FEDERAL UNIVERSITY OYE-EKITI
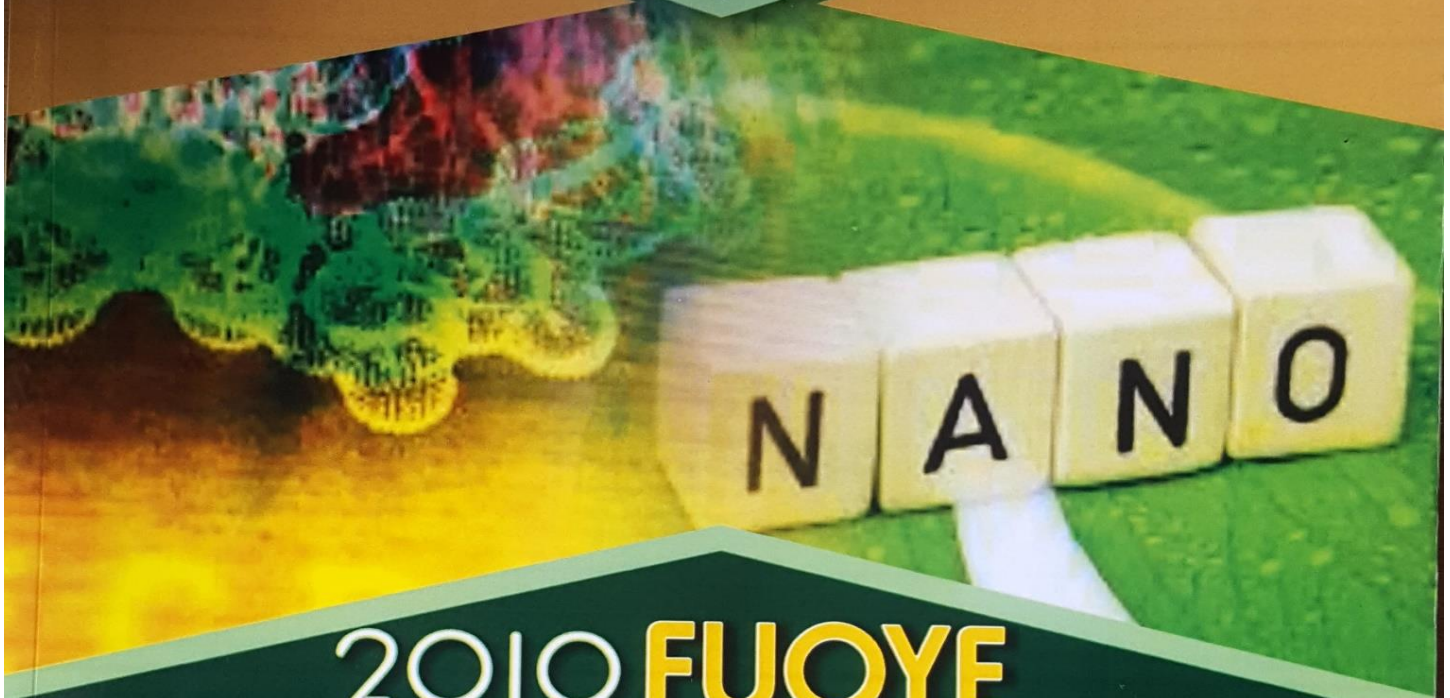### EKITI STATE, NIGERIA

FACULTY OF SCIENCE

2019 FUOYE
INTERNATIONAL SCIENCE CONFERENCE

CONFERENCE PROCEEDINGS

theme INNOVATION **&** ADVANCEMENT IN NANOSCIENCE & NANOTECHNOLOGY

Date: MONDAY 8TH - WEDNESDAY 10TH JULY 2019

Time: 9:00am-4:00pm daily

Venue:
Faculty of Science Auditorium, Federal University Oye-Ekiti, Ekiti State.

2019 FUOYE
INTERNATIONAL SCIENCE CONFERENCE

CONFERENCE PROCEEDINGS

THEME:
INNOVATION AND ADVANCEMENT IN NANOSCIENCE AND NANOTECHNOLOGY
08-10 JULY, 2019

Federal University Oye-Ekiti

Km 3, Are-Afao Road , Oye Ekiti,

371010, Ekiti State, Nigeria

www.fuoye.edu.ng

The papers in this book comprise the proceedings of the 2019 International Science Conference, held at the Faculty of Science Auditorium, Federal University Oye–Ekiti, Ekiti State, Nigeria, on 08–10 July, 2019. They reflect the authors' opinions and, in the interests of timely dissemination, are published as presented and without change. All papers are peer–reviewed.

Table of Contents

Conference Proceedings

A Mini-Review on the Application of Alumina Nanoparticles for Water Treatment

Eletta A.A. Omodele, Adeniyi G. Adewale[*], Magaji M. Mikaila, Ighalo O. Joshua

Chemical Engineering Department, Faculty of Engineering and Technology, University of Ilorin, Ilorin, P. M. B. 1515, Nigeria
adeniyi.ag@unilorin.edu.ng

Abstract

γ-Alumina possesses numerous industrial applications. In this mini-review, the use of alumina as precursors for the development of nanoparticles for water treatment was examined. A cursory examination of alumina as it appears in nature revealed its availability over the country. Alumina nanoparticles were synthesised in a variety of ways which includes precipitation and co-precipitation, sol-gel method, solution combustion and microwave synthesis. Most experimental investigations on alumina nanoparticles as adsorbent utilised batch experiments. From the review of experiments, it was discovered that alumina nanoparticles adsorption is mostly monolayer in nature and according to pseudo-second order kinetics. Though the adsorption capacities are widely varying, the removal efficiencies showed that the particles are very good adsorbents for the pollutants reported in studies.

Keywords: Alumina, wastewater, nanoparticles, pollutant, adsorption

1   Introduction

The pollution of water bodies by industrial effluents is of major environmental concern in developing countries in general and Nigeria in particular [1] and it has been on the rise due to continual industrialization [2]. These effluents contain heavy metals, dyes, pharmaceutically active compounds and a plethora of other inorganic pollutants [3] which are toxic to man and the environment. Conventional methods of water treatment includes adsorption and biosorption [4], chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies, and evaporation recovery [5, 6]. Some of these technologies have several disadvantages. They include high operational and maintenance cost, high energy requirement and generation of toxic sludge [7].

Nanotechnology is an emerging technology in recent years that exploits the potential of nanosized organic or inorganic particles in the removal of pollutants from aqueous medium. Nanoparticles are particles between 1 and 100 nm in size. Nano particles have several key advantages that make them of importance for removing pollutants from aqueous medium. (i) larger surface area than the bulk material, (ii) ability to be functionalised (by adding other chemical groups) to enhance performance [8]. Nanoparticles can be developed for carbonaceous materials, polymer-based substances, silicon dioxide, iron oxides, magnesium oxides, alumina and others [9]. γ-Alumina is a widely used metal oxides with numerous industrial applications. It can be used in plastic composites [10], as catalyst or catalyst support [11], as adsorbent [12] and as precursors for the development of nanoparticles. This paper is a mini-review of the use of alumina as precursors for the development of nanoparticles. The specific application of interest is in the removal of pollutants from aqueous solutions.

2   Alumina

Aluminium oxide (commonly called alumina) is the most commonly occurring oxide of aluminium and is represented by the chemical formula Al_2O_3. Aluminium is the most abundant metallic element and the third most abundant in the earth's crust after oxygen and silicon [13]. Alumina in its natural form is corundum but can be made from bauxite which is rich in hydrated aluminium oxides.
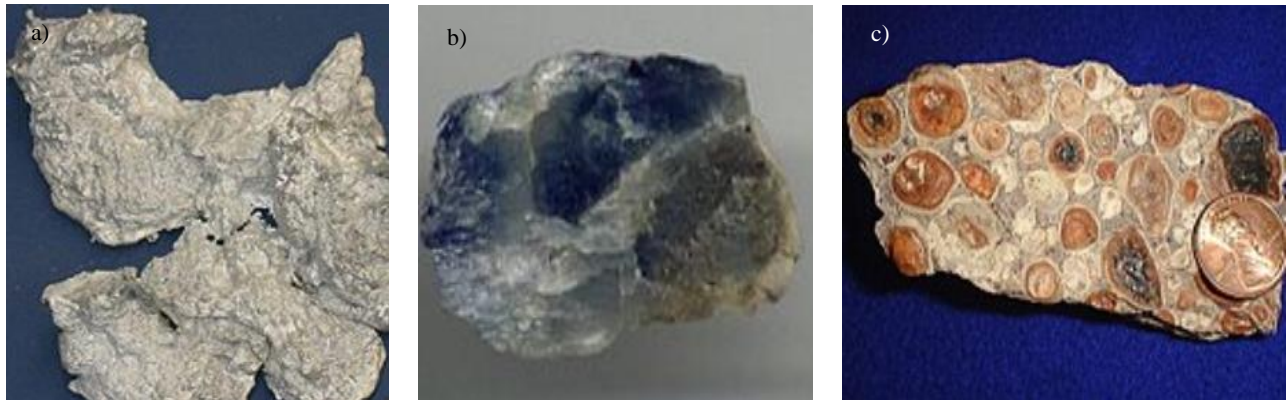
Fig. 1. Images of alumina (a), corundum (b) and bauxite (c).

Aluminium can also occur as silicates such as feldspar, muscovite mica, kaolin, fuller's cart, etc. [13]. Valeton [14] had earlier pin-pointed bauxite deposits in different parts of Africa and Nigeria inclusive. Ademola [13] revealed that there are significant quantities of alumina and bauxite in the east alongside other alumina silicates in Akwa-Ibom, Benue and other parts of North central Nigeria. There are significant amounts of bauxite in the Mambilla plateau in Northeastern Nigeria [15, 16] and in Orin-Ekiti in Southwestern Nigeria [17]. With these, we can confidently say that obtaining bauxite in Nigeria will not be a problem as studies have proven without any doubt. By consequence, alumina and aluminium compounds are cheaply available in Nigeria. In most laboratory experiments, aluminium compounds generally are used for aluminium nanoparticle synthesis. Some of such compounds are aluminium nitrate ($Al(NO_3)_3.9H_2O$), aluminium chloride ($AlCl_3.6H_2O$) and aluminium sulphate ($Al_2SO_4.16H_2O$).

3   Preparation of Alumina Nano-Adsorbent

Alumina nanoparticles have been synthesised in a variety of ways and these includes precipitation and co-precipitation, sol-gel method, solution combustion and microwave synthesis. Banerjee et al. [18] developed γ-Al_2O_3 by controlled precipitation of aluminium nitrate with sodium carbonate. The process was done at a pH of 7.5-8.5 and temperature of 70^0C. The obtained precipitate was kept for ageing at 80^0C for 2 hours before filtration, oven drying (2 h) and calcined in a furnace (600^0C, 3 h). The study obtained an 89% yield of 60-70 nm particles size. Dehghani et al. [19] also used a similar process as described above but with different calcination parameters (550^0C, 5 h). The product was then further precipitated with perfluoro-octanoic acid to obtain nano-perfluoro-octyl alumina particles. They obtained nanoparticles sizes of 2.5 to 3 nm.

Table 1. Summary of alumina nanoparticles preparation

Method	Starting materials	Diameter (nm)	Surface area (m²/g)	Ref
Precipitation	Aluminium nitrate ($Al(NO_3)_3.9H_2O$), Sodium carbonate (Na_2CO_3)	60-70	76	[18]
Sol-gel	Aluminium chloride ($AlCl_3.6H_2O$), liquid Ammonia (NH_3)	30-35	128	[20]
Solution combustion Synthesis	Aluminium nitrate ($Al(NO_3)_3.9H_2O$), Glycine ($C_2H_5O_2N$)	-	-	[21]
Co-precipitation	Aluminium nitrate ($Al(NO_3)_3.9H_2O$), Sodium carbonate (Na_2CO_3)	2.5-3	265.7	[19]
Sol-gel	Aluminium sulphate ($Al_2SO_4.16H_2O$), cetyl tri-methyl ammonium bromide ($N(CH_3)_3Br$), Ammonium hydroxide (NH_4OH)	3-12	58.49	[22], [23]
Microwave synthesis	Aluminium nitrate ($Al(NO_3)_3.9H_2O$), Ammonium hydroxide (NH_4OH), tri-methyl ammonium bromide ($N(CH_3)_3Br$), glacial acetic acid (CH_3COOH)	-	300-375	[12]
Sol-gel	Aluminium chloride ($AlCl_3.6H_2O$), liquid Ammonia (NH_3), Iron chloride ($FeCl_3.6H_2O$)	230	86.08	[24]
Sol-gel	Not stated	15-20	78.79	[25]

Banerjee et al. [20] synthesised the alumina nanoparticles via sol-gel precipitation. Aluminium chloride was dissolved in ethanol whilst the precipitating agent liquid ammonia was added dropwise until a gelatinous white precipitate of aluminium oxide was observed. The resultant solution was oven dried (90^0C, 6 h), calcined (600^0C, 3 h), milled and sieved. The study obtained a 91% yield

of 30-350 nm particles size. Bhargavi et al. [21] utilised a solution combustion technique for their synthesis. The mixture of aluminium nitrate and glycine solution was placed in a muffle furnace ($<45^0C$). There were no further details of yield and particle size obtained. Table 1 gives a summary of the processes and reagents used and some for alumina nanoparticles and the basic characteristics of the product obtained.

4   Application in Water Treatment

Most studies evaluated the application of nanoparticles in water treatment via batch experiments. Experiments are performed in glass-stoppered Erlenmeyer *fl*asks in most cases. A known volume of polluted aqueous solution is introduced into the flask. A weighed amount of adsorbent is added to the water to form a solution of known concentration or dosage. The pH of the solution can be adjusted by small volumes of strong acids and bases and then measured with a pH meter. Agitation can be achieved by an incubator shaker or centrifuge. Agitation speed and temperature can be set on the device. The experiment is terminated at a known time and then taken for analysis.

Banerjee et al. [18] utilised γ-Al$_2$O$_3$ nanoparticles for the scavenging of methylene blue from aqueous solutions. They obtained a maximum removal efficiency of 77% with film diffusion being the major controlling mechanism. Banerjee et al. [20] studied the adsorptive removal of orange G dye using alumina nanoparticles. It was observed that up to 98% of the pollutant could be removed and the process was according to pseudo-second order kinetics. Langmuir isotherm was the equilibrium model of best fit. Bhargavi et al. [21] studied the sorption of Zn(II) and colour black G. Though the nanoparticles had a higher capacity for Zn(II) than colour black G, a lesser removal efficiency was observed. This was probably due to the researchers utilising a starting pollutant concentration for Zn(II) 5 times higher than that of colour black G. For both pollutants, the process was based on pseudo-first order kinetics and Langmuir equilibrium isotherm. Dubey et al. [22] studied the removal of chromium from aqueous solutions using nano-alumina as a nano adsorbent. Their study was more focused on the comparison of different statistical and modelling techniques.

Dubey et al. [23] utilised response surface methodology in the optimisation of nickel adsorption by γ-Al$_2$O$_3$ nanoparticles. Virtually 100% of the pollutants was successfully removed. The process was based on pseudo-second order kinetics and Langmuir equilibrium isotherm. Prathna et al. [24] studied the sorption of fluoride, trivalent arsenic and pentavalent arsenic onto magnetic nano-alumina particles. The adsorption followed pseudo-second order kinetics and Langmuir equilibrium isotherm. Maximum removal efficiencies for fluoride, trivalent arsenic and pentavalent arsenic were 82%, 70% and 99%, respectively. Srivastava et al. [25] examined the sorption of nickel onto alumina nanoparticles. They observed a removal efficiency of 99% at optimum conditions. Yang et al. [26] studied the mass transfer and performance of fluoride removal by magnetic alumina aerogel. A moderately high adsorption capacity of the 32.1 mg/g was observed at an optimum pH of 5. Alumina nanoparticles have also been used to adsorb other pollutants such as methyl tert-butyl ether (MTBE) [19], petroleum sulphur and aromatics [12], oil emulsions [27, 28] with excellent results being obtained.

As revealed by Table 2, alumina nanoparticles adsorption is mostly by monolayer adsorption (Langmuir). This is expected as multi-layers of adsorbate will be difficult to obtain in very small pore spaces present in nanosized particles. Though the adsorption capacities are widely varying, the removal efficiencies give a proper picture of performance. From Fig. 2, we observe that the particles give very good removal efficiencies for most of the pollutants studied. The kinetics of the adsorption is also by second order in most cases. This implies that the process depends both on the amount of pollutant species present in the solution as well as the free adsorption sites on the nanoparticles.

Table 2. Summary of alumina nanoparticles application in water treatment

Target Pollutant	Adsorption capacity (mg/g)	Removal Efficiency (%)	Best fit isotherm	Best fit kinetics	Ref
Methylene blue	6.013	77	Langmuir	Weber-Morris	[18]
Orange G	93.3	98.4	Langmuir	Pseudo second-order	[20]
Zn^{2+}	1047.8	55	Langmuir	Pseudo first-order	[21]
Colour black G	263.2	98	Langmuir	Pseudo first-order	[21]
MTBE	46	-	BET	-	[19]
Cr^{6+}	0.8581	-	Langmuir	Pseudo second-order	[22]
Ni^{2+}	3.92	99.9	Langmuir	Pseudo second-order	[23]
As^{3+}	1.126	70	Langmuir	Pseudo second-order	[24]
As^{5+}	2.513	99	Langmuir	Pseudo second-order	[24]
Fluoride	4	82	Langmuir	Pseudo second-order	[24]
Ni^{2+}	30.82	99	Langmuir	Pseudo second-order	[25]
Fluoride	32.1	-	Freundlich	Pseudo second-order	[26]
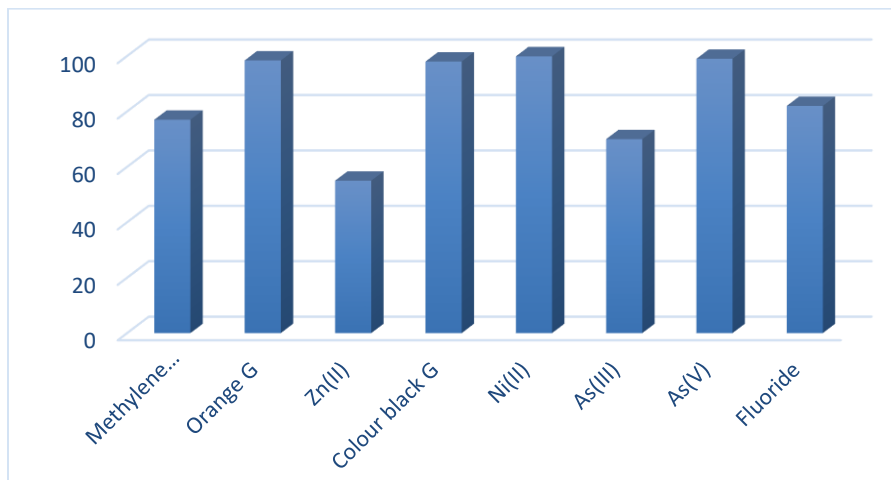
Fig. 2. Removal efficiency of different pollutants by alumina nanoparticles

5 Conclusion

From this mini-review, a cursory examination of alumina as it appears in nature was presented and its availability over the country was established. Alumina nanoparticles was reported in open literature to be synthesised in a variety of ways which includes precipitation and co-precipitation, sol-gel method, solution combustion and microwave synthesis. Most experimental investigations on alumina nanoparticles as adsorbent utilised batch experiments in their studies. From the review of experiments, several observations were made. Alumina nanoparticles adsorption is mostly by monolayer adsorption (Langmuir) and pseudo-second order kinetics. Though the adsorption capacities are widely varying, the removal efficiencies showed that the particles are very good adsorbents for the pollutants reported in studies. Alumina nanoparticles are efficient for treating effluents containing pollutants ranging from heavy metals, dyes, oils amongst others.

References

1. Patwardhan A., 2017. Industrial wastewater treatment. PHI Learning Pvt. Ltd.
2. Chowdhury S. and Balasubramanian R., 2014. Recent advances in the use of graphene-family nanoadsorbents for removal of toxic pollutants from wastewater. Adv. Colloid Interface Sci., 204, 35-56.
3. Chandrakanth G., Antía P.-G., Ganapati D.Y., Inmaculada O., Ibanez R., Virendra K.R. and Kumudini V.M., 2014. Pharmaceutical Industry Wastewater: Review of the Technologies for Water Treatment and reuse. Ind Eng Chem Res.
4. Eletta A.A.O. and Ighalo J.O. 2019. A Review of fish scales as a Source of Biosorbent for the Removal of Pollutants from Industrial Effluents. J Res Info Civ Eng, 16(1), 2479-2510.
5. Ahluwalia S.S. and Goyal D., 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98, 2243–2257.
6. Ramachandra N. Ahalya and Kanamadi R.D., 2003. Biosorption of Heavy Metals. Res J Chem Environ, 7(4), 1-19.
7. Sharma Y.C., Srivastava V., Singh V., Kaul S., and Weng C., 2009. Nano-adsorbents for the removal of metallic pollutants from water and wastewater. Environ Technol, 30(6), 583-609.
8. Kyzas G.Z. and Matis K.A., 2015. Nanoadsorbents for pollutants removal: a review. J Mol Liq, 203, 159-168.
9. Khajeh M., Laurent S., and Dastafkan K., 2013. Nanoadsorbents: classification, preparation, and applications (with emphasis on aqueous media). Chem Rev, 113(10), 7728-7768.
10. Abdulkareem S., Amosa M.K., and Adeniyi A., 2018. Synthesis and Structural analysis of Aluminium-filled Polystyrene Composites from Recycled Wastes. Environ Res Eng Mgt, 74(2), 58-66.
11. Adhikari S., Fernando S., and Haryanto A., 2007. Production of hydrogen by steam reforming of glycerin over alumina-supported metal catalysts. Catal Today, 129(3-4), 355-364.
12. Zaki T., Mohamed N.H., Nessim M.I., and El Salam H.A., 2013. Characterization and application of nano-alumina sorbents for desulfurization and dearomatization of Suez crude petrolatum. Fuel Process Technol, 106, 625-630.
13. Ademola J.A. and Olatunji M.A., 2013. Evaluation of NORM and dose assessment in an aluminium industry in Nigeria. World J Nucl Sci Technol, 3(04), p. 150.
14. Valeton I., 1991. Bauxites and associated terrestrial sediments in Nigeria and their position in the bauxite belts of Africa. J Afr Earth Sci (Middle East), 12(1-2), 297-310.
15. Idris Y., Funtua I., and Umar I., 2004. Rapid analysis with energy-dispersive X-ray fluorescence spectrometry for bauxite investigation on the Mambilla Plateau, North Eastern Nigeria. Chemie der Erde-Geochem, 64(4), 385-398.

16. Schwarz T., 1997. Distribution and genesis of bauxite on the Mambilla Plateau. SE Nigeria. Appl Geochem, 12(2), 119-131.

17. Ademilua O.L., Ajayi O.Z., Ogunniyi S.O., and Talabi A.O., 2013. Preliminary geophysical evaluation of orin bauxite deposit Southwestern Nigeria. J Emerging Trends Eng Appl Sci, 4(3), 432-437.

18. Banerjee S., Gautam R.K., Jaiswal A., Chattopadhyaya M.C., and Sharma Y.C., 2015. Rapid scavenging of methylene blue dye from a liquid phase by adsorption on alumina nanoparticles. RSC Adv, 5(19), 14425-14440.

19. Dehghani A., Ebadi A., Shafiei S., and Aghaeinejad-Meybodi A., 2016. Synthesis, characterization, and application of nano-perfluorooctyl alumina for adsorption of methyl tertiary-butyl ether (MTBE) from aqueous medium. Desalin Water Treat, 57(25), 11807-11819.

20. Banerjee S., Dubey S., Gautam R.K., Chattopadhyaya M., and Sharma Y.C., 2017. Adsorption characteristics of alumina nanoparticles for the removal of hazardous dye, Orange G from aqueous solutions. Arabian J Chem.

21. Bhargavi R.J., Maheshwari U., and Gupta S., 2015. Synthesis and use of alumina nanoparticles as an adsorbent for the removal of Zn (II) and CBG dye from wastewater. Int J Ind Chem, 6(1), 31-41.

22. Dubey S., Gusain D., and Sharma Y.C., 2016. Kinetic and isotherm parameter determination for the removal of chromium from aqueous solutions by nanoalumina, a nanoadsorbent. J Mol Liq, 219, 1-8.

23. Dubey S., Sharma G.C., and Sharma Y.C., 2019. Optimization of Reclamation of Ni (II)-Rich Solutions by γ-Alumina Nanoparticles. J Hazard Toxic Radioact Waste, 23(3), 04019005.

24. Prathna T., Sitompul D.N., Sharma S.K., and Kennedy M., 2018. Synthesis, characterization and performance of iron oxide/alumina-based nanoadsorbents for simultaneous arsenic and fluoride removal. Desalin Water Treat, 104, 121-134.

25. Srivastava V., Weng C., Singh V., and Sharma Y., 2011. Adsorption of nickel ions from aqueous solutions by nano alumina: kinetic, mass transfer, and equilibrium studies. J Chem Eng Data, 56(4), 1414-1422.

26. Yang W., Dou X., Li Y., Mohan D., Pittman C.U., and Ok Y.S., 2016. Performance and mass transfer of aqueous fluoride removal by a magnetic alumina aerogel. RSC Adv, 6(114), 112988-112999.

27. Franco C.A., Cortés F.B., and Nassar N.N., 2014. Adsorptive removal of oil spill from oil-in-fresh water emulsions by hydrophobic alumina nanoparticles functionalized with petroleum vacuum residue. J Colloid Interface Sci, 425, 168-177.

28. Franco C.A., Nassar N.N., and Cortés F.B., Removal of oil from oil-in-saltwater emulsions by adsorption onto nano-alumina functionalized with petroleum vacuum residue. J Colloid Interface Sci, 433, 58-67.

Nanotechnology in Water Treatment - Review

Omolola H. Aremu*, Olushola S. Ayanda, Olusola M. Fasoranti, Cecilia O. Akintayo

Nanoscience Research Unit, Department of Industrial Chemistry, Federal University Oye Ekiti, P.M.B 373, Oye Ekiti, Nigeria
hellenlol@yahoo.com, osayanda@gmail.com

Abstract
Treatment of wastewater has raised a great concern as it has been a rising setback nowadays. Wastewater from pharmaceutical industries, agriculture and textile industries are continually polluting the environment as wastewater are being discharged without proper remediation techniques. This usually affects the inhabitants and ecosystem at large. As conventional treatment of wastewater cannot eradicate all the pollutants in water, nanotechnology has gained ground in providing novel nanomaterials to remove contaminants in wastewater to have environment free of pollutants. One of the special characteristic of nanomaterials is the particle size on a nanoscale which can penetrate on a large surface material. This review paper includes the nanotechnological processes of wastewater treatment, innovations and future insight in nanotechnology for water treatment.

Keywords: Nanotechnology, conventional treatment, nanoadsorbents, nanomaterials, nanocatalyst

1   Introduction

Water is an essential natural substance in life as life cannot exist without consistent and secured water. There is therefore a remarkable sum of demand in shielding the sources of water accessible in the environment as this determines the life quality. It is the most valuable constituent on earth and it covers over 75% of the earth crust. It is consumed by animals, humans and plants and can be used for various purposes such as domestic use, religious functions, commercial use, industrial use, electricity generation, recreation and agricultural use. For water to be fit for use, it must be in a pure state i.e. it must be odourless, colorless, tasteless and free of contaminants. There is no doubt that most of what is been done requires the use of water and improper disposal leads to pollution of the environment without necessary treatment technique. According to World Health Organization (WHO), 16% of the world's population is denied of hygienic water and this is as a result of overcrowded people, unavailability of good water resources, pollution (air, water and land), deficiency of water recycling plants, indiscriminate location of industries in residential areas etc. [1]. Numerous organic materials, pathogens, toxic materials are often released into water during production processes and these often contaminate the environment [2]. The wastewater becomes hazardous to the health of plants, animals, water bodies and the ecosystem at large. To have an environment free of pollutants, wastewater must be treated so as to enhance a healthy living for those whose source of livelihood depends on water.

1.1   Water Treatment

Treatment of water can be done in various ways depending on the type of pollutants that needs to be removed. Water can be treated conventionally and this involves four stages which are preliminary stage, primary stage, secondary stage and tertiary stage (Fig. 1).
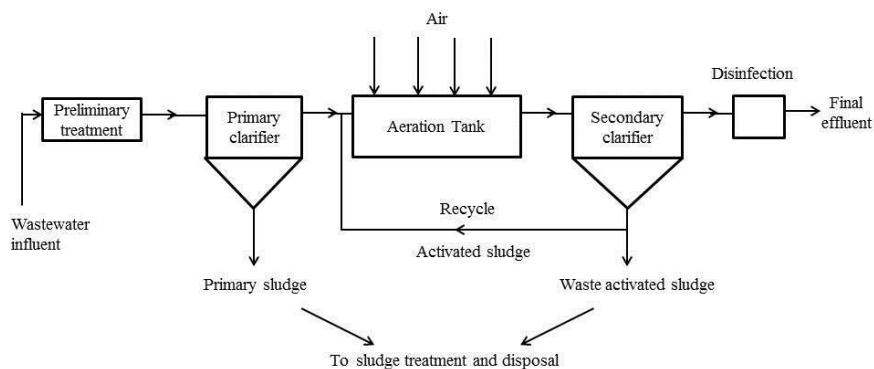

Fig. 1. Schematic diagram of a conventional water treatment [3]

1.1.1 Preliminary Stage

The preliminary treatment involves the removal of coarse solid materials and other large materials in wastewater with the use of grit chambers and bar screens. The water is passed through the bar screen to remove big solid objects such as clothes, plastics, papers, bottles and wood. The water is later passed through the grit chamber to remove smaller heavy objects that cannot be retained by the bar screen such as sand. The objects removed can later be disposed into the environment as landfills.

1.1.2 Primary Stage

The primary stage is a physical operation in conventional treatment of water and it involves the removal of settleable organic and inorganic solids by sedimentation, this is usually done for some hours to allow solid particles to settle at the bottom of the tank while non settleable materials float to the top for removal.

1.1.3 Secondary Stage

This is also known as biological treatment and it involves the removal of biodegradable dissolved and colloidal organic matter by biofilters and activated sludge. The major basis for biological treatment of water is the introduction of microorganisms, especially bacteria. The partly treated water from the primary stage flows into the aeration tank (aerobic) where oxygen consumes the organic matter remnant and changes them into water, carbon dioxide and biomass. The water can also be treated without air (anaerobic) to digest organic pollutants. The end products are methane and biomass.

1.1.4 Tertiary Stage

This stage entails the use of chemicals such as chlorine, alum, ozone, sodium bisulphate or ultraviolet irradiation to disinfect water before recycling. Substances such as phosphorous and nitrogen, inorganic compounds, bacteria, parasites, pathogens and viruses are all removed in this stage. This can later be disposed into the rivers or streams. Conventional treatment of water often results to partial removal of pollutants and there is therefore a necessity to build up a novel technology that can eradicate contaminants from water without secondary pollutants to meet the conditions of pure and hygienic water.

2   Reviews on Water Treatment

A lot of work has been done on the treatment of wastewater to ensure consistent accessibility to clean water in the environment. There is no doubt that water is often scarce during dry season and so the demand for water is always high. Abraham et al. [4] worked on the use of activated charcoal for the treatment of wastewater from bathrooms, kitchen sinks and laundry to remove contaminants and impurities as a means of recycling used water for clean-up and additional purposes. The authors reported that the water achieved by treatment with activated charcoal is pure, free from contaminants, tasteless and odorless. Activated charcoal is always readily available and it is less expensive; this makes it a good adsorbent as it has high porosity to penetrate on a large surface area for pollutants to be adsorbed. The report on the physicochemical parameters of treated refinery wastewater after the removal of naphthenic acids and aromatic naphthenic acids by Wang et al. [5] shows that 16% of naphthenic acids and 24% of aromatic naphthenic acids were removed. Ang et al. [6] reported that the use of chlorine in conventional treatment plants to get rid of bacteria and viruses brings about the development of disinfection by-product which has a harmful effect on the health of human. Conventional treatments often result to partial removal of pollutants; therefore, nanotechnology is seen as a viable method of water purification.

2.1   Applications of Nanotechnology in Water Treatment

Nanotechnology is a division of science that deals with the process of reforming, applying materials and breaking down of substances by an atom or molecule to create novel and distinctive substances in a scale of less than 100 nm [7]. For the past years, nanotechnology has been an up- and coming area of research as it is applied in wastewater treatment, textiles, explosives, car bumpers, propellants, window glasses, sun glasses, cosmetics, coatings, sport goods and paints. It offers a leaping opportunity to expand generations to come in restructuring the availability of water to be more sustainable. The outstanding utilization of nanoparticles such as ZnO and TiO_2 for water treatment has reduced the amount of pollutants in water. Nanotechnology is aggressively trailed to improve the ability of the previous treatment processes and build up novel processes. It is understood that nanotechnology is able to make a remarkable impact in ensuring availability of clean water in undeveloped countries because it is effective and cost effective [8]. The attractiveness of nanomaterials for water purification is dependent on some unique properties such as small size, high surface area, high photocatalytic activity, low-cost, high antimicrobial activity for disinfection and biofouling, etc. The nanotechnological processes of water treatment include nanofiltration, nanoadsorbents, nanocatalysts and nanomaterials.

2.1.1 Nanofiltration

It is certain that the varieties of contaminants seen in water can be removed via membrane filtration and this can serve as an important way of ensuring high level of water decontamination. This membrane filtration is very enormous because it is an important tool for removing selected dissolved organic compounds and variety of pathogens present in water; this is as a result of its efficiency. It is a membrane that is extremely pressured for water treatment procedures in removing pollutants from wastewater. It is essential to know that centrifugal pumps are used in nanofiltration for easy movement of wastewater at an efficient pressure around the nanomembrane. The membranes are of three types namely, organic/inorganic, ceramic and biological membranes [9]. Various membranes are configured in each of the unit inside the nanofiltration machine. Each length of the unit in the nanofiltration machine varies between 0.9 m to 5.5 m while the diameter ranges from 100 to 300 mm [10]. Fig. 2 and Table 1 shows the arrangement of units (vertical and horizontal) and the efficient removal of contaminants in wastewater via nanofiltration process, respectively.

Fig. 2. Horizontal and vertical arrangement of modules

Sources: Pentair X-flow, Membrane filtration pure and simple. http://advancedfiltration.pentair.com/en/products/horizontal-deadend, 05.11.2018.Pentair X-flow, Membrane filtration pure and simple http://xflow.pentair.com/en/products/aquaflex, 05.11.2018.

Table 1. Efficiency of nanofiltration in the removal of pollutants [10]

Pollutants	Removal Efficiency
Total dissolved solids	40 – 60 (%)
Total organic carbon	90 – 98 (%)
Colour	90 – 96 (%)
Hardness	80 – 85 (%)
NaCl	10 – 50 (%)
Sodium-sulphate	80 – 95 (%)
Calcium-chloride	10 – 50 (%)
Magnesium-sulphate	80 – 95 (%)
Nitrates	80 – 85 (%)
Fluorides	10 – 50 (%)
Arsenic	< 40 (%)
Atrazine	85 – 90 (%)
Proteins	3 – 5
Bacteria	3 – 6
Protozoa	> 6
Viruses	3-5

2.1.2 Nanoadsorbents

Nanoadsorbents show a noteworthy development for the remediation of wastewater as it is less expensive against conventional adsorbents due to its high précised surface area, short particle penetrating space and visible pore sizes [11]. The high adsorption ability of nanoadsorbent majorly depends on the high specific précised surface area. In other words, the energetic adsorption sites produced is as a result of the increase surface energy and the nano size attributed to nanoadsorbent. The exposure of the adsorption sites leads to retrieval of contaminants found around the surface of various nanomaterials. These functions of nanoadsorbent have made it to be voluntarily incorporated into other treatment procedures such as filters and sludge reactors. The utilization of nanoadsorbent in water treatment procedure will be of assistance to developing and underdeveloped countries where the reuse of water and other recycling procedures are expensive to maintain. It can guide against the increase in the discharge of wastewater into the environment and unsafe chemical mixtures. Furthermore, nanoadsorbents can serve as an alternative for other treatment reagents that is manufactured with huge amount of raw materials which invariably consume more energy during production or as substitutes to treatment techniques that

often result to environmental pollution. When pollution occur, plants, animals (aquatic or terrestrial) as well as the ecosystem is greatly affected [12].

2.1.3 Nanocatalysts

Metal nanoparticles and metal oxide nanoparticles are known to be practicable catalysts because they have a high catalytic power via which the molecules seen in contaminants undergoes oxidation during oxidation processes to generate harmless materials that can be changed to eco-friendly substances. These metallic and metallic oxide nanoparticles are well-known to have a small size that enables the particle to break in effortlessly on a large surface area and this invariably allow them to be exceedingly reactive in nature. Moreover, the usage of nanoparticles as catalyst during a chemical or photochemical reaction aids the demolition of pollutants that exist in wastewater. The possession of catalytic ability of metal and metallic oxide nanoparticles allows them to be used as an effective treatment of wastewater by the exploitation of nanocatalysts [13].

2.1.4 Nanomaterials

To keep an eye on the quality of water is very exigent due to low concentrations of micro contaminants found in wastewater. There is a need for an effective incorporation of nanomaterials that has a high photocatalytic activity, no secondary pollutants, non-toxic, biologically safe, stable under harsh environment and cost effective. Excellent nanomaterials such as zinc oxide, titanium dioxide and silver nanoparticles have the capacity to endure microbial commotion in water. They are mild oxidants and inert when introduced in water as disinfectants to produce non-destructive substances. Nevertheless, wastewater treatment involving the use of nanomaterials has its deficiency in that it will require a lot of nanomaterials for effective removal of large quantity of wastewater which is not economically safe [14].

3 Innovations in Nanotechnology for Water Treatment

Nanotechnology has improved the treatment of wastewater through various nanotechnological processes of wastewater, unlike the use of conventional treatment of wastewater that result in partial removal of pollutants and also involves the addition of chemicals. The attributes of nanotechnology in wastewater treatment are the small size found on a nanoscale, novel smart filters for different contaminant removal and the photocatalytic ability of the nanomaterials. The small size allows for easier penetration on a large surface of materials. Nanotechnology does not require the use of chemical, nanomaterials can be synthesized effortlessly, and the treatment procedure is fast when compared with other treatment processes for wastewater treatment. With the help of nanotechnology, the combination of ultrasound [15] and ultraviolet radiation in the presence of nanoparticles such as nZnO, nanoFe, and nTiO$_2$ often result to higher degradation of pollutant in wastewater treatment due to the formation of increased cavitation bubbles and hydroxyl radicals.

3.1 Future Insight

It is expected to have good and clean water in the environment for life sustenance. Nanotechnology has explored various fields such as science, engineering, education, medicine, agriculture, food technology, etc. It is vital to note that nanotechnology will soon be easily accessible both in urban as well as rural areas as nanoscience and nanotechnology researchers are seriously developing and implementing novel materials that are cost effective and available to treat drinking water and wastewater.

4 Conclusion

This review paper highlights the nanotechnological processes involved in wastewater treatment as an effective low-cost and readily available technique. Human population has grown extensively, and this has posed a challenge in protecting water resources. The ignorance and nonchalant attitude of people has resulted in water pollution which is as a result of improper disposal of wastewater without necessary remediation techniques and has contributed to environmental hazard. In other to sustain life, water must be consumed in a hygienic state. Therefore, with the help of nanotechnology, high percentage of pollutants can be removed from water and wastewater and this is attributed to the small size of nanomaterials on nano scale which aids easy penetration on large surfaces of materials. The use of nanotechnology is presently being recognized as an efficient and eco-friendly technology in wastewater treatment because it is cost effective, less tedious and has no secondary pollutant.

References

1. World Health Organization, 2004. Meeting the MDG drinking water and sanitation target: A mid-term assessment of progress, WHO, Geneva.

