GROWTH STUDIES OF SOME DERMATOPHYTES ON SIX GROWTH MEDIA

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ABSTRACT
Growth patterns of ten strains of dermatophyte: Epidermohyton fluccosum, Microsporum audouinii, Microsporum ferrugineum, Microsporum nanum, Trichophyton concentricum, Trichophyton mentagrophytes var. quinckeanun,, Trichophyton rubrum, Trichophyton soudanense, Trichophyton tonsurans and Trichophyton violaceum using six different growth media: Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), One percent (1%) Peptone Agar, Malt Extract Agar (MEA), Nutrient Agar (NA) and Yeast Extract Agar (YEA)] were carried out. The results from this study revealed that one percent (1%) peptone agar is the best medium for the cultivation Trichophyton and Epidermaphyton species, while Microsporum species attained their optimal growth on Sabouraud Dextrose Agar (SDA). The growth studies also revealed that nutrient constituents of each medium can interfere with the phenotypic characteristics of dermatophytic species. These findings will help to make the right choice of growth medium to employ in isolating this group of microorganisms and to prevent wrong diagnosis of dermatomycoses in health-care centers.

Keywords: Growth media, Fungi, Dermatophytes, Pleomorphism.

INTRODUCTION

Dermatophytic infection is a disease of worldwide distribution that account for the majority of superficial infections. Clinically, it is important to identify the species causing the skin infections in order to ensure appropriate treatment (Seifert, 2009). However, conventional laboratory methods (*In-vitro* and microscopy) which are the first basic step in the diagnosis and identification of the infection for the selection of the therapeutic approach is slow and time-consuming since dermatophytes grow slowly (Liu *et al.* 2000). In literature, media used for the culturing of dermatophytes include Sabouraud dextrose agar (SDA), Potato dextrose agar (PDA), One percent (1%) Peptone agar, Malt Extract Agar (MEA), Nutrient agar (NA) and to mention a few. In medical mycological laboratories in Lagos State, Nigeria, the most used media for the culturing of dermatophytes are Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA). This study is aim at comparing the growth patterns of some isolated dermatophytes species on different growth media. This is to ascertain the best medium to employ for the cultivation of this group of organisms so as to hasten the growth rate.

MATERIALS AND METHODS

Sample source

Ten strains of dermatophyte which were isolated from different clinical cases of dermatomycoses were collected on prepared Sabouraud Dextrose Agar slants in McCartney bottles from the Mycology Unit of the Department of Medical Microbiology and Parasitology, Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos State, Nigeria. These were stored in the refrigerator prior to use.

Identification

The identities of the isolated fungi were certified using both conventional laboratory methods (*in-vitro* culture and microscopy) and molecular methods (DNA extraction, PCR-RFLP and DNA sequencing). The identification of these fungi by conventional laboratory methods were achieved via their cultural and morphological characteristics as well as comparing them with confirmed representatives of different species in relevant texts such as Alexopolous *et al*. (8) and Ellis *et al*. (9). For the molecular identification approach,

extraction of total genomic DNA was done using the Zymo kit by Zymo Research Corp (Hatfield-Pretoria 0028, South Africa) as reported by Samuel *et al.* (10).

Maintenance of pure cultures of clinical dermatophytes

A little portion of the each of the collected isolates was picked from the McCartney bottle with the aid of a sterilized loop and seeded on a freshly prepared agar (SDA) on different petri plates. These were incubated at 30^0C (Robert and Pihet, 2008). Culture plates were examined at twenty-four hours intervals for morphological traits.

Media preparation

Commercially produced Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), One percent (1%) Peptone Agar, Malt Extract Agar (MEA), Nutrient Agar (NA) and Yeast Extract Agar (YEA) were prepared according to manufacturer specification. Cycloheximide (50 µg/ml) and Chloramphenicol (50 µg/ml) were added to the prepared media (these are to inhibit the growth of many bacterial species and opportunistic moulds that could contaminate the recovery of dermatophytes), before dispensing 15 ml of the melted medium into sterile Petri plates (15-cm diameter Petri plates at a depth of 4.0 mm) and cool at room temperature to solidify.

Growth studies using different types of media

Growth studies of all the isolated fungi were carried out on the six prepared media using the modified methods of Khattab (2006) to determine the best media for their cultivation. Each medium was poured in triplicates into Petri dishes and a cross was drawn with a permanent marker at the reversed side of each Petri dish to indicate the centre as the origin. A bore was made at the centre of each marked Petri dish using a cork borer of 6 mm in diameter. The fresh pure culture of each fungus was aseptically transferred into the bore using sterile loops. The plates were then incubated at 30^0 C and observation was made daily. The growth diameters were measured and recorded.

Statistical analysis

The data were expressed as mean ± Standard Error (S.E) and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan Multiple Range Test (DMRT). Values were considered significant at $p< 0.05$.

RESULTS

The isolated dermatophytes used in this study were *Trichophyton concentricum, Trichophyton tonsurans, Trichophyton mentagrophytes var.quinckeanum, Trichophyton rubrum, Trichophyton soudanense, Trichophyton violaceum, Epidermaphyton fluccosum, Microsporum audouinii, Microsporum nanum* and *Microsporum ferrugineum.* This group of fungi had the optimal growth on 1% peptone agar and on Sabouraud Dextrose Agar. All the *Trichophyton* isolates had the highest growth rate on 1% peptone agar followed by growth on Sabouraud dextrose agar (SDA) (Fig 1, 2 and 3). *Trichophyton concentricum* and *T. tonsurans* had their optimal growth on 1% peptone agar followed by growth on SDA (Fig. 1). In Fig 2, *Trichophyton rubrum* had the same growth rate of 16 mm in diameter on 1% peptone agar and SDA and *T. soudanense* attained its optimum growth rate of 17 mm in diameter on 1% peptone agar followed by growth of 15.5 mm in diameter on SDA. While *Trichophyton mentagrophytes var. quinckeanun* and *T. violaceum* attained *optimal* growth on 1% peptone agar and SDA at the same time (Fig 3). Similarly to the *Trichophyton* species, *Epidermaphyton fluccosum* had its optimum growth on 1% peptone agar followed by growth on SDA (Fig 4).

Unlike the *Trichophyton* species and *Epidermaphyton fluccosum, Microsporum audouinii and M. ferrugineum* attained their optimal growth on Sabouraud Dextrose Agar (Fig 5) and *M. nanum* had its optimum growth rate on Sabouraud Dextrose Agar, Potato Dextrose Agar, Nutrient Agar and Malt Extract Agar (Fig 4).

