3). Forests attract rainfall, but here Hardwar having the noticeable forest diversion, has shown an increasing rainfall trend.

Uttarakhand is famous for religious and adventure tourism. Noticeably the tourist pressure at four major religious centres of the state has been almost doubled during the last twelve years (Table 4).

The three districts – Uttarkashi, Rudraprayag and Chamoli, where these religious centres are located, have recorded very significant warming during past decade. Increasing vehicular pollution seems fuelling temperature rise in these areas. Although growing industrialisation and vehicular density in Dehradun and Hardwar districts have no significant impression on temperature trend on the other hand, it can be said that vehicular pollution is more significantly correlated with temperature patterns in hilly areas while in the plain region, it has less impact on

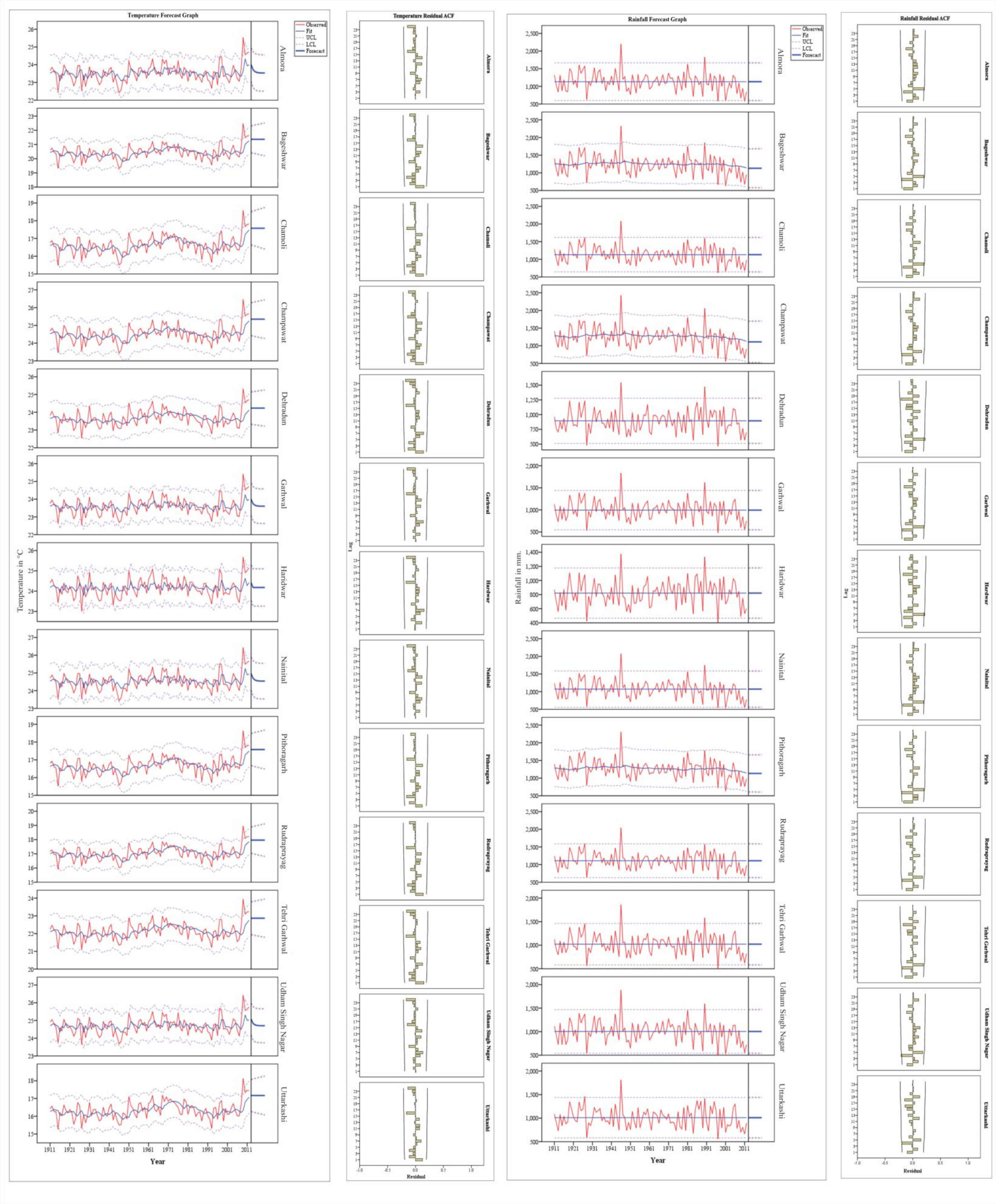

Figure 4. District level temperature and rainfall forecast graph for Uttarakhand.

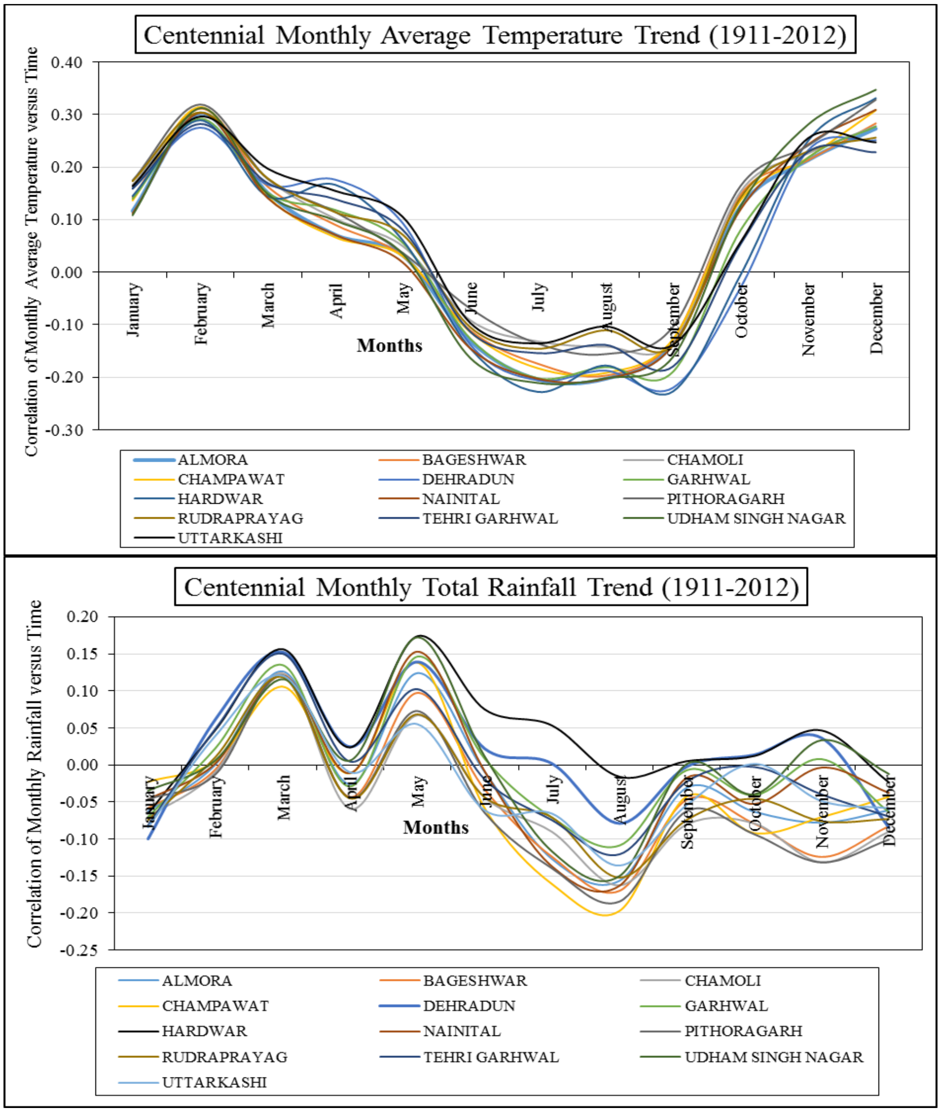

Figure 5. District level centennial monthly temperature and rainfall trend of Uttarakhand (correlation values above 0.19 are significant and above 0.25 are highly significant).

the atmospheric state. In other words, neutralising capacity of plain ecosystem seems greater than mountainous ecosystem.

CONCLUSION

Analysis shows noticeable departures in temperature and rainfall patterns. Months of March and May have recorded more rainfall and significant warming. Temperature of June, July, August and September are at cooler side. Result shows that surface temperatures have risen significantly during the last century, but this may be result of various cooling and warming phases. Besides having significant temperature-time correlations, R^2 values are very weak because of very noisy data. The ARIMA models predicted warming up to 0.3°C till 2035, being maximum for Chamoli district. Results show that this change is almost natural rather than anthropogenic. Warming is unequivocal with decreasing rainfall (except Hardwar), however, temperature and rainfall patterns do not fully support the hypothesis that urbanisation, industrialisation or green cover removal have great bearing on this warming or drying trend. Although increasing vehicular pollution in temperature-sensitive high altitude areas seems to have some impact on these trends, we can say while human interference has fuelled some variations in patterns, natural factors are the major cause behind climatic variability and changes. IPCC's claim that due to human intervention in the Himalayan ecosystem, by 2035 we are going to lose large volume of glaciers, thus, does not seem a real claim.

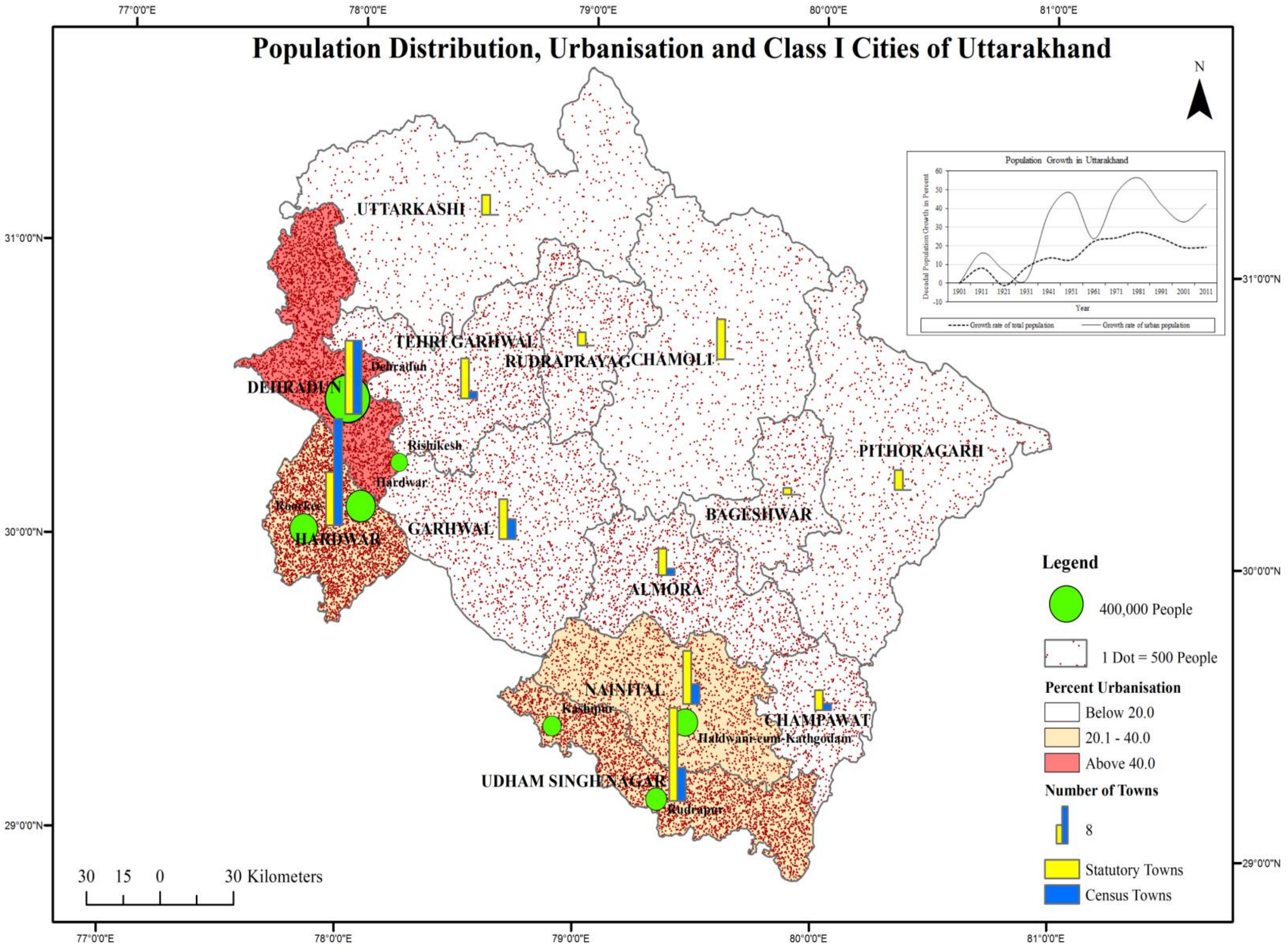

Figure 6. Population distribution in Uttarakhand.

Table 2. Urbanisation in Uttarakhand.

S/N	District	Total population	Percent urbanisation	Statutory towns	Census towns	Village
1	Almora	621927	10.02	4	1	2289
2	Bageshwar	259840	3.5	1	0	947
3	Chamoli	391114	15.11	6	0	1246
4	Champawat	259315	14.79	3	1	717
5	Dehradun	1698560	55.9	11	11	748
6	Garhwal	686527	16.41	6	3	3473
7	Hardwar	1927029	37.77	8	16	612
8	Nainital	955128	38.94	8	3	1141
9	Pithoragarh	485993	14.31	3	0	1675
10	Rudraprayag	236857	4.19	2	0	688
11	Tehri Garhwal	616409	11.37	6	1	1862
12	Udham Singh Nagar	1648367	35.58	14	5	688
13	Uttarkashi	329686	7.35	3	0	707

Source: Census, 2011, GOI and computed.

Table 3. District wise/sector wise details of forest area diverted from 2000 to 2013.

District	Total forest diverted area in hectare	Percent of the total forest diverted area for different purposes						
		Road construction	Managing drinking water	Irrigation	Electricity transmission lines	Hydroelectric power plants	Mining	Other uses
Almora	816.29	88.36	1.7	0.61	2.94	0.01	0	6.39
Bageshwar	559.05	67.73	1.57	0.52	0.99	2.88	20.01	6.3
Chamoli	2097.74	44.85	0.46	0.05	37.45	11.54	0.05	5.59
Champawat	738.57	37.76	0.8	0.28	0.49	0	52.09	8.58
Dehradun	19496.09	2.05	0.05	0.01	0.13	0	8.22	89.54
Garhwal	677.4	43.97	2.95	0.18	24.69	0.43	21.48	6.3
Haridwar	5197.71	1.16	0	0.31	0.16	0	55.79	42.57
Nainital	3165.71	13.71	2.24	0.32	0.61	0	77.14	5.98
Pithoragarh	1667.66	66.42	0.58	0.1	29.6	0.75	0.25	2.3
Rudraprayag	389.56	66.19	1.76	2.36	3.99	16.16	0	9.55
Tehri Garhwal	1591.74	30.35	1.16	0.08	9.88	44.46	0.41	13.67
Udham Singh Nagar	156.23	9.15	0	2.3	3.16	0	0	85.39
Uttarkashi	830.95	52.71	1.1	0.95	13.22	27.59	0	4.44

Source: Uttarakhand forest statistics, Forest Department, Government of Uttarakhand, p. 44.

Table 4. Tourist inflow at selected location in Uttarakhand.

Place	District	Percent increase in tourist Inflow (2001-2012)
Yamunotri	Uttarkashi	240
Gangotri	Uttarkashi	250
Kedarnath	Rudraprayag	378
Badrinath	Chamoli	136

Source: CSE, 2013.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Black G (2009). India Enlightened. On earth, summer edition, pp. 28-29.

Cruz RV, Harasawa H, Lal M, Wu S, Anokhin Y, Punsalmaa, B, Honda Y, Jafari M, Li C, Huu NN (2007). Asia. In ML Parry, OF Canziani, JP Palutikof, PJ Linden, Hanson CE (eds.), Climate Change 2007: Impacts, Adaptation and Vulnerability, Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, UK, pp. 469-506.

NDMA (2007). National Disaster Management Guideline, National Disaster Management Authority, Government of India, p. 1.

Raina VK (2010). Himalayan Glaciers: A State-of-Art Review of Glacial Studies, Glacial Retreat and Climate Change. MOEF Discussion Paper, Ministry of Environment and Forests, Government of India, p. 7.

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Ecotoxicological effects of discharge of Nigerian petroleum refinery oily sludge on biological sentinels

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Ecotoxicological effects of the discharge of Nigerian petroleum refinery oily sludge on biological sentinels were examined. The ecotoxicological effects examined included acute toxicity tests on *Nitrobacter* sp., fresh water shrimp (*Desmoscaris trispinosa)* and brackish water shrimp (*Palaemoneles africanus*) from the aquatic environment. It also covered chronic toxicity tests on microbial nitrogen transformation activity in soil and the growth of the terrestrial fauna, earthworm (*Apporectoda longa*) in pristine soils spiked with predetermined concentrations of the sludge. Analysis of the Nigerian petroleum refinery oily sludge used in this study indicated that the sludge is slightly acidic with a high total petroleum hydrocarbon (TPH) content of 340,000 mg/kg made up mainly between 10-40 carbon unit compounds. The sludge reduced the growth of *Nitrobacter* sp. in aqueous medium and also caused chronic effect on microbial nitrogen transformation activity in soil because it exceeded the 25% inhibition limit for chemicals with the potential to cause chronic effects on soil microbial activities. Similarly, the sludge exhibited toxicity on fresh and brackish shrimp. The freshwater shrimp was however more affected with an LC_{50} of 1097.375 ± 0.62 mg/kg when compared with an LC_{50} of 1590. 37±0.92 mg/kg obtained for the brackish water shrimp. It also reduced the growth rate of the earthworm (*A. longa)* progressively as the sludge concentration increased.

Key words: Toxic effects, petroleum refinery oily sludge, biological sentinels.

INTRODUCTION

Petroleum sludge are oily and viscous residues, which are formed during production, transportation, refining of petroleum and storage. Petroleum refinery oily sludge is composed basically of oil, water and solids (Ururahy et al., 1998). The oil industry is responsible for the generation of high amounts of oily sludge as waste by-product. However, one of the problems faced by the oil industry is the safe disposal of the oily waste generated. It is estimated that approximately 1% of the total oil processed in a refinery is discarded as oily sludge (Ururahy et al., 1998). These oily wastes are expensive to store or destroy and previously contaminated areas have required expensive remediation processes to minimize contaminant dispersion.

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Improper disposal of petroleum refinery oily sludge leads to environmental pollution, particularly soil contamination, and poses a serious threat to ground water. Many of the constituents are carcinogenic and immunotoxicants (Prospt et al., 1999). The polycyclic aromatic hydrocarbons (PAHs) have also been known to impair chemoreceptor functions in aquatic lives and hence lead to extinction of some species. They have also been known to bioaccumulate up the food chain, resulting in cancers and other genetic malfunctioning in man and other higher animals (Atlas and Bartha, 1992).

Several disposal options for petroleum refinery oily sludge disposal include thermal treatment (incineration) (Ururahy et al., 1998), landfills (Mishra et al., 2001) and biotreatment using the following methods: composting, land farming and biopile (Englert et al., 1993). Other conventional biological treatment methods include activated sludge and anaerobic digestion (Hazardous Waste Management, 1997). An interesting alternative to circumvent these problems is the use of bioreactors since optimum process conditions can be easily controlled, allowing higher quality final effluent in shorter times. However, they might have high costs (Oolman et al., 1996).

The Department of Petroleum Resources (DPR), an agency under the Federal Ministry of Environment (FMENV), which regulates activities in both the upstream and downstream petroleum sector, recommends that petroleum refinery oily sludge should be treated and disposed by method that shall not endanger human life and living organisms and cause significant pollution to ground and surface water (DPR, 2002, part iii sec. D 3.6.31, 12). Such approved methods include recycling (resource recovery), incineration, solidification, land farming (bioremediation) and land filling.

Toxicity tests are used to expose test organisms (fish, shrimps, microorganism, earthworms) to a medium– water, sediment, or soil and evaluate the effect of contamination on the survival, growth, reproduction, behavior and or other attributes of these organisms. These tests may help to determine whether the contaminant concentrations in a site's media are high enough to cause adverse effects in organisms. Acute toxicity tests are short term tests that measure the effects of exposure to relatively high concentrations of chemical. Chronic toxicity test, on the other hand, generally are longer– term tests that measure the effects of exposure to relatively lower, less toxic concentrations. Toxicologists have based their selection of test organisms (sentinels) on several factors; sensitivity to variety of substances, availability, representativeness of a variety of ecosystems, and ease of maintenance and culture under laboratory conditions. Sentinels are biological indicators that can help define the ecotoxicological effects of environmental contaminants (USEPA, 2004). In view of the problems encountered in the management of petroleum refinery sludge, this study examined the acute and chronic ecotoxicological effects of Nigerian Petroleum refinery oily sludge on biological sentinels.

MATERIALS AND METHODS

Sample collection

The petroleum refinery oily sludge used for this study was collected from petroleum refinery oily sludge holding tank of Warri Refinery and Petrochemical Company Ltd (WRPC), Warri, Ekpan, Delta State, Nigeria in 2 L glass bottle and preserved at 4°C until required for use. WRPC lies within the coordinates 5° 32' 15''N 5° 41'41''E.

The *Nitrobacter* sp. bacteria used was isolated from Aladja River, Aladja (5° 14'4''N, 60 15' 18''E) Delta State, in southern Nigeria. DSMZ heterotrophic nitrobacter medium was used for the isolation of bacteria. Isolates that were grayish, mucoid, flat, Gram negative, pear shaped and aerobic were selected according to the scheme of Colwell and Zambrushki (1972). Subcultures were made into slants of DSMZ– nitrobacter agar and stored at 4°C until required for use.

The shrimps were collected from the wild at Abua and Bomadi in Rivers and Delta states of Nigeria, respectively. Coordinates for Bomadi, Delta State are 5° 10' 0 N, 5° 56' 0''E. Abua in Rivers State is 10b miles from Port Hacourt with coordinates 5° 18' 30"N, 6° 25' 0''E Transportation of the shrimp to the laboratory was in air-inflated bags with the organisms' habitat water. Physicochemical parameters of the water were determined using standard methods (APHA, 1998).

The earthworm (*Aporrectoda longa)* commonly found in southern Nigeria was collected from a farm at Ubogo, Delta Southern Nigeria. The worms were collected according to the method described by Terhivuo et al. (1994) and Spiegel (2002). They were collected by digging and hand sorting from subsurface litters and taken to the laboratory for identification. They were then washed free of adhering soil particles and left on moist filter paper for voiding. Earthworms were selected based on their maturity (shown by the presence of clitellum) and liveliness (active response when anterior segment is prodded). The physicochemical parameters of the native soil were determined prior to the test.

Acute toxicity of petroleum refinery oily sludge pollution on the bacteria (*Nitrobacter* sp.), brackish water and freshwater shrimps

A range finding test was first conducted using three concentrations after which a definitive test was conducted with five geometric concentrations based on the result of the range finding test. The methods of Duffus (1980), Wang (1984) and APHA (1998) were adapted with some modifications. A fresh dilution and culture was made from the *Nitrobacter* sp. slant. A loopful of the bacteria was collected from the slant and dislodged in 20 ml peptone water and allowed to stand for a few hours at 30°C. The stock culture was prepared by inoculating 180 ml of sterilized peptone water with 20 ml of the activated culture. The test concentrations were prepared using the dilution water from the bacteria's habitat, sterilized at 121°C for 15 min.

Concentrations of sludge used were 5000, 2500, 1250, 625 and 312.5 mg/l. One hundred milliliters (100 ml) of each test concentration was then put into a 250 ml conical flask and sterilized at 121°C for 15 min. On cooling, 10 ml of the cell suspension was added to each flask containing the different sludge concentration and control (sterile dilution water). This was done in triplicate. The flasks were shaken thoroughly to mix and were incubated at 30± 2°C to determine the number of viable cell at 0 (start), 8, 16 and 24 h. 0.1 ml of each test concentration and the control was collected from the test solution and dispersed onto the surface of an already prepared DSMZ nitrobacter agar plate. The plates were then incubated at 30°C for 24 h. The viable cells were counted and recorded. The EC_{50} was determined using the probit method of analysis (Finney, 1978). The following parameters were determined on the test solutions; pH, conductivity, dissolved oxygen, TDS, ammonia, alkalinity and sulphide. The methods used were adapted from APHA (1998).

Acute toxicity of freshwater shrimp (*Desmoscaris trispinosa*) and brackish water shrimp (*Palaemonetes africanus*) exposed to petroleum refinery oily sludge was determined using the method recommended by OECD #218 (2004). The sediment used in the toxicity test was collected with the aid of hand held van Veen type grab. After the collection, the sediment was sieved through a 500 µm mesh using dilution water in order to remove any organisms, which may interfere with the test. The sediment was allowed to settle overnight and the supernatural water decanted. The sediment was then stored in the dark at 4°C until required for the experiment. Approximately 24 h prior to testing, the sediment samples were removed from the refrigerator storage and allowed to equilibrate to room temperature and weighed.

A preliminary range-finding test was conducted prior to the actual test to assist in determining the appropriate dilutions. The range finding test was conducted using a broad concentration range (100, 1000 and 1000 0mg/kg) and the test was terminated in 24 h. In the definitive test, the concentrations selected were based on the mortality values obtained from the range finding and were in appropriate logarithmic dilution series. The 10-day static sediment bioassay was conducted by placing the weighed sediment into triplicate sets of 5 L amber coloured glass tanks. Concentrations of 625, 1250, 2500, 5000 and 10000 mg/kg of sludge were prepared in three replicates and properly homogenized with the sediment and were spread evenly in each tank. The sediment was then overland with 2 L of water from the organism's habitats. The contents of the containers were left to settle for 2 to 3 ho prior to the addition of the test organisms. Shrimps were collected with a 500-µm mesh sieve and placed in dilution water to rinse off any debris. Ten (10) shrimps were gently transferred into each glass test-tank containing test chemical (sludge) and control. The overlaying water was gently aerated for the 10-day's exposure period. Observations for mortality in the test – vessels were made and records taken for the numbers of shrimp which were swimming, crawling on the surface, loss of appendages emergency of organisms from sediment, immobilized (lying on the sediment surface but obviously still alive) or dead. Dead shrimps were removed at each observation. After 10 days, the sediments were sieved and the number of dead shrimps recorded. Average mortality in the bioassay, that is, the total number of organism used on day 0 was used to estimate the average percentage mortality in the bioassay at day 10. The LC_{50} was also determined by the probit method (Finney, 1978). Controls with clean sediment (without sludge) were conducted along with the treated sludge (Environment Canada, 1992). The size of test shrimps used was 0.156±0.03 g and 2.65±0.36 cm in length for the freshwater and brackish water shrimp, respectively. The physicochemical parameters of the test solutions were determined 24 hourly for 96 h.

Chronic toxicity (sublethal) effects of petroleum refinery oily sludge pollution on nitrogen transformation activity in the soil

The OECD TG 216 (2000) test method was used for this test. The effect of the petroleum refinery oily sludge on the nitrifying bacteria, *Nitrobacter* sp. was determined. This test was used to detect long-term (chronic) adverse effects of petroleum refinery oily sludge to the process of nitrogen transformation activity in the soil. Pristine soil was taken from a depth of 0 to 20 cm from a garden in Orhuwhorun town in Delta state, Nigeria and was transported in an ice – chest at 4°C to guarantee the initial soil properties were not significantly altered. In the laboratory soil, samples were kept in the refrigerator at 4± 2°C when they could not be used immediately. The soil was dried, sieved and amended with 5 g/kg compost and treated with five concentrations (375, 6250, 12500, 25000 and 50,000 mg/kg) of petroleum refinery oily sludge or left untreated (control) after day 0, 7, 14 and 28, treated control samples were extracted and analyzed for ammonia, nitrate and *Nitrobacter* sp. counts, the rate of ammonia nitrate formation in treated soil was compared with rate in the controls and the percent deviation of the treated from control was calculated. Enumeration of nitrogen transformation bacteria (*Nitrobacter* sp.) was also done to correlate the microbial growth with the transformed nitrogen. Results from the test of multiple were analyzed using a regression model (ANOVA) and the EC_{50} was calculated. All analyses were done by ASTM method. The control contained only the soil. A geometric series of five concentrations was used. Three replicates for both treatments and control were used.

The test was carried out in the dark at 25± 20°C where 90 ml of sterile distilled water was added to each tank to achieve moisture content of between 40-60%, while 60 ml was added to the 50,000 mg/kg test – tanks to achieve the same percentage of moisture content. The content of the tank was mixed thoroughly and covered with perforated polythene to prevent excessive evaporation of water and volatile fractions. Moisture content of between 40-60% of the maximum water holding capacity was maintained during the test by watering at intervals with distilled water. The duration of the test was 28 days. Composite soil sampling was done on days 7 and 28 and the soils samples were analyzed for some physico-chemical parameters such as pH, total petroleum hydrocarbons, total organic carbon, polyaromatic hydrocarbon nitrate and ammonia. microbiological parameters included enumeration of total heterotrophic bacteria and *Nitrobacter* sp. count. Enumeration of *Nitrobacter* sp. was done with DSMS heterotrophic nitrobacter medium.