2. Turkar S.S., Bharti D.B. and Gaikwad G.S., 2011. Various methods involved in waste water treatment to control water pollution. J Chem Pharm Res, 3, pp. 58.
3. Rakesh S.A., Santosh K.S., Shweta S. and Megha S., 2016. Wastewater Treatment by Effluent Treatment Plants. SSRG Int J Civil Eng, 3, 29-35.
4. Abraham F.A., Thenmozhi R., Sivakumar M., Sivakumar K., Sasikumar G., and Thamaraimuthuayyanraj S., 2018. Wastewater treatment unit using activated charcoal. Int Res J Eng Technol, 5, 312 – 315.
5. Wang B., Yi W., Yingxin G., Guomao Z., Min Y., Song W. and Jianying H., 2015. Occurrences and behaviors of Naphthenic Acids in a petroleum refinery wastewater treatment plant. Environ Sci Technol, 49, 5796-5804.
6. Ang W.L., Mohammad A.W., Hila N., and Leo C.P., 2015. A review on the applicability of integrated/hybrid membrane processes in water treatment and desalination plants. Desalination, 363, 2-18.
7. Kavitha K.S., Syed B., Rakshith D., Kavitha H.U., Yashwantha Rao H.C., Harini B.P. and Satish S., 2013. Plants as green source towards synthesis of nanoparticles. Int Res J Biol Sci, 2(6), 66-76.
8. Gupta K., Bhattacharya S., Chattopadhyay D.J., Mukhopadhyay A., Biswas H., Dutta J., Roy N.R. and Ghosh U.C., 2011. Ceria associated manganese oxide nanoparticles: Synthesis, characterization and arsenic (V) sorption behavior. J Chem Eng, 172, 219-229.
9. Pendergast M.T.M. and Hoek E.M.V., 2011. A review of water treatment membrane nanotechnologies. Journal: Energy Environ Sci, 6(4), 1946-1971.
10. Tchobanoglous G., Stensel H.D., Tsuchihashi R. and Burton F., 2014. Wastewater engineering: Treatment and resource recovery, Fourth edition, New York, McGraw-Hill, Vol. 4, pp. 1-24.
11. Amin M.T., Alazba A.A. and Manzoor U., 2014. A review of removal of pollutants from water/wastewater using different types of nanomaterials. Adv Mater Sci Eng, 1(5), 125-146.
12. Bhattacharya S., Saha I., Mukhopadhyay A., Chattopadhyay D., Ghosh U.C. and Chatterjee D., 2013. Role of nanotechnology in water treatment and purification: Potential applications and implications. Int J Chem Sci Technol, 3(3), 59-64.
13. Nowack B., 2008. Pollution prevention n treatment using Nanotechnology". *In Environmental Aspects.* Edited by Harald Krug, Volume 2 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 1-12.
14. Nora S., and Mamadou S.D., 2005. Nanomaterials and water purification: opportunities and challenges. J Nanopart Res, 7, 331-342.
15. Ayanda O.S., Nelana S.M., Naidoo E.B., 2018. Ultrasonic Degradation of aqueous Phenolsulfonphthalein (PSP) in the presence of nano-Fe/H_2O_2. Ultrason Sonochem, 47, 29-35.

Geochemical and Petrological Evaluation of the Paleogene Strata Exposed at Ibese Quarry, Dahomey Basin, Southwestern, Nigeria

T.A. Bolaji[*], R.A. Akinpelu

Department of Geology, Federal University Oye-Ekiti, Nigeria
taiwo.bolaji@fuoye.edu.ng

Abstract
Paleogene strata freshly exposed by quarry activities at the Dangote Cement, Ibese were mapped, studied, logged and sampled. The twelve samples, which were recovered from the Northern (NM) and Southwestern (SWM) sections, include limestone, shale, ferruginized sandstone and a glauconitic rock, within the Akinbo and Ewekoro formations, respectively. These samples were examined to determine the geochemical characteristics of the sediments, mineralogical composition and provide petrographic interpretation in order to understand the associated diagenetic processes and depositional conditions. A combination of analytical techniques including: geochemical analysis for major elements, X-Ray Diffraction (XRD), and thin section were used in this study. Results showed that high Ca and low Mg content characterized the limestones. CaO content ranged from $4.13 - 94.67$ *wt*.% (*Av.* 65.85 *wt*.%), followed by SiO_2 with values ranging from $1.38 - 59.78$ *wt*.% (*Av.* 30.58 *wt*.%). Loss on Ignition (LOI) values ranged from $5.13 - 12.20\%$. The high Ca/Mg ratio defines a high calcite purity, which is significant in cement production. Calcite ($56.69 - 57.96\%$) dominates the mineralogy followed by quartz, dolomite and kaolinite. Three microfacies identified from petrographic observations are unsorted biosparite (packstone), sparse biomicrite (wackestone), and fossiliferous micrite (mudstone). Various diagenetic processes such as cementation, neomorphism, micritization, compaction, dissolution and dolomitization have affected the limestones. The presence of fossils such as sponges and molluscs in the limestones suggest they are typically deposited in a shallow marine environment, (probably upper deltaic) or brackish water (probably lower deltaic (marginal-marine)) environment. The studied limestones are suitable for cement production based on their elemental composition.

Keywords: Paleogene, limestone, microfacies, Ibese, Dahomey basin

1   Introduction

Paleogene rocks of the Akinbo and Ewekoro formations exposed by quarry activities at Ibese area of the Dahomey basin are composed of clastic materials and carbonates. These formations are dominantly limestone and carbonaceous fissile shale, along with mudstones, ferruginized sandstone and notable presence of a glauconitic bed, which marks the base of the Akinbo formation. The studied sections have variable thickness, 12–23 m, depending on the excavation program at the quarry site. Although limestone is the most economically viable resource within these units, this study evaluates the rocks freshly encountered within the Ibese quarry, operated by Dangote Industries Limited. Previous studies on the structural setting, general geology and hydrocarbon potential of Eastern Dahomey basin have been summarized by Adegoke et al. [1], Jones and Hockey [7], Reyment [13] among several others. Studies such as Fayose and Azeez [5], Ogbe [9], Okosun [10], Olatinsu et al. [11] focus largely on stratigraphy, paleontology, petrology and geochemistry of the limestone of Dahomey. Although some of these authors considered aspects of geochemistry and petrography using well core samples, studies on quarry samples has received less attention with little or no information on the aspects of diagenesis of these limestones.

Dahomey Basin comprises of island coastal and offshore basins, which extends from southeastern Ghana through Togo and Benin Republic to Southwestern Nigeria. It is separated from the Niger Delta Basin by the Okitipupa ridge [9, 12] at the eastern flank. The Nigerian sector of the basin extends from the boundary between Benin Republic and Nigeria to the Benin hinge line, consisting of an arcuate belt roughly parallel to the coastline (Fig. 1) [3, 14]. The Dahomey Basin has been described as an extensive wedge of Cretaceous to Recent sediments, which lies unconformably on the basement [14]. It is a marginal sag or pull-apart basin which developed in the Mesozoic as a result of the drifting of the African and south American plate [14]. The stratigraphy of Dahomey Basin is divided into three chronostratigraphic packages: pre-lower Cretaceous folded sediments, Cretaceous folded sediments and Tertiary sediments [3]. The oldest dated sediments onshore are the Cretaceous sediments, which rest unconformably on the crystalline Basement Complex. Further offshore, the Cretaceous sediments thicken and rest unconformably on the pre-lower Cretaceous sediments. In the Nigerian sector of the basin, the Cretaceous sequence referred to as Abeokuta Group is sub-divided into three formations: Ise, Afowo and Araromi formations [12]. Ise formation (Neocomian-Albian) is the oldest lithic fill and is unconformably disposed on the Basement Complex. It comprises of conglomerate and grit at the base, overlain by coarse-grained loose sand interbedded with kaolinitic clays [12]. Afowo formation is the middle layer of the Cretaceous sequence. It is composed of transitional

sand and sandstone with variable but thick interbedded marine shales, siltstone and claystones. The sandy facies are tar bearing, while the shales are organic-rich. The formation has been dated Turonian – Maastrichtian. Araromi formation (Maastrichtian – Paleocene) is the uppermost unit and is made up of fine to medium-grained sandstone at the base, overlain by shale and siltstone with interbeds of limestone, marl and lignite.
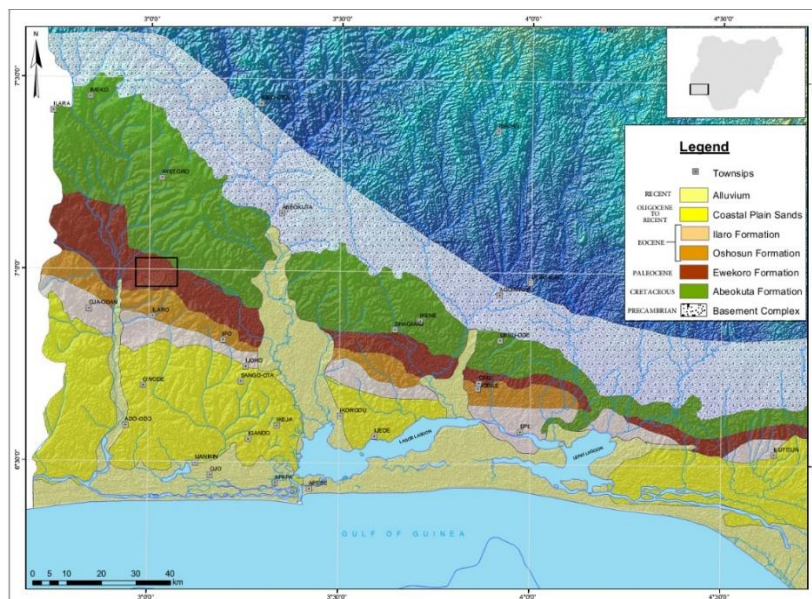
Fig. 1. The geological map of Eastern Dahomey Basin showing the study area

The Tertiary sediments overlying the Abeokuta group conformably consist of the Ewekoro, Akinbo, Oshosun, Ilaro and Benin formations. Ewekoro formation is made up of thick fossiliferous limestone while Akinbo and Oshosun formations are made up of flaggy grey and black shales, glauconitic bands and phosphatic beds, which define the boundary between the Ewekoro and Akinbo formation. The Ewekoro formation is Paleocene in age and associated with shallow marine environment due to abundance of coralline algae, gastropods, pelecypods, echinoid fragments and other skeletal debris [8]. The Akinbo formation overlies Ewekoro formation and it consists of shale, flaggy glauconitic rock bed, and gritty sand to pure grey shale and with little clay [9]. Limestone lenses from Ewekoro formation grades literally into the Akinbo shale towards the base. The base is marked by the presence of a glauconitic unit. The age of the formation is Paleocene to Eocene. The Oshosun formation overlies the Akinbo, which is a sequence of mostly pale greenish grey laminated phosphatic marls, light grey white-purple clay with interbeds of sandstones [10]. It also consists of claystone underlain by argillaceous limestone of phosphatic and glauconitic materials in the lower part of the formation and were deposited during Eocene [2]. The sedimentation of the Oshosun formation was followed by a regression phase, which deposited the sandstone unit of Ilaro formation. The sequence represents mainly coarse sandy estuarine deltaic and continental beds, which show rapid lateral facies change. The coastal plain sands are the youngest sedimentary unit in the eastern Dahomey Basin. It conceivably unconformably overlay the Ilaro formation but lack convincing evidence [7]. It consists of soft, poorly sorted clayey sand and pebbly sands deposited during Oligocene to Recent. Ilaro and Benin formations are predominantly coarse, sandy, estuarine, deltaic and continental beds, which are difficult to identify in the field.

The objectives of this work are to determine the geochemical characteristics of the sediments and integrating the major element geochemistry with the mineralogical composition and petrographic observations in order to understand associated diagenetic processes to which the rocks have been subjected and infer the depositional conditions.

2   Materials and Methods

Field mapping was carried out within the Dangote cement quarry located at Ibese. Rocks were examined in their natural locations *in-situ*, geologically relevant observations of rock features were made and field relationships established. Twelve (12) fresh samples were collected from the studied section. Laboratory studies include geochemical analysis, carried out on pulverized and pelletized samples using ED-XRF Spectrometer (EDX3600B Model). LOI was determined by igniting samples for 2 hours at 1000^0C. Mineralogical analysis was performed using a Shimadzu XDS 2400H diffractometer with Cu anode, λ_{CU} = 1.5406 [A°], on uncompressed powders in order to collect the maximum of the diffraction lines and a better identification of the phases. The prepared sample was placed in a Lucite holder on the goinometer of the instrument, which was configured with a graphite monochromator. The diffraction beam

monochromator operated at 40 KVA and a current of 30 mA with the 2θ values varying from 2^0 to 60^0 with step size of 0.02^0 for 120 minutes to create x–ray patterns with enough intensity to produce lines to identify minerals at the 2θ angles. Scanning rate was 0.75 degree per minute. Minerals were identified using the JCPDFWIN software of the Joint Committee on Powder Diffraction Standard (JCPDS). Thin sections were also prepared for petrographic observations by standard methods.

3 Results and Discussion

3.1 Geochemistry and Mineralogy

Chemical composition (major oxides) of the samples is summarized in Table 1, including loss on ignition (LOI) values. CaO and SiO_2 were the dominant oxides, with the exception of SWM-03 where Fe_2O_3 is present in significant amounts (26.26 *wt.*%). CaO concentration ranges from 4.13 – 94.67 *wt.* %, with an average of 65.85%, while SiO_2 values range from 1.38 - 59.78 *wt.*% with an average of 30.58%. The average percentages of the chemical composition of the major elements in the samples is as follows: SO_3 (6.61%), Al_2O_3 (4.62%), Fe_2O_3 (4.38%), P_2O_5 (1.05%), MgO (0.72%), K_2O (0.59%), TiO_2 (0.03%) and MnO (0.01%). CaO concentration is typically below 50% in SWM-02, 03, 04 and 05 because they are non-carbonates, while the concentration of SiO_2 is generally greater than 20% in these samples. This is consistent with the geology of the Akinbo formation [9]. The high Ca/Mg ratio observed defines high calcite purity, which is significant in the cement production. LOI values range from 5.13 - 12.20% with an average of 8.18%.

Table 1: Major elemental geochemistry of the samples

ELEMENT/ OXIDE	NM-02	NM-03	NM-04	NM-05	NM-06	SWM-02	SWM-03	SWM-04	SWM-05	SWM-06	SWM-07	SWM-08	Min.	Max.	Av.	St. Dev.
MgO	0.00	0.00	0.09	0.00	0.00	0.99	2.66	0.00	3.23	0.21	0.82	0.65	0.00	2.66	0.72	1.10
Al_2O_3	1.13	1.40	2.90	4.88	4.55	13.61	9.39	9.37	4.12	1.24	1.13	1.71	1.13	13.61	4.62	4.09
SiO_2	1.38	1.94	5.10	10.09	9.12	59.78	36.74	31.66	19.89	1.79	1.47	2.36	1.38	59.78	15.11	18.61
P_2O_5	0.72	0.75	0.68	0.58	0.64	0.51	2.13	0.66	3.82	0.70	0.79	0.64	0.66	2.13	1.05	0.97
SO_3	1.19	1.19	1.01	0.49	1.28	11.48	4.87	40.41	9.34	2.30	3.38	2.42	0.49	40.41	6.61	11.20
K_2O	0.00	0.00	0.00	0.00	0.00	1.13	1.54	0.69	3.72	0.00	0.00	0.00	0.00	1.54	0.59	1.12
CaO	94.67	93.63	89.07	76.02	82.59	4.13	14.21	11.92	48.82	92.81	91.40	90.95	4.13	94.67	65.85	35.94
TiO_2	0.00	0.00	0.00	0.00	0.00	0.19	0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.19	0.03	0.06
MnO	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.01	0.02	0.00	0.00	0.00	0.00	0.06	0.01	0.02
Fe_2O_3	0.34	0.47	0.48	5.76	1.09	6.57	26.26	4.09	6.14	0.35	0.48	0.55	0.35	26.26	4.38	7.34
LOI	6.85	7.69	7.63	5.13	9.26	10.20	12.20	10.10	8.62	7.14	7.14	6.25	5.13	12.20	8.18	1.97
Total	106.28	107.07	106.96	102.95	108.53	108.59	110.16	108.92	107.72	106.54	106.61	105.53	13.27	241.31	107.16	1.86

LOI = Loss on ignition

Results of x-ray diffraction (XRD) analysis revealed that the selected limestone samples are composed of calcite as the dominant constituent, while dolomite occur as a minor constituent. Kaolinite is the dominant clay fraction (<2μ) present, while quartz is the only non-carbonate and non-clay mineral recorded. Sharp x-ray peaks of kaolinite indicate its high crystallinity.

3.2 Petrography

The petrological studies of the thin sections were used to evaluate the carbonate microfacies, which consists of the allochems, micrite, sparry cement and ferruginized fragments. On the average, allochems constitute about 33.75% of the rock fabric followed by micrite (23.25%). The average composition of sparry cement is 25.25% and ferruginous fragments of about 22.75%. The results of the petrological classification of the limestones is presented in Table 2. Three microfacies identified based on Folk [6] and Dunham [4] classification schemes are: unsorted biosparite (packstone), sparse biomicrite (wackestone) and fossiliferous micrite (mudstone). 50% of the studied limestones show that they are mainly biosparites (packstones), with the allochems generally elongate and poorly sorted in thin section. The allochems consists mainly of fossils and pellets. Intraclasts are abundant in the limestones. Some of the limestones contain detrital quartz grains (NM-04). The fossils (macroscopically speaking) consist mainly of gastropods and bivalves shell fragments, some of which have been recrystallized to calcite. The fossils contain some nucleus of calcitic replacement or recrystallization of aragonitic shells, which is known as neomorphism (NM-05). The calcitic replacement as well as the absence of

micrite suggests some considerable amount of solution in the limestones. Some of the gastropods and bivalves shells have been recrystallized to calcite. Foliated structures were also observed in some of the thin sections.

Table 2. Petrographic classification of the studied limestones

Sample Location		Composition of Limestone (*wt.*%)			Composition of allochems (*wt.*%)				Folk (1974) Classification Scheme	Dunham (1962) Classification Scheme
Label	Description	Allochems	Micrite	Sparry cement	Ferruginized fragment	Intraclasts	Oolites	Fossils	Pellets		
NM-02	Limestone	70	20	10	30	30	0	27	43	Sparse Biomicrite	Wackestone
NM-03	Limestone	33	3	50	24	40	2	40	28	Unsorted Biosparite	Packstone
NM-04	Limestone	30	50	5	15	50	5	30	15	Sparse Biomicrite	Wackestone
NM-05	Limestone	20	10	50	20	20	10	20	50	Unsorted Biosparite	Packstone
NM-06	Limestone	2	80	2	16	0	0	5	95	Fossiliferous micrite	Mudstone
SWM-06	Limestone	65	20	5	10	50	10	20	20	Packed Biomicrite	Wackestone
SWM-07	Limestone	10	0	60	30	20	30	10	40	Unsorted Biosparite	Packstone
SWM-08	Limestone	40	3	20	37	30	20	30	20	Unsorted Biosparite	Packstone

Table 3. Petrographic classification of non-carbonate samples

Label	Description	Quartz	Microcline	Plagioclase	Biotite	Hornblende	Muscovite	Opaque
SWM-03	Ferrugnized Sandstone	27.7	22.2	32.5	10.0	3.6	3.2	0.8
SWM-05	Glauconitic unit	1.1	-	-	-	96.3	2.1	0.5

The framework of the glauconitic layer (SWM-05) shows predominance of hornblende (Table 3) while feldspar and quartz are dominant components of the sandstone (SWM-03). Attempts to impregnate and lap the shale samples were futile, hence, they were not captured in the petrography.

4   Conclusion

Globally, limestone is one of the industrial minerals quarried for cement production and a huge reserve is available in the study area. Chemical analysis of the studied limestones revealed a very low magnesium content and significantly high calcium content. The average CaO composition ranges between 4.13 and 94.67 *wt. %*. Mineralogically, calcite is the predominant constituent and its of the low-magnesian type. This low Mg is marked by the presence of advanced phyla such as sponges and molluscs in the limestones, rather than simpler organisms like algae. Based on this premise, and the alumina (Al_2O_3)–MgO relationship, a shallow marine environment of deposition is suggested. The presence of such fossils suggest that deposition would have taken place in a shallow marine environment, (probably upper deltaic) or brackish water (probably lower deltaic (marginal-marine)) environment. The presence of detrital input notwithstanding, the limestones are classified as pure, based on Todd scheme and this is further complimented by significantly high Ca/Mg ratio. Three microfacies identified from petrographic observations include: unsorted biosparite (packstone), sparse biomicrite (wackestone), and fossiliferous micrite (mudstone). The rocks have been subjected to extensive diagenetic processes, the most important of which are cementation, neomorphism, micritization, compaction, dissolution and dolomitization have affected the limestones. Petrographic studies of Ibese limestone deposit have shown that the rock is highly fossiliferous with the identified fossils indicating deposition in an open shelf environment. Moreover, the limestone deposit was equally observed to be principally mud supported which is indicative of rocks deposited in quiet water and a low energy environment. From the aforementioned textural characteristics, Ibese limestone deposits can be classified as mainly packstone and wackestone.

References

1. Adegoke O.S., Dessauvagie T.F., Kogbe C.A., and Ogbe F.G.A., 1971. The type section, Ewekoro Formation (Paleocene) of Southwestern Nigeria. Biostratigraphy and microfacies proceeding, 14 African Micropal. Colloquim, Abidjan, 27-39.
2. Agagu O.A., 1985. A Geological Guide Bituminous Sediments in South Western Nigeria. Unpublished Report, Department of Geology University of Ibadan.
3. Billman H.G., 1992. Offshore stratigraphy and paleontology of Dahomey Embayment, West Africa. NAPE Bull., 7 (21), 121 – 130.
4. Dunham R.J., 1962. Classification of Carbonate Rocks according to Depositional Texture. In: Ham, W.E., Ed., Classification of Carbonate Rock, American Association of Petroleum Geologists Memoir, 108-201.
5. Fayose E.A. and Azeez L.O., 1972. Micropaleontological Investigation of Ewekoro Formation, Southwestern Nigeria. Micropaleontology, 18, 69-332.
6. Folk R.L., 1974. Petrography of Sedimentary Rocks. Hemphill Publishing Company, Austin, Texas, pp. 182.
7. Jones M.A., and Hockey R.O., 1964. The Geology of Part of South Western Nigeria. Nigeria Geological Survey Bulletin, 31, 1-101.
8. Maduekwe H.Y., Obasi R.A., Fakolade O.R., and Bassey C.E., 2015. Provenance, Tectonic setting and Source-Area Weathering of the Coastal Plain Sediments, South West, Nigeria. Scientific Research Journal, 3(2), 20 – 31.
9. Ogbe F.A., 1972. Stratigraphy of Strata Exposed in the Ewekoro Quarry Southwestern Nigeria. African Geology, 305-322.
10. Okosun E.A., 1998. Review of the Early Tertiary Stratigraphy of Southwestern Nigeria. Journal of Mining and Geology, 34 (1), 27-35.
11. Olatinsu O.B., Olorode D.O., Clennell B., Esteban L., and Josh M., 2017. Lithotype characterizations by Nuclear Magnetic Resonance (NMR): A case study on limestone and associated rocks from the eastern Dahomey Basin, Nigeria. Journal of African Earth Sciences, 129, 701-712.
12. Omatsola M.E., and Adegoke O.S., 1981. Tectonic Evolution and Cretaceous Stratigraphy of the Dahomey Basin. Journal of Mining Geology, 18, 130-137.
13. Reyment R.A., 1965. Aspect of the Geology of Nigeria: Ibadan University Press, pp. 145.
14. Whiteman A.J., 1982. Nigeria, Its Petroleum Geology, Resources and Potential. Graham and Trottan London, 3949.

Kinetics of Heavy Metals Desorption from Soil Remediated with Hardwood Biochar and Flame of the Forest (*Delonix Regia)* Pods

B. M. Babalola[*], E.G. Olumayede, S.O. Afolabi

Department of Industrial Chemistry Federal University Oye-Ekiti, Ekiti State, Nigeria
bolamorayo@yahoo.com

Abstract

In this study, the efficacy of two treatments (*Delonix regia* pod powder and hard wood biochar) for the remediation of tropical soils contaminated with heavy metal ions (Pb^{2+}, Cu^{2+} and Cd^{2+}) were examined. The objective was to study the stabilization of the treated soils by assessing the available metal ions concentration and the index of resupply (R value). The effect of each treatment on metal availability and metal ions desorption kinetics was assessed using DGT-induced fluxes in soils model (DIFS). Soil dynamics predicted from values of the labile partition coefficient (K_d), response time to depletion (T_c) and rates of exchange or desorption (k_{-1}) of metal ions between soil solid and solution phases were also obtained using the DIFS model. The derived value for the labile partition coefficient, K_d, (Pb^{2+}, Cu^{2+} and Cd^{2+}) showed that metal ions that can be resupplied to soil solution from the soil solid phase was highest in biochar-treated soils. The depletion time (T_c) and desorption rate constant, k_{-1}, values also revealed that the biochar treated soils would be depleted faster than the *Delonix regia* treated soils. Thus, results from this work has shown that dynamics of metals in soils should be considered after treatment of contaminated soils.

Keywords: Hardwood-biochar, *Delonix regia,* metal ions, DGT-induced fluxes in soils (DIFS), labile pool size

1   Introduction

Soils serve as the major reservoir for heavy metals released into the environment as a result of human activities [1], their total concentration persist in soils for a very long time after they are introduced because they cannot be degraded by microorganisms; but available methods that give information on the dynamics and mobility of heavy metals resident in soils are limited. To be able to account for the dynamics of the soil/solute system, the diffusive gradients in thin films technique (DGT) was proposed [2]. The DGT can be used as a powerful tool for assessing bioavailability of metals in soils, taking into account metal concentration in soil solution and the solid phase and at the same time providing useful information on the kinetics of release from the solid phase to solution [3]. DGT induced fluxes in soils (DIFS), a dynamic model of the DGT–medium system, can be used to describe quantitatively the exchange of chemicals between solid phase and solution when the soil is perturbed by a DGT device, allowing the acquisition of kinetic parameters for the exchange process [4, 5].

The aim of this work is to obtain the exchange kinetics of metal ions (R values, from the solid phase to soil solution, and coefficient of distribution of the labile pool, K_d values) in soil contaminated with lead, copper and cadmium, after its remediation with commercially available hardwood biochar and *Delonix regia* material. The kinetics of resupply by response time, T_c and rate of desorption, k_{-1} were assessed using the DIFS model.

2   Materials and Methods

2.1   Materials

Metal solutions of Cu^{2+}, Cd^{2+} and Pb^{2+} prepared from salts of copper chloride dihydrate, $(CuCl_2.2H_2O)$, cadmium nitrate $(Cd(HNO_3)_2)$ and lead nitrate, $(Pb(NO_3)_2)$ obtained from Merck, Germany were used to spike the tropical soils used as samples for the remediation study. Hardwood biochar purchased from Bodfari environmental UK and *Delonix regia* pods harvested from Ekiti State University Ado Ekiti, Ekiti State, Nigeria were added as amendment to the contaminated soils to immobilize the heavy metals ions.

2.2   Methods

Preparation of Chelex binding gels, diffusive gels, the DGT devices and the whole experimental set up were carried out as described by Houben *etal*., 2013 and Zhang and Davison 1999 [6, 7]. All experimental apparatuses and DGT plastic moulds were acid washed in

10% nitric acid overnight and rinse properly with ultra-pure water before use. Triplicate samples of the contaminated soils obtained from the Western part of Nigeria (Ife, Ilesha and Ijero Ekiti) were treated with hardwood biochar and *Delonix regia.*

3   Results and Discussion

3.1   The Index of Resupply

The ratio of DGT-measured analyte concentrations (C_{DGT}) to the independently measured soil solution concentration (C_{soln}) referred to as R value (Equation 1) is an indication of the ability of the solid phase to resupply the analyte to soil solution. The closer the value of the resupply index, R to unity, the rapid the resupply of the metal from the solid phase to solution, it is thus used as a measure of the depletion of soil solution concentration at the DGT-medium interface [7, 8]. Table 1 represented the obtained average R values for the study.

$$R = \frac{C_{DGT}}{C_{soln}}$$ 1

Across all the soils, the average resupply of lead from the solid phase to soil solution is highest in the control soil of most samples (except in Ijero Ekiti). The control samples were able to resupply each of the metal ions studied moderately back to soil solution. The resupply of copper indicate that the buffering capacity of the biochar treated soils for the copper is high and copper in the solid phase could be resupplied to the solution faster in biochar-treated soils than those with *Delonix regia* treatment where the soil buffering capacity is poor. Cd soil solution concentrations were below the detection limit in the biochar treated soils and so its resupply in these soils could not be estimated. From the resupply of cadmium it can be concluded that the index of metal resupply is generally higher in all biochar treated soils.

3.2   Labile Pool Size of K_d Values

The distribution coefficient K_d is an indication of the labile pool size of the metal ions that can be available for DGT measurement; it is represented in Equation 2 as a ratio used to express the relation between the concentration of the labile metal associated with the solid phase to the concentration in soil solution [5, 9, 10].

$$Kd = \frac{C_{solid\ phase}}{C_{soil\ solution}}$$ 2

where the $C_{solid\ phase}$ is the concentration of each metal obtained by extraction with 0.01M $CaCl_2$ and the $C_{soil\ solution}$ is the concentration of the metal ions in soil solution obtained by centrifugation. When K_d value is high, it means that the metal species has been retained by the solid phase through adsorption. Whereas when it is low, it implies that high amount of the metal species are partitioning into soil solution.

The K_d values for Cu, Cd and Pb in this study showed that biochar treated soils have the highest K_d values in all the soil types irrespective of metals ions. The K_d values obtained from biochar treated soils for each metal ion was more than the K_d in the *Delonix regia* treated soils. The K_d values were as follows: Pb in biochar-treated soils was at least 700 times more than in *Delonix regia* treated soils; Cu was at least 2400 times more; while Cd was at least 200 times more in the biochar treated soils. This means that a large proportion of metals ions in the biochar treated soils are in the soil solid phase and can be redistributed to soil solution. Across all soils samples, K_d values for lead was the highest while cadmium had the lowest value.

Comparison of R measurements from DGT deployments for different times with the DIFS modelled R values allows the estimation of parameters such as the soil response time to depletion (T_c), which is the time needed for the disequilibria of labile metals induced by DGT to revert to 63% of the equilibrium value [11].

The response time T_c can be expressed as the inverse of the dissociation rate constants as shown in Equation 3 [5].

$$Tc = \frac{1}{k_1 + k_{-1}}$$ 3

The relationship between R, K_d and T_c can be quantified by the DIFS model if the input parameters of particle concentration (P_c), soil porosity (Φ), and the diffusion coefficients of the metals ions in the soil (D_s) shown in Equations 4 to 6 are supplied [12].

$$Pc = \frac{m}{V} \qquad\qquad 4$$

$$\Phi = \frac{dp}{(Pc+dp)} \qquad\qquad 5$$

$$Ds = \frac{Do}{(1 - ln(\Phi^2))} \qquad\qquad 6$$

with m being the total mass of soil particles; V is the porewater volume in a given volume of total soil; Do is the diffusion coefficient in water and dp is the density of the soil.

The dissociation rate constant k_{-1} is estimated by using the Equation 7 [5].

$$k_{-1} = \frac{1}{((1 + K_d P_c)T_c)} \qquad\qquad 7$$

3.3 Response Time and Resupply Rate Constant

Result obtained for the response time to depletion for the system, T_c, is shown in Table 2, from this Table, biochar treated samples of (Ilesha and Ife) have the highest values; it is observed that soil with high and moderate cation exchange capacity, CEC, (Ilesha and Ife) have highest response times with biochar treatment. The average T_c for copper and cadmium in biochar treated soil were the lowest (copper has T_c of 200 s while cadmium has 0.001 s on Ijero soil). From these results, the rate at which biochar treated soil is able to resupply the metals bound to its solid phase is very fast compared with T_c in *Delonix regia* treated soil samples. Although biochar treated soils have large coefficient of distribution for each of the metals ions, but it also has the fastest resupply, thus, from the kinetic point of view, remediation of heavy metal contaminated soils with biochar will not be considered as effective as *Delonix regia* treated soils over the time frame used in this study. Ilesha soil has the next slower response time probably because it has higher CEC value than other soils; it means it has higher sorption or ability to hold metal to itself than soils with lower CEC. Ife and Ijero soils have the fastest response time depending on the metal ions considered, this could be as a result of the low or moderate CEC, low pH, and poor organic matter content in these soils; these properties must have contributed to the fast response time in these soils. Low CEC and low clay content reduces the sorptive ability of soil for metals ions (Ijero and Ife soils).

3.4 The Desorption Rate Constant

The desorption or resupply rate constant k_{-1} was calculated from T_c, P_c and K_d using Equation 7. The values obtained for all studied soils samples (before and after remediation with 2% biochar and 2% *Delonix regia*) are shown in Table 3. Generally, the highest values of T_c produce minimum values of k_{-1}. Desorption rate constant for lead in the Ijero remediated soils showed that biochar treated soils have the least values except in Ijero soil. Soil with highest k_{-1} is quick to release metal bound to it and the one with lowest k_{-1} signifies the stronger the material retains the metal, and therefore a slower desorption will occur. In the case of cadmium, *Delonix regia* had highest value on Ife soil while Ilesha have highest k_{-1} for Cd in biochar treated soils. Generally, desorption rate constants for lead and copper before remediation were higher than those calculated but for cadmium, the desorption rate constant was in most soils the lowest before remediation was carried out. Lead k_{-1} was higher in biochar treated soils than in *D. regia* treated soils in all soil samples used in this study indicating that the desorption of lead from biochar treated soils is slower than in the *D. regia* treated soils; making biochar treatment more effective than *D. regia* material for lead removal. The Pb k_{-1} obtained for *D. regia* treatment appeared to the highest.

Table 1. Average R values for soil with and without hardwood biochar and Delonix regia treatment

Soil	Cu (R) Value			Cd (R) value			Pb (R) value		
	Control	Biochar	*Delonix regia*	Control	Biochar	*Delonix regia*	Control	Biochar	*Delonix regia*
Ijero	0.12	0.69	0.21	0.10	BDL	0.12	0.11	0.63	0.25
Ilesha	0.63	0.58	0.54	0.42	BDL	0.52	0.40	0.16	0.31
Ife	0.34	0.81	0.52	0.18	BDL	0.23	0.45	0.13	1.44

Table 2. Response time, Tc, (s) values obtained for Cu, Cd and Pb in selected soils with and without hardwood biochar and *Delonix regia* treatment

	Ijero			Ife			Ilesha		
	Cu	Cd	Pb	Cu	Cd	Pb	Cu	Cd	Pb
Day 5									
Control	$1.38*10^4$	0.40	$2.36*10^4$	701.9	1.52	345	210	$6.24*10^2$	4318
2% Bio	63	BDL	286.8	30.03	BDL	5401	133.1	BDL	2037
2% De	1476	$1.45*10^3$	701.5	124.1	230.6	0.02	628.1	470.3	626.2
Day 21									
Control	6330	0.001	5046	289.4	326.6	184.8	450.4	$1.32*10^2$	285.3
2% Bio	199.9	BDL	589.7	589.5	BDL	$3.34*10^4$	BDL	BDL	9505
2% De	3182	3649	$1.69*10^3$	149.6	2.67	0.03	111.7	0.043	2212
Day 42									
Control	6023	4176	4220	651.2	158.2	291.3	BDL	BDL	283.3
2% Bio	BDL	BDL	86.07	31.3	BDL	6978	BDL	DBL	4930
2% De	1965	2885	$9.98*10^2$	283.1	0.02	BDL	BDL	52.84	1043

Key: Bio: Biochar treated sample, De: Delonix regia pod powder treated sample, BDL: Below instrument's detection limit

Table 3. Dissociation rate constant, k_{-1} estimated for Pb, Cu and Cd on control and soil samples remediated with 2% (w/w) Biochar and 2% (w/w) *Delonix regia*

Metal/soil	Pb	Cu	Cd
k_{-1} value in Ijero soil			
Control	1.7e-07	3.40e-06	5.33e+01
Biochar	1.63e-07	1.85e01	2.74e-02
Delonix regia	2.85e-06	3.37e-06	4.37e-05
k_{-1} value in Ilesha			
Control	6.29e-08	2.81e-01	8.55e-03
Biochar	3.54e-09	2.16e-01	9.27e-01
Delonix regia	2.27e-08	1.44e-01	1.76e-02
k_{-1} value in Ife soil			
Control	3.35e-06	1.44e-05	1.64e-02
Biochar	4.63e-09	1.23e-06	2.77e-02
Delonix regia	2.41e+00	8.83e-06	9.51e-01

4   Conclusion

This work focused on the assessment of the remediation treatment of contaminated soils by biochar and *D. regia* using the DGT technique and kinetic modelling by DIFS. The results showed that each of the treatments have remarkable reducing effects on the available concentration of the studied metals in contaminated soils. The labile pool size (K_d) of metals (Pb, Cu and Cd) that can be resupplied to soil solution from the soil solid phase is in this order: Bio-treated soils > *Delonix regia* treated soils > Control soil samples. The response time (Tc) values followed the order: Control samples > *Delonix regia* treated soil > biochar treated soils. This depict that the resupply of available metal concentration back into the soil solution will be fastest in the biochar treated soils. In majority of the soils, the trend in magnitude obtained for the Tc values are the same obtained for the desorption rate constant, k_{-1}, a further confirmation that the control soils release available metal to soil solution much slower than the treated soils. The values obtained for k-1 showed that biochar treatment is effective for lead removal, both treatment types (biochar and *Delonix regia*) are effective for copper while none of the two treatments used in this work is effective for the removal of cadmium.

References

1. Wuana R.A., and Okieimen F.E., 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Network Ecology, pp. 1.
2. Davlson W., and Zhang H., 1994. In situspeciation measurements of trace components in natural waters using thin-film gels. Nature, 367, 546-548.
3. Gu X., Liu Z., Wang X., Luo J., Zhang H., Davison W., Ma L.Q. and Xue Y., 2017. Coupling biological assays with diffusive gradients in thin-films technique to study the biological responses of Eisenia fetida to cadmium in soil. J Hazard Mater, 339, 340-346.

4. Sochaczewski L., Tych W., Davison B., and Zhang H., 2007. 2D DGT induced fluxes in sediments and soils (2D DIFS), Environ. Modell Softw, 22, 14–23.

5. Harper M.P., Davison W. and Tych W., 2000. DIFS—a modelling and simulation tool for DGT induced trace metal remobilisation in sediments and soils. Environ Model Softw, 15, 55-66.

6. Houben D., Evrard L. and Sonnet P., 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere, 92(11), 1450-1457.

7. Zhang H. and Davison W., 1999. Diffusional characteristics of hydrogels used in DGT and DET techniques. Anal Chim Acta, 398, 329-340.

8. Ernstberger H., Davison W., Zhang H., Tye A. and Young S., 2002. Measurement and dynamic modeling of trace metal mobilization in soils using DGT and DIFS. Environ Sci Technol, 36, 349-354.

9. Lehto N.J., Davison W., Tych W. and Zhang H., 2008. Quantitative assessment of soil parameter (K D and T C) estimation using DGT measurements and the 2D DIFS model. Chemosphere, 71, 795-801.

10. Di Bonito M., Breward N., Crout N., Smith B. and Young S., 2008. Overview of selected soil pore water extraction methods for the determination of potentially toxic elements in contaminated soils: operational and technical aspects. Environ Geochem. Site characterization, data analysis and case histories. Nottingham: Elsevier, 213-249.

11. Guan D.X., Zheng J.L., Zhang H., Davison W. and Ma L.Q., 2017. A diffusion gradient in thin-films technique for the assessment of bisphenol desorption from soild. J Hazard Mater, 331, 321-328.

12. Harper M.P., Davison W., Zhang H. and Tych W., 1998. Kinetics of metal exchange between solids and solutions in sediments and soils interpreted from DGT measured fluxes. Geochim Cosmochim Acta., 62, 2757-2770.