A notable observation from the growth studies was that of the variation of colour and texture of colonies of the same organism on different growth media. This was observed with two isolates; *Epidermaphyton flucossum* and *Trichophyton tonsurans* which showed different morphological characteristics on Sabouraud Dextrose Agar and 1% Peptone Agar (Plates 1 and 2).

DISCUSSION

The results from this study revealed that SDA and one percent (1%) peptone agar are the medium of choice that supported the growth of all dermatophytes (Fig 1, 2, 3, 4 and 5). This is due to the fact that the species of the genus *Trichophyton* and *Epidermaphyton* obtained their optimal growth on One percent (1%) peptone agar, compare to the other five media used in this study. Rebell and Tapliin (1970), de-Hoog *et al*. (1995) and Kane *et al*. (1997) had reported that One percent (1%) peptone agar is a rich source of carbon and nitrogen for fungal growth. Also, from the results, Sabouraud Dextrose Agar (SDA) showed to be the medium of choice for the cultivation of *Microsporum* species and this agrees with the reports of Ajello (1977), Sharma and Sharma (2011) and Gumral *et al*. (2015). It was also observed that some dermatophytes have the ability to change into different forms. This brings the subject of "Pleomorphism" to the fore. This phenomenon was noticed with *Epidermaphyton flucossum* and *Trichophyton tonsurans* where the two species exhibited different morphological characteristic on different media under the same condition. This report agrees with the findings of Bistis (1959), Ellis *et al*. (2007) and AL-Janabi (2009). These researchers concluded that pleomorphic was as a result of nutrient constituent of the medium on which the organisms were grown. This was in contrast with the report of Chin and Knight (1957) that pleomorphism is brought about by changes in environmental factors. Sharma and Sharma (2011), also reported that the constituents of the growth medium (e.g. carbon content) used may have interfered with morphological characteristics. Sharma and Sharma further explained that Pleomorphism takes place in dermatophytes during sporulation state.

CONCLUSION

The results from this study revealed SDA and one percent (1%) peptone agar are the medium of choice that supported the growth of all dermatophytic isolates used in this study. It also showed that nutrient constituents of each medium can interfere with phenotypic characteristic of dermatophyes. This can leads to wrong diagnosis of dermatomycoses in health institutions.

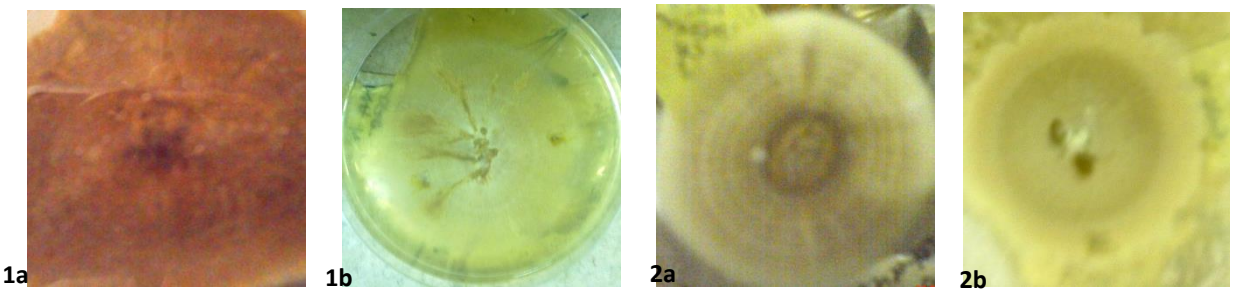

Plate 1&2: Culture photograph of two dermatophytes species exhibiting pleomorphism

Plate 1a: *Epidermaphyton flucossum* on Sabouraud Dextrose Agar
Plate 1b: *Epidermaphyton flucossum* on 1% Peptone Agar

Plate 2a: *Trichophyton tonsurans* on Sabouraud Dextrose Agar medium
Plate 2b: *Trichophyton tonsurans* on 1% Peptone Agar

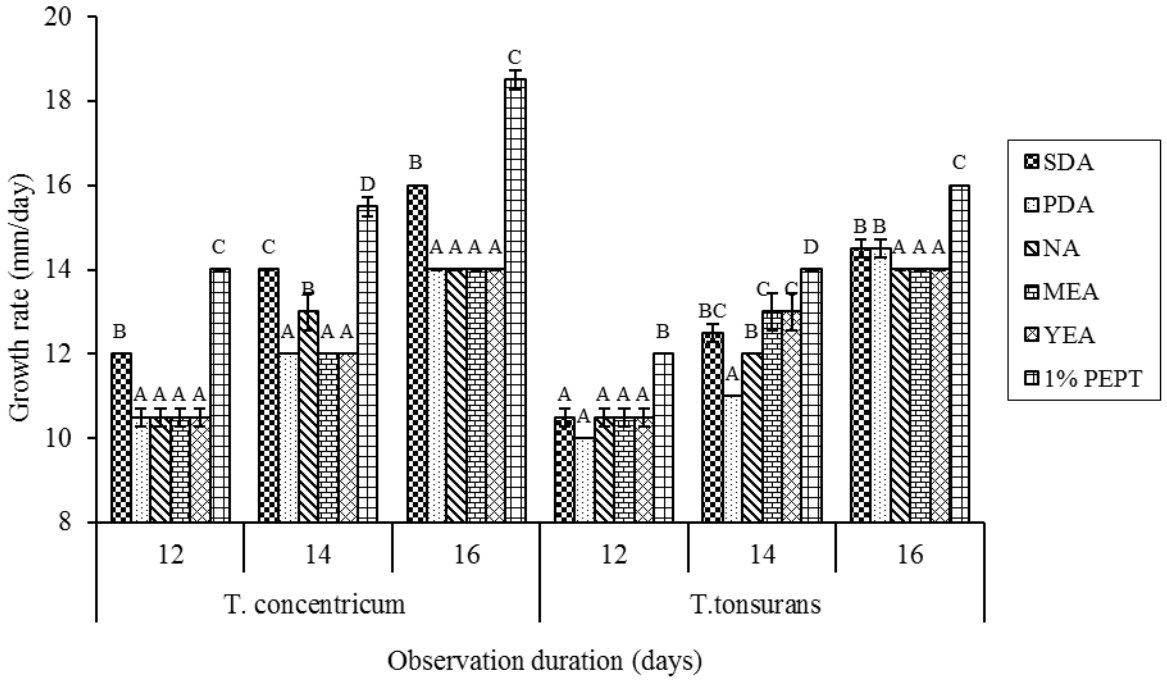

Fig. 1: Growth profile of *Trichophyton concentricum* and *T. tonsurans* on six different media. Means ± SE bars with the same superscript letter in each growth period are not significantly different (p>0.05; DMRT)