The quantity of ammonia and nitrate formed and *Nitrobacter* sp. counts obtained in each replication test soil were recorded, mean values of all replicates were determined and a dose response curve was prepared for the estimation of the effective concentration causing 50% reduction (EC_{50}value). The rate of nitrate formation in treated samples was compared with the rate in the controls, and percent deviation/inhibition of the treated from the control was calculated after 28 days using the formula below (Grunditz and Dalhammar, 2001):

$$\text{Inhibition (\%)} = \frac{C_{ref} - C_{sample}}{C_{ref}} \times 100$$

Where C_{ref} is concentration of nitrate formed in control, C_{sample}

Chronic toxicity effects of petroleum refinery oily sludge on the growth and survival of earthworm (*A. longa*)

Once organisms were obtained, they were identified and maintained in the laboratory using the procedures described in ASTM standard E2172 – 01 (ASTM, 2001). The selected worms were acclimatized for 1-7 days in the soil from the organisms' habitat. During this period, the worms were fed with cellulose. Cellulose was prepared in advance by shredding white, kaolin based paper, followed by converting it to pulp by mixing with distilled water, and subsequently drying at 30°C for 48 h. The weight of the worms was between 300 and 600 mg. Test conditions used were: temperature 20 ± 2°C, light-dark cycles; 16 and 8 h. The test earthworms used for this study were ecologically relevant to the Niger Delta of Nigeria. They possess the following characteristics, red – violet in colour, anterior black segment, a prolobous postonium, 8-14 cm in length, about 149 – 157 segments, a bar-shaped tubercular and alternately paired ternatogential tumescences.

Four concentrations (375, 750, 1500 and 3000 mg/kg) of the petroleum refinery oily sludge were prepared for the definitive test after a preliminary range-finding test was conducted for two days. Five hundred grams (500 g) of soil were mixed with various test concentrations of the sludge and 20 g of cellulose. Cellulose was added to the soil as food for the earthworms. These were manually homogenized and distilled water (80 ml) was added to achieve 45% moisture content in one litre (I L) amber-coloured glass jars. A blank (control) containing only cellulose, water and soil was also prepared. Test tanks were prepared in triplicates per concentration. Prior to use for the test, chosen worm were stored for 24 h on a damp filter paper to void contents of the stomach and intestinal tract. The ten (10) earthworms selected were placed on the surface of the control and test soil samples and were allowed to ingest and burrow into the test medium. The distribution of individual earthworms among the test chambers was randomized. The test medium and control were analyzed for pH, TPH content, total organic carbon (TOC)$_g$ metals (chromium, cadmium, nickel, iron and copper), at the start of the experiment and weekly for 28 day.

Death was the primary criterion used in this test guideline to evaluate the toxicity of the test substance. Earthworm in the test and control chambers were observed weekly for 28 days and the number alive were recorded and the dead removed. In addition to death, weight loss, behavioural symptoms and pathological symptoms were recorded. Each test and control chambers were for dead or affected earthworms and observations recorded on day 7, 14, 21 and 28 days after the beginning of the test. Missing earthworms were considered dead. Mortality was assessed by empting the test medium on a glass or other inert surface and the earthworms were sorted from test mixture and their reactions were tested by a gentle mechanical stimulus. Any adverse effects (eg weight loss, behavioural or pathological symptoms) were noted and reported. The 28 day test result would be unacceptable if more than 20% of control organisms died or the total mean weight of the earthworm in the control containers declined significantly during the test (by 30%). The sublethal effects and growth (fresh weight) data were used to determine the EC50 using the Probit software. ANOVA was used to test for significant differences between treatment means and the control. At the end of the test, worms were removed from each jar, washed, dried, counted and weighed. Observations such as motility, light sensitivity and physical qualities (discolouration), morphology (open wounds) were documented to provide some indication of toxic response. The worms were then depurated for 24 h to void contents of the intestinal tract and subsequently rewashed and reweighed. The worms were analyzed for TPH and metals concentrations.

RESULTS

The physicochemical and microbial qualities of Nigerian petroleum refinery oily sludge shown in Table 1 indicates the sludge was acidic with a pH value of 5.81 ± 0.28 and total petroleum hydrocarbon (TPH) of 340000 ± 50000 mg/kg. Polyaromatic hydrocarbon (PAH) content of the sludge was 0.075 ± 0.02 mg/kg while the value for ammonium was 21.65 ± 1.21 mg/kg. The heterotrophic bacteria and fungi counts were 5.86E + 05 and 4.72E + 05 cfu/g, respectively. Hydrocarbon degrading bacteria and fungi counts were 2.85E + 02 and 2.75E + 02 cfu/g, respectively.

As shown in Table 2, the counts of *Nitrobacter* sp. after 24 h exposure to five concentrations of petroleum refinery oily sludge used dropped from 4.92E + 07 to 2.52E + 06 cfu/ml from the lowest concentration (312.50 mg/l) to the highest concentration (5000 mg/L) of the petroleum refinery oily sludge, respectively. *Nitrobacter* sp. counts obtained in the experimental control were 2.68E + 09 cfu/ml.

As shown in Table 3, the chronic toxicity effects of petroleum refinery oily sludge on nitrogen transformation activities in soils showed that the percentage inhibition of nitrogen transformation in petroleum refinery oily sludge contaminated soils in relation to the control increased with increasing sludge concentration. The increase ranged from 18.7 to 79.38% from the lowest concentration of 3750 mg/kg to the highest of 5000 mg/kg, respectively.

Since in our analysis of metal contents of sludge, zinc recorded a high concentration of 100.62 mg/kg in relation to other metals analyzed, it could have contributed to the observed inhibitory effect of the sludge on the organisms used in this study as observed in similar studies by Wang and Reed (1984). They noted that a high concentration of metal cations inhibits microbial activities by causing damage or inactivating one or more critical enzymes resulting in formation of an inactive complex between the metal cations and an active enzyme. TPH contains toxic compounds such as PAHs and these have also been implicated in the inhibition of nitrification process (Suschka et al., 1996; Dokaniakis et al., 2005)

Chronic toxicity profile of nitrogen transforming bacteria exposed to petroleum refinery oily sludge for 28 days recorded an EC_{50} of 13761.059 mg/kg as show in Table 4. This indicates that at the obtained EC_{50}, there would be 50% inhibition of nitrogen transformation activity in the soil. The recommended limit for non-chronic effect on nitrogen transformation activity in soil is ≤ 25% (ISO/DIs 14248; 1995). This would be a sludge concentration of 502.41 mg/kg. The sludge concentration recorded as the EC_{50} and above would result in serious inhibition of nitrogen transformation activity in soils and subsequently result in soil infertility.

The mean acute toxicity profile of the fresh and brackish water shrimp exposed to varying concentrations of petroleum refinery oily sludge in the sediment shown in Table 5 indicate that there were no death or physiological changes in the negative control for the 10-days test duration. The control shrimps appeared active and healthy (responsive to stimuli) throughout the test period. The test organisms exposed to the various sludge concentration had higher mean percentage mortality by day 10 in the fresh water test (40, 57, 67, 80 and 100%) than in the brackish water test (33, 50 63, 73 and 93%) in 625, 1250, 2500, 5000 and 10,000 mg/kg respectively (Figure 1). These results indicated that mortality increased with increased sludge concentrations and exposure duration. The toxicity profile indicated the estimated mean LC_{50} at day 10 for the fresh water shrimp was 1097.375 ± 0.620 mg/kg while that for brackish water shrimp was 1590.376 ± 0.920 mg/kg. GESAMP rating indicates both LC_{50} to be hazardous (Table 5).

Effect of petroleum refinery oily sludge on organism in the terrestrial environment was also determined with earthworm (*A. longa)* bioassay shown in Table 6.

Table 1. Physicochemical and Microbiological properties of Nigerian Petroleum refinery oily sludge.

Parameter	Mean (± S.E).
pH	5.81± 0.28
Conductivity, us/cm^2	466.65 ± 25.25
Sulphate, mg/kg	4.83 ± 0.64
Nitrate, mg/kg	26.40 ±1.02
Phosphate, mg/kg	7.73 ± 0.88
Total Nitrogen, mg/kg	0.12 ± 0.5
Total Petroleum Hydrocarbon, mg/kg	340,000 ± 50,000
Polyaromatic Hydrocarbon, mg/kg	0.075 ± 0.02
Hydrocarbon Degrading fungi, *cfu/g*	2.85×10^2
Ammonium, mg/ kg	21.65 ± 1.21
Copper, mg/kg	5.53 ±0.20
Chromium, mg/kg	8.68 ± 0.03
Nickel, mg/kg	3.36 ± 0.02
Cadmium, mg/kg	0.32 ± 0.5
Zinc, mg/kg	100.65 ± 2.30
Barium, mg/kg	0.31 ±0.04
Heterotrophic Bacteria, cfu/g	5.86E +05
Heterotrophic Fungi, cfu/g	4.72E+ 05
Hydrocarbon Degrading Bacteria, cfu/g	2.75E + 05

Table 2. Growth of *Nitrobacter Sp* Exposed to Petroleum refinery oily sludge after 24hrs

Exposure time (h)	Control Counts (CFU/ml)	Microbial Counts CFU/ml				
		Concentration of sludge (mg/l)				
		312.50	625	1250	2500	5000
0	2.88E +07	7.40E +05	5.10E + 05	3.60E + 05	2.40E + 05	2.20E + 05
	2.96E + 07	7.22E + 05	5.42E + 05	3.36E + 05	2.62E + 05	1.82E +05
8	6.78E + 08	5.36E + 06	4.88E + 06	2.88E + 06	6.88E + 05	3.96E + 05
	7.26E + 08	6.12E + 06	5.22E + 06	2.24E + 06	7.12E + 05	4.22E + 05
24	2.16E + 09	5.36E + 07	3.64E + 07	1.36E + 07	7.20E + 06	2.40E + 06
	2.68E + 09	4.92E +07	3.12E + 07	1.42E + 07	6.88E + 06	2.52E + 06

Table 3. Percentage (%) inhibition of Nitrate formation in Petroleum refinery oily sludge contaminated soil

Sludge concentration (mg/kg)	% inhibition
3750	18.66
5250	33.04
12500	51.70
25000	59.82
50000	79.38

Earthworms are associated with a healthy soil and their absence is an indication of poor soil health (Doube and Schmidt, 1997; Edwards and Shipitalo, 1998; Parmelee et al., 1998). This chronic effect studies of petroleum refinery oily sludge on earthworms showed that the sludge led to a reduction of growth progressively as the concentration of the sludge increased (Figure 2). The growth rate was inhibited from 86.71 (375 mg/kg) to 37.92 (3000 mg/kg). While 825.03 mg/kg was obtained as the EC_{50} at the end of 28 days (Table 6). This concentration reduced the growth rate of the test earthworms by 50%. In comparison with toxicity ratio of chemicals to earthworms (Davies, 2003), the EC_{50} value indicates the sludge as slightly toxic (Table 6). The values obtained as biocentration factor (BCF) for TPH concentration in the earthworm ranged from 1.22 to 5.17,

Table 4. Chronic Toxicity Profile of Nitrogen Transforming Bacteria Exposed to Petroleum Refinery Oily Sludge for 28 Days.

Days limit	EC_{50} (mg/kg)	Confidence	Probit equation	Slope
7	Could not be determined growth growth inhibition <50%	Could not be determined inhibition <50%	Could not be determined growth inhibition <50%	Could not be determined growth inhibition <50%
14	Could not be determined growth growth inhibition <50%	Could not be determined inhibition <50%	Could not be determined growth inhibition <50%	Could not be determined growthinhibition <50%
21	24627	19317-33858	-0.110 + 1.164X	7.82
28	13761	11796- 17418	-0.881+1.415x	5.005

Table 5. Acute toxicity profile of fresh and brackish water shrimp at day 10 exposure to Petroleum refinery oily sludgein comparison with GESAMP toxicity rating (1997).

Test shrimp	Duration	GESAMP (1997) Toxicity Rating for Damage to Living Resources			
		LC_{50} (mg/kg)	Rating (Days)	Toxicity status	96h LC_5
Fresh water shrimp	**10**	1097.375 ± 5 0.62	Very highly toxic	5	<0.10
Brackish water shrimp	**10**	1590.376 ± 0.92	Highly toxic	4	0.10-1.0
			Moderately toxic	3	1.0-10
			Slightly toxic	2	10-100
			Practically non-toxic	1	100-1000
			Non-hazardous	0	>1000

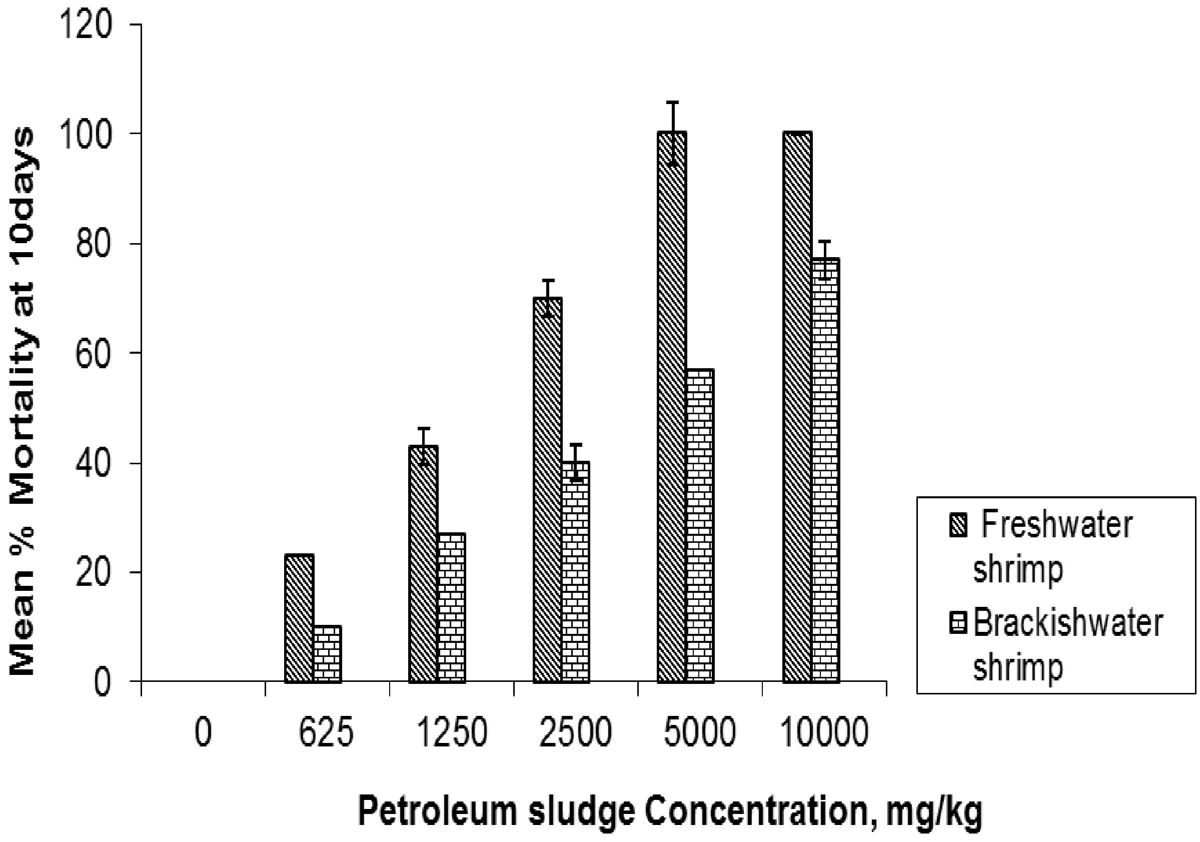

Figure 1. Mean percentage (%) mortality of shrimp exposed to petroleum refinery oily sludge after 10 days.

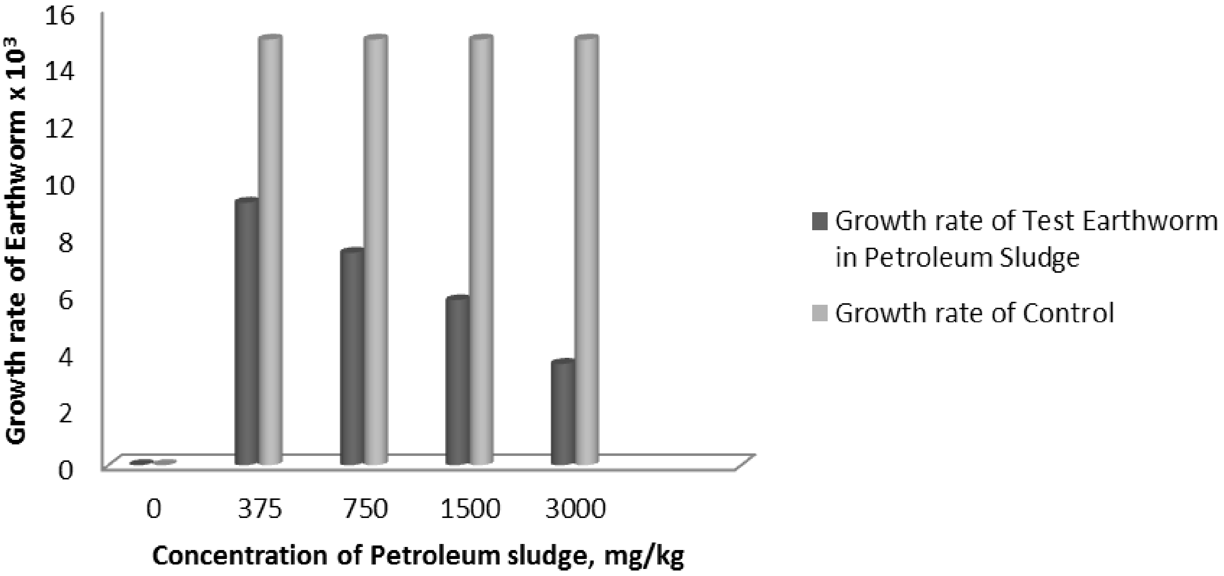

Figure 2. Growth rate of earthworm in petroleum refinery oily sludge.

Table 6. Chronic toxicity profile of Petroleum refinery oily sludge on the growth rate *of Apporectoda longa.*

Test sample	Time (days)	EC_{50}, mg/kg	Earthworm' Toxicity		
			Rating	Designation	EC50 (mg/kg)
Petroleum Sludge	7	Cannot be determined not up to 50% deaths	1	Super toxic	< 1.0
Petroleum Sludge	14	Cannot be determined not up to 50% deaths	2	Extremely toxic	1.0-10
Petroleum Sludge	21	1655	3	Very toxic	10-100
Petroleum Sludge	28	825.04	4	Slightly toxic	100-1000

Table 7. Bioconcentration factor of TPH in Earthworm exposed to Petroleum refinery oily sludge for 28 days

TPH concentration (mg/kg)	TPH concentration in Earthworm	BCF
375	1938	5.168
750	2052	2.738
1500	2660	1.773
3000	3651	1.2173

from the lowest to highest sludge concentration (Table 7). This indicates that the sludge would be bioaccumulated into the tissues of terrestrial organism as sludge concentration increases.

DISCUSSION

The discharge of untreated petroleum refinery oily sludge into the environment has been shown to have acute and chronic effects on the biotic and abiotic components of the aquatic and terrestrial environments (Wang and Reed, 1984; Ma and Ortolano, 2002; Tang et al., 2012). The acute effect of petroleum refinery oily sludge pollution on *Nitrobacter* sp. in the aquatic environment was conducted since the nitrification process is a function of enzyme activity and its measurement has been used as an indicator of pollution (Williamson and Johnson, 1981; Wang and Reed, 1984). The decline in the as an indicator of pollution (Williamson and Johnson, 1981; Wang and Reed, 1984). The decline in the *Nitrobacter* sp. counts as the concentration of petroleum refinery oily sludge increased could be due to the toxic effect of sludge resulting from sludge concentration as earlier reported by Okpokwasili and Odokuma (1997).

The genus *Nitrobacter* belongs to a variety of nitrate-oxidizing bacteria which are responsible for the second step of the nitrification process (oxidation of nitrite to nitrate). This bacterium was used for the chronic toxicity test of petroleum refinery oily sludge on nitrogen transformation activities in soil. This second step of nitrification is particularly sensitive. Inhibition of this step under uncontrolled conditions may lead to accumulation of nitrite nitrogen which is toxic (Dokaniakis et al., 2005).

As stipulated in the test guideline, OECD TG 216 (2000), since the difference between the lowest and highest percentage inhibition is greater than 25%, the sludge has the potential to inhibit nitrogen transformation. The observed increase in inhibition of transformed nitrogen as the concentration of petroleum refinery oily sludge increased could be due to the increase of some physicochemical properties of sludge such as total petroleum hydrocarbon (TPH) and metals (Wilde et al., 1983; Okpokwasili and Odukuma, 1997). Wang and Reed (1984) noted that a high concentration of metal cations inhibits microbial activities by causing damage or inactivating one or more critical enzymes resulting in formation of an inactive complex between the metal cations and an active enzyme. TPH contains toxic compounds such as PAHs and these have also been implicated in the inhibition of nitrification process (Suschka et al., 1996; Dokaniakis et al., 2005).

The obtained LC_{50} values for the acute toxicity test of the test sample on the shrimp indicated that the freshwater test organisms were more adversely affected by the sludge than the brackish water shrimps. Buikema et al. (1982) observed that the higher the LC_{50} value, the lower the toxicity or sensitivity of the test organism and vice versa. The difference in the response of the fresh and brackish water shrimps may be attributed to the osmoregulatory demand of the different environments.

The reduction of growth at higher concentration for the chronic toxicity test of petroleum refinery oily sludge on earthworms showed it reduced growth progressively as the sludge concentration increased and could eventually lead to death. The mechanism of toxicity of hydrocarbon to earthworms was observed to be based on the ability of hydrocarbons to bind to the Polar Regions in biogeneous membrane and to disorganize them (Krab et al., 2000). MacGeer et al. (2003) recorded an inverse relationship between BCF and exposure concentration of the test chemical on earthworms and attributed this to the lipophilic nature of the sludge.

Conclusion

In conclusion, the findings from this research indicate that petroleum refinery oily sludge if not properly treated before disposal into the recipient environment could pose serious threat to the physiological and reproductive functions as well as survival of aquatic and terrestrial organisms. Also, xenobiotic compounds such as PAHs that are bioaccumulated in tissues of aquatic or terrestrial organisms could move up the food chain in higher organisms such as man and lead to conditions such as cancer, infertility among others.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

American Public Health Association (APHA) (1998). Standard, Methods for the Examination of Water and Waste Water 20th Edition Published by American Public Health Association, Washington DC. 1220p.

American Society for Testing and Materials (ASTM). Standard E 2172-01 (2001). Standard guide for conducting laboratory soil toxicity with nematode *Caenorhabditiselegans*. Annual Book of ASTM Standard 11: 1606-1616.

Atlas RM, Bartha R (1992). Hydrocarbon biodegradation and oil spill bioremediation; in KC Marshall (Ed). Advances in Microbial Ecology. New York. Plenum Pres. Vol. 12, pp. 287-338.

Buikema AL, Nederlehner BR, Cairn J (1982). Biological Monitoring Part IV – Toxicity Testing. Water Resources 16:239-262.

Colwell RR, Zambruski MS (1972). Methods of Aquatic Microbiology University Park Press, Baltimore. 461p.

Davies NA, Hodson ME, Black S (2003). The influence of time on lead toxicity and bioaccumulation determined by the OECD earthworm toxicity test. Environ. Pollut. 121(1):55-61.

Department of Petroleum Resources (DPR) (2002). Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN*)* 314p.

Dokaniakis SN, Kornaros M, Lyberatos C (2005). On the effect of xenobiotic bacterial nitrite oxidation. Proceedings of the 9th International Conference on Environmental Sciences and Technology Rhodes Island, Greece.

Doube BM, Schmidt O (1997). Can the abundance or activity of soil macrofauna be used to indicate the biological health of soils? In: Biological Indicators of Soil Health. Eds. C. Pankhurst, B Doube and V. Gupta. CAB International New York. pp. 265-295.

Duffus JH (1980). Environmental Toxicology (Resource and Environmental Science Series). Arnold Publishers Ltd. 26p.

Edwards WM, Shipitalo MJ (1998). Consequences of earthworms in agricultural soils; Aggregation and porosity. In Earthworm Ecology (Eds C.A. Edwards) St. Lucie Press Boca Raton, Florida. pp. 147-161.

Englert CJ, Kenzie EJ, Dragun J (1993) . Bioremediation of petroleum products in soil, Principles & Practice of Petroleum Contamination, in soils. API, 40:111-129.

Environment Canada (1992). Biological Test Method; Acute test for sediment toxicity using fresh and brackish or estuarine amplipods. Conservation and protection, Ottawa, Ontario Report EPSI /RM/26.

Finney DJ (1978). Probit Analysis*,* Cambridge University Press, London. 333p.

Grunditz C, Dalhammar G (2001). Development of nitrification inhibition assays using pure cultures of *Nitrosomonas and Nitrobacter.* Water Res*.* 35:433-440.

Hazardous Waste Management System (1997). Environmental Protection Agency 62:29-32.

Krab J, Mathes K, Breckling B, Lonker O, Schulz – Berend V (2000). Scale – up biologischer Reinigungsvertahren – projeckt – Abachluberícht, bmb .F; Bremen.

Ma X, Ortolano L (2002). Environmental regulation in China, Rowman and little field. Publishers, Inc . 209p.

MacGeer JC, Brix KV, Skeaff JM, Deforest DK, Brigham SI (2003). Inverse relationship between bioconcentration factor and exposure concentration for metals, implication for hazard assessment of metals in the aquatic environment. Environ. Toxicol. Chem*.* 22:1017-1037.

Mishra S, Jyot J, Kuhad RC, Lal B (2001). Evaluation of inoculums addition to stimulate in – situ bioremediation of oily – sludge contaminated soil. Appl. Environ. Microbiol. 67:1675-1681.

Okpokwasili GC, Odokuma LO (1997). Response of Nitrobacter to oilfield dispersants and domestic detergents. Trop. Freshw. Biol. 6:65-74.

Oolman T, Baker RR, Rentro NL, Marshall GE (1996). Refinery Uses Bioslurry Process to Treat RCRA Wasters. Hydrocarb. Process. 71(76):18-20.

Organization for economic co-operation and Development (OECD) (2002). Guidelines for testing of chemical TG 216. C. 21 Soil microorganisms; Test Nitrogen Transform.

Organization for economic cooperation and Development (OECI) (2004) Guidelines for the Testing of Chemicals No 218; Sediment – water chironomid toxicity test using spiked sediment (Adopted April 2004).

Prospt TL, Lochmiller RI, Quals CW, Mcbee K (1999). *In-situ* (mesocosm). Assessment of immunotoxicity risks to small mammals inhabiting petrochemical waste sites. Chemosphere 38:1049-1062.

Spiegel H (2002). Trace element accumulation is selected bioindicators exposed to emission along the industrial facilities of Danube, lowland. Turk. J. Chem. 26:815-823.

Suschka J, Mrowies B, Kuszmider G (1996). Respose of Nitrobacter toxicity of oil field dispersants and domestic detergents. Trop. Freshw. Biol*.* 6:65-74.

Tang J, Lu X, Sun Q, Zhu W (2012). Aging effect of Petroleum Hydrocarbon in Soil under different Attenuation Conditions. Agric. Ecosyst. Environ*.* 149:109-117.

Terhivuo J, Pankakoski E, Hyvarinen H, Koivisto K (1994). PB uptake by ecologically dissimilar earthworms (Lumbricidae) species near a lead smelter in Southern Finland. Environ. Pollut*.* 85:87-96*.*

United State Environmental Protection Agency (USEPA) (2004). United States Environmental Protection Agency, understanding oil spills and oil spill response. htt:// www.fundyforum.com/profile archives/ profile 3.html.

Ururahy AFP, Marins MDM, Vital RL, Therezinha I, Pereira N (1998). Effect of Aeration on Biodegradation of Petroleum Wastes. Rev. Microbiol. 29:4.

Wang W (1984) Time response of Nitrobacter to toxicity. Environ. Int. 10:21-24.

Wang W, Reed P (1984). "Nitrobacter bioassay for aquatic toxicity." Toxicity screening procedures using bacterial systems. 309-325**.**

Wilde EW, Soracce RJ, Mayack LA, Shealy RL, Broadwell TL, Steffen RF (1983). Comparison of chlorine and chlorine dioxide toxicity to fathead Minnows and Bluegill. Water Res. 17(10):1327-1331.

Williamson KJ, Johnson OG (1981). A bacterial bioassay for assessment of wastewater toxicity. Water Res. 15:383-390.

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Full Length Research Paper

Indications of the changing nature of rainfall in Ethiopia: The example of the 1st decade of 21st century

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Climate change was defined as a statistically significant variation in the mean state of climate. Accordingly, with regards to rainfall, a working hypothesis that reads as: there is a statistically significant change (increase/decrease) in the mean annual and seasonal rainfall values at weather stations was forwarded for testing. Eight years (2001-2008) annual and seasonal rainfall data of 17 weather stations from most parts of Ethiopia were used for the purpose. Setting the significance level at 0.05, the simple correlation and regression techniques were used to reach results. Most stations from the western part of Ethiopia were seen to exhibit statistically significant increasing trend in annual rainfall receipt. Albeit not significantly, northward and eastward from here, the receipt was also seen tending first to increase then decrease. This tendency was observed to overlap with the traditional distribution of rainfall in the country. The alternating of dry and wet years and the more localizing of wet years than dry years were identified. The four seasons considered were observed to be statistically significant, but increasing and decreasing trends in seasonal rainfall receipt at the respective stations were not significant. Most conspicuously, 94% of the total number of stations considered in this study show increasing trend in autumn rainfall receipt. Based on the findings of the study, some conclusions policy makers that may be taken into consideration were finally drawn.

Key words: Annual, Ethiopia, rainfall, seasonal, significant.

INTRODUCTION

The hypothesis

Climatologically speaking, Ethiopia is a world in miniature, in that, dictated by its high amplitude of relief (Mesfin, 1970), the largeness of its size, 1,127,127 Km^2 (US Library of Congress, 2005: 4) and its location in the heart of the Horn of Africa, it enjoys varieties of climate that are comparable to the effects of latitude, so much so that the high latitude equivalents can be found along the uplands and the low latitude equivalents along the lowlands (Daniel, 1977).

This fact lends the country to serve as a laboratory for examining change in climate. As per the series of the syntheses reports of the Intergovernmental Panel on Climate Change (IPCC) the working definition of climate change is put as a statistically significant change in the mean state of climate and/or its variability over prolonged period (WMO-IPCC, 2001, 2007, 2013).