Incidence of Nasal and Hand Carriage of *Staphylococcus aureus* among Food Handlers in Lagos, Southwest Nigeria

T.O. Egwuatu[1,*], O.M. Ukhureigbe[2], S. Iyayi[1]

[1]Department of Microbiology, University of Lagos, Akoka, Lagos State, Nigeria
[2]Department of Microbiology, Federal University, Oye Ekiti, Ekiti State, Nigeria
ukhureigbemiriam1983@gmail.com

Abstract

The study was carried out to determine the incidence of nasal and hand carriers of Methicillin Resistant *Staphylococcus aureus* (MRSA) among food handlers. A total of 120 samples were obtained from food handlers in six restaurants in Surulere, Lagos metropolis. A total of 96 *Staphylococcus aureus* representing 59 (61.5%) nasal and 37 (38.5%) hand were isolated and confirmed biochemically. Out of 96 *S. aureus* obtained, 7 were MRSA which comprised of 2 (2.1) from Home food, 1 (1.1%) from African cuisine, 1 (1.1%) from Lerovar, 3 (3.1%) from local joints and none from upper-crust and ideal restaurants. Antibiotic susceptibility test was done using Kirby-Bauer disc diffusion technique with zone of inhibition evaluated. The result showed that 28.1% were resistant to ceftazidime, cefuroxime (25.0%), gentamicin (25.0%), ceftriaxone (17.0%), erythromycin (17.0%), oxacillin (18.8%), ofloxacin (12.5%), augmentin (8.3%), vancomycin (8.3%), oxacillin (6.3%) and cefoxitin (7.2%). Nasal region had the highest level of resistance of antimicrobial. Age, gender and hygiene practice were major factors in distribution of *Staphylococcus aureus*. Out of the 98% of the food handlers that did not practice good hygiene management, 95% of them did not put on apron, 100% of them did not cover their nose while cooking, 100% of the females did not cover their hair, 45% of the male kept Afro, 30% of the female and 15% of the male had injury at one part of their body without proper covering. This study revealed that there was an incidence of MRSA/other antibiotics resistant *Staphylococcus aureus* in the community.

Keywords: Dnase agar, Kirby-Bauer disc, susceptibility, *Staphylococcus aureus*

1   Introduction

Food-borne infection is a major international health problem worldwide with consequent economic reduction [1]. Recognizing this, the World Health Organization (WHO) developed its global strategy for food safety. Food borne diseases result from ingestion of bacteria, toxins and cells produced by micro-organisms present in food. In the developing world, Food-borne infection leads to death of many children and resulting diarrheal disease can have long term effects on children's growth as well as on their physical and cognitive development [2]. The consumption of contaminated or unsafe foods may result in illness, also referred to as food -borne disease. In the industrialized world, food-borne infection causes considerable illness, heavily affecting healthcare systems [3]. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. Food poisoning outbreaks result in huge financial losses to restaurants, in addition to the loss of reputation and confidence among the public. Such disease remains a major public health problem globally, but particularly in developing countries due to difficulties in securing optimal hygienic food handling practices. Staphylococcal food-borne diseases are estimated to cause 6-81 million illnesses and up to 9000 deaths, and accounts for 14-20% of outbreaks involving contaminated food [4]. Most of the Staphylococcal infections are associated with the way food preparation and services are handled, as food handler's proximity to food brings about food contamination from the surrounding. An estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food [5]. The pathogenicity of *S. aureus* is due to the toxins' adherence to host cell proteins antibiotic resistance.

The role of food handlers in the spread of *S. aureus* in Nigeria is well documented by several authors showing the level of staphylococcal contamination and the prevalence of the disease thereafter. Some had worked on the effect of antibiotic on this organism notable in the Federal capital, Abuja, but only few works are available on the antibiotic pattern in Lagos State. Lagos represents good sample space to study the distribution of staphylococcal infection as it is densely populated with people from all spheres from the country. Moreover, the indiscriminate use of antibiotic is high in Lagos State compared to other states in Nigeria which is a basic health problem [6]. The global antibiotics resistance level of *staphylococcus* has increased and the amount being spread by food handlers is alarming. This study will therefore determine the level of the spread of *S. aureus*, and determine the antibiotic susceptibility profile. The source and point of contamination of the isolated organisms will as well be determined.

2   Materials and Methods

2.1   Collection of Samples

A total of 120 samples obtained from 60 food handlers' (made up of 60 nasals and 60 hands from 60 food handlers) working in six restaurants in Surulere, Lagos were obtained by swabbing the sterile swab sticks in between the fingers, after been moistened in sterile saline (0.9% NaCl) solution.

2.2   Isolation of Microorganisms and Characterization of Isolates

The samples were inoculated on solidified mannitol salt sugar (which is highly selective because of its high sodium chloride that inhibits the growth of most microorganisms except halophilic vibrio), and incubated at 37^0C for 24 h, after which the plates were observed for green-yellow coloration.

2.3   Catalase Test

A drop of three percent (v/v) hydrogen peroxide was placed on a clean grease -free glass slide and a sterile loop was used to place a small fraction of the organism on the hydrogen peroxide, after which it was emulsified. The production of glass bubbles indicated a positive reaction.

2.4   Bound Coagulase

Coagulase test was used to identify *S. aureus* which produced the enzyme coagulase. A drop of distilled water was placed on a clean slide; the test-colony from nutrient agar was picked with sterile loop and emulsified with the distilled water, after which a loop full EDTA anti-coagulated plasma was gently mixed with the suspension.

2.5   Free Coagulase

Isolates of 24 h culture were inoculated in a 2 mL prepared sterile tryptone soya broth (Oxoid, UK) in a test-tube, mixed thoroughly, after which equal volumes of plasma were dispensed into the constituent of the tubes and then incubated at 37^0C. Coagulation was checked at intervals of 1 h, 4 h and the preparations were later discarded after 24 h.

2.6   DNase Test

The isolates were confirmed by sub-culturing on DNase agar medium, and incubating for 24 h at 57^0C. The colonies were tested for DNase production by flooding the plates with 1 M HCl solution with precipitation of unhydrolyzed DNA. A positive reaction of DNase showed a distinct clear zone surrounding growth while no clearing indicted negative reaction.

2.7   Storage of Isolates

All confirmed isolates of *S. aureus* were stored in nutrient agar slants.

2.8   Methicillin resistance *Staphylococcus aureus* (MRSA) test

Methicillin resistance was determined by the OPxacillin disk diffusion test. Methicillin resistance test was carried out by inoculation of the isolates on Mueller Hinton agar supplemented with 4% NaCl. The 5 ug Oxacillin discs (Oxoid, USA) were aseptically placed on the surface of the inoculated plates and incubated aerobically at 35^0C for 24 h. The zones of inhibition were measured and compared with Microbiology Performance Standards for Antimicrobial Susceptibility Guidelines.

2.9   Antibiotics Susceptibility Test

Susceptibility to antibiotics was tested by the disc diffusion using the following antibiotics; augmentin (30 ug/disc), gentamicin (10 ug/disc), ofloxacin (5 ug/disc), erythromycin (25 ug/Disc), oxacillin (12.5 ug/disc), ceftazidime (30 ug/disc), ceftriaxone (30 ug/disc) and cefuroxime (30 ug/disc). Isolates were first cultured in Mueller Hinton agar (Oxoid) prepared with 5% human blood according to

the manufacturer's specification. The pure isolates from the blood agar were then subsequently sub-cultured into Mueller Hinton agar; the antibiotics disc were placed at the center and incubated at 37^0C for 24 h, after which the zones of inhibition were measured in millimeters.

2.10   Statistical Analysis

Chi-square test was applied at 95% confidence interval to determine potential risk factors associated with Staphylococcal hand and nasal carriage. The level of significance was set at $\alpha=0.05$ using two tailed method.

2.11   Interpretation of Result

The diameter of the zone of inhibition was measured to the nearest millimeter. Isolates were classified as susceptible, intermediate or resistant according to the guidelines of Clinical Laboratory Standards Institute, while multi-resistance was defined as resistance to at least three classes of antibiotics.

3   Results and Discussion

In this study, a total of 60 people working in restaurants and local eateries were examined to determine the carriage of methicillin resistance *Staphylococcus aureus*. The male: female ratio in the study was even, with mean age of 25. Ninety-six out of the 120 (80%) samples were positive for *S. aureus* based on Gram reaction, microscopy and biochemical reactions. The isolates were Gram-positive and appeared cocci in shape under the microscope, catalase positive with the evolution of bubbles when treated with hydrogen peroxide. Isolates were as well coagulase positive to both slide and tube coagulase reaction, with the formation of clot in the tube after incubation for 1 h and 4 h. All the samples positive to Gram reaction, catalase and coagulase tests were confirmed as *S. aureus* when incubated in Dnase agar for 24 h with the formation of clear zone around the colonies after flooding the plates with 1 M HCl solution.

Of the total 96 isolates of *S. aureus* obtained from the food handlers, the highest number of isolate - 87.5% (14/16) was observed in samples obtained from African cuisine, followed by side way vendors 85.7% (36/42). Others included home food with isolation rate of 83.3% (20/24), Ideal 67.7% (8/12), Lerovar 71.4% (10/14), and then least - Upper crust 6.7% (8/12) (Table 1). The overall performance of MRSA among the study population was 7.29% (7/96) which were obtained from home food restaurants 2(2.1%), 1(1.0%) from African cuisine, 1(1.0%) from Lerovar restaurants and then 3(3.1%) from side way vendor restaurants (Table 1 and 5).

Table 1. Incidence of *Staphylococcus aureus* among the study subjects

Samples source (Restaurants)	No. examined	No. of positive *Staphylococcus aureus*	Percentage isolation (%)	Percentage of MRSA (%)
Home food	24	20	83.33	2(2.08)
Ideal	12	8	66.67	0(0)
African cuisine	16	14	87.50	1(1.04)
Lerovar	14	10	71.43	1(1.04)
Upper crust	12	8	66.67	0(0)
Roadside vendors	42	36	85.71	3(3.13)
Total	129	96		7(7.29)

Key: MRSA represents Methicillin resistant *Staphylococcus aureus*

The age and sex distribution of *S. aureus* as seen in Table 2 showed that out of 96 isolates, 50 (52:1) were isolated from male and 46(47:7) from female. Among the males, the incidence rates were 12(12.5%), 17(17.7%), 13(13.5%), 6(6.3%), 2(2.1%), 0(0%) for ages 11-20, 21-30, 31-40, 41-50, 51-60 and >60, respectively; while that of the females were 7(7.3%), 9(9.4%), 11(11.5%), 8(8.3%), 3(3.1%), 0(0%) for ages 11-20, 21-30, 31-40, 41-50, 51-60 and >60, respectively (Table 2). However, the relationship between female and male was not statistically significant ($x^2=1.340$; p>0.5 and $x^2=2.894$; p>0.1, respectively).

There was statistical association between the different sites of samples obtained and isolation of *S. aureus* from the food handlers ($x^2=11.030$, p<0.01). Isolation of *S. aureus* with respect to site of sample collection showed a high percentage for nasal samples than from hand as 19(38.0%) were isolated from the hands of males and 31(62.0%) from their nasal regions, while 18(39.1%) were isolated from females' hands and 28(60.9%) from their nasal regions as shown in Table 3.

A total of 96 *S. aureus* isolates were tested against antimicrobial agents. Majority of the isolates were susceptible to ceftazidime 71.9% (69/96), cefuroxime 75.0% (72/96), gentamicin 75.0% (72/96), ceftriaxone 81.2% (79/96), erythromycin 81.2% (79/96),

cloxacillin 81.2% (78/96), ofloxacin 87.5% (84/96) and augmentin 91.7% (88/96). The isolates were highly susceptible to cefuroxime 92.7% (89/96) and gentamicin 91.7% (88/96).

Table 1 deduces that the highest number of isolate was recovered from samples obtained from African cuisine, while the lowest was recovered from sample obtained from Ideal/Upper crust. Table 2 shows that among the males, the highest incidence rate of *Staphylococcus aureus* infection was, 17(17.7%), while that of the females was 11(11.5%). Table 3 indicates that with respect to gender, isolation of *S. aureus* was higher in males compared to females. Table 4 shows that among the home food handlers, all females (100%) were infected by this organism, while 87.5% of the males were infected.

Table 2. Age and sex distribution of food handlers with *Staphylococcus aureus*

Age group(Years)	No. of positive sample Male(%)	Female(%)	No of positive sample (%)
11-20	12(12.5)	7(7.29)	19(19.79)
21-30	17(17.71)	9(9.38)	26(27.08)
31-40	13(13.54)	11(11.46)	24(25)
41-50	6(6.25)	8(8.33)	14(14.58)
51-60	2(2.08)	8(8.33)	11(11.46)
>60	0(0)	3(3.13)	3(3.13)
Total	50(52.08)	46(47.72)	96(100)

Table 3. Distribution of *S. aureus* in the nose and hand of the study subjects

Male		Female		
Hand	Nasal	Hand	Nasal	Total
5	9	5	3	22
2	2	3	3	10
2	2	2	4	10
2	4	1	3	10
1	3	2	2	8
7	11	5	13	36
19(38%)	31(62%)	18(39.13%)	28(60.87%)	96

Table 4: Distribution of *Staphylococcus aureus* among subject gender from the different restaurants

Sample source (Restaurant)	No. examined	No. of male	No. of female	Positive male (%)	Positive female (%)
Home food	12	8	4	7(87.5)	4(100)
Ideal	6	2	5	2(100)	3(60)
African cuisine	8	3	5	2(66.7)	3(60)
Lerovar	7	5	2	3(60)	2(100)
Uppercrust	6	3	3	2(66.7)	2(60)
Roasdside Vendors	21	9	11	8(88.9)	9(81.8)
Total	60	30	30	25(83.33)	23(76.67)

This study revealed a high incidence of *S. aureus* among 60 food handlers sampled from Surulere area of Lagos state. The rate of isolation 96(80%) of *S. aureus* in this study corresponds with a particular report [6], where *S. aureus* recorded an incidence of 71.4% among food handlers in a University Community in Southern part of Nigeria. However, this is in contrast with the report of another study [7] with a reported incidence rate of 20.5% among food handlers working at Gondar University, Northwest Ethiopia. The variation in incidence of *S. aureus* in these studies was attributed to the activities and hygiene management of the food handlers: 57 out of 60 (95%) did not put on aprons, 54(90%) did not have their regular bath, 48(80%) kept long finger nails, 54(90%) picked their nose, 39(65%) of the males kept afro hair while 39(15%) of them had cut on the fingers that were not covered. Moreover 18(30%) of the females had uncovered or undressed cut on their fingers. There was a total (100%) observation of non-compliance to hand washing, wearing of hand glove or moth mask before food preparation.

It was observed that the males 25 (83.3%) harboured *S. aureus* more than females 23 (76.7%) but this was not statistically significant (x^2=1.340; p>0.5 and x^2=2.894; p>0.1, respectively). In a report Pertanika [8], there was also no significant relationship between the male and female food handlers (x^2=0.261; p=0.609) despite the fact that 25% of female food handlers harboured *S. aureus* when compared to 18.8% of the male food handlers. The incidence of *S. aureus* was prominent in ages 21-30 years with 26 positive isolates followed by 31-40 years with 24 isolates. The isolation of *S. aureus* was observed in 60 years and above with 3 positive isolates. This

observation was similar to the work of a particular scientist [9]. The low occurrence of *S. aureus* among the aged can be attributed to restricted activities as they were involved in the supervision of the younger ones and it was observed that 90% of them practiced good hygiene of frequent hand washing and general cleanliness. Nasal carriage (59) in this study was higher than hand carriage (37) and this was statistically significant (x^2=11.03, p<0.01). The contamination of the food handlers with *S. aureus* could probably be as a result of hygienic non-compliance of food handlers. It was observed that ninety percent (90%) of the food handlers engaged in picking of their noses while on duty. In a report by Cepoglu *et al.*, [10] a higher incidence of *S. aureus* was also from the nasal region (43.6%) when compared to the hand region (36.8%) of the food handlers.

In this study, isolates showed low antibiotic resistance to augmentin (8.3%), ofloxacin (12.5%), vancomycin (8.3%) which was in agreement with the report by Onanuga and Temedie, [11] in which the resistances were recorded as follows: augmentin (5.0%), ofloxacin (10.0%), and vancomycin (7.5%). Vancomycin, a drug reserved for the treatment of MRSA infection, which in the past *S. aureus* exhibited 100% susceptibility to; but in this study *S. aureus* isolates in healthy individual were seen to have 8.3% resistance. A high antibiotics resistance of *S. aureus* to gentamicin (25.0%), ceftazidime (28.0%) and cefuroxime (25.0%) were also observed in this study, which was in contrast to the work by Onanuga and Temedie, [11] who reported no gentamicin resistant *S. aureus* but observed low occurrence of ceftazidime (5.0%) and cefuroxime (2.5%) resistance among their *S. aureus* isolates. The high resistance to cefuroxime (25.0%) in this study is of public concern because the antibiotic is expensive and not easily available to the common man; however, further work need to be carried out to substantiate this observation. Out of the 96 positive isolates, 7 (7.3%) were MRSA of which 5 (71.4%) were obtained from the nasal region and 2 (28.6%) from the hand region. However, the rate of resistance in this study was in consonance with a particular study in which 9.8% of *S. aureus* were resistant to cefoxitin.

4   Conclusion

A resistance of 7.3% observed in MRSA in the nasal and hand region of food handlers screened in this study portends potential danger to the public, if the organism eventually gets into the hospital environment due to the high infection potentials of the organism.

References

1. Borges L.J., Campos M.H., Andre M.D. and Serafini A.B., 2011. Microbiological quantity and phenotypic characterization of microorganism isolated from enteral feeding, food handlers and environment of two public Brazilian hospitals. J Food Safety, 31, 1125-1131.
2. Arvola T., Ruuska T., Keranen J., Hyoty H.S. and Isolauri E., 2006. Rectal bleeding in infancy: clinical, allergological, and microbiological examination. J Pediatr, 117(4), 760-768.
3. Okonko I.O., Ikpoh I.S., Nkang A.O., Udeze,A.O., Babalola T.A. and Fajobi E.A., 2010. Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria. *EJEAF Che* 9(1), 89-100.
4. Mead P.S., Slutsker L., Dietz V., McCaig L.F., Griffin P.M. and Tauxe R.V., 1999. Food-related illness and death in the Unites States. Emerg Infect Dis, 5, 607-625.
5. Ifeadike C.O., Ironkwe O.C., Adogu P.O.U., Nnebue C.C., Emelumadu F.O., Nwabueze S.A. and Ubajaka C.F., 2012. Prevalence and pattern of bacteria and intestinal parasites among food handlers in the Federal Capital Territory of Nigeria. Niger Med J, 53, 166-171.
6. Chigbu C.O. and Ezeronye O.U., 2003. Antibiotic resistant *Staphylococcus aureus* in Abia State of Nigeria. J Bacteriology 2(10), 374-378.
7. Mulat D., Moges T. and Fekeke M., 2012. Survey of nasal carriage of *Staphylococcus aureus* and intestinal parasite among food handlers working at Gondar University Northwest, Ethopia. BMC Public Health, 12(1), pp. 837.
8. Pertanika J., 2012. *Staphylococcus aureus* in hand and nares of food handlers in Kuala, Malaysia. J Trop Agric Sci, 35(4), 855-862.
9. Ketamkua C. and Tekalign A., 2013. Emergence of a community associated methicillin-resistant *Staphylococcus aureus* strain with a unique resistance profile in Southwest Nigeria. J Clin Microbiol, 97(9), 2975-2981.
10. Cepolgu O.D, Oyediran E.I.O., Adeyemo A.T. and Oyeleke O.T., 2010. Prevalence and antibiotic resistance pattern of methicillin resistance *Staphylococcus aureus* among in-patients at a tertiary health facility in ido-Ekiti, Nigeria. J Lab Med, 4, 21-30.
11. Onanuga A. and Temedie C., 2011. Nasal carriage of multi-drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassonma in Niger Delta region of Nigeria. Afr Health Sci, 11(2), 176-181.

Levels and Risk Assessment of Heavy Metals in Vegetables from Hospital Waste Dumpsite using Energy Dispersive X-Ray Fluorescence Spectrometry

Oketayo Oyebamiji Oyedele[*], Ikpe Uchechukwu Oluwasegun

Department of Physics, Federal University Oye-Ekiti, Ekiti State, Nigeria
oyebamiji.oketayo@fuoye.edu.ng

Abstract

In this study, the levels of nineteen heavy metals (As, Br, Ca, Cr, Cu, Fe, Ga, K, Mn, Ni, Rb, Sc Se, Sr, Ti, V, Y, Zn and Zr) in twelve vegetable samples (*Talinum triangulae and Amaranthus hybridus*) from the hospital waste section of Ado-Ekiti waste dumpsite were determined using Energy Dispersive X-Ray Fluorescence Spectrometry. Identical samples were also collected and analyzed as control. The results indicated that the observed heavy metals were within the range 1.196 - 4426.799 and 0.236 -3614.888 mg/kg in the site and control, respectively. Significant difference ($p <0.05$) also exists between the levels ($t_{values} = 0.01$-0.42) of Cr, Mn, Fe, As, Se and Y in the sites and control. The samples from the site were slightly enriched in As, Br, Cu, Cr, Ga, Mn and V (Enrichment Factors (EF) = 1.00-3.2). The relatively higher levels of Mn, Zn, Cr, Ni and As in the samples from the site compared with WHO/FEPA permissible limits was an indication of contamination. Hence, the consumption of these vegetables could be detrimental to health.

Keywords: Levels and risk assessment, heavy metals, vegetables, hospital wastes, EDXRF

1   Introduction

Over the years, Nigeria to be precise has been lagging behind when it comes to waste management [1]. Hospital waste dumpsites have been known to be a major source for the production of naturally occurring radioactive materials (NORM) and have been in existence for years without having knowledge of the threats posed to the public [2]. Medical waste that is burned with regular trash in incinerators or burned in the open poses a hazard as the smoke creates mercury. Dioxins and other toxic substances that go into the air we breathe. Medical waste thrown in the regular trash ends up in landfills where scavengers may be exposed to diseases caused by medical waste. Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis [3]. Several studies have demonstrated that reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as cadmium, chromium and lead [4]. It has also been reported that heavy metals have negative effects on the human system such as the cell, endoplasmic reticulum, nuclei, membrane and some enzymes that are involved in the metabolism, damage repair and detoxification [5].

This study therefore aimed at determining the baseline concentration of heavy metals in vegetables from a major hospital waste dumpsite and assesses the hazard or risk to the residents within the area of study.

2   Materials and Methods

Twelve (12) samples were collected from the hospital waste section of Ado-Ekiti waste dumpsite (located at Ilokun Ado-Ekiti). Two (2) vegetables (*Amaranthus hybridus* - African spinach and *Taliium triangulae* - water leaf*)* at various distances from the source were collected from the site. Identical samples were also collected as control. Samples were properly rinsed with distilled water to remove the sand and dust and stalk of the plants were carefully removed using nylon gloves and rinsed properly, freeze dried, labeled accordingly and kept separately. The same procedure was used for the control. Sample was first pulverized into fine powder. About 300 mg of the pulverized samples were each measured via a Sartorius micro-weighing balance. The measured sample was mechanically turned into a 13 mm target via the Carver model pelletizing machine before irradiating the samples in the external target chamber of the XRF system. The irradiation was done with an X-Ray Fluorescence Spectrometer (equipped with a silver (Ag) anode) at a voltage of 25 kV and current of 50 µA for 1200 counts or 20 minutes. The equipment model is PX 2CR Power Supply and Amplifier for XR-100CR Si-pin Detector. Characteristic X-ray of the sample was detected by the solid state Si-pin detector system and spectrum acquisition was done using an Amptek model multi-channel analyzer while elemental analysis was done using the thick target mode of the International Atomic Energy Agency (IAEA) Quantitative Analysis of X-ray Iterative Least (Q-Axil) square software. Determination of all the heavy metals in the vegetable samples was done using Energy Dispersive X-Ray Fluoresence

(EDXRF) Spectroscopy at the Centre for Energy and Research Development Obafemi Awolowo University Ile-Ife, Nigeria. This analytical technique is non-destructive, fast, sensitive, and multi-elemental.

The enrichment factor (EF) for each of the elements was determined by using crustal values and Fe as the reference element (Equation 1). Thus, Equation 1 indicates how enriched the samples are in heavy metals.

$$EF = \frac{\frac{X}{Conc\ of\ Fe(sample)}}{\frac{Y}{Conc\ of\ Fe(reference)}} \qquad\qquad 1$$

where X is the concentration of the element in the sample, and Y is the concentration of the element in the reference. The reference used in this research is the non-contaminating site (i.e. the control).

2.1 Quality Control and Statistical Analysis

Certified/ reference material (NIST 1515) was digested and analyzed for some heavy metals to ascertain the precision and accuracy of the results obtained in this study. Strong correlation calculation ($r = 0.99$) was obtained between the certified and experimental values. This ascertained the precision and reliability of the results obtained in this study. Significant differences were determined between the levels of these heavy metals in the site and control at $p < 0.05$ using t-test (SPSS 17)

3 Results and Discussion

The levels of heavy metals in vegetable samples from the site and control were presented in Table 1 while Table 2 shows the comparison of the results obtained in this study with WHO limits [6] and the control. Table 3 depicts the enrichment factors of heavy metals in the vegetable samples from the site.

Table 1. Descriptive statistics of heavy metals in the samples

Elements	*Talinum triangulare* (mg/kg)	Control (mg/kg)	*Amaranthus hybridus* (mg/kg)	Control (mg/kg)
K	7.061 ± 0.350	2.650 ± 0.133	2.819 ± 0.175	2.627 ± 0.165
Ca	4426.799 ± 211.745	3614.888 ± 140.974	1.251 ± 0.055	1.285 ± 0.049
Sc	349.506 ± 78.715	255.125 ± 20.390	812.859 ± 216.355	999.558 ± 200.153
Ti	21.832 ± 3.515	6.936 ± 3.435	49.075 ± 5.864	47.972 ± 3.512
V	13.628 ± 2.224	5.253 ± 5.864	21.544 ± 6.377	16.353 ± 4.544
Cr	12.000 ± 3.515	3.443 ± 1.330	11.766 ± 3.515	13.661 ± 0.556
Mn	106.766 ± 9.008	40.074 ± 1.482	57.546 ± 6.555	26.320 ± 5.529
Fe	123.356 ± 8.109	111.801 ± 2.802	374.431 ± 21.128	223.602 ± 29.283
Ni	18.275 ± 3.515	17.589 ± 0.888	31.749 ± 3.754	23.371 ± 4.558
Cu	22.347 ± 4.075	32.841 ± 1.182	20.661 ± 3.329	25.814 ± 4.488
Zn	79.003 ± 4.770	81.752 ± 1.532	88.371 ± 6.920	69.168 ± 10.987
Ga	10.089 ±5.864	3.969 ± 0.343	14.980 ±5.864	3.171 ±5.612
As	11.217 ±0.004	3.585 ±1.345	13.721 ±5.777	3.683 ±7.210
Se	11.966 ±1.347	5.504 ±0.344	17.513 ± 4.653	5.298 ± 1.309
Br	15.064 ±1.347	6.872 ± 1.347	15.940 ± 4.554	11.619 ± 1.419
Rb	19.667 ± 5.820	19.002 ± 1.619	27.078 ± 5.228	25.368 ± 1.848
Sr	16.333 ±5.864	7.333 ± 1.684	28.889 ± 5.864	34.222 ± 1.928
Y	17.154 ± 1.045	6.481 ±5.864	18.668 ±1.488	7.011 ±0.878
Zr	17.714 ± 2.334	5.450 ± 0.567	21.108 ±3.445	5.905 ±2.112

Table 2: Comparison of the levels (mg/kg) of heavy metals in the vegetables with WHO limits

Elements	*Talinum triangulare* (site)	*Talinum triangulare* (Control)	*Amaranthus hybridus* (site)	*Amaranthus hybridus* (Control)	WHO (2011) limits
Ca	4426.799	3614.888	1.251	1.285	1000
Sc	349.506	255.125	812.859	999.558	1000
Fe	123.356	111.801	374.431	223.602	420
Mn	106.766	40.074	57.546	26.320	6.64
Zn	79.003	81.752	88.371	69.168	60
Cu	22.347	32.841	20.661	25.814	40
Ti	21.832	6.936	49.075	47.972	1000
V	13.628	5.253	21.544	16.353	1000
Cr	12.000	3.443	-	13.661	2.3
Ni	18.275	17.589	31.749	23.371	10
Ga	10.089	3.969	14.980	3.171	-
As	11.217	3.585	13.721	3.683	1.4
Se	11.966	5.504	17.513	5.298	-
Br	15.064	6.872	15.940	11.619	-
Rb	19.667	19.002	27.078	25.368	-
Sr	16.333	7.333	28.889	34.222	-
Y	17.154	6.481	18.668	7.011	-
Zr	17.714	5.450	21.108	5.905	-

Table 3. Enrichment factors of the heavy metals in the samples

Element	Enrichment Factor (TT)	Enrichment Factor (AH)
Ca	1.11	0.58
Sc	1.24	0.49
Fe	1.00	1.00
Mn	2.41	1.30
Zn	0.88	0.76
Cu	1.10	0.48
Rb	0.93	0.64
K	2.41	0.64
Ti	0.30	0.59
V	2.34	0.79
Cr	3.20	0.51
Ni	0.94	0.81
Ga	2.30	2.82
As	2.83	2.22
Br	2.00	1.24

Key: TT = *Taliium triangulare* (water leaf), AH = *Amaranthus hybridus* (African spinach); E<1 (not enriched), 1-10 (slightly enriched), 10+ (enriched)

The results indicated that the concentrations of Ca, Sc, Fe, Rb, K, Ti, V, Cr, Ni, Ga, As, Se, Br, Sr, Y, Zr and Mn on the site were within 1.196-4426.799 mg/kg. Figs. 1 and 2 are the pictorial representations of the levels of the observed heavy metals in the site considered and the control. These results were found to be relatively higher than the control for heavy metals Cr, Mn, Fe, As, Se and Y (t = 0.01-0.42) as shown in Figs. 1 and 2. Generally in the site, the mean concentration of Ca was higher in *Talinum triangulare* indicating the variability in the rate of absorption of these heavy metals by the two vegetables. This implies that the heavy metal could be easily absorbed by *Talinum triangulare* than *amaranthus hybridus*. The same trend was also observed for Mn in those samples. In most cases, the concentrations of these metals decreases with respect to distances (m) from the site. However, the results obtained for Br, Cr, Cu, Ni and Zn in this work were higher but lower for Fe and Fe compared with a similar study by Muhammad et al. [7].

The mean concentration (mg/kg) of Mn, Zn, Cr, Ni and As in *Talinum triangulare* and *Amaranthus hybridus* were higher than the maximum permissible limits set by WHO (Table 2). This was an indication that the vegetables at this site were contaminated. Their consumption could be detrimental to health. Although, Manganese is very essential for photosynthesis but its continuous consumption could result into bio-accumulation in the body system. Generally and also relative to the control, the results obtained from t-test indicated that significant difference exists (t<0.05) in the concentrations of these heavy metals. This variation was also an evidence of contamination by the hospital waste dumpsite. These vegetable samples from the site were slightly enriched in Mn, Cu, K, V, As, Br, Ca, Se (EF = 1.00-3.20) but not enriched in Zn, Rb, Ti and Ni.
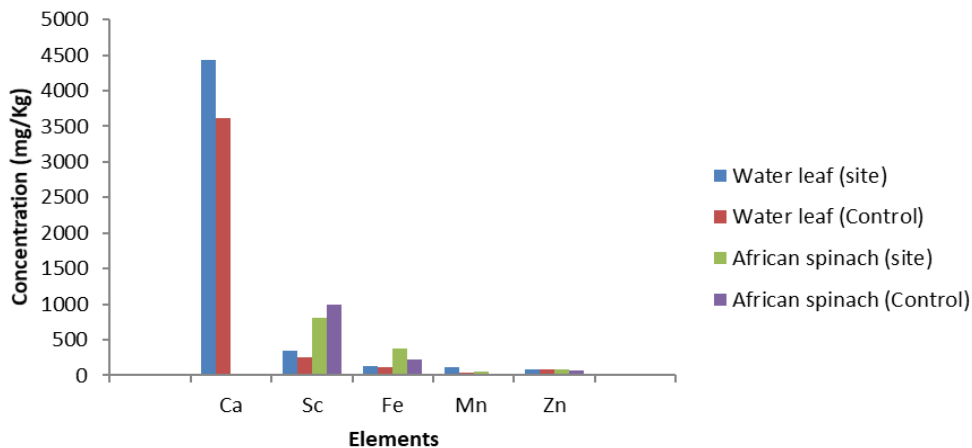
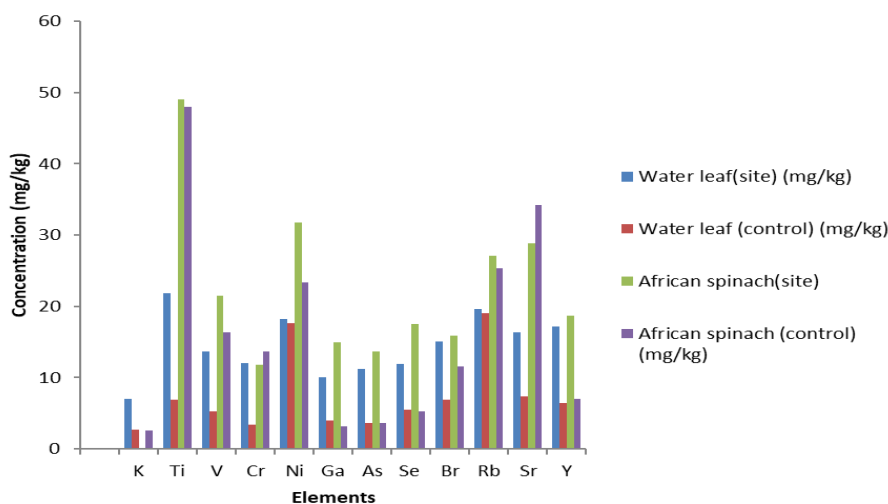Fig. 1. Levels of heavy metals in the samples and control (mg/kg)

Fig. 2. Levels of heavy metals in the samples and control (mg/kg)

4   Conclusion

The levels of nineteen heavy metals (Ca, Sc, Fe, Rb, K, Ti, V, Cr, Ni, Ga, As, Se, Br, Sr, Y, Zr, Mn, Zn and Cu) in vegetable samples have been determined using Energy Dispersive X-Ray Fluorescence spectrometry (EDXRF). The results obtained gave the baseline levels of these metals in vegetable samples from the hospital waste dumpsites. Using t-test, the result showed that significant differences exists between the levels of these heavy metals in the site and control ($p < 0.05$, $t = 0.01$-0.42). The two different vegetable samples were also found to be slightly enriched in Mn, Zn, Cr, Ni and As (EF = 1.00-3.20). The relatively higher levels of heavy metals (compared with control and WHO) observed in this study was an indication of pollution/contamination of these edible plant samples at the dumpsites.

References

1. Nwachukwu Nkechi Chuks, Orji Frank Anayo and Ugbog Ositadinma Chinyere, 2013. Health Care Waste Management – Public Health: Benefits, and the Need for Effective Environmental Regulatory Surveillance in Federal Republic of Nigeria, In: Current Topics in Public Health, Intech, http://dx-doi.org/105772/53196, 150-178.
2. Uwadiegwu B.O., 2013. Strategies for effective urban solid waste management in Nigeria. European Scientific Journal, 9 (8), 296-298.
3. Tchounwou P.B., Ishaque A., Schneider J., 2001. Cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2) exposed to cadmium chloride. Molecular Cellular Biochemistry, 222, 21-28.
4. Vousta, D., Grimanis A., Samara C., 1996. Environmental Pollution, 94, 325-335.
5. Wang S., Shi X., 2001. Molecular mechanisms of metal toxicity and carcinogenesis. Molecular Cellular Biochemistry, 222, 3-9.

6. WHO, 2011. Evaluation of certain contaminants in food (Seventy-Second Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959.
7. Muhammad F., Anwar F. and Rashid U, 2008. Appraisal of Heavy Metal Contents in Different Vegetables Grown in the Vicinity of an Industrial Area. Pak J Bot, 40, 2099-2106.

Near Field Communication (NFC) Based Lecture Attendance Management System on Android Mobile Platform

Comfort Y. Daramola*, Olaiya Folorunsho, Bosede A. Ayogu, Lawrence Adewole

Department of Computer Science, Federal University Oye-Ekiti, Ekiti State, Nigeria
daramola.bola@gmail.com

Abstract

Automatic identification system (AIS) has been employed by several organizations for keeping track of the attendance record of their employees. In higher institutions in Nigeria, the usage of AIS has not been fully adopted despite the tremendous benefits of using this system for recording attendance. The manual method of calling names and marking attendance list in the universities consume quality time and distract students whenever lectures are going on. Also, impersonation on the part of the student; a situation whereby students sign attendance for their friends, which is not helping students to build their integrity. The difficulty experienced during manual method could be addressed by adopting technology like Near Field Communication (NFC)-based attendance management system. Using this technology, there would be significant reduction in the paper work and manual data input that may lead to data inconsistency and loss. This work, therefore, provides an efficient way of capturing students attendance during lectures that will be less prone to both human manipulations and error-free using NFC on android mobile platform.

Keywords: Near Field Communication (NFC), attendance, management system, mobile platform

1 Introduction

In present-day institution, attendance registration is posing a great challenge in academic setting as a result of hurdles surrounding the process adopted. Attendance is an integral part of students' academic record; in most institution students are required to meet-up with some percentage attendance to be eligible to write examination or used as part of continuous assessment in each of their respective courses. However, the traditional method of passing an attendance paper or calling names is tedious, error prone, time consuming and inefficient as some students may double-dip or attendance paper misplaced. These challenges in recent time has imposed a need for an improved and more efficient process of attendance registration. Since user authentication and verification is the major aspect of an attendance system, improved technology is required. Few of the technologies that can be in place for simplification and improvement of attendance system are Radio Frequency Identification (RFID), Biometric, and Near Field Communication (NFC) [1].

In the recent decade, attention has been drawn to the usage of RFID and NFC contactless technologies for the communication and exchange of data with their contactless identification associated with these device [2]. These two devices made use of active reading devices to gather data stored on passive, wireless tags which could be attached to objects. RFID is best known for the tagging of consumer products and its use in contactless smartcards for identification, ticketing or mobile payment. Although both RFID and NFC use the same contactless identification, NFC is mobile devices compliance [3].

Nowadays, the number of mobile phones that come with NFC functionality has continue to increase, there is tendency for adoption of NFC technology mobile contactless interaction. NFC works on short/close operating distance between the devices and (passive) tags of about 3 to 5 cm. To establish communication between any mobile phone and tag, either the tag is touched with the reading device or closely holding them together. Adopting the technology of either NFC or RFID in managing student attendance in higher institutions in Nigeria will of no thought eradicate the wastage of quality time, errors and manipulations associated with traditional attendance collection.

The NFC is variant of an RFID, both are based on contactless identification technology. NFC has a relatively short-range radio communication while RFID has a longer range. Despite that both NFC and RFID can identify objects at a range, the NFC is more secure to RFID due to its shorter range, whereas the RFID has a greater potential for unauthorized tag readings, known as eavesdropping. NFC's short-range reading capacity lowers its potential for eavesdropping, even if the tags are not sufficiently secured [4]. There are currently several terminal devices for RFID (e.g., PDAs and laptops) that have been integrated with an RFID reader. However, RFID does not yet occupy enough space in the smartphone area compared to NFC, and having an NFC reader and tag in a smartphone gives it benefits in terms of availability and usability.

2   Review of Related Works

For the purpose of managing and improving attendance, researchers have come up with different perspective on the enhancement of the system. Jain et al. [5] developed a desktop application recording student attendance, whenever the application is launched, it displays the list of students that registered for a particular course. To mark students that were present, both the check box close to the student' name and register button is clicked. Zhang et al. [6] developed web-based attendance management system that used card technology for identifying students and taking attendance. Their system was developed using Oracle and VisualStudio.Net. Basheer and Raghu [7] developed attendance system that is authenticated using fingerprint. To record student attendance, the student placed his or her finger on the device's sensor. Geng et al. [8] developed RFID reader using components such as transceiver chip, microcontroller, LCD, serial communication IC, power supply module, USB interface, and power supply module. Whenever the staff card is brought in contact with the reader, data is sent to PC manager application which will authenticate the data and extract information like staff ID and access time into the database. Lim et al. [3] work was similar to Geng et al. [9], when a student touches the reader, it sends the data to the microcontroller for comparison with the ID stored in the microcontroller's memory; if ID exists, the name, ID and attendance will be presented on the LCD then transfer the data to PC via RS323 port. Kassim et al. [10] also developed attendance system based on RFID, the terminal of RFID reads and stores into the online server's database the student ID, date and time.

