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CONTENTS

Model for Calculating the Concentration of Upgraded Iron Designated for Production of Stainless Steel Based Biomedical Devices Used in Orthopaedics

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Abstract: Successful attempt has been made to derive a model for calculating the concentration of upgraded iron designated for production of stainless steel based biomedical devices used in orthopaedics. The iron component of the iron oxide ore was upgraded during the pyrobeneficiation of the ore using powdered potassium chlorate. The model-predicted %Fe upgrades were found to agree with a direct relationship between %Fe values and weight-input of $KClO_3$ as exhibited by %Fe upgrades obtained from the experiment. It was found that the model; $\%Fe = \gamma[(\ln(T/\beta))^{2.1277}]$ is dependent on the weight-inputs of $KClO_3$, iron oxide ore and the treatment temperature. The validity of the model is rooted in the expression $(\%Fe/\gamma)^N = \ln(T/\beta)$ where both sides of the expression are correspondingly approximately equal to 3. The maximum deviation of the model-predicted values of %Fe from those of the corresponding experimental values was found to be less than 21% which is quite within the range of acceptable deviation limit of experimental results.
[C. I Nwoye, G. C. Obasi, U. C Nwoye, K. Okeke, C. C. Nwakwuo and O. O Onyemaobi. Model for Calculating the Concentration of Upgraded Iron Designated for Production of Stainless Steel Based Biomedical Devices Used in Orthopaedics. Life Science Journal 2010;7(4):1-6]. (ISSN: 1097-8135).

Keywords: Model, Upgraded Iron, Production, Stainless Steel, Biomedical Devices, Orthopaedics.

1. Introduction

Biomaterials such as metals are widely used due to their strength and toughness. While the widely used implant metals (stainless steel, titanium and cobalt alloys) are generally biocompatible, some people are allergic to ions released from these metals. The major problem with metals is the generation of fine wear particles in service that can lead to inflammation and implant loosening [1].

Biometals and associated biomedical devices are used in some parts of the human body. Therefore biocompatibility is expected to prevail in the biometal-biomedical device interaction within the body system. Medical practice has shown that placing a prosthetic device into the body, goes with two considerations which include: functional performance & biocompatibilty and nature of the physiological environment [2].

Functional performance considers the effect of the physiological environment on the biometal/device [2]. This implies that the biometal must satisfy its design requirements with respect to the environment where it serves with time. The varied functions of biometal include: control of blood and fluid flow; eg artificial heart, electrical stimuli; eg pacemaker, light transmission; eg implanted lenses and sound transmission; eg cochlear implant. The material is not expected to degrade in its properties within the environment of the body and must not cause any adverse reactions within the host body. This is in line with the requirement and expectation for biocompatibility [2].

The physiological environment on which biometals and associated biodevices can operate favourably is made up of 0.9M NaCl aqueous solution containing organic acids, proteins, enzymes, biological macromolecules, electrolytes and dissolved oxygen, nitrogen compounds, and soluble carbonate [2]. It was found [2] that pH ≈ 7.4 is normal for physiological extracellular fluid. It has been discovered that cells (eg. inflammatory cells and fibrotic cells) secrete several complex compounds that may significantly affect an implanted biomaterial. Applications of these biometals/devices have been found [3] to be also dependent on mechanical environment: static, dynamic, stress, strain and friction which degrades the metal through corrosion, dissolution and leaching. Medical practice has shown that the resultant degradation affects the materials adversely in terms of strength, fracture toughness and wear resistance [3].

It has been found [4] that pure Ti is useful for dental implants. Ti-6Al-4V has also been found [4] to be useful for investment cast hip and knee implants, dental implants and pacemaker housings due to its mechanical properties (ultimate tensile strength; 825Mpa, elongation; 8%). Ti alloy has also been discovered [4] to have coherent stable passive layer which offers excellent corrosion resistance. They are also resistance to stress corrosion cracking and corrosion fatigue in body fluids. These alloys were also found [4] to permit bone growth at the interface .However, titanium has unsatisfactory wear resistance and may produce wear debris.

Cobalt alloys have been found [5] to possess superior mechanical properties (ultimate tensile strength; 720-890Mpa, elongation; 5-17%) and chemical properties due to finer grain sizes and a more homogenous microstructure. They show excellent wear resistance and have coherent stable passivation layer which gives excellent corrosion resistance.

Steel has been found [6] to be chiefly made up of over 95% Fe and carbon less than 1%. Increasing addition of Cr into the Fe and C matrix and structure re-designs the steel to stainless steel. Addition of nickel to the stainless steel microstructure causes the austenite structure to be maintained at room temperature hence producing austenitic stainless steel [7]. It has been found [7] that 316 stainless steel finds application in early hip implants due to its good strength, ability to work harden and pitting corrosion resistance. The mechanical properties of stainless steel includes: ultimate tensile strength; 190-690Mpa and elongation; 12-40% [7]. Stainless steel usage in biomedical engineering is restricted to temporary device such as screws, plates, fittings and wires for orthopaedics due to potential long term release of Ni^{2+}, Cr^{3+}, and Cr^{6+} into the body [7].

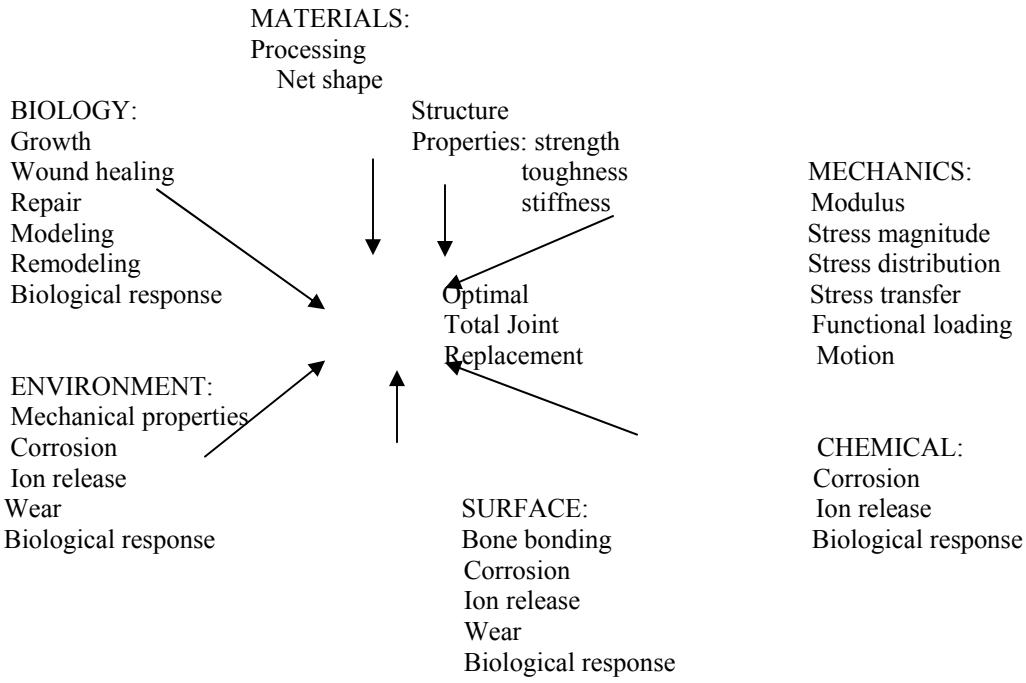

Fig. 1 Joint Replacement Design [5]

Metals dominate the bulk of the implant structure. Co-based alloys are specifically ideal for joint construction because of its high tensile strength, excellent corrosion resistance and excellent fatigue strength [5].

Iron oxide ore from Agbaja (Nigeria) being a primary raw material for the production of Fe used for making stainless steel (used as biomedical device) was found to contain 45.6%Fe and principally goethite, with minor hematite, maghemite, siderite, kaolinite and guartz [8].

An intensive and selective oil agglomeration of the iron ore has been carried out [9]. The researcher, starting from the crude ore Fe content (45.6%), concentrated (upgraded) the ore by oil agglomeration technique to 90% Fe

recovery and 65% Fe assay. He stated that the ore require grinding to minus 5μm to effect adequate liberation. These results were obtained at optimum pH 9. Successful studies on the effect of temperature on magnetizing reduction of Agbaja iron ore have been carried out [10]. The results of the investigation showed that the fine-grained oolitic Agbaja iron ore, which is not responsive to conventional processing techniques, can be upgraded by the magnetizing reduction method with an Fe recovery of 87.3% and Fe assay of 60% at 600^0C.

Attempt has been made to upgrade concentrate Fe recovery [11]. The researchers stated that concentrate Fe recovery decreases progressively below pH 8. In this pH region, oleate used is present as dispersion of oleic acid, and its adsorption on the surface of the iron oxides is similar to the process of hetero-coagulation involving positively charged iron oxide particles and negatively charged oleic acid droplet.

The aim of this work is to derive a model for calculating the concentration of upgraded iron designated for production of stainless steel based biomedical devices used in orthopaedics. The upgrading process stems on the pyro-beneficiation of Agbaja (Nigerian) iron oxide ore using powdered potassium chlorate as oxidant.

2. Model

The solid phase (ore) is assumed to be stationary, contains some unreduced iron remaining in the ore. It was found [12] that oxygen gas from the decomposition of $KClO_3$ attacked the ore in a gas-solid reaction, hence removing (through oxidation) the sulphur present in the ore in the form of SO_2. Equations (1) and (2) show this.

$$2KClO_{3\,(s)} \longrightarrow 2KCl_{\,(s)} + 3O_{2\,(g)} \quad (1)$$

$$S_{(s)}\ \text{Heat} \longrightarrow S_{(g)} + O_{2\,(g)} \longrightarrow SO_{2\,(g)} \quad (2)$$

Nwoye [12] posited that when sulphur inherent in the iron ore is removed in this stance; the concentration of iron present in the ore is upgraded since sulphur is an impurity element.

2.1 Model Formulation

Experimental data obtained from studies [13] carried out at SynchroWell Research Laboratory, Enugu were used for this work.

Results of the experiment as presented in report [13] and used for the model formulation are as shown in Table 1. Computational analysis of these experimental results [13] shown in Table 1, gave rise to Table 2 which indicate that;

$$(\%Fe/\gamma)^N = \ln(T/\beta) \quad \text{(approximately)} \quad (3)$$

Introducing the values of N and β into equation (3);

$$(\%Fe/\gamma)^{0.47} = \ln(T/\beta) \quad (4)$$

Since the inverse of 2.1277 = 0.47

$$(\%Fe/\gamma)^{1/2.1277} = \ln(T/\beta) \quad (5)$$

Multiplying the indices of both sides by 2.1277;

$$\%Fe/\gamma = (\ln(T/\beta))^{2.1277} \quad (6)$$

$$\%Fe = \gamma\,[(\ln(T/\beta))^{2.1277}] \quad (7)$$

Introducing the values of T and β into equation (7) reduces it to;

$$\%Fe = 7.8837\gamma \quad (8)$$

Where

%Fe = Upgraded concentration of iron during the beneficiation process

N= 0.47 (Decomposition coefficient of $KClO_3$ during the beneficiation process) determined in the experiment [13]

(β) =Weight of iron oxide ore added during the beneficiation process (g).

(γ) = Weight of $KClO_3$ added during the beneficiation process (g)

T =Treatment temperature (^{0}C)

N_e =2.1277(Assumed Temperature-Ore Interaction Factor)

I_e = 7.8837(Assumed Iron Enhancement Factor)

Equation (7) or (8) is the derived model**.**

Table 1: Variation of upgraded concentration of iron with weight-input of $KClO_3$ [13]

%Fe	(γ)	(β)
59.71	6	50
62.20	7	50
64.60	8	50
66.40	9	50
67.28	9.5	50
68.50	10	50

Table 2: Variation of $(\%Fe/\gamma)^N$ with ln(T/β)

$(\%Fe/\gamma)^N$	ln(T/β)
2.9445	2.6391
2.7918	2.6391
2.6691	2.6391
2.5581	2.6391
2.5094	2.6391
2.4704	2.6391

3. Boundary and Initial Condition

Consider iron ore (in a furnace) mixed with potassium chlorate (oxidant).The furnace atmosphere is not contaminated i.e (free of unwanted gases and dusts). Initially, atmospheric levels of oxygen are assumed just before the decomposition of $KClO_3$ (due to air in the furnace).Weight of iron oxide ore used; (50g), and treatment time; 360secs. were used. Treatment temperature; 700^{o}C, ore grain size; 150μm, and range of weight of $KClO_3$ used; (6-10g) were also used. These and other process conditions are as stated in the experimental technique [13].

The boundary conditions are: furnace oxygen atmosphere due to decomposition of $KClO_3$ (since the furnace was air-tight closed) at the top and bottom of the ore particles interacting with the gas phase. At the bottom of the particles, a zero gradient for the gas scalar are assumed and also for the gas phase at the top of the particles. The reduced iron is stationary. The sides of the particles are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of %Fe values predicted by the model and those obtained from the experiment for equality or near equality.

Analysis and comparison between these %Fe values reveal deviations of model-predicted %Fe values from those of the experiment. This is attributed to the fact that the surface properties of the ore and the physiochemical interactions between the ore and the oxidant (under the influence of the treatment temperature) which were found to have played vital roles during the oxidation-beneficiation process [13] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted %Fe values to those of the experimental %Fe values (Table 3).

Deviation (Dv) (%) of model-predicted %Fe values from experimental %Fe values is given by

$$Dv = \frac{Dm - De}{De} \times 100 \quad (9)$$

Where Dm = Predicted %Fe values from model
De = Experimental %Fe values

Correction factor (Cr) is the negative of the deviation i.e

$$Cr = -Dv \quad (10)$$

Therefore

$$Cr = -\left(\frac{Dm - De}{De}\right) \times 100 \quad (11)$$

Substitution of the values of Cr calculated from equation (11) into the model (equation (7) or (8)) gives exactly the corresponding experimental %Fe values [13].

5. Results and Discussion

The derived model is equation (7) or (8). A comparison of the values of %Fe from the experiment and those from the model shows maximum deviation of the model-predicted values (from experimental values) less than 21% which is quite within the acceptable deviation limit of experimental results hence depicting the reliability and validity of the model. This is shown in Table 3. Table 2 also agrees with equation (3) following the values $(\%Fe/\gamma)^N$ and $In(T/\beta)$ evaluated from Table 1 as a result of corresponding computational analysis. The validity of the model is rooted in equation (3) where both sides of the equation are correspondingly approximately equal to 3.

It is believed that since 2.1277 is the index of the expression $\ln(T/\beta)$ (as in equations (6)and (7)), the values of the process parameters; T and β as applied to the beneficiation process are simultaneously affected by the constant 2.1277 towards iron upgrade. The constant is therefore assumed to be the interaction factor between the treatment temperature and the iron oxide ore (Temperature-Ore Interaction Factor N_e since it is common to both T and β mathematically.

Also 7.8837 was obtained following evaluation of the expression; $[(\ln(T/\beta))^{2.1277}]$ (in equation (7)) which consists of input process parameters; T and β . Based on the foregoing, the constant is assumed to be the Iron Enhancement Factor I_e. In addition, the model-predicted %Fe upgrades were found to show a direct relationship with the weight-input of $KClO_3$ (agreeing with %Fe from the experiment as in Table 1) where I_e acts a multiplying constant of proportionality hence contributing to the iron upgrades mathematically.

Table 3: Comparison between %Fe upgrade as predicted by model and as obtained from experiment [13].

$\%Fe_{exp}$	$\%Fe_M$	Dv (%)	Cf (%)
59.71	47.3022	-20.78	+20.78
62.20	55.1859	-11.28	+11.28
64.60	63.0696	-2.37	+2.37
66.40	70.9533	+6.86	-6.86
67.28	74.8952	+11.32	-11.32
68.50	78.8370	+15.09	-15.09

Where

$\%Fe_{exp}$ = %Fe upgrade from experiment [13]
$\%Fe_M$ = %Fe upgrade predicted by model

6. Conclusion

The model calculates the concentration of upgraded-iron designated for production of stainless steel based biomedical devices used in orthopaedics. The maximum deviation of the model-predicted %Fe values from those of the experiment is less than 21% which is quite within the acceptable deviation limit of experimental results. The validity of the model is rooted in equation (3) where both sides of the equation are correspondingly approximately equal to 3.

Further works should incorporate more process parameters into the model with the aim of reducing the deviations of the model-predicted %Fe values from those of the experiment

Acknowledgement

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8/8/2010

Tracheary elements characteristics of *Kigelia africana* (Lam) Benth and *Newbouldia laevis* (P. Beauv) Seemann ex. Bureau growing in rainforest and derived savanna regions of Edo State, Nigeria.

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ABSTRACT: Variations in dimensions of tracheary elements of *Kigelia africana* (Lam) Benth. and *Newbouldia laevis* (P.Beauv.) Seemann ex Bureau growing in the rainforest and derived savanna areas of Edo State are reported. Both taxa exhibit short vessel lengths (<350µm), with medium-sized (100-200µm) diameters in the two vegetation zones. Vessels are thick-walled (>3.0µm) and decrease in wall thickness from derived savanna to the rainforest area. Taxa vessels are tailless having simple perforation with simple pits arranged in rows. Fibres are long (1600 µm), medium (900-1600µm) except in *K. africana* growing in the derived savanna areas where short lengths (<900µm) were encountered. Variations in fibre dimensions were significant at 1% and 5% probability levels between *K. africana* growing in the two ecological zones but were only significant at 5% probability level in fibre diameter of *N. laevis* growing in the two ecological zones. Taxa fibres were non-pitted and septations were only encountered in species found in the rainforest zone. Fibre /vessel length ratio in both taxa is greater than 1 and ratios approaching 10 are phylogenetically more advanced and specialized. Higher mean maximum values were obtained in vessel element lengths of *K. africana* and *N. laevis* growing in the rain forest zone. *N. laevis* from the derived savanna had thicker fibre walls than its counterpart in rain forest. Both species are suitable for various end-uses but are however not suitable for high grade pulp because of their relative low fibre length and runkel ratio (<1) which was low.
[Okoegwale. E.E, Ogie-Odia E and Idialu J.E. Tracheary elements characteristics of *Kigelia africana* (Lam) Benth and *Newbouldia laevis* (P. Beauv) Seemann ex. Bureau growing in rainforest and derived savanna regions of Edo State, Nigeria. Life Science Journal 2010;7(4):7-13]. (ISSN: 1097-8135).

Key words: Tracheary elements, *Kigelia africana*, *Newbouldia laevis*, rainforest, derived savanna, ecological zones.

INTRODUCTION

Kigelia africana (Lam) Benth. and *Newbouldia laevis* (P.Beauv.) Seemann ex Bureau belong to the Bignoniaceae family which has world distribution of 120 genera and 800 species and is primarily tropical in distribution. In West Africa it contains 17 genera and 20 species (Gill 1988). According to Gill (1992), only 50 timber species are being coµmercially exploited. The possible reason for this low value is that not enough is known about the characteristics, qualities and the uses to which the others could be put. An important characteristic which determines the end use of timber species is the tracheary element. Previous contributions to the tracheary elements characteristics of hard wood include Akachuku (1987), Baas (1973, 1976), Baas *et al.* (1983), Gill and Okoegwale (1990), Gill *et al.* (1985), Okoegwale and Idialu (1998), Outer and Van-Veenendaal (1976). Baas *et al*. (1983) reported almost consistent trend for vessel member to be shorter in arid region species and longer in the hygrophylic regions of Isreal and adjacent regions. They also reported higher maximum vessel diameter in hygrophylic regions and higher vessel wall thickness in the arid flora.

Gill and Onuja (1982) reported medium (100-200µm) to large – sized (>200µm) vessel diameters in the family Bignoniaceae but Gill *et al*. (1985) reported short vessels with extremely narrow diameter (mean 30.43µm) and medium length (mean 1519.88µm) in *Jacaranda acutifolia.* Okoegwale and Idialu (1998) reported higher maximum and minimum values for vessels and fibre lengths of woody leguminous plants in rain forest than in derived savanna counterparts, and these are important parameters for the determination of strength qualities and end-uses of wood. They also reported significant variations in fibre wall thickness in the same plant found in the rainforest and derived savanna areas which according to them are of relevance in comparing density or strength qualities. According to Akachuku (1987), density is largely determined by diameter and wall thickness of cells and the proportion of thick-walled tissues (vessels and fibres) and is the best singular indication of wood quality and its suitability for various purposes.

Outer and Van-Veenendaal (1976) reported short vessel (>350µm) of medium sized diameter (100-200µm) in *Kigelia africana* in the rainforest and savanna areas of Cote d' Voire. They however

reported non-significant variations in vessel lengths between *K. africana* in rainforest and savanna areas.

The purpose of this study was to ascertain the level of plasticity of the dimensions of tracheary elements (fibres and vessels) of both *Kigelia africana* (Lam). Benth and *Newbouldia laevis (*P. Beauv) Seemann ex Bureau growing naturally in the rainforest and derived savanna areas of Edo State, Nigeria. It was also to assess the effects of ecological variations on the dimensions of their tracheary elements on qualities and potentialities of their wood for various uses and to ascertain their phylogenetic trend.

METHODOLOGY.