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With respect to rainfall, its variability has been a topic of discussion by several research works both from outside Ethiopia (Otun and Adewumi, 2009; Hendrix and Salehyah, 2010; Wolff et al., 2011) and from within Ethiopia (Woldeamlak, 2009; Hongwei et al., 2011; Rientiger et al., 2013).

Otun and Adewumi (2009) looked at rainfall variability in the Sudano-Sahilian regions of Nigeria, and its connection with the sahelian drought. They used daily rainfall series from seven stations that spanned over 30 year climate interval (1940 – 1970 and 1970 – 2000). Their conclusion includes the fact that the drought condition of 1970's might be recurring in the future (Otuned and Adewuni, 2009). Hendrix and Salehyan (2010), established relationship between rainfall variability and socio political conflict in Africa. They particularly found out that dry and wet years are associated with all types of small scale and large scale instances of political conflict (Hendrix and Salehyan, 2010). Based on their evidence from laminated lake sediment record from Southern Kenya Wolff et al. (2011) reported of changes in El-Nino related Variability of East Africa's rainfall during the last three millennia (Wolff et al., 2011)

Studies on rainfall trends and variability made in Ethiopia include Weldeamlak (2009) and Hongwei et al. (2011). Using rainfall data spanning from 1975-2003 for 12 stations from the Amhara region, Woldeamlak (2009) reported, among other things, of lower variability of rainfall in the western part of the region than the eastern. From their trend analysis of 53 years' daily precipitation data at Debremarkos Hongwei et al. (2011) found no increasing trend in the extreme precipitation at Debre Markos. In their assessment of the diurnal cycle of rainfall across the Upper Blue Nile basin Rientjes et al. (2013) revealed the fact that over most parts of the basin rainfall is highest between mid-and-late afternoons. They used seven years (2002-2008) satellite derived participation data (Rientjes et al., 2013).

With regards to rainfall again, research works done in Ethiopia in the style amenable to the definition of climate change given above includes Yilma and Zanke (2004), as quoted by Cheung et al. (2008), and Cheung et al. (2008). Using decadal rainfall records of 134 gauging stations with differing study period (ranging between 35 and 43 years) and employing the regression and correlation techniques, Cheung et al. (2008) analyzed trends in seasonal and annual rainfall at national, watershed, and gauging station levels. In their seasonal analysis, they considered two seasons – *kiremt* (July to September) and *belg* (March to May) (Cheung et al., 2008). At the seasonal level, their findings brought in a significant decline in *kiremt* rainfall for some watersheds eg. the southern Blue Nile watershed (Cheung et al., 2008), and at gauging station level – their finding include a significant increase at Hirna and a significant decrease at Robe from the eastern region of the country (Cheung et al., 2008). Yilma and Zanke (2004), as quoted by Cheung et al. (2008) reported of the decline in annual rainfall in southwestern and eastern Ethiopia (Cheung et al., 2008). Much earlier, Conway and Hulmes (1993), as quoted by Yilma (1996), reported of decrease in annual rainfall in north central highlands, later on confirmed by Yilma (1996).

Unlike such a previous work like Cheung et al. (2008) which based its analyses on two seasons, *kiremt* and *belg*, and non-uniform, but large number of years (35 to 43 years), this research work is based on mean annual and four seasons' mean rainfall of 17 weather stations[1] drawn from most parts of Ethiopia (Figure 1), and spread over eight uniform years (2001-2008) almost the first decade of the twenty first century, would endeavor to test one central hypothesis: that there is statistically significant change (increase/decrease) in mean annual and seasonal rainfall values at the stations.

Although this paper has several shortcomings including the briefness of the study period and the unevenness in the distribution of the stations considered, it is my conviction that the paper may serve two purposes, (a) that it may be indicative of the general direction on the changing nature of rainfall in Ethiopia and (b) it may be a modest addition to the related works in Ethiopia and serve as a reference material for future researches.

METHODOLOGY

Data source

All the eight years mean annual and seasonal values of the 17 stations used in this study were based on the monthly rainfall records collected from the National Methodological Agency[2] (NMA). The years and seasons considered in this study were meteorological years and meteorological seasons. A meteorological year comprises four seasons each composed of three months (Ahrens, 1988). In the northern hemisphere the meteorological definition of winter would be December, January and February; spring would be March, April, and May; summer would be June, July, August; and autumn, September, October, and November (Ahrens, 1988). In order to fit to the meteorological definition, the first month of winter, December, considered here, in each case, was the previous year's December. This procedure was strictly observed throughout. Setting up the mean annual and seasonal

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[1] Originally the writer intended to consider more than seventeen stations distributed over the northern, southern, eastern, western and central parts of Ethiopia, and a minimum study period of 10 years, complete with twelve calendar months of rainfall data. After excluding station with incomplete data and taking cognizance of their distribution over most parts of the country, the author, finally, concentrated on the seventeen stations with comparable data from 2001 to 2008, nearly the first decade of the twenty first century. Monthly rainfall data in two cases, however, i) Dessie for November and December (2006); and ii) Goba, for January and February (2005), and May (2006) were missing. In all these cases, nevertheless, the missing data were filled with the respective arithmetic average of the immediate proceeding and succeeding months with data.)

[2] It would be appropriate to mention here the fact that most of the data on the monthly rainfall of the seventeen stations were secured from the National Meteorological Agency (NMA) by my students for a course work on GeEs 1012 (Applied climatology). The author also obtained a good stock of data straight from the same agency for cash.

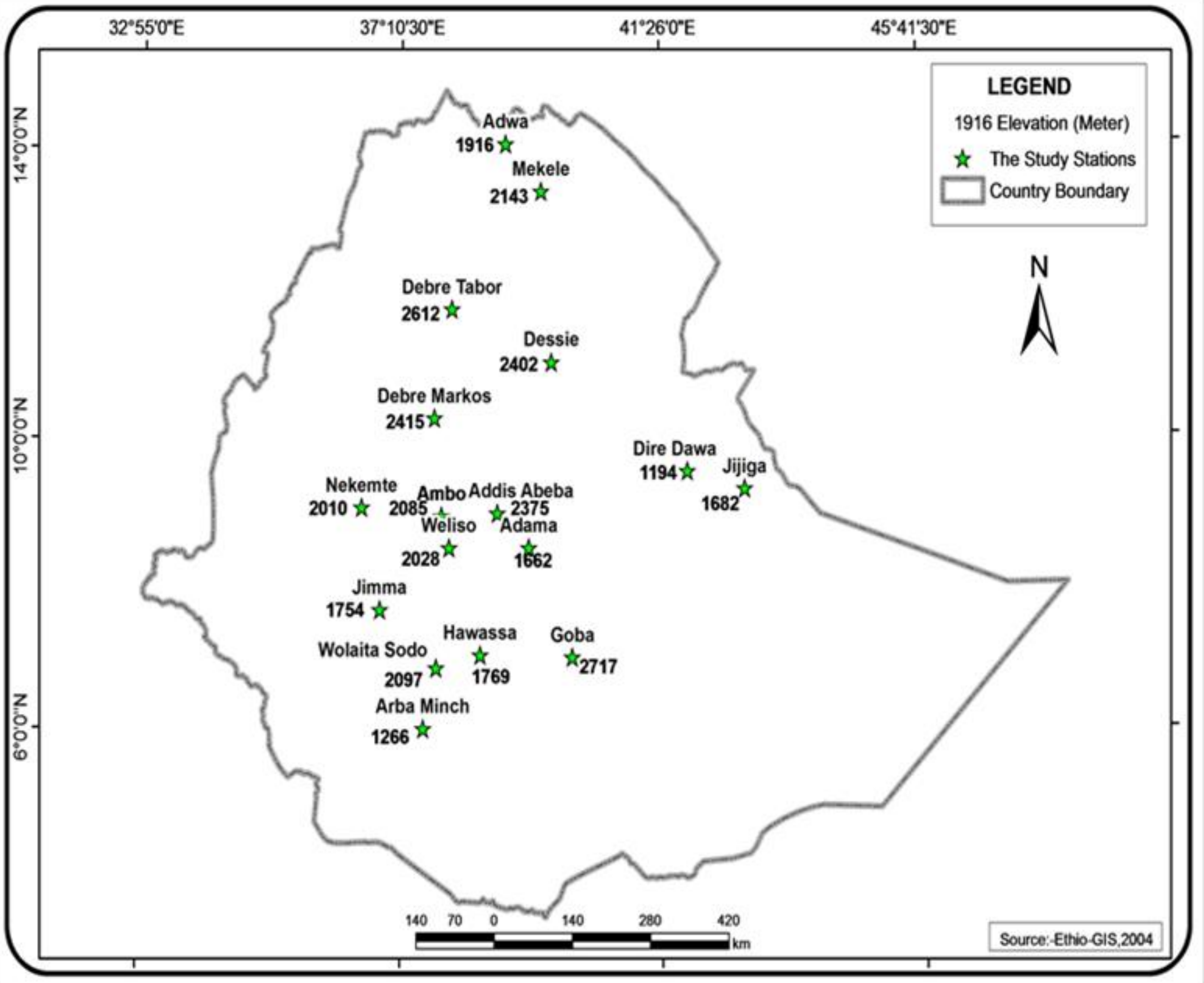

Figure 1. The study stations.

rainfall values as dependent variables: the years and the seasons there in as independent variables; and putting the significance level at 0.05, the regression and correlation techniques (Berenson and Levine, 1983) were used to arrive at the results.

RESULTS AND DISCUSSION

Annual increases and decreases

Table 1 provides a summary of the correlation and regression analyses done on the mean annual rainfall of the fourteen stations. Three distinct groups can be identified. The first group comprises Ambo, Debre Tabor, Nekemte, and Woliso, all but Debre Tabore, from the western part of the Ethiopia, where mean annual rainfall exhibited statistically significant increase over the study period. The second group has Adama, Addis Ababa, Arba Minch, Debre Markos, Goba, Hawassa, Jijiga, Jimma, and Wolaita Sodo – most of which are from the central and southern Ethiopia – which observed increased annual rainfall, albeit not significantly. The third group composed of Dessie, from north east Ethiopia that showed a statistically significant decrease in the mean annual rainfall, plus Adwa and Mekele from northern and Dire Dawa for eastern Ethiopia, which showed decreased rainfall, though not statistically significant again.

It appears, then when, in general annual rainfall receipt in the western part of the country is tending to experience statistically significant increase-northward and eastward from here the tendency is first to continue to increase (in almost all cases not significantly) then decrease (in almost all cases not significantly again). There is the established fact that amount of rainfall in the country is decreasing as one goes northward and eastward from the western part of Ethiopia to modestly increase over the northern and eastern highlands, and decrease thereafter (Kebede, 1964; Mesfin, 1972; Daniel, 1977; Nigtu, 2011), a fact which can also be read from the data on the mean annual rainfall of the 17 stations given in Table 2 and illustrated in Figure 2. Adwa and Mekele (northern most

Table 1. Correlation co-efficient, slope (b_1) decreases/increases and t-test statistics for seventeen stations in Ethiopia (2001 – 2008).

Station	r	Slope (b_1)	I=increased D=decreased	t_{cal}	t_{tab}	R=Reject H_0 A=Accept H_0
Adama	0.500	+3.412	I	1.410	1.9432	A
Addis Ababa	0.261	+1.375	I	0.657	1.9432	A
Adwa	-0.204	-0.979	D	-0.208	1.9432	A
Ambo	0.690	+3.451	I	2.350	1.9432	R
Arba Minch	0.272	+1.645	I	0.689	1.9432	A
Debre Marcos	0.349	+1.040	I	1.196	1.9432	A
Debre Tabor	0.660	+4.400	I	2.200	1.9432	R
Dessie	-0.346	-6.930	D	-2.327	1.9432	R
Dire Dawa	-0.011	-0.043	D	-0.028	1.9432	A
Goba	0.083	+0.587	I	0.206	1.9432	A
Hawassa	0.385	+1.801	I	1.007	1.9432	A
Jijiga	0.176	+0.712	I	0.434	1.9432	A
Jimma	0.106	+0.657	I	0.259	1.9432	A
Mekele	-0.161	-0.820	D	-0.403	1.9432	A
Nekemte	0.835	+7.332	I	3.752	1.9432	R
Wolaita Sodo	0.461	+3.747	I	1.274	1.9432	A
Woliso	0.674	+2.910	I	2.243	1.9432	R

H_1 = There is significant increase/decrease in the mean annual rainfall; $H_0 = H_1$ is not true. Significance level =0.05. Source: Original data from NMA, compiled by the writer, r = regression.

Table 2. The annual mean, the wettest and driest years for seventeen weather stations is Ethiopia (2001 – 2008).

Station	Annual mean (mm)	Wettest year (W)	Driest year (D)	Difference in N^0 years between W and D
Adama	898.2	2007	2002	5
Addis Ababa	1277.2	2001	2002	1
Adwa	778.4	2001	2002	1
Ambo	990.2	2007	2002	5
Arba Minch	738.5	2007	2002	5
Debre Marcos	1336.9	2006	2002	4
Debre Tabor	1411.1	2006	2002	4
Dessie	1390.0	2003	2008	5
Dire Dawa	602.1	2007	2005	2
Goba	1031.1	2006	2002	4
Hawassa	989.3	2007	2003	4
Jijiga	591.9	2006	2008	2
Jimma	1415.2	2001	2002	1
Mekele	476.7	2006	2004	2
Nekemte	2036.3	2008	2002	6
Wolaita Sodo	1385.0	2007	2002	5
Woliso	1196.6	2006	2001	5

Source: Original data from NMA, compiled by the writer.

stations) reported mean annual rainfall of 778.4 and 476.7 mm, respectively; and Dire Dawa and Jijiga (eastern most stations) revealed mean annual rainfall of 602.1 and 591.9 mm, in that order Nekemte and Jimma (western most stations) presented 2036.3 and 1415.22 mm of annual rainfall, the highest and the second highest, respectively, among the stations considered in this study. The other stations, in general, took the

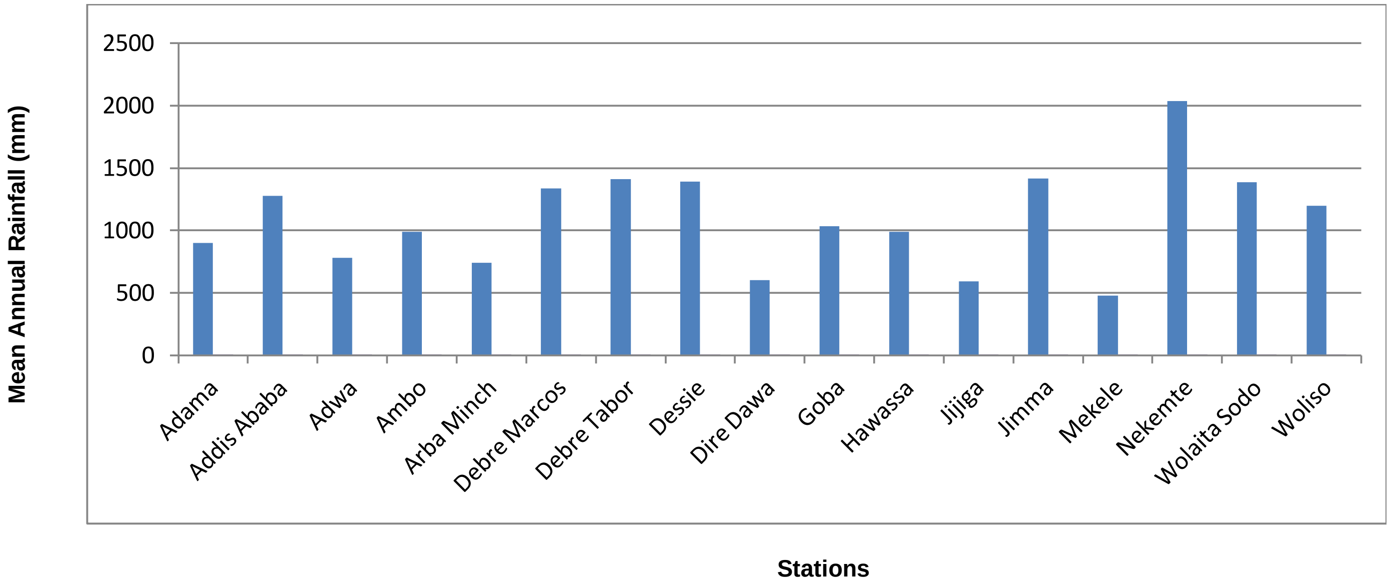

Figure 2. Graphic rrepresentation of the mean annual rainfall of the 17 Stations (2001-2008).

intermediate position between these two extremes. It can be said then that the behaviour of the 17 stations' increases and decreases in the mean annual rainfall amounts observed over the study period, somehow, tends to overlap with the traditional distribution of rainfall in Ethiopia.

Exceptions to this general trend were, however, presented by some stations. For instance, when Jimma from mid-west Ethiopia experienced an increase in the annual rainfall receipt which was not statistically significant; most of the neighbouring stations revealed a statistically significant increase. Likewise, Debre Tabor and Dessie both from northern highlands and at about the same distance from mid-west Ethiopia produced contrasting annual results-statistically significant increase for Debre Tabor and statistically significant decrease for Dessie. Difference in annual values was also observed between the eastern most stations – Dire Dawa, decreasing (not significantly), and Jijiga, increasing (not significantly). These differences may be decidedly explained by local circumstances including elevation factor.

Table 2 also provides a list of the wettest and driest years each station experienced during the study period together with the interval in years between these extreme events. The first feature that may be deciphered from the table would be the fact that wetness tends to be more localized than dryness. This may be learned from the fact that, when in six cases-at Adama, Ambo, Arba Minch, Hawassa, Dire Dawa and Wolaita Sodo or about 35% of the total number of stations, 2007 was the wettest year; and in another six cases-at Debre Marcos; Debre Tabor, Goba, Jijiga, Mekele and Woliso or about 35% of the total again, 2006 was the wettest year; in 11 cases-at Adama, Addis Ababa, Adwa, Ambo, Arba Minch, Debre Marcos, Debre Tabor, Goba, Jimma, Nekemte, and Wolaita Sodo or about 65% of the total number of stations, 2002 was the driest year. The other feature worth mentioning would be the difference in years between the wettest and driest years. The average gap for the 17 stations was put at three and – half years. At some stations, however, that is Addis Ababa, Adwa and Jimma the driest and the wettest years occurred consecutively, while at Nekemte a gap of six years was required.

Seasonal increases and decreases

Table 3 shows the chief points of the statistical analyses done on the seasonal data. Two outstanding features can be learned from the table. First, the compatibility in the pattern of mean annual rainfall increases and decreases with the traditional spatial distribution of rainfall in the country mentioned above, in some way, seems to be repeated here. This is so, for again, mostly the same group of stations with statistically significant annual rainfall increase records, Ambo, Debre Tabor, Nekemte and Woliso emerged with the largest number of seasons with increased rainfall; Three seasons had increase in the case of Ambo, Nekemte and Woliso; and all the four seasons had increase in the case of Debre Tabor. Ambo observed increases in spring (significant), summer (significant) and autumn (significant at 0.10 level); Nekemte in spring (significant), in autumn (significant), and summer (not significant); Woliso in autumn (signify cant at 0.10 level), spring (not significant), and summer

Table 3. Increases and decreases in seasonal rainfall receipt and t-test statistics for seventeen stations in Ethiopia (2001-2008).

Station	Season			
	Winter	Spring	Summer	Autumn
Adama	I(A)	D(A)	I(A)	I(R)
Addis Ababa	I(A)	D(A)	D(A)	I(R)
Adwa	I(A)	I(A)	D(A)	I(R)
Ambo	D(A)	I(A)	I(R)	I(R)*
Arba Minch	D(A)	D(A)	I(A)	I(R)
Debre Marcos	D(A)	I(A)	I(A)	I(R)
Debre Tabor	I(R)	I(R)	I(A)	I(A)
Dessie	I(A)	D(A)	D(A)	I(A)
Dire Dawa	D(A)	I(A)	D(A)	I(A)
Goba	D(A)	I(A)	I(A)	I(A)
Hawassa	D(A)	D(A)	I(A)	I(R)
Jijiga	D(A)	I(A)	D(A)	I(A)
Jimma	I(A)	I(A)	D(A)	I(A)
Mekele	D(A)	I(A)	D(A)	D(A)
Nekemte	D(A)	I(R)	I(A)	I(R)
Wolaita Sodo	D(A)	I(A)	I(A)	I(R)
Woliso	D(A)	I(A)	I(A)	I(R)*

H_1=There is significant increase/decrease in the mean seasonal rainfall; H_2=H_1 is not true. Significance level= 0.05. Source: Original Data from NMA, Compiled by the writer. I = Increased; D = Decreased; A = Accept H_0; R = Reject H_0; * = Reject H_0 at 0.10 significance level.

(not significant). Debre Tabor which can be regarded as a 'moisture island' of the northern highlands witnessed increase in winter (significant), spring (significant), summer (not significant), and autumn (not significant). Other stations distributed over most parts of the country also joined the ranks of stations with three seasons of increased rainfall, which includes Adama, Adwa, Debre Markos, Goba, Jimma, and Wolaita Sodo. In contrast to all these Mekele, from Northern Ethiopia, experienced three seasons of decreased rainfall-in summer, autumn and winter (in all cases not statistically significant). The other stations, including Adama and Addis Ababa, from central Ethiopia, Dire Dawa and Jigjiga from Eastern Ethiopia, and Hawassa from south central part of the country, observed two seasons of decreased rainfall.

Indeed, here too, exceptions to the general trend mentioned earlier were observed, locally produced. The comparison of Adwa's and Mekele's four seasons increasing and decreasing trends present good example. Adwa, about 100 kilometers north west of Mekele, is at a lower elevation (1916 meters above sea level) than Mekele (2143 meters above sea level), (vide the map attached here with) and still Adwa experienced three seasons of increased rainfall (though not significant in all cases), while Mekele observed three seasons of decreased rainfall (in all the cases not significant again). Besides, Mekele reported of an eight year mean annual rainfall of 476.7 mm, when Adwa reported 778.4 mm (vide Table 2). Facts that exclude elevation factor like geographical position may explain this.

The second feature would be the season to season differences - six stations – Debre Tabor (significant), Adama, Addis Ababa, Adwa, Dessie and Jimma (not significant in the last five), or about 35 percent of the total, witnessed increasing trend in winter rainfall. Seven stations or about 41 percent of the total exhibited decreasing tread in summer. This includes – Addis Ababa, Adwa, Dessie, Jimma and Mekele. (in all cases not significant). Twelve stations or about 71 percent of the total number of stations which includes Deble Tabor (significant), Jijiga (not significant), Dire Dawa (not significant) saw increasing trend in spring rainfall. Lastly and probably most strikingly sixteen stations or about 94 percent of the total showed increasing trend in autumn rainfall. Nine stations, or about 51 percent of these comprising Adama, Addis Ababa, Arba Minch, Hawassa, Debre Markos, Plus Ambo and Woliso (both significant at 0.10 level), exhibited statistically significant increase in autumn.

Conclusion

The eight – year period mean annual and seasonal data of the 17 stations considered in this study supported the rejection and acceptance of the null hypotheses of no significant increase/decrease in the annual and seasonal rainfall. On the annual scale, most stations from western part of Ethiopia show statistically significant increasing trend in annual rainfall. In almost all directions from here the tendency, though inconsistently, was first to increase then to decrease albeit not significantly. This appeared to be in close parallelism with the normal distribution of rainfall in Ethiopia. The alternating recurrence of dry and wet years and the more localizing of wet years than dry years were recognized. At the seasonal level, every station witnessed either statistically significant or not significant increasing and/or decreasing trend in the respective seasonal rainfall amount, the pattern of which, taken as a unit, coincided with the traditional distribution of rainfall in the country. Moreover, more than half of the weather stations considered in this study, observed a statistically significant increase in autumn rainfall receipt.

The following conclusions, which policy makers may take into consideration, are drawn from the findings of the study mentioned above:

a) The establishment of the close parallelism in the behaviour of the increases/decreases in the mean annual and seasonal rainfall values with the traditional distribution of the rainfall in Ethiopian as a fact may persuade one to say that rainfall distribution in the country would continue to be as it was and as it is now with the wet and dry areas growing wetter and drier, respectively.
b) The alternating recurrence of dry and wet years and the localizing of wet years than dry years would mean that the resultants, positive or negative, would be local in scale when it comes to wetness and regional or beyond when it comes to dryness.
c) Autumn comes after summer-the observation of statistically significant increase in autumn rainfall at more than 50% of the total weather stations considered in this study could, therefore, mean (i) the continuation of summer rainfall into autumn when it comes to parts of central, northern and western Ethiopia; and (ii) the further augmentation of autumn rainfall when it comes to the eastern and southern Ethiopia.
d) Finally, it can be said that, climate change as associated with rainfall in Ethiopia would confront decision makers with decrease or increase in annual and seasonal amounts. What needs to be done is not hard to forward – all the possible means should be designed to combat all the possible consequences of the events.

Conflict of interests

The author did not declare any conflict of interest.

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REFERENCES

Ahrens D (1988). *Meteorology Today* St. Paul: West Publishing Company.
Berenson M, Levine D (1983). Basic Business Statistics Concepts and Application Englewood Cliffs: Prentice-hall.
Cheung W, Senay G, Singh A (2008). “Trends and spatial distribution of annual and seasonal rainfall in Ethiopia,” Int. Metrol. J.
Daniel G (1977). Aspects of climate and Water Budget in Ethiopia Addis Ababa: University press.
Hendrix CS, Salehyan (2010). ‘After the rain: Rainfall Variability, Hydro-Meteorological Disasters, and Social Conflict in Africa’ in http://ssrn.com/abstract=1641312.
Hongwei S, Yan J, Gebremichael M, Ayalew SM (2011). Trend analysis of extreme precipitation in the Northwestern Highlands of Ethiopia with a case study of Debre Markos. Hydrology and Earth System Sciences. 15(6):1937-1944.
Kebede T (1964). “Rainfall in Ethiopia” Ethiopian Geo. J. 2, 28-36.
Mesfin WM (1970). An Atlas of Ethiopia Asmera: Poligrafico.
Mesfin W M (1972). Ethiopia. Addis Ababa: Berhanena Selam HSI Printing press.
Nigatu M (2011). “Rainfall Intensity Duration Frequency (RIDF) Relationship Under Changing Climate” (Case study on upper Blue Nile River Basin, Ethiopia). Msc Thesis.
Otun JA, Adewumi (2009). ‘Rainfall Variablity and Drought Inference in Sudano-Saheliah Region of Nigeria’. An Int. J. Agric. Sci. Technol. 8:2. anaals.edu.hg/index.php/series-B/article/view/429/0.
Rientjes T, Alemseged TH, Ayele AF (2013). ‘Diurnal Rainfall Variability Over the Upper Blue Nile Basin; A Remote Sensising Based Approach’. Int. J. Appl. Earth Observation and Geo information. 21:311-326. www.sciencedirect.com.
US Library of Congress (2005). “Ethiopia country profile” :ttp;//covervit.oc.gov/frd/es/profiles/Ethiopia.
Wolff C, Haug GH, Timmermann A, Damsté JSS., Brauer, A., Sigman, DM, Mark AC, Verschuren D (2011). Reduced interannual rainfall variability in East Africa during the last ice age. Science, 333(6043), 743-747.
WMO, IPCC 2001, Climate Change: synthesis Report.
WMO, IPCC 2007, Climate Change: synthesis Report
WMO, IPCC, 2013, Climate Change: synthesis Report
Woldeamlak B (2009). “Rainfall variability and crop production in Ethiopia case study in the Amhara Region” proceedings of the 16th international conference of Ethiopian studies ed. by Ege S, Harald, A, Birhanu T, Shiferaw BT. 823-836.
Yilma S (1996). Stochastic predictions of summer rainfall amounts over northeastern Africa Highlands and over India. Brusels

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Full Length Research Paper

A comparative study of the defluoridation efficiency of synthetic dicalcium phosphate dihydrate (DCPD) and lacunar hydroxyapatite (L-HAp): An application of synthetic solution and Koundoumawa field water

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This paper deals with the comparison of defluoridation efficiency of two defluoridation agents by the use of dicalcium phosphate dihydrate (DCPD) and lacunar hydroxyapatite (L-Hap) as a fluoride sorbents. The DCPD and L-HAp are characterized by using XRD and FTIR techniques. Defluoridation of synthetic solution of sodium fluoride (NaF) and natural waters of Koudoumawa are studied. The fluoride removal capacity is as follows: DCPD: (26.37 mg.g^{-1}; 0.0174 g, 9.81 mg.g^{-1}; 0.1012 g) and L-Hap: (18.96 mg.g^{-1}; 0.0174 g, 8.00 mg.g^{-1}; 0.1012 g). The optimum 0.0623 g of DCPD/100 mL dosage of synthetic solution could bring down the level of fluoride within the tolerance limit, [F$^-$] = 0.38 mg/l (WHO guideline value = 0.8 mg/l), the pH rise is 5.10 and the defluoridation time is 72 h. For L-Hap, it is 0.1012 g of L-Hap/100 mL, [F$^-$] = 1.98 mg/l in the same conditions. For Koundoumawa natural waters, 0.0527 g of L-Hap/100 mL of solution could bring down the level of fluoride, [F$^-$] = 0.84 mg/l. New mechanisms of fluoride removal by DCPD and L-HAp are proposed from which it is established that this material removes fluoride by ion-exchange, adsorption process, dissolution, precipitation and co-precipitation.

Key words: Defluoridation, dicalcium phosphate dihydrate (DCPD), lacunar hydroxyapatite (L-Hap), adsorption, ion-exchange, dissolution-precipitation.

INTRODUCTION

To provide water requirements for the rural population and to fight against poverty, Niger government exploits waters from wells and drillings. In 1996, more than 6207 drillings and 10005 wells were done. These drillings play

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Table 1. Physicochemical composition of Koundoumawa waters.