In university setting, contactless identification technology like NFC can be utilized to record and manage attendance easily. Benyo et al. [11] developed NFC based student attendance monitoring system for the Budapest University of Technology and Economics (BME). The system assisted the university to take the percentage of student's attendance for the purpose of allowing student that meet up with allowable attendance to write exam. In order to avoid impersonation by the student, biometric identification was introduced and incorporated to the system. The students register their attendance from a terminal that has NFC and fingerprint reader using only card that stores student ID and fingerprint. The terminals store the attendance data and it periodically send the data during the day to a back-office system. The terminals as well as back-office store timetable and identification policy. The back-office generates the report from the data received from the terminals. The terminal is capable of storing attendance data for a complete semester. Patela and Jainb [12] developed a mobile phone attendance system for small office employees. The employee will touch the NFC-enabled mobile device with their card, and then the employee data (ID, Photo, Time in/Time out, and Date) will be sent to the backend system.

3   System Architecture

The system used an NFC enabled android smartphone and an NFC Tag (optional). NFC, is a form of contactless communication between devices like smartphones or tablets. Contactless communication allows a user to wave the smartphone over an NFC compatible device to send information without needing to touch the devices together or go through multiple steps setting up a connection. Evolved from RFID technology, an NFC chip operates as one part of a wireless link. Once it is activated by another chip, small amounts of data between the two devices can be transferred when held a few centimeters from each other.
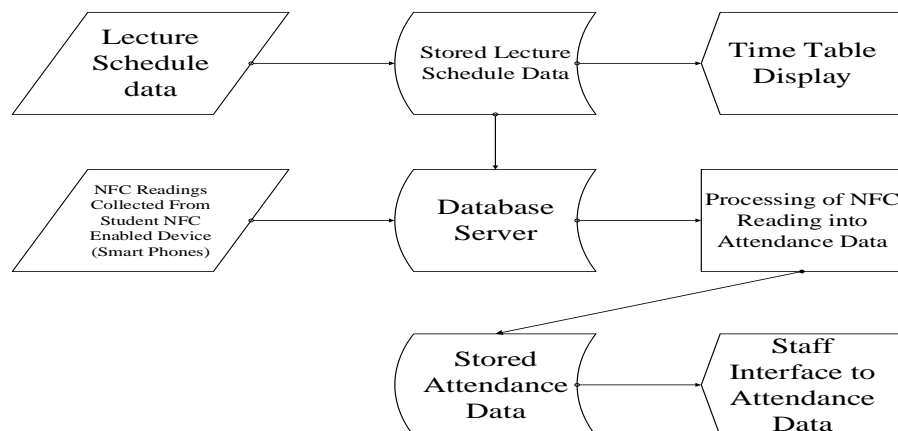
Fig. 1. Data flow diagram

The technology behind NFC allows a device, known as a reader, interrogator, or active device, to create a radio frequency current that communicates with another NFC compatible device or a small NFC tag holding the information the reader wants. Passive devices, such as the NFC tag in smart posters, store information and communicate with the reader but do not actively read other devices. Peer-to-peer communication through two active devices is also a possibility with NFC. This allows both devices to send and receive

information. The system implemented is based on series of distributed terminals the managing lecture schedule, store lecture schedule, time table display, database server, generate report, view student and NFC reading collected from student NFC enabled device as shown in Fig. 1.

3.1   Application Description

This system can be implemented for the Server and other modules via the following hardware requirement:

2.0 GHz Intel Pentium and above.	Minimum of 500GB of available disk space.
4 GB available RAM.	Input devices (Keyboard and Mouse).
Minimum of 500GB of available disk space.	A USB port for USB cords and flash drive.
Input devices (Keyboard and Mouse).	An NFC enabled android device.
A USB port for USB cords and flash drive.	A USB cable for software debugging.
2.0 GHz Intel Pentium and above.	Android smartphone (API 14 and above).
4 GB available RAM.	NFC Tag (Optional).

3.2   Software Requirements

The software required for the system are Android Studio, Dreamweaver and WAMP Server. For the programming aspect, JavaScript, XML, PHP and SQL were utilized

4   Software Interface

4.1   Admin Section

The function of the admin is to create entries for the students and the lecturers. The admin provides in the system the login credentials. Fig. 2a provides the option to which the student or the lecturer can sign in. The surname of the course lecturer is inputted for each course as shown in Fig. 2b.
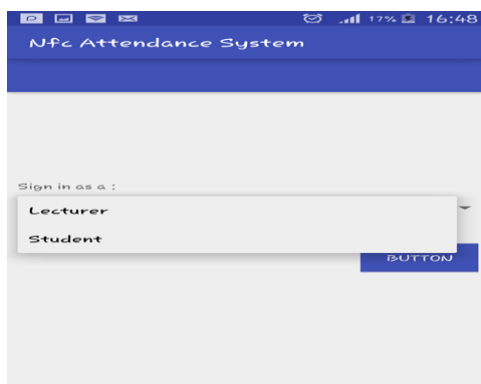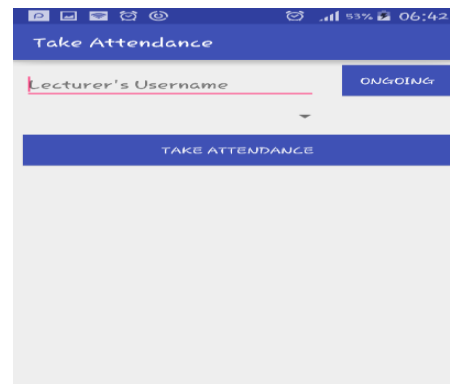
Fig. 2a. Login in page   Fig. 2b. Course lecturer's name

4.2   Student's Registration and Login Page

This allows the student to register on the application by entering the requested information. If the student already has a user account, they can click on the text 'Already have an account? Login' to take them to the login page. Fig. 3a show the registration page for student details. The student login page allows the student to get verified to use the application. To gain access to the application functions, a student must have done their registration on the application. Fig. 3b show the student's login page
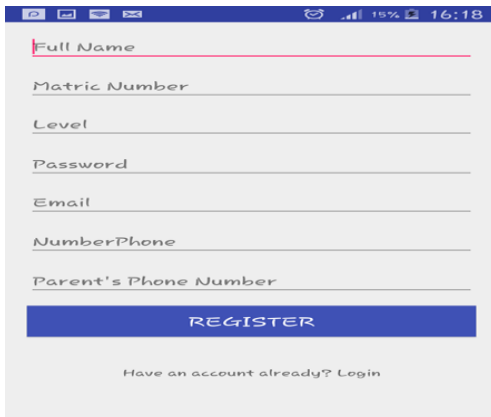
Fig.3a. Student registration page
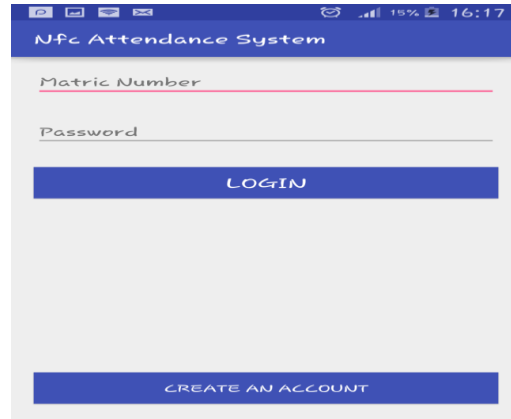
Fig. 3b. Student login page

4.3 Lecturer's Registration and Login Page

This allows the lecturer to register on the application by entering the requested information, one of which is the 'Admin Identification' that must be given to the lecturer by the backend administrator so as to verify that the person registering is actually a lecturer. If the lecturer already has a user account, they can click on the text 'Already have an account? Login' to take them to the login page as shown in Fig. 4a.

Fig. 4a. Lecturer's registration page

Fig. 4b. Course Registration Page

4.4 Student Course Registration Page
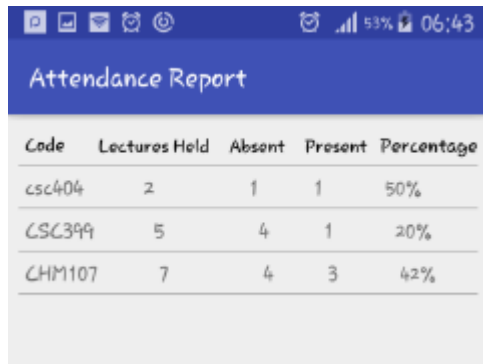
The course registration page allows the students to register for their courses by clicking on the check button at the corresponding course of the appropriate student courses and then click on 'Register Courses' button to register the ticked courses to the back-end. Fig. 4b shows course registration page.

4.5 Student Attendance Report

This shows the student attendance report based on the number of lectures they attend and their equivalent percentages. Which allows the student to either take the Exam or not, according to the University Examination Regulation. Student attendance page is shown on Fig. 5a.

4.6 Lecturer's Lecture Schedule

The lecture schedule aspect of the application allows the lecturer to schedule lecture, by picking the course from the list of courses in the spinner and to either turn on lecture or turn it off so that students can either view the lecture as ongoing lecture or it never show up as ongoing lecture. The Lecturer is also allowed to set time and date for the lecture. On clicking the button, the data in the fields are transported to the backend to be saved as shown on Fig. 5b.

Fig. 5a. Student Attendance Report

Fig. 5b. Lecture Schedule

5   Conclusion

The system has been implemented for use in recording attendance for students of Federal University Oye Ekiti, Nigeria for all their lectures in both first and second semesters. The work took advantage of the auto identification specifically using NFC to capture student attendance for lecturers without having the student write their name, matric number and signing against it anymore and attendance report and other student information can be accessed anytime anywhere without stress. The ability to write computer programs and the availability of network has contributed greatly to the success of this work. This system is of immerse benefits to the department as it is a better student attendance management, reduce administrative work, and improve student attendance ratio.

References

1. Ayu A.A. and Ahmad B.I., 2014. Touch In: An NFC supported attendance system in a university environment. International Journal of Information and Education Technology, 4(5), 448-452.
2. Want R., 2006. An Introduction to RFID Technology. IEEE Pervasive Computing, 5(1), 25-30.
3. Lim T.S., Sim S.C. and Mansor M.M., 2009. RFID based attendance system, IEEE Symposium on Industrial Electronics & Applications (ISIEA 2009), 2, 778-782.
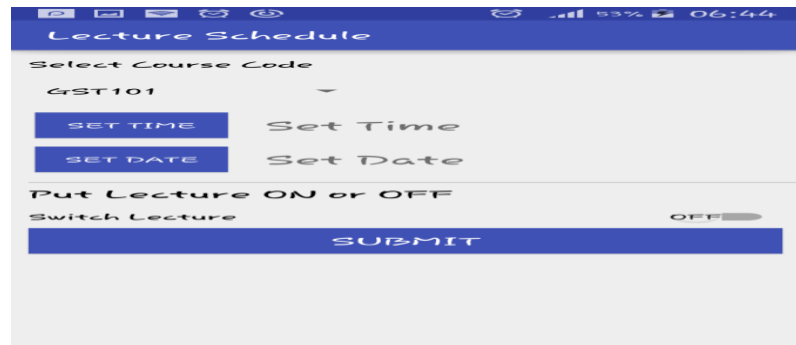4. Iglesias R., Parra J., Cruces C. and Segura N.G., 2009. Experiencing NFC based touch for home healthcare. In Proceedings of the 2nd International Conference on Pervasive Technologies Related to Assistive Environments, Corfu. In PETRA 2009.
5. Jain S.K., Joshi U. and Sharma B.K., 2008. Attendance Management System, Masters Project Report, Rajasthan Technical University, Kota.
6. Zhang Z.-G., Gong P., Cao L.J., and Chen Y.L., 2007. Information technologies and applications in education. 1st IEEE International Symposium on Digital Object Identifier, 606–609.
7. Basheer M.K.P. and Raghu C.V., 2012. Fingerprint attendance system for classroom needs. In Proceedings India Conference (INDICON), Annual IEEE, 433-438.
8. Geng S.H., Li G.M. and Liu W., 2012. Design and implement of attendance management system based on contactless smart IC card. In Proceedings International Conference on Computer Science and Electronics Engineering (ICCSEE), 3, 290-294, 23-25.
9. Kassim M., Mazlan H., Zaini N. and Salleh M. K., 2012. Web-based student attendance system using RFID technology, In Proceedings IEEE Control and System Graduate Research Colloquium (ICSGRC 2012), 213-218.
10. Benyo B., Sodor B., Doktor T. and Fordos G., 2012. University life in contactless way - NFC use cases in academic environment. In Proceeding IEEE 16th International Conference on Intelligent Engineering Systems (INES), 511-514.
11. Patela S.B. and Jainb N.K. 2013. Near field communication (NFC) based mobile phone attendance system for employees. International Journal of Engineering Research & Technology, 2(3), 52-58.

Cross-sectional Prevalence of Gastrointestinal Helminth Parasites in Two Cattle Holding Farms in Ekiti State, Nigeria

H.O. Mogaji[*], A.A. Bamigboye

Parasitology and Epidemiology Unit, Department of Animal and Environmental Biology, Federal University Oye-Ekiti, Nigeria
mogajihammed@gmail.com

Abstract
This study investigated the prevalence of gastrointestinal helminth infections in two cattle holding farms in Ekiti State, Nigeria. Faecal samples were collected directly from the rectum of cattle in Ayegbaju and Ikole holding farms. Samples were processed using ether concentration method and further examined under the microscope for helminths ova or larva. Demographic data on the gender, age and breed of the examined cattle were also obtained. Data were analyzed using SPSS 20.0 software for descriptive and chi square statistic. Confidence interval was set at 95%. Findings showed an overall prevalence of 88.0% for gastrointestinal helminth infection in both farms, with species' prevalence of 54.2% recorded for *Ascaris spp.,* 52.0% for hookworm and 6.0% for *Trichuris spp.* By location, there were no significant differences in the number of infected cattle across the holding farms (p > 0.05). However, significant differences exist across the age categories for infection with *Trichuris spp*. This study has shown that gastrointestinal helminth infections are endemic in the two holding farms surveyed and the high prevalence recorded may have negative implication on productivity of the cattle. Therefore, improving farm management system and routine deworming of farm animals is recommended.

Keywords: Cattle, helminth infections, farms, Ekiti State, Nigeria

1 Introduction

Gastrointestinal helminth infections (GHI) is one of the most prevalent parasitic diseases of cattle in Sub Saharan Africa (SSA) [1]. Infection occurs when cattle ingest parasitic eggs and/or the infective larvae via contaminated pasture, water and/or soil [2]. Usually, GHI is asymptomatic in cattle with light parasite load. However, heavier infections can be symptomatic with clinical morbidities such as anemia, malnutrition, malabsorbtion of nutrients, abdominal discomfort, tiredness, loss of appetite, reduced nutritional intake, reduced physical fitness and impaired physical development [3].

Of the 13.9 million cattle in Nigeria, about 11.5 million are reared under intensive care system and 2.4 million in rural villages where water, sanitation and hygiene resources are lacking [4, 5]. GHI therefore remains a threat to the health and productivity of cattle [6,7]. Although, there are published reports on the prevalence of GHI among cattle in Nigeria [8, 9]. However, to the best of our knowledge, there are no published report on the prevalence of GHI among cattle holding farms in Ekiti State, Nigeria. Providing such evidence would assist in prioritization of appropriate intervention measures to improve health conditions and productivity of cattle.

2 Materials and Methods

2.1 Study Area

Ekiti State is one of the six Southwestern State in Nigeria. The state has 16 Local Government Areas (LGA), with its capital located at Ado-Ekiti. Generally, the state enjoys a tropical climate with two distinct seasons; rainy season (April – October) and the dry season (November – March).

2.2 Selection of Sampling Area

A purposeful sampling method was employed in the selection of cattle holding farms. Two local governments areas (LGAs) were purposefully selected in the Northern region of the state. In each of the LGA's, the existing cattle holding farm closest to the research laboratory, at the Federal University Oye-Ekiti was selected; Ayegbaju holding farm in Oye LGA and Ikole holding farm in Ikole LGA.

2.3   Collection of Feacal Samples

A total sampling method was employed in recruiting cattle in both farms during collection of samples. Feacal samples were collected directly from the rectum of study cattle and transferred into properly labelled sterile bottles using disposable gloves. 10 mL of sodium-acetate-acetic-acid formaldehyde (SAF) solution were added to each sterile bottle to preserve the faecal samples; the bottles were covered and agitated vigorously to suspend the stool in the solution. The specimens were transported to the laboratory for parasitology examinations.

2.4   Parasitological Diagnosis of Gastrointestinal Helminths Ova

The stool suspensions were further strained into a centrifuge tube using double gauze of about 13 mm diameter placed in a funnel. The residue was discarded and the filtrates were centrifuged at 2000 rpm for 1 minute. The supernatant was discarded after centrifuging. 7 mL of normal saline was later added to the sediment, after which 3 mL of ether was finally added to the suspension. A stopper was placed on the tube, and the mixture was shaken vigorously before centrifuging for another 5 minutes at 2000 rpm. The first three layer of the suspension observed after centrifuging were discarded, leaving the last layer of the sediment. This sediment was pipetted on a clean microscope slide and examined for microscopic ova or larvae of STH under a compound microscope.

2.5   Data Analysis

Data obtained from the study were entered into Microsoft excel and analyzed using SPSS 20.0 software. Descriptive statistics such as frequency and percentages were used to describe demographic variable statistic. Chi square was used to test for significant difference between variables. Significance level was set at 95%.

3   Results and Discussion

3.1   Demographic Characteristics

Table 1 shows the demographic characteristics of surveyed cattle in Ayegbaju and Ikole holding farm in Ekiti state. A total of 50 cattle were surveyed; 21(42%) and 29(58%) from Ayegbaju and Ikole LGA, respectively. By sex category, majority of surveyed cattle were male, 18(85.7%) in Ayegbaju and 18(62.1) in Ikole. However, there was no significant difference between the sexes of the cattle surveyed at the two locations. By age category, majority of the age 25-48 were sampled; 11(52.4%) and 16(44.8%), respectively in both Ayegabju and Ikole whereas the age >6 has the lesser population; 1(4.8%) and 0(0.0%) when sampled. However, there was no significant difference between the ages of cattle surveyed. By breed category, the Bokolo breed has the highest number of cattle sampled in the population; 11(52.4%) and 29(100%) and 10(47.6%) and 0(0.0%) for the Yakana breed in both Ayegbaju and Ikole, however there was significant difference between the two breed ($p < 0.05$).

3.2   Prevalence of Gastrointestinal Helminth Infections (GHI)

An overall prevalence of 88.0% was recorded for any GHI in this study. By species' categories, a total of 3 species were identified i.e. *Ascaris spp, Trichuris spp* and hookworm. By species' prevalence, *Ascaris spp* was the most prevalent with 54.2%, followed by hookworm with 52.0% and *Trichuris spp* with 6.0%. By location categories, the cattle holding farm in Ayegbaju had more infected cattle compared to that in Ikole; 57.1% vs 51.7% for *Ascaris spp*, 30.0% vs 0.0% for *Trichuris spp* and 52.4% vs 51.7% for hookworm and 90.5% vs 86.2% for any GHI, respectively (Table 2).

3.3   Prevalence of Gastrointestinal Helminth Infection (GHI) by demographic variables

By sex categories, there were more infected male cattle than females with 16(59.3), 3(100), and 20(76.9) for *Ascaris spp, Trichurus* spp and hookworm, respectively. By age categories, cattle within the age range 25-48 months old were more infected; 14(51.9), 2(66.7), 13(50.0) for *Ascaris spp, Trichurus* spp and hookworm, respectively than other ages examined. However, age was not significantly associated with prevalence of GHI ($P > 0.05$), except for *Trichuris spp.* ($p < 0.05$). By breed, the bokolo breed has specific prevalence' of 22(81.5%), 0(0.0%), 20(76.9%) for *Ascaris spp, Trichurus* spp and hookworm, respectively. The overall prevalence recorded for this breed was 77.3%. However, for yakana breed, an overall prevalence of 22.7% was recorded for GHI, while *Ascaris spp., Trichurus spp.* and hookworm had prevalence estimate of 5(18.5%), 3(100%), 6(23.1%), respectively. There was also no significant association between GHI and breed status of the examined cattle ($p > 0.05$).

Table 1. Demographic characteristics

Variables	Ayegbaju (%)	Ikole (%)	Total (%)
Sex			
Male	18(85.7)	18(62.1)	36(72.0)
Female	3(14.3)	11(37.9)	14(28.0)
Total	21(100)	29(100)	50(100)
X^2, d$_f$, pvalue	3.378, 1, 0.06		
Age (in months)			
<6	1(4.8)	0(0.0)	1(2.0)
7-24	8(38.1)	13(44.8)	21(42.0)
25-48	11(52.4)	16(44.8)	24(48.0)
>48	1(4.8)	3(10.3)	4(8.0)
Total	21(100)	36(100)	50(100)
X^2, d$_f$, pvalue	2.132,3,0.546		
Breed			
Bokolo	11(52.4)	29(100)	21(80.0)
Yakana	10(47.6)	0(0.0)	10(20.0)
Total	21(100)	29(100)	30(100)
X^2, d$_f$, pvalue	17.262,1,0.00		

Table 2. Prevalence of gastrointestinal helminth infection (GHI)

	Ayegbaju		Ikole		Total	
	NE (%)	NI (%)	NE (%)	NI (%)	NE (%)	NI (%)
Ascaris spp	21	12(57.1)	29	15(51.7)	50	27(54.2)
Trichuris spp	21	3(30.0)	29	0(0.0)	50	3(6.0)
Hookworm	21	11(52.4)	29	15(51.7)	50	26(52.0)
Any GHI	21	19(90.5)	29	25(86.2)	50	44(88.0)

*NE: Number Examined; NI: Number Infected

Table 3. Prevalence of gastrointestinal helminth infection (GHI) by demographic variables

	Ascaris spp NI(%)	*Trichuris spp* NI(%)	*Hookworm* NI(%)	Any GIH NI(%)
Sex				
Male	16(59.3)	3(100)	20(76.9)	30(68.2)
Female	11(40.7)	0(0.0)	6(23.1)	14(31.8)
Total	27(100)	3(100)	26(100)	44(100)
x^2, d$_f$, pvalue	4.726,1,0.030	1.241,1,0.265	0.651,1,0.419	2.652,1,0.103
Age (in months)				
6	0(0.0)	1(33.3)	1(3.8)	1(2.3)
7-24	11(40.7)	0(0.0)	9(34.6)	17(38.9)
25-48	14(51.9)	2(66.7)	13(50.0)	22(50.0)
>48	2(7.9)	0(0.0)	3(11.5)	4(9.1)
Total	27(100)	3(100)	26(100)	44(100
x^2, d$_f$, pvalue	1.403,3,0.705	17.494,3,0.001	2.519,3,0.472	2.652,1,0.103
Breed				
Bokolo	22(81.5)	0(0.0)	20(76.9)	34(77.3)
Yakana	5(18.5)	3(100)	6(23.1)	10(22.7)
Total	27(100)	3(100)	26(100)	44(100)
x^2, d$_f$, pvalue	0.081,1,0.77	12.766,1,0.00	0.321,1,0.571	1.705,1,0.192

The prevalence estimate observed in this study is higher compared to the report of Ogudo et al. [8] and Oluwole et al. [9] in Ogun and Oyo States, respectively. The dissimilarities in findings may be due to difference in the two ecological zones and associated

management practices. The high prevalence observed could be attributed to the poor management system in the holding farms, as cattle were allowed to graze on pastures within and around the farm, which might have been contaminated with infective stages of these parasites. In this study, majority of the infected cattle were males. This is in agreement with earlier findings by Raza et al. [10] who indicated that the male cattle are more aggressive when feeding and thus likely to pick up more ova with pasture. Furthermore, significantly higher prevalence rate was recorded in adult animals. This is in contrast with existing literatures because adult animals are much less susceptible to most parasites, unless they are in poor living conditions [11]. In Nigeria, despite the enormous contribution made by the livestock sector to the economic wellbeing of the citizenry, little or no attention has been given to the burden of GHI among cattle. This study therefore reiterates the importance of regular de-worming activity, health education and environmental cleanliness in the phase of controlling GHI infections among cattle in holding farms.

4   Conclusion

This study has shown that the two cattle holding farms surveyed in this study are endemic for GHI. There is thus a need for regular de-worming activity, health education and ensuring environmental cleanliness.

References

1. WHO, 2012. Eliminating soil-transmitted helminthiases as a public health problem in children: Progress Report 2001–2010 and Strategic Plan 2011–2020.
2. Greenland K., Dixon R., Khan S.A., Gunawardena K., Kihara J.H, Smith J.L., Drake L., Makkar P., Rama S., Singh S. and Kumar S., 2015. The epidemiology of soil-transmitted helminths in Bihar state, India. PLoS Neglected Tropical Diseases, 9(5), e0003790.
3. WHO, 2017. WHO Fact Sheet on Soil Transmitted Helminth Infections. http://www.who.int/soil%20helminthiasis/en/
4. Lawal-Adebowale O.A., 2012. Dynamics of ruminant livestock management in the context of Nigerian. Agricultural System, 4, 61-80.
5. Okoli I.C., Enyinnia N.C., Elijah A.J., Omede A.A. and Unam-Opara C.I., 2012. Animal reproduction management pratices of Fulani patoralist in the umid rainforest of Imo State Nigeria. Journal of Animal Science Advances, 2(2), 221-225.
6. Dimaner S.O., Höglund J., Spörndly E and Waller P.J., 2000. The impact of internal parasites on the productivity of young cattle organically reared on semi-natural pastures in Sweden. Veterinary Parasitology, 90(4), 271-284.
7. Johannes C., Johan H., Georg V.S., Pierre D. and Jozef V., 2009. Gastrointestinal nematode infections in adult dairy cattle: Impact on production, diagnosis and control. Veterinary Parasitology, 164(1), 70-79.
8. Ogudo U., Oluwole A., Mogaji H., Adeniran A., Alabi O. and Ekpo U., 2015. Gastrointestinal helminth infections in a ruminant livestock farm in Abeokuta, South Western Nigeria. Annual Research & Review in Biology, 8(4), 1-8.
9. Oluwole A., Adeniran A., Mogaji H., Shittu E., Alabi O., Odoemene S., Ekpo U., 2016. Survey of gastrointestinal parasites among nomadic cattle herds in Eruwa, Oyo State, Southwestern Nigeria. Annual Research & Review in Biology, 10(6), 1-7.
10. Raza M.A., Ayaz M., Murtaza M., Murtaza S., Akhtar M.S., Muhammed N., Ali M. and Bachaya H.A., 2013. Prevalence of GIT helminths in cattle at the vicinities of Tehsil Jatoi, Punjab. Science International (Lahore), 25(2), 305–309.
11. Keyyu J.D., Kassuku A.A., Kyvsgaard N.C. and Willingham A.L., 2003. Gastrointestinal nematodes in indigenous Zebu cattle under pastoral and nomadic management systems in the lower plain of the southern highlands of Tanzania. Veterinary Research Communication, 27, 371-380.

Determination of Lipid Profile in Inflammation-Induced Wistar Rats Administered *Ficus sur* Flavonoid-Rich Extract

S.A. Emaleku[1,*], I.G. Adanlawo[2], M.T. Gbadegesin[1], E.A. Adeola[1], O.D. Omueti[1], G.O. Emaleku[1], A.F. Adeyemi[1], A.D. Ogunbodede[3]

[1]Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State
[2]Department of Biochemistry, Ekiti State University, Ado Ekiti, Ekiti State
[3]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State
crownsage@yahoo.com, sunday.emaleku@aaua.edu.ng

Abstract

Inflammation has been implicated in the prevalence and occurrence of many diseases including cardiovascular diseases (CVDs). CVDs are one of the leading causes of mortality in the world and dyslipidemia is a risk factor for CVDs. Presently, there is the use of statin drugs (HMG-CoA inhibitors) as therapeutic measures in the management of these diseases but their adverse effects have necessitated the development and use of natural drug products e.g. plants. Therefore, this study aimed at determining the effect of flavonoid-rich extract of *Ficus sur* (FEFS) on serum lipid profile of acetic acid-induced inflammation in male Wistar rats. 6%, 3% and 6% acetic acid at 20 ml/kg, 15 ml/kg and 10 ml/kg respectively were used to orally induce inflammation in animals within one week. The acetic acid-induced inflammatory animals were divided into six (6) groups of five (5) animals each in addition to the control animal group (non-inflammatory animals). The test groups were administered 15 mg/kg, 30 mg/kg, 60 mg/kg and 120 mg/kg of FEFS. Findings indicated that FEFS, most especially at 60 and/or 120 mg/kg non-significantly ($p \leq 0.05$) reduced serum triglycerides, low-density lipoprotein cholesterol (LDL-C) and total cholesterol, but increased high density lipoprotein-cholesterol (HDL-C) in comparison with other groups. It was therefore concluded that FEFS had heart-healthy benefiting effects since it had positive effect on lipid profile parameters in the inflammation-induced Wistar rats and would tend to ameliorate lipid profile-associated disorders/diseases like CVDs.

Keywords: Cardiovascular diseases, dyslipidemia, flavonoid-rich extract, inflammation, lipid profile, statin drugs

1   Introduction

Survival of man from various diseased conditions remains major a challenge globally. Despite the introduction of plethora of pharmacological drugs and other modalities into the society, cardiovascular diseases (CVDs) are still one of the principal causes of mobility and mortality in Western society [1]. The disease imposes tremendously heavy socio-economic burdens on man worldwide. Also, there are varieties of risk factors in literatures that increase the incidence of CVDs, and this includes hyperlipidemia [2]. According to Thomas *et al.* [3], disorder of lipid metabolism is one of the main determinants of cardiovascular risk. Furthermore, it has also been reported that inflammation is a fundamental cause of pathogenesis of cardiovascular diseases [4]. It is reported that inflammation leads to oxidative stress, which in turn causes dysregulation in lipid metabolism. It is thus no longer news that increased level of low density lipoprotein cholesterol (LDL-C), triglyceride (TG), total cholesterol (TC) and decreased level of high density lipoprotein cholesterol (HDL-C) are associated with atherosclerosis [5], a form of CVDs. Therefore, any therapeutic strategy that can reduce LDL-C, TC and TG but increase HDL-C would bring about the needed remedy against this life-threatening disease.

Fortunately, interest in phytomedicines has been rekindled in recent years. The potential side effects associated with lipid-lowering substances (e.g. statins and fibrates) coupled with great drug dependence has led to the hunt for medicinal plants as alternative therapy for the treatment of CVDs and its related diseases [6]. This study therefore investigates the effects of *Ficus sur* flavanoid-rich extract (FSFE) on serum lipid profile in acetic acid-induced inflammatory rats.

2   Materials and Methods

2.1   Chemical and Reagents

All chemicals and reagents used in the study were of analytical grade and were purchased from Sigma-Aldrich, France and Merck KGaA Darmstadt, Germany. Randox kits such as Randox cholesterol kit (Product number: 424660), Randox triglyceride kit (Product

number: 435212) and Randox HDL kit (Product Number: 269477) for lipid profile assay were obtained from Randox Laboratories, UK.

2.2   Collection and Authentication of Plant Material

Ficus sur leaves were gotten from Iwaro-Oka bush in Akoko area of Ondo State. It was identified and authenticated by a competent curator at Ekiti State University, Ado Ekiti. The plant specimen was deposited in the University's Herbarium and assigned Voucher Number of UHAE2018033.

2.3   Extraction and Solvent-Solvent Partitioning

The extraction and solvent-solvent partitioning technique were done according to Agbo *et al.* [7] protocol with slight modifications. Fresh leaves of *Ficus sur* were washed with water to remove dirt or dust, air dried for three weeks and pulverized into powder using electric blender. The powder leave (625 g) were macerated with 2 L of methanol at room temperature for 72 h and filtered by Whatman number 1 filter paper. It was concentrated using rotary evaporator at 45°C. The crude extract was further purified using partitioning technique with water and n-hexane at 2:1, and then water and ethyl acetate at 1:1 successively. The resulting extracts were freeze-dried, and the ethyl acetate extract rich in flavonoids was used for animal study.

2.4   Animal Used for the Study

A total number of 50 healthy male Wistar rats, average weight of 108.4 g were purchased from animal house of the Institute of Advanced Medical Research and Training (IAMRAT), University College Hospital (UCH), Ibadan. The animals were acclimatized for two weeks and maintained in line with National Institute of Health [8] guide for the care and use of laboratory animals. They were kept in an iron cage housed in the animal's house of the Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, and fed standard pellets feed and water *ad libitum* at room temperature of 22 ± 2°C and $55 \pm 5\%$ relative humidity where equal period (hour) of light and darkness was maintained (i.e. 12 h light/12 h dark).

2.5   Inflammation Induction Process

Modified method of Tannahill *et al*. [9] was used to induce intestinal inflammation in the animals within a week. The Wistar rats were first orally administered 20 ml/kg of 6% acetic acid (AA). These dosage and/or concentration were reduced to 15 ml/kg of 3% AA and administered three days after the first one *via* the same route. Finally, two days after the second administration, 10 ml/kg of 6% AA was orally administered for three consecutive days. Administration was done once a day in the morning around 7.30 am within a week. These inflamed animals were then used for the experiment.

2.6   Experimental Design

A total of thirty (30) inflammation-induced Wistar rats were randomly divided into six groups of five animals each in addition to the normal control group with the same number of animals (n=5). i) Group I – Normal control (NC) group, orally administered 1 mL distilled water; ii) Group II - Positive control (PC) group, orally administered 15 mg/kg Celecoxib; iii) Group III - Negative control (NegC) group, orally administered 1 ml distilled; iv) Group IV – FEFS treatment group, orally administered 15 mg/kg FEFS; v) Group V - FEFS treatment group, orally administered 30 mg/kg FEFS; vi) Group VI - FEFS treatment group, orally administered 60 mg/kg FEFS; and vii) Group VII - FEFS treatment group, orally administered 120 mg/kg FEFS. The treatment lasted for two weeks before experimental animals were sacrificed and blood collected in plain bottles for processing into serum for lipid biochemical assay (lipid profile).

2.7   Lipid Profile Assay

Lipid profile, which includes estimation of TC, HDL-C, LDL-C, and TG in experimental animals' serum, was determined by enzymatic end point method as in randox kits manual's instructions reported by Emaleku *et al*. [10].

2.8   Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) and differences in means between groups were determined by Tukey's Krammer Least significant difference (LSD) test using SPSS version 20. Values are presented as Mean ± standard mean of error (SEM), and values of $p < 0.05$ were considered statistically significant.

3   Results and Discussion

Table 1 shows that there is no significance difference ($p > 0.05$) in body weights of animals between groups at week 0 (i.e. before treatments) and even at week 2 (i.e. after treatments). Although there was no significant difference ($p > 0.05$) in body weights of experimental animals between groups at week 0 (i.e. before treatments) and even at week 2 (i.e. after treatments), but there was moderate increase (51.40 g) in body weight observed in animals administered 120 mg/kg FEFS, which is close to 47.40 g increase observed in normal control group. These observations indicate that FEFS did not stimulate/mediate unnecessary weight gain that can lead to overweight (obesity), a risk factor for CVDs. It could further be reasoned that FEFS did not impair lipid metabolism nor cause dyslipidemia e.g. hyperlipidemia (as shown in Table 3) that could adversely increase or raise body weights of animals (i.e. overweight) as a result of accumulated fats. Conversely, the observed high increase in body weight (66.90 g) of NegC animals is a worrisome sign that could lead to overweight overtime as a result of imbalance in lipid metabolism of the inflammatory animals. Ridker *et al.* [11] reported that inflammation causes dyslipidemia, which is a risk factor for obesity [12]. More also, the high weight gain by NegC animals could be a sign of possible growth of tumour due to inflammation. According to Coussens and Werb [13], there is a positive correlation between inflammation and cancer.

Table 1. Effects of flavonoid-rich extract of *Ficus sur* on weights of experimental animals

Groups/Weeks	Week 0	Week 1	Week 2
		Weight (g)	
NC	111.00±5.70	144.60±3.17	158.40±4.33
PC (15 mg/kg CXB)	114.40±24.14	141.60±14.61	153.00±16.75
NegC	110.60±25.67	155.25±9.86	177.50±10.57
FEFS (15 mg/kg)	114.20±22.94	138.25±15.09	143.75±5.95
FEFS (30 mg/kg)	112.60±34.56	120.60±16.78	143.33±14.26
FEFS (60 mg/kg)	116.60±23.47	139.20±14.39	152.80±16.07
FEFS (120 mg/kg)	108.60±18.76	136.60±9.75	160.00±11.00

Note: Comparison is strictly between groups. No alphabet superscript because there is no statistically significant difference
Key: NC – Negative control; PC – Positive control; CXB – Celecoxib; NegC – Negative control; FEFS – Flavonoid-rich extract of *Ficus sur*

Table 2. Effects of flavonoid-rich extract of *Ficus sur* on organs' weights of animals

Groups/Organs	Liver	Kidney	Heart
		Weight (g)	
NC	5.80±0.46	1.27±0.08	0.60±0.07
PC (15 mg/kg CXB)	6.08±0.57	1.07±0.10	0.59±0.06
NegC	6.50±0.41	1.34±0.08	0.70±0.07
FEFS (15 mg/kg)	5.66±0.21	1.09±0.07	0.50±0.02
FEFS (30 mg/kg)	6.05±0.30	1.23±0.11	0.50±0.07
FEFS (60 mg/kg)	5.66±0.47	1.23±0.06	0.60±0.06
FEFS (120 mg/kg)	6.09±0.57	1.21±0.14	0.59±0.02

Note: Comparison is strictly between groups. No alphabet superscript because there is no statistically significant difference
Key: NC – Negative control; PC – Positive control; CXB – Celecoxib; NegC – Negative control; FEFS – Flavonoid-rich extract of *Ficus sur*

Table 2 shows that there is no significant difference ($p > 0.05$) in the organs' weights (i.e. liver, kidney and heart) between groups. Despite the non-significant difference in organs' weights, it was observed that FEFS-treated animals have lesser organs' weights than NegC animals but almost the same weights (i.e. close to) with the normal control animals. This result further suggests the potential of FEFS to maintain organs' weights in addition to maintaining body weights of inflammation-induced animals after treatments. It is a further testimony that there is no likelihood of fat accumulation typical of dyslipidemia (risk factor of CVDs) or tumour growth that could adversely increase the organs' weights. Just as observed in body weights, high organs' weights (liver: 6.50±0.41; kidney: 1.34±0.08; heart: 0.70±0.07) was also observed in NegC animals to reestablish the possibility or tendency of derangement in lipid

45

metabolism that resulted in fats accumulation and/or likelihood of tumour growth (cancerous growth) in this untreated inflamed group as earlier opined.

Table 3. Effects of flavonoid-rich extract of *Ficus sur* on serum lipid profile parameters

Groups/Parameters	CHOL	TG (mg/dl)	HDL-C	LDL-C
NC	65.72±10.72	102.11±5.54	24.38±4.8	35.95±2.32
PC (15 mg/kg CXB)	71.29±7.25	111.67±17.77	29.96±3.77	34.31±4.27
NegC	82.46±6.70	114.44±12.67	25.06±6.01	41.32±5.69
FEFS (15 mg/kg)	61.88±6.45	103.72±21.17	24.69±7.90	22.00±3.08
FEFS (30 mg/kg)	56.69±15.73	135.01±37.35	31.76±1.34	19.06±5.95
FEFS (60 mg/kg)	48.54±9.80	89.31±21.33	37.59±4.63	24.69±3.90
FEFS (120 mg/kg)	58.71±7.69	129.74±41.36	41.36±7.57	18.87±4.00

Note: Comparison is strictly between groups. No alphabet superscript because there is no statistically significant difference
Key: NC – Negative control; PC – Positive control; CXB – Celecoxib; NegC – Negative control; FEFS – Flavonoid-rich extract of *Ficus sur*

Table 3 shows that there is no significant difference ($p > 0.05$) in lipid profile parameters (TC, LDL-C, TG and HDL-C levels) between groups. However, TC and LDL-C levels of FEFS-treated groups are lesser than that of other groups (i.e. NC, PC and NegC) but the FEFS-treated animals' HDL-C levels, especially at 120 mg/kg are higher than that of other groups.