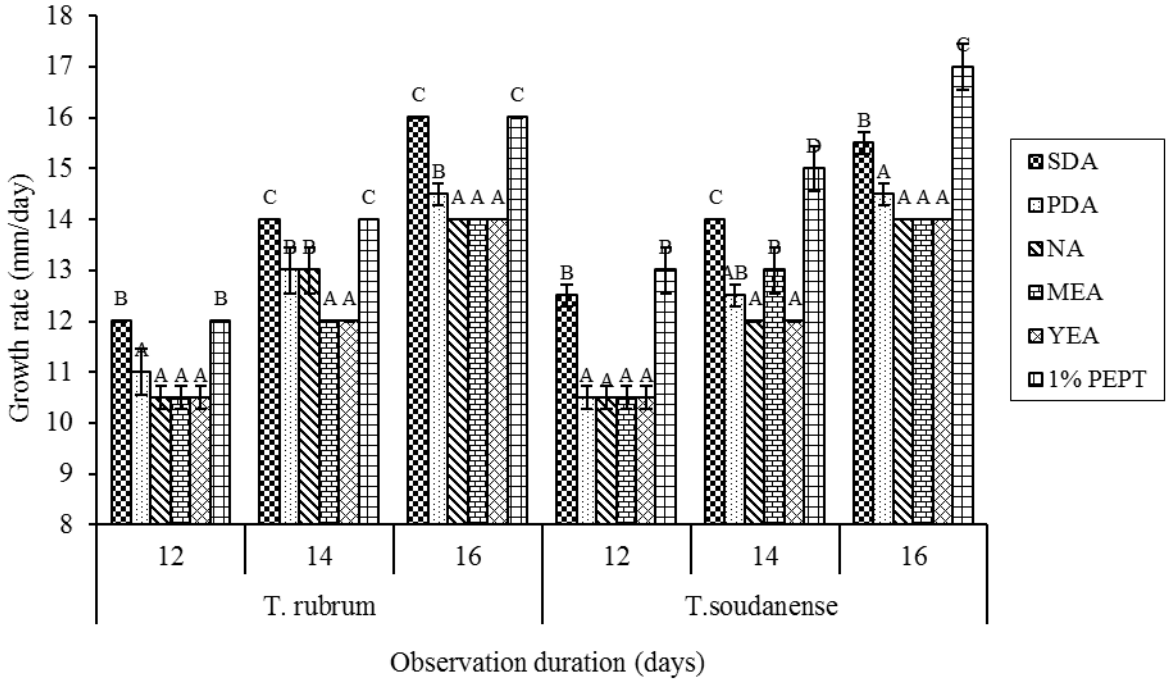

Fig. 2: Growth profile of *T. rubrum* and *T. soudanense* on six different media. Means ± SE bars with the same superscript letter in each growth period are not significantly different (p>0.05; DMRT)

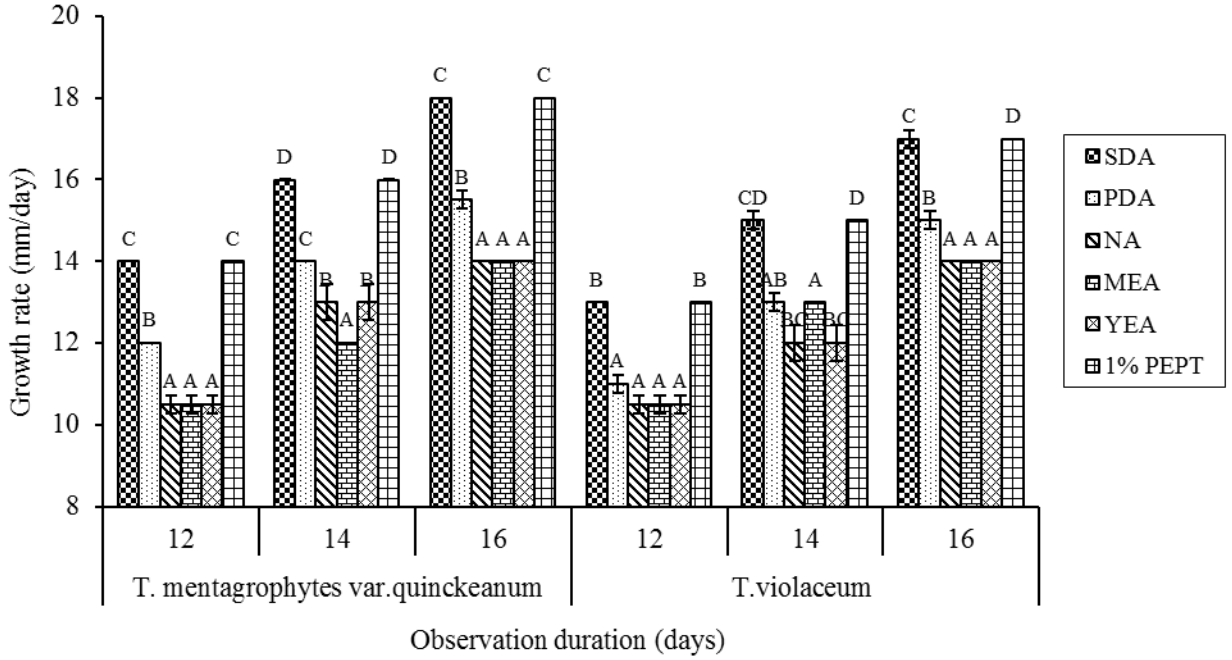

Fig. 3: Growth profile of *T.mentagrophytes var. quinckeanum* and *T. violaceum* on six different media. Means ± SE bars with the same superscript letter in each growth period are not significantly different (*p*>0.05; DMRT)