Parameter	pH	Conductivity ($\mu S.cm^{-1}$)	Concentrations (mg/l)								
			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	$S0_4^{2-}$	HCO_3^{-}	NO_3^{-}	NO_2^{-}	F^{-}
Koundoumawa well water (summer)	7.1	384	42.1	24.45	87	5	25	216.08	8	0.02	4
Koundoumawa well water (rainy)	7	381	20.8	03	85	06	20	200.1	28	0.28	2.3
Koundoumawa drilled water	7.5	379	39.1	22.15	69	03	29	216.70	9	0.01	5

a primary role in water supply to the rural population. More than 90% of rural population in Niger depends on underground water as their drinking waters sources and fluoride excess is generally found in underground waters. A study carried out by the Ministry in charge of Waters, Environment and Fight against desertification (MHE/FAD) in 2005 on the physico-chemical analysis of raw water showed a high fluoride concentration according to World Health Organization (WHO) guidelines. WHO has set a guideline value of 0.8 mg/l for hot countries as the maximum permissible level of fluoride for drinking water to avoid health effects of fluoride water poisoning (WHO, 1996). In over a total of 211 studied drillings, 38% presented a fluoride concentration higher than 0.8 mg/l. The highest concentrations are recorded in Tibiri, Koundoumawa (in the Eastern part of Niger) and in Ingall (in the Northern part of Niger) with concentrations varying from 5 to 8 mg/l. Table 1 gives the chemical composition of Koundoumawa well and drilled waters during the summer rainy season. It has fluoride concentration of about 2 to 5 mg/l, its pH is about 7 to 7.5, and a bicarbonate concentration of about 167.7 to 216.08 mg/l.

A study carried out by UNICEF in 1998 classified Niger among the countries presenting fluorosis endemic contaminations. Several processes of defluoridation of drinking water have been reported in literature such as: ion-exchange, adsorption, and coagulation and precipitation process. Based on these processes, several defluoridation methods have been proposed by using nanosized hydroxyapatite (Yu et al., 2011), hydroxyapatite (Mourabet et al., 2011; Jiménez-Reyes and Solache-Ríos, 2010), surface coated hydroxyapatite powders (Subbaiah and Sankaran, 2014). Dicalcium phosphate dihydrate (DCPD) has been reported to be efficient for water defluoridation (Sekar et al., 2009; Masamoto and Tetsuji, 2004; Moubaret et al., 2011; Taewook et al., 2012). Several methods based on coconut shell carbon (Anirudhan et al., 2007; Amit et al., 2011), bone char (Medellin-Castillo et al., 2014), hybrid precipitation-microfiltration process (Nash and Liu, 2010), precipitated fluorhydroxyapatite nanoparticles (Kevin, 2014), Al (III) modified calcium hydroxyapatite, nano-hydroxyapatite/chitin composite (Yulun and Chun, 2012), alginate bioencapsulated nano-hydroxyapatite composite (Kalimuthu and Natrayasamy, 2014), and recently other defluoridation methods including membrane process based on reverse osmosis and nanofiltration (Simons, 1993; Rao et al., 1998; Mameri et al., 1998) and cellulose anhydroxyapatite nanocomposites (Xiaolin et al., 2013), glass derived hydroxyapatite (Wen et al., 2011), nano-hydroxyapatite/chitosan (Sundaram et al., 2008) were reported. Because of the socio-economic conditions in Niger, it is impossible to set up the defluoridation processes mentioned above. Thus, the rural populations are obliged to consume this fluoride poisoning water with its consequence, the appearance of the dental and skeletal fluorosis. The most dramatic example is the case of the children from Tibiri (Maradi, in the middle-east of Niger) where more than 450 children are affected by various forms of fluorosis, which include dental, skeletal and non-skeletal forms (Rapport Mission Internationale d'Enquête, 2002). However, for developing countries, precipitation and adsorption are the most accessible methods. Indeed the management and maintenance of current and proposed defluoridation technologies require expensive chemicals and/or a high level of technological skill and can be applied only in centralized water distribution systems (Rao et al., 1998). Precipitation methods are based on the addition of chemicals to water and removal of insoluble fluoride compounds as precipitates or co-precipitates or adsorbed onto the formed precipitates (Nash and Liu, 2010). In adsorption processes, fluoride is removed either by ion exchange, physical or surface chemical reactions with the adsorbent material. Hydroxyapatite (HAp) was used to remove cadmium, oxovanadium, cobalt, lead and zinc (Lusvardi et al., 2002; Vega et al., 2003; Hammari et al., 2004; Smiciklas et al., 2006; Sandrine et al., 2007). It appears that with hydroxyapatite (HAp), the adsorption and ion-exchange mechanisms are the most favorable mechanisms for fluoride removal (Meenakshi et al., 2007). The removal of fluoride using HAp has been reported earlier (Fan et al., 2003; Hammari et al., 2004). Sairam et al. (2008) have used nano-hydroxyapatites for water defluoridation. Lacunar hydroxyapatite or calcium-deficient hydroxyapatite (CDHA) nano-crystals incorporated with bovine serum albumin (BSA) to form BSA-loaded nano-carriers were synthesized via both *in situ* and *ex situ* processes (Tse-Ying et al., 2005). Spherical Ca-deficient hydroxyapatite (HA) granules are expected to be useful drug carriers in bony sites because of their bone regeneration and adsorption ability (Masanobu et al., 2013). To study the effects of nanocrystalline calcium deficient hydroxyapatite incorporation in glass ionomer cements, bioactive nanocrystalline calcium deficient hydroxyapatite (nCDHA) with improved mechanical and resorption properties was synthesized (Sumit et al., 2011). The objective of the present study was to investigate the performance of synthesized DCPD and Lacunar hydroxyapatite as feasible and suitable adsorbent. Synthesized L-HAp and DCPD are synthesized in the laboratory by precipitation method. Defluoridation studies are carried out under various equilibrating conditions like the amount of adsorbent, the effect of contact time and the pH. Detailed precisions during the defluoridation mechanism by L-HAp and DCPD and the kinetic studies are presented.

EXPERIMENTAL SET UP AND PROCEDURES

Synthesis of DCPD and L-Hap

The synthesis of L-HAp and DCPD involved adding variable volumes of 0.1 M of monosodium phosphate dihydrate to 100 mL of 0.025 M of calcium chloride monohydrate in a closed tricol balloon reaction vessel. The calcium phosphate precipitation is being controlled by the pH level adjustment with 1 M NaOH solution, using a pH-meter TACUSSEL giving an accuracy of 0.01 unit of pH. The synthesis of DCPD involves the reaction of calcium chloride monohydrate and monosodium phosphate dihydrate with a Ca/P ratio close to 1. pH level of the reaction should be maintained at pH ranging from 5.8 to 6.6 (Manzola et al., 2013), otherwise it may lead to the formation of OH-Apatite (Legeros et al., 1983; Casciani et al., 1980). From pH 7 to 7.8 it precipitates Lacunar hydroxyapatite (L-Hap) (Manzola et al., 2013). The medium agitation is carried out using a magnetic stick at the ambient temperature of 22 to 25°C. The precipitated solution is poured into a 250 mL bottle and kept for 1, 3 or 7 days, then filtered. The precipitated solid is dried from 60 and 70°C for 24 h to get DCPD ($CaHPO_4.2H_2O$) or L-Hap.

Characterisation of DCPD and L-Hap

The solid phases obtained at different pH are characterized by powder X-ray diffraction (XRD) and the phase identification is made by using a JCPDS cards (Joint Committee on Powder Diffraction Standards) and Fourier Transform Infrared Spectrometer (FTIR). The XRD patterns are obtained by using a PW 1050/37 diffractometer with a monochromatic radiation $K\alpha 1$ of Cu (λ = 1.5418; 1.5405 A°). The FTIR spectra are performed by using the KBr pellet technique in a Shimadzu Fourier Transform-8300 in the range of 4500-400 cm^{-1} at a resolution of 4 cm^{-1}. Also, the results of FTIR spectrometer and XRD are used to confirm the fluoride uptake by the DCPD or L-Hap precipitates.

Adsorption experiments

Synthetic solution

The fluoride experimental solutions are prepared by a quantitative dilution of stock solution. The stock solution of 1000 mg/l fluoride is obtained by dissolving an appropriate amount of sodium fluoride in distilled water.

A 100 ml of the fluoride experimental solution (10 mg/l as initial fluoride concentration) is taken into a 150 mL of PVC conical flask and a known weight of adsorbent material (0.0174; 0.0318; 0.0527; 0.0623 and 0.1012 g) is added in this solution, and then kept for stirring at 150 rpm on a horizontal rotary shaker for 0; 2; 21; 48; 72 and 92 h in order to reach the equilibrium. After that, the solution is then filtered through Millipore filter paper 22 µm and the filtrate is analyzed for residual fluoride concentration by ion selective electrode (ISE) using field ion meter 340I/ION. In order to regulate the total ionic strength, the TISAB adjusting buffer is added to the sample and standard solutions. The TISAB buffer was added in order to maintain pH constant, to decomplex metal-F complexes contained in the sample during measurement and allowed us to get the total free fluoride concentration in the solution. The fluoride removal experiments are studied over different operational conditions including effect of adsorbent dose, the nature of adsorbent and the effect of contact time. All the experiments were carried out at 30°C, room temperature. During the experiments, the fluoride ion concentration and the pH solution were recorded.

Fluoride poisoning field water of Koundoumawa

In the second serial experiments, the fluoride removal ability of L-HAp and DCPD was tested in field water collected from KOUNDOUMAWA drilling (Zinder, in the far eastern part of Niger) at the same conditions as the synthetic water. The physicochemical characteristics of field water samples are determined before batch adsorption study. The detailed characteristics of field waters are given in Table 1.

RESULTS AND DISCUSSION

Characterization of DCPD sorbent

In order to characterize DCPD, XRD and FTIR were carried out on precipitated DCPD and DCPD samples mixed with fluoride solution. The XRD patterns of precipitated DCPD and the DCPD samples mixed with fluoride solution are respectively presented in Figures 1 and 2.

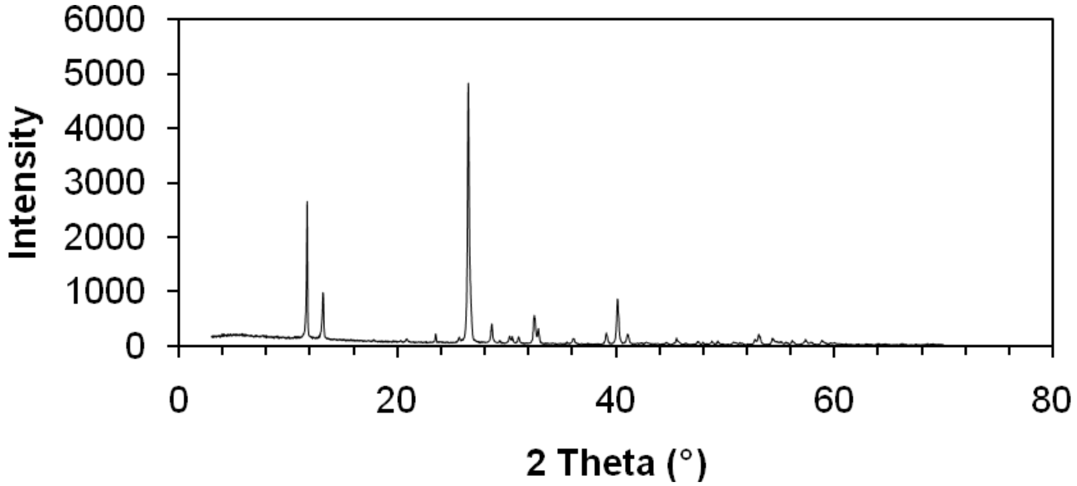

Figure 1. XRD patterns of precipitated DCPD.

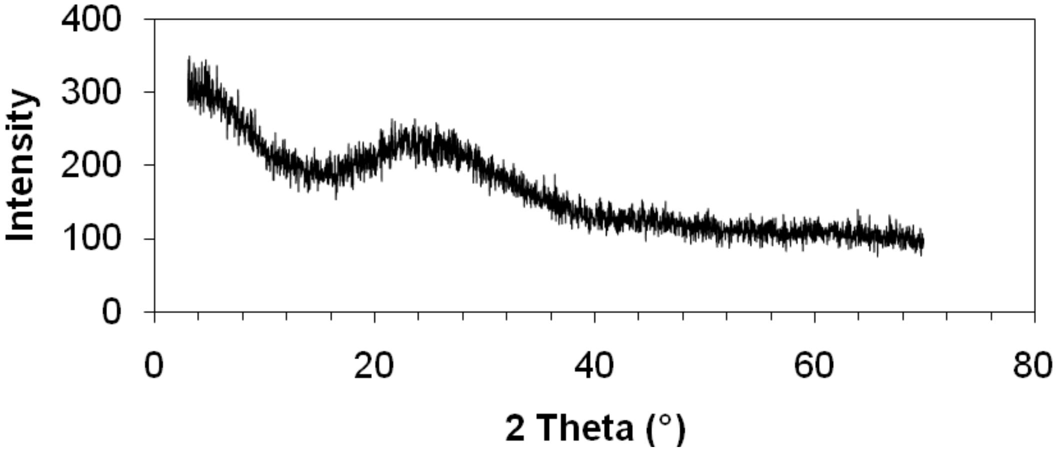

Figure 2. XRD patterns of fluoride sorbed precipitated DCPD.

The identification of crystal phases was done by using a JCPDS cards (Joint Committee on Powder Diffraction Standards). The XRD patterns shown in Figure 1 indicates the DCPD peaks at 2θ values of about 11.6°, 13.2°, 20.9°, 23.6°, 26.5°, 29.3° which are in the JCPDS cards (card number 09–0077). The maximum peak intensity is 26.5 degree theta. The spectra confirmed that the products obtained are mainly composed of the DCPD form of calcium phosphate (Figure 1). There is a meaningful change in the XRD patterns of DCPD after treatment with fluoride (Figure 2). The results of X-ray diffraction of treated product show amorphous product. Similar results are reported (Larsen et al., 1993) while studying the fluoride sorption on brushite, calcium hydroxide, and bone char. The fine powder obtained was poorly crystallized apatite.

Figure 3 represents FTIR spectra of the samples before and after treatment with fluoride. In Figure 3, the absorption peaks appeared at 3540 and 3488 cm^{-1}, characteristics of valence vibrations of free H_2O (ν_{H_20}), at 3290 and 3161 cm^{-1}, characteristics of valence vibrations of associated H_20 (ν_{H_20}), at 2367 cm^{-1}, characteristic of valence vibrations $\nu_{O\text{-}H}$ of HPO_4^{2-} groups, at 1649 cm^{-1} characteristic of valence vibrations of H_20 of constitution (ν_{H_20}), at 1220 and 790 cm^{-1}, characteristics of bond elongation vibrations $\delta_{P\text{-}O\text{-}H}$ at 1135, 1059 and 985 cm^{-1}, characteristics of valence vibrations ν_{PO} at 873 cm^{-1},

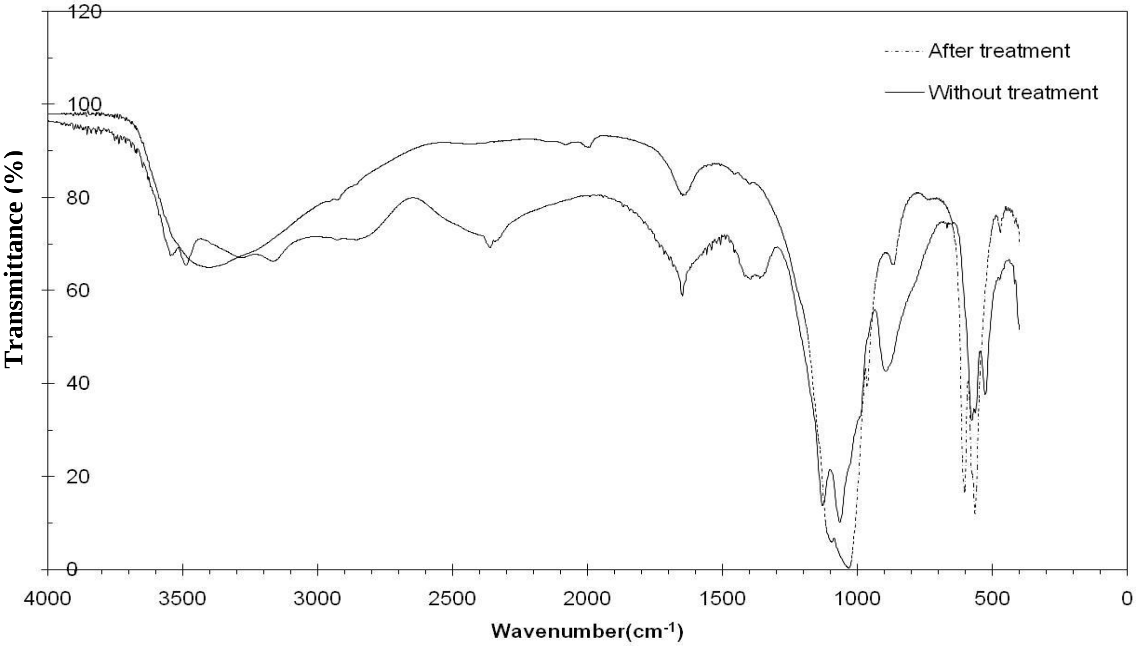

Figure 3. FTIR spectra of DCPD (without treatment and after treatment with fluoride).

characteristics of valence vibrations $\nu_{P\text{-}OH}$ and at 525 cm^{-1}, characteristics of valence vibrations $\nu_{O\text{-}P\text{-}O}$ (Legeros et al., 1983; Casciani et al., 1980). We can observe that the spectrum of precipitated solid is perfectly identical to infra-red spectrum of $CaHPO_4 \cdot 2H_2O$ (Legeros et al., 1983), and the characteristic bands are in conformity with those obtained (Legeros et al., 1983; Casciani et al., 1980).

There is a meaningful change in the FTIR spectrum of the sample after treatment with fluoride. In the presence of F^- ions, the DCPD is transformed into ″OH-Apatite″, then into fluorinated hydroxyapatite FAp (Maiti et al., 1981; Takahashi et al., 1978). Indeed for the infra-red spectrum of ″OH-Apatite″ (Legeros et al., 1983; Casciani et al., 1980), absorption peaks appeared at 3561 cm^{-1} characteristics of valence vibrations of ν_{OH^-}, at 3473 cm^{-1} characteristic of valence vibrations $\nu_{H\text{-}O\text{-}H}$ of free H_2O, at 1647 cm^{-1} characteristic of valence vibrations $\nu_{H\text{-}O\text{-}H}$, at 1112, 1033 and 960 cm^{-1} characteristic of valence vibrations $\nu_{PO_4^{3-}}$, at 873 cm^{-1} characteristic of valence vibrations $\nu_{P\text{-}OH}$, at 602 and 562 cm^{-1} characteristics of elongation vibrations $\delta_{PO_4^{3-}}$. The apparition of two new bands at 775 and 468 cm^{-1} may be due to the fluoride adsorption/exchange, indicating the incorporation of fluoride into the solid.

Characterization of L-Hap sorbent

The identification of crystal phases was done by using a JCPDS cards (Joint Committee on Powder Diffraction Standards). The crystalline peaks at 2θ = 25.9°, 32°, 33° and 40° (Figure 4) indicating the L-Hap peaks which are in the JCPDS cards (card number 9-432). The maximum peak intensity is 32 degree theta. The spectra confirmed that the products obtained are mainly composed of the L-Hap form of calcium phosphate (Figure 4). There is no significant change in the XRD pattern of L-HAp after treatment with fluoride (Figure 5). Similar results were reported (Diaz-Nava et al., 2002) while studying the fluoride sorption on zeolites.

In Figure 6, there is a meaningful change in the FTIR spectrum of the sample after treatment with fluoride. The presence of F^- ions makes the L-Hap transformed into fluorinated hydroxyapatite FAp (Maiti et al., 1981; Takahashi et al., 1978). For the infra-red spectrum of ″OH-Apatite″ (Legeros et al., 1983; Casciani et al., 1980), absorption peaks appear at 3561 cm^{-1} characteristics of valence vibrations of ν_{OH^-}, at 3473 cm^{-1} characteristic of valence vibrations $\nu_{H\text{-}O\text{-}H}$ of free H_2O, at 1647 cm^{-1} characteristic of valence vibrations $\nu_{H\text{-}O\text{-}H}$, at 1112, 1033 and 960 cm^{-1} characteristic of valence vibrations $\nu_{PO_4^{3-}}$, at 873 cm^{-1} characteristic of valence vibrations $\nu_{P\text{-}OH}$, at 602

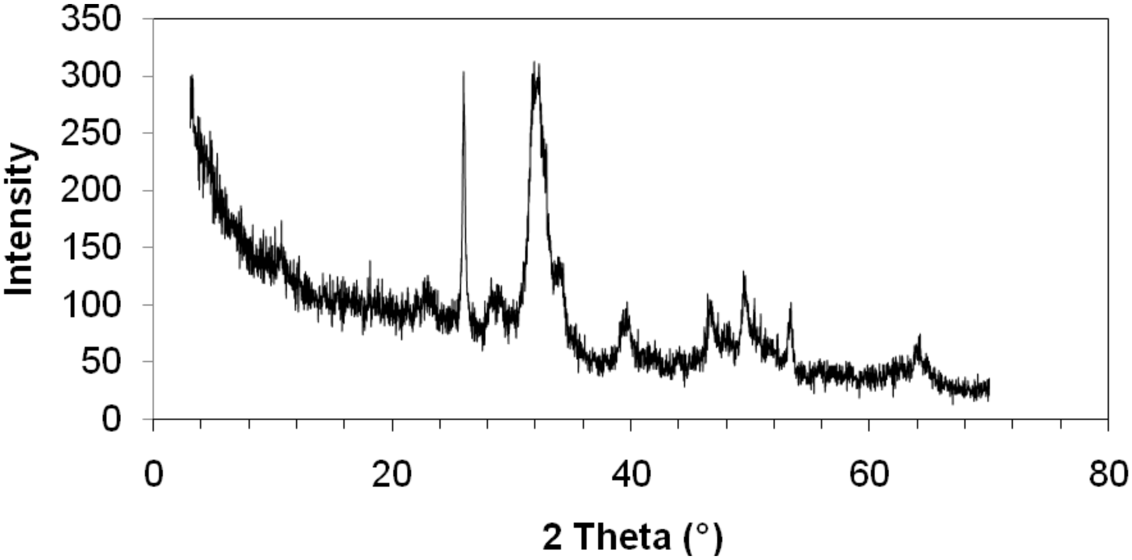

Figure 4. XRD patterns of precipitated L-HAp.

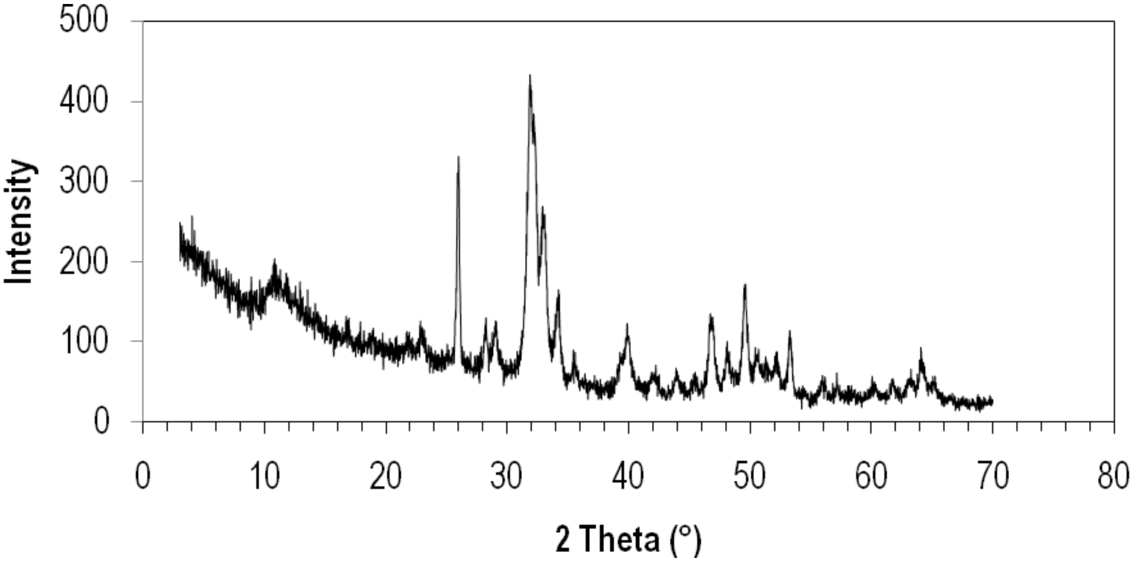

Figure 5. XRD patterns of fluoride sorbed precipitated L-HAp.

and 562 cm^{-1} characteristics of elongation vibrations $\delta_{PO4}{}^{3-}$. The presence of some OH$^-$ with F$^-$ disturbs the OH band frequencies of the pure calcium ″OH-Apatite″. There is a reduction in the intensity of OH bands at 3570 and 602 cm^{-1} with some displacement with lower frequencies in fluoride treated L-HAp which may be due to fluoride adsorption/exchange. The apparition of two new bands at 701 and 468 cm^{-1} for fluorinated hydroxyapatite FAp may be due to the fluoride adsorption/exchange, indicating the incorporation of fluoride into the solid. It is known that FAp is more stable in solution than ″OH-Apatite″ (Chow et al., 1997). For the initial fluoride treated ″OH-Apatite″, the new band appears at 701 cm^{-1}. The displacement of the new band from 775 to 701 cm^{-1} confirms the formation of O–H···F bond in agreement with other authors (Okazaki et al.,

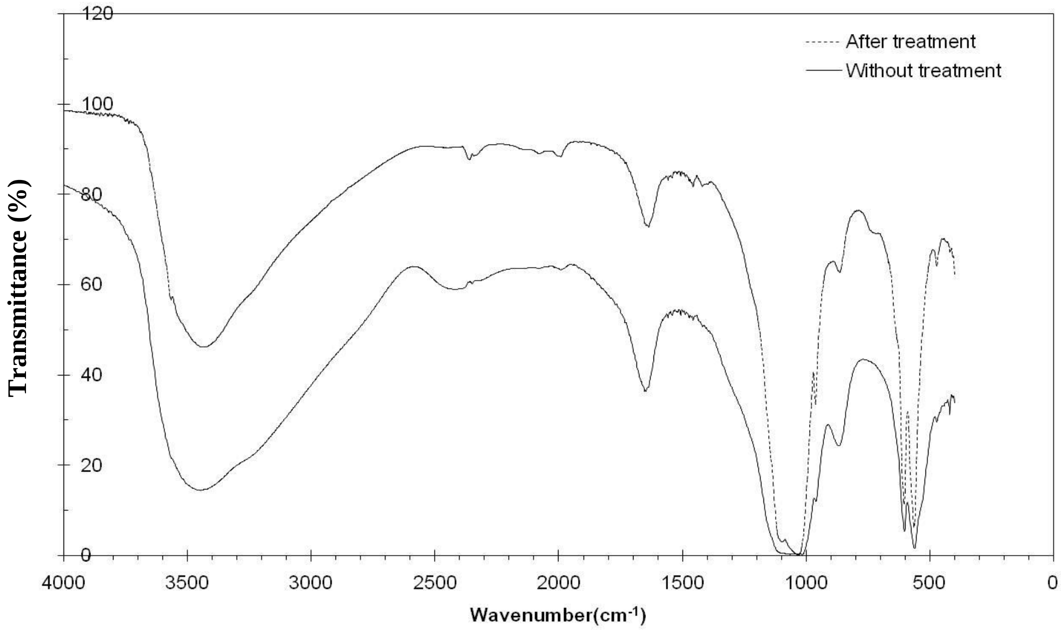

Figure 6. FTIR spectra of L-Hap (without treatment and after treatment with fluoride).

1981; Okazaki, 1992).