This non-significant decrease in TC, LDL-C and TG (only in FEFS at 60 mg/kg for TG) but increase in HDL-C levels in FEFS-treated animals shows the plant's tendency to ameliorate dyslipidemia or hyperlipidemia. Decreased LDL-C levels observed in FEFS-treated animals imply less transportation of cholesterol to the heart to cause atherosclerosis [14] while the increased HDL-C levels imply more transportation of cholesterol away from extrahepatic tissues e.g. heart to the liver for excretion [15], which prevents CVDs. The observed results further suggest that FEFS would have restored and/or maintained balances in lipid metabolism in inflammation-induced animals treated with FEFS to bring about these desired effects (i.e. reductions in TC, LDL-C and TG, and increase in HDL-C levels) since higher TC, LDL-C and TG levels but lower HDL-C level were observed in NegC (untreated) group. This hypolipidemic tendency is a good omen for the heart and could help alleviate the problem of CVDs. According to Ritchie and Connell [16], high fat accumulation, which has the hallmark of high TC, LDL-C and TG serum levels, is associated with dyslipidemia, a risk factor of CVDs. Similarly, Franssen and Kastelein [17] reported dyslipidemia to be the major risk factor for CVDs, and could cause increase in BMI due to fat accumulation. So, the low serum levels of TC, LDL-C and TG and high HDL-C levels in animals administered FEFS ultimately demonstrate the non-dyslipidemic or anti-hyperlipidemic potential of the plant extract. Finally, these moderate values of lipid parameters in FEFS-treated animals provide the underlying reason for the moderate body and organs weights observed in these animals and conversely, the high values of these parameters in NegC animals, which signals dyslipidemic tendency, account for the high body and organs weights observed in the group.

4   Conclusion

Based on the findings of this study, FEFS could be a potential hypolipidemic agent of natural source that could be used in the treatment of CVDs and associated diseases since it reduced serum TC, LDL-C and TG but increased HDL-C in administered animals.

References

1. Harchaoui K.E.L., Visser M.E., Kastelein J.J.P., Stroes E.S. and Dallinga-Thie G.M., 2009. Triglycerides and cardiovascular risk. Current Cardiology Review, 5, 216-222.
2. Kumar L. and Las Das A., 2018. Assessment of serum lipid profile in patients of coronary artery disease: A case-control study. International Journal of Contemporary Medical Research, 5 (5), 59-62.
3. Thomas A., Gaziano M.D., Asaf B., Shuchi A., Shafika A. and Adrianna M., 2010. Growing epidemic of coronary heart disease in low- and middle-income countries. Current Problems in Cardiology, 35, 72–115.
4. Jones D.P., True H.D. and Patel J., 2017. Leukocyte trafficking in cardiovascular disease: Insights from experimental models. Mediators of Inflammation, 974, 61-69.
5. Koley S. and Sur A., 2018. Association of lipid profile parameters with high-sensitive C–reactive protein (hsCRP) in patients with dyslipidemia. Annals of Medical and Health Science Research, 8, 105-107.
6. Zeni A.L.B., Moreira T.D., Dalmagro A.P., Camargo A., Bini L.A., Simionatto E.L. and Scharf D.R., 2017. Evaluation of phenolic compounds and lipid-lowering effect of *Morus nigra* leaves extract. Annals of the Brazilian Academy of Sciences, 1-11.

7. Agbo M.O., Lai D., Okoye F.B.C., Osadebe O.P. and Proksch P., 2013. Antioxidative polyphenols from Nigerian mistletoe *Loranthus micranthus* (Linn.) parasitizing on *Hevea brasiliensis*. Fitoterapia, 86, 78-83.

8. National Institutes of Health, 2011. Guide for the care and use of laboratory animals. National Research Council (US) Committee for the update of the guide for the care and use of laboratory animals. National Academy of Sciences. 8th Edition. Washington (DC): National Academies Press (US).

9. Tannahill C.L., Stevenot S.A., Campbell-Thompson M., Nick H.S. and Valentine J.F., 1995. Induction and immunolocalization of manganese superoxide dismutase in acute acetic acid-induced colitis in the rat. Gastroenterology, 109, 800–811.

10. Emaleku S.A., Omueti O.D. and Emaleku G.O., 2018. *Talinum triangulare*: Whole wheat meal fortified with soy flour consumed with *Talinum triangulare* (gbure) soup glycemic index and the test human subjects' lipid profiles. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 12, 831-837.

11. Ridker P.M., Everett B.M., Thuren T., MacFadyen J.G., Chang W.H., Ballantyne C., Fonseca F., Nicolau J., Koenig W., Anker S.D., Kastelein J.J.P., Cornel J.H., Pais P., Pella D., Genest J., Cifkova R. and Lorenzatti A., 2017. Antiinflammatory therapy with Canakinumab for atherosclerotic disease. New England Journal of Medicine, 377, 1119-1131.

12. Feingold K.R. and Grunfeld C., 2019. The effects of inflammation and infections on lipids and lipoproteins. Endotext (Internet). www.ncbi.nlm.nih.gov/books/NBK326741/. (Accessed 7th February, 2019).

13. Coussens L.M. and Werbs Z., 2002. Inflammation and Cancer. Nature, 420, 860-867.

14. Yoshida H. and Kisugi R., 2010. Mechanisms of LDL oxidation. Clinica Chimica Acta, 411, 1875 -1882.

15. Navab M., Srinivasa T., Brian R., Van Lenten J. and Alan M.F., 2011. HDL and cardiovascular disease: Atherogenic and atheroprotective mechanisms. Nature Reviews of Cardiology, 8, 222-232.

16. Ritchie S.A. and Connell J.M., 2007. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. Nutrition, Metabolism & Cadiovascular Diseases, 17(4), 319-326.

17. Franssen H.M. and Kastelein J.J., 2007. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. New England Journal of Medicine, 356, 1620–30.

Understanding Walls of Gene Regulatory Networks from Focal Points of Adjacent Regulatory Domains

Ngwu Benitho A.[1,*], Mmaduakor C.O.[1], Ajewole R.O.[1] , Iorkua M.[2]

[1]Department of Mathematics, Federal university Oye-Ekiti, Ekiti State, Nigeria
[2]Department of Statistics, Benue State Polytechnic, Ugbokolo, Nigeria
benamaobi@gmail.com

Abstract

Walls in gene regulatory networks are determined by the concentration threshold of regulatory elements. Each element in the network has different threshold levels at which it interacts with different elements in the network. The thresholds divide the state space into open rectangular boxes. The boundaries of these boxes are known as threshold walls and are responsible for the switch-like properties exhibited by the regulatory elements in the networks. In this work, we propose a method that simplifies the characterization of walls using the focal points of adjacent regular domain and by so doing we hope to bridge the gap existing in literature.

Keywords: Cardiovascular diseases, dyslipidemia, flavonoid-rich extract, inflammation, lipid profile, statin drugs

1 Introduction

Gene regulatory networks (GRN) are considered switched system because the elements of the network behave in a switch-like manner. The switch-like behaviour of these elements is due to their concentration. At different concentration levels, they interact with one another in a complex and intertwined manner, thereby regulating the activities of one another. These concentration levels are considered the thresholds of the network and they divide the phase space into open rectangular boxes [1-6]. GRN has been investigated for periodic solutions, limit cycles, chaos, steady points and qualitative properties. For a study of walls in GRN, Gouze and Sari [7] investigated some walls (white and black) for sliding motion and stability. Their study was based on the nature of walls which uses the direction of focal points of adjacent rectangular boxes [2]. Our findings as shall be demonstrated show that defining the nature of a wall based on mere direction of focal point of adjacent rectangular boxes to the wall is not realistic. One of the reasons why this may not be correct is because of the fact that biological systems are prone to relapses. This, in other words means that such flow can be reversed due to either gain or loss in function of the elements close to a threshold wall.

Defining sliding motions on threshold walls requires Filippov's concatenation approach at the threshold wall. A definition of how to do this was also given in Gouze and Sari [7] and we shall make use of in our method. Definition of walls based on direction of focal flow is provided by Filippov's first order theory. This theory provides computation that may not be easily used by researchers with weak mathematical background. To make it readily available, we show by practical examples, how to find the nature of a wall especially transparent ones from the focal point of adjacent regulatory domains.

2 Gene Regulatory Networks

The mathematical expression for gene expression is defined as Equation 1.

$$\dot{x}_i(t) = F(z(t)) - \lambda G(z(t)) \qquad\qquad 1$$

In Equation 1, $X = (x_1, x_2, \cdots, x_n)$ is an n-dimensional vector that indicates the concentration of the variables. The functions F and G are multinomial and depend on $z_i(x_i, \theta_{ij}) = (z_1, z_2, \cdots, z_n)$ which defines the concentration threshold (represented with θ here) at which each variable interacts with self (possibly) and others in the network. These threshold functions can be step function, logoid function or sigmoid function. Threshold function which we shall consider here is the step function which is given as Equation 2a.

$$z^+(x, \theta) = 1 \text{ if } x > \theta \text{ and } z^-(x, \theta) = 0 \text{ if } x < \theta . \qquad\qquad 2a$$

$$z^-(x,\theta) = 1 - z^+(x,\theta) \qquad\qquad 2b$$

Note that step functions are piecewise continuous. An example of logoid and sigmoid functions can be found in Plahte et al [8]. Threshold hyperplanes partition the phase space into n-dimensional open rectangular domains called boxes. Within each of these boxes, the right-hand side of Equation 1 (or the vector field) is just a first order ordinary differential equation.

$$\dot{x}_i(t) = \beta_i - \lambda_i x_i(t) \qquad\qquad 3$$

Consider for instance a network that has just two variables and each variable has just one threshold. The number of boxes here is $B_1^{00} = x_1 < \theta_1 \times x_2 < \theta_2$, $B_2^{10} = x_1 > \theta_1 \times x_2 < \theta_2$, $B_3^{01} = x_1 < \theta_1 \times x_2 > \theta_2$ and $B_1^{11} = x_1 > \theta_1 \times x_2 > \theta_2$. It can be proved that the number of such boxes in an n-dimensional network where each variable has p_i, $i = 1, 2, \cdots, n$ thresholds is

$$\prod_{i=1}^{n} (p_i + 1)$$ [9, 10]. The superscript on the box refers to the state of the variable as defined in Equation 2a. Equation 3 with initial

point and time $x(t = t_0) = x_0$ has a solution given by:

$$x_i(t) = \phi_i + (x_i - \phi_i)\ell^{-\lambda_k(t-t_0)} \qquad\qquad 4$$

The limit of Equation 4 in forward time as $t \to \infty$ is ϕ_i. This limit is what is called the focal point [8]. A box is considered stable if it contains its focal point but unstable otherwise. Walls are defined in Plahte et al [8] with respect to the relationship between focal points and threshold wall of interest. This we believe fails for reasons such as relapses as observed before. Here we shall define walls based on the focal points of adjacent boxes as follows.

3 Types of Threshold Walls in GRN.

Filippov motion on threshold walls can be treated in a similar way as that in boxes [7]. Consider that threshold wall θ_i separates two walls B_1 and B_2. Then a vector field on such wall (which is linear) can be defined as Equation 5 [7] as:

$$f^W = \alpha f^{B_1} + (1-\alpha)f^{B_2}, \text{ where } \alpha = \frac{f_k^{B_1} - \lambda_k \theta_k^j}{f_k^{B_2} - f_k^{B_1}} \qquad\qquad 5$$

From the form of Equation 5, it is evident that the constant α does not exist if the two boxes have the same focal point, which is when the denominator is zero. In other words, existence of such a constant is essential for a motion to slide on any wall. As a result of this we propose the following:

Proposition 1. Let θ_i be the threshold wall separating the boxes B_i and B_{i+1}, where $B_i = \{x \in X : x_i < \theta\}$ and $B_{i+1} = \{x \in X : x_i > \theta_i\}$. Let the focal points of the boxes be ϕ_i and ϕ_{i+1}, respectively. It follows from Equation 5 that a wall is:

    (i) black if $f_k^{B_{i+1}} < f_k^{B_i}$ 5a

    (ii) transparent if $f_k^{B_{i+1}} = f_k^{B_i}$ 5b

    (iii) white if $f_k^{B_{i+1}} > f_k^{B_i}$.   5c

Observe that f_k is the kth-component of F in Equation 1 where x_k attains a threshold value. Following the definition given by Plahte et al [8] we can have the following characterization of wall:

Proposition 2. Let θ_i be the threshold wall separating the boxes B_i and B_{i+1}, and let the focal points of the ith-variable in the boxes be ϕ_i and ϕ_{i+1}, respectively. Then we say that a wall is:

  (i) black if $\phi_i \in B_{i+1}$ and $\phi_{i+1} \in B_i$ 5d

  (ii) transparent if $\phi_i = \phi_{i+1}$ in either of the boxes   5e

  (iii) white if $\phi_i \in B_i$ and $\phi_{i+1} \in B_{i+1}$.   5f

Remark 1. The proof of these propositions is part of other works submitted for review elsewhere. However, the proof is not difficult because $\phi_i = \dfrac{f_1^{\,B}}{\lambda_i}$ and since λ_i is constant inside each box, it follows from proposition 1.

The nature of these definitions can serve as a practical guide to studying walls and their exit conditions. Proposition 1 has is a bridge between the reports of Plahte et al. [8] and, Gouze and Sari [7] because it combines the definition of a wall in principle and practice. In the next section we shall consider the application of this to some examples in literature.

4   Illustrative Examples

We shall consider three examples here. First let us consider the two-dimensional network given in Plahte and Kjoglum [11] as follows:

$$\dot{x}_1 = z_1 + z_2 - 2z_1 z_2 - \lambda_1 x_1 \qquad\qquad 6a$$
$$\dot{x}_2 = 1 - z_1 z_2 - \lambda_1 x_1 \qquad\qquad\qquad 6b$$

The variables in this network have just one threshold each.
Another network with one threshold for each variable is given in Snoussi and Thomas [12] as:

$$\dot{x}_1 = k_1(z_3 - z_2 z_3) - \lambda_1 x_1 \qquad\qquad 7a$$
$$\dot{x}_2 = k_2 z_1 z_3 - \lambda_2 x_2 \qquad\qquad\qquad 7b$$
$$\dot{x}_3 = k_3(1 + z_1 - z_2 z_1) - \lambda_3 x_3 \qquad 7c$$

The third network we shall study is a modified form of Equations 7a – 7c given as Equations 8a – 8c.

$$\dot{x}_1 = k_1(1 - z_1 + z_2 z_3) - \lambda_1 x_1 \qquad 8a$$
$$\dot{x}_2 = k_2(1 + z_3 + z_2 z_1) - \lambda_2 x_2 \qquad 8b$$
$$\dot{x}_3 = k_3(z_2 + z_1 z_3) - \lambda_3 x_3 \qquad\quad 8c$$

We shall begin by identifying the walls in each example before exploring their nature with our proposition. The example network described by equation (4.1) has four boxes. These are $B_1 = (x_1 < \theta_1, x_2 < \theta_2)$, $B_2 = (x_1 < \theta_1, x_2 > \theta_2)$, $B_3 = (x_1 > \theta_1, x_2 < \theta_2)$ and $B_4 = (x_1 > \theta_1, x_2 > \theta_2)$. Each of these boxes can be represented by the state of the variables in them as $B_1(0,0) = B_1^{00}$, $B_2(0,1) = B_2^{01}$, $B_3(1,0) = B_3^{10}$ and $B_4(1,1) = B_4^{11}$.

This example has two threshold hyperplanes which are $x_1 = \theta_1$ and $x_2 = \theta_2$. Each hyperplane is divided into two walls and each wall is bounded by two boxes. One of this wall is $w_1^{\,1}$ which divides the boxes $B_1^{\,1} = (0,0)$ and $B_1^{\,2} = (1,0)$ for the domain $x_1 = \theta_1$ and $x_2 < \theta_2$. The other wall that divides the boxes $B_2^{\,1} = (0,1)$ and $B_2^{\,2} = (1,1)$ is $w_1^{\,2}$, which is for the domain

$x_1 = \theta_1$ and $x_2 > \theta_2$. Similarly, the threshold hyperplane $x_2 = \theta_2$ has the following two walls: $w_2{}^1 : B_1{}^1 = (0,0), B_1{}^2 = (0,1)$ and $w_2{}^2 : B_1{}^1 = (1,0), B_1{}^2 = (1,1)$.

Note: In the box $B_i{}^1 = (1,0)$, the state of z_1 is 1 and that of z_2 is 0. This is how $f_i{}^{B_k{}^j}$, j = 1,2; i = 1, 2, . . . , n and k = 1, 2 , . . . , p (where p refers to the number of walls that emanate from the threshold hyperplane $x_i = \theta_i$).

The wall $w_1{}^1$ is white because $f_1{}^{B_1{}^1} = 0$, and $f_1{}^{B_1{}^2} = 1$, thus $f_1{}^{B_1{}^1} - f_1{}^{B_1{}^2} > 0$. The wall $w_1{}^2$ is black since $f_1{}^{B_1{}^1} = 1$ and $f_1{}^{B_1{}^2} = 0$, thus $f_1{}^{B_1{}^1} - f_1{}^{B_1{}^2} < 0$. Recall that $f_k{}^B$ is the value of the vector field of Equation 1 corresponding to x_k.

On the other hand, the wall $w_2{}^1$ is transparent because $f_2{}^{B_1{}^1} = 1 = f_2{}^{B_1{}^2}$ and as such $f_1{}^{B_1{}^1} - f_1{}^{B_1{}^2} = 0$. The last wall $w_2{}^2$ is black because $f_2{}^{B_1{}^1} = 1$ and $f_2{}^{B_1{}^2} = 0$, thus $f_2{}^{B_1{}^1} - f_2{}^{B_1{}^2} < 0$. This result agrees with that of Plahte and Kjoglum [11].

For the example network of Equations (7a, b, and c) there are twelve walls, four for each threshold hyperplane, in the network. The walls corresponding to the hyperplane $x_1 = \theta_1$ are the following: $w_1{}^1 : B_1{}^1(000), B_1{}^2(100)$; $w_1{}^2 : B_2{}^1(010), B_2{}^2(110)$, $w_1{}^3 : B_3{}^1(001), B_3{}^2(101)$ and $w_1{}^4 : B_3{}^1(011), B_3{}^2(111)$. These walls are all transparent. For instance, $f_1{}^{B_1{}^1} = 0$ and $f_1{}^{B_1{}^2} = 0$; $f_1{}^{B_2{}^1} = 0$ and $f_1{}^{B_2{}^2} = 0$; $f_1{}^{B_3{}^1} = 0$ and $f_1{}^{B_3{}^2} = 0$ and $f_1{}^{B_4{}^1} = 1$ and $f_1{}^{B_4{}^2} = 1$.

The walls belonging to the hyperplane $x_2 = \theta_2$ walls are $w_2{}^1 : B_1{}^1(000), B_1{}^2(010)$; $w_2{}^2 : B_2{}^1(100), B_2{}^2(110)$, $w_2{}^3 : B_3{}^1(001), B_3{}^2(011)$ and $w_2{}^4 : B_4{}^1(101), B_4{}^2(111)$. These walls behave the same way as the walls of $x_1 = \theta_1$ above and as such are transparent.

The hyperplane $x_3 = \theta_3$, have the following walls: $w_3{}^1 : B_1{}^1(000), B_1{}^2(001)$; $w_3{}^2 : B_2{}^1(100), B_2{}^2(101)$, $w_3{}^3 : B_3{}^1(010), B_3{}^2(011)$ and $w_3{}^4 : B_4{}^1(110), B_4{}^2(111)$. These walls are all transparent but unlike the two walls seen above only $w_3{}^2$ has $f = 0$ as the rest of the walls has $f = k_3$.

Equations (8a, b, and c) have the same number of thresholds as that of Equation (7a, b and c). The walls belonging to the hyperplane $x_1 = \theta_1$ and the boxes adjacent to them are $w_1{}^1 : B_1{}^1(000), B_1{}^2(100)$; $w_1{}^2 : B_2{}^1(010), B_2{}^2(110)$, $w_1{}^3 : B_3{}^1(001), B_3{}^2(101)$ and $w_2{}^4 : B_4{}^1(101), B_4{}^2(111)$. It is easily seen that all the walls of this hyperplane $x_1 = \theta_1$ are black.

For the hyperplane $x_2 = \theta_2$, the walls are $w_2{}^1 : B_1{}^1(000), B_1{}^2(010)$; $w_2{}^2 : B_2{}^1(100), B_2{}^2(110)$; $w_2{}^3 : B_3{}^1(001), B_3{}^2(011)$ and $w_1{}^4 : B_4{}^1(011), B_4{}^2(111)$. The walls $w_2{}^1$ and $w_2{}^3$ are transparent. The other two walls $w_2{}^1$ and $w_2{}^3$ are black.

Finally, the hyperplane $x_3 = \theta_3$, have the following walls: $w_3{}^1 : B_1{}^1(000), B_1{}^2(001)$; $w_3{}^2 : B_2{}^1(100), B_2{}^2(101)$, $w_3{}^3 : B_3{}^1(010), B_3{}^2(011)$ and $w_3{}^4 : B_4{}^1(110), B_4{}^2(111)$. The walls $w_3{}^1$ and $w_3{}^3$ are transparent whereas $w_3{}^2$ and $w_3{}^4$ are white.

An example that illustrates distinctly the advantage of this method over existing ones [7, 13] is the two-dimensional network proposed by Snoussi and Thomas [12] and investigated for regular and singular stationary points in Gouze and Sari [7]. The diagram of the walls and their nature is shown in Fig 1.
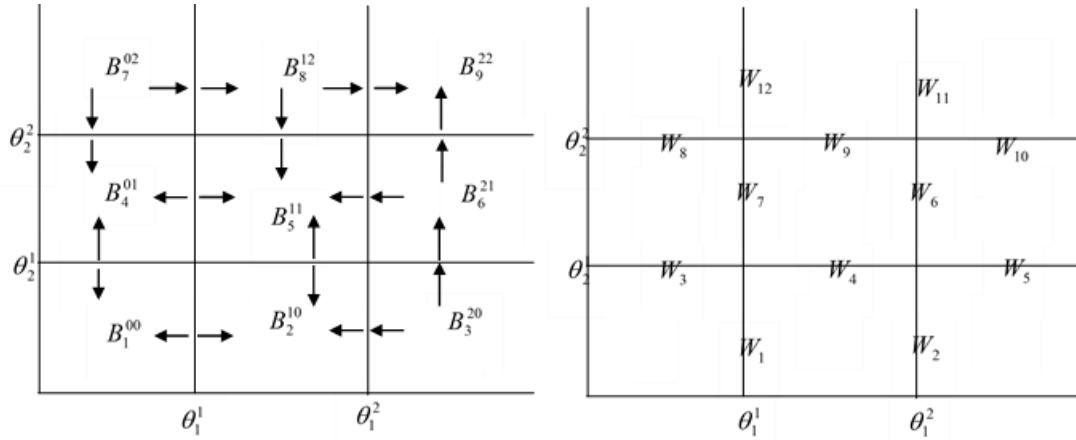
Fig. 1. Walls of equation (4.4) and their nature based on focal flow

From the diagram and as reported in Gouze and Sari [7], the wall w_5 is transparent because focal points are expected to cross the wall. However, our method shows that $f_2^{B_2^2} - f_2^{B_2^1} = k_4 > 0$, showing that the wall is white rather than transparent. This is not the only wall as w_{12} is not transparent but white since $f_1^{B_1^2} - f_2^{B_1^1} = k_1 > 0$.

5 Results and Discussion

The nature of walls reported in previous literature [7, 8] does not bring out the intrinsic properties and qualities of interactions existing in GRN. These qualities are hidden in the regulatory interactions of the variables which either activate or repress each other. This action is not conspicuous going by the earlier definitions of these walls. For instance, $[n^T(x)f_1(x)][n^T(x)f_2(x)] > 0$ implies that the wall is transversal [13] and as reported in Gouze and Sari [7] that "a wall is transparent if trajectories enter one box from the wall and leave through the other box". Neither of this tells at a glance if there is a gain or loss across the wall. Part of the goal of this work is to expose these intrinsic interactions that exist at the threshold walls from the nature of the walls; and as discussed above, the nature of transparent walls can be inferred easily without any mathematical rigors or exercise. The transparent walls in the examples studied in the last section clearly tell whether there is a gain or loss in concentration across a transparent wall. For instance, consider the two transparent walls belonging to the threshold hyperplane $x_2 = \theta_2$ of Equations 8b. Applying Equation 5b to Equation 8b is $f_2^{B_1^i} = k_1$ and $f_2^{B_3^i} = 2k_1$ showing an increase across the walls w_2^1 and w_2^3, respectively. On the other hand, the walls w_3^1 and w_3^3 of Equation 8c have $f_3^{B_1^i} = 0$ and $f_3^{B_3^i} = k_3$, respectively showing that the variable's concentration decreases across w_3^1 but increases across w_3^3.

Proposition 2 will still yield the same result as proposition 1, only that it uses trajectories of adjacent boxes rather than the production function $f(Z)$. It has one edge over proposition 1 which is that one will not have to seek trajectories in case it is needed. However, when one is not interested in the trajectories of the boxes but nature of walls just as we did here, proposition 1 is preferable. It is pertinent to state that the importance of this work is highly appreciated when one wants to study the dynamics of trajectories on a wall, which is possible since the work of Plahte and Kjoglum [11] shows that trajectories can belong to threshold walls. It will provide an investigator with the correct nature of the wall first and this in turn will tell which walls to investigate and which to ignore.

6 Conclusion

In conclusion, we have seen that nature of walls should not depend on the direction of trajectory of adjacent boxes but on the result derived from concatenating the trajectories about the walls. To do this requires some level of mathematical involvement; however, we have shown by the methods in this work that such mathematical demand can be circumvented. This therefore serves as a bridge for investigators with weak mathematical background.

References

1. Plahte E., Mestl T., and Omholt S.W., 1998. A methodological basis for the description and analysis of systems with complex switch-like interactions. J Math Biol, 36, 321-348.
2. Mestl T., Plahte E. and Omholt S.W., 1995. A mathematical framework for describing and analysing gene regulatory networks. Journal of Theoretical Biology, 176(2), 291-300.
3. Mestl T., Lemay C. and Glass L., 1996). Chaos in high-dimensional neural and gene networks. Physica D, 98, 33-52.
4. Ropers D., de Jong H., Page M., Schneider D. and Geiselmann J., 2006. Qualitative simulation of the carbon starvation response in Escherichia coli. BioSystems, 84, 124–152.
5. de Jong H., 2002. Modeling and simulation of genetic regulatory systems: a literature review. J Comput Biol, 9, 67-103.
6. Etienne F., 2005. Geometric properties of a class of piecewise affine biological network models. J Math Biol, doi: 10.1007/s00285-005-0360-4.
7. Gouze J.-L., Sari T., 2001. A Class of Piecewise Linear Differential Equations Arising in Biological Models.RR-4207, INRIA. <inria-00072415>
8. Plahte E., Mestl T. and Omholt S.W., 1994. Global analysis of steady points for system of differential equations with sigmoid interaction. Dynamics and Stability of Systems: An International Journal, 9(4), 275-291.
9. Porreca R., Drulhe S., de Jong H. and Ferrari-Trecate G., 2008. Structural identification of Piecewise-Linear models of genetic regulatory networks. J Comput Biol, 15(10), 1365-80.
10. Grognard F., De Jong H. and Gouzé J.-L., 2007. Piecewise-linear models of genetic regulatory networks: theory and example. Isabelle Queinnec, Sophie Tarbouriech, Germain Garcia, SilviuIulianNiculescu. Biology and Control Theory: Current Challenges, 357, Springer, pp.137 - 159, Lecture Notes in Control and Information Sciences.
11. Plahte E. and Kjoglum S., 2005. Analysis and generic properties of gene regulatory networks with graded response functions. Physica D 201, 150-176.
12. Snoussi E.H. and Thomas R., 1993. Logical identification of all Steady States: the concept of feedback loop characteristic states. Bulletin of Mathematical Biology, 55(5), 973-991.
13. Dieci L. and Lopez L., 2009. Sliding motion in Filippov differential systems: theoretical results and a computational approach. SIAM Journal on Numerical Analysis, 47(3), 2023-2051.

Rule-Based Expert System for Classification of Hospitality Industry

Akinpelu S.A.[1], Olabode O.[2], Daramola C.Y.[1], Ogunleye G.O.[1], Adeoti E.A.[1]

[1]Department of Computer Science, Federal University Oye-Ekiti, Nigeria
[2]Department of Computer Science, Federal University of Technology Akure, Nigeria
samson.akinpelu@fuoye.edu.ng

Abstract

The conventional method of classification in hospitality domain is bedeviled with problems such as inaccuracy, low speed of processing, prejudice and lack of unifying standards. To eradicate these problems, a rule-based expert system capable of imitating human cognitive reasoning was introduced into hospitality industry. In this paper, hospitality knowledge obtained from experts was codified into electronic form, in order to automate and preserve knowledge into a central shareable form. Hospitality industry was characterized with diversity of types of hotel accommodations, a diversity that is constantly increasing at a fast rate, which invariably renders a conventional approach of classification unsuitable. Thus, an expert system becomes an ideal approach of classification in hospitality domain.

Keywords: Expert system, knowledge base, artificial intelligence, hospitality industry, inference engine

1   Introduction

The growth of knowledge management in the world has made it crucial to codify knowledge into the work process [1]. Codified knowledge is knowledge that has been obtained from a domain expert and transferred into electronic form. This transference of knowledge then helps businesses automate, streamline, and preserve knowledge into a central shareable source. Decisions and training specifics can then be derived from the knowledge using analysis tools or other domain experts [2]. One way to implement this knowledge is through the use of rule-based expert systems. A rule based expert system is a program or hardware configuration that uses a set of preconditions and rules to come up with expert answers to a situation [3]. An "Expert system" is an intelligent computer program that uses knowledge and inference procedures to solve problems that are difficult enough to require human expertise for their solution. The knowledge necessary to perform at such a level plus the inference procedures used can be thought of as a model of the expertise of the best practitioners in the field [4]. Rule base is therefore one of the viable approach of expert system, in which knowledge from a domain are represented inform of human linguistic terms.

The hospitality industry is one of the leading stakeholders in the tourism sector. With roughly 11% of the world's total employment or GDP, tourism often presented as the first global industry, and Europe is by far the first tourist continent [5]. The World Tourism Organization predicts that by 2020, tourist arrivals around the world will increase by over 200%. An important factor therefore, for the visitors of a country is the quality of the hotels in that country [6]. The quality of hotel facilities offered during visitors stay at a hotel directly and significantly affects the Gross National Product (GNP) share of the country dedicated to tourism industry [7]. In the light of the above, the conceptual work deals with developing an Expert System for classification of hospitality industry so as to provide a unified standard in the industry [8]. The need to acquaint hotel customer or guest with what to expect in hotel of their choice is a strong motivation for presenting this paper. More so that hotel classification is a prerequisite for the world's fastest growing industry whose customers' needs and demands change with developmental phase. Hospitality is the act of kindness in welcoming and looking after the basic needs of customers or strangers, mainly in relation to food, drink and accommodation [9]. Hotel ratings are often used to classify hotels according to their quality. From the initial purpose of informing travelers on basic facilities that can be expected, the objectives of hotel classification have expanded into a focus on the hotel experience. Today the terms 'grading', 'rating', and 'classification' are used to generally refer to the same concept, that is to categorize hotels [10].

Researchers in hospitality domain have tried to describe the hospitality industry in different ways. Some tried to summarize the scope of the industry and its characteristics of involving both tangible and intangible features in the service delivery process. Others attempted to describe the industry by exploring the stakeholders involved, mutual benefits generated and the industry's impacts to the society and economy [11]. Balfe et al [12] proposed an expert system approach to hospitality company diagnosis using Linkert scale and Crystal shell. The operating environment of hospitality industry was described as an increasing difficult business environment in which to survive, they must constantly review all aspects of their business, ensure that each unit is operating at an optimum level in order to achieve satisfactory profitability, but could not state how the industry could be rated or classified so as to ensure a unified standard in the industry. The WTO [13] pointed out the benefits of hotel classification to travel agency, tour operators, hotel industry, government and consumers in facilitating travel agents' tasks of hotel selection. The research also measures the perceived influences

of the hotel classification system on the hotel industry in general as well as hotel properties, through the use of a self-administered questionnaire. It was discovered that the standard of classification among hospitality industry cannot be based on questionnaire alone because of level of diversity. Yeamdao [14], opined that an inseparable level interdependence exists between hotel classification and quality of service. Thus, hotel classifications systems were said to have offered benefits to various sectors, especially the tourism sector. Although the level of interdependence of service quality is essential in hotel classification. It cannot alone form the basis of classification, but rather a robust system that can emulate human cognitive reasons in classifying hospitality industry. Ángel et al. [15] proposed a semantic-based expert system to provide recommendation in tourism domain. The system only make recommendation to tourist based personal interest. Also, the method of data collection for customer feeling about recommendation for the system was too narrow in scope. Amir et al. [16] proposed a model through which factors affecting hotel classification were identified and a domestic model for classifying hotels in Iran was presented. But the model was not implemented with any real life system in other to ascertain its functionality. In all, none could yield a unified standard of classification of hospitality industry without human intervention, hence the necessity of expert system for classification in hospitality domain.

2 Architecture of the Expert System

The proposed system is a rule-based expert which is implemented using Java Expert System Shell (JESS), as shown in Fig. 1. The system makes use of forward chaining for the inference engine and uses the RETE algorithm to search the knowledge base. The system has a graphical user interface where the user is presented the option to answer in yes or no. The set of questions are prepared according to various hotel facilities to which a hotel unit may be categorized.
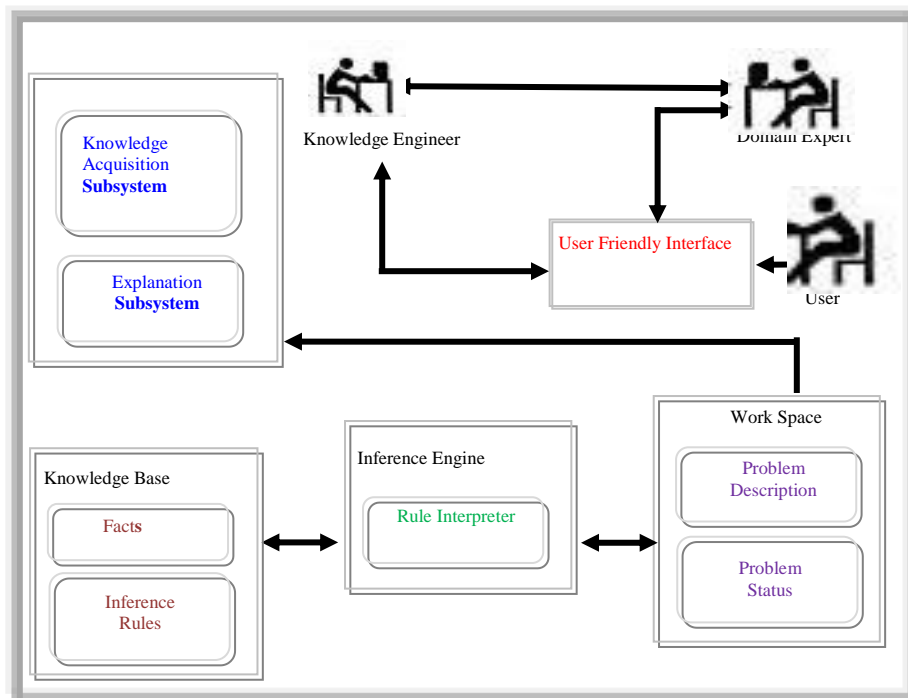
Fig. 1. Architecture of the expert system for hotel classification

Now, according to the feedback given by the user, the RETE algorithm (pattern matcher) searches the knowledge base for possible pattern matches. If there is a rule in the knowledge base which matches the hotel facilities, the system shows the possible classification (hotel star) in the recommendation window.

2.1 Knowledge Acquisition

The knowledge base can be considered as the heart of the Expert system as all the required facts for building the rules are contained resides in it. The first step in building an expert system is the elicitation of the necessary knowledge about the domain from the human expert by the knowledge engineer. With the knowledge acquisition facility, application experts can add knowledge about their domain of expertise. Taking this knowledge as source, Expert System rules can be formed [18]. The primary source of knowledge acquisition

for the Expert System for inferencing the domain knowledge of Hospitality Industry was consultation with expert in hospitality domain such as hotel managers, hotel customers', Hotelier Association, and Ministry of Tourism in Ekiti State, Nigeria.

2.2 Knowledge Representation

The knowledge base was represented in the form of production rules using an expert system tool called Java Expert System Shell (JESS). JESS is called an expert system tool because it is a complete environment for developing expert systems which includes features such as an integrated editor and a debugging tool. The word shell is reserved for that portion of JESS which performs inferences or reasoning. The JESS shell provides the basic elements of an expert system [19]. JESS matches facts in the fact base to rules in the rule base (Fig. 2). Here the user of the system will first be presented with a list of questions which the user has to answer in yes or no. With the help of the questions the available facilities of hotels are acquired. Then by using the RETE algorithm, which is a pattern matching algorithm, the knowledge base is searched for matching the features of the hotel with those already present in the knowledge base. The RETE algorithm is usually implemented as a directed acyclic graph which is used to match rules to facts. Let us suppose a hotel is to be classified as One Star, the rules for one star is thus:
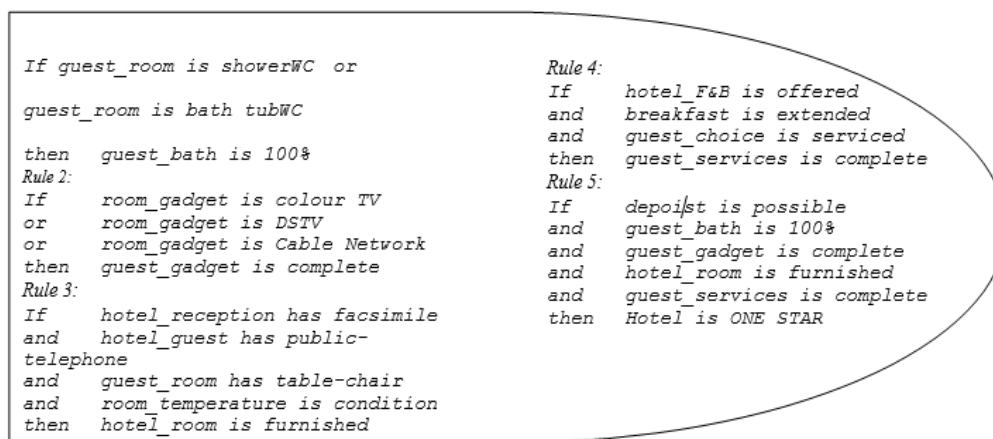

Fig. 2. Knowledge representation

2.3 Inference engine

Inference engine (IE) is concerned with carrying out the relevant inductions and deductions on the knowledge base. It is the adoption of appropriate line of reasoning, leading to the solution of a given problem or the formulation of a body of consultative advice on a given demographic phenomenon [20]. A rule based expert system using forward chaining usually consists of if-then rules, a bunch of facts and an interpreter controlling the application of rules, given facts. A single If-Then rule (Fig. 3) assumes the form 'if X is A then Y is B'; if part of the rule 'X is A' is called antecedent and then part of the rule 'Y is B' is called consequent.
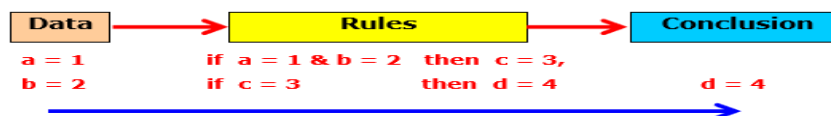
Fig. 3. Forward chaining inference

3 Methodology

The expert system for classification of hospitality industry was implemented using forward chaining approach to reach the conclusion. In order to execute a rule-based expert system using this method, a rule merely need to be fired and the corresponding action be triggered. Suppose, a hotel has facilities ranges from daily room cleaning, table and chair, reception service, extended breakfast, daily room cleaning, to deposit possible. The expert system starts with the assumption that the hotel is One Star. On being queried about other available facilities, if the user answers yes, the expert system will discard the assumption and then the expert system proceed further on its queries to ascertain other facilities that are available. After all the inputs from the user, the expert system will use the

RETE algorithm to search the knowledge base for matching the pattern of the facilities. If the pattern matches with One Star, the rule for One Star will be fired and the system will display the corresponding hotel classification as indicated in following section.

4   Result and Discussion

The proposed expert system for classification of hospitality industry has its inference engine implemented with JESS. There is room for updating the expert system with new knowledge and new rules without changing the whole configuration and adding only the specific rules. A java Swing was used for the graphical interface as shown in Figs. 4, 5 and 6.

Fig. 4. Hotel registration

The registration module is critical to the expert system as it establish the fact that an industry operator is well registered and licensed by the regulating body. The importance of hotel registration cannot be overemphasized as it pivotal to classification on which this paper is based. Once the registration is completed, the system proceeds to actual classification.