Wood samples from *Kigelia africana* and *Newbouldia laevis* growing naturally in two ecological zones (rain forest and derived savanna) of Edo State Nigeria and whose ages were not ascertained were obtained. The ecological zones are located between longitude 5°04' East and 6°43' East and latitude 5°44' North and 7°34' North. Wood samples were collected from plants whose girths ranged from 9.0-11.0 centimeters at 1.3 meters above ground level i.e. 1.3 meters diameter at breast height (d.b.h).Wood samples were air-dried for 10 days before they were made into chips. Maceration of chips was carried out using the procedures of Gill *et al*. (1983) and Okoegwale and Gill (1990). Wood chips obtained, were placed in a test tube containing 10-15ml of 60% nitric acid and left overnight. It was then boiled for 5-10 minutes. The macerated materials were washed several times with distilled water. By modification, macerated materials were not centrifuged as described by Okoegwale and Gill (1990). A diluted (1%) drop of 1:1 glycerol-safranin solution was added before placing a cover slip. Linear measurements (length, diameter, lumen diameter and wall thickness) of vessels and fibres were made on calibrated microscope. Average values were based on 100 measurements. A t-test distribution was used to analyze the data.

RESULTS

Table 1. Morphological characteristics of vessels and fibres of *K. africana* (Lam) Benth growing in rainforest and derived savanna regions of Edo State.

	RAINFOREST	**DERIVED SAVANNA**
Plant tissue type	**Morphological characteristics**	**Morphological characteristics**
VESSELS		
Length	Short, ranging from 104.3-316.8µm mean 245.0µm	Short, ranging from 100.0-304.70µm. mean 200.0µm.
Diameter	Medium-sized, ranging from 75.66-177.8µm mean 126.0µm	Medium-sized, ranging from 62.68-141.60µm. mean 118.40µm.
Wall thickness	Thick-walled, ranging from 2.90-9.50µm mean 5.42µm.	Thick. Ranges from 3.14-11.70µm. mean 7.0µm.
Tail	Absent	Absent
Perforation plate	Simple and obliquely located at the end walls	Simple, obliquely located at the end walls.
Pit	Simple, round, arranged in rows	Simple, round, arranged in rows.
FIBRE		
Length	Medium, ranging from 1018.61-1640.11µm. mean 1450.15µm.	Short, ranges from 11.01-18.66µm. mean 13.71µm.
Diameter	Ranging from 11.4-31.11µm. mean 26.0µm.	-
Lumen diameter	Ranging from 11.46-21.70µm. mean 19.04µm.	-
Wall thickness	Moderate, ranging from 3.61-6.0µm. mean 4.10µm.	-
Pit	Absent	Absent
Septae	Present	-
Fibre vessel: Length ratio	5.92	3.55
Runkle ratio	0.43	0.50

Table 2. Morphological characteristics of vessel and fibres of *Newbouldia laevis* (P. Beauv) Seemann ex Bureau growing in rainforest and derived savannah regions of Edo State.

	RAINFOREST	**DERIVED SAVANNAH**
Plant tissue type	**Morphological characteristics**	**Morphological Characteristics**
VESSELS		
Length	Short, ranging from 212.60-441.20µm mean 320.50µm	Short, ranges from 210.4- 356.0µm mean 292.0µm
Diameter	Medium-sized, ranging from 61.40-216.40µm mean 130.0µm	Medium size, ranges from 55.8-194.3µm mean 123.10µm
Wall thickness	Thick-walled, ranging from 2.82-11.10µm mean 6.40µm.	Thick. Ranges from 3.60-12.60µm. mean 8.0µm.
Tail	Absent	Absent
perforation plate	Simple, obliquely located at the end walls	Simple, obliquely located at the end walls.
Pit	Simple, round, arranged in rows	Simple, round, arranged in rows.
FIBRE		
Length	Long, ranging from 1178.13-1660.40µm. mean 1620.22µm.	Medium, ranges from 1105.10-1630.90µm. mean 1596.78µm
Diameter	Ranging from 10.55-23.16µm. mean 21.48µm.	Ranges from 9.82-18.92µm mean 16.61µm
Lumen diameter	Ranging from 9.88-14.71µm. mean 12.58µm.	Ranges from 9.22-13.98µm mean 10.11µm
Wall thickness	Moderate, ranging from 3.11-6.44µm. mean 4.18µm.	Moderate, ranges from 3.0-6.05µm mean 5.0µm
Pit	Absent	Absent
Septae	Present	Absent
Fibre Vessel	Length ratio = 5.06	Length ratio = 5.47
Runkle ratio	0.67	0.99

DISCUSSION

In the presently investigated plants, vessel members are of medium lengths (350-800µm), medium- sized diameter (100-200µm), thick-walled (>3.0µm), tailless with simple perforation plates obliquely situated and simple round pits arranged in rows (Table 1)

Mean length of vessel members ranged from 245.0±102.08µm in *Kigelia africana* to 320.50±133.3µm in *Newbouldia laevis* growing in the rain forest area. In the derived savanna habitats, mean length ranged from 200.0±83.3µm in *K. africana* to 292.0±121.67µm in *N. laevis*.

Diameter was of the mean range 126.0±72.5µm in *K. africana* to 130.0± 54.17µm in *N. laevis* growing in the rain forest habitat while it ranged from 118.40±69.3µm in *K. africana* to 123.10+51.25µm in *N. laevis* growing in the derived savanna area. The presence of short vessels in the presently investigated species is in line with Gill *et al* (1985) and Outer and Van-Veenendaal (1976) who reported similar trends in *J. acutifolia* and *K. africana* respectively. Occurrence of medium sized vessel diameter is also in agreement with Outer and Van-Veenendaal (1976). Gill *et al* (1985), however, reported extremely small-sized diameter (mean 30.45µm) in *J. acutifolia.*

Vessel wall thickness ranged from 5.42±2.95µm in *K. africana* to 6.40±2.14µm in *N. laevis* growing in the rain forest habitat; it ranged from 7.01±4.93µm in *K. africana* to 8.0±3.34µm in *N. laevis* in the derived savanna area. Variations in vessel element wall thickness between *N. laevis* obtained from the two ecological zones were significant at 1% and 5% probability levels. The derived savanna plants have higher mean maximum vessel dimensions to counterbalance the water stress prevalent in that environment.

Taxa investigated possessed vessels with simple perforation plates confirming the previous report by Gill *et al.* (1985). The presence of simple round pits arranged in rows is also reported in the vessels of the taxa under study. Gill *et al* (1985)

however reported simple pits of no definite pattern of arrangement in *J. acutifolia*, while Outer and Van-Veenendanl (1976) neither reported the presence nor absence of this feature nor the pitting pattern in *K. africana*. Pit membranes affect the penetration of liquids, preservatives and gases in timber. It is important as research on pit membranes provides interesting applications in the field of wood technology, including the paper and pulp industry (Watanabe *et al.*, 1999, Singh *et al.*, 1999 and Flynn 1995). Taxa fibres are of short (>900µm) medium (900-1600µm) and long (>1600µm) types. Moderately thick-walled (3-5µm), septate; non-septate and non-pitted Fibre mean length ranged from 1450.15±534.0µm to 1620.22±611.0µm in *N. laevis* growing in the rain forest zone. In the derived savanna habitat, it ranged from 710.0±344.11µm in *K. africana* to 1595.78±543.16µm in *N. laevis.* Fibre mean diameters in the rain forest zone range from 21.48±9.36µm in *N. laevis* to 26.0±12.55µm in *K. africana*. Average fibre diameters in the derived savanna habitat ranged from 16.61±7.31µm in *N. laevis* to 18.0±7.99µm in *K. africana*.

Fibre mean lumen diameter ranged from 12.58±5.0µm in *N. laevis* to 19.04± 7.10µm in *K. africana* growing in the rainforest zone while it ranged from 10.11 ±4.77µm in *N. laevis* to 13.71±7.11µm in *K. africana* growing in the derived savanna area. Occurrence of medium –sized fibres ?is in line with Gill *et al*. (1985) on *J. acutifolia*. Variations in fibre length of taxa are not however significant. Higher mean maximum values in fibre element length were obtained in *N. laevis* occurring in the rain forest area, thus justifying the assumption of Okoegwale and Idialu (1998) on fibre lengths of some leguminous woody plants.

The thickness of the fibre walls ranged from 4.10±2.11µm in *K. africana* to 4.18±2.15µm in *N. laevis* obtained from the rain forest habitat. In the derived savanna habitat, fibre wall thickness ranged from 3.40±1.30µm in *K. africana* to 5.0±2.33µm in *N. laevis*. However, Gill *et al*. (1985) reported very thin-walled fibres in *J. acutifolia*, but Outer and Van-Veenendaal (1976) gave no description on the fibres of *K. africana*. On comparison, *N. laevis* of the derived savanna habitat has thicker fibre walls than its counterpart in the rain forest. Fibre wall thickness is of relevance in the determination of wood density and its end-uses (Okoegwale and Idialu, 1998).

In this investigation only *K. africana* and *N. laevis* growing in the rain forest habitat had septate fibre. Taxa fibres were non-pitted but Gill *et al.* (1985) reported simple pits with a row-like pattern of arrangement in *J. acutifolia.* Fibre vessel length ratio of taxa investigated ranges from 3.55 to 5.92µm in *K. africana* growing in the derived savanna and rain forest area respectively. Although the species growing in the rain forest area has higher ratio than its derived savanna counterpart, presence of septate may be an indication that such species has not lost all its primitive traits. The fibre/vessel length ratio of *K. africana* and *N. laevis* growing in the two ecological zones are presented in Fig 1. This ratio is an important parameter for the assessment of phylogeny of taxa. The ratio of taxa approaching 10 is more advanced and specialized and suitable for different uses as timber.

There were no significant variations in vessel element lengths and diameters ($P<0.05$) of these species growing in the two ecological zones. However, on comparison higher mean maximum value were obtained in vessel element lengths of *K. africana* and *N. laevis* growing in the rain forest zone. This is in agreement with Okoegwale and Idialu (1998) who reported similar trends in some leguminous woody taxa occurring in two different ecological zones. According to Baas *et al*. (1983), the advantage of high mean maximum length is for increased efficiency for water conduction.

CONCLUSION

From a technology point of view, the shape of the vessel and fibre cells, their lengths and wall thickness are some important parameters for the determination of wood properties. The present investigation on woods of lesser known plants viz: *Kigelia africana* and *Newbouldia laevis* growing in the rain forest and derived savanna area of Edo State, Nigeria indicated that vessel and fibre dimensions show plasticity and so these plants are suitable for various end-uses but are however not suitable for high grade pulp because of their relatively low fibre length and runkel ratio (<1). Plasticity in the tracheary elements had hitherto not been reported in these species.

Table 3: Vessel and fibre characteristics of *Kigelia africana* and *Newbouldia laevis*

Plant species	*Kigelia africana* (Lam) Benth		*Newbouldia laevis* (p. Beauv) Seemann ex Bureau	
Habitat	F	DS	F	DS
d.b.h.(cm)	10	11	10	9
Vessel means length ±SD (µm)	245.0±10 2.8	200.0±83. 3	32.0.5±13 3.3	292.0±12 1.67
Level of significance	NS 1.82		NS 1.28	
Vessel mean diameter ± SD (µm)	126.0 ±72.5	118.4 0±69. 3	130.0 ±54.1 7	123.1 ±51.2 5
Level of significance	Ns 0.41		Ns 0.34	
Vessel wall thickness ± SD(µm)	5.42±2. 95	7.0±4.9 3	6.40±2. 14	8.0±3.3 4
Level of significance	NS 1.51		**3.0	
Vessel tail length (µm)	-	-	-	-
Vessel perforation type	SIMPLE / OBLIQUE			
Pits/pattern of arrangement	Simple round in rows	Simple round in rows	Simple round in rows	Simple round in rows
Fibre mean length ±SD(µm)	1450. 15±5 3.4.0	710.0 ±344. 11	1620. 22±6 11.0	1596. 78±5 43.16
Level of significance	** 6. 38		NS 0. 16	
Fibre mean diameter ±SD(µm)	26.0± 12.55	18.0± 7.99	21.48 ±9.63	16.61 ±7.31
Level of significance	2.94		2.24	
Fibre mean lumen diameter ±SD(µm)	19.04 ±7.10	13.71 ±7.11	12.58 ±5.0	10.11 ±4.77
Fibre mean wall thickness ± SDµm)	4.10±2.1 1	3.40±1.3 0	4.18±2.1 5	5.0±2.32
Level of significance	Ns 0.91		NS 1.56	
Pits	-	-	-	-
Septate	+	-	+	-
F/v length ratio	5.92	3.55	5.06	5.47
Runkel ratio	0.43	0.50	0.67	0.99

F - rainforest habitat
DS - derived savanna habitat
d.b.h. - diameter at breast height
M (µm - microns
\+ - present
\- - absent
NS - Not significant
SD - Standard deviation

* Significant at 5% probability level
** Significant at 1% probability level

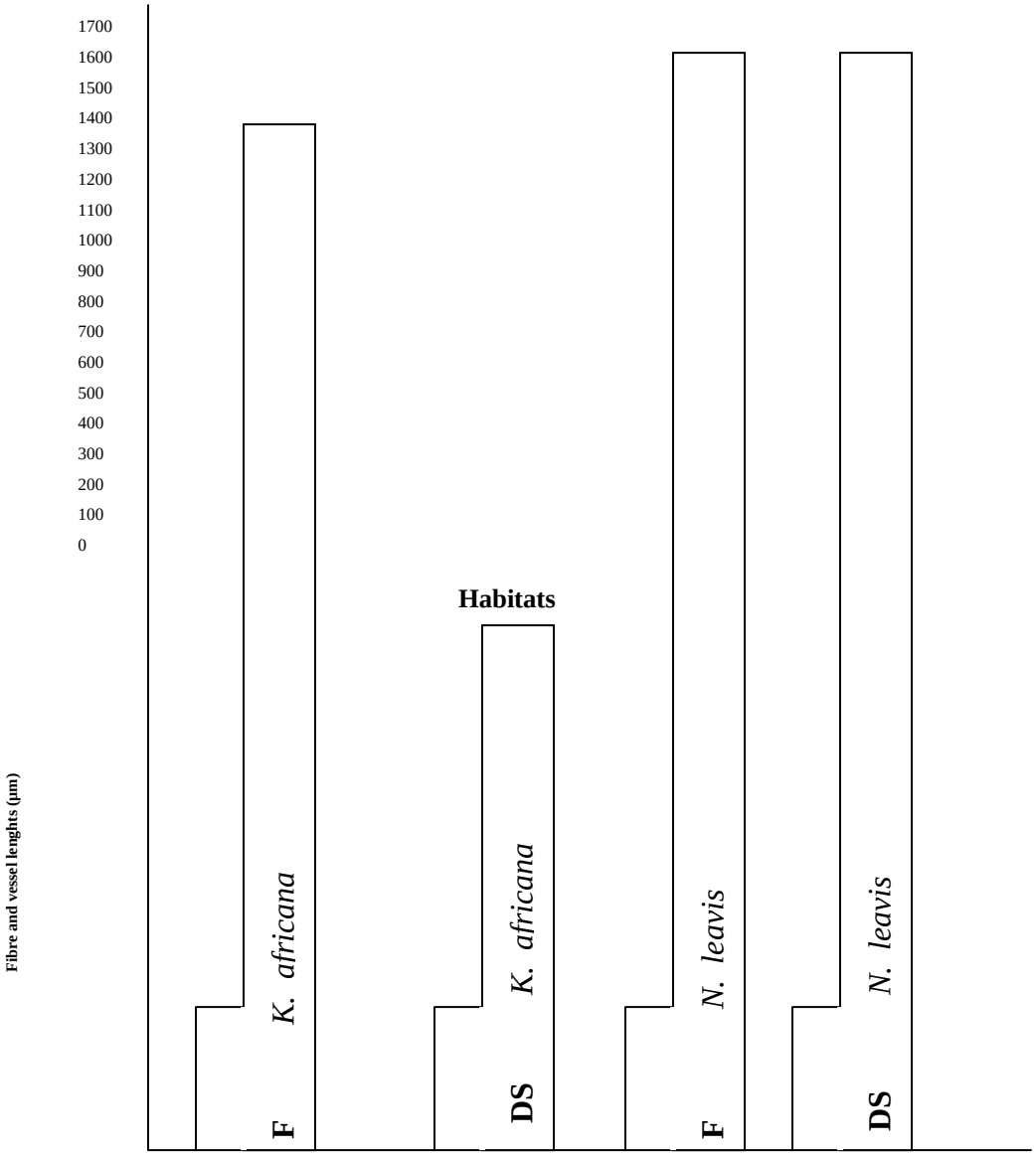

Fig. 1. Fibre/vessel length ratio of *Kigelia africana* and *Newbouldia laevis* in rainforest and derived savannah habitat

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Plant Biotechnology on a flight: Is Africa on board?

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Abstract: The rate of development of plant biotechnologies has been huge in recent times, especially in the developed countries. The technologies have created a new branch of biotechnology known as molecular farming, where plants are engineered to produce pharmaceutical and technical proteins in commercial quantities. An evaluation of the status of plant biotechnology development in Africa revealed that the very few countries, with the exception of South Africa, that are involved in biotechnology activities are still at the level of tissue culture applications. This calls for sincere commitments on the part of various stakeholders in Africa, especially the governments, to the development of biotechnology capacity.

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Introduction

Since the first report of plant genetic transformation in the 80s, the technology has been deployed to produce the first-generation genetically modified (GM) plants, the herbicide-tolerant (Ht) and insect-resistant (*Bacillus thuringiensis* [Bt]) crops, that were engineered basically to increase farmers productivity. The first-generation GM crops have proven to be of tremendous benefits to the countries that have adopted them, including the developing ones. For example, as high as 70 to 85% reduction in the application of herbicides and pesticides were reported for India and China (Bennett *et al*, 2006; Huang *et al*, 2003), hence impacting positively on the cost of these chemicals and overall production costs. The introduction of the Bt crops has led to reduction in insect damage, and has reduced the labor costs by about half in South Africa (Morse *et al*, 2005) and even by a higher percentage (66%) in Australia (Fitt, 2003). It was estimated that Bt-cotton in the US led to 860,000 kg reduction in pesticide use and increased farmers' net income by $100 million (Gianessi *et al*, 2002, cited in Konde, 2006). Additionally, these first generation GM crops have increased the yield at unprecedented level, for example, a striking 87% yield increase was recorded in the field trial of Bt cotton in India (Qaim, 2003) and additional 1.6 million Metric tons maize production was achieved with Bt-maize in the US (Gianessi *et al*, 2002, cited in Konde, 2006).

Plant biotechnology has since moved on to engineering second-generation GM crops, which incorporate traits that lead to enhanced nutritional contents of the farm products, for example, the engineering of the β-carotene biosynthetic pathway in rice for enhanced provitamin A content (Ye *et al*, 2000) and the engineering of tomatoes for increased folate production (Diaz de la Garza *et al*, 2007). The technology is actually on a flight at the moment, with the third-generation GM crops that are engineered as bio-factories for the production of different kinds of recombinant proteins for pharmaceutical and industrial applications. This plant-based production of biopharmaceuticals and technical proteins is known as molecular farming (Schillberg *et al*, 2005). Interestingly, there appears to be somewhat favorable public perception about the plant molecular farming (PMF) crops, possibly because of the potential benefits of the products, and also probably because most of these crops are not intended for consumption but are only being used as vehicles for high-value molecules. With the huge technological advances within short period, plant biotechnology is indeed poised to be the leading plant science of the century, but the question is, is Africa part of these

developments? This review discusses the evolution and the development of PMF. It then gives an overview of the various plant-derived recombinant pharmaceutical and non-pharmaceutical proteins, which are at different stages of research, clinical and commercial developments. Lastly, I shall assess the status of development of PMF technologies in the developing countries, with particular emphasis on Africa and then discuss issues bothering on capacity building in African biotechnology development generally.

The evolution and development of PMF

Plants have been used right from the dawn of ages, as sources of natural medicaments for treating various ailments. In addition to being the major resource for drug exploration, plants are still being used hugely in complementary and alternative medicine in many developing countries and nowadays, in the developed countries (Fønnebø *et al*, 2007). Up till early 1970s, bioactive compounds of drugs were being extracted, purified and synthesized solely from plants. However, synthetic drugs, whose evolution started with the production of aspirin by the drug company Bayer in 1899, took the centre stage in pharmaceutical production, for the greater parts of the 20th century. Since the advent of genetic engineering technologies in the 1970s, living systems, such as bacteria, yeast, and animal cells have been used as production systems for many valuable therapeutic and diagnostic proteins (Andersen *et al,* 2002; Harvey *et al,* 2002; Heyer, 2005; Jones *et al,* 2003; Kuroiwa, 2002), thereby complementing chemical synthesis and extraction of bioactive compounds from living materials. However, due to the production constraints of these systems, which include poor quality, low yield, and non-flexible scalability, the development of plant-based expression systems for recombinant proteins has been well-accepted as a promising cost-effective alternative platform for the production of safer and cheaper biopharmaceutical proteins. The comparative advantages of the plant-based system over the existing expression systems, which are are summarized in Table 1 below, are the incentive for this.

Table 1. Comparison of different production systems for expression of recombinant pharmaceutical proteins (Data adapted from Biemelt and Sonnewald, 2005).