Adsorption experiments

Synthetic solution

DCPD sorbent - Effect of contact time and dose: In order to obtain the optimum DCPD sorbent dose defluoridation and contact time, experiments were carried out with various dosages of DCPD ranging from 0.0174 to 0.1012 g with 10 mg/l as initial fluoride concentration. We investigated the sorption of fluoride ion on DCPD as a function of contact time in the range of 0 to 92 h at room temperature and as a function of sorbent dose. The effect of fluoride removal capacity of DCPD with contact time and sorbent dose is shown in Figure 7. It was observed that fluoride removal capacity increases with contact time and contrary to the fluoride ion concentration (Figure 8), the fluoride removal capacity decreases with the raise of sorbent dose, where saturation is reached after 72 h for 0.0174; 0.0318; 0.0527 and 0.0623 g amount of DCPD sorbent dose. The maximum fluoride removal capacity is found to be 26.37 mg.g^{-1} at a sorbent dose of 0.0174 g. For 0.1012 g amount of DCPD sorbent dose, the fluoride removal capacity kept increasing and reached over 92 h 9.81 mg.g^{-1}. Indeed at low sorbent dose, the fluoride uptake capacity is high because of the better utilization of the available active sites and at high sorbent dose, too many sites are available for limited quantity of fluoride and the lower equilibrium concentration of fluoride for sorption becomes negligible (Sanjay et al., 2009). Similar behavior has also been reported previously for other adsorbent (Kamble et al., 2007). The evaluation of fluoride ion concentration with contact time and sorbent dose is shown in Figure 8. We observed that the fluoride ion concentration decreased rapidly and continuously with the increase in the dose of the sorbent. This phenomenon was still observed for 72 h. From 72 to 92 h, it stayed steady for 0.0174; 0.0318; 0.0527 and 0.0623 g amount of DCPD sorbent dose. For 0.1012 g amount of DCPD sorbent dose, the fluoride ion concentration continued to decrease. For this amount, the concentration of fluoride ion reached for 92 h was [F$^-$] = 0.15 mg/l. The optimum dosage can be fixed as 0.0623 g for further studies as this dosage was found to bring down the level of fluoride within the tolerance limit, [F$^-$] = 0.38 mg/l for this amount (WHO guideline value = 0.8 mg/l). The steady state of DCPD sorbent was reached after 72 h for 0.0174; 0.0318; 0.0527 and 0.0623 g amount of DCPD sorbent dose. The uptake of fluoride can be controlled by adsorption or the dissolution-and-recrystallization mechanism. DCPD reached steady state only after 72 h, suggesting that the process is also governed by adsorption and mainly by dissolution-and-recrystallization mechanisms which are slow processes (Sairam et al., 2008; Meenakshi et al., 2007; Low et al., 1995; Taewook et al., 2012). For 0.1012 g amount of

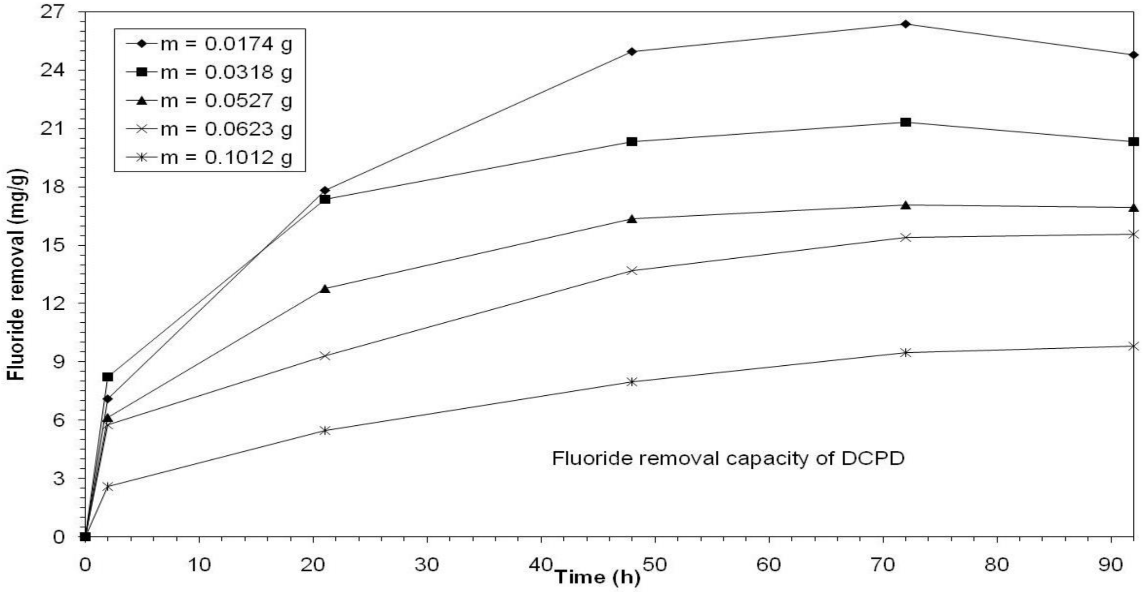

Figure 7. Effect of contact time and sorbent dose on the fluoride removal capacity of precipitated DCPD.

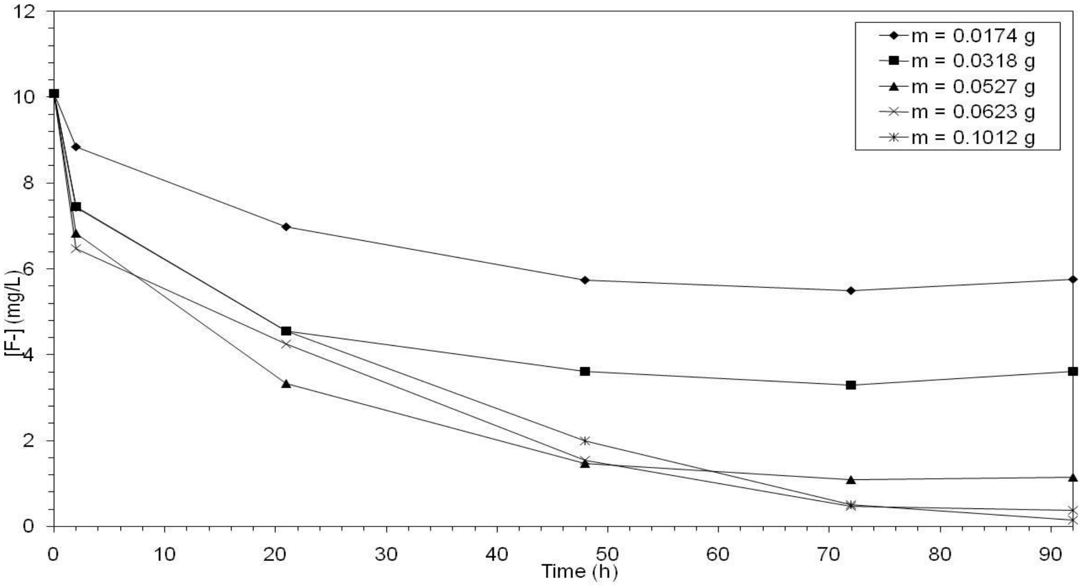

Figure 8. The evaluation of fluoride concentration during defluoridation: Effect of contact time and sorbent dose on precipitated DCPD.

DCPD sorbent dose, the steady state is not reached after 72 h.

Figure 9 shows the evaluation of the pH of the aqueous solution. The evaluation of pH fits the evaluation of fluoride ion concentration (Figure 9). We observed that the pH solution decreases rapidly and continuously with an increase in the dose of the sorbent (Lerch et al., 1966) while studying the hydrolytic conversion of DCPD to apatite. Indeed in aqueous solution, the following reactions are proposed:

*Quick and partial dispersion of phosphate in aqueous medium:

$CaHPO_4.2H_2O \Leftarrow\Rightarrow Ca^{2+} + HPO_4^{2-} + 2H_2O$

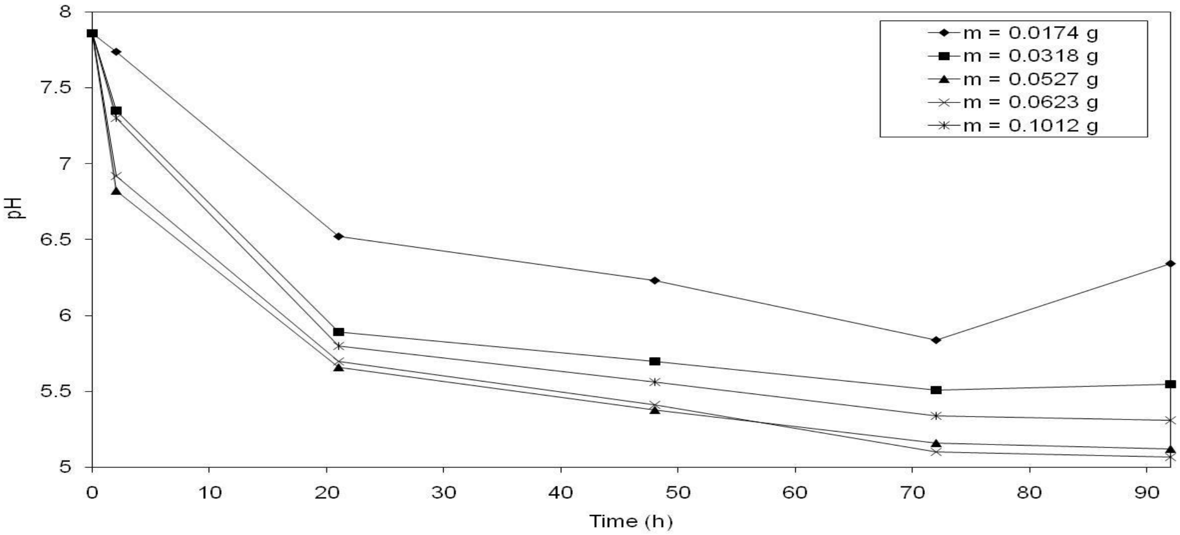

Figure 9. The evaluation of pH solution during the defluoridation: Effect of contact time and sorbent dose on precipitated DCPD.

*Quick hydrolytic conversion of HPO_4^{2-} ions:

$HPO_4^{2-} + H_2O \Leftarrow\Rightarrow H_2PO_4^- + OH^-$

*Low and concurential dismutation reaction:

$2HPO_4^{2-} \Leftarrow\Rightarrow PO_4^{3-} + H_2PO_4^-$

$2HPO_4^{2-} + 2H_2O \Leftarrow\Rightarrow 2H_2PO_4^- + 2OH^-$

*Precipitation of PO_4^{3-} ions:

$PO_4^{3-} + Ca^{2+} + HPO_4^{2-} + OH^- \Leftarrow\Rightarrow$ Apatitic calcium phosphate.

The decrease of pH is due to the presence of $H_2PO_4^-$ ions. Indeed this phosphate is acidic; its aqueous dissolution gives H_3PO_4 and a residual $CaHPO_4.2H_2O$ (Brown et al., 1959), reaches steady state at pH 5.5. In our experiments, DCPD reaches steady state after 72 h at pH 5.51 for 0.0318 g of sorbent; at pH 5.34 for 0.1012 g of sorbent; pH 5.16 for 0.0527 g of sorbent; pH 5.10 for 0.0623 g of sorbent. The pH solution plays an important role by controlling the adsorption at the water adsorbent interface and the dissolution-reprecipitation mechanism (Meenakshi et al., 1991). In the presence of fluoride ions and at these low pH, hydroxyapatite is faster when converted to fluorapatite because the solubility of the two salts differs increasingly with lower pH (Larsen et al., 1992). At high pH, the solubility of the two salts does not differ greatly (Featherstone et al., 1990), which explains why the uptake of fluoride in apatite is slow in this pH range. The dissolution of hydroxyapatite-reprecipitation of fluorapatite principle would be expected to operate most efficiently at low pH. As we have seen above, one disadvantage of such a defluoridation mechanism is that phosphate may be left in solution (the formation of H_3PO_4) and thus may favor bacterial growth.

L-Hap sorbent - effect of contact time and dose: In order to obtain the optimum L-Hap sorbent dose defluoridation and contact time, experiments were carried out with various dosages of L-Hap ranging from 0.0174 to 0.1012 g with 10 mg/l as initial fluoride concentration. We have investigated the sorption of fluoride ion on L-Hap as a function of contact time in the range of 0 to 72 h at room temperature and as a function of sorbent dose. The effect of fluoride removal capacity of L-Hap with contact time and sorbent dose is shown in Figure 10. It was observed that fluoride removal capacity increases with contact time and contrary to the fluoride ion concentration (Figure 11), the fluoride removal capacity decreases with increase in sorbent dose, where saturation is reached after 48 h for 0.0174; 0.0318; 0.0527 and 0.0623 g amount of L-Hap sorbent dose. The maximum of fluoride removal capacity is found to be 18.96 $mg.g^{-1}$ at a sorbent dose of 0.0174 g. For 0.1012 g amount of DCPD sorbent dose, the fluoride removal capacity stays increasing and reached 72 h for 8.00 $mg.g^{-1}$. Indeed at low sorbent dose, the fluoride uptake capacity is high because of the better utilization of the available active sites and at high sorbent dose, too many sites are available for limited quantity of fluoride and the lower equilibrium concentration of fluoride for sorption becomes negligible (Sanjay et al.,

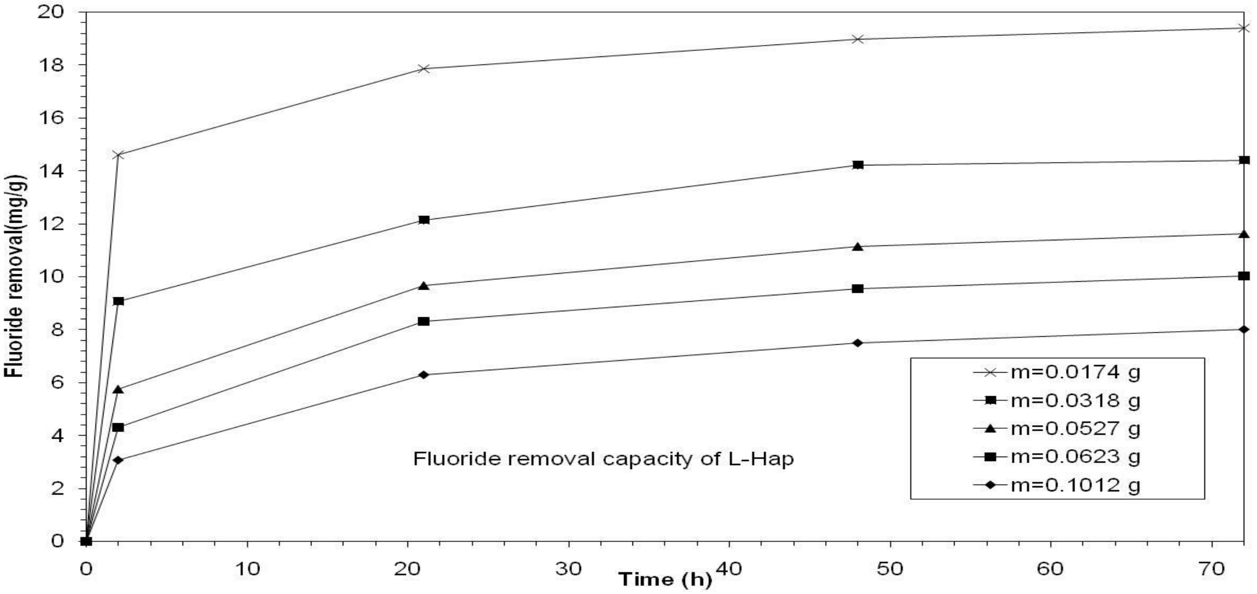

Figure 10. Effect of contact time and sorbent dose on the fluoride removal capacity of precipitated L-Hap.

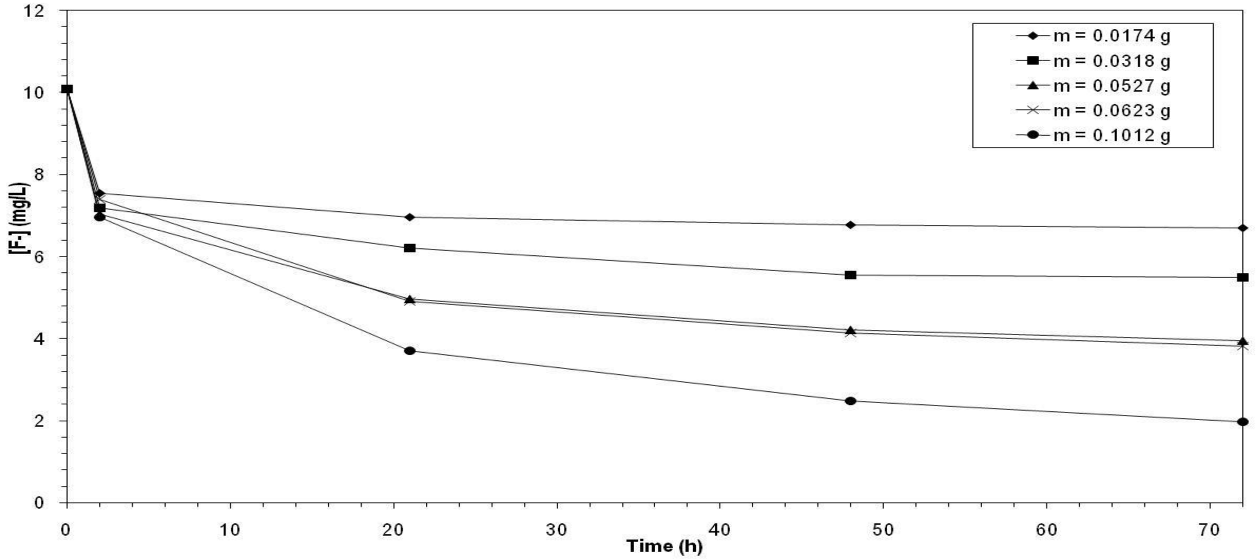

Figure 11. The evaluation of fluoride concentration during defluoridation: Effect of contact time and sorbent dose on precipitated L-HAp.

2009). Similar behavior has also been reported previously for other adsorbent (Kamble et al., 2007). The evaluation of fluoride ion concentration with contact time and sorbent dose is shown in Figure 11. We observed that the fluoride ion concentration decreases instantaneously and continuously within 2 hours, due to more active sites when increasing the dose of sorbent (Boualia et al., 1993). Within the first two hours, the sorption process is essentially controlled by ion exchange mechanism because of the rapidity of the phenomenon and because of the pH solution increases instantaneously (Figure 12). This phenomenon can be described by the following reaction:

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- \Rightarrow Ca_{10}(PO_4)_6F_2 + 2OH^-$$

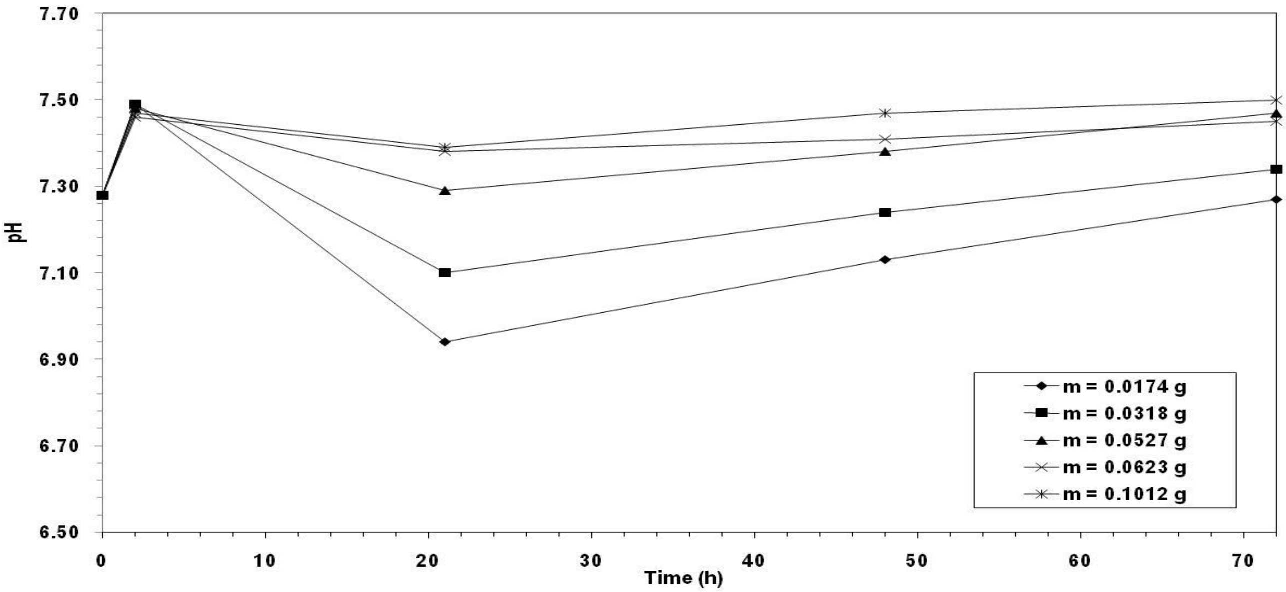

Figure 12. The evaluation of pH solution during the defluoridation: Effect of contact time and sorbent dose on precipitated L-HAp.

The uptake of fluoride can be controlled by adsorption or the dissolution-and-recrystallization mechanism. From 2 to 20 h, the fluoride ion concentration decreases with a slow rate with an increase in the dose of the sorbent, suggesting that the process is controlled by the dissolution-and-recrystallization mechanism because of the pH solution decreases in the same shape (Figure 12). This phenomenon can be described by the following reactions:

*Dissolution of Apatitic calcium phosphate:

Apatitic calcium phosphate $\Leftarrow\Rightarrow PO_4^{3-} + Ca^{2+} + HPO_4^{2-} + OH^-$

*Conversion of HPO_4^{2-} ions:

$2HPO_4^{2-} \Leftarrow\Rightarrow PO_4^{3-} + H_2PO_4^-$

*Formation of fluoroapatite:

$10Ca^{2+} + 6PO_4^{3-} + 2F^- + H_2PO_4^- \Rightarrow Ca_{10}(PO_4)_6F_2 + H_2PO_4^-$

The decrease of pH is due to the presence of $H_2PO_4^-$ which renders the solution acidic (Brown et al., 1959). From 20 to 72 h, the fluoride ion concentration decreases with a average rate with the growth in the dose of the sorbent, suggesting that the process is again controlled by ion exchange mechanism because of the pH solution increase in the same shape (Figure 11). This phenomenon can be described by the following reaction:

$Ca_{10}(PO_4)_6(OH)_2 + F^- \Rightarrow Ca_{10}(PO_4)_6FOH + OH^-$

These schemes have been proposed by Sairam et al. (2008) without precision during the process. The fluoride ion concentration reached at 72 h is $[F^-]$ = 6.70 mg/l for 0.0174 g amount of L-Hap sorbent dose, $[F^-]$ = 5.5 mg/l for 0.0318 g amount of L-Hap sorbent dose, $[F^-]$ = 3.83 mg/l for 0.0623 g amount of L-Hap sorbent dose, $[F^-]$ = 3.83 mg/l for 0.0527 g amount of L-Hap sorbent dose and $[F^-]$ = 1.98 mg/l for 0.1012 g amount of L-Hap sorbent dose. According to these results, the minimum fluoride ion concentration reached at 72 his $[F^-]$ = 1.98 mg/l for 0.1012 g amount of L-Hap sorbent dose. This value does not satisfy the level of fluoride within the tolerance limit (WHO guideline value = 0.8 mg/l).

At high pH, the solubility of the two salts does not differ greatly (Featherstone et al., 1990) (Figure 12), which explains why the uptake of fluoride in apatite is slow in this pH range.

Fluoride poisoning Koundoumawa field water

A second set of tests, with fluoride poisoning Koundoumawa field water during rainy season (Table 1) ($[F^-]$ = 2.3 mg/l) has been performed in the laboratory at the same conditions as the synthetic water. With the aim

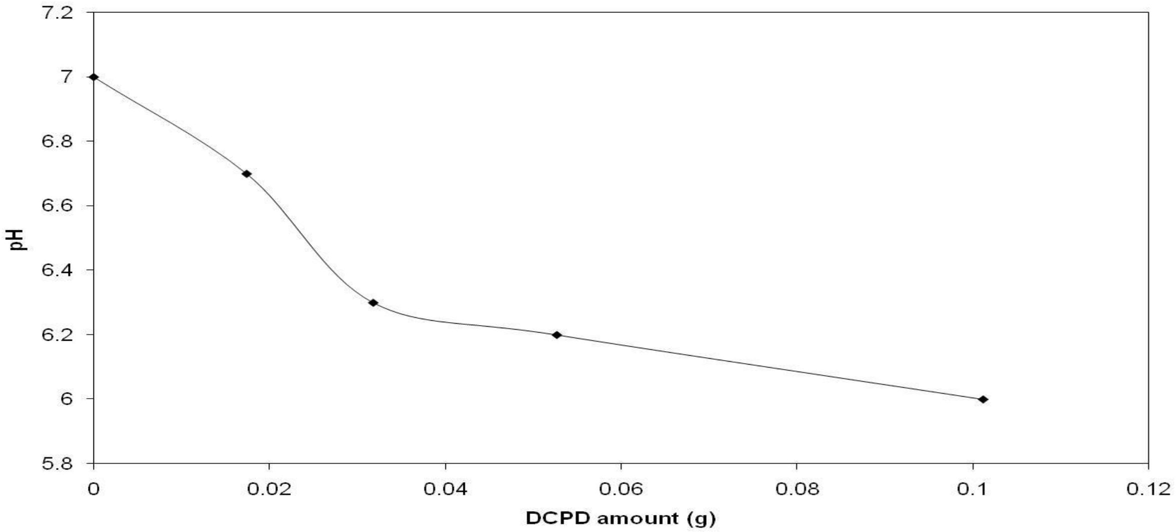

Figure 13. The evaluation of pH Koundoumawa field water during defluoridation: Effect of sorbent dose on precipitated DCPD.

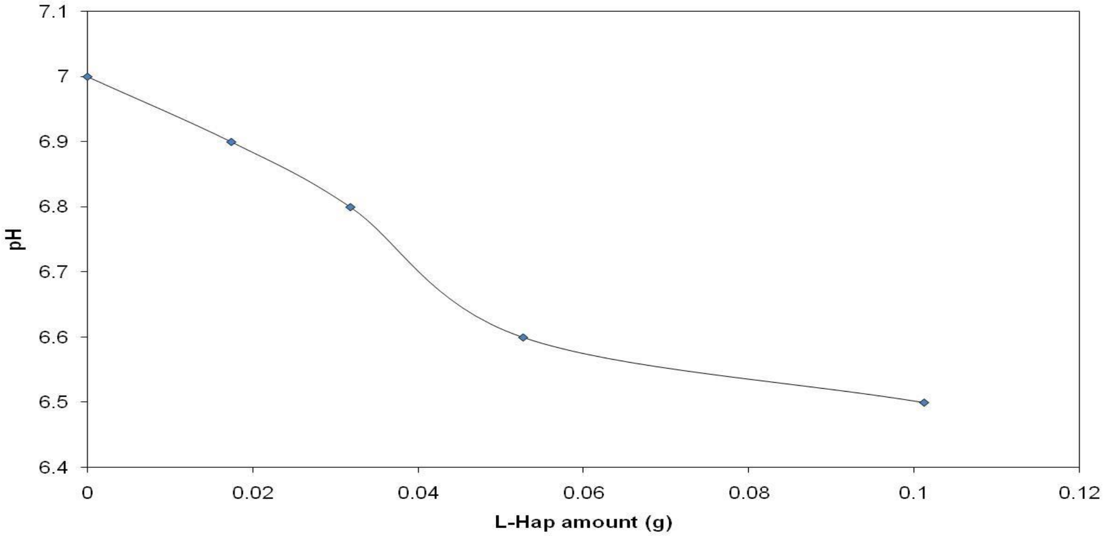

Figure 14. The evaluation of pH Koundoumawa field water during defluoridation: Effect of sorbent dose on precipitated L-HAp.

to obtain the maximum defluoridation, the contact time was fixed at 72 h. The results are presented in Figures 13, 14 and 15.

DCPD sorbent - Effect of amount: Figure 13 gives the evaluation of the pH of the aqueous solution. We observed that the pH solution decreases rapidly and continuously with increase in the dose of the DCPD sorbent. This phenomenon observed during synthetic solution defluoridation is in conformity with the observation (Lerch et al., 1966), suggesting that the process is controlled by the dissolution-and-recrystallization mechanism. The minimum pH obtained is 6.0 for 0.1012 g of sorbent.

L-Hap sorbent - Effect of amount: Figure 14 shows the evaluation of the pH of the aqueous solution. We observed that the pH solution decreases rapidly and

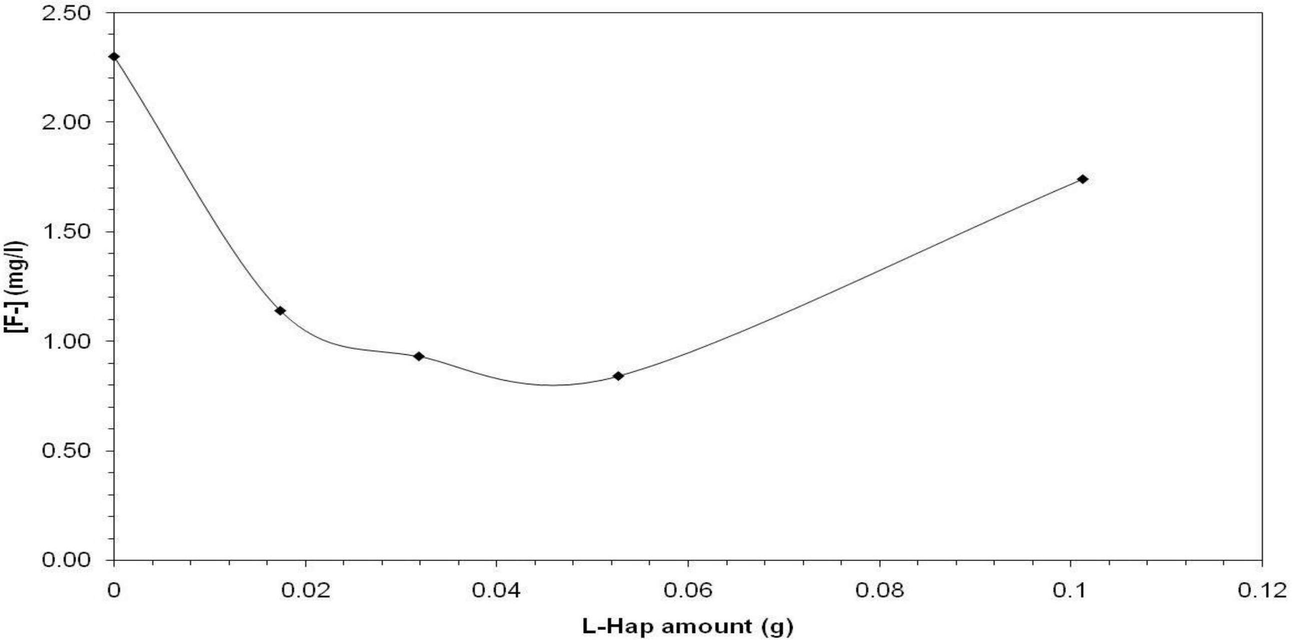

Figure 15. The evaluation of fluoride concentration of Koundoumawa field water during the defluoridation: Effect of sorbent dose of precipitated L-HAp.

continuously with an increasing dose of the L-Hap sorbent, contrary to synthetic solution. With the fluoride poisoning Koundoumawa field water, the defluoridation by L-Hap sorbent is mainly controlled by the dissolution-and-recrystallization mechanism, contrary to synthetic solution, where ion exchange is the dominant phenomenon. The minimum pH obtained is 6.5 for 0.1012 g of sorbent.

The evaluation of fluoride ion concentration with sorbent dose is shown in Figure 15. We observed that the fluoride ion concentration decreases with an increasing dose of sorbent. This phenomenon is still observed until the addition of 0.0527 g amount of L-Hap sorbent dose. The minimum fluoride ion concentration reached is 0.84 mg/l. This value satisfies the level of fluoride within the tolerance limit (WHO guideline value = 0.8 mg/l). After this dose, there is an increase of fluoride concentration, confirming the recrystallization-and-dissolution mechanism. For 0.0527 g amount of L-Hap sorbent with contact time of 72 h, the fluoride removal capacity of L-Hap is 11.63 mg/g for synthetic water and 2.80 for Koundoumawa field water. We can conclude that the presence of high concentration of bicarbonate ion in Koundoumawa field water is responsible of this low fluoride removal capacity of L-Hap during defluoridation of this water. Similar results for bicarbonate ion have also been reported (Dilip et al., 2010; Sairam et al., 2008; Sanjay et al., 2009).