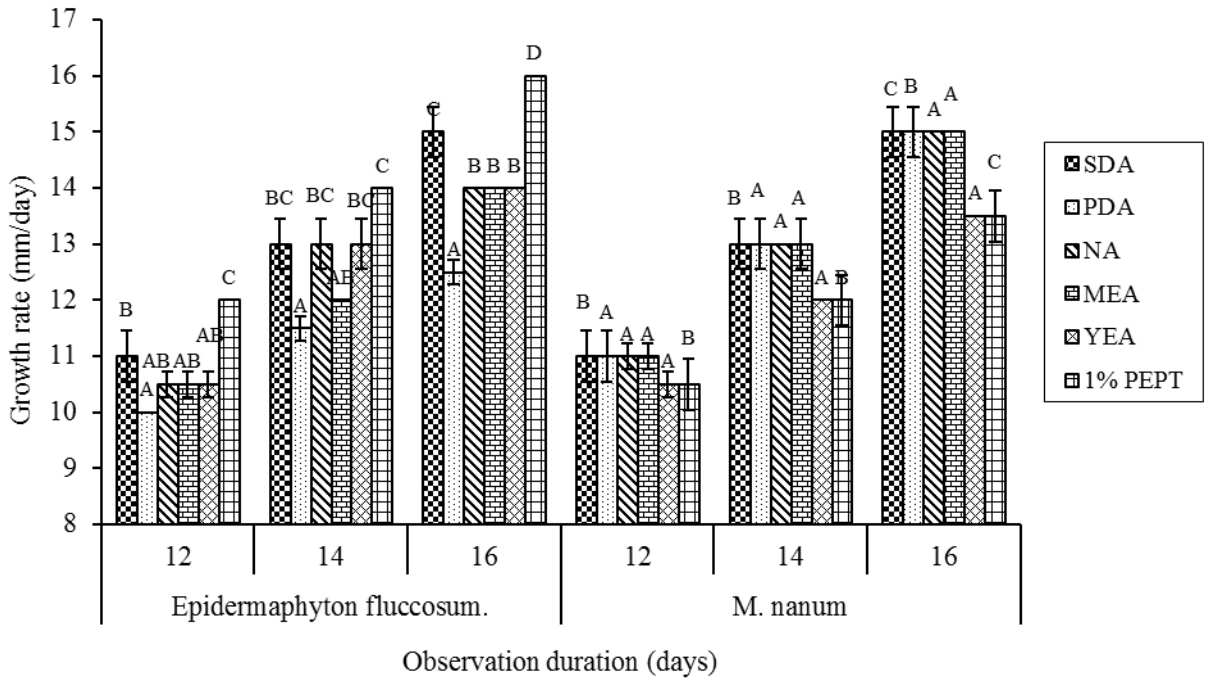

Fig. 4: Growth profile of *Epidermaphyton fluccosum* and *Microsporum nanum* on six different media. Means ± SE bars with the same superscript letter in each growth period are not significantly different (*p*>0.05; DMRT)

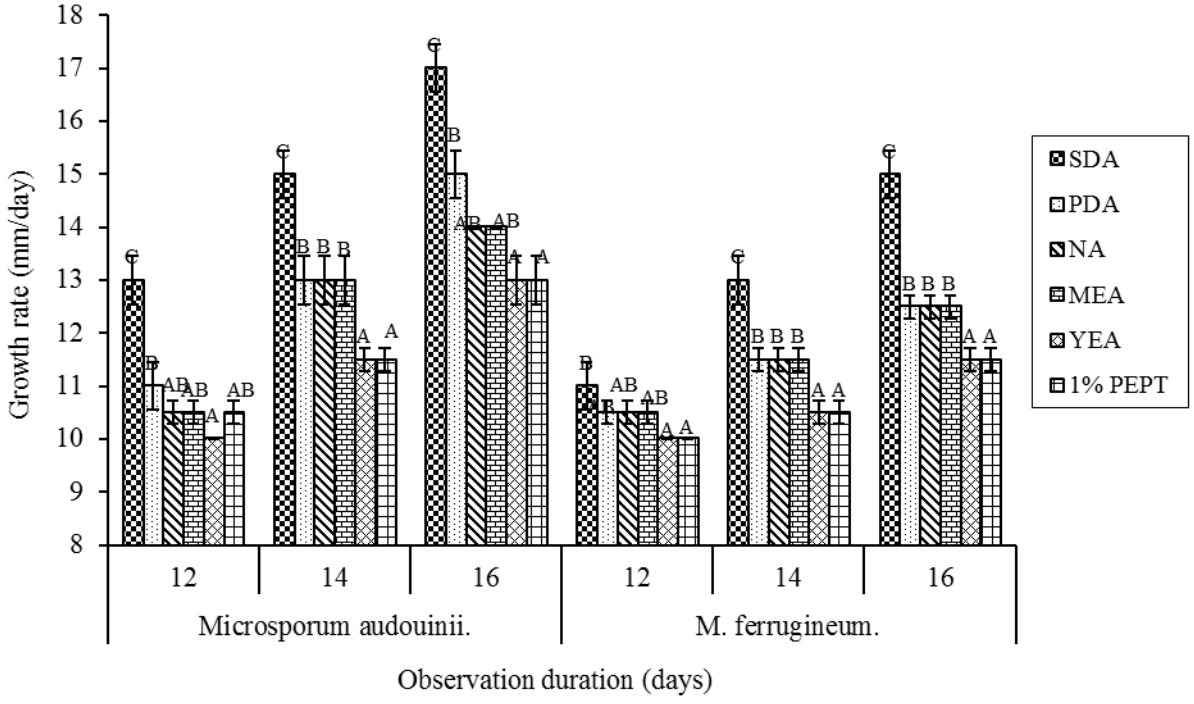

Fig. 5: Growth profile of *M.audouinii* and *M.ferrugineum* on six different media. Means ± SE bars with the same superscript letter in each growth period are not significantly different (p>0.05; DMRT)

REFERENCES

Ajello, L. (1977). Milestones in the history of medical mycology: the dermatophytes. In: Iwata, K. (Ed.) *Recent advances in medical and veterinary mycology*. University of Tokyo Press, Tokyo. pp 3-11.

AL-Janabi, A.A.H.S. (2009). Study of Characteristic Features of Pleomorphic *Epidermophyton floccosum. Global Journal of Environmental Research* 3(2):132-134.

Alexopolous, C.J., Mims, C.W. and and Blackwell, M. (2007). Introductory mycology. 4th ed. New Delhi: Wiley India, pp869.

Bistis, G.N. (1959). Pleomorphism in the dermatophytes. *Mycologia* 51(3): 440 - 452.

Chin, B., and Knight, S. G. (1957). Growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum* in increased carbon dioxide tensions. *Journal of General Microbiology* 16:642-646. de-Hoog, G.S., Gueho, E., Masclaux, F., Van den Ende, A.H.G., Kwon-Chung, K.J. and McGinnis, M.R. (1995), Nutritional physiology and taxonomy of human-pathogenic *Cladosporium-Xylohypha* species. *Journal of Medical and Veterinary Mycology* 33:339 - 347.