System	Production costs	Time effort	Scale-up capacity	Product quality	Glycosyla-Tion	Contamination risk	Storage	Social acceptance level
Bacteria	Low	Low	High	Low	None	Endotoxins	Medium/ -20°C	High
Yeast	Medium	Medium	High	Medium	incorrect	Low	Medium/ -20°C	High
Mammalian cell culture	High	High	Very Low	Very high	Correct	Viruses, oncogens	Difficult/ Liquid N_2	Medium
Transgenic animals	High	High	Low	Very High	Correct	Viruses, oncogens	Difficult	Low
Plant cell cultures	Medium	Medium	Medium	High	Minor differences	Low	Medium/ -20°C	High
Transgenic plants	Low	High	Very high	High	Minor differences	Low	Easy/Room temperature	Medium

Since the first recombinant plant-made pharmaceutical protein, the human growth hormone was expressed in tobacco and sunflower (Barta *et al*, 1986), there have been significant advances in the development of PMF technologies, which have largely demonstrated that plants could be turned into bio-factories for the large-scale production of recombinant proteins. Major advances in the development of PMF are shown in table 2. Moreover, plants are now being engineered to mimic mammalian pattern of protein processing (including N-glycosylation), that make these recombinant proteins fold properly and maintain their structural and functional integrity. As such they are being made to produce even more complex functional mammalian proteins with therapeutic activity, such

as human serum proteins and growth regulators, antibodies, vaccines, hormones, cytokines, enzymes and antibodies (Liénard *et al*, 2007). With increasing demand for bio-pharmaceuticals, coupled with the high costs and inefficiency of the established production systems (Knablein, 2005), there is now pressure to increase production capacity. Hence attention is being shifted to transgenic plants as the new generation bio-reactors.

Table 2. Major advances in the development of PMF (data adapted from Schillberg and Twyman, 2007).

Year	Major Advance	Reference
1986	Human growth hormone produced in tobacco and sunflower – the fiirst plant-derived recombinant therapeutic protein and the first proof-of-concept of plants as bioreactors	Barta *et al*, 1986
1989	Full-size IgG produced in tobacco – the first plant-derived recombinant antibody and the first demonstration of the ability of plants to assemble heterologous complex biomolecules	Hiatt *et al*, 1989
1990	Human serum albumin produced in tobacco and potato – the first native human protein produced in plants	Sijmons *et al*, 1990
1992	Hepatitis B virus surface antigen produced in tobacco – the first plant-derived vaccine candidate	Mason *et al*, 1992
1992	α-amylase produced in tobacco – the first plant-derived industrial enzyme	Pen *et al*, 1992
1995	First secretory IgA produced in tobacco	Ma *et al*, 1995
1995	E. coli heat-labile enterotoxin (LT-B) expression in tobacco and potato – the first proof-of-concept of a plant edible vaccine	Haq *et al*, 1995
1996	Artificial elastin expression in tobacco– the first plant-derived protein polymer	Zhang *et al*, 1996
1997	First clinical trial using recombinant bacterial antigen delivered in a transgenic potato	Tacket *et al*, 1998
1997	Avidin produced in maize – the first commercialized plant-derived protein	Hood *et al*, 1997
1999	First glycan analysis of plant-produced recombinant glycoprotein	Cabanes-Macheteau *et al*, 1999
2000	Human growth hormone produced in tobacco chloroplasts	Staub *et al*, 2000
2000	Triple helix assembly and processing of human collagen produced in tobacco	Ruggiero *et al*, 2000
2001	Highest recombinant protein accumulation achieved in plants so far – 46.1% total soluble protein for *Bacillus thuringiensis* Cry2Aa2 protein	De Cosa *et al*, 2001
2001	First multi-component vaccine candidate expressed in potato – cholera toxin B and A2 subunits, rotavirus enterotoxin and enterotoxigenic *Escherichia coli* fimbrial antigen fusions for protection against several enteric diseases	Yu and Langridge, 2001
2001	Glycan modification of a foreign protein produced in a plant host using a human glycosyltransferase	Bakker *et al*, 2001
2003	Expression and assembly of a functional antibody in algae	Mayfield *et al*, 2003
2003	Bovine trypsin – the first marketed plant-derived protein, targeted towards a broad market	Woodard *et al*, 2003
2004	Glyco-engineered moss strains – the first plant system to be commercialized as bioreactor	Decker and Reski, 2004 (http://www.greenovation.com)
2005	First demonstration of most 'humanized' protein glycosylation patterns in plant production system	Huether *et al*, 2005
2006	HN proteins of Newcastle disease virus – the world's first regulatory approval (USDA) for a plant-made vaccine for animals (poultry)	Dow AgroSciences, 2006
2006	Antibody against Hepatitis B – the first commercialized plant-derived antibody (marketed in Cuba)	Pujol *et al*, 2005
2006	Rapid high-yield (transient) expression of full-size IgG antibodies in	Giritch *et al*, 2006

	plants	
2008	Caro^{RX} – the first antibody vaccine for human application (prevention of tooth decay), to be approved by the EU	Kaiser, 2008
2009	Highest transient expression of full-sized IgG antibody in plants	Vézina *et al*, 2009

Overview of plant-derived recombinant proteins

Plant-derived vaccines

Several candidate recombinant proteins with potential use as vaccines have been expressed in plants, since the first plant-derived vaccine-relevant protein was reported 20 years ago (Tiwari *et al*, 2009). Recent examples include the hepatitis B surface antigen (He *et al*, 2008; Qian *et al*, 2008), the heat labile enterotoxin B subunit (LTB) of *Escherichia coli* (Moravec *et al*, 2007; Rosales-Mendoza *et al*. 2009), and the cholera toxin B subunit (CTB) of *Vibrio cholera* (Nochi *et al*, 2007; Sharma *et al*, 2008). Others include the L1 protein of human papillomavirus type 11 and 16 (Liu *et al*. 2005; Maclean *et al*. 2007), the Norwalk virus capsid protein (Tacket *et al*, 2000), and the Hemagglutinin protein from measles virus (Marquet-Blouin *et al*. 2003). It should be noted that the US Defense Department has been sponsoring research and development of various bio-defense vaccines against lethal bioterror agents including anthrax and plague (Hull *et al*. 2005; Mett *et al*. 2007; Santi *et al*. 2006).

Several plant-produced antigens have been reported to induce immune responses, conferring protection against challenge in mouse model systems (Satyavathi *et al*. 2003; Streatfield and Howard 2003). Oral administration of several plant-produced antigens has also been reported to induce protective immune response in mice and humans (McCormick *et al*. 2008; Tregoning *et al*. 2005). However, much lower dose of vaccine administered orally is required by injection delivery (Streatfield and Howard 2003). Several plant-produced vaccine candidates, which are at different stages of clinical trials, are summarized in Table 3 below. To date only one veterinary vaccine, the NDV vaccine for poultry, developed by Dow AgroSciences, has been approved by the US Department of Agriculture (USDA) (www.thepoultrysite.com).

Table 3. Plant-derived pharmaceuticals in clinical stages of development or on market

Product	Disease	Plant	Clinical trial status	Company
Vaccines				
Hepatitis B antigen (HBsAg)	Hepatitis B	Lettuce Potato	Phase I Phase II	Thomas Jefferson University, USA Arizona State University
Fusion proteins, including epitopes from rabies	Rabies	Spinach	Phase I completed	Thomas Jefferson University, USA
Cancer vaccine	Non-Hodgkin's lymphoma	Tobacco	Phase II	Large Scale Biology, USA[a]
Vibrio Cholerae	Cholera	Potato	Phase I	Arizona State University
Heat-labile toxin B subunit of *Escherichia coli*	Diarrhea	Maize Potato	Phase I Phase I	ProdiGene[a], USA Arizona State University
Capsid protein Norwalk virus	Diarrhea	Potato, Tomato	Phase I	Arizona State University
Antigen	Feline parvovirus (Dogs)	Tobacco	Advanced	Large Scale Biology, USA[a]
Antigen	Papilloma virus (Rabbit)	Tobacco	Early	Large Scale Biology, USA[a]
HN protein of Newcastle disease virus	Newcastle disease (Poultry)	Tobacco suspension cells	USDA Approved	Dow Agro Sciences, USA
Viral vaccine	Diseases of horses,	Tobacco suspension	Phase I	Dow Agro Sciences,

mixture	dogs, and birds	cells		USA
Poultry vaccine	Coccidiosis infection	Canola	Phase II	Guardian Biosciences, Canada
Gastroenteritis virus (TGEV) capsid protein	Piglet gastroenteritis	Maize	Phase I	ProdiGene[a], USA
Antibodies				
CaroRX	Dental caries	Tobacco	EU approved as medical advice	Planet Biotechnology, USA
DoxoRX	Side-effects of cancer therapy	Tobacco	Phase I completed	Planet Biotechnology, USA
RhinoRX	Common cold	Tobacco	Phase I completed	Planet Biotechnology, USA
Fv antibodies	Non-Hodgkin's lymphoma	Tobacco	Phase I	Large Scale Biology, USA[a]
IgG (ICAM1)	Common cold	Tobacco	Phase I	Planet Biotechnology, USA
Antibody against Hepatitis B	Vaccine purification	Tobacco	On market	CIGB, Cuba
Therapeutic human proteins				
Gastric lipase	Cystic fibrosis	Maize	Phase II trials, commercialization expected for 2010	Meristem Therapeutics France
α-Galactosidase	Fabry disease	Tobacco	Phase I	Planet Biotechnology, USA
Lactoferon™ (α-interferon)	Hepatitis B & C	Duckweed	Phase II	Biolex, USA
Interleukin	Crohn's disease	Tobacco	Field trails	Southern Crop Protection and Food Research Centre, Canada
Fibrinolytic drug (thrombolytic drug)	Blood clot	Duckweed	Phase I	Biolex, USA
Human glucocerebrosidase (prGCD)	Gaucher disease	Carrot suspension cells	Marketing expected for 2010	Protalix Biotherapeutics, Israel
Insulin	Diabetes	Safflower	Commercialization expected for 2010	SemBioSys, Canada
Apolipoprotein	Cardiovascular	Safflower	Phase I	SemBioSys, Canada
Nutraceuticals				
Human intrinsic factor	Vitamin B12 deficiency	*Arabidopsis*	EU Approved	Cobento Biotech AS (EU)
Human lactoferrin	Anti-infection, anti-inflammatory	Rice	Advanced, on market as fine chemical	Ventria, USA
Human lysozyme	Anti-infection, anti-inflammatory	Rice	Advanced, on market as fine chemical	Ventria, USA
Immunosphere™	Food additive for shrimps	Safflower	Marketing expected for 2010	SemBioSys, Canada

Data adapted from Basaran and Rodríguez-Cerezo (2008), Spök *et al.* (2008), Kaiser (2008), Key *et al.* 2008 and Lau and Sun (2009).
[a]The firm has filed for bankruptcy and since winded up activity

Plant-derived antibodies

Two main approaches are being employed to produce biologically active full antibodies in plants. These are cross-pollination of individually transformed plants expressing light or heavy chains (Huang *et al.* 2001) and co-transformation of the heavy and light chain genes (Nicholson *et al.* 2005; Villani *et al.* 2008). One of such plant-derived antibodies, a surface antigen of *Streptococcus mutans* has been developed into a clinical product, CaroRX^{TM}, and has recently been approved by the EU to be used as medical advice, for the prevention of tooth decay (Kaiser, 2008). Moreover, a maize seed-produced HIV-specific monoclonal antibody was found to exhibit high antigen-binding activity (Ramessar *et al.* 2008), as such has proven to be a good candidate for clinical development as HIV microbicide for topical vaginal application, to prevent HIV transmission . There are five different plant-derived monoclonal antibodies presently being tested in the clinical trials, as listed in Table 3. To date, only one plant-made antibody for production of Hepatitis B Virus vaccine has been commercialized (Pujol *et al.* 2005).

Therapeutic and nutraceutical proteins

Several human proteins have been expressed in the plants, including the epidermal growth factor (Bai *et al,* 2007), α-, β- and γ-interferons, which are used in treating hepatitis B and C (Arlen *et al.* 2007; Edelbaum *et al,* 1992; Sadhu and Reddy, 2003), erythropoietin, which promote red blood cell production (Musa *et al,* 2009; Weise *et al,* 2007), interleukin, which is used in treating Crohn's disease (Gutiérrez-Ortega *et al,* 2005; Elias-Lopez *et al,* 2008), insulin, which is used for treating diabetes (Nykiforuk *et al*, 2006) and several others. Contained in Table 3 is the list of some of these therapeutics, which are at various stages of clinical trials or at the verge of being commercialized.
Plant-produced antimicrobial nutraceutics, such as human lactoferrin and lysozymes are now commercially available, but only as fine chemicals (Table 3). The Cobento Biotechnology's human intrinsic factor, produced in transgenic *Arabidopsis* plants, and which is to be used against vitamin B12 deficiency has just been approved by the EU (Key *et al.* 2008). Other nutraceutical products at various stages of development are listed in Table 3.

Plant-derived industrial proteins

The plant-derived industrial proteins, most of which are enzymes, including avidin, trypsin, β-glucuronidase, peroxidase, laccase, cellulase and so on (Basaran and Rodríguez-Cerezo, 2008) are now commercialized. The molecular farming of cellulases as well as other cell-wall deconstructing enzymes such as hemicellulases and ligninases holds great promise for the biofuel industry, with respect to the production of cellulosic ethanol (Lee *et al,* 2008; Sticklen, 2008). Non-hydrolytic proteins with cell wall disrupting and loosening properties, such as the carbohydrate binding modules of cell wall deconstructing enzymes and the expansins, which have been demonstrated to alter cell wall structure (Obembe *et al,* 2007a; Obembe *et al,* 2007b), are potential candidate PMF proteins for the production of cellulosic ethanol.

Status of PMF development in the developing countries

It is now exactly a decade since one of the foremost campaigners for plant biotechnology in Africa, Florence Wambugu gave a wakeup call to all stakeholders in Africa to rally and stimulate research and development in Plant Biotechnology for solving, especially, the food insecurity problem of the continent (Wambugu, 1999). An assessment of the status of plant biotechnology development for generating the first and the second generation GM crops shows that Africa does not seem ready to catch up with the trends in other parts of the world, as most of the counties are still at the stage of tissue culture applications, while genetic engineering is limited to only three countries, South Africa, Kenya and Zimbabwe (Ayele *et al*, 2006; Cohen, 2005**)**, with South Africa as the clear leader (Thomson, 2008). The group of Prof EP Rybicki at the University of Cape Town is the trail blazer for that country and indeed for the entire continent. Recently, they reported the world's first maize streak virus (MSV) resistant transgenic maize (Shepherd *et al*, 2007). This MSV-resistant maize is also the first all-African produced transgenic crop plant, as well as the first genetically engineered crop developed wholly by a developing country (Sinha, 2007).

With respect to the development of PMF technologies in the developing countries, an analysis of recent data compiled by Basaran and Rodríguez-Cerezo (2008) indeed revealed that the developing countries account for 37% of the world PMF activities while the developed countries account for 63%. Although, this seems encouraging, however, on further analysis, the margin between the two worlds widens remarkably with respect to the actual numbers of PMF centers. The analysis shows that 87% of the world PMF centers are actually located in the developed countries, while the developing countries can only boast of 13%, which is just about one-third of the number of centers located in the US (Figure 1).

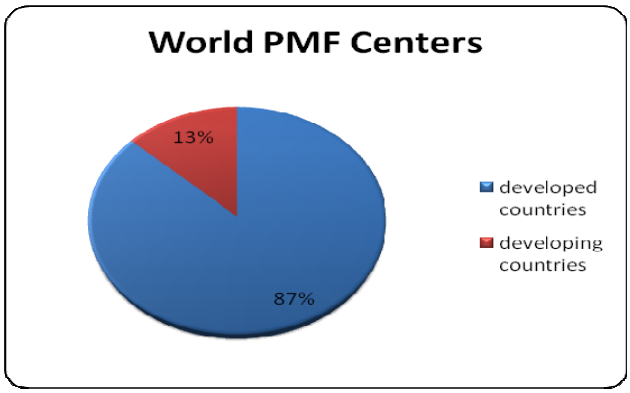

Figure 1. Pie Chart showing the distribution of PMF centers between the developed and the developing countries.

It should be noted that Africa, through the sole activities of Prof. Rybicki's group, accounts for less than 1% of the world PMF centers. The research activities of his laboratory in the development of plant-derived vaccines have secured a seat for Africa on the plant biotechnology flight! In his review article, published in the January 2009 issue of Drug Discovery Today, and titled "Plant-produced vaccines: promise and reality", Prof. Rybicki illustrated the evolution of the PMF activities in his laboratory, which dates back to 1997 (Rybicki, 2009). It was such a delight to read the success story of PMF technologies in his laboratory, from the early years of little beginnings to the landmark advances in recent times, in the development of plant-produced tumour vaccine, papillomavirus vaccines (Maclean *et al*, 2007; Varsani *et al*, 2006), which could be made available at affordable prices, thereby placing them at the reach of the poor patients. This feat is particularly inspiring, as we all hope that these pioneering activities would eventually rob off on the rest of the continent, with time, especially when biotechnology capacity improves generally.

Building Capacity for Plant biotechnologies in Africa

Discussion on the problem of wide spread inadequacy in infrastructural capacity for biotechnology generally, in Africa cannot be over flogged and the solution to the prevailing dearth of plant biotechnology research and development activities, in particular, in most of the continent, is believed not to be beyond reach. Several models and recommendations have been proposed for taking the continent out of the woods, with respect to plant biotechnology development, in particular (Ayele *et al*, 2006; Delmer, 2005; Konde, 2006; Machuka, 2001; Ozor, 2008; Singh and Daar, 2008; Wambugu, 1999). It remains to be seen whether the various stakeholders in Africa really have the strong will, like other developing countries in Asia and Latin America, to drive this through, and not to be disinterested further by recent external negative attitudes against GM crops, which is keeping the technology out of Africa (Paarlberg, 2008). The impacts of biotechnology on the economic growth of these emerging economies are glaring for all. As such, I believe that biotechnology can also work in Africa if it is working elsewhere. The strategies adopted by some of these other developing economies are worth publicizing, to serve as good templates for Africa's biotechnology development. In this regard, a paper on the biotechnology exploits and bio-economic growth of India was presented at the Knowledge Management Africa (KMA) Conference 2009, which was held in Dakar, Senegal from 4th through to 7th May, 2009 (Obembe and Dike, 2009). Some of the recommendations presented at the Conference, based on India's strategic plans are highlighted below.

i. Deliberate and aggressive awareness campaigns about the new technology, with respect to the potential benefits and to allay public fear over their safety.
ii. Revisiting the educational policies to encourage and stimulate interest of young people in Science and Technology at the primary and secondary level. Also, the redesigning of the curricula at the tertiary level to make Biotechnology courses compulsory component. This agrees other viewpoints that the development of man power base for biotechnology should be long

term local and international trainings and not through workshop and seminars.

iii. Provision of basic infrastructures for low-techniques, such as tissue culture and nucleotide analyses for the Universities, as well as funding of research activities of researchers in these Universities.

iv. Provision of motivation and incentives in order to retain the highly educated human resources and to make those trained overseas return home.

v. Investment in basic infrastructures – reliable power supply, portable water, roads, modern information and telecommunications facilities. ICT Infrastructures in particular, will enhance acquisition of knowledge and its application, and also reduce transaction costs.

vi. Setting up specialized biotechnology centers. This is in line with the NEPAD initiatives of establishing four specialized biotech centers of excellence across Africa. This initiative will ensure capacity building in core and priority areas where expertise and resources already exist.

vii. Establishment of collaborative ventures / Technology Park / incubators with private companies, to facilitate that biotech products get to the market. This sort of venture will eventually be self-funded and also ensure placements for trained workforce. Alternatively, the Government can provide loans to small and medium scale companies that might be interested in such ventures.

viii. Attraction of foreign investments and fostering international partnership and linkages, all of which can only be established when there are functional basic facilities on ground.

ix. Setting up / Strengthening of existing biosafety, regulatory and Intellectual property bodies to formulate more efficient biotechnology policies and guidelines, and also to set up testing and certification facilities.

By and large, for any meaningful change to happen at the national level for instance there must be substantial financial commitment on the parts of the various governments, as there is no segment of the field that is cheap to run. The African countries cannot expect to get the same results as other developing countries when most of them are committing less than 0.01% of their GDP to Science and Technology on the whole, while other countries like India, Korea, Brazil and Cuba are committing more than 1.0% of their GDPs to biotechnology activities alone. The transformations that are being celebrated in the bio-economies of these countries today attest to the saying that "your harvest is a proportion of your sowing".

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Model for Predicting the Concentration of Phosphorus Removed as Impurity during Hydro-Processing of Iron Oxide Ore Designated for Production of Orthopedics Devices

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Abstract: A model for predicting the concentration of phosphorus removed as impurity during leaching of iron oxide ore in sulphuric acid solution has been derived. The model; $P = 10^{4.86\gamma}$ was found to depend on the value of the final pH of the leaching solution which varies with leaching time It was observed that the validity of the model is rooted in the expression $LogP = N\gamma$ where both sides of the relationship are approximately equal to 2.3. The maximum deviation of the model-predicted phosphorus concentration (removed) from the corresponding value obtained from the experiment was found to be less than 8% which is quite within the acceptable deviation limit of experimental results.
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Keywords: Model, Phosphorus Removal, Sulphuric Acid, Iron Oxide Ore, Leaching.