Fig. 5. Catalogue of expert system query for classification

After which, the system engages the user in some question through which conclusion is drawn as to which classification a hotel falls into. The questions revolve around physical and service quality that exists in a hotel which forms the basis for classification.

This interface is the actual module that classifies a hotel base on the physical facilities and services that exist in the hotel as presented in Fig. 6. The user selects various facilities and services that are available in the hotel from a drop-down menu. Internally, the expert system through its inference engine classifies available facilities as "yes/no". Then, the exact rule that matches the hotel features from the knowledge base is fired, which eventually will lead to a hotel being classifies as One star (*), two star (**), three star (***), four star (****) and five star (*****) as the case may be as shown in Fig. 6. As indicated above, the key purpose that necessitates this

classification as presented in this paper is to remove the aged long problem of the fact that guests are not given value for money and are being ripped off by hospitality industries operators.

ID	NAME	ADD	CAC	PHONE	RATING
SUNV/13	SUNVIEW Hotel	Futa Road Akure	RCC340009	0809887467	1 Star
Prop/3	Properous Royal Hotel ...	Km, 14 Iworoko Road A...	RC23001	01-2334489884	3 Star
Moni/15	Monilac Hotel	Ondo Road Akure	RCC679000	0908999990	2 Star
Iati/6	Iatin Hotel	Km 4 Ondio Road Akure	Rc3455	0984847447	1 Star
IITA/17	IITA Hotel	Ibadan City, Ibadan, Oy...	Rc20007172	07023345598	1 Star
Hepz/14	Hepzico Hotel	12, Odo Lane Ado-Ekiti	RC102334	09088874444	1 Star
FABI/10	FABIAN HOTEL	NTA ILAWE ROAD ADO...	RCC023456	08097766866	1 Star
Deli/5	Delight Hotel	Iyin Road Ado-Ekiti	RC0956	01-34778009	1 Star
Davi/16	Davies Hotel	Adeyi Avenue, Old Bodij...	RCC045866	090788474000	1 Star
Dave/4	Dave Hotel	Adebayo Aread Ado-Ekiti	RC0201	02-898374	1 Star
Aval/7	Avalon Hotel	12, Temiridire Road, Ak...	RC23456	080675432343	1 Star

Extended breakfast
Beverage offer in the hotel
Deposit possibility
Gym
Breakfast buffet
Reading light next to the bed
Bath essence or shower gel
Bath towels
Linen shelves
Offer of sanitary products (e.g. toothbrush, toothpaste, shaving kit)
Swimming Pool

Fig. 6. Hotel classification

5   Evaluation of the Expert System

In order to assure the functionality of the proposed system, expert users' rating observed from some experts were used to determine the degree of confidence (DoC) of the classification made by the expert system. In information retrieval context, standard measures, like mean accuracy (MA) and efficiency have been used to determine the performance of several systems. In this study, DoC is taken as a measure to establish the confidentiality of the system. This is achieved by performing relational-join operation on the result of the proposed system and the ratings by experts after demonstration. The DoC of a ranking is determined using Equation 1.

$$DoC_i = \frac{\#False\ Positives}{\#True\ Positives} * 100 \qquad\qquad 1$$

where #True Positive is the number of hotel classification by the system and an expert agrees with, and #False Positive is the number of hotel classification by the system but an expert does not agree with. The data obtained from experts after assessment through a score sheet is presented in Table 1, and the DoC of first user u_i is computed as thus:

$$DoC_i = \frac{52}{56} * 100 = 92.85\% \qquad\qquad 2$$

$$Error_i = \frac{100 - DoCi}{100} \qquad\qquad 3$$

Table 1. Data showing domain expert rating

Id	001	002	003	004	005	006	007	008	009	010
#True +Ve	55	56	57	55	53	57	54	55	56	58
#False +Ve	52	49	51	53	44	48	43	54	44	50
DoC (%)	94.55	87.50	89.47	96.30	83.01	84.21	79.63	98.18	78.57	86.21
Error$_i$	0.05	0.13	0.11	0.04	0.17	0.16	0.21	0.02	0.21	0.14
1 - Error$_i$	0.95	0.87	0.89	0.96	0.83	0.84	0.79	0.98	0.79	0.86

Therefore, the MA is calculated as:

$$MA = \frac{\sum_{i=1}^{n}(1 - Error_i)}{n} \qquad\qquad 4$$

$$MA = \frac{8.76}{10} = 0.876$$

$$Efficiency = MA^* 100 \qquad\qquad 5$$

$$Efficiency = 0.876^* 100 = 87.6\%$$

Therefore, it can be inferred from the computation that the proposed system is 87.60% efficient in providing accurate classification of hotels.

6   Conclusion

In this paper, a rule-based expert system for classifying and rating of hospitality industry was developed purposefully to ensure proper standardization in terms of facilities, services, pricing and healthy competition in the industry. The result has shown that any form of self-acclaim status without requisite facilities and services by an industry operator can be abolished. Hotel guests are better informed of what to expect in a hotel of their choice. The system codified experts' knowledge in hospitality domain and used pattern matcher algorithm to reach its conclusion. The system was tested, and it perform as expected.

References

1. Cernik J., 2009. Framework for an expert system generator, Master thesis presented to graduate faculty, University of Akron.
2. Abraham A., 2005. Rule based Expert System: Handbook of measuring system design.Peter H, syndenhan and Richard thorn, eds. John Willey & Sons Ltd., Oklahoma State University, USA.
3. Jaja K., 2009. Issues in grading and classification of Nigeria's hospitality sector, The Economy Newspapers.
4. Russell S. and Norvig P., 2002. Artificial Intelligence: A Modern Approach, Prentice Hall, Second Edition.
5. World Tourism Organization (WTO) & International Hotel and Restaurant Association (IH&RA), 2004. The Joint WTO & IH&RA Study on Hotel Classification.
6. Amir M., Ghadami R. and Sarafizadeh D., 2012. Designing a domestic model for classification and grading hotels in Iran. International Journal of Business and Social Science, 3(8), 306-310.
7. Rajdeep B. and Suagta S., 2012. Rule based expert system for diagnosis neuromuscular disorder, retrieved from https://arxiv.org/ftp/arxiv/papers/1207/1207.2104.pdf on 10th April, 2018.
8. Benny C. and Murray M., 2013. Introduction to hospitality. Tourism and Hospitalities Studies, Hong Kong Polytechnic University.
9. Henses W., 2010. Guest review criteria on tripadvisor compared to conventional hotel rating systems to assess hotel quality, Stenden University, SA.
10. Yeamdao N., 2007. The relationship of the hotel rating system and service quality. Ph.D Thesis, Submitted to the Faculty of the Graduate College of the Oklahoma State University.
11. Olabode O. and Olabode B., 2008. Projection manager: A knowledge based cohort component model for population projection in Nigeria. Journal of Information Technology Impact, 8(1), 11-24.
12. Balfe A., O'Connor P. and McDonnell C., 1994. An expert system-based approach to hospitality company. HITA Conference. Dallas, Texas.
13. Ángel G., López-Cuadrado J., Ricardo C., González-Carrasco I. and Belén R., 2011. Sem-Fit: A semantic based expert system to provide recommendations in the tourism domain. Journal of Expert Systems with Applications, 38(10), 13310-13319.
14. Bullinaria A., 2005a. IAI: Production systems, http://www.cs.bham.ac.uk/~jxb/IAI/w7.pdf, accessed on 29th March, 2018.
15. Chakraborty R., 2010. Expert System: AI course lecture 35-36. www.myreaders.info/html/artificial_intelligence.html, accessed on 7th September, 2018.
16. Bullinaria A., 2005b. IAI: Expert systems, http://www.cs.bham.ac.uk/~jxb/IAI/w9.pdf, accessed on 29th March, 2019.
17. Orthodox T. and Krishna G., 2015. Hotel grading, service quality, satisfaction and loyalty – Proposing a theoretical model and relationship, African Journal of Hospitality, Tourism and Leisure Vol. 4 (Special edition), ISSN: 2223- 814X.
18. Hunt V., 1986. Artificial Intelligence & Expert Systems Sourcebook. Springer US.
19. Morris J., 2005. Introduction to Java Expert System Shell, Seminar "AI Tools" Winter semester, Universit at des Saarlandes 2006/2007.
20. Mostafa A., Ahmad A., Mohammed M. and Obaid O., 2012. Implemeting an expert diagnostic assistance system for car failure and malfunction. Intl Journal Comp Sci, 9(2), 116-125.

Review on the Impact of Nanotechnology in M-Learning Devices

Bamidele V. Aremu[1],*, Olufemi V. Adeoluwa[1], Omolola H. Aremu[2]

[1]Department of Vocational and Technical Education, Ekiti State University, Ado-Ekiti, P.M.B 5363, Ado-Ekiti, Ekiti State, Nigeria
[2]Nanoscience Research Unit, Department of Industrial Chemistry, Federal University Oye-Ekiti, P.M.B 373, Oye-Ekiti, Nigeria
helebami@gmail.com

Abstract

M – learning is a form of learning that supports both classroom and distance learning experiences. The educational institutions in Nigeria have not been able to admit all the candidates that are available for learning in Nigeria due to limitation in space and other resources. This has been negatively affecting those candidates emotionally and psychological even the country at large as the working population is less than dependent population. m – learning makes learning activities to be easily accessed. Nanotechnology has been able to come up with components of m-learning devices in nanoscale (1-100 nm) which are smaller but have great potentials in performance than the non–nanoscale components. This paper reviews the impacts of carbon nanotubes on production of chips, transistors and the rechargeable batteries that form part of the components of m – learning devices.

Keywords: Nanotechnology, mobile learning, mobile devices, teaching aids

1 Introduction

Globalization had made the world to be interested in the search for devices that can link the world together. The linking search led to the development of mobile devices which are now the easiest and faster channels of connecting the societies within the world. It is obvious that mobile devices have been experiencing increase and continuous transformation even as they become an integral part of human livelihood because novel models of the technologies that are faster, portable, presentable and affordable are been produced. Cellular phones, smart phones, tablets, computers and personal digital assistants (PDAs) are some of the available mobile devices [1]. Learning is another part of human livelihood that is often performed in the classroom with teaching aids. Teaching aids are materials needed to support learning and it can be grouped into visual (e.g. placard, pictures, projectors, telex, film strip,), audio (e.g. radio, tape recorder, public address system) and audio-visual (e.g. television, video-sound strip, and some mobile devices) teaching aids. Mobile devices are good materials that bring flexibility and interactivity during learning experience [2], when these mobile devices are incorporated into the act of learning, the learning process is tagged mobile learning (m - learning).

Nanotechnology that is known for its ability to manufacture devices in a scale ranging from 1 to 100 nanometers is considered to be useful in factually all the fields of study and that is why this review is on the impact of nanotechnology on the field of education.

2 Overview of Nanotechnology in M - devices and M - learning

Jeroh [3] was of the opinion that nanotechnology has immensely enhanced the competence of mobile phones and computers (mobile devices) by increasing their speed and efficiency. It is further stated that nanotechnology has the potential needed to manufacture computer chips, energy batteries and sensors that are appreciably smaller, faster, powerful and cheaper [4]. However, Brown and Mbati [5] opined that mobile learning (m-learning) involves the use mobile devices in teaching and learning experiences. One will then, admit that the relevancy of mobile devices in education is to realize a better and flexible channel of learning and assimilation. More so, this channel of learning entails that mobile devices be intelligently incorporated into the learning activities of the Nigerian educational system as well as the daily living of individual citizens in order to make learning to be easily accessed.

There is no doubt that nanotechnology has been growing since its inception and it has been relevant in many professional fields; such as sciences, engineering, pharmacy, medicine and surgery etc. Parts of the various impact of nanotechnology in this contemporary world as revealed by Jeroh [3] are:

- The reduction of the size of chips as well as the enlargement of its memory.
- The administration of pharmaceuticals to a precise part of the body with a level of accuracy that brings in the most efficient kind of treatment.
- The production of tennis balls through carbon nanotubes in order to help them gain more elasticity.
- The improvisation of knee cap as well as the knee implants itself during the knee replacement surgery.

- Presently, vehicle manufacturers employ nanotechnology in manufacturing vehicles parts such as car bumpers, windscreens etc.

3 Application of Nanotechnology to M-learning devices

Nanotechnology uses nanomaterials in the production of mobile technologies; especially, those ones that possess high intelligent qualities which make them function in teaching and learning communication; it is certain that this contemporary world of technology is working hard to ensure that mobile devices are gradually transforming in state so as to function more and be relevant in all human societies. The various functions of m - learning devices as aided by nanotechnology has made the input and output activities of the mobile devices to be friendly to their users in learning environment. More so, the cleverness of m - learning devices and their functions in educational activities creates a new platform that aids intelligent activities such as educational games, tutorials and subject tests. Furthermore, the application of nanotechnology to the production of m - learning devices brings in such an opportunity that makes m - learning device users to partake in learning even outside the walls of the classroom. The speed and accuracy level of the mobile devices incorporated into teaching - learning platform is to help with connectivity; this is why nanotechnology is used in the production of smaller gadgets with high speed limit and large storage capacity [6].

It is observed that nanotechnology can be useful in the production of m -learning devices through the involvement of any of these; carbon nanotube, vacuum tube, microscopic microphones and liquid lenses [7]. This review paper will reveal the impacts of carbon nanotubes in the production of mobile learning devices.

3.1 Carbon Nanotubes

Carbon nanotubes are one of the nanomaterials that belong to the Fullerenes family. The fullerenes nanomaterials are known to have the composition of carbon either in the hollow- sphere, ellipsoid or tube shapes. Consequently, the fullerenes in spherical shapes are profoundly referred to as fullerenes (C_{60}) or Bucky balls. They are considered useful in electronics and other applications [8]. However, the fullerenes in tube form are generally known as carbon nanotubes, and they are seen as mainly the prominent nanomaterials used in nanotechnology. Carbon nanotubes are tiny elongated carbon tubes of carbon, which were made popular in 1991 when it was found in an insoluble material of arc-burned graphite rods. The cylindrical shape carbon nanotubes composed of only carbon atoms that make hexagonal lattice on the sheet of its cylinder. This nanotechnological material possesses some unique distinctiveness because of their form and size [9].

Carbon nanotubes have a very wide collection of electronic, thermal, and structural qualities that transform base on their different diameter, lengths, and coils. It is also noted that carbon nanotubes exist in two forms, namely single wall carbon nanotubes and multi-wall carbon nanotubes. Single-wall nanotubes consist of a single graphite layer while multi-wall nanotubes consist of multiple concentric layers [10]. Fig. 1 shows the diagram of the two forms of carbon nanotubes. The arrangement of the carbon molecules of carbon nanotubes in the tube determines either the carbon nanotube will be a strong conductor or a semi-conductor. It has been established that the arrangement that made carbon nanotubes to be metallic, also account for why carbon nanotubes acquire electrical conductive capacity that is 1000 times higher than the electrical conductive capacity of copper. In order to reduce high electrical conductivity level of carbon nanotubes, polymers are wrapped with it [11].

3.1.1 Application of Carbon Nanotubes in the Production of Chips for M-learning Devices

The process of manufacturing of micro-chips entails metallic components that work as the channel for the interconnectivity between the transistors placed on the micro cards. It is noted that the metallic substance that was initially imprinted on chips was aluminum and this was later changed to copper; meanwhile, the need for higher performance chips combined with more tightly packed transistors require interconnects less than 40 nanometers wide later surfaced and copper could not stand the capacity needed for such chips as it relate to high conductivity as well as small dimensions. This is what led to the initiative of using carbon nanotubes to serve as a substitute for conducting substance of copper [13].

Since, the introduction of carbon nanotubes as the connecting channel between the transistors of micro-chips, it has been serving its purposes and have been a viable nanomaterial for the production of electronic micro-chips. In fact, the result of a research conducted revealed that carbon nanotubes functions at 1Ghz on a chip of size of 1/100th square inch that has 11000 transistors. It is also noted that carbon nanotubes make the production of micro-chips to be easier since it does not require special lining on the circuit of the micro-chip [14]. In addition, a high density nonvolatile random access memory chip has been created using carbon nanotubes which serve as active memory elements as well as semiconductors [15].

3.1.2 Application of Carbon Nanotubes in the Production of Transistors for M-learning Devices

Transistors are vital components of the circuit board of any electronic device. They mainly perform the underlying work of any electronics because the circuits of the devices function through the connected transistors. It has been raised that carbon nanotube can also be used in the production of transistors by making a single carbon nanotube molecule into semi-conductive substance. A single walled nanotube can act as transistors when carbon nanotubes are constructed to act as such. Endo et al. [16] revealed that this can be done by locating a molecule inside a carbon nanotube in order to influence the flow of electron of the electrical current that passes through it with the resultant effect of creating a molecular gate; this controls the flow of the electrical current within the carbon nanotube. According to them, such gate will be very small compared to a silicon chip and will operate under room temperature.
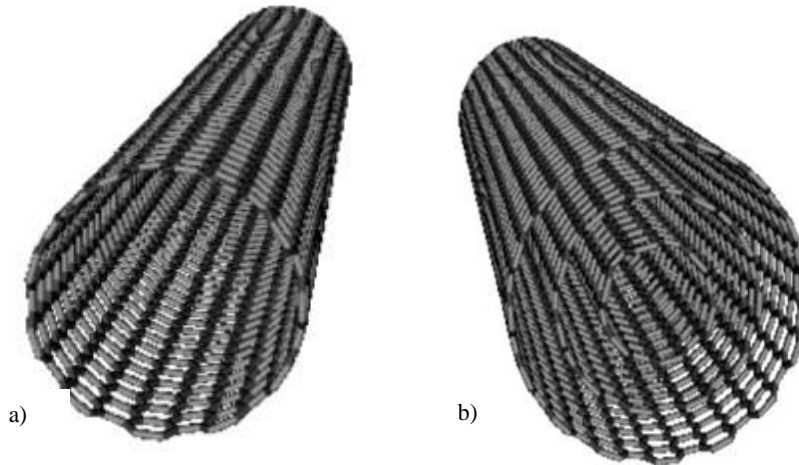

a)   b)

Fig. 1. Diagram of single wall (a) and double wall (b) carbon nanotubes [12]

3.1.3 Application of Carbon Nanotubes in the Production of Batteries for M-learning Devices

One of the features that made m - learning devices to be users friendly is the possession of rechargeable batteries. It has been identified that carbon nanotubes have qualities that make them to function as anode materials that are relevant in the production lithium-ion (Li-ion) battery systems [16]. It is further stressed that carbon nanotubes can be used to produce electrodes that are very thin with great conductivity potentials. Gröning [10] wrote that the predominant part of commercially produced carbon nanotubes is used for the manufacturing of porous conductive electrodes for Li-ion batteries that can serve any m - learning devices better.

3.2   Future Insights

Nanotechnology is well known to have impacts in almost all professional fields; the workings of nanotechnologists have sparked up revolution in the field of engineering, science, medicine, agriculture, food processing, telecommunications and education. The education industry will be drastically improved in the nearest future as nanotechnology will invents small, sophisticated and economical educational gadgets that are more flexible, more portable, and easy to use for educational purposes both in rural and urban areas. The future impacts of nanotechnology will be to produce nanoscale materials that have more memory, more sustaining battery power, more computing strength, easy sensing and easy usage by both students and teachers in order to offer effective and efficient learning even outside the classroom.

4   Conclusion

This paper has shown the application of nanotechnology in the production of mobile electronic devices that is useful, effective, inexpensive and accessible for teaching and learning experiences. The quest for learning is on the increase in the present-day Nigerian and this has already created the challenge of overpopulation in Nigeria educational institutions. In fact, some who desire to learn could not gain entrance into school to learn and they end up creating menace to society. Consequently, nanotechnology can help in the production of m - learning devices that are smart and active for learning, in order to accommodate learning outside the school premises.

References

1. Liu M., Scordino R., Geurtz R., Navarrete C., Ko Y. and Lim M., 2014. A look at research on mobile learning in k-12 education from 2007 to the present. Journal of Research on Technology in Education, 46(4), 325-372.

2. Crompton H., 2013. A historical overview of mobile learning: Toward learner-centered learning in Z L Berge & L Y Muilenburg (Eds.), Handbook of mobile learning, Florence, KY Routledge, Italy, 3-14.

3. Jeroh M.D. (2017). The impact of nanotechnology on mobile phones and computers. J. Nano. Adv. Mat. (5)1, 17-22.

4. Ali Ahmed E.S. and Sohal H.S., 2017. Nanotechnology in communication engineering: issues, applications, and future possibilities. World Science News, 66, 134-148.

5. Brown T.H. and Mbati L.S., 2015. Mobile learning: moving past the myths and embracing the opportunities. International Review of Research in Open and Distributed Learning, 16(2) 115-135.

6. Akyildiz F., Pierobon M., Balasubramaniam S. and Koucheryavy Y., 2015. The internet of bio-nanothings. IEEE communications magazine - communications standards supplement.

7. Jamthe D.V. and Bhande S.A., 2017. Nanotechnology in 5g wireless communication network: an approach. International Research Journal of Engineering and Technology, 4 (6), 58-61.

8. Flamant G., Robert J.F., Marty S., Gineste J.M., Giral J., Rivoire B. and Laplaze D., 2004. Solar reactor scaling up the fullerene synthesis case study. Energy, 29, 801- 809.

9. Lam C.W., James J.T., McCluskey R., Arepalli S. and Hunter R.L., 2006. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. Crit Rev Toxicol, 36, 189-217.

10. Gröning P., 2005. Nanotechnology: an approach to mimic natural architectures and concepts. Adv Eng Mater, 7 (5), 279-291.

11. Whatmore R.W., 2006. Nanotechnology – what is it? Should we be worried? Occupational Medicine – Oxford 56 (5), 295-299.

12. Purohita R., Purohitb K., Rana S., Rana R.S. and Patel V., 2014. Carbon nanotubes and their growth methods. Procedia Materials Science, 6, 716-728.

13. Corso D., Crup I., Ammendola G., Lombardo S. and Gerardi C., 2003. Programming options for nanocrystal MOS memories. J Mater Sci Eng, 23, 687-689.

14. Stanford University, 2008. A 1 GHz integrated circuit with carbon nanotube interconnects and silicon transistors: http://nano.stanford.edu/publication.php?id=135 (Accessed on 21st April, 2019).

15. Nantero, 2006. Press release: Nantero announces routine use of nanotubes in production CMOS fabs. Carbon nanotube electronics era has begun: http://www.nantero.com/pdf/Press_Release_11_06%20.pdf (Accessed on 3rd May, 2019).

16. Endo M., Hayashi T., Kim Y.A. and Muramatsu H., 2006. Development and application of carbon nanotubes. Japanese J Appl Phys, 45 (6A), 4883-4892.

Foliar Anatomy of the Genus *Pterocarpus* Jacq. (Papilionaceae) in Nigeria

Chukwuma Deborah Moradeke[1,*], Ayodele Abiodun Emmanuel[2]

[1]Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria
[2]Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria
deborah.chukwuma@fuoye.edu.ng

Abstract

Comparative foliar anatomical study of six species of the genus *Pterocarpus* was carried out with a view to elucidating their taxonomic significance in their proper identification. Fresh leaves of the six indigenous species - *Pterocarpus osun, P. erinaceus, P. mildbraedii, P. soyauxii, P. lucens* and *P. santalinoides* were obtained from different geographical locations in Nigeria. Stripes of epidermal layers were obtained from the adaxial and abaxial surfaces of the leaves and viewed under a light microscope. Quantitative and qualitative characteristics such as, epidermal cell shape, epidermal length and width, cell wall thickness, number and type of stomata, stomata length and width amongst others were carefully studied. Findings showed that epidermal cell shapes were polygonal and irregular in all except *P. soyauxii* and *P. mildbraedii* where they were predominantly irregular. Stomata were generally anomocytic and observed on abaxial surfaces alone except in *P. mildbraedii* where it was present on both surfaces, that is, *P. mildbraedii* is amphistomatic. Grandular trichomes were observed on the abaxial surfaces of *P. osun, P. soyauxii* and *P. lucens*. The number of stomata vary with as much as 114 per mm^2 (*P. lucens*) and fewer trichomes, just about 1 - 19 per mm^2. The length of the cells ranges between 14.3 µm (*P. santalinoides*) and 67.9 µm (*P. erinaceus*). The dendrogram generated from the observed characters divides the genus into three groups - *P. osun, P. soyauxii* and *P. lucens* (group 1), *P. erinaceus* and *P. santalinoides* (group 2), and *P. mildbraedii* (group 3) as a distinct species from the others.

Keywords: Foliar, adaxial, abaxial, trichomes, dendogram

1   Introduction

The genus *Pterocarpus* Jacq. is pantropical comprising trees and belongs to the family Papilionaceae or Fabaceae, subfamily Faboideae, and monophyletic clade *Pterocarpus* within the tribe Dalbergieae [1]. The word "*Pterocarpus*" is derived from two Greek words: "pteron" (wing) and "karpos" (fruit) [2] which refers to the unusual shape of the seed pods of members of this group. According to Lavin et al. [3], root nodules of the members of this tribe are distinctive and therefore referred to as an "aeschynomenoid" or "dalbergioid" nodule. *Pterocarpus* legumes were circumscribed as a pantropical group of Papilionaceae or Fabaceae based on molecular and morphological data [4]. They grow in a variety of habitat, including moist and dry tropical forests, savannas, costal dunes and rocky outcrops and contain a number of woody life forms such as shrubs, tree and climbing lianas as noted by De Carvalho [5]. Among flowering plants, the Fabaceae (Leguminosae) is the third largest family with over 19,300 species found in the world's vegetation types. In Nigeria, it is the largest family comprising 143 genera and 529 species [6]. Vatanparast et al. [7] reported the exceptionally high economic and ecological importance of dalbergioid legumes because they are sources of food, fodder, woods, fuels, medicines and their ability to enrich soils. A valuable timber known as paduak (or paduok) is produced by most species of *Pterocarpus* [2] and these timbers are prized for their hardiness, firmness in use and in decorations. Their woods contain water- or alcohol-soluble substances that can be used as dyes [5]. However, there is dearth of information on the taxonomic characters of members of this genus occurring in Nigeria except for a few work done on some of their economic properties. Avwioro et al. [8] discussed the staining ability of *P. osun* on tissue sections, while Ajiboye et al. [9] reported its free radical scavenging property. In 2011, Abubakar et al. [10] described the antifungal potential of *P. erinaceus*. Ndukwe and Ikpeama [11] examined the phytochemical and proximate constituents of the leaves of *P. soyauxii* and *P. santalinoides* amongst others.

A total of 35 species of *Pterocarpus* are currently accepted with six of these existing in West Africa and all six indigenous to Nigeria [6, 12, 13]. These include *Pterocarpus erinaceus* Poir., *Pterocarpus lucens* Lepr. ex Guill. et Perr., *Pterocarpus milbraedii* Harms, *Pterocarpus osun* Craib, *Pterocarpus santalinoides* L'Her. ex DC. and *Pterocarpus soyauxii* Taub. These species have not been adequately studied for their taxonomic characters; hence, the present study identifies, scrutinizes and evaluates the epidermal layers of these underutilized legumes in an attempt to clarify the similarities, differences and distinctiveness from each other.

2 Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents used in the study were obtained from the Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Ekiti and these include concentrated nitric acid (100% HNO_3), concentrated ethanol (100% C_2H_5OH), safranin O, glycerol, and distilled water.

2.2 Collection of samples

Fresh leaves of each species were collected from different locations and vegetative zones across Nigeria. *Pterocarpus mildbraedii* and *P. soyauxii* were collected from Umauhia (Abia State), *P. erinaceus* and *P. santalinoides* from Olokemeji (Ogun State), *P. osun* from the Aboretum of Forestry Research Institute of Nigeria, Ibadan (Oyo State) while *P. lucens* was from Mokwa (Niger State). All collected samples were carefully identified at the Forest Herbarium Ibadan [14].

2.3 Epidermal preparations

All leaves of individual species were separately revived in boiling water for 30 minutes, with average heat intensity. Thereafter, about 2-5 cm^2 of three pieces each of the specimens from the standard median portion were cut and soaked in separate Petri dishes containing concentrated solution of nitric acid, and then placed under the sun for about 2-3 hr to allow tissue disintegration. The samples were transferred unto a clean Petri dish and carefully rinsed in distilled water, before the epidermises were separated using a pair of camel brush which had been moistened in water. Debris and skeleton of veins were brushed off carefully from the epidermal layers. The separated tissues/epidermises (adaxial and abaxial layers) were then transferred to different Petri dishes containing distilled water, with each labeled appropriately and again rinsed. Unused tissue sections were transferred to vials (specimen bottles) containing 50% ethanol, indicating the adaxial tissues and abaxial tissues separately for all plant leaves [15, 16].

2.4 Mounting of tissues

Abaxial and adaxial tissues were stained with safranin O for about 2-3 minutes, and rinsed in continuous changes of distilled water to remove excess stain. Afterwards, they were carefully mounted in 15% glycerol unto clean microscopic glass slides, covered with cover slips and the edges ringed with nail vanish to prevent dehydration. All microscopic slides were appropriately labeled for each species, and studied under Olympus light microscopes at different magnifications. Observations were taken from 20 different fields of view, while photo-micrographic images were taken with an attached camera mounted on the light microscope [16, 17].

2.5 Data Analysis

A dendrogram was generated using Minitab 17.0 to further reveal the level of relatedness among the species studied, based on the foliar micro-characters identified.

3 Results and Discussion

The qualitative result shows that there are similarities among the six species of *Pterocarpus* studied (Table 1). It was also observed that *P. erinaceus, P. santalinoides* and *P. lucens* possess thin-walled, irregular straight walls on both the adaxial and abaxial surfaces; *P. mildbraedii* has thin-walled, irregular straight walls only on the adaxial surface; *P. osun* and *P. soyauxii* possess thin-walled irregular wavy walls on both adaxial and abaxial surfaces while *P. mildbraedii* possesses thin-walled wavy walls on the abaxial surface. All six species possess anomocytic stomata (i.e. the surrounding epidermal cells have no special arrangement, they are all similar and there are no subsidiary cells, so all the cells are normal epidermal cells without any modification) and are hypostomatic (they possess stomata on the abaxial surface only) except *P. mildbraedii* (Table 1 and Plate 2).

Some of the species studied possess grandular trichome on abaxial surfaces, while some lack trichome completely (Plate 1). Metcalfe & Chalk [18] reported that trichomes have been used widely for taxonomic purposes. It has been used in the classification of genera and even some certain families. Trichome plays a vital role in plant defense, protection of parts and help in retention of moisture on the plant body. The result of the qualitative anatomical attributes of species considered in this study does not show much variation among the species.

Table 1. Qualitative characteristics of foliar epidermal surfaces of *Pterocarpus* species

Species	Surface	Stomata type	Shape of epidermal cell
P. erinaceus	Abaxial	Anomocytic	Thin-walled, irregular straight walls
	Adaxial	Absent	Thin-walled, irregular straight walls
P. lucens	Abaxial	Anomocytic	Thin-walled, irregular straight walls
	Adaxial	Absent	Thin-walled, irregular straight walls
P. mildbraedii	Abaxial	Anomocytic	Thin-walled,irregular wavy walls
	Adaxial	Anomocytic	Thin-walled, irregular straight walls
P. osun	Abaxial	Anomocytic	Thin-walled, irregular wavy, walls
	Adaxial	Absent	Thin-walled, irregular wavy, walls
P. santalinoides	Abaxial	Anomocytic	Thin-walled, irregular straight walls
	Adaxial	Absent	Thin-walled, irregular straight walls
P. soyauxii	Abaxial	Anomocytic	Thin-walled, irregular wavy, walls
	Adaxial	Absent	Thin-walled, irregular wavy, walls

Table 2. Quantitative characteristics of foliar epidermal surfaces of *Pterocarpus* species

Species	Surface	Cell length (µm)	Cell width (µm)	Cell wall thickness (µm)	No. of stomata/mm^2	Stomata length (µm)	Stomata width (µm)	No. of trichome/mm^2	Trichome length (µm)
P. erinaceus	Abaxial	24.7-67.9 35.0±3.9	9.3-17.7 13.1±0.8	1.6-3.3 2.4±0.2	38-59 46.5±1.8	13.1-17.2 15.2±0.4	5.6-10.6 7.1±0.5	4-8 5.5±0.4	63.1-113.4 86.1±4.7
	Adaxial	18.5-33.7 28.2±1.5	12.0-23.4 17.2±0.9	1.4-3.9 2.8±0.2	-	-	-	-	-
P. lucens	Abaxial	17.7-30.3 24.4±1.3	6.1-10.3 9.4±0.8	1.3-3.8 2.2±0.2	71-114 92.1±4.4	10.0-14.9 12.2±0.4	8.6-10.2 9.6±0.2	11-19 13.9±0.9	42.7-124.1 74.0±8.5
	Adaxial	22.5-34.6 28.5±1.3	12.9-19.9 15.3±0.7	1.2-1.7 1.5±0.1	-	-	-	-	-
P. mildbraedii	Abaxial	32.2-61.9 51.9 ±2.5	19.3-39.4 28.9±2.1	0.4-1.4 1.0±0.1	0-2 1.2±0.2	27.3-33.5 30.1±0.6	14.3-19.2 16.9±0.4	-	-
	Adaxial	38.0-62.0 47.6±2.3	18.2-41.6 26.5±2.0	1.3-2.8 1.9±0.2	5-11 8.7±0.6	25.9-36.5 31.2±1.0	14.4-23.5 18.9±0.8	-	-
P. osun	Abaxial	17.6-34.0 27.3±1.7	8.9-16.7 13.3±0.9	0.9-3.2 1.9±0.3	25-37 32.5±1.2	15.2-19.2 17.0±0.4	7.3-11.1 9.6±0.4	1-4 2.0±0.3	116.1-183-9 145.0±7.6
	Adaxial	22.6-42.1 33.2±2.0	13.9-27.0 18.7±1.2	1.1-2.9 2.0±0.2	-	-	-	-	-
P. santalinoides	Abaxial	14.3-35.5 26.9±2.1	10.4-16.6 13.3±0.7	0.8-2.0 1.4±0.1	35-56 43.1±1.8	16.5-21.7 18.1±0.5	7.2-12.2 8.7±0.3	1-2 1.2±0.1	42.3-88.2 68.7±7.2
	Adaxial	25.3-44.5 36.5±1.9	19.1-26.1 21.3±0.7	0.9-3.2 1.7±0.3	-	-	-	-	-
P. soyauxii	Abaxial	19.5-39.6 25.8±2.4	9.9-21.4 15.2±1.1	1.1-2.7 1.8±0.2	35-50 42.2±1.7	14.9-20.7 17.9±0.8	9.8-12.4 11.1±0.3	1-2 1.3±0.2	51.6-250.9 128.7±20.6
	Adaxial	25.6-39.3 31.0±1.4	12.7-23.9 18.6±1.1	1.6-3.3 2.5±0.2	-	-	-	-	-

Key: measurements presented as: min – max above, mean ± standard error beneath

However, results of the quantitative characteristics such as the cell length, cell width, cell wall thickness, number of stomata, stomata length, stomata width, number of trichome and trichome length (Table 2) show that there are differences among the species studied. On the abaxial surface, *P. erinaceus* has the longest epidermal cell (67.7 µm) but *P. mildbraedii* has the highest mean value (51.9 µm). However, on the adaxial surface, *P. mildbraedii* has the longest cell (62.0 µm) as well as the highest mean value (47.6 µm). The species with the thickest cell wall are *P. lucens* (3.8 µm on the abaxial surface) and *P. erinaceus* (3.9 µm on the adaxial surface).
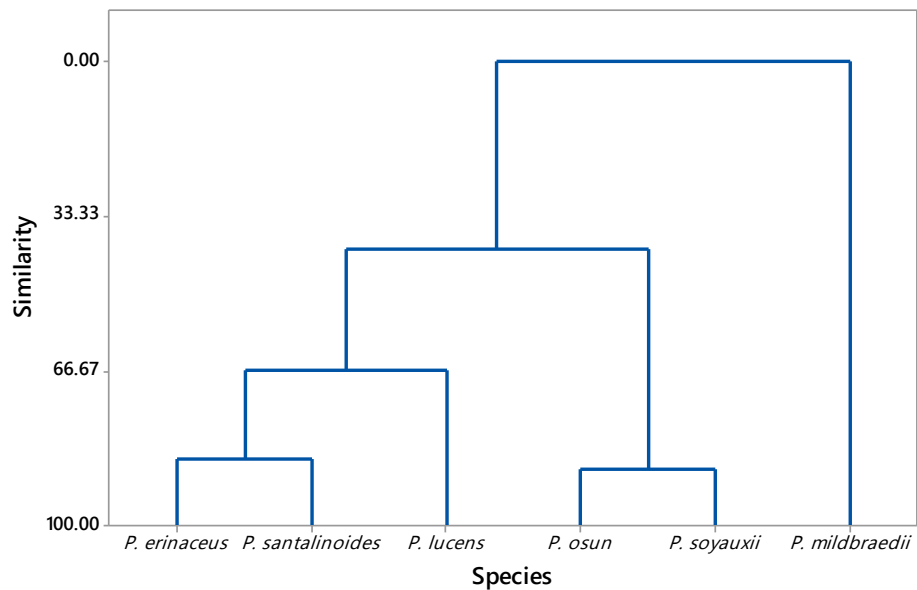
Fig. 1. Dendrogram of species based on complete linkage and Euclidean distance
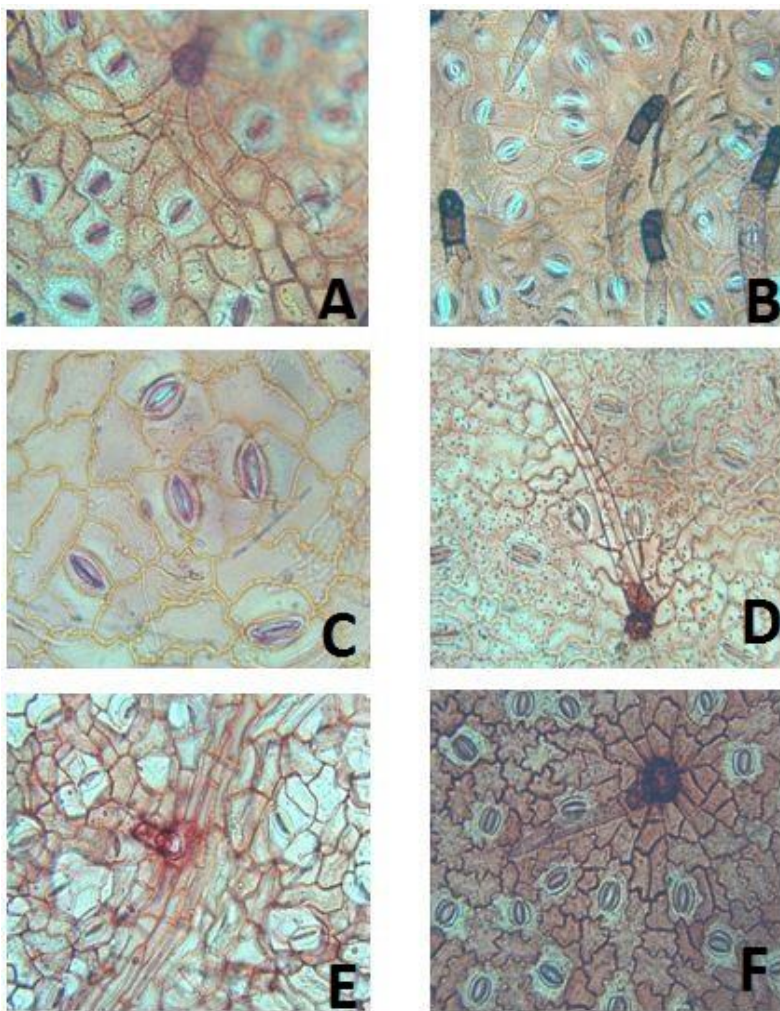

Plate 1. Photomicrographs of Abaxial surfaces of *Pterocarpus* species studied x40. A - *P. erinaceus* ; B - *P. lucens* ; C - *P. mildbraedii* ; D - *P. osun* : E - *P. santalinoides* ; F - *P. souyauxii*
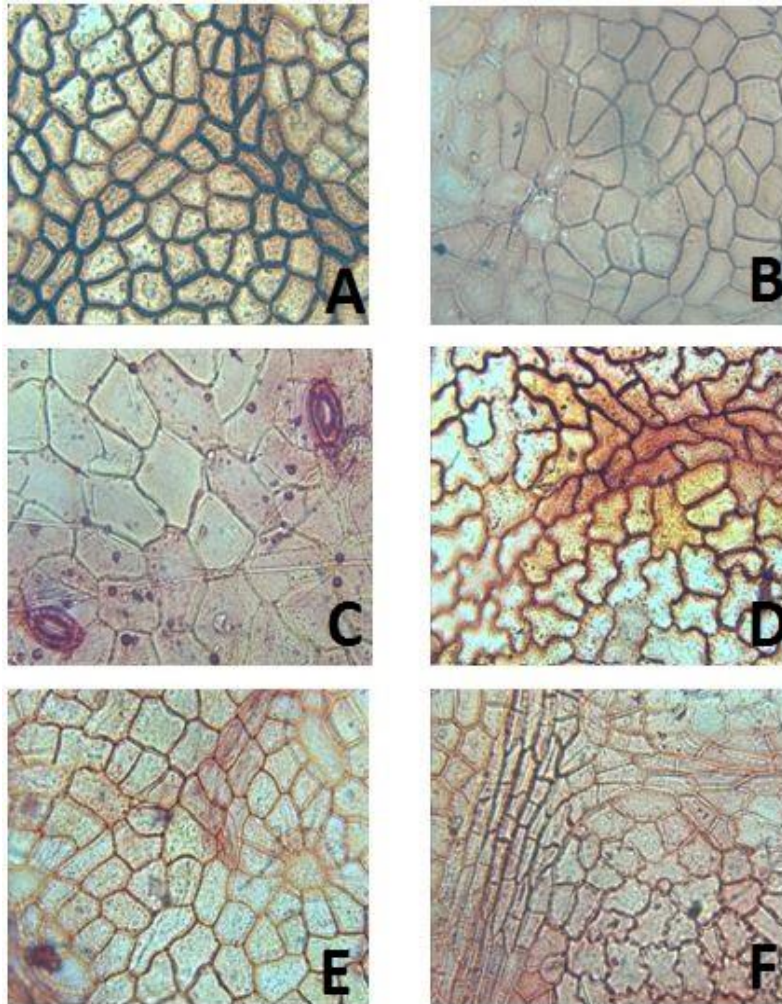
Plate 2. Photomicrographs of Adaxial surfaces of *Pterocarpus* species studied x40. A - *P. erinaceus* ; B - *P. lucens* ; C - *P. mildbraedii* ; D - *P. osun* : E - *P. santalinoides* ; F - *P. souyauxii*

The highest number of trichomes was observed on the abaxial surface of *P. lucens*, which was between 11 and 19, while *P. mildbraedii* was completely void of trichome. Further illustrations shown in Figure 1 revealed that *P. osun* and *P. soyauxii* appears to be the close species based on the micro-characters observed in this study. This is closely followed by *P. erinaceaus* and *P. santalinoides* but these two species also share some affinity with *P. lucens.* Nevertheless, *P. mildbraedii* appears to be an outlier as it occupies an extreme position on the dendrogram.