Ellis D, Davis S, Alexiou H, Handke R, Bartley R. (2007). *Descriptions of medical fungi*. 2nd ed. Underdale, South Australia: Nexus Print Solutions 198pp.

Gumral, R. Dogen, A. Ilkit, M.M. (2015). Comparison of the contamination rates of culture media used for isolation and identification of dermatophytes. *Turkish Journal of Medical Sciences* 45: 587-592

Kane, J., Summerbell, R.C., Sigler, L., Krajden, S. and Land, G. (1997). *Laboratory handbook of dermtophytes.* Star Publisher, Belmont, USA. pp232.

Khattab, O.H. (2006). Factors affecting growth and pycnidial production of aquatic pycnidial fungi. *Journal of Agriculture and Social Sciences* 2(4): 234-237.

Liu, D., Coloe, S., Baird, R. and Pedersen, J. (2000). Application of PCR to the identification of dermatophytes fungi. *Journal of Medical Microbiology* 49:493 - 497.

Rebell, G. and Tapliin, D. (1970). *The Dermatophytes*. 2nd revised edition. University of Miami Press, Coral Gables, Florida. USA. 124pp.
Robert, R. and Pihet, M. (2008). Conventional methods for the diagnosis of dermatophytosis. *Mycopathologia* 166(5-6): 295-306.
Samuel, T.O., Adekunle, A.A. and Ogundipe, O.T. (2013). Prevalence of dermatomycoses in tertiary health institutions in Lagos State, Nigeria. *Journal of Public Health Epidemiology* 5(2): 101-109
Seifert, H. (2009).The Clinical importance of microbiological findings in the diagnosis and management of bloodstream infections. *Clinical Infectious Diseases* 48(4):238 – 245.
Sharma, M. and Sharma, M. (2011). Influence of culture media on mycelial growth and sporulation of some soil dermatophytes compared to their clinical isolates. *Journal of Microbiology and Antimicrobials* 3(8):196 - 200.

REDUCTION OF CHEMICAL OXYGEN DEMAND AND POTENTIALLY TOXIC METALS IN LEACHATES GENERATED FROM ISOLO DUMPSITE USING DIFFERENT COAGULANTS

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ABSTRACT
Leachate samples were collected from Isolo dumpsite within a seven month period, at eight different times after rainfall. The characterization of the leachate shows that they were sourced from an old landfill. The highest concentration of chemical oxygen demand (COD), biological oxygen demand (BOD) and total organic carbon (TOC) in the most polluted leachate samples were 392 mg/L, 203 mg/L and 5090 mg/L respectively. The levels of Fe, Pb, Cu and Zn were 19.7 mg/L, 2.06 mg/L, 2.18 mg/L and 3.50 mg/L respectively. Three different coagulants: ferric chloride, ferrous sulphate and aluminium sulphate were used to treat the most polluted leachate sample. Different pH and concentrations were investigated to obtain the optimal dosage for the most effective coagulant. The experimental results showed that at a neutral pH, 96% COD, 66.2% Fe, 94.3% Zn reductions were achieved at the lowest concentration of 1000 mg/L of $Al_2SO_4.14H_2O$ while $FeCl_3$ and $FeSO_4$ gave <96% reduction at a high concentration of 25,000 mg/L. The pH of 3, 5, 9 and 11 at optimal dosage of $Al_2SO_4.14H_2O$ gave 14%, 78%, 82%, 88% reduction of COD respectively. The result therefore indicates that $Al_2SO_4.14H_2O$ is the most effective of the three coagulants with optimal pH and dosage of 7 and 1000 mg/L respectively.

Keyword: coagulant, COD, leachate, potentially toxic metals

INTRODUCTION

One of the simplest and cheapest methods of disposing waste is the landfill which is adopted by most low-to medium- income developing nations. The management of landfill has become one of the main focus of waste management because decomposing organic wastes in the landfills generate greenhouse gases (methane and carbon dioxide) with attendant impact on climate and potential health hazard (Aljaradin and Persson, 2012). In addition to air pollution, solid waste disposed in landfills is usually subjected to series of complex biochemical and physical processes which lead to the production of leachates (Olsson et al, 2009). Leachates are generated by direct or indirect mixing of rainwater, snowmelt and groundwater together with liquids produced from the waste itself through hydrolysis and solubilisation, brought about by a series of complex biochemical reactions during decomposition of organic wastes. Leachate refers to any liquid percolated through deposited waste and seeped from or contained within a landfill consisting of many different organic and inorganic compounds that may either be dissolved or suspended. The landfill leachate is a secondary contamination related to landfills. Leachate spends many years infiltrating through the landfill and during this time, it will make contact with various substances such as paints, plastic, oil among others within the landfill. The resulting liquid leaches and dissolves various constituents until it contains a load of heavy metals, chlorinated organic compounds and other substances. The leachate water quality reduces after rainwater washes the landfill (Abbas *et al*., 2009, Kangsepp and Mathiasson, 2009).

When leachate becomes highly concentrated, the wastewater that results is a potential threat to the quality of groundwater as it could migrate or seep from the landfill either through direct infiltration on site or by infiltration of leachate-laden runoff offsite. The extent of the risk posed to groundwater-fed drinking water sources is usually considered in terms of waste composition, quality of leachate produced and leachate migration - attenuation and dilution. This implies that water bodies around the landfill site are likely to be polluted with various constituents of the leachate. The presence of the non- biodegradable compounds in leachate after biological treatment, calls for an alternative treatment method that reduces both biodegradable and non-biodegradable constituents. The treatment of such leachates by physical or chemical methods achieves this. This treatment method is sometimes, applied after biological treatment method to improve leachate treatment efficiency (Aziz *et al*., 2007).

Coagulation and flocculation treatment process is a widely used technique effective in removing high concentration of organic pollutants, heavy metal and anions (Wang *et al*., 2002). The process involves destabilization and aggregation of colloidal dispersion to permit particle removal by sedimentation and filtration (Dewall and Chain, 2006). The chemistry of coagulation reaction may be considered in two stages: dissociation of the coagulant into positively charged ions which combine with the negatively charged colloids, neutralizing their charge thereby permitting agglomeration and formation of insoluble complexes (floc) by the reaction of the metal ions with the hydroxide ions (and other available ions such as phosphates, sulphates and

chlorides). The most widely used coagulants for wastewater treatment are the salts of iron and aluminum (both supply cations and have positive Zeta potential (Amokrane *et al*., 1997). The objectives of this research are to examine the efficiency of coagulation-flocculation processes for the removal and/or reduction of metals and COD in the landfill leachate and to study the optimal conditions for the removal using three coagulants.