1.0 Introduction

Steel has been found [1] to be chiefly made up of over 95% Fe and carbon less than 1%. Increasing addition of Cr into the Fe and C matrix and structure re-designs the steel to stainless steel. Addition of nickel to the stainless steel microstructure causes the austenite structure to be maintained at room temperature hence producing austenitic stainless steel [2]. It has been found [2] that 316 stainless steel finds application in early hip implants due to its good strength, ability to work harden and pitting corrosion resistance. The mechanical properties of stainless steel includes: ultimate tensile strength; 190-690Mpa and elongation; 12-40% [2]. Stainless steel usage in biomedical engineering is restricted to temporary device such as screws, plates, fittings and wires for orthopaedics due to potential long term release of Ni^{2+}, Cr^{3+}, and Cr^{6+} into the body [2].

Presence of phosphorus in iron designated for production of engineering materials has been found to enhance embrittlement when these materials are put into use. Furthermore, application of iron with high phosphorus content for production of orthopedic devices endangers the health of the patient involved since phosphorus is very poisonous. This formed the basis for several attempts already made to reduce the concentration of phosphorus inherent in iron oxide ores designated for production of engineering materials.

Several works [3-8] have been carried out to remove phosphorus from steel during steel making. All these works carried out, pointed out low treatment temperature and high oxygen activity as the only essential and unavoidable process conditions which can enhance the rate of dephosphorization. High activity of CaO; a product of decomposition of $CaCO_3$ and a slag forming material is required for enhancement of the dephosphorization process with the phosphorus forming part of the slag. This process involves pyrometallurgy and is capital intensive.

It has been reported [9] that the removal of phosphorus from iron can be achieved only by oxidation during steel making, under a basic slag.

Nwoye [10] derived a model for predicting the time for dissolution of pre-quantified concentration of phosphorus during leaching of iron oxide ore in oxalic acid solution as:

$$\tau = \frac{\text{Log}\left(\frac{P^{1/4}}{1.8}\right)}{\text{LogT}} \qquad (1)$$

Where

T= Leaching temperature (^{0}C) in the experiment [11], taken as specified leaching temperature (^{0}C) aiding the expected dissolution of phosphorus .

N= 1.8 (Dissolution coefficient of phosphorus in oxalic acid solution during leaching of iron oxide ore) determined in the experiment [11].

P = Concentration of dissolved phosphorus (mg/Kg) in the experiment [11], taken as pre-quantified concentration of phosphorus expected to dissolve after a leaching time t (mg/Kg) in the model.

τ = Leaching time (sec.) in the experiment [11], taken as time for dissolution of the pre-quantified concentration of phosphorus (hrs) in the model.

The model was found to depend on a range of specified leaching temperatures (45-70^{0}C) for its validity. It was found [11] that the time for dissolution of any given concentration of phosphorus decreases with increase in the leaching temperature (up to 70^{0}C), at initial pH 5.5 and average grain size of 150μm.

Nwoye et al. [12] also formulated a model for predicting the concentration of phosphorus removed during leaching of iron oxide ore in oxalic acid solution. The model is expressed as;

$$P = 150.5/\mu\alpha \quad (2)$$

It was found to predict the removed phosphorus concentration, with utmost dependence on the final pH of the leaching solution and weight input of the iron oxide ore. The model indicates that the concentration of phosphorus removed is inversely proportional to the product of the weight input of the iron oxide ore and the final pH of the leaching solution. Process conditions considered during the formulation of the model [12] include: leaching temperature of 25^{0}C, initial solution pH 5.5 and average ore grain size; 150μm).

Nwoye [13] derived a model for the evaluation of the concentration of dissolved phosphorus (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution. It was observed that the validity of the model is rooted in the relationship $\ln P = N/\alpha$ where both sides of the expression are approximately equal to 4. The model expressed as;

$$P = e^{(12.25/\alpha)} \quad (3)$$

depends on the value of the final pH of the leaching solution which varies with leaching time. In all, the positive or negative deviation of the model-predicted phosphorus concentration from its corresponding value obtained from the experiment was found to be less than 22%.

Nwoye [14] also derived a model for predicting the concentration of phosphorus removed during leaching of iron oxide ore in oxalic acid solution. The model is expressed as;

$$P = [(1.8(T)^{\tau})]^4 \quad (4)$$

was found to be dependent on leaching temperature ranging from 45-70^{0}C and specified leaching time of 0.1381hr (497secs.) recorded during experiment, for its validity. It was found that the validity of the model is rooted in the expression $(P^{1/4})/N = (T)^{\tau}$ where both sides of the expression are correspondingly almost equal. The maximum deviation of the model-predicted values of P from the corresponding experimental values was found to be less than 29% which is quite within the range of acceptable deviation limit of experimental results.

Model for predictive analysis of the concentration of phosphorus removed (relative to the initial and final pH of the leaching solution) during leaching of iron oxide ore in sulphuric acid solution has been derived by Nwoye and Ndlu [15]. It was observed that the validity of the model is rooted in the mathematical expression; $(P/N)^{1/3} = (e^{\gamma/\alpha})$ where both sides of the relationship are almost equal. The model;

$$P = 4.25(e^{\gamma/\alpha})^3 \quad (5)$$

shows that the concentration of phosphorus removed is dependent on the values of the initial and final pH of the leaching solution.

Biological processes for phosphorus removal have also been evaluated based on the use of several types of fungi, some being oxalic acid producing. Anyakwo and Obot [16] recently presented their results of a study on the use of Aspergillus niger and their cultural filtrates for removing phosphorus from Agbaja (Nigeria) iron oxide ore. The results of this work [16] show that phosphorus removal efficiencies at the end of the 49 days of the leaching process are 81, 63 and 68% for 5, 100 and 250 mesh grain sizes respectively.

An attempt has been made in the past [17] to leach Itakpe iron oxide ore using oxalic acid solution in order to determine the maximum concentration of phosphorus that is removable. Results of chemical analysis of the ore indicate that the percentage of phosphorus in the ore is about 1.18%, which from all indication is quite high and likely to affect adversely the mechanical properties of the steel involved; hence the need for dephosphorization. It was reported [17] that phosphorus can be removed from this iron oxide ore through a process associated with hydrometallurgy. Phosphorus was removed at a temperature of 25^{0}C and initial solution pH 2.5, leading to the dissolution of the phosphorus oxide formed. This involved using acid leaching process to remove phosphorus from the iron oxide ore in readiness for steel making process.

The aim of this work is to derive a model for predicting the concentration of phosphorus removed (as impurity) relative to the final pH of the solution during leaching (hydrometallurgical processing) of Itakpe (Nigerian) iron oxide ore using sulphuric acid solution. This derivation is embarked on in furtherance of the previous work. [17]

The solid phase (ore) is assumed to be stationary, contains the un-leached iron remaining in the ore.

2. Model

Hydrogen ions from the sulphuric acid attack the ore within the liquid phase in the presence of oxygen.

2.1 Model Formulation

Experimental data obtained from research work [17] carried out at SynchroWell Research Laboratory, Enugu were used for this work.

Results of the experiment as presented in report [17] and used for the model formulation are as shown in Table 1.

Computational analysis of the experimental data [17] shown in Table 1, resulted to Table 2 which indicate that;

$$\text{LogP} = N\gamma \quad \text{(approximately)} \qquad (6)$$

$$P = 10^{N\gamma} \qquad (7)$$

Introducing the value of N into equation (7)

$$P = 10^{4.86\gamma} \qquad (8)$$

where

P = Concentration of phosphorus removed during the leaching process (mg/Kg)

N= 4.86; (pH coefficient for phosphorus dissolution in sulphuric acid solution) determined in the experiment. [17]

γ = Final pH of the leaching solution at the time t when the concentration of phosphorus removed is evaluated.

Equation (8) is the derived model**.**

3. Boundary and Initial Condition

Consider iron oxide ore in cylindrical flask 30cm high containing leaching solution of sulphuric acid. The leaching solution is stationary i.e (non-flowing). The flask is assumed to be initially free of attached bacteria. Initially, atmospheric levels of oxygen are assumed. Varying weights (4-14g) of iron oxide ore were used as outlined in Table 1. The initial pH of leaching solution; 0.52 and leaching time of 30minutes were used for all samples. A constant leaching temperature of 25^{o}C was used. Ore grain size; 150µm, volume of leaching solution; 0.1 litre and sulphuric acid concentration; 0.1mol/litre were used. These and other process conditions are as stated in the experimental technique [17].

The boundary conditions are: atmospheric levels of oxygen (since the cylinder was open at the top) at the top and bottom of the ore particles in the liquid and gas phases respectively. At the bottom of the particles, a zero gradient for the liquid scalar are assumed and also for the gas phase at the top of the particles. The leaching solution is stationary. The sides of the particles are taken to be symmetries.

Table1: Variation of final solution pH with concentration of phosphorus removed. [17]

M (g)	γ	P (mg/Kg)
4	0.48	214.00
6	0.47	203.00
8	0.48	199.75
10	0.48	232.25
12	0.47	198.00
14	0.47	183.00

Where M = Mass of iron oxide ore used for the leaching process (g).

Table 2: Variation of LogP with Nγ

M (g)	LogP	Nγ
4	2.3304	2.3328
6	2.3075	2.2842
8	2.3005	2.3328
10	2.3660	2.3328
12	2.2967	2.2842
14	2.2625	2.2842

4. Model Validation

The formulated model was validated by direct analysis and comparism of the model-predicted P values and those from the experiment [17] for equality or near equality. Analysis and comparison between these P values reveal deviation of model-predicted P values from the corresponding experimental P values. This is believed to be due to the fact that the surface properties of the ore and the physiochemical interactions between the ore and leaching solution which were found to play vital roles during the leaching process [17] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted P values to that of those of the experiment.

Deviation (Dv) (%) of model- predicted P values from experimental P values is given by

$$Dv = \left(\frac{Dp - DE}{DE} \times 100\right) \qquad (9)$$

Where Dp = Model-predicted P values

DE = Experimental P values

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \qquad (10)$$

Therefore

$$Cf = -100 \left[\frac{Dp - DE}{DE} \right] \quad (11)$$

Introduction of the corresponding values of Cf from equation (11) into the model gives exactly the corresponding experimental P values. [17]

5. Results and Discussion

The derived model is equation (8). A comparison of the values of P from the experiment and those from the model (Table 3) shows very insignificant positive and negative deviations, hence depicting the reliability and validity of the model. The maximum deviation of the model-predicted P values from those of the experiment is less than 8% which is quite within the acceptable deviation limit of experimental results. The validity of the model is believed to be rooted on equation (6) where both sides of the equation are approximately equal to 2.3. Table 2 also agrees with equation (6) following the values of LogP and Nγ obtained after statistical and computational analysis were carried out on the experimental results in Table 1. A comparison of these two concentrations of removed phosphorus (Tables 1 and 3) from the iron oxide ore shows proximate agreement. The close alignment of the curves; ExD and MoD in Figure 1 also shows proximate agreement between experimental [17] and model-predicted values of removed phosphorus. This indicates a very high degree of validity for the model as a reliable tool for predicting the concentration of phosphorus removed as impurity during hydrometallurgical processing of iron oxide ore designated for production of orthopedic devices.

Table 3: Variation of the model-predicted concentration of removed phosphorus with the associated deviation and correction factor.

P_M (mg/Kg)	Dv (%)	Cf (%)
215.18	+0.55	-0.55
192.40	-5.22	+5.22
215.18	+7.72	-7.72
215.18	-7.35	+7.35
192.40	-2.83	+2.83
192.40	+5.14	-5.14

P_M = P values predicted by model.

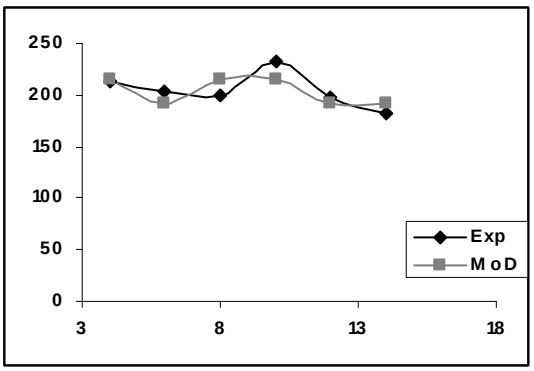

Figure 1. Comparison of the concentrations of phosphorus removed as obtained from experiment (line ExD) [17] and derived model (line MoD).

6. Conclusion

The model predicts the concentration of phosphorus removed (relative to the final solution pH) as impurity during hydrometallurgical processing of iron oxide ore designated for production of orthopedic devices. The validity of the model is believed to be rooted on the expression $LogP = N\gamma$ where both sides of the expression are approximately equal to 2.3. It was observed that the maximum deviation of the model-predicted P values from those of the experiment is less than 8% which is quite insignificant and within the acceptable deviation limit of experimental results.

Further works should incorporate more process parameters into the model with the aim of reducing the deviation of the model-predicted P values from those of the experiment.

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Sorption Energies Estimation Using Dubinin-Radushkevich and Temkin Adsorption Isotherms

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Abstract: In this study, we add to scholarly knowledge in simple terms, the forces or energy defining certain adsorption phenomenon, using isotherm models. GCMS measurement of equilibrium phase atrazine after adsorption onto Sheanut shells (SS) acid derived activated carbon were fitted into the D-R and Temkin isotherm relationships for energy data estimation. Sorption energy value (B_D), mean free energy (E_D) and heat of sorption (B). They were estimated as $0.7600 mol^2KJ^{-2}$, 0.8111 $kjmol^{-1}$ and 0.790$Jmol^{-1}$ respectively. The parameter predicting the type of adsorption was evaluated B_D , B < 20kJ/mol and E_D<8 which is an indication that physisorption (Non specific adsorption) dominates chemisorption and ion exchange. The D-R model with a higher correlation coefficient values, R^2 = 0.979 proves a better choice in explaining sorption energies. Generally, sheanut shells can be used as alternative precursors for activated carbon production via the two steps and acid treatment schemes.

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Key words: Dubinin-Radushkevich , Temkin, Adsorption, GCMS, Isotherm, Sorption energy

1. Introduction

It was reported that quality evaluation of activated carbon with adsorption capacity, intensity ,thermodynamics and kinetics parameters among others are as vital as the estimation of certain energy parameters namely mean free energy, sorption energy etc. An approximation from such energy parameter gives a clue as to the type of adsorption in question.

Previous studies showed that the herbicide, atrazine disrupt the production and functionality of human hormones and a higher incidence was reported for cases of cancer in humans and laboratory animals (Zhongren *et al*., 2006). The use of active carbon was prescribed by USEPA as the best available technology for the removal of atrazine from drinking water. Many studies have revealed adsorption of atrazine using conventional activated carbon granules and fibres.

A chemical activation using activating agents is a new generation of adsorption fibre development sorbents obtained with this method provides higher yield, high surface area, high mesopores volumes and some unusual pore surface chemistries (Zhongren *et al*., 2006; WHO, 1999; Hertrick *et al*., 2000).The sorption of herbicide aqueous phase by activated carbon has been reported (Agdi *et al*.,2000;WHO,1999; Zhongren *et al*., 2006). This present study reports atrazine sorption, not in a micro quantity but within range that could account for both the topical and systemic poisoning reportedly associated with atrazine (Raymond, 2003).

Basics of adsorption : Adsorption is actually a mechanism in which the forces of interaction between surface atoms and the adsorbate molecules are similar to Van der Waals forces that exist between all adjacent molecules .There are both attractive forces and repulsive forces with the net force depending on the distance between the surface of the adsorbent and the adsorbate molecule (Cooney,1999).

In other words,adsorption is a process in which a soluble chemical (the adsorbate) is removed from a fluid (liquid or gas) by contact with a solid surface (the adsorbent).It is the trapping of impurities by strong physical bond within the porous structure of Carbon. It is one of the many processes used to purify, concentrate, or separate component (Glenn,1993) and competes with other processes like evaporation, solvent extraction, distillation, sublimation, drying, sedimentation, filteration, screening, ion exchange, centrifugation, and absorption (Glenn,1993).It is used in industry for product separation and waste treatment. In general, adsorption is the process by which a component moves from one phase to another while crossing

some boundary. It was found that the observed effect of adsorption was achieved within porous solids and that adsorption was the result of interactive forces of physical attraction between the surface of porous solids and component molecules being removed from the bulk phase (Arun, 2002).

Improved Adsorption via Surface Activation: The surface of an adsorbent is typically composed of various surface functional groups (SFG). Adsorption of organic adsorbate is greatly dependent on the amount and nature of surface oxide groups (Cooney, 1999). Surface functional groups (carbon/oxygen) are created by oxidation occurring during the activation process of an adsorbent. Some of the common basic functional groups created are lactones, quinones, and carboxylates (Arun, 2002). Some of the common acidic functional groups created are phenolic, hydroxyl, carbonyl, and carboxylic acids (Arun, 2002). The presence of oxygen-containing basic groups such are a key factor in promoting irreversible adsorption (Vidic *et al.*, 1993).Strongly dissociated adsorbates are weakly adsorbed when compared to nondissociated adsorbates (Cooney, 1999). The more non polar an adsorbate, the higher the adsorption capacity. This is attributed to the fact these adsorbate molecules tend to prefer the adsorbent surface rather than being in the solution (Cooney, 1999). It has also been shown that an increase in the molecular weight of the adsorbate will generally enhance adsorption until the size of the adsorbate is larger than the pore size of the adsorbent. Typically, aromatic compounds are more adsorbable than aliphatic compounds of similar molecular size and branched-chain molecules are generally more adsorbable than straight-chain molecules (Cooney, 1999). In addition, solubility of the adsorbate is also an important factor. In general, the lower the solubility of the adsorbate, the higher the adsorption capacity since the forces of attraction between the adsorbate molecules and the adsorbent surface molecules will be greater than the forces of attraction between the adsorbate and the solvent molecules (Cooney, 1999).

Types of Adsorption based on Energy values

1. Chemisorption: This is a shorter way of writing chemical adsorption. It is also called specific adsorption and limited to monolayer coverage of the substrate. Here, a covalent bond is formed between the adsorbate and adsorbent. The enthalpy of chemisorption is within the range of 200kJ/Mol (Atkins, 1999)

2. Physisorption: This stands for physical adsorption. It is also called non specific adsorption which occur as a result of long range weak Van der Waals forces between adsorbates and adsorbents. The energy released when a particle is physisorbed is of the same magnitude as the enthalpy of condensation. The enthalpy of physisorption is measured by monitoring the rise in temperature of a sample of known heat capacity. Typical values of in the region of 20kJ /Mol (Atkins, 1999).

Chemisorption occurs at high temperatures with a significant activation energy, which involves strong bonds and is not reversible. The heat of adsorption is typically high in chemisorption and is similar to heat generated during a chemical reaction. There are several factors that impacts physical adsorption (LaGrega *et al.*, 1994; Cooney, 1999). The major factors which affect physical adsorption include the surface area of the adsorbent, pore structure of the adsorbent, surface chemistry of the adsorbent, nature of the adsorbate, pH of the solution, and the presence of competing adsorbates. It is due to these factors, physical adsorption is considered to be a complex phenomena. Surface area of the adsorbent is one of the most important factors on which adsorption greatly depends. The surface area is comprised of two types, the external surface area and the internal surface area (pore walls). When molecules are larger than the pore diameter, lesser adsorption would take place because of steric hindrances.

Adsorption isotherm: Isotherm are empirical relationship used to predict how much solute can be adsorbed by activated carbon (Steve and Erika, 1998). Chilton *et al.*,(2002) defined Adsorption isotherm as a graphical representation showing the relationship between the amount adsorbed by a Unit weight of adsorbent (eg activated carbon) and the amount of adsorbate remaining in a test medium at equilibrium. It maps the distribution of adsorbable solute between the liquid and solid phases at various equilibrium concentration (Chilton *et al.*, 2002).The adsorption isotherm is based on data that are specific for each system and the isotherm must be determined for every application. An adsorption isotherm beside providing a panorama of the course taken by the system under study in a concise form indicate how efficiently a carbon will allow an estimate of the economic feasibility of the carbons' commercial application for the specific solute (Chilton *et al.*, 2002).

Dubinin-Radushkevich isotherm: This isotherm model was chosen to estimate the characteristic porosity of the biomass and the apparent energy of adsorption. The model is represented by the equation 1 below:

$qe = q_D \exp(- B_D [RT \ln (1 + 1/Ce)$ (1)

Where, B_D is related to the free energy of sorption per mole of the sorbate as it migrates to the surface of the biomass from infinite distance in the solution and q_D is the Dubinin-Radushkevich isotherm constant related to the degree of sorbate sorption by the sorbent surface (Horsfall *et al.*, 2004;Itodo *et al*.,2009b). The linear form of equation is given as 2;

$\ln qe = \ln q_D - 2B_D RT \ln (1 + 1/Ce)$ (2)

A plot of ln qe against RT ln (1 + 1/Ce) for modified sorbents, yielded straight lines and indicates a good fit of the isotherm to the experimental data. The apparent energy (E_D) of adsorption from Dubinin-Radushkevich isotherm model can be computed using the relation given as 3 below (Horsfall *et al.*, 2004).

$E_D = \sqrt{1/2B_D}$ (3)

Temkin adsorption Isotherm: The Temkin was tested for equilibrium description at room temperature. The model was respectively represented by equations 4 and 5 below. Therefore, by plotting *q*e versus ln *C*e , enables the determination of the constants *A* and B.B is the Temkin constant related to heat of sorption (J/mol), *A* is the Temkin isotherm constant (L/g), *R* the gas constant (8.314 J/mol K), *b* is Temkin isotherm constant and *T is* the temperature (K). (Nunes *et al*., 2009; Hameed,2009).