Conclusion

From the present results, we can conclude as follows:

1. DCPD and L-Hap sorbents are appropriate for the defluoridation of synthetic solutions and Koundoumawa fluoride poisoning field waters.
2. The DCPD shows higher fluoride uptake capacity for defluoridation of synthetic water as compared to L-HAp. The uptake of fluoride in acidic pH is higher as compared to alkaline pH for synthetic solution.
3. The L-HAp shows higher fluoride uptake capacity for fluoride poisoning Koundoumawa field waters as compared to DCPD.
4. Within the first two hours, the sorption process is essentially controlled by ion exchange mechanism.
5. From 2 to 20 h, the sorption process is controlled by the dissolution-and-recrystallization mechanism.
6. From 20 to 72 h, the sorption process is again controlled by ion exchange mechanism.
7. The fluoride removal capacity of DCPD and L-hap decreases with a raise in sorbent dose contrary to the fluoride ion concentration.
8. With the fluoride poisoning Koundoumawa field water, the defluoridation by L-Hap sorbent is mainly controlled by the dissolution-and-recrystallization mechanism, contrary to the synthetic solution, where ion exchange is the dominant phenomenon.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Amit B, Eva K, Mika S (2011). Fluoride removal from water by adsorption - A review. Chem. Eng. J. 171(3):811-840.

Anirudhan TS, Maya RU (2007). Arsenic(V) removal from aqueous solutions using an anion exchanger derived from coconut coir pith and its recovery. Chemosphere, 66(1):60-66.

Boualia A, Mellah A, Aissaoui T, Menacer K, Silem A (1993). Adsorption of organic matter contained in industrial H_3PO_4 onto bentonite: batch-contact time and kinetic study, Appl. Clay Sci. 7:431-445.

Brown WE, Lehr JR (1959). Applictation of phase rule to the chemical behaviour of MCPM in soils. Soil Sci. Soc. Amer. Proc. 23:7-12.

Casciani FS, Condrate SrRA (1980). The infrared and Raman Spectra of Several Calcium Hydrogen Phosphates, Proc. 2^{nd} Internatl., Congress on Phosphorus compounds, Boston, pp.175-190.

Chow LC, Markovic MI (1997). Calcium phosphates in biological and industrial systems. Boston: Kluwer Academic Publishers, pp. 67-84.

Diaz-Nava C, Olguin MT, Solache-Rios M (2002). Water defluoridation by Mexican heulandite-clinoptilolite, Sep. Sci. Tech. 37:3109-3128.

Dilip T, Sadhana R, Raju K, Siddharth M, Subrt J, Nitin L (2010). Magnesium incorporated bentonite clay for defluoridation of drinking water. J. Hazard. Mater. 180:122-130.

Fan X, Parker DJ, Smith MD (2003). Adsorption kinetics of fluoride on low cost materials, Water Res. 37:4929-4937.

Featherstone JDB, Glena R, Shariati M, Shields CP (1990). Dependence ofiln vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. J. Dent. Res. 69:620-625.

Hammari LEL, Laghzizil A, Barboux P, Lahlil K, Saoiabi A (2004). Retention of fluoride ions from aqueous solution using porous hydroxyapatite: structure and conduction properties, J. Hazard. Mater. 114:41-44.

JCPDS (Joint Committee on Powder Diffraction Standards) cards, (DCPD card number 09-0077).

JCPDS (Joint Committee on Powder Diffraction Standards) cards, (L-Hap card number 9-432).

Jiménez-Reyes M, Solache-Ríos M (2010). Sorption behavior of fluoride ions from aqueous solutions by hydroxyapatite, J. Hazard. Mater. 180(1-3):297-302.

Kalimuthu P, Natrayasamy V (2014). Synthesis of alginate bioencapsulated nano-hydroxyapatite composite for selective fluoride sorption. Carbohydrate Polymers, 112:662-667.

Kamble SP, Jagtap S, Labhsetwar NK, Thakare D, Godfrey S Devotta S, Rayalu SS (2007). Defluoridation of drinking water using chitin, chitosan and lanthanum-modified chitosan. Chem. Eng. J. 129:173-180.

Kevin JR, Kenneth TS (2014). Measurement of fluoride substitution in precipitated fluorhydroxyapatite nanoparticles. J. Fluorine Chem. 161:102-109.

Larsen MJ, Pearce EIF, Jensen SJ (1993). Defluoridation of Water at High pH with Use of Brushite, Calcium Hydroxide, and Bone Char. J. Dent. Res. 72:15-19.

LeGeros RZ, Lee D, Quirolgico G, Shirra WP, Reich L (1983). In vitro formation of Dicalcium Phosphate Dihydrate $CaHPO_4.2H_2O$, Scanning Electron Microscopy, pp. 407-418.

Lerch P, Lemp R, Kraheebuhl U, Bosset J (1966). Etude de l'hydrolyse de l'orthophosphate dicalcique dihydrat´e. Chimia, 20:430-432.

Low KS, Lee CK, Leo AC (1995). Removal of metals from electroplating wastes using banana pith, Bioresour. Technol. 51:227-231.

Lusvardi G, Malavasi G, Menabue L, Saladini M (2002). Removal of cadmium ion by means of synthetic hydroxyapatite,Waste Manag. 22:853-857.

Maiti GC, Freund F (1981). Influence of fluorine substitution on the proton conductivity of hydroxyapatite. J. Chem. Soc. Dalton Trans. 4:949-955.

Mameri N, Yeddou AR, Lounici H, Lounici D, Belhocine H, Grib BB (1998). Defluoridation of serpentrional Sahara water of North Africa by electrocoagulation process using bipolar aluminium electrodes, Water Res. p.32.

Manzola AS, Mgaidi A, Laouali MS, El Maaoui M (2014). On precipitated calcium and magnesium phosphates during hard waters softening by monosodium phosphate. Desalination and water treatment: 52(25-27):4734-4744.

Masamoto T, Tetsuji C (2004). Reaction between calcium phosphate and fluoride in phosphogypsum. J. Eur. Ceramic Soc. 26(4-5):767-770.

Masanobu K, Ryohei I, Koji I (2013). Preparation and evaluation of spherical Ca-deficient hydroxyapatite granules with controlled surface microstructure as drug carriers. Mat. Sci. Eng. C. 33(4):2446-2450.

Medellin-Castillo NA, Leyva-Ramos R, Padilla-Ortega E, Ocampo-Perez R, Flores-Cano JV, Berber-Mendoza MS (2014). Adsorption capacity of bone char for removing fluoride from water solution. Role of hydroxyapatite content, adsorption mechanism and competing anions. J. Indus. Eng. Chem. 20(6):4014-4021.

Meenakshi S, Anitha P, Karthikeyan G, Appa Rao BV (1991). The pH dependence of efficiency of activated alumina in defluoridation of water, Indian. J. Environ. Prot. 11:511-513.

Meenakshi S, Viswanathan N (2007). Identification of selective ion exchange resin for fluoride sorption, J. Colloid Interface Sci. 308:438-450.

Mourabet MM, El Rhilassi A, El Boujaady H, Bennani-Ziatni M, El Hamri R, Taitai A (2012). Removal of fluoride from aqueous solution by adsorption on hydroxyapatite (HAp) using response surface methodology, J. Saoudi Chem. Soc. doi:10.1016/j.jscs.2012.03.003.

Nash CL, Liu JC (2010). Removal of phosphate and fluoride from wastewater by a hybrid precipitation–microfiltration process. Separation and Purification Technology, 74(3):329-335.

Okazaki M, Aoba T, Doi Y, Takahashi J, Moriwaki J (1981). Solubility and crystallinity in relation to fluoride content of fluoridated hydroxyapatites. J. Dent. Res. 60:845-849.

Okazaki M (1992). Heterogeneous synthesis of fluoridated hydroxyapatites. Biomat.13:749-754**.**

Rapport Mission Internationale d'Enquête de la Fédération Internationale des Ligues des Droits de l'Homme (2002), N° 341 octobre.

Rao NV, Mohan R, Bhaskaran CS (1998). Studies on defluoridation of water, J. Fluorine Chem. 41:17-24.

Sairam CS, Natrayasamy V, Meenakshi S (2008). Defluoridation chemistry of synthetic hydroxyapatite at nano scale: Equilibrium and kinetic studies, J. Hazard. Mater. 155(1-2):206-215.

Sandrine B, Ange N, Didier B, Eric C, Patrick S (2007). Removal of aqueous lead ions by hydroxyapatite: equilibria and kinetic process, J. Hazard. Mater. 139:443-446.

Sanjay PK, Priyadarshini D, Sadhana SR, Nitin KL (2009). Defluoridation of drinking water using chemically modified bentonite clay. Desalination 249(9):687-693.

Sekar C, Kanchana P, Nithyaselvi R, Girija EK (2009). Effect of fluorides (KF and NaF) on the growth of dicalcium phosphate dihydrate (DCPD) crystal. Mater. Chem. Phys. 115(1):21-27.

Smiciklas I, Dimovic S, Plecas I, Mitric M (2006). Removal of Co^{2+} from aqueous solutions by hydroxyapatite, Water Res. 40:2267-2274.

Sumit G, Rajkamal B, Sampath KTS (2012). Effects of nanocrystalline calcium deficient hydroxyapatite incorporation in glass ionomer cements. J. Mechan. Behav. Biomed. Mater. 7:69-76.

Sundaram CS, Natrayasamy V, Meenakshi S (2008). Uptake of fluoride by nano-hydroxyapatite/chitosan, a bioinorganic composite. Bioresource Technology, 99(17): 8226-8230.

Taewook Y, Chulki K, Jaeyoung J, Il Won K (2012). Regulating fluoride uptake by calcium phosphate minerals with polymeric additives. Colloids and Surfaces A: Physicochem. Eng. Aspects 401:126-136.

Takahashi T, Tanase S, Yamamoto O (1978). Electrical conductivity of some hydroxyapatites. Electrochim Acta. 23:369-373.

Tse-Ying Liu, San-Yuan Chen, Dean-Mo Liu, Sz-Chian Liou (2005). On the study of BSA-loaded calcium-deficient hydroxyapatite nano-carriers for controlled drug delivery. J. Contr. Release 107(1):112-121.

UNICEF (1998). Fluoride in water: An overview." WES (Warter Environment & Sanitation) Section, Programme Division, UNICEF 3 UN Plaza, New York, NY, USA 10017 Phone: (212) 824-6000 Fax: (212) 824-6480

Vega ED, Pedregosa JC, Narda GE, Morando PJ (2003). Removal of oxovanadium (IV) from aqueous solutions by using commercial crystalline calcium hydroxyapatite, Water Res. 37:1776-1782.

World Health Organisation (WHO) (1996). Fluoride, Guidelines for Drinking Water Quality, vol. II, 2nd ed., Geneva, pp. 231-237.

Wen L, Lei Z, Longhua P, Christian R (2011). Fluoride removal performance of glass derived hydroxyapatite. Mater. Res. Bull. 46(2):205-209.

Xiaolin Y, Shengrui T, Maofa G, Junchao Z (2013). Removal of fluoride from drinking water by cellulose@hydroxyapatite nanocomposites. Carbohydr. Polymers 92(1):269-275.

Yulun N, Chun H, Chuipeng K (2012). Enhanced fluoride adsorption using Al (III) modified calcium hydroxyapatite. J. Hazard. Mater. pp. 233-234:194-199.

Yu W, Ningping C, Wei W, Jing C, Zhenggui W (2011). Enhanced adsorption of fluoride from aqueous solution onto nanosized hydroxyapatite by low-molecular-weight organic acids, Desalination, 276(1-3):161-168.

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Full Length Research Paper

Biodegradation of petroleum oil by fungi isolated from *Treculia africana* (Dec'ne) seeds in Nigeria

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Petroleum crude oil biodegrading fungi were isolated from *Treculia africana* seeds in the presence and absence of petroleum fumes. An assessment of the relative ability of each fungus to biodegrade petroleum crude oil, kerosene, diesel, unspent engine oil, spent engine oil and extracted oil from *T. africana* seeds on minimal salt solution was investigated using changes in optical density read on a spectrophotometer and gas chromatographic analyses. Ten fungi were isolated from *T. africana* seeds in the presence and absence of petroleum fumes. These included one species each of *Mucor*, *Paecilomyces*, *Rhizopus* and *Syncephalastrum*, four species of *Aspergillus* and two species of *Penicillium*. It was evident that the fungi used in this research work were capable of biodegrading the petroleum and extracted *T. africana* seed oil hydrocarbon, though at different rates. *Rhizopus* had the highest degrading ability in kerosene, unspent engine oil, crude oil and the extracted oil from the seed, while *Penicillium pinophyllum* had the lowest ability to degrade the oil. The gas chromatogram (GC) showed that *Paecilomyces* biodegraded the hydrocarbons in the crude oil compared to the control (crude without fungi) using up some carbon atoms (C_{12}-C_{24}) after the 40 days of incubation, suggesting n-alkane biodegradation. Also the GC analysis of the seed oil of *T. africana*, after 40 days of incubation, showed a reduction in the seed oil hydrocarbons, removing C_{10}- C_{15}.

Key words: Hydrocarbon utilization, *Treculia Africana*, seeds, petroleum crude oil, fungi.

INTRODUCTION

Various microorganisms have been reported to possess the capability for utilizing hydrocarbons as their source of carbon and energy (Atlas, 1981). The ability to degrade petroleum hydrocarbons is not restricted to a few microbial genera. A diverse group of bacteria and fungi have been shown to have this ability. In a review made by Zobell (1946), more than 100 species representing 30 microbial genera have been shown to be capable of utilizing hydrocarbons. Recent studies continue to expand the list of microbial species, which have been demonstrated to be capable of degrading petroleum hydrocarbons (Nwachukwu, 2000). Microorganisms available for bioremediation include a range of bacteria like Arthrobacter, *Pseudomonas*, *Flavobacterium* and fungi such as *Penicillium, Cladosporium,*

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Aspergillus etc, isolated from water (freshwater, brackish and marine) or soil (Atlas and Bartha, 1972).

Treculia africana (Dec'ne) belongs to the family Moraceae. It contains many seeds, which are buried in the spongy pulp of the fruit. *T. africana* is a riverine fruit tree of the tropical African rain forest. The seeds are extracted after macerating the fruit in water. Analysis of the hexane extracts of *T. africana* seed indicate that it contains a stearine solid fat fraction, resembling that of palm-kernel oil, and an oleine fraction with a composition similar to that of cotton seed oil (Burkill, 1997).

Microorganisms used for biodegradation have been isolated from the soil or water (Amund et al, 1978; and Salminen et al., 2004). No report has been on the use of fungi isolated from *T. africana* seeds in the biodegradation of crude petroleum. The aim of this paper was to isolate pathogenic fungi from *T. africana* seeds. These pathogenic fungi were then used to biodegrade petroleum hydrocarbon to assess the potential ability of the fungi to biodegrade the hydrocarbons using optical density and gas chromatographic methods.

MATERIALS AND METHODS

Collection of seeds

African bread fruit (*T. africana*) seeds were collected from three different markets, Agege and Oyingbo markets in Lagos state, and Ogere market in Ogun state, Nigeria (Long 8° N Lat 4°E). Diseased seeds were separated from the healthy seeds. The visually diseased seeds were used in this experiment. The seeds were sampled from the markets monthly for 9 months. About 1000 seeds were collected from each market at every sampling period. The various oil used here were: crude oil, spent engine oil, unspent engine oil, kerosene, diesel and extracted oil from the seeds of *T. africana*.

Isolation, identification and screening for hydrocarbon degrading fungi

Sixty diseased seeds of *T. africana* were surface sterilized by soaking them in a solution of common bleach (sodium hypochloride) and sterilized distilled water in a ratio of 3:2, for 2 min and then rinsed with three changes of sterilized distilled water. To isolate fungi from the seeds under petroleum crude' oil fumes, the modified methods of Amund et al. (1987) was adopted. Sixteen filter papers (Whatman No. 1001125) were sterilized in the oven at 40°C for 15 min. Eight of the dried filter paper was dipped in 250 ml petroleum crude oil contained in a 500 ml beaker for about 15 s with the help of a sterile forcep, and drained. The petroleum crude oil was obtained from Shell Escravos Port Harcourt, Nigeria. Each of the eight crude oil treated filter papers was placed on the cover of 8 Petri dishes containing solidified potato dextrose agar (PDA) and sterilized diseased seeds (seven seeds per plate) under sterile conditions. The aim of the petroleum fumes was to supply the fungi with hydrocarbons through vapour transfer from petroleum fumes. Another set of eight Petri dishes with the PDA and *T. africana* surface sterilized seeds, were without the 'oiled' filter papers, but contained the 8 non-oil treated filter papers and they served as control. All the plates were incubated at room temperature (28 -31°C) in the incubator, and observed daily for fungal growth. This process was carried out monthly for the 9 months sampling period. The developing fungal colonies were sub cultured aseptically into fresh PDA plates to get pure culture of isolates. A part of each pure culture was then aseptically transferred into sterilized PDA slants, which was previously prepared in the 14 ml McCartney bottles, and served as stock culture.

To identify the fungi, light microscopic examination was carried out and cultural characteristics such as colour of the fungal colony, number of days taken for the fungi to reach maximum growth or diameter (9 cm) of the Petri dish, and texture of the fungal growth were noted. The morphological and cultural features of each fungus were compared with descriptions given by Talbot (1971); Deacon (1980); Domoschet et al. (1980) and Bryce (1992) for identifycation. Some mycologist (Prof N.U Uma) within the department of Botany and Microbiology, University of Lagos, was consulted for confirmatory identification of the fungi.

Extraction of oil

The method of extraction of oil of *T. africana* seeds was adopted from the oil extraction methods of Egan et al. (1981). The visually healthy seeds were ground using a ceramic pestle and mortar before blending in an electric blender. An amount of 200 g of the ground seed was packed into the extraction thimble before covering with a small ball of cotton wool. The thimble was inserted in a quick fit plain body soxhlet extractor. Petroleum ether in the quantity of 200 ml (60 - 80°C) was poured in a 250 ml round-bottom flask of known weight, which was connected to the extractor and refluxed on an electric thermal heater for 5 h. The other was then collected in the plain body extractor and then separated from the flask that contained the oil. The flask containing the oil was then heated in an oven at 103°C for 30 min. It was cooled and weighed to get the final weight. The percentage oil content of the sample was calculated using the ratio of the amount of oil produced to the weight of sample used expressed as a percentage. The procedure was repeated until at least 250 ml of the oil was extracted from the seed.

Confirmatory test for hydrogen utilization potential of the fungi

The estimation of hydrocarbon utilizers were obtained using enrichment procedure as described by Nwachukwu (2000). A minimal salt solution (MSS) containing 2.0 g of Na_2HPO_4, 0.17 g of K_2S0_4, and 0.10 g of $MgS0_4.7H_20$, 4 g of NH_4NO_3 and 0.53 g K_2S0_4 dissolved in 1000 ml distilled water and sterilized in an autoclave. Fifty-six test tubes were sterilized, plugged with cotton wrapped in aluminum foil, and placed on test-tube racks. There were 7 test-tube racks, containing eight test-tube each. In each test-tubes 10 ml of the minimal salt solution (MSS) was added and each of 6 racks had either 2 ml of petroleum crude oil, diesel, kerosene, spent engine oil, unspent engine oil or extracted oil from *T. africana* seed. The seventh rack served as control, it had only the MSS in its test-tubes. Six fungi (choice was based on their growth from seed in the petroleum fumes), were inoculated in each test-tube in the rack. The last tube (seventh tube) on each rack served as a second control, and was not inoculated with any fungus. Each of the test tubes was plugged with sterilized cotton wool wrapped with aluminum foil to ensure maximum aeration and prevent cross contamination. All the test tubes were then inoculated at the room temperature in an incubator for 40 days. Constant shaking of the test-tubes was ensured to facilitate oil/cell phase contact. The ability to degrade petroleum crude, diesel, kerosene, spent engine oil, unspent engine oil and extracted oil from *T. africana* seed (based on growth of the organism on the MSS medium) were measured every 5 days using the visual method based on the turbidity of the MSS. The turbidity was measured using the spectrophotometer at a wavelength of 530 and 620 nm. This experiment was repeated twice. Results were statistically analyzed using T-test, Anova (F-test) and Duncan multiple range test as described by Parker (1979). The percentage contribution of each fungus in biodegrading the various oil

Table 1. Fungi Isolated from diseased seeds of *Treculia africana* in the presence and absence of petroleum fumes.

***Treculia africana* seeds without petroleum fumes**	***Treculia africana* seeds with petroleum fumes**
Aspergillus flavus	*Aspergillus flavus*
Aspergillus niger	*Aspergillus niger*
Aspergillus japonicus	*Aspergillus wentii*
Aspergillus wentii	*Paecilomyces* sp
Mucor sp	*Penicillium chrysogenum*
Paecilomyces sp	*Penicillium pinophyllum*
Penicillium chrysogenum	*Rhizopus* sp
Penicillium pinophyllum	
Rhizopus sp	
Syncephalastrum sp	

(kerosene, crude oil, diesel, unspent engine oil, spent engine oil and extracted oil from seed) after the 40th day of incubation was calculated. To determine the percentage contribution of each fungus in each oil, after the 40th day, the optical density (OD) of each fungus was divided by the total OD of the various fungi in particular oil and expressed as a percentage.

Gas chromatographic (GC) analysis of some oil samples

Gas chromatographic analysis were carried out to assess and further confirm the ability of each fungi, *Paecilomyces* and *Aspergillus niger* isolated from *T. africana* seeds to biodegrade the hydrocarbons. After incubating for 40 days, as done above, the degraded hydrocarbons were extracted based on the physical changes and OD that were observed in the test-tubes after the 40 days incubation. These tubes were: (a) MSS + crude oil; (b) MSS + crude oil + *Paecilomyces*; (c) MSS + extracted oil and (d) MSS + extracted oil + *A. niger*.

The method of Song and Bartha (1990), Kampfler and Steoif (1991) and Salminen et al. (2004) was used for extraction of samples and GC analysis. For the extraction process about 20 ml hexane was used. Each sample was poured into a separating funnel and 20 ml of hexane was added and shaken well and the different oil collected. Column was prepared to get pure extracts. This was done using a 150 ml burette; the base of the burette was blocked with cotton wool to provide a base for the silca gel. About 2 0ml of hexane was poured into a beaker and about 5 g of Na_2CO_3 was added, which was the drying agent. The drying agent was poured into the burette. The silca gel helped to remove impurities.

The extract was poured into the burette and 20 ml hexane was added. The extract diffused down the column and was collected in a 14 ml McCartney bottle. All traces of hexane in the extract were allowed to evaporate by leaving the McCartney bottle opened, and the final extract was used subsequently. Gas chromatographic analysis was carried out using Perkin Elmer Auto-system GC equipped with flame ionization detector. A 30 m- fused capillary column with internal diameter of 0.25 nm and 0.25 m thickness was used, and the peak areas were analyzed with a SRT model 203 peak simple chromatography Data system. About 1-2 ml of extracted sample was injected. The column temperature was 60°C for 2 min to 300°C programmed at a rate increase of 120°C/min. Nitrogen was used as carrier gas with pressure of 30 ml. Hydrogen and air flow rates were 30 ml/min respectively.

RESULTS AND DISCUSSION

Table 1 shows the fungal species isolated from diseased seeds of *T. africana* in the presence and absence of petroleum fumes. Ten fungal species were isolated which included one species each of *Mucor, Paecilomyces, Rhizopus* and *Syncephalastrum* , four species of *Asperigillus* and two species of *Penicillium*.. More fungal species were isolated from the diseased seeds incubated without petroleum fumes. The growth pattern of fungi in the MSS and oil shows that the growth of each fungus had different maximum growth peaks (Figures 1 and 2), providing a fluctuation in the growth pattern of the fungi in the oil media. The growth pattern thus shows that the utilization of the different hydrocarbon used varied widely among the fungi. *Rhizopus* had the highest percentage contribution in the biodegradation of 33.13% in unspent engine oil while *P. pinophylum* had the least of 6.80% in kerosene (Table 2). Generally, *Rhizopus* ranked highest in the degradation of oil in all the oil used except diesel and spent engine oil (Table 3). The least in ranking in the biodegradation was *P. pinophylum* for all the oil used except in the spent engine oil as shown in Table 3.

The results of this work indicate that many of the fungal species isolated from *T. africana* seeds in the presence and absence of petroleum fumes were capable of biodegrading petroleum hydrocarbon. This *T. africana* seeds may be added to the known sources of hydrocarbon degrading fungi. The results further prove that fungi could also play a role in surviving in hydrocarbon rich environment, supporting the reports of Plante-Cunny (1993). An interesting observation in this study was the growth of each fungus in the presence of oil compared to when oil was absent. This probably means the fungi used the oil for its growth. Shaw (1995) found that microorganism breakdown hydrocarbons and use the energy to synthesize cellular components. After being completely broken down the reaction releases $C0_2$, H_20 and energy used to create cellular biomass (Keeler, 1996). It is evident from the results obtained that the fungi were more active in the extracted oil than in other oil (Figure 2). This may be due to the fact that the fungi were isolated from the *T. africana* seed where the oil was extracted, and these fungi have being adapted to using the seed oil hydrocarbon as their source of carbon. Also the

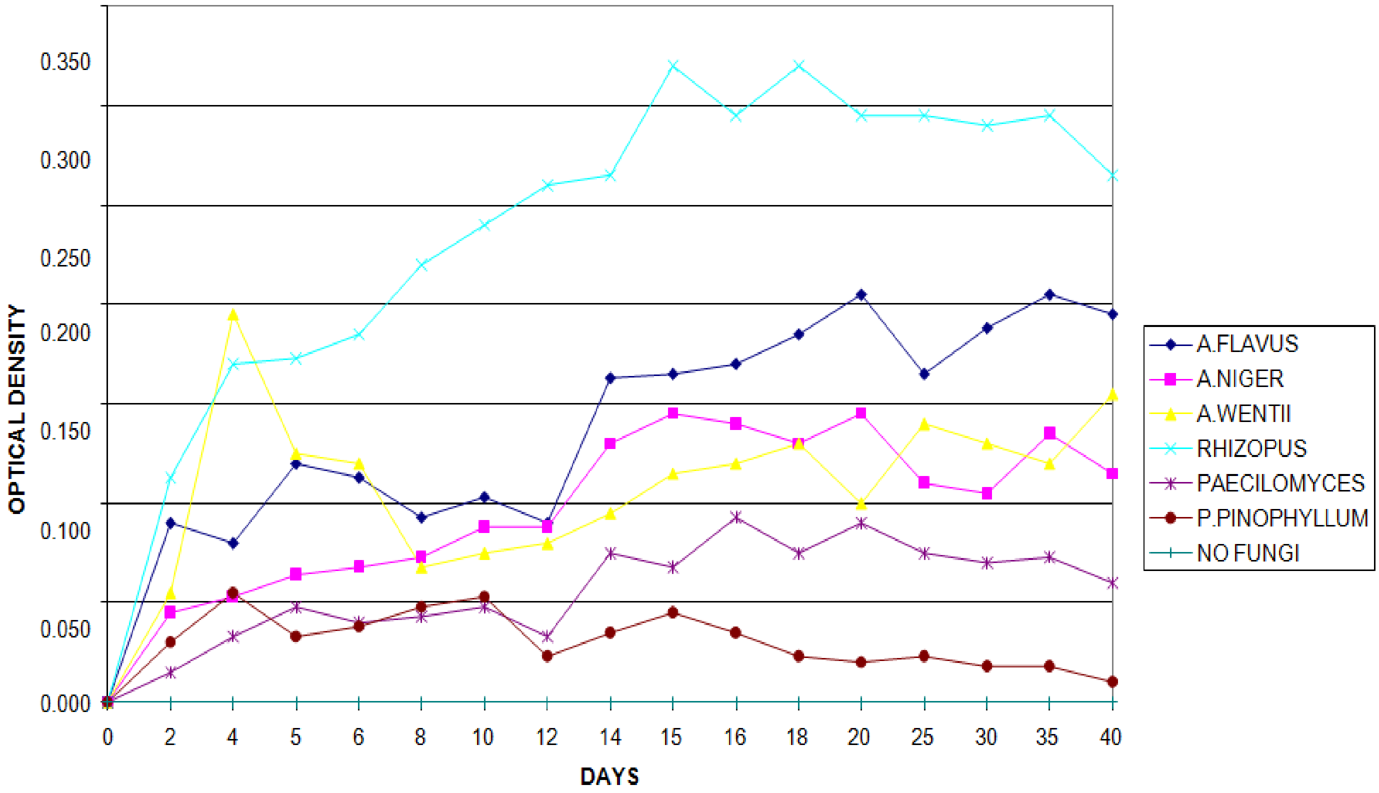

Figure 1. The growth pattern of fungi in minimal salt solution and unspent engine oil using 530 nm.