MATERIAL AND METHODS

Study Site

Isolo dumpsite is an inactive landfill situated close to the General Hospital, Isolo, Lagos with GPS coordinates of 6º 31´ 52´´N and 3º 19´ 4´´E. The total area of the dumpsite is about 0.3km^2. The dumpsite shares boundary with an abattoir, a mechanic workshop and food stuff shops. Solid and liquid wastes from the dumpsite and other areas connected to it, find their ways into the Oke-Afa canal which is a few meters away from the dumpsite. This dumpsite is not well-designed and has no leachate collection point.

Sampling

Eight leachate samples were collected at the sampling site between September 2013 and March 2014. The leachate samples were collected from two point sources connecting drainage point to the Oke-Afa canal. The leachate samples were collected in clean dry plastic containers and stored in the refrigerator (< 4 ºC) prior to analysis to keep the microbial load constant. Physicochemical analysis was carried out on all the leachate samples according to standard methods (Ademoroti, 1996). Before the analysis, the leachate samples were removed from the refrigerator and conditioned for about three hours under ambient temperature. All chemical reagents used for analysis were of analytical grade. The coagulants used were $FeCl_3.6H_2O$, $FeSO_4$ and $Al_2(SO_4)_3.14H_2O$. After the physico-chemical analysis, the leachate samples were treated with the different three coagulants.

Physicochemical analyses

The methodology for the determination of the physico-chemical parameters such as, pH, total hardness, total dissolved solids, chlorides, chemical oxygen demand, conductivity, alkalinity, sulphates, phosphates, nitrates. such as copper, Iron, zinc, copper, nickel and lead were determined using standard methods (Oluseyi et al, 2011) as shown in Table 1.

Table 1. Analytical methods applied for the determination of physico-chemical parameters

Parameter	Unit of measurement	Analytical method
pH	-	Potentiometry
Conductivity	µs/cm	Conductimetry
Total hardness	mg/L	Titrimetry
Chemical oxygen demand (COD)	mg/L	Titrimetry
Total dissolved solids (TDS) And Total Solids (TS)	mg/L	Gravimetry
Alkalinity	mg/L	Titrimetry
Chlorides	mg/L	Titrimetry
Sulphates	mg/L	UV Spectrophotometry
Phosphates	mg/L	UV Spectrophotometry
Nitrates	mg/L	UV Spectrophotometry
Potentially toxic metals (Fe, Pb, Cu, Zn and Ni)	mg/L	Atomic absorption spectrometry

Determination of Chemical oxygen demand

50.0 mL of leachate sample was placed in a reflux flask containing antibumping granules. 5.0 mL of conc. H_2SO_4 and powdered $HgSO_4$ was added and swirled until the mercuric sulphate has dissolved. The reflux flask was placed in an ice bath and 25.0 mL of 0.025 N $K_2Cr_2O_7$ was slowly added with swirling. This was later followed by addition of 70 mL of sulphuric acid-silver sulphate solution to the cooled reflux flask, using slow addition with swirling motion. Heat was applied to the flask and refluxed for 2 hours. The flask was allowed to

cool and the content was transferred to a 500 mL Erlenmeyer flask, washing out the reflux flask 3 times with distilled water. The acid solution diluted to about 300 mL with distilled water and the solution allowed to cool to room temperature. 8 to 10 drops of ferroin indicator was added to the solution and titrated with 0.25 N ferrous ammonium sulphate solution to the end point with a colour change from a blue-green to a reddish hue.

Leachate treatment

The initial pH of the sample was 6.63 and later adjusted to pH 7 by adding an appropriate amount of NaOH solution. At constant pH, four different concentrations of each coagulant were added to the 100 mL of leachate sample, shaken vigorously, centrifuged at 100 rpm for five minutes and then filtered. The required filtrate volume was withdrawn with the aid of a pipette. This solution was characterized and the reduction levels of the pollution parameters were compared with the raw leachate sample. Each of the three coagulants was used to treat the leachate firstly at constant pH of 7 and varying concentration of 1000, 5000, 10000 and 25,000 mg/L respectively. The coagulant with a high COD removal was selected and the pH was varied at 3, 5, 9, and 11 to obtain the optimal pH while maintaining a constant concentration of the coagulant. The sample with the highest COD removal employing the optimal pH and concentration was digested with aqua regia and analysed to ascertain the reduction level for the metals.

RESULTS AND DISCUSSION

The characterizations of the raw leachate samples are shown in Table 1. It was observed that these leachate samples had pH which ranged from 6.6 – 7.1, indicating a weakly acidic to a slightly alkaline leachate sample. This is due to the fact that leachate generally tends towards alkaline as the age of the landfill increases. The concentration of pollution parameters, chemical oxygen demand (COD) and potentially toxic metals (PTMs) in the leachates were also found to be high and above permissible limits when compared to the LASEPA discharge standard.

Table 2: Physicochemical parameters of untreated leachate samples

Physicochemical Parameters	A1	A2	A3	A4	B1	B2	B3	B4
pH	7.1	7.0	6.6	6.9	7.1	6.9	6.9	6.6
Temperature (ºC)	26.9	27.3	27.3	27.0	27.3	27.1	27.6	26.5
Conductivity (µs/cm)	1190	1330	1580	1610	1340	2160	1700	1100
TDS (mg/L)	600	650	790	380	670	1100	840	500
TS (mg/L)	1560	1790	2300	5950	1060	1980	1340	1370
Chloride (mg/L)	234.0	230.0	298.0	284.0	268.0	504.0	376.0	255.0
Total hardness (mg/L)	12.0	16.0	14.80	56.0	12.40	38.0	33.20	40.0
Sulphate (mg/L)	79.0	49.6	35.8	28.3	104.0	158.0	101.0	56.4
Phosphate (mg/L)	2090	3090	2360	1980	5450	1140	1350	1570
Nitrate (mg/L)	41.4	53.4	21.0	13.8	64.2	28.5	44.4	ND
COD (mg/L)	220.0	152.0	392.0	104.0	44.0	36.0	52.0	160.0
Alkalinity (mg/L)	7.44	8.80	10.10	4.81	7.61	8.01	6.64	5.61
Iron (mg/L)	16.60	10.60	19.70	9.40	3.74	3.22	3.52	ND
Lead (mg/L)	1.40	1.50	2.06	ND	0.18	ND	0.48	1.70
Copper (mg/L)	1.94	1.92	2.18	2.44	0.54	1.22	2.72	1.62
Zinc (mg/L)	3.24	2.98	3.50	3.00	0.92	1.58	2.22	2.77
Nickel (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND = not detected.