$q_e = B \ln (A+C_e)$ (4)

Where $B = RT/b$ (5)

Aim of this work: In this present work, chemically activated carbon was formulated to adsorb atrazine traces from water. This research was based on an initial qualitative study based on atrazine sorption as earlier predicted by FTIR analysis (Itodo *et al*.,2009a). The specific objectives include; Generation of activated carbon thereby adding values to the wastes. Sorption energy studies, (Evaluation of the mean free and sorption energies as would be predicted by Temkin isotherm and R-D isotherm models.

2. MATERIALS AND METHODS

Choice of equipment: The gas chromatographic technique is at best a mediocre tool for qualitative analysis. It is best used with other technique to answer the question of what is present in a sample. Besides the simplicity of the instrument, ease of operation, GC also provides the answer to how much? It is an excellent quantitative analytical tool in quantifying micrograms in a litre or one volume in millions of volumes (Robert and Eugene, 2004).The sample herbicide (containing atrazine) is a multicomponent mixture containing atrazine (test sample) and other organochlorine moieties, which are very similar to atrazine. Secondly, the GC column has a very high efficiency which was claimed to be in excess of 400,000 theoretical plates. The column is about 100m long, a very dispersive type of stationary phase retaining the solute approximately in order of increasing boiling point. Helium carrier gas was selected since it can realize high efficiencies with reasonable analysis time (Raymond, 2003). Techniques of external standardization entails the preparation of standards at the same levels of concentration as the unknown in the same matrix with the known. These standards are then run chromatographically under ideal conditions as the sample. A direct relationship between the peak size and composition of the target component is established and the unknown was extrapolated graphically

Sample treatment and preparations: The method of sample treatment by Fan *et al*., (2003); Itodo *et al* .,(2009a&b) were adopted. The samples were washed with plenty of water to removes surface impurities and sundried, then, dried in an oven at $105^{O}C$ overnight (Omonhenle *et al.,* 2006). The samples were separately pounded/ grounded followed by sieving with a <2mm aperture sieve. The less than 2mm samples were stored in airtight containers. About 3g of each pretreated biosolid (< 2mm mesh size) were introduced into six (6) different clean and pre weighed crucibles. They were introduced into a furnace at $500^{O}c$ for 5 minutes after which they were poured from the crucible into a bath of ice block. The excess water was drained and the samples were sun dried. This process was repeated until a substantial amount of carbonized samples were obtained (Gimba *et al.,* 2004). The carbonized sample was washed, using 10% HCl to remove surface ash, followed by hot water wash and rinsing with distilled water to remove residual acid (Fan *et al*., 2003) the solids were then sun dried, then, dried in the oven at $100^{O}C$ for one hour . Accurately weighed 2g each of already carbonized samples were separately mixed with $2cm^3$ of each 1M activating agent (H_3PO_4 and $ZnCl_2$). The samples were introduced into a furnace, heated at $800^{O}c$ For 5 minutes. The activated samples were cooled with ice cold water. Excess water was drained and samples were allowed to dry at room temperature (Gimba *et al.,* 2004,). The above procedure was

repeated for different residual time (5min and 15 min).Washing of the above sample was done with 10% HCl to remove surface ash, followed by hot water and rinsing with distilled water to remove residual acid (Fan *et al*, 2003). Washing was completed when pH of the supernatant of 6-8 was ascertained (Ahmedna *et al.*, 2000). The sample were dried in an oven at 110^{O}C overnight and milled or grounded, followed by filtration to different mesh size and stored in air tight container.

Atrazine standard solution for equilibrium studies: For the sorption/equilibrium studies, several concentrations viz; 10, 20, 30, 40 and 50g/L Herbicide equivalent of 5, 10, 15, 20 and 25g/L Atrazine was prepared by respectively dissolving 0.25, 0.5, 0.75, 1.0 and 1.25g of herbicide into a conical flask, poured gently into a 25cm^3 volumetric flask, homogenized and made to the mark with chloroform (i.e. 5,000ppm – 25,000ppm atrazine).A three point calibration curved based on external standard method was prepared with the GC/MS run.

Batch equilibrium experiment: 5g of substrate was diluted to the mark of 100cm^3 volumetric flask. This concentration of 50g/L herbicide is equivalent to 25g/L or 25,000ppm atrazine stock. 10cm^3 of the atrazine solution was interacted with 0.1g of each sorbent and allowed to stand for 12hours. The mixture was filtered and the filtrate was analyzed with a gas chromatography (coupled with a mass spectrophotometer detector) for atrazine equilibrium phase concentration (Min and Yun, 2008; Agdi *et al.*, 2000). The amount of atrazine at equilibrium, q_e was calculated from the mass balance equation given in equation 6 by Hameed *et al.* ,(2006).

$$q_e = (C_O - C_e)\, V/W \qquad (6)$$

where C_o and C_e are the initial and final Dye concentrations (mg/L) respectively. V is the volume of dye solution and M is the mass of the acid catalyzed Poultry waste sorbent (g). while t is the equilibrium contact time, when q_e = qt, equation 6 will be expressed as equation 7 below:

$$q_t = (C_o - C_t)v/w \qquad (7)$$

where $q_e = q_t$ and C_t is the concentration at time,t. The percent dye removal (RE %) was calculated for each equilibration by the expression presented as equation 8

$$R\,E(\%) = (C_0 - C_e)/Co \times 100 \qquad (8)$$

Where RE (%) is the percent of dye adsorbed or removed. The % removal and adsorption capacities were used to optimize the activation condition. The test were done at a constant temperature of 27 ± 2^0C.(Rozada *et al.*,2002). The equilibrium concentration of atrazine (herbicide), q_e and Adsorption efficiency (% Removal) were estimated. The extent of atrazine removal (by difference) from chloroform spiked with 25g/L of atrazine was expressed as equation 3 (Hameed *et al.*, 2006).

3. Results

The chromatogram shown as Figure 1 stands for unadsorbed sorbate out of the 5g/dm^3 atrazine which was interacted with SS/A sorbent. The chromatogram was characterized by a baseline disturbance. This is caused by either hydrocarbon impurities or by impure carrier gas (Robert and Eugene, 2004).The former could be linked to the fact that the sorbate concentration (5g/dm^3) is too low for the 0.1g carbon dose. Unoccupied pore size could as well, lead to desorption of the sorbate with a resultant poor percentage removal (46.08%).

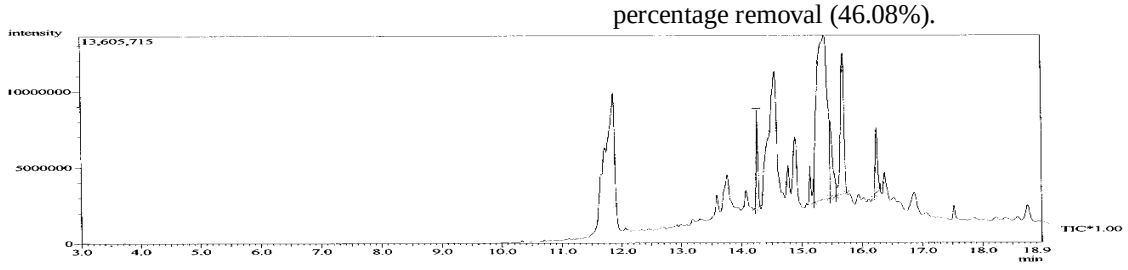

Figure 1: GC/MS chromatogram of equilibrium concentration atrazine after adsorption onto SS/A/5gL^{-1} sorbent (*Carrier gas-Helium 100.2kpa,Column temperature –60^{o}c,Injection temperature-250^{o}c,Injection volume—1µL,Flow rate-1.61mL/min, Injection method- split, Linear velocity- 43.6cm/sec.*)

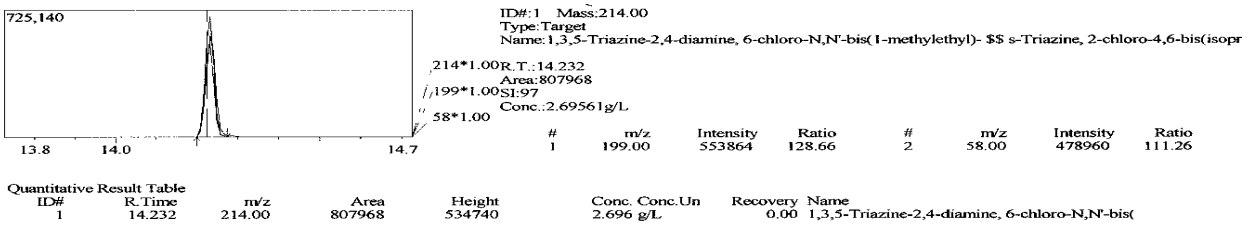

Figure 2: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/5gL^{-1} sorbent

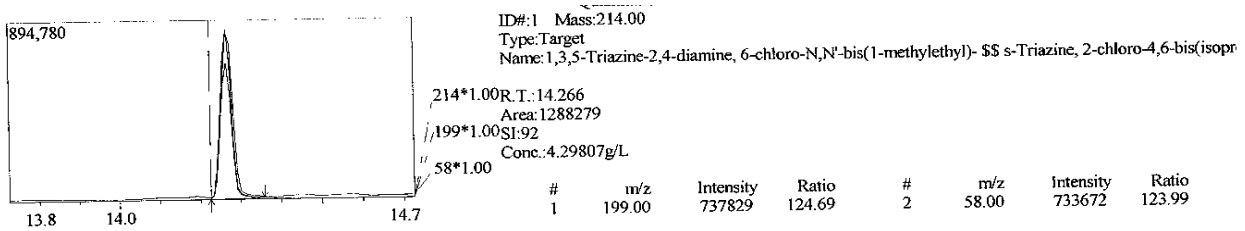

Figure 3: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/10gL^{-1} sorbent

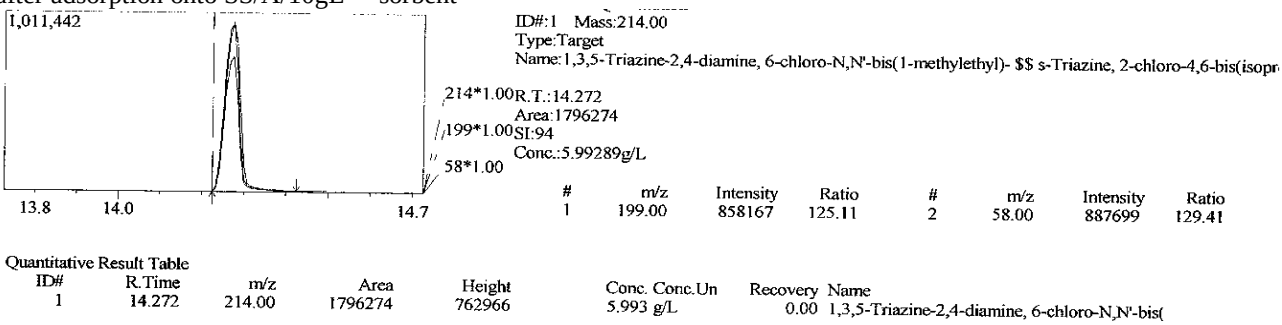

Figure 4: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/15gL^{-1} sorbent

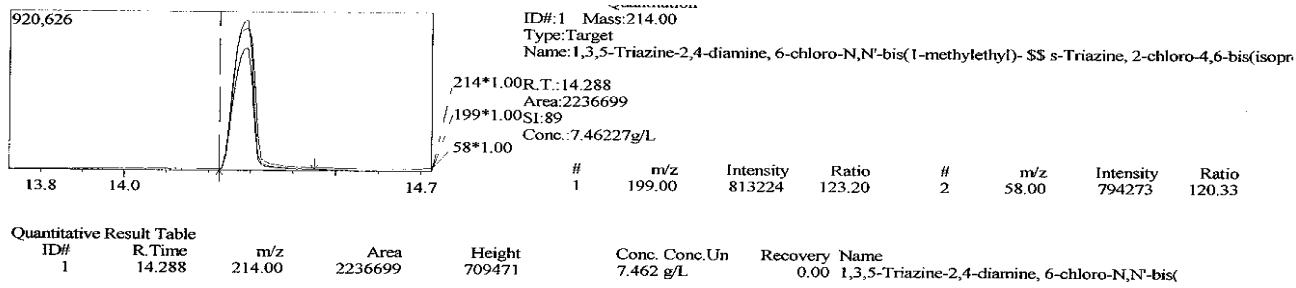

Figure 5: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/20gL^{-1} sorbent

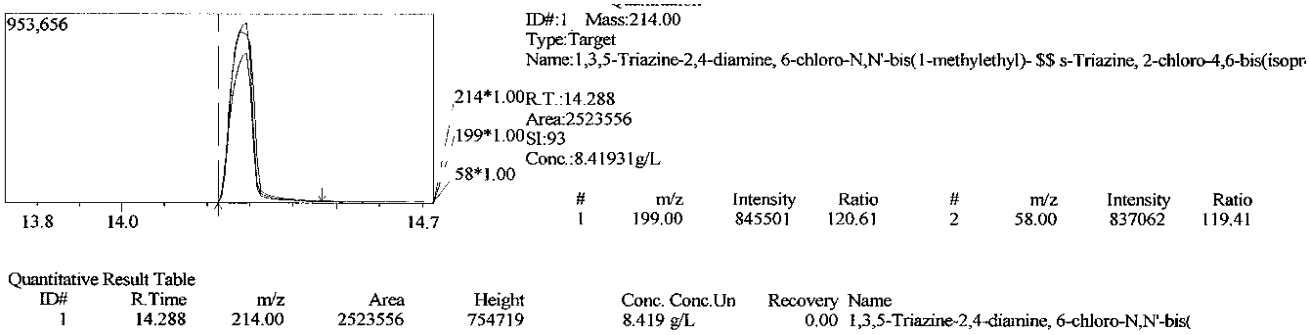

Figure 6: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/25gL^{-1} sorbent

Equilibrium experimental data from Figures 2 to 6 were treated as Table 1

Table 1: Adsorption experimental data of atrazine uptake by fixed mass of SS-Sorbents at different initial sorbate concentration, using GC/MS

Sorbent	Co (g/dm^3)	Ce (g/dm^3)	Ca (g/dm^3)	% RE	Ads.m (mg.10^{-3})	q_e (mg/g x 10^{-3})	Kc	ΔG (kJ/mol)
SS/A/5	5	2.696	2.304	46.08	0.0230	0.230	0.854	+393.941
SS/A/10	10	4.298	5.702	57.02	0.0570	0.570	1.327	-705.381
SS/A/15	15	5.993	9.007	60.047	0.0901	0.901	1.503	-1016.670
SS/A/20	20	7.462	12.558	62.69	0.1254	1.254	1.680	-1294.989
SS/A/25	25	8.419	16.581	66.324	0.1658	1.658	1.969	-1691.330

SS/A/15 -Sheanut shells, treated with H_3PO_4,activated for 15 minute dwell time, **SS/A/25** -Sheanut shells, treated with H_3PO_4,activated for 25 minute dwell time

4. Discursion

Effect of initial atrazine concentration on removal efficiency

Table 1 presents the role played by initial sorbate concentration and its effect on sorption efficiency. The highest percentage atrazine removal was observed with the interaction of 0.1g atrazine with 10Cm^3 of a 25g/L atrazine solution. Hence, out of 25g/L, 20g/L, 15g/L, 10g/L and 5g/L initial atrazines concentration, a total of 16.581, 12.538, 9.007, 5.702 and 2.696g/L atrazine was attracted onto the sheanut shell (SS) bioadsorbent. These accounts for a 66.324, 62.69, 60.007, 57.020 and 46.080% removal efficiency respectively. Findings in this research showed that for the selected time (1hour interaction) and within the 0.1g sorbent dose on 10Cm^3 sorbate solution, (i) Adsorption efficiency increases with initial sorbate concentration.

(ii) Adsorption within the low sorbate concentration (5 – 10g/dm^3) range could possibly be followed by desorption. Hence, a less than 50% adsorption was investigated.

(iii) Adsorption of fairly high concentrated atrazine (15 – 25g/L) could be governed by a multilayer adsorption with resultant intraparticle attraction. In light of this, sorption efficiency or percentage sorbate uptake is greater than 60%.

Sorption isotherm modeling

The regression equation and R^2 values for Dubinin-Radushkevich model was observed that this isotherm also gave very good description of the sorption process, over the range of concentration studied. The apparent energy of adsorption and Dubinin-Radushkevich isotherm constants are shown on Table 2 and obtained from the plot of type in figure 7. The high values of q_D shows high sorption capacity. The values of the apparent energy of adsorption also depict physisorption process.

Table 2 - Temkin and R-D adsorption experimental GC/MS data for atrazine uptake by chemically modified SS-sorbent.

ISOTHERMS	Relationship (Y=)	R^2	Parameters (constant)	Values.
Temkin	0.790x _ 0.947	0.927	b (Unitless) B(J/mol) A(Lg^{-1})	3158.794 0.790 3.316
R-D	-9.466x-2.360	0.979	q_D (mgg^{-1}) B_D ($Mo1^2$ KJ^{-2}) E_D(kjmo1-2)	$4.37x10^{-3}$ 0.7600 0.8111

The two isotherms experimental data on Table 2 were used to estimate certain energy parameters. The Dubinin-Radushkevich, (R-D) isotherm model is more general than the Langmuir isotherm as its deviations is not based on ideal assumptions such as equipotential of sorption sites, absence of steric hindrances between sorbed and incoming particles and surface homogeneity on microscopic level (Monika *et al*,. 2009). The estimated constant, B_D, related to adsorption energy was presented as 0.7600 mol^2kJ^2. This constant gives an idea about the mean free energy which was valued as E_D= 0.811 $kJmol^{-1}$. E_D is a parameter used in predicting the type of adsorption. An E_D value < 8 $kJmol^{-1}$ is an indication of physisorption (Monika *et al*., 2009).

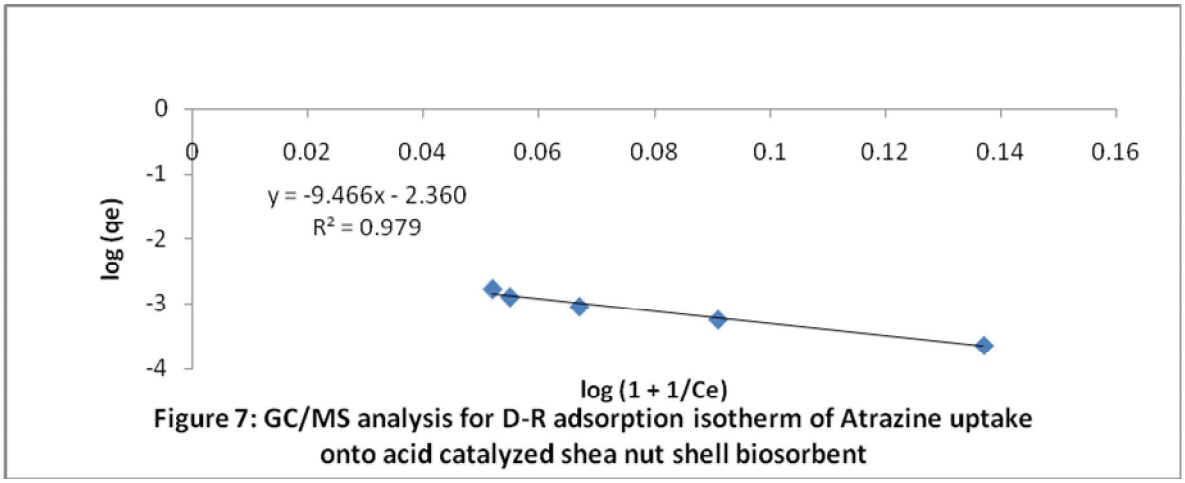

Figure 7: GC/MS analysis for D-R adsorption isotherm of Atrazine uptake onto acid catalyzed shea nut shell biosorbent

The R-D Theoretical saturation capacity, q_D and the Langmuir maximum adsorption capacity, q_m were both estimated as q_D = $4.37x10^{-3}mgg^{-1}$ and q_m = $0.772x10^{-3}$ mgg^{-1}. No known and proven reference could make us conclude that the theoretical saturation capacity, q_D is always higher than the maximum adsorption capacity as the case was made evidence in this research.

The Temkin constant related to heat of sorption, B was estimated as 0.790 J/mol. The unit less quantity, b (3158.794) and Temkin constant, A (3.3159 Lg^{-1}) were in good agreement with values presented by Hameed, (2009) on the Evaluation of papaya seed as a non conventional low cost adsorbent for removal of dye.