According to Adedeji and Jewoola [19], foliar anatomy is one of the most noteworthy taxonomic characters which have been used extensively in taxonomic studies of families and genera. Rejdali moh [20] noted that any taxonomic monographs produced without including the microscopic features of the epidermis may be regarded as incomplete. Leaf epidermal characters are of great taxonomic importance [15, 21], and this had earlier been reviewed by some workers [22, 23]. In the course of this study, the use of light microscope has made it possible to evaluate foliar features such as shape of epidermal cells, stomata and trichome types.

4   Conclusion

This study has shown that the genus *Pterocarpus* exhibits some interesting micro-characteristics that could be useful in species identification especially in fragmentary conditions. All the qualitative foliar characters observed on both adaxial and abaxial leaf surfaces of the species studied showed significant similarities. The species are hypostomatic except *P. mildbraedii.* The fact that these epidermal attributes show significant similarities among the six species is an indication that all the traits are good taxonomic indicators. Findings have shown that the examined species are likely to share common ancestors, which corroborates the fact that the genus is a monophyletic clade.

References

1. Cardoso D., Pennington R.T., de Queiroz L.P., Boatwright J.S., Van Wyk B-E, Wojciechowskie M.F. and Lavin M., 2013. Reconstructing the deep-branching relationships of the papilionoid legumes. S Afr J Bot, 89, 58-75.
2. Thomson, Lex A.J., 2006. Species profiles for Pacific island agroforestry, ver. 2.1 www.traditionaltree.org
3. Lavin M., Pennington R.T., Klitgaard B.B., Sprent J.I., de Lima H.C. and Gasson P.E., 2001. The dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade. Am J Bot, 88 (3), 503-533.
4. Lavin M., Thulin M., Labat J. and Pennington R., 2000. Africa, the odd man out: molecular biogeography of dalbergioid legumes (Fabaceae) suggests otherwise. Sys Bot, 25, 449-467.
5. De Carvalho A.M., 1997. A synopsis of the genus *Dalbergia* (Fabaceae: Dalbergieae) in Brazil. Brit, 49, 87-109.
6. Ayodele A.E. and Yang Y., 2012. Diversity and distribution of vascular plants in Nigeria. Qingdao Publishing House, Qingdao, China. pp.350.
7. Vatanparast M., Klitgaard B.B., Adema Frits A.C.B. and Pennington R.T., 2013. First molecular phylogeny of the pantropical genus *Dalbergia*: Implications for infrageneric circumscription and biogeography. S Afr J Bot, 89, 143-149.
8. Avwioro O.G., Aloamaka P.C., Ojianya N.U., Oduola T. and Ekpo E.O., 2005. Extracts of *Pterocarpus osun* as a histological stain for collagen fibres. Afr J Biotech, 4 (5), 460-462.
9. Abubakar K., Halilu M.E., Jimoh A.O. and Hudu A.S., 2011. Preliminary phytochemical and antifungal screening of the aqeous extract of leaves of *Pterocarpus erinaceous.* Research Journal of Pharmacology, 5 (3), 40-42.
10. Ajiboye T.O., Salau A.K., Yakubu M.T., Oladiji A.T., Akanji M.A. and Okogun J.I., 2010. Acetaminophen perturbed redox homeostasis in Wistar rat liver: protective role of aqueous *Pterocarpus osun* leaf extract. Drug and Chemical Toxicology, 33(1), 77-87.
11. Ndukwe O.K. and Ikpeama A., 2013. Comparative evalutation of the phytochemical and proximate constituents of OHA (Pterocarpus soyansii) and Nturukpa (Pterocarpus santalinoides) leaves. International Journal of Academic Research in Progressive Education and Development 2(3), 22-31.
12. Keay R.W.J., 1989. Trees of Nigeria. A revised version of Nigerian trees (1960, 1964) by R.W.J. Keay, C.F.A. Onochie and D.P. Stanfield. Clarendon Press, Oxford, United Kingdom. pp. 476.
13. Soladoye M.O. and Lewis G.P., 2003. A checklist of Nigerian Legumes. Center for Environmental Research and Development (CENRAD), Ibadan. pp. 141.
14. Holmgren P.K., Keuken W. and Schofield E.K., 1990. Index Harbariorum Part I. The Herbaria of the World. 8th ed. Reg. Veg., New York.
15. Adedeji O. and Illoh H.C., 2004. Comparative foliar anatomy of ten species in the genus *Hibiscus* Linn. in Nigeria. New Botanist, 31, 147-180.
16. Chukwuma D.M. and Ayodele A.E., 2016. Taxonomic studies of the genus *Tephrosia* Pers. (Papilionaceae) in Nigeria. *Pl Sci Today*, 3(1), 9-18.
17. Chukwuma E.C., Soyewo L.T., Okanlawon T.F. and Ugbogu O.A., 2017. Foliar and petiole anatomy of *Pterygota* (Sterculioideae; Malvaceae) species and their distribution in Nigeria. Anales de Biología, 39, 103-109.
18. Metcalfe C. R. and Chalk L., 1979. Anatomy of the dicotyledons. Vol. 2, Oxford U.K.
19. Adedeji O. and Jewoola O.A., 2008. Importance of Leaf Epidermal Characters in the Asteraceae Family. Not Bot Hort Agrobot, 36 (2), 7-16.
20. Rejdali moh F.L.S., 1991. Leaf micromorphology and taxonomy of North African species of *Sideritis* L. (Lamiaceae). Bot J Linn. Soc, 107, 67-77.
21. Jayeola A.A., Thorpe J. R. and Adenegan J.A., 2001. Macromorphological and micromorphological studies of the West African *Rhizophora* L. Feddes Repertorium 112, 349-356.
22. Wilkinson H.P., 1979. The plant surface (mainly leaf). In Metcalfe, C.R. and Chalk, L. (eds.) Anatomy of the dicotyledons, Oxford: Clarendon Press. pp. 97-165.
23. Stace C.A., 1989. Plant taxonomy and biosystematics, London: Edward Arnold Publishers, pp. 264.

Molecular Interaction of GC-MS Products of *Trichosanthes cucumerina* and *Solanium lycopersicum* with Selected Enzymes in *Drosophila melanogaster*

Adeniran O.Y.[*]

Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria
olyni2001@yahoo.co.uk

Abstract

Medicinal plants have historically proven their values as a source of molecules with therapeutic potential. These natural products possess enormous structural and chemical diversity that may have adverse effect on the body tissues. This study therefore sought to examine the molecular interactions between the phytochemical compounds present in Snake tomato (*Trichosanthes cucumerina*), Hausa and Yoruba strains of tomatoes (*Solanum lycopersicon esculentum spp.*) and some selected enzymes in *Drosophila melanogaster*. Antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT)), glycerol-3-phosphate dehydrogenase and insulin-like receptor which represent a complex class of proteins (receptors) were modelled into 3 dimensional structures using the automated homology modeling by computational method. *S. lycopersicum and T. cucumerina* were subjected to GC/MS analyses that generate compounds (ligands) with varying glide score. The ligands prepared were docked into the receptors. Cytidine, phenylglyoxal and nonanoic acid were found to exhibit great pharmacological potential based on the *insilico* study as attested to by the binding affinity which predicts the strength of the molecular interaction of the ligand-protein complex and significantly satisfied the Lipinski's rule of five as to their drug-likeness with less side effect. Hence, they are presented to the scientific community for further investigational confirmation.

Keywords: Phytochemicals, tomato, GC-MS, molecular docking, *Drosophila melanogaster*

1 Introduction

Nutrition plays a role in the prevention of diabetes and chronic diseases related to oxidative stress such as cancer, cardiovascular, neurodegenerative, or inflammatory diseases [1]. Epidemiological studies have shown that regular consumption of plant-foods, which are natural sources of dietary antioxidants, is associated with reduced risk of such diseases [2]. From various researches, it has been proposed that the antioxidant potential of foods and diets may arise from additive and synergistic actions of the different anti-oxidant phytochemicals present in the food matrix [3]. Tomato (*Solanum lycopersicum*) is one of the most consumed vegetables in the world. In recent decades, its consumption has been associated with the prevention of several diseases mainly due to its content of antioxidants, including carotenes (lycopene as well as β-carotene), ascorbic acid, tocopherol, and phenolic compounds [4]. Snake tomato (*Trichosanthes cucumerina*) is an underutilized plant in Asia and Africa, which has gained popularity for its low cost of cultivation, rapid growth, health promotion, and as suitable replacement for *Lycopersicum esculentum* in many countries of Africa [5, 6]. The fruit fly, *Drosophila melanogaster* is chosen as an experimental model in biological research as it shares many genes that are orthologous to humans [7].

Molecular docking, an *in silico* technique enables the extraordinary structural diversity of natural products to be harnessed in an efficient manner. It aids in virtual screening for the identification of bioactive molecules from natural product databases. To the best of my understanding, there is no readily available information in the literatures on the possible interactions of the compounds in these fruits with biological proteins/enzymes. This study is designed to reveal such interactions and possible therapeutics agent(s) among these compounds.

2 Materials and Methods

2.1 Protein Preparation for Docking

The crystallized form of the protein was taken from the Protein Data Bank (PDB) repository, co-crystallized with homo-sapiens (http://www.rcsb.org/). The complete sequence of the protein was gotten from Pubmed fasta file, and modelled in 3-Dimensional structure using the automated homology modelling known as Swiss model [8]. The 3D-modelled structure was downloaded and extracted as separate file for docking. Water and ligand coordinate were deleted; the active site of the protein was identified using

Pymol Autodock vina [9] to visualize the interaction of the co-crystallized ligand when docked into the protein's active site to get the binding affinity.

2.2   Creating Ligands Library

The GC-MS products of *Trichosanthes cucumerina* roots and *Lycopersicum esculentum* fruits were downloaded [10]. A library of these compounds was prepared by Marvinsketch. The 2D structure of the ligands were retrieved from the NCBI PubChem database and saved in a folder. The folder was catenated in the 3D pdb format.

2.3   Molecular Docking of Ligands

Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands [11]. Active sites were generated for binding of ligands and receptor using BSP-SLIM on line (job i.d Q440, Q443). Using the Autodock vina [12] compiled under Ubuntu 14.04 LTS, the ligands were docked into the orthoseric site to get the respective binding affinity. The binding affinity predicts the strength of the molecular interaction of the ligand-protein complex.

2.4   Docking Receptor-Ligand Complex Structure

The binding results were validated using the ChEMBL Database. The fasta file gotten from Pubmed was blasted on www.ebi.ac.uk/chembl/ and the search result with chembl ID of 210 was downloaded in the txt format, using the EC50 chembl activity type. The smile format of the compounds was converted to sdf using Data warrior software and saved as 2D. These 2D structures were converted to pdb and pdbqt using babel and lig prep command lines, respectively to generate the 3D structures of the compounds.

3   Result and Discussion

Tomatoes are of great economic importance because of their use not only as food but as a major source of therapeutic active compounds used in the treatment of the various diseases of man and animal. Tomato possess a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, anti-hypertensive, anti-diabetic, anti-microbial [13] and anti-asthmatic properties [14]. Molecular docking of the phytochemical extracts of *Trichosanthes cucumerina* and *Solanium lycopersicum* into four different enzymes (Catalase, Insulin-like-receptor, Sod and G3PDH) showed the presence of some very active compounds. Table 1and 2 show relative docking scores of the phytochemicals compared to the normal substrate/ligands. Among these compounds, cytidine had lower docking score and hence higher affinity which has potential of modulating the target enzymes [15].

Table 1. Docked results of *Trichosanthes cucumerina* and *Solanium lycopersicum* phytochemicals to modelled structure of catalase

Compound Name	Docking score (Kcal/mol)
Standard	**-5.532**
Phytol acetate	-7.723
Henecosanoic acid methyl ester	-7.077
Cytidine	-6.707
Hepto acetate	-6.686
1-Naphthalene acetic acid	-6.454
Phenylglyoxal	-6.409
Lavandulyl acetate	-5.692
1,1-Dimethyl-3-dueteriopropyl thioether	-5.514
Heptacosanol	-5.054
Azulene	-4.793
3-Buten-1-01,2-phenyl	-4.735
2,3-Heptanodione	-4.446
2-phenyl ester	-4.429
Isopropylbenzene	-4.423
Hept-enyl-2-acetate	-4.339
Ethylcyclohexane	-4.034
2-Methyl-4,6-octadiyn-3-one	-3.789

Table 2. Docked results of *Trichosanthes cucumerina* and *Solanium lycopersicum* phytochemicals to modelled structure of Insulin-like receptor

Compound Name	Docking score (Kcal/mol)
Standard	**-4.3201**
Cytidine	-5.205
Phenylglyoxal	-2.958
3-Buten-1-01,2-phenyl	-2.656
1,1-Dimethyl-3-dueteriopropyl thioether	-2.287
2-phenyl ester	-2.233
Lavandulyl acetate	-1.977
Isopropylbenzene	-1.948
2,3-Heptanodione	-1.873
Ethylcyclohexane	-1.855
Neopentyl alcohol	-1.541
3-Hexen-2-one	-1.422
3-Buten-1-01,2-phenyl	-1.298
Isooctane	-1.232
Hept-enyl-2-acetate	-1.23
3-Methylheptane	-0.503
Azulene	-0.436
1-Naphthalene acetic acid	-0.166
2-Methyl-4,6-octadiyn-3-one	-0.044

Table 3. Pharmacological properties of *Trichosanthes cucumerina* and *Solanium lycopersicum* phytochemicals

Compound Names	mol MW	DHB	AcceptHB	QPlogPo/w	QPlogBB	QPlogKhsa	HOA	ROF
1-Naphthalene acetic acid	186.21	1	2	2.644	-0.339	-0.245	3	0
1,1-Dimethyl-3-dueteriopropyl thioether	180.307	0	0.5	3.535	0.385	0.366	3	0
2-Methyl-4,6-octadiyn-3-one	134.177	0	2	2.155	0.024	-0.199	3	0
2-phenyl ester	164.204	0	2	2.475	-0.237	-0.088	3	0
2,3-Heptanodione	128.171	0	4	0.494	-0.415	-0.949	3	0
3-Buten-1-01,2-phenyl	148.204	1	1.7	2.614	-0.064	-0.223	3	0
3-Hexen-2-one	98.144	0	2	1.285	0.013	-0.572	3	0
3-Methylheptane	114.23	0	0	4.727	0.964	0.205	3	0
9-Octadeconioc acid	282.465	1	2	5.847	-0.9	0.606	3	1
Azulene	128.173	0	0	3.315	0.426	0.14	3	0
Cytidine	243.219	5	10.8	-2.063	-1.999	-0.895	2	0
Ethylcyclohexane	112.214	0	0	4.074	0.891	0.165	3	0
Henecosanoic acid methyl ester	340.588	0	2	7.759	-1.368	1.664	1	1
Hept-enyl-2-acetate	156.224	0	2	2.601	-0.323	-0.036	3	0
Heptacosanol	396.739	1	1.7	9.47	-1.79	2.117	1	1
Hexyl propionate	158.24	0	2	2.746	-0.354	-0.025	3	0
Isooctane	114.23	0	0	4.181	0.908	0.208	3	0
Isopropylbenzene	120.194	0	0	3.308	0.526	0.168	3	0
Lavandulyl acetate	196.289	0	2	3.348	-0.251	0.273	3	0
Methyl 15-methylhexadecanoate	284.481	0	2	6.103	-0.992	1.14	1	1
Methyl tridecanoate	228.374	0	2	4.616	-0.703	0.592	3	0
Neopentyl alcohol	104.149	2	3.4	-0.117	-0.328	-0.802	3	0
Neophytadine	278.52	0	0	9.453	1.486	1.688	1	1
Nonanoic acid	158.24	1	2	2.642	-0.815	-0.324	3	0
Octadenioc acid methyl ester	298.508	0	2	6.602	-1.152	1.278	1	1
Pentadecanal	226.401	0	2	4.678	-1.018	0.589	3	0
Phenylglyoxal	134.134	0	4	0.165	-0.482	-1.05	3	0
Phytol acetate	338.573	0	2	7.299	-1.076	1.681	1	1
Tridecanal	198.348	0	2	3.841	-0.868	0.309	3	0

M.W: Molecular Weight of compounds; DonorHB: Hydrogen Bond donor; AcceptHB: Hydrogen Bond acceptor; QPlogPo/w: octanol/water partition coefficient; HOA: Human Oral Absorption. 1, 2, or 3 for low, medium, or high; QPlogBB: Prediction of blood-brain barrier penetration (Normal range between -3.0 to 1.2); QPlogKsha: Prediction of binding to human serum albumin (Normal range between -1.5 to 1.5); ROF: Rule of Five Violation.
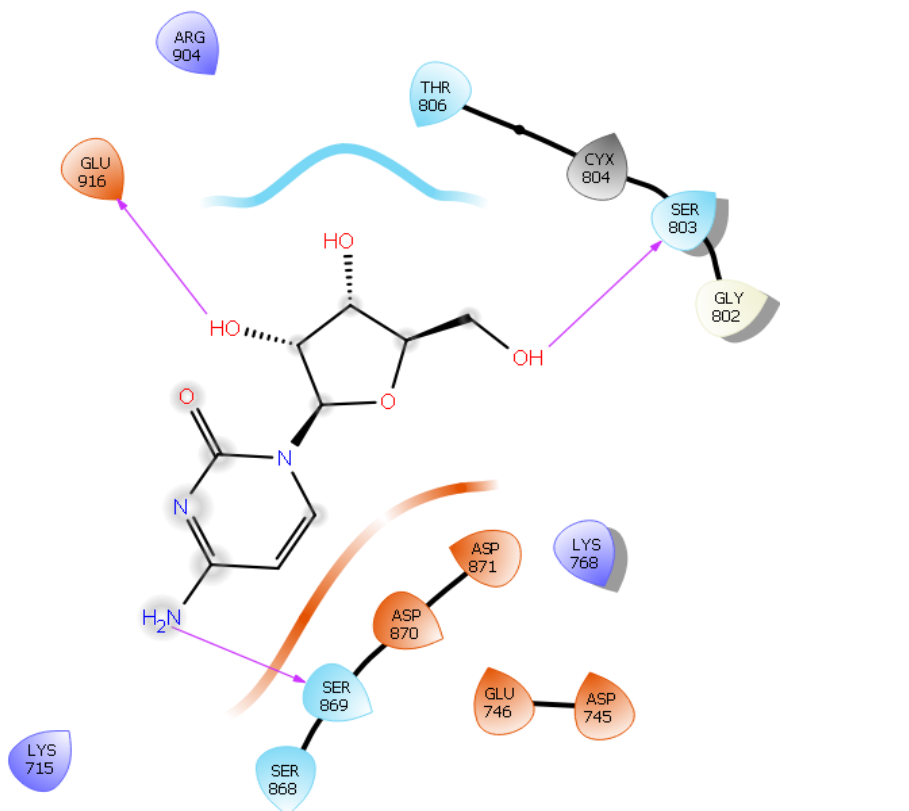
Fig. 1. Binding pose of cytidine with modelled structure of Insulin-like receptor

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans [16]. Based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules, the rule describes molecular properties important for drugs pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion. Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria: (a) No more than 5 hydrogen bond donors (the total number of nitrogen – hydrogen and oxygen–hydrogen bonds); (b) No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms); (c) A molecular mass less than 500 daltons; (d) An octanol-water partition coefficient log P not greater than 5.

Table 3 reveals the pharmacological properties of these compounds. Based on this set of rules and the molecular interaction with the active site of the protein (Fig. 1), the following sets of compounds listed in Table 4 are the best set of compounds to evaluate for when determining for drug-likeness or when determining if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans. This conforms to similar research by Metibomu *et al*. [17].

Table 4 Compounds with desirable pharmacological properties

Compound Name	mol MW	DonorHB	AcceptHB	QPlogPo/w	QPlogBB	QPlogKhsa	HOA
Cytidine	243.219	5	10.8	-2.063	-1.999	-0.895	2
Nonanoic Acid	158.240	1	2	2.642	-0.815	-0324	3
Phenylglyoxal	134.134	0	0	0.165	-0.482	-1.05	3

4   Conclusion

These compounds can be suggested as a potent drug with less or no side effect. It is therefore proposed that *T. cucumerina* and *S. lycopersicum* possess valuable nutraceutical properties that man must take advantage of.

References

1. Mursu J., Virtanen J.K., Tuomainen T.P., Nurmi T., Voutilainen S., 2014. Intake of fruit, berries, and vegetables and risk of type 2 diabetes in Finnish men: The kuopio ischaemic heart disease risk factor study. Am J Clin Nutr, 9, 328–333.
2. Agbor G.A., Moumbegna P., Oluwasola E.O., Nwosu L.U., Njoku R.C., Kanu S., Emekabasi E.I., Akin F., Obasi A.P. and Abudei F.A., 2011. Antioxidant capacity of some plant foods and beverages consumed in the Eastern region of Nigeria. Afr J Tradit Complement Altern Med, 8(4), 362–369.
3. Wang L., Liu S., Manson J.E., Gaziano J.M., Buring J.E., Sesso H.D., 2012. The consumption of lycopene and tomato-based food products is not associated with the risk of type 2 diabetes in women. Journal of Nutrition, 136, 620-625.
4. Panigrahy S.K., Bhatt R., and Kumar A., 2016. Reactive oxygen species: Sources, consequences and targeted therapy in type 2 diabetes. Journal of Drug Targeting, 25, 1-9.
5. Adebooye O.C., Oloyede F.M., and Onagoruwa O.O., 2016. Fruit characteristics and nutrient composition of landrace morphotypes of snake tomato. J Veg Sci, 11, 5-16.
6. Velioglu Y.S., Mazza G. and Oomah B. D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agri Food Chem 46, 4113-4117.
7. Sandhya S., Vinod K., Chandra S., Aradhana R. and Vamshi S., 2010. An updated review on Trichosanthes cucumerina L. International Journal of Pharmaceutical Sciences Review and Research, 1, 56-58.
8. Pandey U.B., Nichols C.D., 2011. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev, 63(2), 411-36.
9. Bordoli L., Kiefer F., Arnold K., Benkert P., Battey J., Schede T., 2009. Protein structure homology using SWISS-MODEL workspace. National Protocol 4(1), 1-13.
10. Ambethkar A. and Ananthalakshmi S., 2014. GC-MS analysis on the methanolic extract of Trichosanthes anguina L. root. Int J Pharm Life Sci, 5(3), 3389-3393.
11. Bikadi Z., Hazai E., 2009. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminf, 1, 1-15.
12. Trott O., Olson J.A., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry, 31(2), 455-461.
13. Ammal M.R. and Bai V.S. 2013. GC-MS determination of bioactive constituents of Heliotropium indicum leaf. *Journal of Medicinal Plants Studies*, 1(6), 30-33.
14. Ushasri S., Ranjith K.J., Sudha Bhargavi C.H., Spoorthi L. and Pushpa Sai A., 2013. Antiasthmatic herbal Drugs-A Compilation. International Journal of Pharmaceutical and Chemical Sciences, 2(1), 383-392.
15. Adeniran O.Y. and Onigbinde A.O., 2018. Molecular docking study of secondary products of oxidized soy bean oil on selected tissue enzymes. NISEB Journal, 18(2), 105-117
16. Lipinski C.A., Lombardo F., Dominy B.W and Feeney P. J. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanceed Drug Delivery Reviews, 46, 3-26.
17. Metibemu D.S. Adeniran O.Y., Akinsiku E.O., Akinloye O.A., Ugbaja R.N., Akamo A.J., Metibemu A.O., Olusola A.O. and Omotuyi I.O., 2019. Homo Sapien Bcr-Abl-Interacting Scaffolds from Bryophyllum pinnatum and Cantharanthus roseus: Computational Studies NISEB Journal, 19, 47-54.

Flownet Construction and its Hydrogeological Implications: A Case Study of Parts of Ilorin Crystalline Rocks, Southwestern Nigeria

Olatunji J.A.[1,3,*], Omonona O.V.[2], Odediran O.A.[1], Akinrinmade A.O.[3], Olasehinde D.A.[4]

[1]Department of Minerals and Petroleum Resources Engineering Technology, Kwara State Polytechnic, Ilorin
[2]Department of Physics/Geology/Geophysics, Alex Ekwueme Federal University, Ndufu Alike Ebonyi
[3]Department of Geology and Mineral Science, Kwara State University Malete
[4]Department of Agricultural and Biosystems Engineering, Landmark University, Omu-Aran, Kwara State, Nigeria
olatwise@yahoo.com

Abstract

Water is considered an inevitable resource on which human life depends. Groundwater plays important roles in sustaining and supporting human continuous existence. This study examined hydrogeological implications of flownet construction and analysis on groundwater exploration. It is to ensure the delineation of appropriate locations that can be considered for further and more extensive groundwater exploratory activities. This research has become necessary in view of the dwindling surface water sources and the wide spread occurrences of borehole failure, both of which can be attributed to phenomena of climate change and the ever-increasing exorbitant cost of dam construction and ancillary services. The area of study is underlain by crystalline rocks which consist of migmatite - gneiss, banded gneiss, granite gneiss, augen gneiss, quartzites and granites. Static water levels, total depths and coordinates of hand dug wells were measured using deep meter, GPS, and tape, respectively. Flownet is constructed by plotting flow lines against equipotential lines orthogonally. Analysis and interpretation of flownet shows that the western part of the study area which covers areas such as Olorunsogo, Agbooba and Gbagba have very wide divergent equipotential lines which imply easy groundwater movement. The southeastern part which include places such as Oko-Erin, Baba-Ode, Gaa-Akanbi and northeastern parts such as Fate, Tanke reflect convergent equipotential lines, an indication of difficulty (less permeable zones) in the water movement within the media. The greater the distance between contours, the slower the flow. The primary technique of constructing the groundwater flownet is considered basic since it is cheap and fast as a reconnaissance tool. Other exploratory activities are considered after flownet construction. Generally, from hydrogeological point of view, the study area has been divided into divergent and convergent areas using inferences from flownet construction and analysis.

Keywords: Flownet, equipotential lines, flowlines, convergent, divergent

1   Introduction

The evaluation of groundwater potential in the crystalline basement complex has been problematic due to the complex nature of rocks and tectonic activities that have affected them [1, 2]. This study has become necessary in view of the dwindling surface water sources in the face of climate change and economic recession militating against the construction and maintenance of surface water sources through dams and weirs. The use of flownet analysis for aquifer identification was first proposed by Stallman [3]. Equipotentials are the loci of points of equal potential (or head), and flow lines (or stream lines) correspond to directions of groundwater flow [4-6]. Constructing flownet map is a well-accepted practice in investigation of groundwater flow directions [7, 8]. Water table surface is a representation of the surface of saturated zone, below which all the geological formation voids are fully filled with water [9]. The position of the water table is as a result of natural processes controlling the rate at which water enters and leaves the saturated zone. If the rate at which water enters the saturated zone (recharge) exceeds the rate of water leaving (discharge) the aquifer, the water table rises and vice versa. The water table surface is not static, nor flat, but reflects the climatic, vegetative and geomorphic conditions. The groundwater water table could be subdued replica of the land surface [10].

Rice et al. [11] employed graphical inverse method as a hydrogeological tool that can be used for site characterization and flow system conceptualization, understanding and defining the subsurface geologic structures and flow system controls.

2 Materials and Methods

2.1 Study Location

The study location is parts of Ilorin metropolis (Fig. 1). The area is bounded by longitudes $4^028'0''E$ and $4^038'0''E$ and latitudes $8^027'0''N$ and $8^034'15''N$. The area covered is about 242.79 km². Weather condition in the region is of two broad types (i.e. rain season and dry season). The rain season commences around March and ends in October with annual average rainfall of 1,200 mm, while dry season begins in November and ends in March. The humidity ranges between 60 % and 89% and mean annual temperature is between 27^0C and $30°C$. The area is well drained by various streams and their tributaries. The tributaries show dendritic drainage pattern. The main rivers are Asa and Agba Rivers, while minor rivers include Oyun and Aluko Rivers. The terrain is undulating and dissected by rivers and streams. The highest altitude is about 1200 m above sea level corresponding to the top of Sobi Hill (migmatite), while along major streams the altitude is about 250 m above sea level. The vegetation cover is basically Guinea savannah with ruminant tropical forest [12].
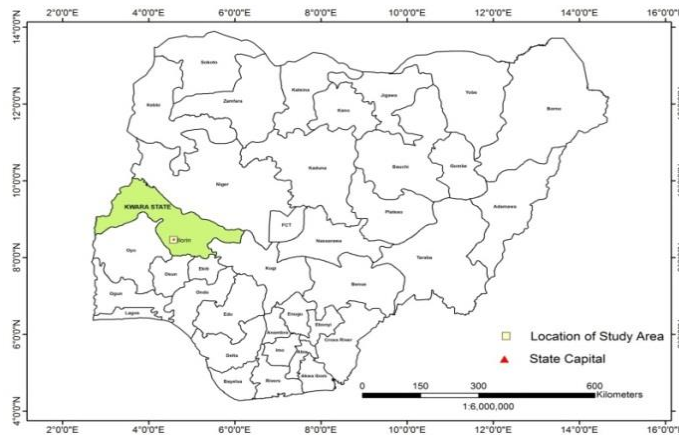

Fig. 1. Map of Nigeria showing Ilorin, Kwara State Nigeria

2.2 Geology and Hydrogeology of Ilorin

The study area falls in the basement complex of Southwestern part of Nigeria, which is of Precambrian to lower Paleozoic in age [13, 14]. This Precambrian crystalline basement complex consists of gneisses and migmatities; metasediments i.e schists, quartzites and metavolcanics; Pan-African (older) granite and late-stage minor pegmatitic and aplitic intrusives [15]. According to Olasheinde [16], Ilorin is situated on the undifferentiated Precambrian Basement Complex rocks of granitic and metamorphic origin. These rocks represent the deeper, fractured aquifer which is partly overlain by a shallow, porous aquifer within the lateritic soil cover [17]. The rock units form part of the regional Southwestern highlands of Nigeria running NW-SE parallel to the River Niger [18, 17]. The subsurface comprises the weathered, slightly weathered and fresh (fractured or unfractured) crystalline basement rocks. The oldest rocks in the area comprise gneiss complex whose principal member is biotite-hornblende gneiss with intercalated amphibolites. This underlies over half of the city. Other rock types are the older granite mainly porphiritic granite, gneiss and granite-gneiss and quartz schist. Ilorin is underlain by crystalline rocks mainly gneisses and migmatite with pegmatite veins. Rock types within the study areas include; migmatite- gneiss, banded gneiss, granite gneiss, augen gneiss, and quatzites granites as shown in Fig. 2 [19, 20].

2.3 Field Work

The coordinates and elevations of hand dug wells were measured with the aid of Garmin Global Positioning System (GPS). The static water levels of the wells were also measured, and this helps to calculate the corrected water level in the subsurface. A total of 900 wells were covered to give information on the groundwater flow dynamics of the study area. Well positions and their respective elevation relative to the main sea level were determined using (GPS). These helped in determining the hydraulic water head in wells and thus groundwater head elevation contour which gave information on the groundwater flow direction.
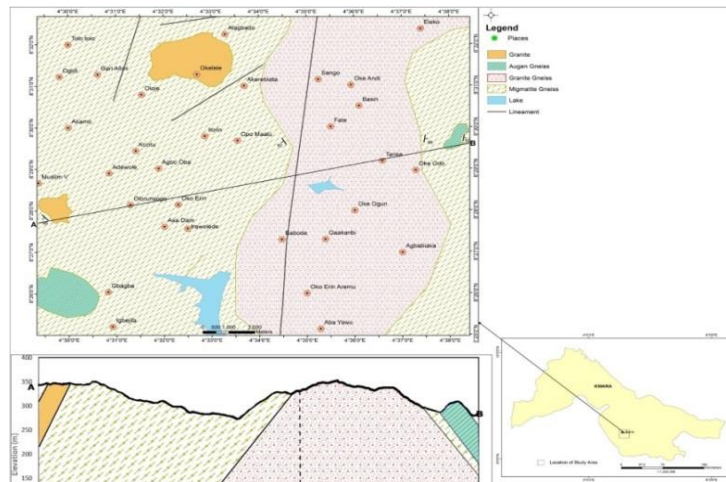
Fig. 2. Geological map of Ilorin modified after NGSA 2007

3 Results and Discussion

3.1 Analysis and Evaluation of the Flownet

Well data collected from Ilorin metropolis were used to construct flownet for the study area. Fig. 3 shows the groundwater flow direction of parts of Ilorin the study area. Under certain conditions such as homogeneous, isotropic and orthogonal system, the set of equipotential lines and flow lines so exposed constitutes a flownet. From Fig. 3, it was observed that the western part of the study area which covers area such as Olorunsogo, Agbooba and Gbagba has very high divergent equipotential lines which imply easy groundwater movement while the southeastern and northeastern part reflect convergent equipotential lines, an indication of difficulty in the water movement within the aquifer. This in turns implies less permeable zone. The greater the distance between contours, the slower the flow. This supports the assertion by Sen [5]. Similarly, the southern part of the study area also shows closed equipotential lines an indication of recharge (domes) or recharge zones (depression). This area is considered to be a depression otherwise a catchment area where water collects and further exploratory activities can be concentrated [21-24].
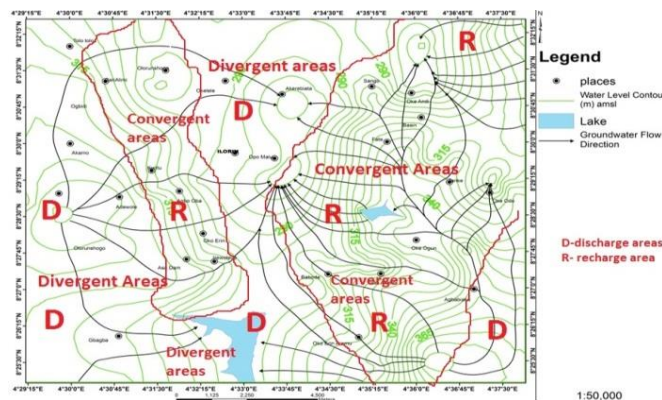

Fig. 3. Flownet contour map analysis and evaluation

Fig. 3 shows the flownet constructed for study area and its hydrogeological implications. This helps in identification of divergent and convergent areas within a location. Divergent areas have widely spaced equipotential lines while convergent areas have closely spaced equipotential lines. The western part and the north southern parts of the study area which covers areas such as Olorunsogo, Gbagba, Ogidi, Baba-ode and Opo-Malu have very high divergent equipotential lines which imply easy groundwater movement. The greater the distance between contours, the slower the flow. Such areas are considered to be highly permeable, have better accumulation and discharge rate and can be explored for further groundwater exploratory activities. Similarly, NE-SE parts of the study area reflect a closely spaced equipotential lines otherwise refers to as convergent area, an indication of difficulty (less permeable zones) in the water movement within the aquifer [5, 23]. Locations such as Tanke, Fate, Basin, Sango, part of Gaa-Akanbi, Kuntu, Eko-Erin areas are considered to be within the convergent areas (Table 1).

Table 1. Flownet classification within the study area

Divergent areas	Convergent Areas
Ogidi	Fate
Akerebiata	Agbabiaka
Baba –ode	Oke-odo
Adewole	Basin
Gbagba	Gaa-akanbi
Okelele	Tanke
Opo- malu	Oke andi
Oko-erin	Sango
Olorunsogo	

4   Conclusion

The study examines the use of flownet construction analysis in aquifer identification and its hydrogeological implications in groundwater exploration aimed at delineate an appropriate location that can be considered for further groundwater exploratory activities. Static water levels, total depth, coordinates of hand dug well were measured using deep meter, GPS and tape, respectively. From the data collected, analysis and interpretation of flownet shows that the western part of the study area which covers areas such as Olorunsogo, Agbooba and Gbagba has very wide divergent equipotential lines which imply easy groundwater movement. The southeastern part which include places such as Oko-Erin, Baba-Ode, Gaa-Akanbi and northeastern parts such as Fate, Tanke reflect convergent equipotential lines, an indication of difficulty (less permeable zones) in the water movement within the media. The greater the distance between contours, the slower the flow. The primary technique of constructing the groundwater flownet is considered basic since it is cheap and fast as a reconnaissance tool. Other exploratory activities are considered after flownet construction. Generally, from hydrogeological point of view, the study area has been divided into divergent and convergent areas using inferences from flownet construction and analysis.