A1, A4, A3and A2 represents leachate sample collected at same point source in 13th September 2013, 20th November 2013, 17th February 2014 and 12th March 2014 respectively.

B1, B4, B3 and B2 are for the other point source on the same date as for sample A.

From the results shown in Table 2, Sample A3 which had the highest concentration of COD of 392.0 mg/L and the PTMs, so it was chosen for further treatment with the three coagulants with the pH was maintained at 7. The percentage reduction of COD using the three coagulants are shown in Table 3.

Table 3: COD values of sample A3 at constant pH 7 after treatment with the coagulants

Coagulant concentration	**Treatment with $Al_2(SO4)_3.14H_2O$**	**Treatment with $FeCl_3$(mg/l)**	**Treatment with $FeSO_4$(mg/l)**
1000 mg/L	16.0	352.0	816.0
5000 mg/L	32.0	336.0	384.0
10,000 mg/L	64.0	272.0	192.0
25,000 mg/L	384.0	96.0	64.0

Note: Initial COD value before treatment was 392 mg/L

COD reduction was 96% and 2% using 1,000 mg/L and 25,000 mg/L $Al_2(SO4)_3.14H_2O$ respectively, $FeCl_3$ achieved 10% and 76% reduction respectively. Also 84% reduction was achieved at high concentration of 25,000 mg/L for $FeSO_4$, while at low concentration of 1,000 mg/L an approximately three (3) times fold increase of initial COD was observed (to 816 mg/L) because at low concentration of $FeSO_4$ its floc is not yet formed and the solubility of ferric hydroxide is not exceeded and therefore no reduction of COD at low concentration. This implies that for $FeSO_4$ treatment, an increase in concentration (that is more quantity of $FeSO_4$), will achieve 84% COD reduction. The disadvantage of this is that more dosage will increase the chemical residue of the solution, thereby interfering with coagulation process during filtration and as such it will not be economical.

The result for $FeSO_4$ and $FeCl_3$ at pH 7 indicates that a high reduction of COD can only be achieved at a high concentration (25,000 mg/L). Even at this, the percentage COD reduction is less compared to that obtained at low concentration using $Al_2(SO_4)_3.14H_2O$. The reason $Al_2(SO_4)_3.14H_2O$ usage gave a better reduction at least concentration investigated (1000 mg/L) and pH 7 is that at this concentration, the solubility limit of aluminium hydroxide has been exceeded favouring the formation of floc. It is this floc that adsorbs particles in the wastewater. This condition therefore enables the activity of aluminium floc and hence the consequent reduction of COD value to 96%. At a higher concentration the activity of aluminium floc begins to reduce thereby resulting to a lower COD reduction value as observed in 5000, 10,000 and 25,000 mg/L, which gave 92%, 84% and 2% COD reduction respectively. As shown in Figure 1 the highest COD reduction was achieved at pH 7 and 1000 mg/L of $Al_2(SO_4)_3.14H_2O$.

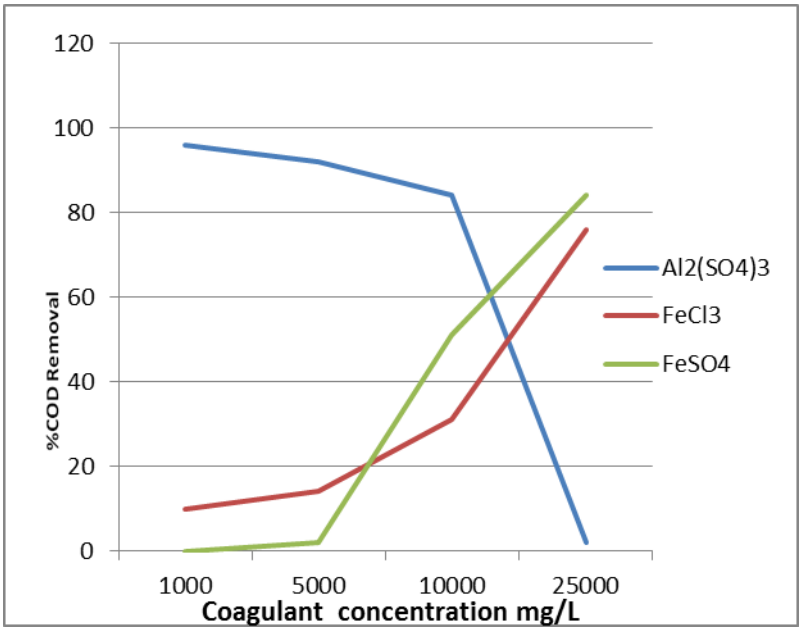

Figure1: Percentage COD Reduction at different coagulant concentrations

When the concentration of the coagulant was kept constant at 1000 mg/L and the pH was varied between 3 and 11, COD reduction of 14%, 78%, 88% and 82% were observed. At pH values of 3 and 5, COD reduction increased, while a decrease was observed in pH 7 and 9. It also further confirmed that at low pH, COD reduction is minimal, and at a higher pH beyond 7, it starts decreasing steadily. The COD removal efficiency thus increases with increasing pH up to the pH of 7 beyond which the removal efficiency starts decreasing gradually as shown in Figure 2. This confirms that coagulation treatment process is pH dependent as observed by Trebouet *et al*. (2001) and Silva *et al*. (2004).