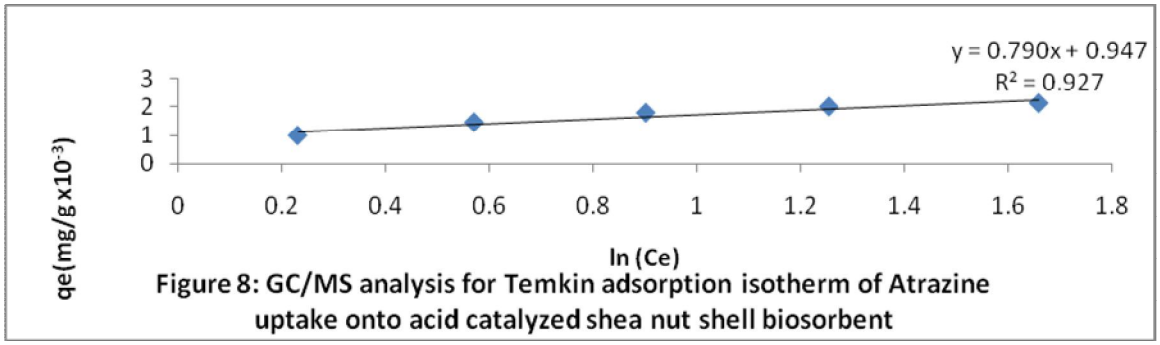

Figure 8: GC/MS analysis for Temkin adsorption isotherm of Atrazine uptake onto acid catalyzed shea nut shell biosorbent

In the energy parameter models, R-D and Temkin were used to present sorption energy value (B_D), mean free energy (E_D) and heat of sorption (B). They were estimated as 0.7600mol^{2}KJ^{-2}, 0.8111 kjmol^{-1} and 0.790Jmol^{-1} respectively, the parameter predicting the type of adsorption was evaluated as E_D which is an indication of physisorption dominates chemisorption, ion exchange etc. The R-D model with a higher correlation coefficient values, R^2 = 0.979 proves a better choice in explaining sorption energies. Besides the E_D validation test, The mean free and energy values and heat of sorption values (in kJ/mol) were lower than 20KJ/mol. This ,according to Atkins,(1999) is characteristics for physisorption. In their report, physisorption is also called non specific adsorption which occur as a result of long range weak Van der Waals forces between adsorbates and adsorbents. The energy released when a particle is physisorbed is of the same magnitude as the enthalpy of condensation. The enthalpy of physisorption was however measured by monitoring the rise in temperature of a sample of known heat capacity to give typical values of in the region of less than 20kJ /Mol (Atkins, 1999).He also argued that Chemisorption is a specific adsorption and limited to monolayer coverage of the substrate. Here, a covalent bond is formed between the adsorbate and adsorbent. The enthalpy of Chemisorption is within the range of 200kJ/Mol (Atkins, 1999).Reports (Unpublished) on surface coverage in this experiment fitted best into the Freundlich Isotherm (sorption on heterogeneous surface).Hence, adsorption is not restricted to monolayer coverage as purposed for Chemisorption.

Conclusion

Generally, sheanut shells can be used as alternative precursors for activated carbon production via the two steps and acid treatment method. On the same fashion, sorption quantification was feasibly observed for a multicomponent system (Herbicide) with a gas chromatography coupled with mass spectrophotometer as detector when the simulated concentration was established via the external standard method. A physorption type of adsorption was predicted for the process.

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Periphyton Algae Dynamics at the University of Lagos Shoreline In Relation To Physico-chemical characteristics

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ABSTRACT: Periphyton algae dynamics at the University of Lagos shoreline in relation to physico-chemical parameters were investigated for a period of 12 weeks (26th January – 13th April 2007). The physical and chemical factors showed weekly variation judged to be linked to tidal sea water incursion associated with the Lagos lagoon and rain events in the dry season. Water quality was brackish (mesohaline) and alkaline throughout the study. Of the 30 periphytic algal species recorded, 18 were diatoms, 9 blue-green algae, 3 green algae and 1 euglenoid species. With regard to density of species, the blue-green algae recorded 92.89%, while the green algae recorded 5.95% and the diatom and euglenoids recorded 1.11 and 0.06% respectively. Species abundance was generally higher at periods of reduced rainfall volume. It is possible that hydrological stability was favourable at this time and assisted the development of the periphyton community (January through April). Conversely micro-algal diversity generally reduced in the same period. The more important species quantitatively were *Oscillatoria limnosa*, *Lyngbya limnetica* (blue green algae) and *Cladophora glomerata* (green algae). The flood water inputs and regime cum dilution, probably lead to unfavorable hydrological stability. Salinity appeared to be a major factor regulating the growth and dynamics of periphytic algae on the concrete slab. Generally, increases in salinity correlated with reduction in nutrient levels, oxygen related parameters, chlorophyll *a*, periphytic algae diversity and abundance as the dry season advanced. Whereas *Oscillatoria formosa* dominated initially, it was subsequently succeeded by *Lyngbaya limnetica* (to a large extent) and *Cladophora glomerata*.
[Onyema, I.C., Onwuka, M. E., Olutimehin, A.O., Lawal, S.T., Babalola, R.M., Olaniyi, A.J., Morgan, P. and Suberu, T.B. Periphyton Algae Dynamics at the University of Lagos Shoreline In Relation To Physico-chemical characteristics. Life Science Journal 2010;7(4):40-47]. (ISSN: 1097-8135).

Keywords: Periphyton, water chemistry, Lagos lagoon, physico-chemical, attached algae.

INTRODUCTION

An intricate network of creeks, rivers and lagoons exist in south-western Nigeria which eventually connects to the sea via the Lagos harbour only, in the Lagos area. Flood waters associated with rainfall are known to enrich these coastal environments, dilute the ionic concentrations and breakdown existing environmental gradients (Olaniyan, 1969; Nwankwo, 1996; Onyema, 2008). Conversely, in the dry season, fresh water inflow is greatly reduced and seawater enters the lagoon through the harbour giving rise to marine conditions near the harbour and proximate creeks and adjoining waters (Hill and Webb, 1958; Nwankwo, 1996; Onyema, 2009).

Any object in water is a suitable foci for the attachment of algae. Algae exists in the brackish waters of the Lagos lagoon primarily as phytoplankton or periphyton when it attaches to fish fences, submerged stones on the littoral zone, foot of bridges and floating objects or materials in water (Nwankwo, 2004). Periphyton form the food base for most aquatic fauna directly or indirectly (Sladeckova, 1962; Nwankwo and Onitiri, 1992). Periphytic algae hence are primary producers and play an important role in the trophic structure and productivity of lagoons.

Information on the periphyton algae of the Lagos lagoon is scanty. A few studies have been reported with regard to attached algae, their composition and dynamics on attached surfaces in the Lagos lagoon and environs. For instance, whereas Nwankwo and Akinsoji (1988) reported on the periphytic algae of an eutropic creek, Nwankwo and Akinsoji (1992) reported on the epiphytic community on the water hyacinth for an array of aquatic environs in Lagos. Furthermore, Nwankwo and Onitiri (1992) reported on the peiphyton community on two submerged aquatic macrophytes in Epe lagoon before the incursion of the water hyacinth to Lagos, Nigeria. Additionally, Nwankwo *et. al*. (1994) reported on periphyton algae on floating timberlogs in the Lagos lagoon. More recently are investigations by Onyema and Nwankwo (2006) on the epiphytic algae of a polluted tidal creek, Onyema (2007a) on the mudflat algae of the only bay in Nigeria and Onyema and Nwankwo (2009) on an incidence of substratum discolouration of part of the Lagos lagoon

substratum. Studies on the food and feeding habits of fish in the Lagos lagoon complex have shown that algae (phytoplankton and periphyton) are especially important components in the diet of phytophagous fish (Fagade 1971, Fagade and Olaniyan 1972, Ikusemiju and Olaniyan 1977). Apart from the key role periphyton algae play in aquatic herbivore diet (Brook, 1975) their use in aquatic biological monitoring system has been proven (Shadeckova, 1962; Patrick 1976, 1973; Nwankwo, 2004). Moreso, the Lagos lagoon has continued to be under intensifying pressure from pollution (Onyema, 2007b, 2009; Nwankwo *et al*., 2010).

The aim of this project was to investigate the periphyton algae dynamics on concrete slabs at the University of Lagos water front within the Lagos lagoon in relation to variations in the physico-chemical characteristics.

MATERIALS AND METHODS

Description of Study Site

The Lagos lagoon has an area of 208km^2 and an average depth of 1.5m (FAO, 1969; Brown, 1998). It is situated in South-western Nigeria and falls within the rainforest zone, which experience a well marked dry and wet season. Two peaks of rainfall linked with excessive floods are generally associated with this area, a major peak in June and a lesser peak in September. The brackish environment is therefore a consequence of tidal seawater incursion and freshwater discharge from the adjoining creeks and rivers (Nwankwo, 1991). The University of Lagos shoreline on the Lagos lagoon (Fig. 1) is occasionally used for recreational activities which eventually constitute debris at the water front. Semi-diurnal tidal regime is operational in the area and the activities of boatmen and fishermen are high in the area. The use of brush packs (Acadja) and sand minning within the lagoon and the shoreline are also common practices.

Notable riparian flora around the shoreline include *Paspalum orbliquilare*, *Cocos nucifera* and *Elaeis guineesis*. Notable shore fauna include *Periopthalmus* sp., *Balanus pallidus*, *Uca tangeri*, *Sersama huzardii*, *Gryphea gasar*, *Pachymelania aurita* and *Typanotonus fuscatus* var. *radula* (Emmanuel and Onyema, 2007a).

Collection of Samples

Surface water samples were collected weekly for 12 consecutive weeks (26-01-07 to 13-04-07) (every Friday) for both physical and chemical analysis with 75cl plastic container with screw cap at the University of Lagos waterfront shoreline between 13 and 16hr. The co-ordinates for collection were Longitude 3^o 24' 05.72"E and Latitude 6^o 31' 09.99"N. All water samples were transported directly to the laboratory after collection for determination of water quality parameters. Periphyton samples were collected from a concrete slab at the shoreline by scrapping with a spatula at the lowest of tide and washed into a 250ml plastic container with distilled water. The scrapping for each collection was done on an area of 25cm^2 (5cm by 5cm) (Onyema, 2007). Samples were properly labeled and preserved with 4% unbuffered formalin in a screw capped container. A similar method have been reported by Onyema (2007a) for the Tarkwa bay mudflat investigation.

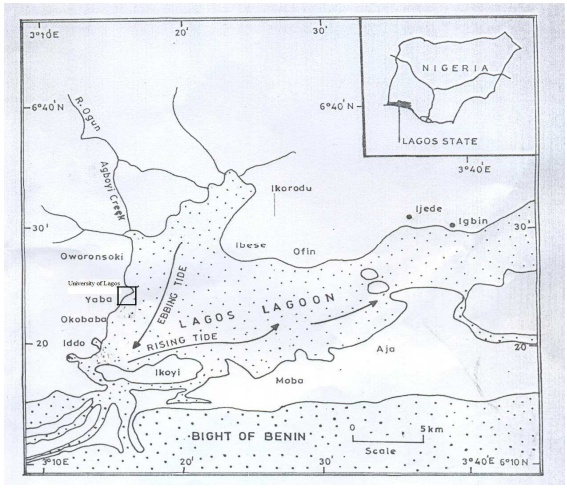

Fig. 1: Lagos lagoon showing the University of Lagos shoreline and study area.

Biological Parameter Analysis

Chlorophyll *a*

Chlorophyll *a* was estimated using the acetone and florimetric method as described by APHA (1998).

Periphyton Analysis

After the preservation of the periphytic sample, they were concentrated to 20ml and at least 5 drops from each sample was examined thoroughly under an Olympus binocular light microscope. The periphyton algae counts are expressed as number of species per ml. Five drops of each concentrated sample were investigated from each sample at different magnifications. Organisms were recorded as numbers of organisms per ml. Entire views of the sample mount were investigated.

Identification aids used included Hustedt (1927 - 1930), Desikachary (1959), Hendey (1958, 1964), Patrick and Reimer (1966-75), Barber and Harworth (1981), Vallandingham (1982), Whitford and Schmacher, 1973.

Community Structure Analysis

A number of ecological indices were employed to analyze the data further. They include Species richness (d), Shannon and Wiener (Hs), Equitability (j) and Menhinick (D) indices (Ogbeibu, 2005).

RESULTS

Physico-chemical

Physical and chemical characteristics between 26-01-07 and 13-04-07 are presented in Table 1, Figs. 2 and 3. These parameters showed weekly variations in values as the study progressed. Whereas Depth, Air Temperature, Total Suspended Solids, Rainfall, pH, Alkalinity, Chloride, Magnesium, Total Hardness, Calcium, Salinity, Conductivity and Iron generally increased as the study progressed, Acidity, Dissolved Oxygen, Biological Oxygen Demand, Chemical Oxygen Demand, Phosphate, Sulphate, Copper and Chlorophyll *a* recorded reduced values in the same episode. Fig. 2 shows weekly variation in Calcium, Nitrate, Sulphate, Biological Oxygen Demand and Chemical Oxygen Demand at the University Of Lagos Shoreline.

Biological characteristics

Chlorophyll *a* (µg/L)

Chlorophyll *a* concentration ranged from 8mg/L recorded in the 12th week to 27mg/L recorded in the 2nd week. The trend of values observed for this parameter was generally higher with reduced rainfall volumes and as the dry season progressed.

Table 1: Weekly Variation in Physico-Chemical Parameters at the University Of Lagos Shoreline from 26-01-07 to 13-04-07.

PARAMETER	26-01-07	02-02-07	09-02-07	16-02-07	23-02-07	02-03-07	09-03-07	16-03-07	23-03-07	30-03-07	06-04-07	13-04-07
Air temperature (^{0}C)	31	32	29	30	30	32	34	33	34	32	33	31
Surface water temperature (^{0}C)	28.8	31	30	31	30	33	29	29	31	29	31	28.9
Depth (cm)	29	20	29	26	28	32	26.6	23.5	27	30	23	20
Total Dissolved Solids (mg/L)	9560	10132	10094	10200	10095	7400	10098	13380	10030	7795	13990	13470
Total Suspended Solids (mg/L)	78	25	20	22	26	24	28	60.1	69	55.1	80.5	502
Rainfall (mm)	0.0		8.4					2.5			43	
pH at 25^{0}C	7.05	7.20	7.15	7.22	7.22	7.20	7.20	7.20	7.25	7.16	7.20	7.30
Acidity (mg/L)	6.3	6.0	6.0	5.8	5.5	5.9	6.2	6.4	6.0	6.8	5.3	6.0
Alkalinity (mg/L)	68	75.1	70.5	78	76.5	76.9	73.8	280	210.6	280.4	190.3	190.1
Salinity ($^O/_{OO}$)	13.3	17.6	17.2	17.45	17.2	13.35	17.1	23.25	17.11	12.45	23.26	20.21
Conductivity (uS/cm)	12185	15710	15655	15810	15655	11470	15650	19520	16460	10624	18384	18488
Dissolved Oxygen (mg/L)	4.4	4.6	4.7	4.6	4.8	4.6	4.5	3.6	3.8	3.6	3.4	3.3
Biological Oxygen Demand (mg/L)	8	9	11	13	9	12	12	8	7	9	6	8
Chemical Oxygen Demand (mg/L)	23	30	35	36	28	33	31	22	20	22	16	18
Total hardness (mg/L)	3560	3560	1390.5	3562	4340	3560	3560	6950	5560	3690	9730	5560
Chloride (mg/L)	10020	10020.2	10340	10370	10360	10360	10168	12900.1	9460.3	6880	12900.1	11180.3
Nitrate (mg/L)	8.5	8.1	6.3	9.3	5.4	4	6.3	3.8	4.1	3.6	2.9	4
Phosphate (mg/L)	0.16	0.24	0.18	0.22	0.10	0.08	0.14	0.08	0.06	0.10	0.15	0.06
Sulphate (mg/L)	80.4	88	92.1	98.1	86.4	80.3	86.6	81.2	75.3	70.1	80.4	78.8
Calcium (mg/L)	350.2	350	317	355	400.2	355.1	356	988	801.1	389	1125.2	705.3
Magnesium (mg/L)	654.9	654.9	145.2	652.4	814.6	652	652	1092.7	562.8	610.1	1689.2	926.2
Zinc (mg/L)	0.003	0.004	0.004	0.004	0.004	0.003	0.004	0.004	0.003	0.003	0.004	0.003
Iron (mg/L)	0.18	0.18	0.20	0.16	0.20	0.22	0.24	0.26	0.22	0.25	0.20	0.28
Copper (mg/L)	0.002	0.003	0.003	0.003	0.003	0.002	0.003	0.003	0.001	0.002	0.002	0.003
Chlorophyll *a* (ug/L)	20	27	16	11	21	18	12	18	24	14	11	8

Table 2: The Composition and Abundance Of Periphyton Algae Dynamics at The University of Lagos Shoreline From 26/01/07 to 13/04/07.

PERIPHYTON TAXA	26-01-06	02-02-07	09-02-07	16-02-07	23-02-07	02-03-07	09-03-07	16-03-07	23-03-07	30-03-07	06-04-07	13-04-07
DIVISION: BACILLARIOPHYTA												
CLASS: BACILLARIOPHYCEAE												
ORDER I: CENTRALES												
Coscinodiscus maginatus Ehrenberg	-	-	10	-	-	-	-	-	-	-	-	-
Cyclotella menighiana Kutzing	-	5	-	-	-	5	60	-	5	-	-	-
Melosira nummuloides Agardh	-	5	25	70	-	-	-	-	-	-	-	-
Paralia sulcata Ehrenberg	-	-	5	-	-	-	-	-	-	-	-	-
ORDER II: PENNALES												
SUB-ORDER I: ARAPHIDINEAE												
Achnanthes longipes	10	70	45	-	15	15	-	715	375	-	15	5
Synedra crystallinia Kutzing	5	-	-	15	10	-	-	30	-	-	-	-
Synedra ulna Ehrenberg	-	5	5	-	-	-	-	-	-	-	-	-
SUB-ORDER II: MONORAPHIDINEAE												
Cocconeis placentula Ehrenberg	-	5	-	-	-	-	-	-	-	5	-	-
SUB-ORDER III: BIRAPHIDINEAE												
Gyrosigma balticum Ehrenberg				5								
Gyrosigma spenceri Grunow	-	5	-	-	-	105	-	-	-	-	-	-
Navicula bicapitata Cleve										5		
Navicula sp.	15	15	5	5	-	-	-	20	-	-	-	-
Nitzschia amphiosus	-	10	15	-	-	-	-	-	-	-	-	-
Nitzschia clostrium Ehrenberg	-	-	-	-	-	-	5	-	-	-	-	-
Pleurosigma angulatum Smith	-	5	15	-	-	-	-	-	-	-	-	-
Pleurosigma sp. Quekett	-	-	-	-	5	-	-	-	-	-	-	-
Pinnularia actosphaeria Smith	-	-	-	-	-	5	-	-	-	-	-	-
DIVISION: CHLOROPHYTA												
CLASS: CHLOROPHYCEAE												
ORDER: CLADOPHORALES												
Cladophora glomerata Kutzing	15	40	20	10	15	3300	50	180	155	5500	5	75
Cladophora sp.	-	-	-	-	5	10	5	-	35	-	15	20
Microspora sp.	-	-	-	-	-	-	5	5	-	-	-	-
DIVISION: CYANOPHYTA												
CLASS: CYANOPHYTA												
ORDER I: CHROCCOCALES												
Aphanocapsa sp.	30	45	80	45	25	-	140	70	80	15	-	5
ORDER II: HORMOGONALES												
Lyngbya limnetica Lemmerman	95	13200	20920	5500	6600	1650	9900	2200	1650	3300	645	530
Lyngbya martensiana Meneghini	5	-	-	10	-	8250	-	2750	75	5500	-	-
Oscillatoria chelabyea Gomont	-	-	-	-	-	10	-	-	-	-	-	-
Oscillatoria formosa Bory	11000	26400	13200	6600	3850	-	1650	-	1650	70	10	-
Oscillatoria limnosa Agardh	-	-	-	5	15	-	15	10	20	-	-	-
Phormodium tenue Meneghini	-	-	-	-	-	-	10	-	-	-	-	-
Scytonema crustaceum Agardh	-	-	5	-	-	-	-	-	-	-	-	-
Spirulina plantensis Geitler	-	-	-	-	5	-	-	-	-	-	-	-
DIVISION: EUGLENOPHYTA												
CLASS: EUGLENOPHYCEAE												
ORDER: EUGLENALES												
Eutreptia sp.	-	-	90	-	-	-	-	-	-	-	-	-
Species Diversity (S)	**8**	**13**	**14**	**10**	**10**	**9**	**10**	**9**	**9**	**7**	**5**	**5**
Abundance (N)	**11175**	**39810**	**34440**	**12265**	**10545**	**13350**	**11840**	**5980**	**4045**	**14395**	**690**	**635**

Table 3: Periphyton community composition parameter.