References

1. Olasehinde P.I., 2010. An integrated geological and geophysical exploration techniques for groundwater in the basement complex of West Central part of Nigeria. Water Resources Journal of Nigerian Association Hydrogeologists, 10(1), 46-49.
2. Olasehinde P.I. and Bayewu O.O., 2011. Evaluation of electrical resistivity anisotropy in geological mapping: A case study of Odo Ara, West Central Nigeria. African Journal of Environmental Science and Technology 5(7), 553-566.
3. Stallman, R.W. 1956 Numerical analysis of regional water levels to define aquifer hydrology. Transaction of the American Geophysical Union, 37(4), 451-460.
4. Ferris J.G, Knowles D.B., Brown R.H. and Stallman R.W., 1962. Groundwater hydraulics- theory of aquifer test. US Geological Survey Water Supply Paper 1536E. pp 139-144.
5. Sen Z., 2015. Practical and applied hydrogeology. ITU Hydraulics Lab. Maslak, Istanbul, Turkey. First edition, pp. 47-100.
6. Auduson E.A., 2018. Concise applied geophysics: a practical approach. Delizon Publishers Limited. First Edition, pp. 241-246.
7. Braga A.C., Filho W.M. and Dourado J.C., 2006. Resistivity (DC) method applied to aquifer protection studies. Revista Brasileira deGeofˊısica, 24(4),573-581.
8. Driscoll, 1986. Groundwater and wells. Johnson Screens, St Paul FD (ed).
9. Heath R.C., 1988. Hydrogeologic settings of regions. In: Back W, Rosenshein J.S, Seaber P.R (eds) Hydrogeology. Geological Society of America, Boulder.
10. Domenico P.A. and Schwartz F.W., 1990. Physical and chemical hydrogeology. Wiley, New York.
11. Rice W.A. and Gore lick S.M., 1985. Geologic inference from "flow net" transmissivity determination: Three case studies. Water Resources Bulletin of the American Water Resources Association, 21(6), 919-930.
12. Abolarin A.T. and Ibrahim S., 2015. Evaluation of groundwater occurrences in the Precambrian Basement Complex of Ilorin metropolis, Southwestern Nigeria. RMZ – M &G, 62, 117-132.
13. Rahaman M.A., 1976. Review of the basement geology of South-Western Nigeria. Geology of Nigeria (C.A. Kogbe, ed): Elizabethan Publishing Co. Lagos.
14. Oluyide P.O., 1979. Report on the Igbeti Marble, Oyo State of Nigeria. Report GSN, 1569, (Unpublished).
15. Oluyide P.O, Nwajide C.S., and Oni A.O., 1998. The Geology of the Ilorin Area. Bulletin No. 42 of Geological Survey of Nigeria, Published by Federal Government of Nigeria.
16. Olasehinde P.I., Virbka P. and Esan A., 1998. Preliminary results of hydrogeochemical investigations in Ilorin area, Southwestern Nigeria- quality of hydrochemical analysis. Water Resources Journal of Nigerian Association of Hydrogeologist, 11, 55-61.
17. Annor A.E. and Olasehinde P.I. 1996. Vegetational niche as a remote sensor for subsurface aquifer: A geological-geophysical study in Jere area, Central Nigeria. Water Resources Journal of Nigerian Association of Hydrogeologist, 7, 26-30.
18. Offodile M.E., 1987. An approach to groundwater study and development in Nigeria. Published by Mecon Services Ltd, Jos, Nigeria.

Proceedings of the 2019 International Science Conference

Innovation and Advancement in Nanoscience and Nanotechnology, Federal University Oye-Ekiti (www.fuoye.edu.ng), 2019.

19. Olasehinde P.I., and Taiwo K.A., 2000. A correlation of schlumberger array geoelectric log with borehole lithologic log in South Western part of Nigeria Basement Complex Terrain. Water Resources Journal of Nigerian Association Hydrogeologists, 11, 55-61.

20. Nigerian Mining and Geosciences Society, 2018. Unlocking the investment opportunities in Kwara State through solid minerals development. Workshop on Solid Minerals of Kwara State, Organized by Kwara State NMGS Chapter. Book of programme and proceedings, 2-20.

21. Afework D., 2011. Groundwater potential evaluation and flow dynamics of Hormat-Golina River Catchment, Kobo Valley, Northern Ethiopia, Addis Ababa University.

22. Fetter C.W., 1994. Applied hydrogeology, 3rd ed. Macmillan College Publishing, Inc., New York, 616.

23. Fetter C.W., 2001. Applied hydrology. Prentice-Hall, inc. Upper Saddle River, New Jersey.

24. Genet M.M., 2017. Groundwater flow direction, recharge and discharge zones identification in lower Gidabo Catchment, Rift Valley Basin, Ethiopia. Journal of Environment and Earth Science, 7(2), 32-39.

Trees and Shrubs of Oba Hills Forest Reserve, Osun State, Nigeria

Emmanuel C. Chukwuma[1], Opeyemi A. Agbo-Adediran[2], Deborah M. Chukwuma[3,*]

[1]Forest Herbarium Ibadan (FHI), Forestry Research Institute of Nigeria, Jericho Hill, Ibadan, Oyo State, Nigeria
[2]Department of Forestry Technology, Federal College of Forestry, Jericho Hill, Ibadan, Oyo State, Nigeria
[3]Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Ekiti State, Nigeria
deborah.chukwuma@fuoye.edu.ng

Abstract

Species composition and diversity were assessed in Oba hills forest reserve located in Osun State, Nigeria, in an attempt to update existing records of the country's flora diversity, following standard procedures. A preliminary survey of the ecosystem recorded 294 individual stands of 57 angiosperm species in 27 families, comprising trees and shrubs, with an average height and DBH of 41.2 m and 9.3 cm, respectively. The trees dominated the list with 51 species while the shrubs had 6 species. The families Papilionaceae, Mimosaceae and Combretaceae were the most represented, with 5 species (8.7%) each, while Euphorbiaceae was next with 4 species (7.0%). Furthermore, Verbenaceae, Hymenocardiaceae, Combretaceae and Caesalpiniaceae were the dominant families of the total enumeration, and comprised 56 (19%), 28 (9.5%), 26 (8.8%) and 25 individuals (8.5%), respectively. Nevertheless, the avalanche of Verbenaceae species predominantly represented by *Gmelina arborea* and *Tectona grandis* is also an indication that the ecosystem has over the years been encroached with human activities, which has in turn reduced the size and species richness of the forest. It thus suggests that the study area needs serious attention if its genetic resources are to be restored and protected in a bid to avoid total loss of valuable species therein.

Keywords: Nigeria, species diversity, flora, conservation

1   Introduction

The Nigerian rain forests have experienced irreparable destruction resulting from urbanization and general farming activities. Today, many of the previously known forested areas have become derived savanna areas largely due to grazing and logging activities and this scenario has been witnessed in Southwestern Nigeria. It is noteworthy that floristic studies are essential for the provision of information in species diversity and richness and even ecosystem changes which occurs overtime. Floristic studies can also be useful for the management of our forest areas [1, 2]. As noted by Olapade and Bakare [3], the existence of plant species in any habitat is important to man and other components of the ecosystem as all plants are valuable for one purpose or the other. It is almost impossible to live without plants, especially as we continue to rely on them for food, improved rural livelihood, household constructions, employment generation and income supplements. Isichie [4] noted that if Nigeria plant resources are properly harnessed, they would ensure food security. Nevertheless, a large number of these species are faced with threat of extinction as pressure on them increases. Such threat also means a threat to the survival of man, especially the rural poor.

The present study thus aimed at identifying the trees and shrubs of Oba hills forest reserve, a threatened vegetation in Osun State, Nigeria in an attempt to document the remaining angiosperm records that could serve as baseline information for future regeneration activities and management of the area.

2   Materials and Methods

2.1   Study Site

Oba hills forest reserve is located in Osun State, Southwestern Nigeria and lies on Latitude $7^{\circ}47^{'}$N and Longitude $4^{\circ}5'$E (Fig. 1). It covers a 52 km^2 hilly terrain with deep gorges and about 12% of the site had been converted to teak plantation [5]. The site is currently composed of savanna and forest species.

2.2 Species Enumeration

The survey involved repeated visits to the study area for the collection of existing trees and shrubs. The species were identified on the field using taxonomic keys provided in Hutchinson *et al.* [6-8] and Keay [9], while others were taken to the Forest Herbarium Ibadan [10] for proper identification. The families and habits are also reported.
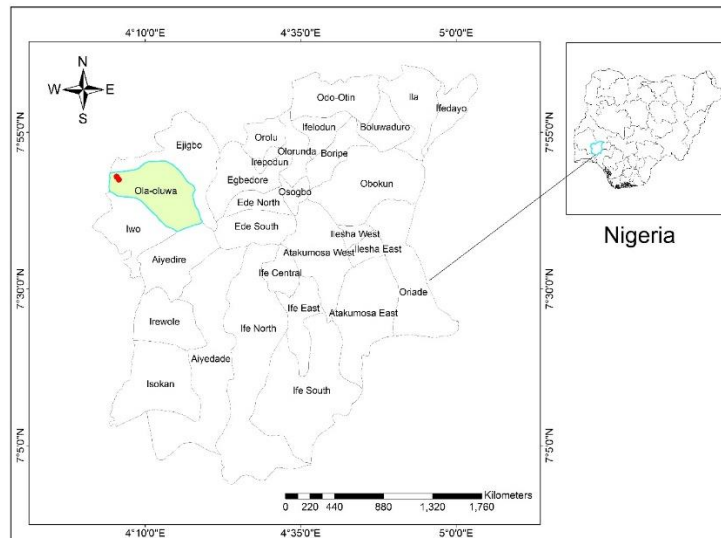

Fig. 1. Location map of study area

3 Results and Discussion

A total of 56 different species comprising trees and shrubs, belonging to 27 families were identified (Table 1). In all, 254 individual stands of these species were recorded, with Verbenaceae, Hymenocardiaceae, Combretaceae and Caesalpiniaceae being the dominant families, and comprised 56 (19%), 28 (9.5%), 26 (8.8%) and 25 individuals (8.5%), respectively.

Table 1. Identified trees and shrubs of Oba hills reserve

Species	Family	Habit
Afzelia africana	Caesalpiniaceae	Tree
Albizia adianthifolia	Mimosaceae	Tree
Albizia ferruginea	Mimosaceae	Tree
Albizia zygia	Mimosaceae	Tree
Alchornea cordifolia	Euphorbiaceae	Shrub
Annona senegalensis	Annonaceae	Shrub
Anogeissus leiocarpus	Combretaceae	Tree
Anthocleista djalonensis	Loganiaceae	Tree
Anthocleista vogelii	Loganiaceae	Tree
Blighia sapida	Sapindaceae	Tree
Bombax buonopozense	Bombacaceae	Tree
Bridelia ferruginea	Euphorbiaceae	Tree
Ceiba pentandra	Bombacaceae	Tree
Cleistopholis patens	Annonaceae	Tree
Cochlospermum planchoni	Cochlopsermaceae	Tree
Cola gigantea	Sterculiaceae	Tree
Combretum platypterum	Combretaceae	Shrub
Crossopteryx febrifuga	Rubiaceae	Tree
Daniellia oliveri	Caesalpiniaceae	Tree
Elaeis guineensis	Palmae	Tree
Ficus exasperata	Moraceae	Tree
Ficus sur	Moraceae	Tree
Gardenia aqualla	Rubiaceae	Shrub
Gmelina arborea	Verbenaceae	Tree
Harungana madagascariensis	Clusiaceae	Shrub

81

Holarrhena floribunda	Apocynaceae	Tree
Hymenocardia acida	Hymenocardiaceae	Shrub
Kigelia africana	Bignoniaceae	Tree
Lannea sp.	Anacardiaceae	Tree
Lonchocarpus sericeus	Papilionaceae	Tree
Lophira lanceolata	Ochnaceae	Tree
Malacantha alnifolia	Sapotaceae	Tree
Margaritaria discoidea	Euphorbiaceae	Tree
Milicia excelsa	Moraceae	Tree
Millettia thonningii	Papilionaceae	Tree
Nauclea latifolia	Rubiaceae	Shrub
Newbouldia laevis	Bignoniaceae	Tree
Parinari polyandra	Chrysobalanaceae	Tree
Parkia biglobosa	Mimosaceae	Tree
Pericopsis laxiflora	Papilionaceae	Tree
Piliostigma thonningii	Caesalpiniaceae	Tree
Prosopis africana	Mimosaceae	Tree
Pterocarpus erinaceus	Papilionaceae	Tree
Securidaca longepedunculata	Polygalaceae	Shrub
Securinega virosa	Euphorbiaceae	Shrub
Spondias mombin	Anacardiaceae	Tree
Strychnos spinosa	Loganiaceae	Shrub
Syzygium guineense	Myrtaceae	Tree
Tectona grandis	Verbenaceae	Tree
Terminalia avicennioides	Combretaceae	Tree
Terminalia macroptera	Combretaceae	Tree
Terminalia sp.	Combretaceae	Tree
Vitellaria paradoxa	Sapotaceae	Tree
Vitex doniana	Verbenaceae	Tree
Ximenia americana	Olacaceae	Shrub
Zanthozylum zanthoxyloides	Rutaceae	Tree

Table 2. Species distribution across plant families

Family	No. of species	% occurrence
Anacardiaceae	2	3.6
Annonaceae	2	3.6
Apocynaceae	1	1.8
Bignoniaceae	2	3.6
Bombacaceae	2	3.6
*Caesalpiniaceae	3	5.4
Chrysobalanaceae	1	1.8
Clusiaceae	1	1.8
Cochlopsermaceae	1	1.8
Combretaceae	5	8.9
Euphorbiaceae	4	7.1
Hymenocardiaceae	1	1.8
Loganiaceae	3	5.4
*Mimosaceae	5	8.9
Moraceae	3	5.4
Myrtaceae	1	1.8
Ochnaceae	1	1.8
Olacaceae	1	1.8
Palmae	1	1.8
*Papilionaceae	4	7.1
Polygalaceae	1	1.8
Rubiaceae	3	5.4
Rutaceae	1	1.8
Sapindaceae	1	1.8
Sapotaceae	2	3.6
Sterculiaceae	1	1.8
Verbenaceae	3	5.4
Total	56	100.0

82

The legumes (Caesalpiniaceae, Mimosaceae and Papilionaceae) collectively contributed the highest number of species diversity with a total of 12 species (21%) of the total enumeration. Others include Combretaceae and Euphorbiaceae with 5 and 4 species each, Loganiaceae, Moraceae, Rubiaceae and Verbenaceae with 3 species each. Five families had 2 species represented while the remaining thirteen (13) families were represented with only 1 species each, thus, indicating their importance in ecosystem balancing (Table 2). It was also evident that the trees were more abundant than the shrubs as shown in Fig. 2.
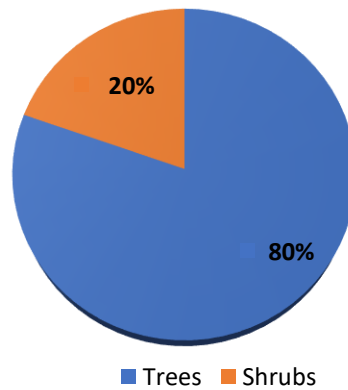

Fig. 2. Species composition across plant habits

Further observations during our study showed that the site area has been largely cultivated with *Gmelina arborea* and *Tectona grandis*, and this corroborates with previous reports by Greengrass [11] who noted that almost all of the reserve had been converted to plantations and farms, with only two gullies remaining forest-covered. The abundance of trees also reflects the original composition of the forest, which had over the years become a derived savanna vegetation resulting from human activities noted above such as farming, logging, animal grazing, etc. The continued exploitation of the species especially for fuelwood, is brought about by an increase in the prices of alternative sources of energy, and this is in line with the report of Food and Agriculture Organization (FAO) [12] which pointed out clearly that wood fuel plays significant roles in the day to day lives of rural people. It was also noted that *Daniellia oliveri, Lophira lanceolata, Pericopsis laxiflora* and *Terminalia avicennioides* are some of the preferred species for fuelwood by the community dwellers. This is similar to the observations of Jimoh et al. [13]. In general, it is considered that immediate economic benefits exceed those for conservation, as clearly put by Ferraro and Kiss [14]. While the continued dependence on plant for survival is inevitable, we advocate for the establishment of sustainable management strategies to salvage our rich but endangered ecosystems. Our position therefore, is not different from that of Soladoye *et al.* [15], who earlier suggested the provision of basic socio-economic amenities such as electricity, schools, good roads, portable water, and hospitals as incentives that could ensure successful conservation of an area.

4   Conclusion

The present study has revealed 56 species comprising trees and shrubs belonging to 27 angiosperm families. It has also shown the importance of biodiversity assessment and monitoring, and has no doubt added to the existing records of similar study in Southwestern Nigeria. With the continued degradation of the study area, we suggest quick intervention by the authorities and implementation of forest/conservation laws to avoid an eventual disappearance of species.

References

1. Addo-Fordjour P., Obeng S., Anning A.K. and Addo M.G., 2009. Floristic composition, structure and natural regeneration in a semi-deciduous forest following anthropogenic disturbances and plant invasion. Int J Biodiv Conserv, 1(2), 021-037.
2. Pappoe A.N.M., Armah F.A., Quaye E.C., Kwakyeand P.K. and Buxton G.N.T., 2010. Composition and stand structure of a tropical moist semi-deciduous forest in Ghana. Int Res J Plant Sci, 1(4), 095-106.
3. Olapade O.E. and Bakare O.A., 1992. Medicinal plants in Ibadan under threat of genetic erosion; Our Forest, Environment and Heritage; Challenges for Our People, Akinsanmi FA (ed). Proceedings of 22nd Annual Conference of Forestry Association of Nigeria Held in Kano, Kano State, Nigeria, pp. 55-58.
4. Isichei A.O., 2005. The role of plant resources in Nigeria's economic recovery agenda. Nigeria Journal of Botany, 18, 1-22.

5. Kormos R., 2003. IUCN/SSC Primate Specialist Group (2003). West African chimpanzees: status survey and conservation action plan. IUCN. pp. 127.
6. Hutchinson J., Dalziel J.M. and Keay R.W.J., 1954. Flora of West Tropical Africa. Vol. 1(1), 2nd ed. pp 1–295. Crown Agents for Oversea Governments and Administrations, London.
7. Hutchinson J., Dalziel J.M. and Keay R.W.J., 1958. Flora of West Tropical Africa. Vol. 1(2), 2nd ed. pp. 297–828. Crown Agents for Oversea Governments and Administrations, London.
8. Hutchinson J., Dalziel J.M., Keay R.W.J. and Hepper F.N., 1963. Flora of West tropical Africa. Volume II. pp. 544. Crown Agents for Oversea Governments and Administrations, London.
9. Keay R.W.J., 1989. Trees of Nigeria. Oxford Science Publication, New York, pp. 476.
10. Holmgren P.K., Keuken W. and Schofield E.K., 1990. Index harbariorum Part I. The Herbaria of the World. 8th ed. Reg. Veg., New York.
11. Greengrass E.J., 2009. Chimpanzees are close to extinction in Southwest Nigeria. Primate Conservation, 24, 77-83.
12. FAO. (2007). State of the world forest, Rome. pp 5-98.
13. Jimoh S.O., Adebisi L.A.A. and Ikyaagba E.T., 2009. Biodiversity and ethnobotanical potentials of plant species of University of Agriculture Makurdi Wildlife Park and Ikwe Games Reserve, Benue State, Nigeria. Int J Biol Chem Sci 3(6), 1375-1385.
14. Ferraro P.J. and Kiss A., 2002. Direct payments to conserve biodiversity. Science, 298, 1718-1719.
15. Soladoye M.O., Asafa B.A., Sonibare M.A., Ibhanesebhor G.A. and Chukwuma E.C., 2011. Angiosperm flora of Kamuku National Park: A northern guinea savanna protected area in Nigeria. European Journal of Scientific Research, 58(3), 326-340.

Ameliorative Effect of Aqueous Extract of *Chromoleana odorata* Leaves on Alloxan-induced Diabetic Rats

Bamisaye F.A.[1,*], Ajuwon O.R.[1], Ajani E.O.[2], Sulyman A.O.[2]

Department of Biochemistry, Faculty of Science, Federal University Oye-Ekiti, P.M.B.373, Oye-Are Road, Oye-Ekiti, Ekiti State, Nigeria
Department of Biochemistry, School of Basic Medical Sciences, College of Pure and Applied Sciences, Kwara State University, Malete, Nigeria
sayesayo@gmail.com, fisayo.bamisaye@fuoye.edu.ng

Abstract

This work sought to investigate the antidiabetic effect of aqueous extract of *Chromoleana odorata* leaves by monitoring the daily changes in blood glucose level of alloxan-induced diabetic rats. Qualitative phytochemical screening of the extract was also investigated. Thirty-five (35) rats were grouped into five (A-E) containing seven rats each. Group A were given 1 ml of distilled water twice daily. Groups B to E were alloxan-induced diabetic rats. Group B was also given 1 ml of distilled water twice daily. Groups C and D were treated with 100 mg and 200 mg/kg body weight of the leaves aqueous extract, respectively while group E were treated with 5 unit/Kg body weight of insulin twice daily. The treatment lasted for 14 consecutive days. The bioactive substances present in the extract include saponins, alkaloids, cardenolides, triterpenes and flavonoids, while anthraquinone, phlobatannins, tannins, phenolics and steroids were not detected. One or more of these secondary metabolites present in this aqueous extract may be responsible for the amelioration of alloxan-induced diabetes in rats since there was a decrease in blood glucose level in these rats treated with 100 mg and 200 mg per Kg body weight of the extract. Furthermore, the aqueous extract of *C. odorata* leaves showed high reduction potential of blood glucose level than insulin in alloxan-induced diabetic rats in this study. Collectively, the results suggest that the aqueous extract of *C. odorata* leaves elicits antidiabetic effect at 100 mg better than either 200 mg/Kg or 5 unit of insulin /Kg body weight in alloxan-induced diabetic rats.

Keywords: Aqueous extract, *Chromoleana odorata*, alloxan, diabetes, antidiabetic activity, albino rats

1   Introduction

One of the most prominent public health threats in modern societies of today is diabetes mellitus; and its prevalence is rapidly increasing [1]. The cause of diabetes mellitus may be due to a variety of different metabolic disorders. These include the inability to produce or use insulin, which then result to elevation of plasma glucose (blood sugar) levels [2]. This abnormally high level of glucose in the blood is called hyperglycaemia. Diabetes is of two types: a disease in which the pancreas produces insufficient amounts of insulin (type 1), or the body's cells fail to respond appropriately to insulin (type 2). Diabetes leads to the production of increasing amounts of acidic compounds in the blood called ketone bodies. These acidic compounds interfere with respiration of cells thereby leading to less energy production [3]. This leads to glucose to build up in the blood. When this excess blood glucose passes through the organs (the kidneys) that remove blood impurities, they cannot absorb all of the excess glucose. This excess glucose will spill into the urine, accompanied by water and electrolyte ions, which are required by cells to regulate the electric charge and flow of water molecules across the cell membrane. These cascades of reactions cause frequent urination to get rid of the additional water drawn into the urine leading to excessive thirst in order to trigger replacement of lost water [4]. Complications of diabetes ranging from heart disease, kidney failure, and other conditions lead to deaths. Prolonged high blood glucose level leads to changes in shape of lenses of the eyes, resulting in vision changes collectively known as neuropathy [5].

There are many therapeutic attempts against diabetes, but unfortunately, there is still no cure for it; the disease has only been managed. There is therefore, an urgent need to double the herbal therapeutic efforts aimed at finding solutions and selecting remedies for diabetes mellitus. In pursuit of this goal, several medicinal plants are being investigated for their antidiabetic efficacies. Of the several indigenous plants used in the local treatment of diabetes mellitus include the extracts of *Sarsaparilla* [6], *Beta vulgaris* [7], *Aloebarbadensis* [8, 9] and *Morinda lucida* [10]. *Chromolaena odorata,* popularly known as Christmas Bush (also commonly called Siam weed and Common floss flower), is a species of flowering shrub that belongs to the kingdom of Plantae; Order: Astereles; Family: Astereceae; Genus: Chromoleana; Species: *C. Odorata* [11]. This flowering plant has been introduced to tropical Asia, West Africa and parts of Australia [11] as a medicinal and ornamental plant. It is an herbaceous perennial plant that forms dense tangled bushes 1.5-2.0 m in height [12] with small seeds (3-5 mm long, 1 mm wide, and weigh about 2.5 mg /seed) [13]. It has been suggested as carcinogenic because it contains pyrrolidizine alkaloids [14], which are produced by the plants as a defense mechanism against insect infestation and herbivores [15]. Indonesia has used *C. odorata* as folkloric traditional medicine where its leaves aqueous extract are applied locally to treat skin wounds, burns, hemorrhages, hemorrhoids, indigestion, skin diseases, edema, fracture and infection

[16]. However, in the present findings, aqueous extract of *C. odorata* leaves was used to monitor the changes of the blood glucose levels in alloxan-induced diabetic rats.

2   Materials and Methods

2.1   Chemicals and Reagents

Chromoleana odorata leaves were obtained at the premises of Kwara State University, Malete, Nigeria. The albino rats used in the study were obtained from the Animal House of Biochemistry Department, College of Pure and Applied Sciences, Kwara State University, Malete, Ilorin. Alloxan was obtained from Sigma St, Louis, MO, USA**.** All other chemicals and reagents were of analytical grade.

2.2   Preparation of Aqueous Extract

Fresh leaves of *C. odorata* (180 g) were blended with a blender and soaked in 2 litres of distilled water for 24 hours. It was filtered with white clean muslin cloth and the suction pump used to reduce the volume of filtrate. Thereafter, the filtrate was evaporated to obtain brown oily substance (14.2 g). The filtrate was kept at $4^{o}C$ for further use.

2.3   Phytochemical Screening

The methods described by Odebiyi and Sofowora [17] and Sofowora [18] were used for the qualitative phytochemical screening of aqueous extract of C. *odorata* leaves.

2.4   Animal Protocol

Thirty-five (35) albino rats weighing between 140 and 150 g were divided into 5 groups (A – E) containing 7 rats each. Group A served as a negative control and received 1 ml of distilled water twice daily. Groups B, C, D, and E were induced with diabetes with a single intraperitoneal injection of 1 ml of alloxan per kg body weight of rats [19], which was prepared by freshly dissolving in citrate buffer (0.1 m, pH 4.5) [20]. After seven days of alloxan induction, diabetes was confirmed in these rats with fasting blood sugar concentration equal or greater than 200 mg/dL. Group B was also treated with 1 ml of distilled water while groups C and D were treated with 100 mg and 200 mg/kg body weight of aqueous extract of *C. odorata*, respectively and group E received 5 unit/kg body weight of insulin twice daily. The animals were maintained on growers feed and water *ad libtum*. The experiment lasted for 14 days and the change in blood glucose level was read daily with glucometer throughout this period.

All the animals that were used received humane care according to the guide, care and Use of Laboratory Animals prepared by the Nigeria Academy of Science. Ethic regulations have been followed in accordance with Nigeria and institutional guidelines for the protection of animal welfare during experiments.

2.5   Statistical Analysis

Data were expressed as means ± S.D. of seven determinations. The statistical tools used were one-way analysis of variance (ANOVA) and Duncan Multiple Range Test. Differences were considered statistically significant at $p < 0.05$ [21].

3   Results and Discussion

Qualitative phytochemical screening revealed the presence of saponins, alkaloids, cardenolides, flavonoids and triterpenes while anthraquinones, phlobatanins, steroids, phenolics and tannins were not detected in the aqueous extract of *C. odorata* leaves (Table 1).

The doses of aqueous extract of *C. odorata* leaves and insulin used in this study ameliorate the glucose levels of alloxan-induced diabetic rats. Group E had their blood glucose levels dropped below 200 mg/dl on the 8th day of treatment. Groups D and C had their blood glucose levels dropped below 200 mg/dl on the 9th and 10th day, respectively. Furthermore, group E had the highest drop in blood glucose level at the end of the experimental period. Also, the 100 mg/Kg body weight aqueous extract has better ameliorative effect than the 200 mg/Kg body weight of aqueous extract at the end of the experiment (Table 2).

Table 1. Qualitative phytochemical screening of the aqueous extract of *Chromoleana odorata* leaves

Phytochemicals	Status
Saponins	+
Alkaloids	+
Cardenolides	+
Anthraquinones	-
Phlobatanins	-
Triterpenes	+
Flavonoids	+
Steroids	-
Phenolics	-
Tannins	-

Key: (+) = present, (-) = absent

Table 2. Hypoglycemic potential of aqueous extract of *C. odorata* leaves (mg/dl)

Days	Group A	Group B	Group C	Group D	Group E
1	90.03 ± 2.22	366±65.64[a]	320.2±47.06[a]	321.6±56.81[a]	242.0±17.32[a]
2	95.66 ± 0.45	366.0±65.20[a]	325.6±42.46[a]	317.0±55.41[a]	240.2±17.89[a]
3	98.08 ± 1.05	359.4±62.74[a]	304.6±41.09[a]	304.0±54.64[a]	235.8±18.54[a]
4	108.01 ± 0.09	361.4±64.51[a]	289.0±37.93[a]	296.8±53.79[a]	229.6±17.24[a]
5	112.06 ± 0.14	359.6±63.57[a]	281.0±37.69[a]	285.8±52.90[a]	222.8±14.39[a]
6	99.21 ± 1.47	357.0±62.55[a]	267.0±38.70[ab]	270.6±51.35[ab]	207.0±9.58[b]
7	89.66 ± 2.06	353.0±61.81[a]	260.2±37.91[ab]	243.4±40.21[ab]	200.0±9.95[b]
8	104.32 ± 0.87	350.2±61.33[a]	234.6±26.83[b]	202.4±25.24[b]	189.4±8.45[b]
9	102.54 ± 0.99	343.6±59.38[a]	204.4±22.08[b]	185.8±28.28[b]	169.6±15.46[b]
10	86.88 ± 3.01	339.6±57.12[a]	183.2±18.69[b]	166.4±24.04[b]	174.6±6.87[b]
11	72.01 ± 2.88	328.8±51.71[a]	123.4±10.70[b]	154.4±13.16[b]	136.6±8.89[b]
12	75.00 ± 0.33	336.6±56.45[a]	104.4±5.70[b]	110.2±1.66[b]	103.2±4.62[b]
13	76.01 ± 0.96	332.6±55.33[a]	90.6±3.33[b]	102.0±1.14[b]	84.2±3.75[b]
14	72.00 ± 3.01	330.0±55.38[a]	88.0±2.83[b]	95.8±2.15[b]	80.2±1.83[b]

Results are means ±Standard Errors of Means of seven determinations; Values with different superscripts on the same row are significantly ($p<0.05$) different

At the end of the experiment, all animal groups gained weights except group B, which showed loss of body weight. Furthermore, the extract-treated groups C and D gained more weights (22.98 and 19.27 g, respectively) compared with the standard drug-treated (13.97 g) group E. However, negative group A witness the highest weight gain (25.94 g) compared with all other experimental groups (Table 3).

Table 3. Body weights of alloxan-induced diabetic rats treated with aqueous extract of *Chromoleana odorata* leaves (g)

Groups	Initial body weight	Final body weight	Weight gained/loss
A	133.52±5.69[a]	150.90 ±14.25[b]	+ 25.94[a]
B	140.02± 10.08[a]	130.40±5.99[b]	- 13.71[e]
C	112.24±34.85[a]	155.76±14.31[b]	+ 22.98[b]
D	115.06±25.39[a]	144.74±14.98[b]	+ 19.27[c]
E	146.28±2.82[a]	152.18±10.89[b]	+ 13.97[d]

Values with different superscripts on the same row are significantly ($p<0.05$) different

Diabetes has no cure yet, but can be managed by exercise, diet and pharmaceutical drugs. However, these drugs are either too expensive or may have undesirable side effects or contraindications on the users [22, 23]. Herb remedies have been known from history to be used to manage diabetes, which is one of the fastest growing degenerative diseases in the world today [24]. Due to the above probably, researchers are encouraged by the World Health Organisation (WHO) Committee on Diabetes Mellitus to investigate the hypoglycemic actions from plants which were originally used in traditional medicine especially in countries, such as Nigeria, where access to conventional treatment of diabetes is not pronounced [25]. It has been established that medicinal plants possess herbal therapeutic effects [23]. Some of these plants have been predicted as anti-diabetic agents when locally administered. Our present investigation showed a correlation between the previous reports which observed that, alloxan can induce hyperglycemia in normal healthy rats [22]. The present finding also revealed that the continuous treatment of alloxan-induced diabetic rats with aqueous extract of *C. odorata* leaves resulted into a significant reduction/decrease in blood glucose concentrations. The possible underlying mechanism responsible for this observed hypoglycemic action of aqueous extract of *C. odorata* leaves may be likened to the

potentiality of the insulin. This may be by increasing the pancreatic secretion of insulin from the cells of the islet of Langerhans by ameliorating the damage done by alloxan on the cells or it is released from the bound insulin by the extract [23, 26].

Some research works have shown that saponins and flavonoids possess hypoglycemic, hypotensive, anti-inflammatory and other pharmacological properties in diabetic-induced animals [27-29]. Therefore, the presence of these phytochemicals in the aqueous extract of *C. odorata* leaves may be responsible for its hypoglycemic effect. One of the possible mechanisms of this extract may be that one or both of these phytochemicals, saponins and flavonoids help to repair the damaged Islet of Langerhans cells.

The alloxan-induced diabetic rats treated with the extract of 100 and 200 mg/kg gained absolute body weight better than insulin-treated rats. This may be attributed to the fact that hepatic tissues were able to metabolise excess glucose generated by alloxan and supply enough energy to various epithelial tissues which resulted into increased growth in aqueous extract treated-rats better than those insulin-treated rats. However, this metabolism of excess glucose generated may be *C. odorata* leaves aqueous extract concentration dependent. This is noticed in more body weight gained in rats treated with 100 mg/Kg body weight (group C) compared to higher concentration of 200 mg/Kg body weight of the extract (group D).

Glucose levels in blood can be measured by metabolic processes in key tissues (liver, kidney and adipose). These tissues are regulated by hormones such as insulin, glucagon and diabetogenic hormones [30]. The present study showed similar significant reduction in the glucose level of these diabetic rats treated with the extract and insulin. This thus indicates tendency towards fine hormonal control following extract treatment as these diabetogenic hormonal interaction play a fine role in glucose control.

4   Conclusion

Leaves of *Chromoleana odorata,* a folkloric medicinal plant has hypoglycemic properties. These hypoglycemic properties may be due to the medicinal properties of one or some of the phytochemical substances that were detected in the leaves aqueous extract of the plant. Therefore, aqueous extract of *C. odorata* leaves may be a better option in nearest feature for the treatment of diabetes mellitus since it has ameliorative activity on blood glucose level, and the rats treated with it have more weight gain compared with the standard drug, insulin used in this research. However, this work merits further research to ascertain, isolate and quantify the bioactive compound(s) responsible for its anti-diabetic activities.

References

1. Steven S., Elizabeth D. and Agabegi M.D., 2008. Step-up to medicine (Step-Up Series). Hagerstwon, MD: Lippincott Williams & Wilkins.
2. Ripsin C.M., Kang H. and Urban R.J., 2009. Management of blood glucose in type 2 diabetes mellitus. Am Fam Physician, 79(1), 29-36.
3. Cooke D.W. and Plotnick L., 2008. Type 1 diabetes mellitus in pediatrics. Pediatr Rev, 29(11), 374-384.
4. King H., 2004. Global prevalence of diabetes: Estimates for 2000 and projections for 2030. Diabetes Care, 27(5), 1047-1053.
5. Williamson D.F., Narayan K.M., Boyle J.P., Thompson T.J. and Sorensen S.W., 2003. Lifetime risk for diabetes mellitus in the United States. Journal of American Medical Association, 290(14), 1884–1890.
6. Augusti K.T. and Mathew P.T., 1973. Effect of long term feeding of the aqueous extracts of Sarsparilla and garlic (Allium sativum Linn.) on normal rats. Indian J Exp Biol., 11, 239-241.
7. Yoshikawa M., Murakami T., Kadoya M., Matsuda H., Muraoka O., Yamahara J. and Murakami N., 1996. Medicinal foodstuff. III. Sugar beet. Hypoglycemic oleanolic acid oligoglycosides, betavulgarosides I, II, III, and IV, from the root of Beta vulgaris L. (Chenopodiaceae). Chem Pharm Bull (Tokyo), 44(6), 1212-1217.
8. Al-Awadi F.M. and Gumaa K.A., 1987. Studies on the activity of individual plants of an antidiabetic plant mixture. Acta Diabetologica Latina, 24, 37-41.
9. Grover J.K., Vats V., Rathi S.S. and Dawar R., 2001. Traditional Indian anti-diabetic plants attenuate renal hypertrophy, urine volume and albuminuria in streptozotocin induced diabetic mice. J Ethnopharmacol, 76(3), 233-238.
10. Bamisaye F.A., Odutuga A.A., Minari J.B., Dairo J.O., Oluba O.M. and Babatola L.J., 2013. Evaluation of hypoglycemic and toxicological effects of leaf extracts of *morindalucida*in hyperglycemic albino rats. International Research Journal of Biochemistry and Bioinformatics, 3(2), 37-43.
11. Schmidt, G.J. and Schilling EE., 2000. Phylogeny and biogeography of Eupatorium (Asteraceae:Eupatorieae) based on nuclear ITS sequence. Am J Bot. (Botanical Society of America), 87(5), 716-726.
12. Cruttwell McFadyen R.E.,1996. The ecology of *Chromolaena odorata* in the neotropics. Proceedings of the 2nd International Workshop on Biological Control of *Chromolaenaodorata*. Bogor 4-8, (Muniappan.R; Ferrar.P., Special Publication, 44,1-9.
13. Vanderwoude C., Scanlan J.C., Davis B. and Funkhouser S., 2005. Plan for national delimiting survey for Siam weed. Natural Resources and Mines Land Protection Services: Queensland Government, 224-300.
14. Yang Y.C., Xia Q., Chou M.C., Cui Y.Y. and Lin G., 2002. Pyrrolizidine alkaloids-tumorigenic components in Chinese herbal medicines and dietary supplements. J Food Drug Anal, 10(4), 198-211.

Proceedings of the 2019 International Science Conference

Innovation and Advancement in Nanoscience and Nanotechnology, Federal University Oye-Ekiti (www.fuoye.edu.ng), 2019.

15. Raimundo R.L.G., Fonseca R.L., Schachetti-Pereira R., Peterson A.T. and Lewinsohn T.M., 2007. Native and exotic distributions of Siam weed (Chromolaena odorata) modeled using the genetic algorithm for rule-set production. Weed Sci, 55 (1), 41-48.

16. Struhsaker T.T., Struhsaker P.J. and Siex K.S., 2005. Conserving African's rain forest: problems in protecting areas and possible solutions. Biol Conserv, 123(1), 45-54.

17. Odebiyi A. and Sofowora A.E., 1978. Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria. J Sustain Agric Environ, 6, 30-34.

18. Sofowora L.A., 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibaban, Harborne. pp. 55-71.

19. Cetto S.M., Vrushabendra B., Gopkumar M., Dhanapal P., Chandrashekara R., 2000. Leaf extracts in alloxan induced diabetics Rats: Iran J Pharm Therap, 4 (1), 38-39.

20. Ozsoy M.L., Rodrignes B., Yuen V.G. and McNeil J.H., 2000. Treatment and pharmacological interventions in Alloxan diabetes. Experimental models of diabetes, (McNeil J.H, Ed), pp. 195-216.

21. Mahajan B.K., 1997. Significance of difference in means. In: Methods in biostatistics for medical and research workers, 6th edition. New Delhi. JAYPEE Brothers Medical Publishers, pp. 130-155.

22. Adewole S.O. and Ojewole J.A., 2006. Effect of insulin on pancreatic β-cells of Streptozotocin-treated diabetic rats. Experimental observations on immuno-histochemical and morphological changes. Pharmacol, 1, 120-134.

23. Odutuga A.A., Dairo J.O., Minari J.B. and Bamisaye F.A., 2010. Anti-diabetic effect of *Morindalucida* stem bark extracts on alloxan-induced diabetic rats. Res J Pharmacol., 4(3), 78-82.

24. Akhtar M.S. and Ali M.R., 1984. Study of antidiabetic effect of a compound medicinal plant prescription in normal and diabetic rabbits. J Pak Med Assoc, 34, 239-244.

25. World Health Organisation (WHO), 1980.Second report of the WHO expert committee on diabetes mellitus. World Health Organisation Technical Report Series, pp. 646.

26. Pari L. and Amarnath S., 2004. Antidiabetic activity of Boerheaviadiffusa L.: Effect on hepatic enzymes in experimental diabetes. J Ethnopharmacol, 91, 109-113.

27. Akah P.A. and Okafor C.I., 1992. Blood sugar lowering effect of *Vernoniaamygdalina* Del, in an experimental rabbit model. Phytother Res, 6, 171-173.

28. Ojewole J.A.O., 2004. Evaluation of analgesic, anti-inflammatory and anti-diabetic properties of *Sclerocaryabirrea* (A Rich) Hochst stem- bark aqueous extract in mice and rats. Phytother Res, 18, 601-618.

29. Ojewole J.A., 2005. Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllumpinnatum (Crassulaceae)* leaf aqueous extract. J Ethnopharmacol, 99, 13-19.

30. Hill AF., 1952. Economic botany. A textbook of useful plants and plant products. 2nd edn. McGraw-Hill Book Company Inc, New York. pp54-67.