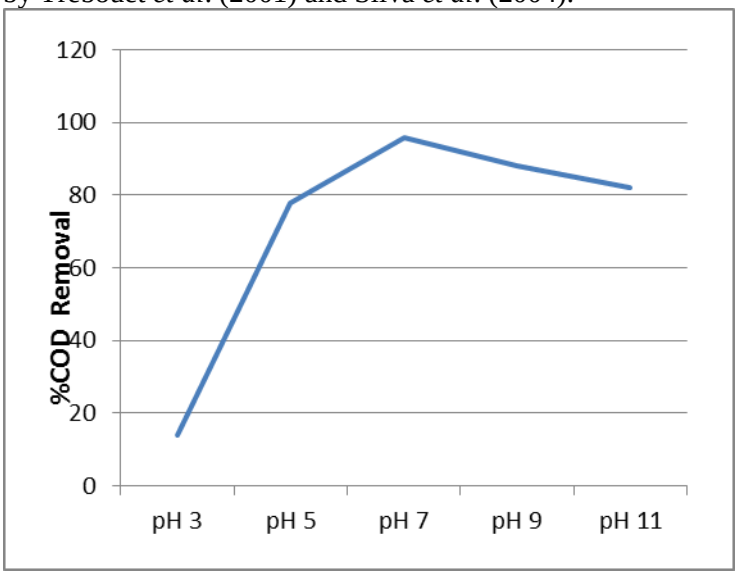

Figure 2: Percentage COD reduction at different pH values

The pH influence on the coagulation treatment process is evident by a competition between hydrogen ion, hydroxide ion and the metal species on the adsorption site of the metal. At low pH, H^+ competes with the metal species generating substances that do not easily precipitate. The resultant effect is a poor removal. While at a neutral pH, there is equal balance between OH^- and H^+, giving the coagulant active action on the water and also since the region of best coagulant activity for $Al_2SO_4.14H_2O$ range from 6.5 to 7. At a higher pH, OH^- competes with the other compounds for metal adsorption site causing precipitation of metal hydroxide to occur by precipitation (Silva *et al*., 2004).

Therefore, the optimal dosage and pH for achieving the highest COD reduction was 1000 mg/L and pH 7 using $Al_2SO_4.14H_2O$ as coagulant. At this same coagulant condition, the concentration of TDS, TS, conductivity and metals (Zn, Pb, Ni, Cu and Fe) also reduced close to various discharge limits set by LASEPA as shown in Table 4. The results showed that 24%, 66% and 94% reductions were observed in the TS, Fe and Zn concentration respectively. The concentrations of Pb, Ni, and Cu were too low to be detected after the treatment. The reduction in TS was greater than LASEPA discharge limit, due to the fact that addition of coagulant must have increased the total solid content in the wastewater. Also the TDS content increased after treatment due to the fact that addition of coagulant also increased the dissolved salt content of the water (Adlan *et al.*, 2004).

Table 4: Results of leachate after treatment with 1000 mg/L $Al_2(SO_4)_3.14H_2O$.

Physicochemical parameters	**Leachate A3 before treatment**	**Leachate A3 after treatment**	**LASEPA discharge Limit**
pH	6.63	7.0	6.5-8.8
TDS (mg/L)	790	880	500
TS (mg/L)	2,300	1760	525

COD (mg/L)	392.0	16	30
Zn (mg/L)	3.50	0.02	2
Fe (mg/L)	19.70	0.60	10
Pb (mg/L)	1.06	ND	0.5
Ni (mg/L)	ND	ND	0.05
Cu (mg/L)	2.18	ND	0.5

ND = not detected.

CONCLUSION

From the results obtained in this experiment, the application of coagulation-flocculation treatment process for untreated leachate collected from Isolo dumpsite was effective. The treatment process performed at fixed centrifuging condition, constant pH, varying coagulant dosage and at varying pH showed that $Al_2(SO_4)_3.14H_2O$ as coagulant gave the best reductions when compared with other coagulants used. $Al_2(SO_4)_3.14H_2O$ when conditioned at an optimal pH of 7 and concentration of 1000mg/L gave a 96% COD reduction, 94% Zn reduction, 66% iron reduction, and subsequently a crystal clear solution (compared to the dark colour before treatment) was achieved. Pb, Ni, and Cu were not detected after treatment. This reduction level achieved is below LASEPA discharge standard. $FeCl_3$ and $FeSO_4$ coagulants on the other hand gave 76% and 84% COD reduction respectively at pH 7 and a high dosage of 25,000 mg/L. Their usage is not economical and will increase chemical sludge in solution whereas $Al_2(SO_4)_3.14H_2O$ gave a better reduction at low concentration of 1000 mg/L approved to be the more economical coagulant.

REFERENCES

Abbas A, Jingsong G, Ping L, Ya P and Al-Rekabi W.S (2009). Review on landfill leachate treatments, *J. Appl. Sci. Res.*, **5**:534-545.

Ademoroti, C.M.A. (1996). Standard Method for Water and Effluent Analysis, 34-87.

Aljaradin M and Persson K.M (2012) Environmental Impact of municipal solid waste landfills in semi – arid climates - Case study – Jordan. *The Open Waste Management Journal*, **5**: 28-39

Amokrane, A., Comel, C. and Veron, J. (1997). Landfill leachate pretreatment by coagulation-flocculation, *Water Research*, **31**(11):2775- 2782.

Aziz, H.A, Alias, S, Assari, F and Adlan, M .N (2007). The use of alum, ferric chloride and ferrous sulphate as coagulants in removing suspended solids, colour and COD from semi-aerobic landfill leachate at controlled pH. *Waste Management and Research*, **25**: 556-565

Dewall, F.B. and Chain, E.S. (2006). Sanitary landfill leachate and their treatment, *J.Enviro.Eng division*, **120**:411-431.

Kangsepp, P and Mathiasson, L (2009) Performance of a full-scale biofilter with peat and ash as a medium for treating industrial landfill leachate: a 3 year study of pollutant removal efficiency. *Waste Manage. Res*.,**27**: 147 – 158

Olsson, S., Gustafsson, J.P, Berggren, K., Bendz, D and Persson I (2009) Size fractionation and characterization of landfill leachate and the improvement of Cu adsorption capacity in soil and aged refuse, *Waste Management*, **29**: 506 – 512

Oluseyi, T., Olayinka, K and Adeleke, I (2011) Assessment of ground water pollution in the residential areas of Ewekoro and Shagamu due to Cement production. *African Journal of Environmental Science and Technology*. **5**(10) pp 786 - 794

Silva, A.C., Dezotti, M. and Sant'anna, G.L. (2004). Treatment and detoxification of a sanitary landfill leachate, *Chemosphere* **55**:207-214.

Trebouet, D., Schlumpf, J.P., Jaounen, P. and Quemeneuer, F. (2001). Stabilized landfill leachate treatment by combined physicochemical-nanofiltration process, *Water Research*, **35**:2935-2942.

Wang, Z.P, Zhang, Z, Lin Y.J, Deng N.S, Tao T and Zhuo K (2002). Landfill leachaye treatment by coagulation – photo oxidation process. *J. Hazard Matter*, **95:** 153 – 159