Bio-index	26-01-06	02-02-07	09-02-07	16-02-07	23-02-07	02-03-07	09-03-07	16-03-07	23-03-07	30-03-07	06-04-07	13-04-07
Total species diversity (S)	8	13	14	10	10	9	10	9	9	7	5	5
Total abundance (N)	11175	39810	34440	12265	10545	13350	11840	5980	4045	14395	690	635
Log of Species diversity (Log S)	0.90	1.11	1.15	1.00	1.00	0.95	1.00	0.95	0.95	0.85	0.70	0.70
Log of abundance (Log N)	4.05	4.60	4.54	4.09	4.02	4.13	4.07	3.78	3.61	4.16	2.84	2.80
Shannon-Wiener Index (Hs)	0.04	0.29	0.32	0.34	0.31	0.42	0.24	0.52	0.57	0.48	0.14	0.26
Menhinick Index (D)	0.08	0.07	0.08	0.09	0.10	0.08	0.09	0.12	0.14	0.06	0.19	0.20
Margalef Index (d)	0.75	1.13	1.24	0.96	0.97	0.84	0.96	0.92	0.96	0.63	0.61	0.62
Equitability Index (j)	0.05	0.26	0.28	0.34	0.31	0.44	0.24	0.55	0.59	0.57	0.20	0.37
Simpson's Dominance Index (C)	0.97	0.55	0.52	0.49	0.53	0.46	0.72	0.36	0.34	0.34	0.88	0.71

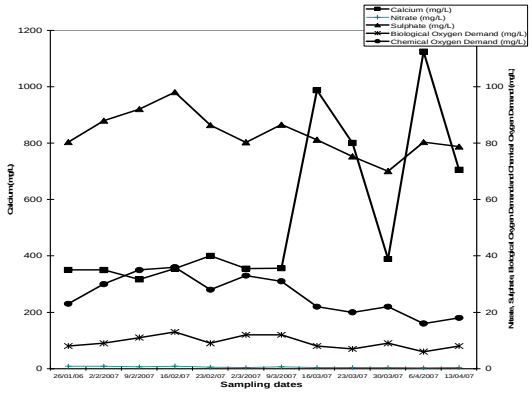

Fig. 2: Weekly variation in Calcium, Nitrate, Sulphate, Biological Oxygen Demand and Chemical Oxygen Demand at the University Of Lagos Shoreline

Biological Characteristics

The periphyton algae dynamics at the University of Lagos shoreline between 26th of January till 13th April 2007 are represented in Table 2. Throughout the sampling period four classes of periphytic algae were identified at the study site. They are: Cyanophyceae (blue green algae), Bacillariophyceae (diatoms), Chlorophyceae (green algae) and Euglenophyceae (euglenoid). Diatoms were clearly the dominant algae group in terms of diversity with the pennate form being more abundant in term of diversity. More taxa occurred in the 3rd week. The more abundant genera were Lyngbya *limnetica* (41.6%), *Oscillatoria formosa* (40.5%), *Lyngbya martensiana* (10.43%), *Aphanocapsa* (0.29%) and *Achnanthes longipes* (0.8%). Species diversity (S) and abundance of individual organisms (N) generally increase as the dry season becomes more pronounced. Fig 3. shows the weekly composition and abundance of Periphyton algae, Chlorophyll *a* and Salinity at the University of Lagos Shoreline.

Community structure analysis

Weekly variation occurred in all indices investigated. Generally higher values were recorded between the 1st week and the 10th week for most parameters. Table 3 shows the periphytic community composition parameters for the study.

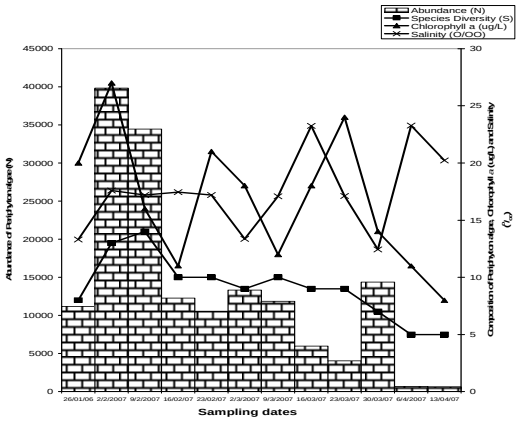

Fig. 3: Weekly variation in the Composition and abundance of Periphyton algae, Chlorophyll a and Salinity at the University Of Lagos Shoreline.

DISCUSSION

The observed variation in the physico-chemical characteristics of the Lagos lagoon agrees with earlier records (Nwankwo, 1996, 1998; Onyema *et al*., 2006, 2007, 2008). Physical and chemical factors in the Nigeria coastal waters create serious environmental limitations and have influence on organism, development and diversity (Nwankwo, 2004).

Hitherto most studies on the physico-chemical parameters and mirco-algal composition of the lagoon area have reported from monthly not weekly samplings. Hence a more subtle trend for the variation of these parameter are elucidated by this study. For instance, the physico-chemical characteristics reflected more of the influence of semi-diurnal tidal regime viz-a-viz rain events periodically. Additionally, the pH was observed to be alkaline throughout the study. A number of authors have reported high temperatures and alkaline pH in our coastal waters (Nwankwo, 1996, 1998; Nwankwo and Kasimu-Iginla, 1997; Onyema *et al.*, 2006, 2007, 2008, 2009). High pH levels observed in coastal waters of Nigeria has been linked to the buffering effects impacted by tidal inflow of sea water (Onyema *et. al*., 2003).

Whereas pH values generally increased with the dry season along with Calcium, Magnesium among others, Biological oxygen demand, Chemical oxygen demand, Nitrate, Sulphate and Phosphate showed a decline in the same period. Consequently, a negative association. Conductivity values also increased all through the period. These were likely occurrences influenced by the reduction / cessation of flood waters during the dry season. Similar observations were made by Onyema (2008).

The increase in biomass in terms of numbers of the periphytic algae in the dry season may be due to the relative stability of the lagoon water with regards to flow conditions and higher transparency which enhanced light penetration and hence greater periphyton production in the lagoon during the dry season. According to Onyema and Nwankwo (2009), increased insolation, especially reaching the lagoon floor, low salinity, absence of flood conditions, suitable sediment type (fine – medium sand) and high nutrient (PO4 - P > 0.24 mg/L; NO3 – N > 4.40mg/L) levels possibly encouraged the algal proliferation and subsequent substratum discoloration of the Lagos lagoon. Onyema and Nwankwo (2006) also reported a high abundance of epipelic algal forms in the dry months at the polluted Ijora creek in Lagos. Flooding associated with the wet season probably dislodges attached algae, increased turbidity, reduce light penetration and limited growth of periphyton algae on fish fences in the Lagos lagoon (Nwankwo and Amuda, 1993).

Diatoms dominated the concrete slabs periphytic community with regards to diversity possibly because of their ability to develop rapidly on newly submerged surface or due to their ability to successfully adapt to fresh / saline water habitats (Kadiri, 2010). Similar observations have been made in the coastal water of south-western Nigeria (Nwankwo and Akinsoji 1992) and in Lake Volta, Ghana (Obeng Asamoa, 1977).

The abundance of diatoms could also suggest their use as indicators. According to Vanlandingham (1982), diatom communities are not indigenous to waters of radically excess pollution and are thus valuable in the indicators community concept. Similarly, Onyema (2007) is of the view that algae in water satisfy conditions to qualify as suitable indicators capable of quantifying changes in water quality. More so micro algal components respond rapidly to perturbations and are suitable bio-indicators of water condition. The possession of a raphe has been described as an asset in pennate diatoms (Nwankwo and Akinsoji, 1989; Onyema and Nwankwo, 2006). They are more likely to attach to the concrete surface if favourable conditions exist. This may have contributed to the predominance of pennate forms on the concrete slabs. This may also explain the occurrence of some centric species usually observed in the lagoon plankton (Nwankwo, 1988, 1996; Onyema *et al*., 2003, 2007). These members may be termed visitors as they are not usually known components of attached micro-algal assemblages. Furthermore, the occurrence of *Coscinodiscus* and *Cyclotella* species which are well known planktonic forms may be an indication that some organisms may have been trapped in the mesh formed by already existing periphytic community. A mesh of *Beggiatoa alba* (an algal-like bacteria) have been reported by Onyema and Nwankwo (2006) to 'filter' and retain planktonic organism previously and continuously in the plankton. This takes place over time and may contain species / communities that were more prominent in the immediate past in the plankton spectrum. Hence a way of biologically assessing the past situations with trapped planktonic forms. *Lyngbya limnetica, Cladophora glomerata* and *Oscillatoria formosa* were the key species recorded for this study in terms of numbers and are known periphytic forms (Vanlandingham,1982; Nwankwo *et al*., 1994). These species are also known to exist is fresh and brackish water situations (Nwankwo, 2004; Onyema, 2008; Nwankwo *et al*., 2010).

The abundance of *Lyngbya limnetica, Cladophora glomerata* and *Oscillatoria formosa* confirm the polluted nature of the Lagos lagoon. This agrees with Nwankwo (2004) who noted that *Oscillatoria formosa* and *Lyngbya limnetica* are pointers to moderate to high levels of organic pollution. It is possible that as the dry season progressed and salinity and pH generally rose, the amount of nutrients and pollution (nitrate, phosphate, chemical and biological oxygen demand) generally reduced. The chlorophyll *a* levels also followed the

general downward trend of the nutrients and oxygen related parameters (Biological oxygen demand and Chemical oxygen demand). Connectedly, this trend also correlated positively with the periphytic algae diversity and abundance on the artificial (slab) surfaces.

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Gas Chromatography – Mass Spectroscopic analysis of *Lawsonia inermis* Leaves

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Abstract: Due to uniqueness of *Lawsonia inermis* leaf property in curing different ailments this part was selected for the study. Hence the present investigation was carried out to determine the possible chemical components from *Lawsonia inermis* leaves by GC-MS. This analysis revealed that *Lawsonia inermis* leaves contain mainly á-D-Glucopyranoside, methyl (51.73%) and 1,4-Naphthalenedione, 2-hydroxy- [Synonyms: Henna] (19.19%), which were used in curing skin ailments caused due to Environmental Pollution of Air and Water.
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Keywords: *Lawsonia inermis*, GC-MS analysis, 1,4-Naphthalenedione, 2-hydroxy- (Henna), Skin ailments caused due to Environmental Pollution of Air and Water.

Introduction:

Lawsonia inermis is commonly known as Henna. Henna has been used since the Bronze Age to dye skin (including body art), hair, fingernails, leather, silk and wool. In several parts of the world it is traditionally used in various festivals and celebrations. There is a mention of henna as a hair dye in Indian court records around 400 CE, (Auboyer *et. al*., 2002) in Rome during the Roman Empire, and in Spain during Convivienca. (Fletcher *et. al*., 1992) It was listed in the medical texts of the Ebers Papyrus (Bryan *et. al*., 1974) and by Ibn Qayyim al-Jawziyya (14th c CE (Syria and Egypt) as a medicinal herb. (Ibn Qayyim al-Jawzīyah *et. al*., 1998).

For skin dyeing, a paste of ground henna (either prepared from a dried powder or from fresh ground leaves) is placed in contact with the skin from a few hours to overnight. Henna strains can last a few days to a month depending on the quality of the paste, individual skin type, and how long the paste is allowed to stay on the skin. Henna also acts as an anti-fungal agent. (Ibn Qayyim al-Jawzīyah *et. al*., 1998).

Henna's coloring properties are due to lawsone, a burgundy organic compound that has an affinity for bonding with protein. Lawsone is primarily concentrated in the leaves, especially in the petioles of the leaf. Lawsone content in leaves is negatively associated with the number of seeds in the fruits. (Bosoglu *et. al*., 1998).

Pre-mixed henna body art pastes may have ingredients added to darken stain, or to alter stain color. The health risks involved in pre-mixed paste can be significant. The United States Food and Drug Administration (FDA) considers these to be adulterants and therefore illegal for use on skin. (Singh *et.al*., 2005).

Some pastes have found to include: Silver nitrate, Carmine, Pyrogallol, disperse orange dye, and Chromium. (FDA, 2009) These have been found to cause allergic reactions, chronic inflammatory reactions, or late-onset allergic reactions to hairdressing products and textile dyes. (Kang *et. al*., 2006; Dron *et. al*., 2007).

The FDA has not approved henna for direct application to the skin. It is unconditionally approved as a hair dye, and can only be imported for that purpose. (Federal Register, 2009)

Natural henna stains only a rich red brown. Products sold as "black henna" or "neutral henna" do not contain henna, and may be derived from indigo (in the plant *Indigofera tinctoria*) or *Cassia obovata*, and may contain unlisted dyes and chemicals. (Singh *et. al*., 2005).

"Black henna" may contain p-phenylenediamine (PPD), that can stain skin black quickly but can cause severe allergic reactions and permanent scarring. The FDA specifically forbids PPD to be used for that purpose. PPD can cause severe allergic reactions, with blistering, intense itching, permanent scarring, and permanent chemical sensitivities.

Estimates of allergic reactions range between 3% and 15%. Henna does not cause these injuries. Henna boosted with PPD can cause lifelong health damage. PPD sensitivity is lifelong, and once

sensitized, the use of synthetic hair dye can be life-threatening.

Materials and Methods:

Plant material – *Lawsonia inermis* was collected in IICPT, Thanjavur Dist. of Tamilnadu.

Plant Sample Extraction

5 gm powdered plant material was soaked in 20ml of Absolute alcohol overnight and then filtered through Whatmann filter paper No.41 along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytocomponents.

GC-MS Analysis: GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm ID ×1 µMdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; Helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 2 µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.

Identification of Components: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

Results and Discussion:

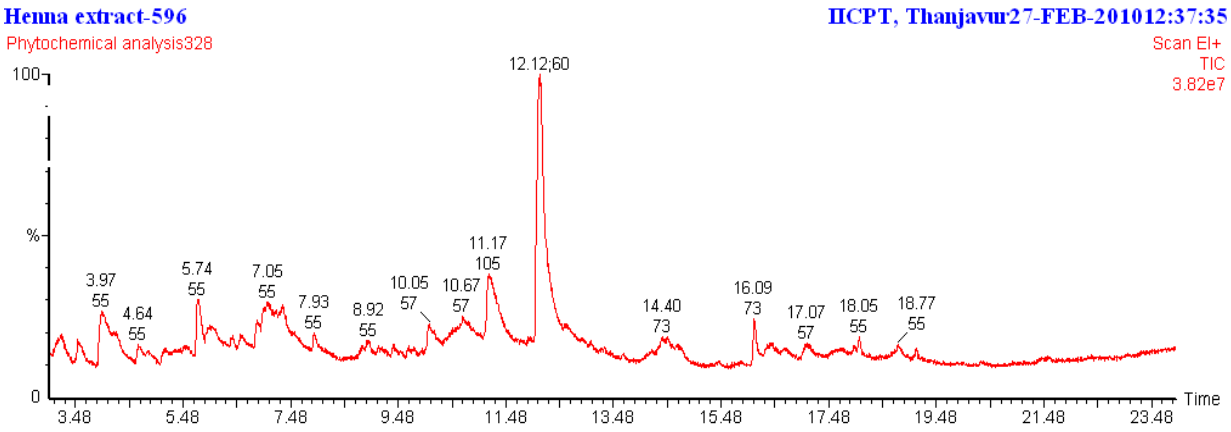

Table 1: Components Identified in *Lawsonia inermis*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	3.97	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	112	16.97
2	5.74	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	6.34
3	6.86	Benzene, (ethenyloxy)-	C_8H_8O	120	2.55
4	11.17	1,4-Naphthalenedione, 2-hydroxy- [Synonyms: Henna]	$C_{10}H_6O_3$	174	19.19
5	12.12	á-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194	51.73
6	16.09	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.21

Six compounds were identified in *Lawsonia inermis* by GC-MS analysis .The active principles with their Retention time(RT), Molecular formula, Molecular weight (MW) and Concentration (%) are presented in (Table 1 and Fig 1).The prevailing compounds were á-D-Glucopyranoside, methyl (51.73%) and 1,4-Naphthalenedione, 2-hydroxy- [Synonyms: Henna] (19.19%).

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Comparative efficiencies of the degradation of C.I. Mordant Orange 1 using UV/H_2O_2, Fenton, and photo-Fenton processes

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Abstract: The kinetics of the photo degradation of C.I. Mordant Orange 1 imparted by UV/H_2O_2, Fenton and photo Fenton process were investigated. Negligible effects were observed in the presence of either UV light or H_2O_2 alone. As expected the Fenton-mediated degradation occurred much faster than the photolytic process. Photo Fenton showed higher in treatment efficiency than that of Fenton process. The effects of dye concentrations, H_2O_2 concentration, pH values and the presence of Fe^{2+} concentration on the degradation rate constant were also studied. The rate constant of dye degradation that occurred in both the phtolytic as well as in the Fenton and photo-Fenton processes was found to pseudo first-order kinetics.
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Keywords: C.I. Mordant Orange 1, Degradation, Kinetic, UV/H_2O_2 , Fenton, photo-Fenton processes.

1 Introduction

Hydrogen peroxide is increasingly favored as an environmentally acceptable bleaching agent both in domestic and industrial situations[1,2]. The combination of H_2O_2 as a bleaching agent, and solar or UV radiation for photochemical degradation of textile dyes without using solid catalyst as titanium, iron, or manganese oxides, were the main objectives of many studies, H_2O_2 is a safe, efficient and easy to use chemical oxidant suitable for wide usage on concentration prevention. Discovered by Thenard in 1818, it was first used to reduce odor in waste water treatment plants, and from then on , it became widely employed in waster water treatment[3]. However, since H_2O_2 itself is not an excellent oxidant for many organic pollutants, it must be combined with UV light, salts of particular metals or ozone to produce the desired degradation results.

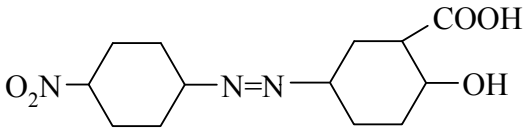
Many different types of synthetic dyes such as azo, vat, nitre, indigo, etc., are widely used for different purposes in paper and pulp rnanufacturing, plastics, printing and textile industry[4-5]. The effluent from these industries, when it contains substantial amounts of such dyes, causes not only coloration of water, but also poses a threat to aquatic life[6] furthermore; their presence in drinking water constitutes a potential human health hazard[7,8]. It is therefore essential either to remove the dyes from water or to treat them in such a way so as to minimize their effects on the environment and also to decolorize the water.

Different approaches have been suggested to remove dyes from aqueous solution including adsorption, biological degradation, coagulation, advanced oxidation processes (AOP), photo-Fenton reaction, ozone treatment and hypochlorite treatment[9-16]. A very simple approach which can be commonly utilized is- the photolytic oxidation of such solutions. In many cases, this method is readily applicable due to the UV content of sunlight. On the other hand, the degradation of dye using Fenton's reagent has been reported in the literature and has shown promising results[17-19]. In this paper, we wanted to examine the relative efficiencies of two different AOP methods, namely UV/H_2O_2 and Fenton's for the degradation of the Alizaration yellow G dye, C.I. Mordant Orange 1 many of the parameters that affect dye degradation rate, such as dye concentration. H_2O_2 concentration, and pH were also evaluated. Since many electrolytes are present in dye effluent, the kinetics of dye degradation in both the presence and absence of various ions were determined[20,21].

2. Experimental

2.1. Reagents

C.I. Mordant Orange 1 (Fig. 1) was procured from CIBA-GEIGY (Switzerland) and was used as received distilled water was used for all dye solutions. Hydrogen peroxide (35%) was obtained from merck and ferrous sulphate heptahydrate (98%) was obtained from Merck.



C.I. Mordant Orange 1
Mol. Wt. 287.00 g/mol
Fig. (1)

2.2. Apparatuses

2.2.1. UV-visible spectroscopy

The absorption spectra are recorded with UV-9200 UV-Vis spectrophotometer. The absorbance of solutions measured at λ_{max} (485 nm) with using a 1cm quartz cell.

2.2.2. Batch type photoreactor

All experiments are conducted in a 500 ml thermostated bath glass reactor equipped with a magnetic stirrer. The light source is low pressure mercury lamp (254 nm 2 lamp each of 4 watt).

2.2.3. pH measurements

The pH values of the solutions are adjusted using Multimeter; WTW (Wissenshaftlich-Technische werkstatten GmbH) Imbolab Multi Lev 11, ba 12237 de Germany.

2.3. Preparation of solutions

2.3.1. Preparation of dye solutions

5.0 x 10^{-3}M stock solution of C.I. Mordant Orange 1 is prepared by dissolving 1.435gm of the dye in 1000 ml bi-distilled water from which working solutions are prepared (0.3-7.0 x 10^{-5}M) by dilution.

2.3.2. Preparation of H_2O_2 solutions

Different concentrations of H_2O_2 are freshly prepared (1.0, 2.0, 10.0, 30.0, 50.0, 70.0, mM). The concentrations of H_2O_2 are large excess in comparison to dye solutions, so that H_2O_2 concentration can be considered constant during the whole experiments.

2.3.3. Preparation of Fe^{2+} solution

1.0 x 10^{-2} M freshly prepared stock solutions of $FeSO_4$. 7 H_2O are prepared and different concentrations (5.0 x 10^{-6},1.0, 3.0, 5.0,7.0x10^{-5}M) are prepared by dilution.

2.4. Homogenous photodegradation

The experiments are carried out in a bath-type photoreactor. H_2O_2, Fenton (Fe^{2+}+ H_2O_2) are acted as photocatalyst and UV light as illuminating light source. Reaction system is setup by adding the photocatalysts into 250ml dye solutions prepared in appropriate concentrations using bi-distilled water. The dye solutions are stirred and 5 ml samples are withdrawn at regular time intervals and the dye concentrations are measured spectrophotometrically.