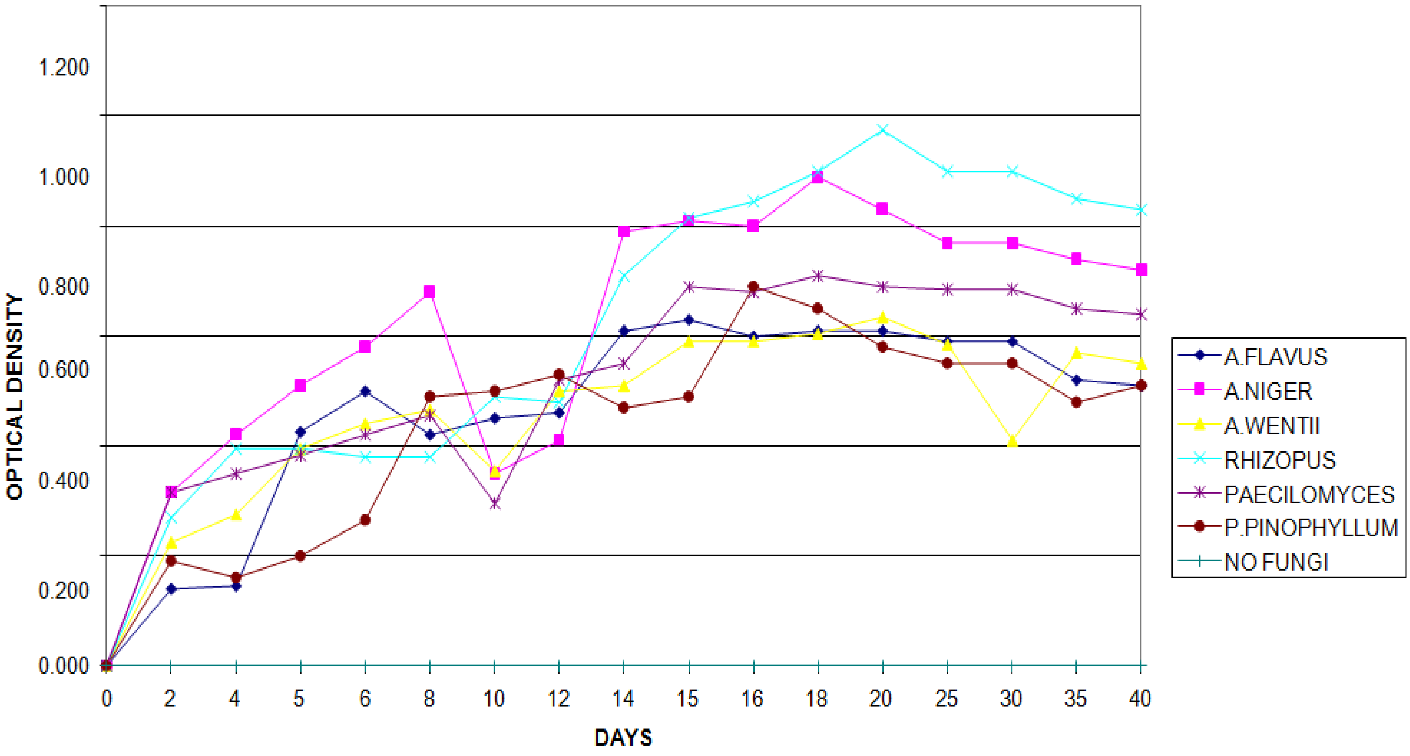

Figure 2. The growth pattern of fungi in minimal salt solution and extracted oil from *Treculia africana* seed using 530. nm

Table 2. Percentage contribution of Biodegradation of each fungus in the different oil after 40 days incubation.

Oil	Percentage contribution of biodegradation of each fungus (%)					
	Aspergillus flavus	*Aspergillus niger*	*Aspergillus wentii*	*Rhizoipus*	*Paecilomyces*	*Penicillium pinophyllum*
Kerosene	20. 87	14.08	16.51	27.19	14.56	6.80
Diesel	17.83	20.93	11.63	18.61	20.61	10.85
Unspent engine oil	24.38	14.38	19.38	33.31	7.50	1.25
Spent engine oil	14.63	18.58	21.74	19.37	11.46	14.23
Crude oil	17.65	18.70	17.85	19.26	14.16	12.45
Extracted oil from the *Treculia africana* seed	13.56	19.15	14.63	22.08	17.02	13.56

Table 3. The order of biodegradation of the various fungi on the different oil after 40 days of incubation.

Oil	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus wentii*	*Paecilomyces* sp	*Penicillium pinophyllum*	*Rhizoipus* sp.
Kerosene	2*	5	3	4	6	1
Diesel	4	1	5	2	6	3
Unspent engine oil	2	4	3	5	6	1
Spent engine oil	4	3	1	6	5	2
Crude oil	4	2	3	5	6	1
Extracted oil from the *T. africana* seed	5	4	4	3	6	1

* Ranking 1 to 6 represents the order of biodegradation from the highest to the lowest.

various fungi were less active in kerosene compared to other oil, which is probably due to the hydrocarbon present in kerosene. Amanchukwu et al. (1989) observed that most microorganisms find it difficult biodegrading kerosene, attributing this to its type of hydrocarbon chain. Increased turbidity and emulsification of the oil was observed during the course of these investigations. Emulsification is a known part of hydrocarbon degradation (Geyer, 1980), and might be a probable indication of hydrocarbon utilization by the fungi.

The chromatogram of the MSS and crude oil (control), shows the detection of C_{12} - C_{24}; the peaks for these carbons were obvious (Figure 3). The growth of *Paecilomyces* in MSS and crude oil indicates a complete disappearance of the peaks in C_{12} - C_{24}, after 40 days incubation (Figure 4). It was only C_{12} peak that was not completely absent but reduced in the chromatogram (Figure 4). Also the chromatogram for the MSS and extracted oil from seed (control), C_{12} - C_{24} were detected and their peaks were obvious (Figure 5). The chromatogram of the growth of Aspergillus *niger in* MSS and the extracted oil from *T. africana* seed after 40 days incubation showed that the peaks of C_{10}-C_{16} were absent (Figure 6). The peak system chromatogram could not detect some of the carbons in the oil artificially inoculated with *A. niger*, and P*aecilomyces* probably due to the biodegradation of the oil by these fungi suggesting n-alkane biodegradation. This observation is similar to the work of Salminen et al. (2004) who worked on the potential for aerobic biodegradation of petroleum hydrocarbons in boreal subsurface, suggesting an n-alkane degradation due to the removal of C_n-C_{15} in their study.

In conclusion, it is evident from the results in this work that all the fungi isolated from *T. africana* seed have the potential to biodegrade petroleum crude oil and petroleum products.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

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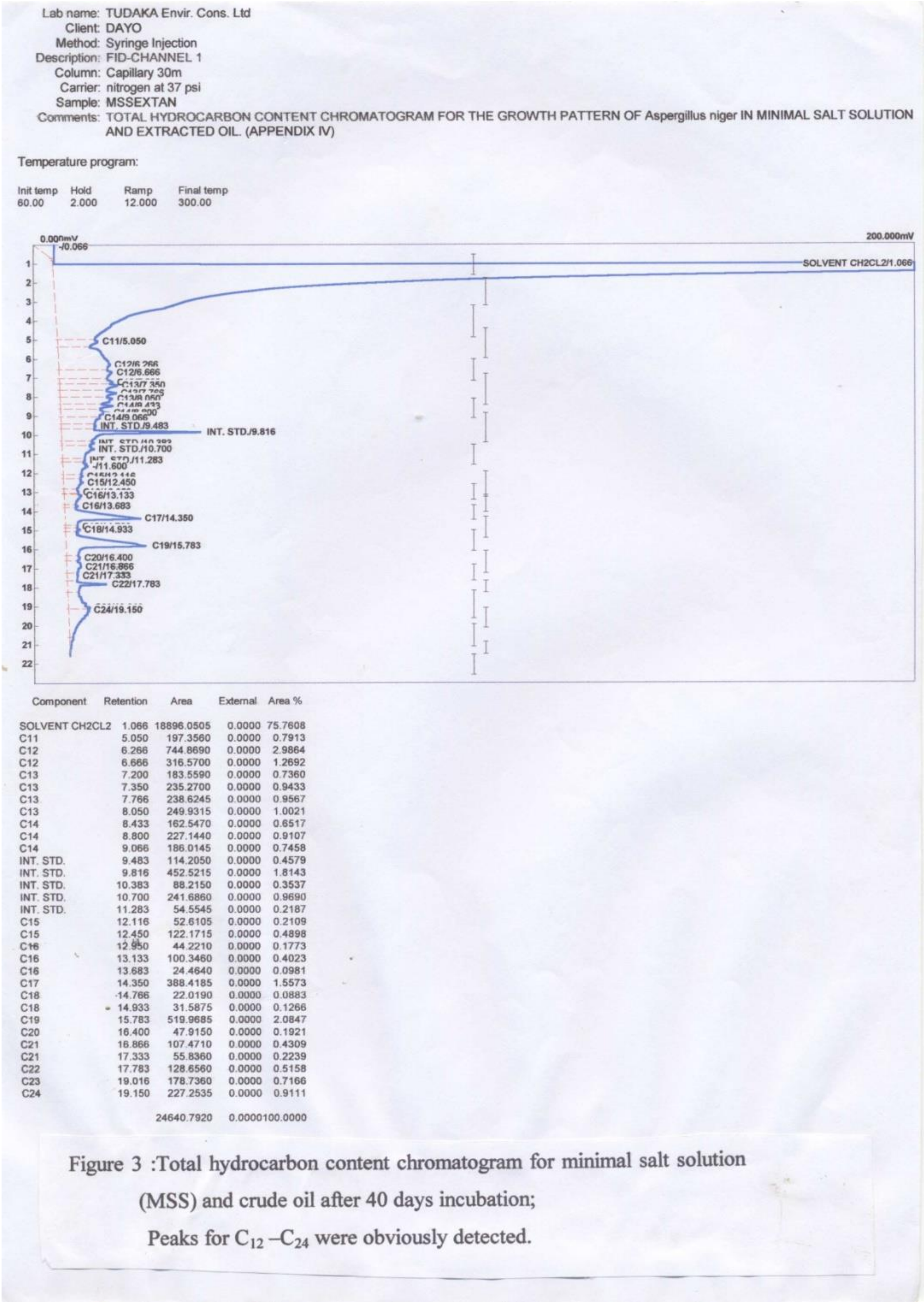


Lab name: TUDAKA Envir. Cons. Ltd
Client: DAYO
Method: Syringe Injection
Description: FID-CHANNEL 1
Column: Capillary 30m
Carrier: nitrogen at 37 psi
Sample: MSSEXTAN
Comments: TOTAL HYDROCARBON CONTENT CHROMATOGRAM FOR THE GROWTH PATTERN OF Aspergillus niger IN MINIMAL SALT SOLUTION AND EXTRACTED OIL. (APPENDIX IV)

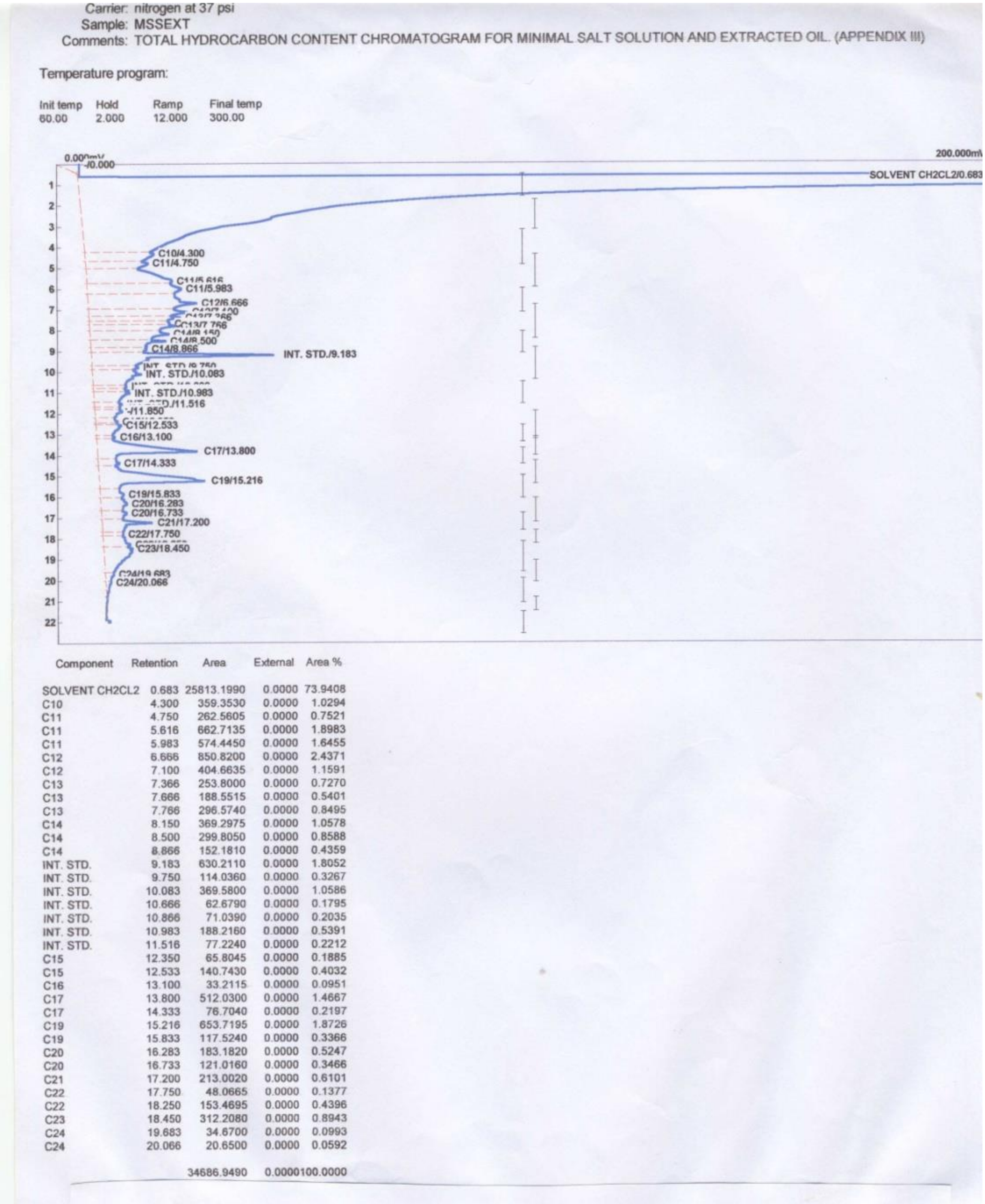
Temperature program:

Init temp	Hold	Ramp	Final temp
60.00	2.000	12.000	300.00

Component	Retention	Area	External	Area %
SOLVENT CH2CL2	1.066	18896.0505	0.0000	75.7608
C11	5.050	197.3560	0.0000	0.7913
C12	6.266	744.8690	0.0000	2.9864
C12	6.666	316.5700	0.0000	1.2692
C13	7.200	183.5590	0.0000	0.7360
C13	7.350	235.2700	0.0000	0.9433
C13	7.766	238.6245	0.0000	0.9567
C13	8.050	249.9315	0.0000	1.0021
C14	8.433	162.5470	0.0000	0.6517
C14	8.800	227.1440	0.0000	0.9107
C14	9.066	186.0145	0.0000	0.7458
INT. STD.	9.483	114.2050	0.0000	0.4579
INT. STD.	9.816	452.5215	0.0000	1.8143
INT. STD.	10.383	88.2150	0.0000	0.3537
INT. STD.	10.700	241.6860	0.0000	0.9690
INT. STD.	11.283	54.5545	0.0000	0.2187
C15	12.116	52.6105	0.0000	0.2109
C15	12.450	122.1715	0.0000	0.4898
C16	12.950	44.2210	0.0000	0.1773
C16	13.133	100.3460	0.0000	0.4023
C16	13.683	24.4640	0.0000	0.0981
C17	14.350	388.4185	0.0000	1.5573
C18	14.766	22.0190	0.0000	0.0883
C18	14.933	31.5875	0.0000	0.1266
C19	15.783	519.9685	0.0000	2.0847
C20	16.400	47.9150	0.0000	0.1921
C21	16.866	107.4710	0.0000	0.4309
C21	17.333	55.8360	0.0000	0.2239
C22	17.783	128.6560	0.0000	0.5158
C23	19.016	178.7360	0.0000	0.7166
C24	19.150	227.2535	0.0000	0.9111
		24640.7920	0.0000	100.0000

Figure 3 :Total hydrocarbon content chromatogram for minimal salt solution (MSS) and crude oil after 40 days incubation; Peaks for C_{12} –C_{24} were obviously detected.

Figure 3. Total hydrocarbon content chromatogram for minimal salt solution (MSS) and crude oil after 40 days incubation; peaks for C_{12}- C_{24} were obviously detected.



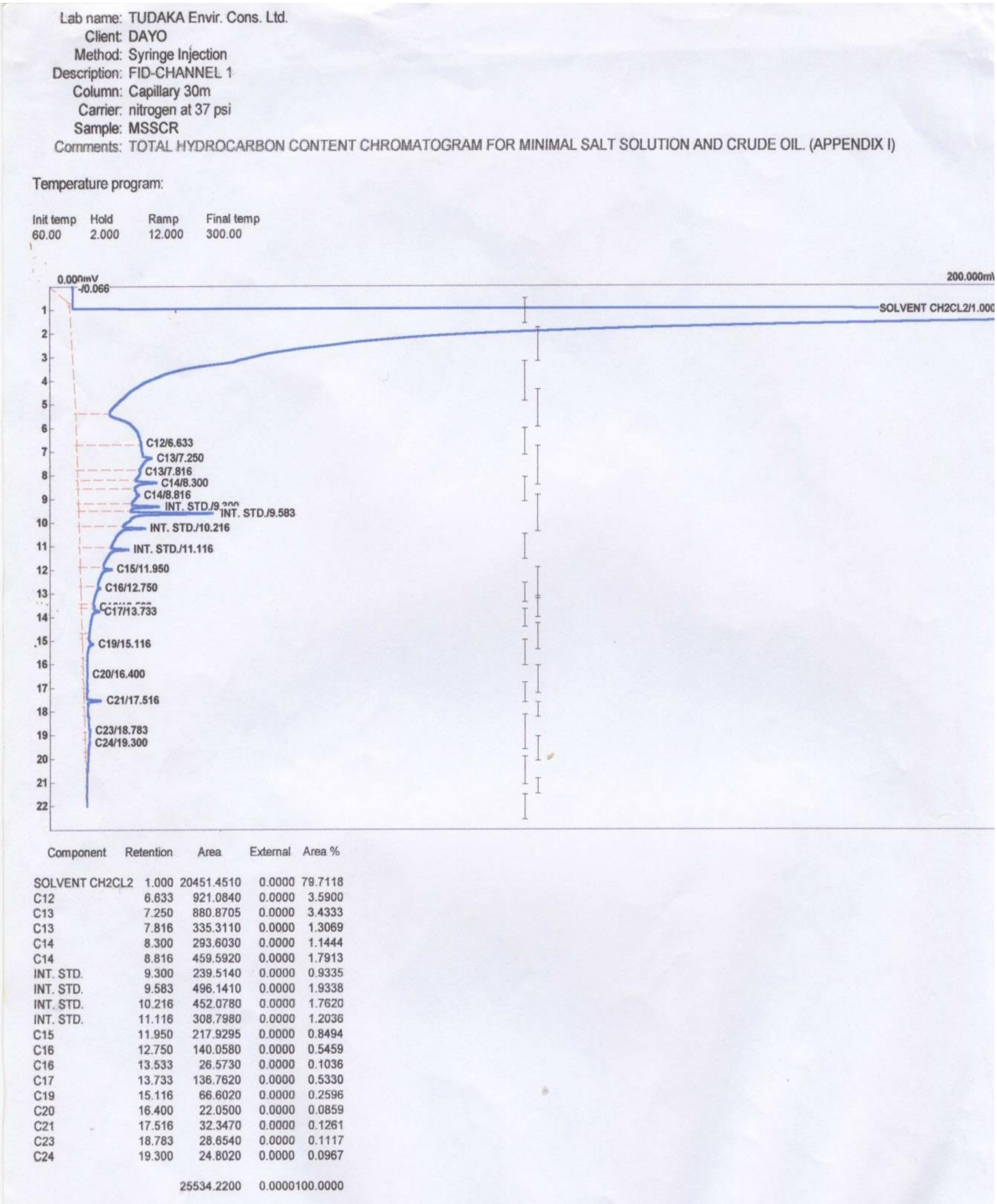
Carrier: nitrogen at 37 psi
Sample: MSSEXT
Comments: TOTAL HYDROCARBON CONTENT CHROMATOGRAM FOR MINIMAL SALT SOLUTION AND EXTRACTED OIL. (APPENDIX III)

Temperature program:

Init temp	Hold	Ramp	Final temp
60.00	2.000	12.000	300.00

Component	Retention	Area	External	Area %
SOLVENT CH2CL2	0.683	25813.1990	0.0000	73.9408
C10	4.300	359.3530	0.0000	1.0294
C11	4.750	262.5605	0.0000	0.7521
C11	5.616	662.7135	0.0000	1.8983
C11	5.983	574.4450	0.0000	1.6455
C12	6.666	850.8200	0.0000	2.4371
C12	7.100	404.6635	0.0000	1.1591
C13	7.366	253.8000	0.0000	0.7270
C13	7.666	188.5515	0.0000	0.5401
C13	7.766	296.5740	0.0000	0.8495
C14	8.150	369.2975	0.0000	1.0578
C14	8.500	299.8050	0.0000	0.8588
C14	8.866	152.1810	0.0000	0.4359
INT. STD.	9.183	630.2110	0.0000	1.8052
INT. STD.	9.750	114.0360	0.0000	0.3267
INT. STD.	10.083	369.5800	0.0000	1.0586
INT. STD.	10.666	62.6790	0.0000	0.1795
INT. STD.	10.866	71.0390	0.0000	0.2035
INT. STD.	10.983	188.2160	0.0000	0.5391
INT. STD.	11.516	77.2240	0.0000	0.2212
C15	12.350	65.8045	0.0000	0.1885
C15	12.533	140.7430	0.0000	0.4032
C16	13.100	33.2115	0.0000	0.0951
C17	13.800	512.0300	0.0000	1.4667
C17	14.333	76.7040	0.0000	0.2197
C19	15.216	653.7195	0.0000	1.8726
C19	15.833	117.5240	0.0000	0.3366
C20	16.283	183.1820	0.0000	0.5247
C20	16.733	121.0160	0.0000	0.3466
C21	17.200	213.0020	0.0000	0.6101
C22	17.750	48.0665	0.0000	0.1377
C22	18.250	153.4695	0.0000	0.4396
C23	18.450	312.2080	0.0000	0.8943
C24	19.683	34.6700	0.0000	0.0993
C24	20.066	20.6500	0.0000	0.0592
		34686.9490	0.0000	100.0000

Figure 4. Total hydrocarbon content chromatogram for the growth of paecilomyces in MSS and crude oil after 40 days incubation; peaks for C_{12}- C_{24} were completely absent except C_{21} peak that was only reduced.



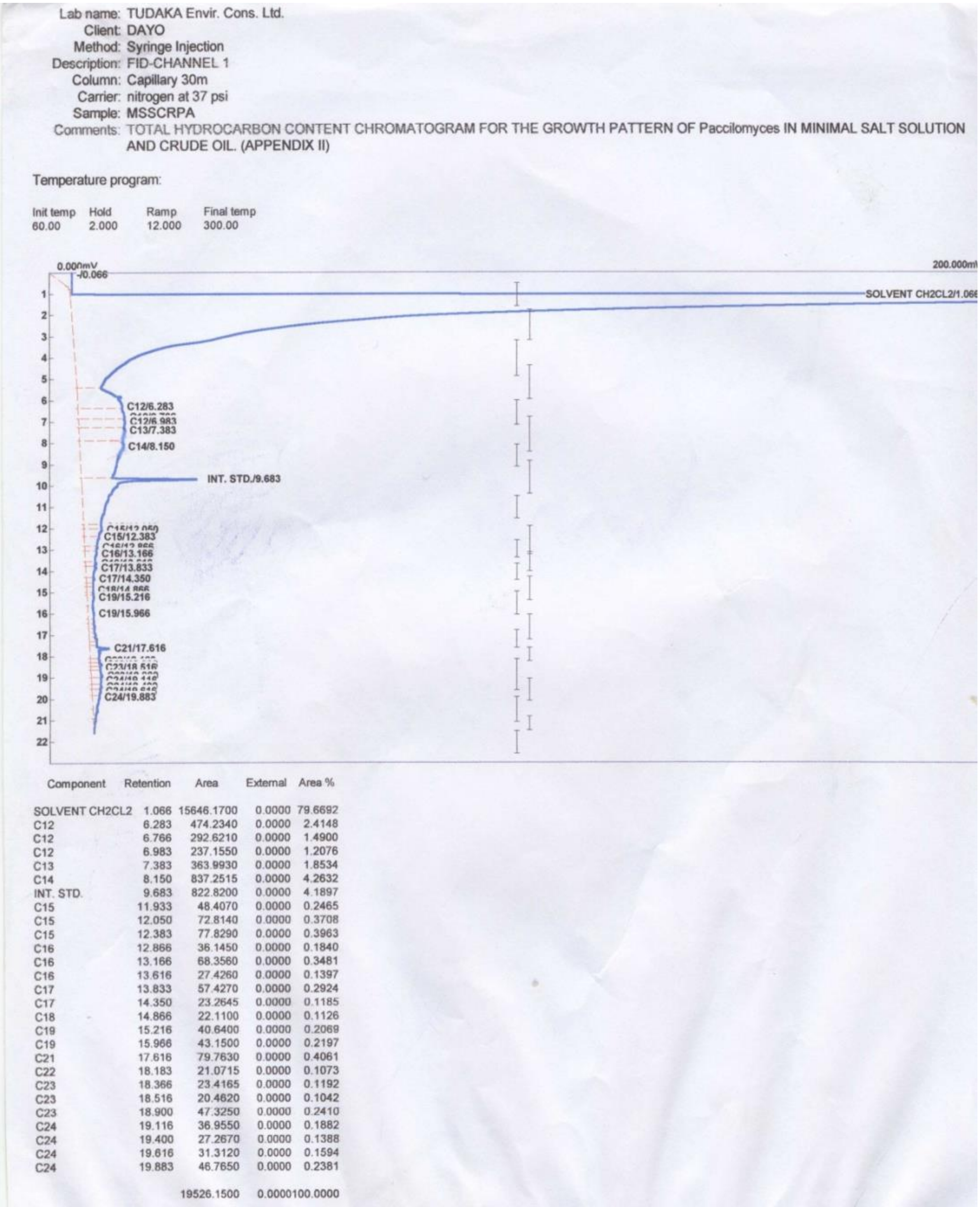
Lab name: TUDAKA Envir. Cons. Ltd.
Client: DAYO
Method: Syringe Injection
Description: FID-CHANNEL 1
Column: Capillary 30m
Carrier: nitrogen at 37 psi
Sample: MSSCR
Comments: TOTAL HYDROCARBON CONTENT CHROMATOGRAM FOR MINIMAL SALT SOLUTION AND CRUDE OIL. (APPENDIX I)

Temperature program:

Init temp	Hold	Ramp	Final temp
60.00	2.000	12.000	300.00

Component	Retention	Area	External	Area %
SOLVENT CH2CL2	1.000	20451.4510	0.0000	79.7118
C12	6.633	921.0840	0.0000	3.5900
C13	7.250	880.8705	0.0000	3.4333
C13	7.816	335.3110	0.0000	1.3069
C14	8.300	293.6030	0.0000	1.1444
C14	8.816	459.5920	0.0000	1.7913
INT. STD.	9.300	239.5140	0.0000	0.9335
INT. STD.	9.583	496.1410	0.0000	1.9338
INT. STD.	10.216	452.0780	0.0000	1.7620
INT. STD.	11.116	308.7980	0.0000	1.2036
C15	11.950	217.9295	0.0000	0.8494
C16	12.750	140.0580	0.0000	0.5459
C16	13.533	26.5730	0.0000	0.1036
C17	13.733	136.7620	0.0000	0.5330
C19	15.116	66.6020	0.0000	0.2596
C20	16.400	22.0500	0.0000	0.0859
C21	17.516	32.3470	0.0000	0.1261
C23	18.783	28.6540	0.0000	0.1117
C24	19.300	24.8020	0.0000	0.0967
		25534.2200	0.0000	100.0000

Figure 5. Total hydrocarbon content chromatogram for minimal salt solution (MSS) and extracted oil after 40 days incubation; peaks for C10-C24 were obviously detected.



Lab name: TUDAKA Envir. Cons. Ltd.
Client: DAYO
Method: Syringe Injection
Description: FID-CHANNEL 1
Column: Capillary 30m
Carrier: nitrogen at 37 psi
Sample: MSSCRPA
Comments: TOTAL HYDROCARBON CONTENT CHROMATOGRAM FOR THE GROWTH PATTERN OF Paccilomyces IN MINIMAL SALT SOLUTION AND CRUDE OIL. (APPENDIX II)

Temperature program:

Init temp	Hold	Ramp	Final temp
60.00	2.000	12.000	300.00

Component	Retention	Area	External	Area %
SOLVENT CH2CL2	1.066	15646.1700	0.0000	79.6692
C12	6.283	474.2340	0.0000	2.4148
C12	6.766	292.6210	0.0000	1.4900
C12	6.983	237.1550	0.0000	1.2076
C13	7.383	363.9930	0.0000	1.8534
C14	8.150	837.2515	0.0000	4.2632
INT. STD.	9.683	822.8200	0.0000	4.1897
C15	11.933	48.4070	0.0000	0.2465
C15	12.050	72.8140	0.0000	0.3708
C15	12.383	77.8290	0.0000	0.3963
C16	12.866	36.1450	0.0000	0.1840
C16	13.166	68.3560	0.0000	0.3481
C16	13.616	27.4260	0.0000	0.1397
C17	13.833	57.4270	0.0000	0.2924
C17	14.350	23.2645	0.0000	0.1185
C18	14.866	22.1100	0.0000	0.1126
C19	15.216	40.6400	0.0000	0.2069
C19	15.966	43.1500	0.0000	0.2197
C21	17.616	79.7630	0.0000	0.4061
C22	18.183	21.0715	0.0000	0.1073
C23	18.366	23.4165	0.0000	0.1192
C23	18.516	20.4620	0.0000	0.1042
C23	18.900	47.3250	0.0000	0.2410
C24	19.116	36.9550	0.0000	0.1882
C24	19.400	27.2670	0.0000	0.1388
C24	19.616	31.3120	0.0000	0.1594
C24	19.883	46.7650	0.0000	0.2381
		19526.1500	0.0000	100.0000

Figure 6. Total hydrocarbon content chromatogram for the growth of *Aspergillus niger* in minimal salt solution (MSS) and extracted oil after 40 days incubation; peaks for C10- C16 were absent or reduced detected.

REFERENCES

Amanchukwu SC, Obafemi A,Okpokwasili AN (1989). Hydrocarbon degradation and utilization by a palm wine yeast isolate. FEMS microbiology 57:151-154.

Amund DO, Adebowale AA, Ugoji EO (1987). Occurrence and characteristics of hydrocarbon utilizing bacteria in Nigerian soils contaminated with spent motor oil. Ind. J. Microbiol. 27:63-87.

Atlas RM (1981). Microbial degradation of petroleum hydrocarbon: An Environment Perspective. Microbiol Rev 45: 180-209.

Atlas RM, Bartha R (1972). Biodegradation of Petroleum in seawater low temperatures. Canadian Journal of Microbiology .22:1851-1855.

Bryce K (1992). The Fifth Kingdom. Mycologue publications, Ontario. 412pp.

Burkill HM (1997). The useful plants of West Tropical Africa. Vol. 4. Royal Botanic gardens, Kew. 960 pp.

Deacon JW (1980). Introduction to modern mycology. Blackwell scientific publications, London. 197 pp.

Domoschet KHW, Gams W, Anderson T (1980). Compendium of Soil fungi. Vol.1. Academic Press, London. 859 pp.

Egan H, Kirk HS, Sawyer R (1981). Pearson's Analysis of Foods. Churchill Living Stone Publishers, London, 596 pp.

Geyer RA (1980). Marine Environmental Pollution. Elsevier Company, New York. 35 pp.

Kampfer P, Steoif M (1991). Microbiological characteristics of a fuel oil contaminated site, including a numerical identification of heterotrophic water and soil bacteria. Microb. Ecol. 21:227-251.

Keeler R (1991). 'Bioremediation' Healing the environment naturally. Research and Development Magazine 2:139-40.

Nwachukwu, S.C.W. (2000). Enhanced rehabilitation of tropical Aquatic Environments polluted with crude petroleum using *Candida utilis*. J. Environ. Biol. 21 (3):241-250.

Parker RE (1979). Introductory Statistics for Biology. 2nd Edition. Edward Arnold, London. 112 pp.

Plante - Cunney MR (1993). Experimental field study of crude oil, drills, cuttings and natural biodeposits on mirco-phyto communities in a Mediterranean Area. Marine Biol. 117:355 - 366.

Salminen, J.M; Tuomi, P.M.; Suortti, P.M and Jorgensen, K.S. (2004). Potential Biodegradation of Petroleum hydrocarbons in boreal subsurface. Biodegradation. 15(1):29-39.

Shaw B (1995). Microbes safety, effective bioremediation oil field pits. Oil and gas J. 4:85-88.

Song, H and Bartha, R. (1990). Effects of jet fuel spills on the microbial community of a soil. Appl. Environ.Microbiol. 56:646-651.

Talbot OHK (1971). An Introduction to Mycology. Leonard Hill, London. 252 pp.

Zobell CE (1946). Action of Microorganisms on hydrocarbons. Bacterial. Rev. 10:1-49.

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Full Length Research Paper

Effectiveness of neem, cashew and mango trees in the uptake of heavy metals in mechanic village, Nigeria

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The concentrations of heavy metals were determined from the soil of the mechanic village, in Abeokuta and a control farmland located at Federal University of Agriculture, Abeokuta (FUNAAB). The soil sample collected at the base of different species of tree showed that the heavy metals were below permissible levels (FAO/WHO and EC/CODEX standard) and show no significant difference in the range of mean. Absorption of heavy metals by the bark of the trees in the mechanic village was evident when compared relatively to the presence and uptake of the heavy metals from the soil by tree in the farmland. The mean concentrations of the heavy metals in the soil of the farmland are in this order of magnitude Cd>Cu>Pb, while the mean concentration of the heavy metals in the soil of the mechanic village are in the order of magnitude Pb>Cu>Cd. Lead has the least concentration in the farmland, while in the mechanic village, it is the predominant heavy metal detected which also shows greater significant different at $p<0.05$ with a value of 24.34 mg/kg indicating area of high mechanic activities. The concentration values of heavy metals in the barks in comparison to the standard shows that the concentration of the heavy metals in those vicinities is within the permissible range for cadmium and copper, while lead present was above the WHO/FAO standard at 0.299 mg/kg and close to the EC/CODEX standard. It can also be concluded that the uptake efficiency of heavy metal under study of the three species are in the order magnitude Mango>Cashew>Neem.

Key words: Absorption, concentration, farmland, magnitude, phytoremediation.

INTRODUCTION

Mechanic Villages or workshop engage in the finishing processes of oil, paints, fuels and other Heavy metals which are inevitably discharge into the soil and render the soil derelict and infertile for live except if proper remediation is done to revert the already damage soil to fertility. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body (Hinchman et al., 1995). Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soil (Bieby et al., 2011).

Heavy metals are the most dangerous contaminants since they are persistent and accumulate in water, sediments and in tissues of the living organisms, through

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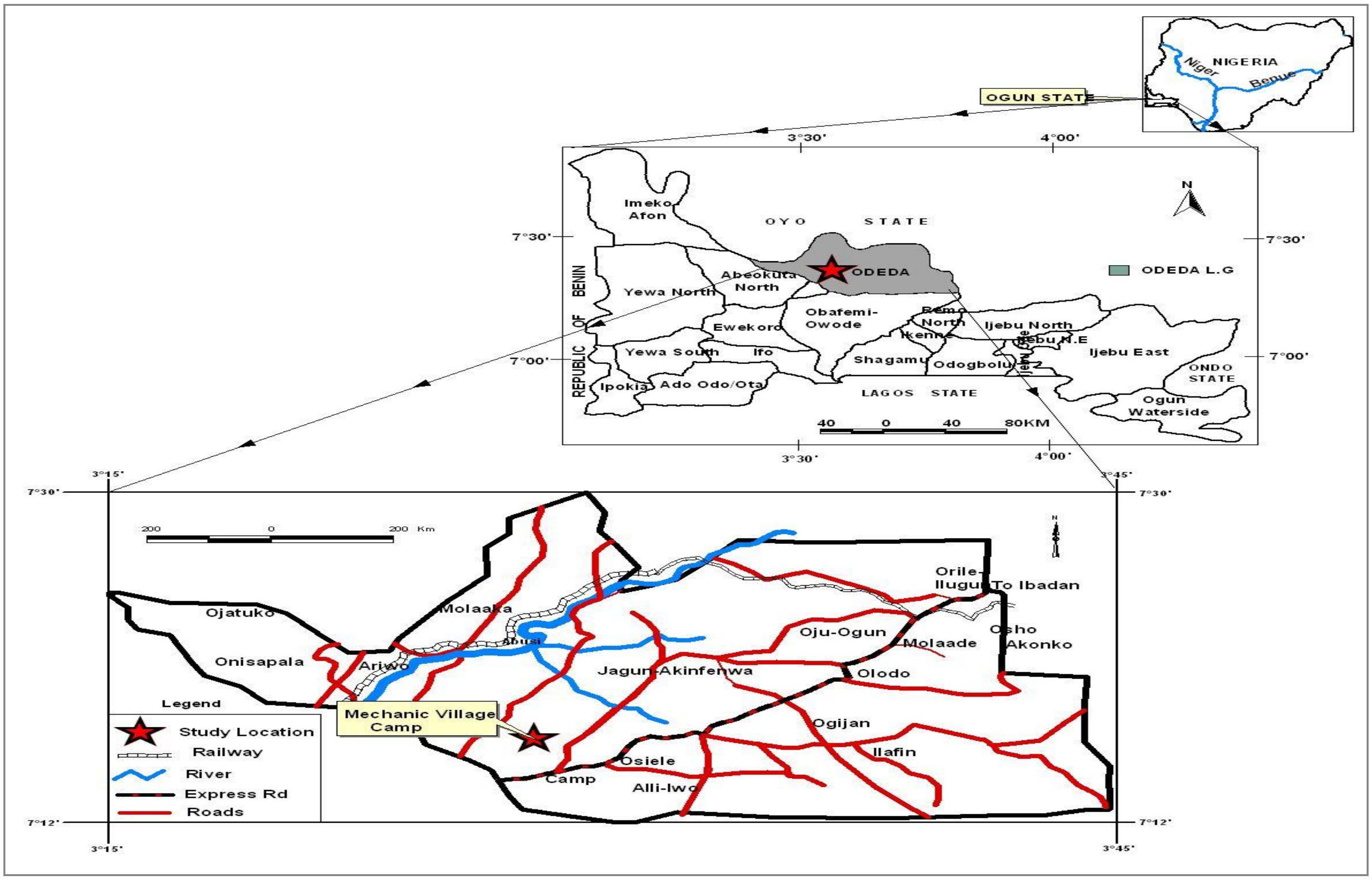

Figure 1. Map of Odeda local government, indicating the mechanic village. Source: GIS laboratory, Federal University of Agriculture Abeokuta, 2014.

two mechanisms, namely "bioconcentration" (uptake from the ambient environment) and "biomagnification" (uptake through the food chain) (Chaphekar, 1991). Hyperaccumulators are plants that can absorb high levels of contaminants concentrated either in their roots, shoots and/or leaves (Penkala, 2005). Baker and Brooks have defined metal hyperaccumulator as plants that contain more than or up to 0.1% that is more than (1000 mg/g) of copper, cadmium, chromium, lead, nickel cobalt or 1% (> 10,000 mg/g) of zinc or manganese in the dry matter.

Various plants have been used as bioindicators to assess the impact of a pollution source on the vicinity which is due to high metal accumulation of plants (Onder and Dursun, 2006). Devendra et al. (2013), have investigated in their study of the bioindicators: A comparative study on uptake and accumulation of heavy metals in some plant's leaves in India. Majolagbe et al. (2010), had investigated 10 different species of trees from 42 sampling locations taken in Oyo town, southwest, Nigeria using Corn (Zea Mays) Grown on Contaminated Soil in Heavy Metal Uptake with comparable results of that of Akhionbare et al. (2010). Lawal et al. (2011) carried out an estimation of heavy metals in Neem tree leaves along Katsina – Dutsinma – Funtua Highway in Katsina State of Nigeria. Bieby et al. (2011) conducted a review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Raskin et al. (1997) understudied phytoremediation of metals using plants to remove pollutants from the environment. Thus establishing the importance for the study of Neem (*Azadirachta indica*), cashew (*Anacardium occidentale*), and mango (*Manifera indica*) trees as possible bioindicators of heavy metals in the environment.

The mechanic village studied is located along the Abeokuta – Ibadan expressway, Odeda local government, Ogun state, Nigeria. It covers a large span of land and serves as the major mechanic workshop for the Abeokuta metropolis and other neighboring states as shown in Figure 1. The landscape is covered with the presence of cover trees such as Neem ((*Azadirachta indica*), cashew (*Anacardium occidentale*), and mango (*Manifera indica*) trees in the land area, and it serve as shades to the mechanics and raw material for the production of herbal medicine.

But due to the activities in the mechanic village which majorly produces non-biodegradable waste that contains heavy metals such as Lead (Pb), Cadmium (Cd) and Chromium (Cr), trees now becomes one of the major avenue of absorbing these substances from the soil and because they are not bio-degradable it posses adverse effect on the plants development (Garba et al., 2013). The objectives of this research was to determine the effectiveness of Neem, Cashew, and Mango trees in uptake of heavy metals in mechanic village as well as evaluate the concentration of heavy metals by the barks of the trees in comparison to a control and WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residue and finally to examine the effectiveness of the uptake of heavy metals as a prospect of phytoremediation in the study area.

MATERIALS AND METHODS

Soil samples was collected 10 m from the base of each species of trees to be sampled, at a depth of 0–15 cm (top soil) and 15-30 cm (subsoil) of the soil, using a soil auger and collected in polythene bags, then transported to the laboratory where it is air dried. The same procedure was repeated for FUNAAB farmland which is act as the controlled to the experiment.

Tree barks was also collected by cutting from the top, middle and bottom of the trunk of the tree with the aid of pre-washed stainless knife, and further washed after each sampling with 10% nitric acid to avoid cross contamination. The bark sample was wrapped with paper, and kept in a polythene material and thereafter transported to the laboratory.

Physical and chemical properties of the soil samples such as pH, electrical conductivity, temperature using pH metre, and total dissolved solid was first analyzed, followed by the determination of total content of Copper (cu), Lead (Pb), and Cadmium (Cd) using spectrophotometric method. Same parameters were analyzed for the bark of the different species of trees.

A composite samples of the tree bark of the trees analyzed which includes Neem, Cashew, and Mango tree were collected by cutting from the top, middle and bottom of the trunk of the tree with the aid of pre-washed stainless knife, and further washed after each sampling with 10% nitric acid to avoid cross contamination. The bark sample was wrapped with paper, and kept in a polythene material and thereafter transported to the laboratory. Random samples are carefully chosen to reflect the areas of high mechanic activities in the mechanic village.

Soil physical parameters analysis

5 grams of air dried and 2 mm sieved soil sample was weighted into 100 ml sampling bottles and 100 ml of distilled water was added. The sampling bottles where then arranged on an Edmund Bühler KS-A SWIP Orbital shaker and allowed to shake for 30 min. The mixture was poured into distilled water rinsed beaker, then the temperature, electrical conductivity and pH, where determined using HANNA combo pH and EC meter.

Soil digestion and heavy metal determination

Two (2) grams of air dried and 2 mm sieved soil was weighted of each soil sample into a BÜCHI k-424 digestion unit. 2 ml of concentrated sulphuric acid/selenium spec solution and 4 ml of concentrated hydrogen peroxide was dosed into each sample. The sample was allowed to digest at 300-400°C until content changes from black to colourless or light golden yellow in the digestion tubes. Digestion was complete when the solution became clear with appearance of white fumes (Audu and Lawal, 2005). The digest was allowed to cool to room temperature and carefully made-up to 100 ml with deionized water in a standard flask. The digest was stored in a 100 ml sample bottle. Heavy metals where determined by aspirating samples into a calibrated Thermo S4 Atomic Absorption spectrometer (AAS) with a digital read out system. Calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. The digested solutions were aspirated individually and atomized in an air-acetylene flame. All samples were run in triplicates and average values taken for each determination.

Bark analysis

One (1) g of each of the samples collected and oven dried at a temperature of 105°C for about 3 h (Majolagbe et al., 2010), was measured into BÜCHI k-424 digestion unit. 2 ml of concentrated sulphuric acid, 4 ml of perchloric acid and 25 ml of concentrated nitric acid was dosed into the sample in the digestion tube. The sample was allowed to digest at 300-400°C until brown fumes of nitric acid disappear and digest becomes colourless or light golden yellow. Digest was allowed to cool to room temperature and made-up to 100ml with deionized water in a standard flask. The digest was stored in a 100 ml sample bottle. Heavy metals where determined by aspirating samples into a calibrated Thermo S4 Atomic Absorption spectrometer (AAS) with a digital read out system. Calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. The digested solutions were aspirated individually and atomized in an air-acetylene flame. All samples were run in triplicates and average values taken for each determination.

Data were reported as mean value and descriptive analysis and graphs were used to analyze the data using SPSS version 17.0.

RESULTS AND DISCUSSION

The mean pH and the temperature of the soils samples collected from both the farmland and the mechanic village was 7.14 and 7.17, respectively, which is neutral and the mean temperature of 27.9°C was found for both the farm land and the mechanic village.

The variation in the mean electrical conductivity between the farmland and the mechanic village were 94.83 μS/cm at 25°C and 136.94 μS/cm at 25°C shows significant difference in their mean at 0.05 level of significant (Figure 2). It indicates that the mineral salts present in the mechanic village, is higher in comparison to the average farmland, hence higher conductivity.

Table 1 results indicates that there is an increase in the mean level of the concentration of cadmium, lead and copper in the soil due to the mechanic activities going on in the mechanic village.

The mean concentrations of the heavy metals in the

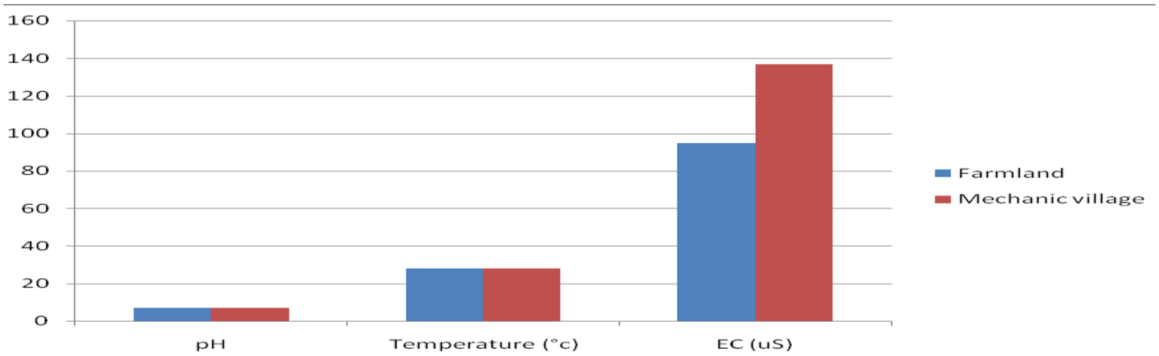

Figure 2. Comparison of the physiochemical parameters of soils from the farmland and mechanic village.

Table 1. The variation of some heavy metals in soil between the control and the mechanic village.

Parameter	Cd (mg/kg)	Pb (mg/kg)	Cu (mg/kg)
Allowable Limit	100	600	100
Farmland (Control)	0.062	0.000	0.018
Mechanic Village	0.069	2.959	0.137

Table 2. Soil concentration range and regulatory guidelines for some heavy metals.

Metal (mg/kg)	Soil concentration range (mg/kg)	Regulatory limits(mg/kg)
Pb	0.00 - 24.34	600
Cd	0.06 - 0.08	100
Cu	0.00 - 1.20	100

soil of the farmland are in this order of magnitude Cd>Cu>Pb, while the mean concentration of the heavy metals in the soil of the mechanic village are in the order of magnitude Pb>Cu>Cd (Table 2). Lead has the least concentration in the farmland, while in the mechanic village, it is the predominant heavy metal detected which also shows greater significant different at $p<0.05$ concuring with the work of Lawal et al. (2011). Also, the maximum statistical value for the concentration of lead in the soil at a point in the mechanic village, which is at 24.34 mg/kg as shown in Figure 3 indicates area of high mechanic activity. Opaluwa et al. (2012) noted that the spread of these metals over a large span of land and the continuous usage of these farmlands for growing crops could lead to bioaccumulation, hence the need for reduction in the concentration of the metals.

In comparison to international standard for permissible level of heavy metals in soil as recommended by World Health Organization (WHO) (2010), the concentration of lead, copper, and cadmium is below the permissible level. Hence, the level of pollution of the area is still minimal.

Table 3 shows the uptake of heavy metal in the bark of the trees. Mean concentration values of heavy metals in the barks in comparison to the FAO/WHO and EC/CODEX standard for these heavy metals shows that the concentration of the heavy metals in those vicinities is within the normal range for cadmium and copper, but the lead present is above the WHO/FAO standard at 0.299 mg/kg and close to the EC/CODEX standard.

The percentage uptake of the heavy metal in the bark of the plant was calculated using the Lawal et al. (2011) formula:

% Conc. of uptake = Conc. of bark ÷ (Conc. of bark + Conc. of soil) × 100

The determination ensured the level of bioaccumulation of the heavy metals by each tree.

The percentage uptake of the three heavy metals as shown is, lead and copper by Neem, Mango and Cashew were (36.63, 0.77, 33.67%), (38.37, 13.63, 75.32%) and cashew was (34.37, 33.70, 44.24%) (Table 4) respectively also showing alliance with the work of Lawal

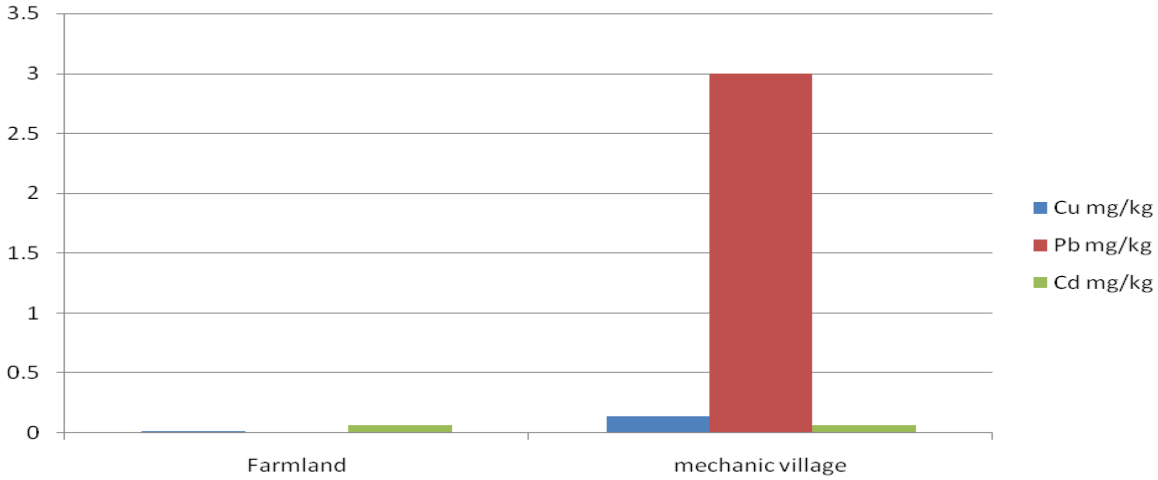

Figure 3. Comparison of the concentration of heavy metals in soils.

Table 3. Comparing the Values of Heavy Metals in Bark of the Trees Under Study with Standards.

Metals	WHO/FAO (mg/kg)	EC/CODEX (mg/kg)	MEAN concentration in plant bark (mg/kg)
Cd	0.05-0.10	0.200	0.042
Cu	0.10	0.300	0.052
Pb	0.10	0.300	0.299

Source: Authors Field Work 2014 and FAO/WHO (2011) Standard.

Table 4. The percentage uptake efficiency of neem, mango and cashew.

Plant	Percentage Uptake Efficiency (%)		
	Cadmium	Lead	Copper
Neem	36.63	0.77	33.67
Mango	38.37	13.63	75.32
Cashew	34.37	33.70	44.24

et al. (2011). These results indicate that different tree species have different uptake efficient capacity with respect to different metals. The three species has almost between 30-40% uptake capacity efficiency for cadmium with no significant difference but the uptake of lead and copper show large significant difference with respect to the mean values which range between 0.77- 33.67% and 33.70-75.32% respectively. These percentages indicate that Mango have greater uptake efficiency with respect to copper follow by Cashew. It can also be concluded that the uptake efficiency of heavy metal under study of the three species are in the order of magnitude of Mango>Cashew>Neem (Figure 4). We can also deduce that for economy importance and uptake or phytoremediation it will be better to plant Mango for steady rate of uptake then Neem could be of great importance.

Conclusion

The concentrations of heavy metals determined from the soil of the mechanic village collected at the base of different species of tree showed that the heavy metals were below permissible levels (FAO/WHO standard) but increased deposition of waste generated from mechanic activity will increase the presence of the heavy metal as areas characterized by high activities showed high concentration of metals especially lead but abandoned or unused areas showed lower concentration relatively.

Absorption of heavy metals by the bark of the trees in the mechanic village was evident when compared relatively to the presence and uptake of the heavy metals from the soil by tree in the average farmland (FUNAAB, COLANIM Farm). The presence of lead in the soil is recorded in high quantity even though it is still within the

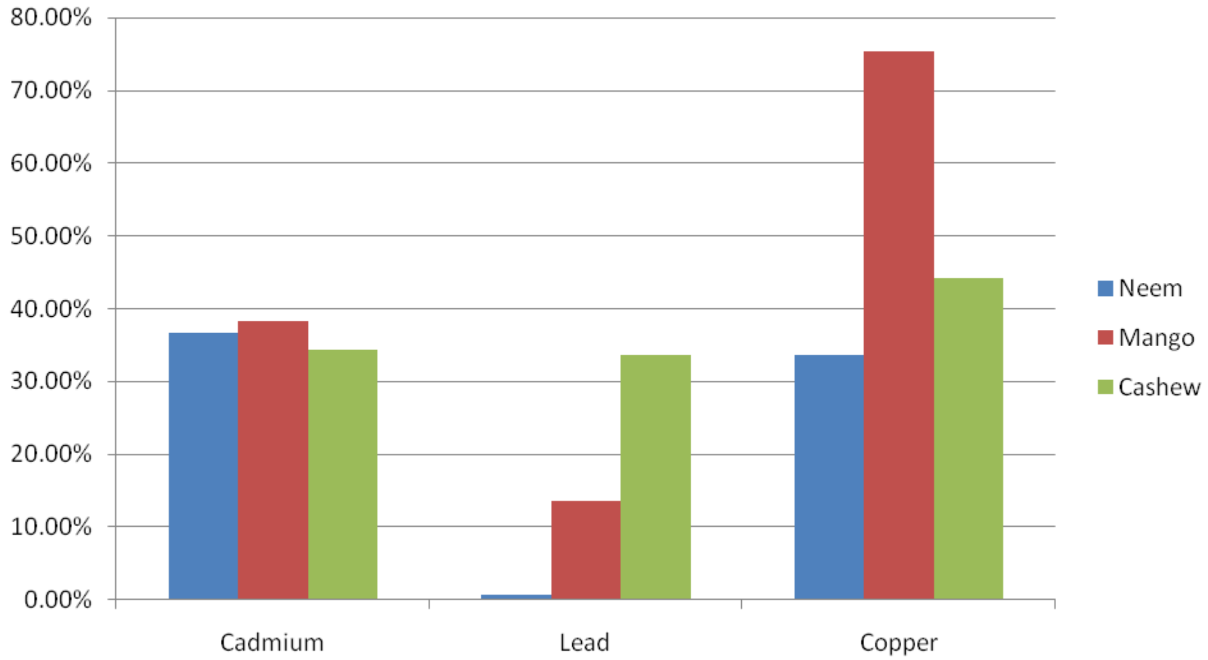

Figure 4. Comparison of the percentage of the uptake of heavy metals by the bark of trees from the soil.

permissible limit while the concentration values of heavy metals in the barks in comparison to set standards for these heavy metals shows that the concentration of the heavy metals in those vicinities is within the normal range for cadmium and copper, but the lead present is above the WHO/FAO standard at 0.299 mg/kg and close to the EC/CODEX standard.

It can also be concluded that the uptake efficiency of heavy metal under study of the three species are in the order of magnitude of Mango>Cashew>Neem. We can also recommend that for better efficient cleanup especially where an area is polluted with copper, mango is best bet for the uptake of such metal while if an equal proportion of many heavy metals is evident then it will be better to use Neem as a phytoremediation plant.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Akhionbare SMO, Ebe TE, Akhionbare WN, Ac-Chukwuocha N (2010). Heavy metal uptake by corn (*Zea Mays*) grown on contaminated soil. Res. J. Agric. Biol. Sci. 6(6):993-997, 2010.

Bieby VT, Siti RSA, Hassan B, Mushrifah I, Nurina A, Muhammad M (2011). A Review on heavymetals (As, Pb, and Hg) uptake by plants through phytoremediation, Hindawi Publishing Corporation. Int. J. Chem. Eng. Volume 2011, Article ID 939161, 31pp, doi:10.1155/2011/939161

Chaphekar SB (1991). An overview on bioindicators. J. Environ. Biol. 12:163-168

Devendra KV, Anek PG, Rajesh D (2013). Bioindicators: A comparative study on uptake and accumulation of heavy metals in some plant's leaves of M.G. road, Agra city, India Columbia International Publishing. Int. J. Environ. Pollut. Solut. 2013:37-53.

Garba NN, Yamusa YA, Isma'ila A, Habiba SA, Garba ZN, Musa Y, Kasim SA (2013). Heavy metal concentration in soil of some mechanic workshops of Zaria-Nigeria. Int. J. Phy. Sci. 8(44):2029-2034.

Hinchman RR, Negri MC, Gatliff EG (1998). "Phytoremediation: using green plants to clean up contaminated soil, groundwater, and wastewater," Argonne National Laboratory Applied Natural Sciences, Inc, 1995, http://www.treemediation.com/Technical/Phytoremediation 1998.pdf

Joint FAO/WHO (2011). Food standards programme codex committee on contaminants in foods, fifth session, The Hague, the netherlands, 21 - 25 march 2011

Lawal AO, Batagarawa SM, Oyeyinka OD, Lawal MO (2011). Estimation of Heavy Metals in Neem Tree Leaves along Katsina – Dutsinma – Funtua, Highway in Katsina State of Nigeria. J. Appl. Sci. Environ. Manag. June, 2011, Vol. 15 (2) 327 – 330, JASEM ISSN 1119-8362. Full-text Available Online at www.bioline.org.br/ja

Majolagbe AO, Paramole AA, Majolagbe HO, Oyewole O, Sowemimo MO (2010). Concentration of heavy metals in tree barks as indicator of atmospheric pollution in Oyo Town, Southwest, Nigeria. Scholars Research Library Archives of Applied Science Research. 2010, 2 (1):170-178.

Opaluwa OD, Aremu MO, Ogbo LO, Abiola KA, Odiba IE, Abubakar MM, Nweze NO (2012). Heavy metal concentrations in soils, plant leaves and crops grown around dump sites in Lafia Metropolis, Nasarawa State, Nigeria. Adv. Appl. Sci Res. 2012, 3 (2):780-784.

Onder S, Dursun S (2006). Air borne heavy metal pollution of Cedrus libani (A. Rich.) in city center of Konya (Turkey). Atmosphere. Environ. 40 (6):1122-1133.

Penkala A (2005). Applied Ecology and Environmental Research. Budapest, Hungary: 2005; 3(1):1-18p

Raskin I, Robert DS, David ES (1997). Phytoremediation of metals using plants to remove pollutants from the environment. ISSN 0958-1669.

WHO (2011). World Health Organization Guildlines for heavy metal WHO Geneva.

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