3. Results and discussion

3.1. Photocatalytic degradation with H_2O_2

3.1.1. Effect of initial dye concentration

The effect of different concentrations of C.I. Mordant Orange 1 (0.3-7.0 x 10^{-5}M) in presence of 30.0mM H_2O_2 and pH 3.0 are studies. Initially, a large degree of removal is observed. This is due to fast decomposition of H_2O_2 producing the hydroxyl radicals. Moreover, dcoloriaztion of dye is mainly due to hydroxyl radicals generated. Azo bonds are more active and C.I. Mordant Orange 1 mountain one azo bond and dcolorization of dye is sue to the initial electrophilic cleavage of its chromophoric azo (–N=N–) bond attached to naphthalene ring[22].

The values of photodegradation Pseudo-first-order rate constant for different concentrations of C.I. Mordant Orange 1 calcualted from the linear plots of ln A/A_o against irradiation time[23] and the results of k are given in Table (1).

Taking into account that, the life-time of hydroxyl radical is very short (only few nanoseconds), they can only react where they are formed[24]. Increasing the quantity of C.I. Mordant Orange 1 molecules per volume unit logically enhance the probability of collision between organic matter and oxidizing species, leading to an increase in the degradation rate so that a rise in concentration induces an inner filter effect, i.e., incident light would largely be wasted for dye excitation rather than for the hydroxyl radical precursor excitation. Consequently, the solution becomes more and more impermeable to UV radiation. As the rate of hydrogen peroxide photolysis directly depends on the fraction of incident light absorbed by H_2O_2 molecules, the degradation rate slows down.

3.1.2. Effect of initial pH

To study the effect of pH on photodegradation experiments are conducted for 1.0 x 10^{-5}M C.I. Mordant Orange 1 in presence of 30.0 mM H_2O_2 at different initial pH values (2.03-8.15). The calculated pseudo first-order rate constants and the values of K are given in Table (2).

The results show that high degradation rate constant values are observed at pH 3.00. The alkaline medium, hydrogen peroxide undergoes decomposition leading to dioxgen and water rather than producing Hydroxyl radicals under UV irradiation[25]**.** Therefore the instantaneous concentration in °OH is lower than that as expected.

The base-catalyzed decomposition involves the HO_2^- anion: the conjugated base of H_2O_2 reacts with a non dissociated molecule of H_2O_2 according to the (eq. 1).

$$HO_2^- + H_2O_2 \longrightarrow H_2O + O_2 + {}^-OH \quad (1)$$

Furthermore, the deactivation of $^\bullet OH$ is greater when the pH of the solution is high (alkaline media), i.e. its expected that the reaction of °OH with H_2O^- being approximately 100 time faster than its reaction with H_2O_2[26].

$$^\bullet OH + HO_2^- \longrightarrow H_2O + O^\bullet{}_2^- \quad (2)$$
$$H_2O_2 + {}^\bullet OH \longrightarrow H_2O + HO_2^- \quad (3)$$

The reactivity of HO_2^- and or its basic from $O^\bullet{}_2^-$ with organic compounds is very weak. They preferentially disproportionate producing some hydrogen peroxide and oxygen, according to the equation.

$$HO^\bullet{}_2^- + O^\bullet{}_2^- + H_2O \longrightarrow H_2O_2 + O_2 + {}^-OH \quad (4)$$

On the whole, the results demonstrate that it is very important to set a suitable pH in order to optimize the operating conditions.

3.1.3. Effect of initial H_2O_2 dose on photo degradation .

The effect of varying the initial H_2O_2 dose (1.0 – 70.0 mM) for C.I. Mordant Orange 1 concentration 1.0 x 10^{-5} and at pH 3.0 for the application of the UV/H_2O_2 initiated degradation of dye effluent, it was necessary to initially change both the initial dye concentration and hydrogen peroxide concentration so as to optimize the conditions of maximum degradation.

Experiments were carried out in the presence of either UV light or H_2O_2 alone: neither of these treatments had an effect on the absorption maxima of the dye solution and imparted no degradation. However degradation of the dye occurred when it was subjected to UV light in the presence of H_2O_2, presumably due to the production of hydroxyl radicals, as shown below:

$$H_2O_2 + h\nu \longrightarrow 2OH^\bullet \quad (5)$$

The hydroxyl radicals can in turn react with the dyes and lead to dye degradation.

$$^\bullet OH + Dye \longrightarrow {}^\bullet Dye + H_2O \quad (6)$$

Further reactions in solution can then take place as a part of the overall scheme:

$$^\bullet Dye + O_2 \longrightarrow Dye\text{–}O_2^\bullet \quad (7)$$
$$2\ Dye\text{–}O_2^\bullet \longrightarrow 2Dye\text{–}O^\bullet + O_2 \quad (8)$$
$$Dye\text{–}O^\bullet + O_2 \longrightarrow HO_2^\bullet + \text{products} \quad (9)$$

Additionally the peroxy radicals can also further react with the dye as follows:

$$HO_2^\bullet + Dye \longrightarrow {}^\bullet Dye + H_2O_2 \quad (10)$$

Table (3) shows the effect of increasing hydrogen peroxide concentrations on degradation rate with the most rapid degradation occurring at the highest H_2O_2 concentration tested. However, it can be seen that the apparent rate seemed to be reaching at higher H_2O_2 concentrations. For example, doubling the H_2O_2 concentration from (1.0, 70.0 mM). This can be explained to be due to the self – to quenching reaction of $^\bullet OH$ radicals with H_2O_2.

$$^\bullet OH + H_2O_2 \longrightarrow H_2O + HO_2^\bullet \quad (11)$$

Therefore, 30.0 mM was selected as the optimum concentration of H_2O_2 for subsequent study. Similarly, the initial concentration of the dye affected the degradation of the dye; Table (1) shows that as the dye concentration increased, an almost linear decrease in the degradation rate of the dye observed[27]. However, as we were unable to find an optimum concentration of C.I. Mordant Orange 1 that would give the best degradation of the dye, it therefore appeared that the apparent decoloriton rate was independent of dye concentration in the range of 0.3-7.0x10^{-5} mM.

Examination of the effect of pH on dye degradation revealed that dye decoloraiton was affected on pH 3.0. In order to optimize the Fenton-mediated degradation of C.I. Mordant Orange 1 the concentrations of dye, hydrogen peroxide and Fe^{2+} were varied results are summarized Table (4-7) and Figs. (2a-5a).

3.2.: Fenton and photo-Fenton processes

The Fenton's reagent involves reaction of ferrous ions with hydrogen peroxide to produce hydroxyl radicals, which are strong oxidizing reagents that react with the dye solution and degrade it[28].

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\bullet}OH + OH^{-} + Fe^{3+} \quad (12)$$

The hydroxyl radical propagates the reaction by reacting with the organic dye to produce further radicals, which can then react in many different steps.

$$^{\bullet}OH + + Dye \longrightarrow H_2O + Dye^{\bullet} \quad (13)$$

$$Dye^{\bullet} + H_2O_2 \longrightarrow Dye\text{–}OH + HO^{\bullet} \quad (14)$$

Additionally many other reaction are also possible, which include the radical-radical reaction or the reaction of the OH radical with H_2O_2.

$$^{\bullet}OH + {}^{\bullet}OH \longrightarrow H_2O_2 \quad (15)$$

The peroxide radicals ($HO_2^{\bullet}$) produced in the above case can further oxidize other species present in the solution[19].

$$HO_2^{\bullet} + Fe^{2+} \longrightarrow O_2 + Fe^{3+} + H^{+} \quad (16)$$

The rapid deplection of H_2O_2 that is often observed with Fenton's reagents it probably due to the combined reactions (12-16). However, since reaction(13) has the highest rate constant, it is responsible for the degradation of the dye. Also, it is possible for Fe^{2+} to be auto regenerated in this system, and act as a catalyst(14).

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} H^{+} + HO_2^{\bullet} \quad (17)$$

Once can see from Table (4) that the rate C.I. Mordant Orange 1 degradation in the Fenton process was proportional to the amount of H_2O_2. This can be explained on the basis that at higher concentrations of H_2O_2, more OH radicals are available which can degrade more dye molecule. Similarly, at a fixed concentration of H_2O_2, the extent of dye degradation decreased with increasing concentration of the dye solution (Table 5). Lastly, Table (6) shows that as the amount of the Fe^{2+} increased, the apparent rate of dye degradation increased proportionally, presumably because of more OH radicals being generated.

It seems that unlike the photolytic process, the Fenton-mediated degradation of C.I. Mordant Orange 1 and H_2O_2 concentrations. Lastly Table (7) shows that as pH: 3.0 the apparent rate of dye degradation is large but on increase the values of pH apparent rate is decrease. The photo-Fenton (Fe^{2+}/UV) system is widely known Apos and is practical use[29]. The results are shown in Tables (4-7), and Figs. (2b-5b) illustrate that C.I. Mordant Orange 1 decolourization was excellent for photo-Fenton process instead of Fenton process. Due to the restriction of the power of UV-light used. The treatment efficiency improved slightly for dye waste water by photo-Fenton process. All pH values also dropped to acidic condition[30].

Conclusions

- The results obtained showed that H_2O_2/UV system could be efficiently used to degrade the C.I. Mordant Orange 1.
- Photodegradation efficiency of dye was negligible when photolysis was carried out in the absence of H_2O_2 and UV light.
- The results indicated that the degree of degradation of C.I. Mordant Orange 1 was obviously affected by pH value and the amount. The optimum amount of Photocatalyst at pH: 3.0 and 30.0mM and at dye concentration of 1.0×10^{-5}M.
- It was observed that using of Fe^{2+} as a photocatalyst it increased the rate of degradation. The plot of ln A/A_o dye versus irradiation time for C.I. Mordant Orange 1 was linear which showed that the photodegradation reaction obeys the pseudo-first-order kinetics reaction.
- The results show that the rate of reaction "k" min^{-1} in photo-Fenton large than Fenton.

Table (1) : Values of K (min^{-1}) for the different concentration of the dye in presence of 30.0mM H_2O_2 and pH 3.0 .

Concentration of dye M	K min^{-1} $x10^{-2}$	R
0.30 x 10^{-5}	13.410	0.991
0.50	14.019	0.993
1.0	17.809	0.996
2.0	12.647	0.967
3.0	10.000	0.977
5.0	7.648	0.985
7.0	5.8954	0.995

Table (2) : Values of K (min^{-1}) for pH in presence of 1.0×10^{-5}M C.I. Mordant orange 1, 30.0 mM H_2O_2 .

pH	K min^{-1} $x10^{-2}$	R
2.03	5.243	0.957
3.00	8.112	0.932
4.00	6.210	0.961
5.10	4.250	0.952
7.09	3.198	0.960
8.15	2.609	0.981

Table (3) : Values of K (min^{-1}) for the different concentration of the H_2O_2 in presence of 1.0×10^{-5}M C.I. Mordant orange 1 and pH 3.0.

H_2O_2 mM	K min^{-1} $x10^{-2}$	R
1.00	0.0598	0.996
2.00	0.09184	0.996
10.00	0.2625	0.942
30.00	0.6432	0.956
50.00	0.4502	0.943
70.00	0.3200	0.956

Table (4) : Effect of H_2O_2 concentration on C.I. Mordant orange 1 degradation in the Fenton, photo–Fenton processes .

Concentration of H_2O_2 mM	pH	Concentration of dye M	Concentration of Fe^{2+}M	Fenton		Photo-Fenton	
				K min^{-1} $x10^{-2}$	R	K min^{-1} $x 10^{-2}$	R
2.00	3.00	1.00×10^{-5}	3.00×10^{-5}	36.702	0.952	47.23	0.976
10.00				42.660	0.993	56.27	0.982
30.00				48.010	0.989	59.40	0.909
50.00				41.000	0.986	50.11	0.956
70.00				37.112	0.992	47.21	0.990

Table (5) : Effect C.I. Mordant orange 1 concentration on its degradation in the Fenton, Photo-Fenton Processes.

Concentration of dye M	Concentration of H_2O_2 mM	pH	Concentration of Fe^{2+} M	Fenton		Photo-Fenton	
				K min^{-1} $x10^{-2}$	R	K min^{-1} $x10^{-2}$	R
1.00 x 10^{-5}	30.00	3.00	3.00×10^{-5}	16.67	0.952	35.72	0.986
3.00				15.84	0.954	28.16	0.994
5.00				13.66	0.964	20.90	0.996
7.00				5.51	0.990	8.44	0.943

Table (6): Effect of Fe^{2+} concentration on C.I. Mordant orange 1 degradation in the Fenton, photo-Fenton processes.

Concentration of Fe^{2+} M	Concentration of H_2O_2	pH	Concentration of dye	Fenton		Photo-Fenton	
				K min^{-1} x 10^{-2}	R	K min^{-1} x 10^{-2}	R
5.00×10^{-6}	30.00	3.00	1.00×10^{-5}	1.034	0.959	2.66	0.996
1.00×10^{-5}				4.239	0.972	7.14	
3.00				25.081	0.989	36.040	0.972
5.00				13.721	0.953	20.66	
7.00				7.174	0.983	11.09	

Table (7) : Effect of pH on C.I. Mordant orange 1 degradation in the Fenton, photo-Fenton processes .

pH	Concentration of H_2O_2 mM	Concentration of dye M	Concentration of Fe^{2+} M	Fenton		Photo-Fenton	
				K min^{-1} x10^{-2}	R	K min^{-1} x10^{-2}	R
3.00	30.00	1.00×10^{-5}	3.00×10^{-5}	14.227	0.988	19.723	0.980
4.00				9.261	0.981	11.166	0.969
5.00				5.130	0.973	8.589	0.996
7.00				2.810	0.984	5.634	0.973

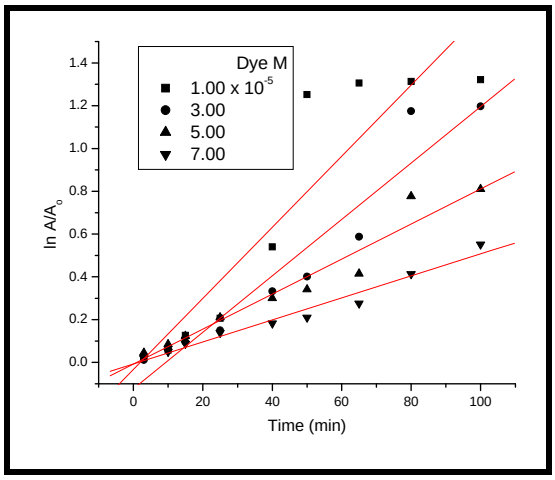


Fig. (2a) Kinetic of photodegradation of Fe^{2+} 3.0 x 10^{-5} at different initial concentrations of C.I. Mordant orange 1 in presence of 30.00 mM H_2O_2 in Fenton process and pH: 3.00.

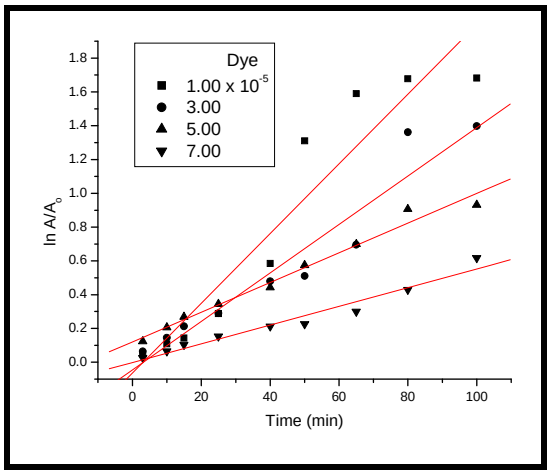


Fig. (2b) Kinetic of photodegradation of Fe^{2+} 3.0 x 10^{-5} at different initial concentrations of C.I. Mordant orange 1 in presence of 30.00 mM H_2O_2 in photo-Fenton process and pH: 3.00.

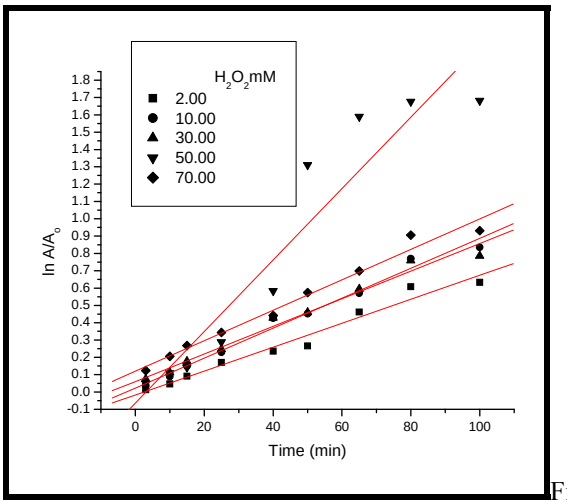


Fig. (3a): Kinetic of photodegradation of C.I. Mordant Orange 1 1.0 x 10^{-5} M at different initial concentrations of H_2O_2 in presence of Fe^{2+}: 3.0 x 10^{-5} in Fenton process and pH: 3.00.

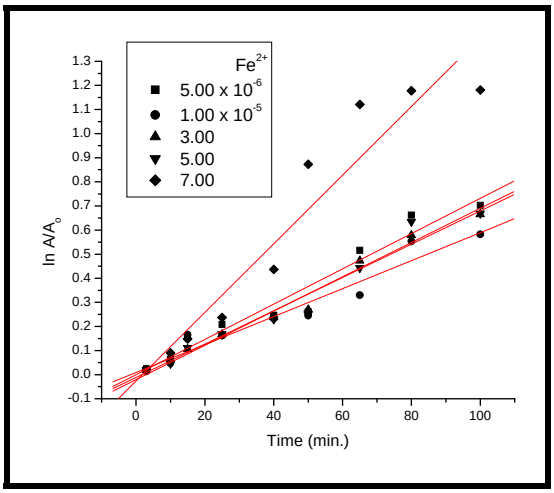


Fig. (4a): Kinetic of photodegradation of C.I. Mordant Orange 1 1.0 x 10^{-5} M at different initial concentrations of Fe^{2+} in presence of 30.00 mM H_2O_2 Fenton process and pH: 3.00.

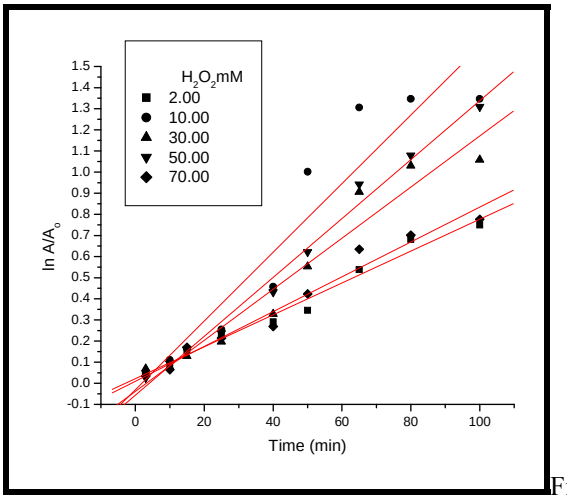


Fig. (3b): Kinetic of photodegradation of C.I. Mordant Orange 1 1.0 x 10^{-5} M at different initial concentrations of H_2O_2 in presence of Fe^{2+}: 3.0 x 10^{-5} in Photo-Fenton process and pH: 3.00.

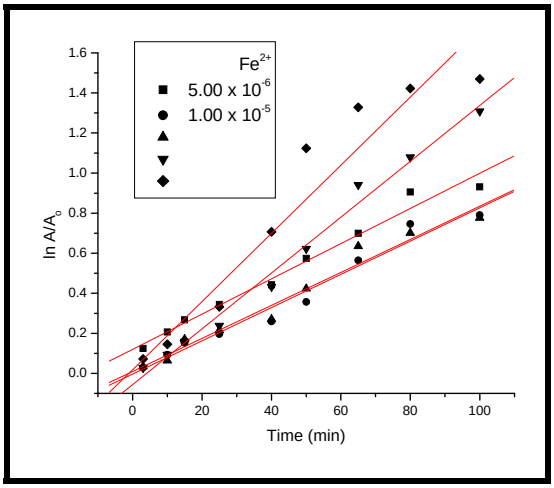


Fig. (4b): Kinetic of photodegradation of C.I. Mordant Orange 1 1.0 x 10^{-5} M at different initial concentrations of Fe^{2+} in presence of 30.00 mM H_2O_2 photo-Fenton process and pH: 3.00.

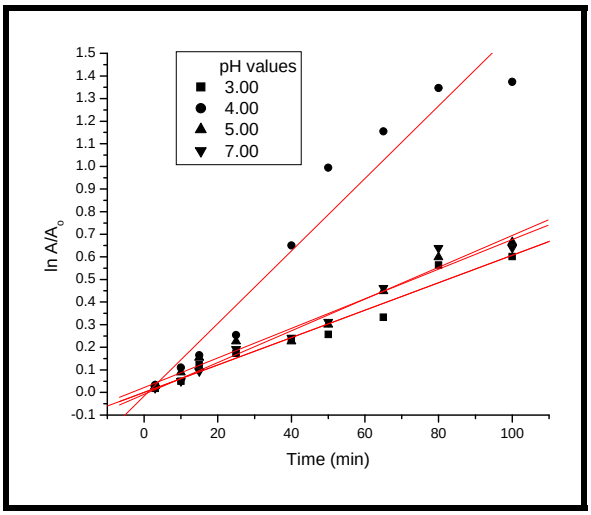


Fig. (5a): Kinetic of photodegradation of C.I. Mordant Orange 1 1.0 x 10^{-5} M at different initial concentrations of pH values in presence of 30.00 mM H_2O_2 Fenton process and Fe^{2+} 3.0 x 10^{-5} M

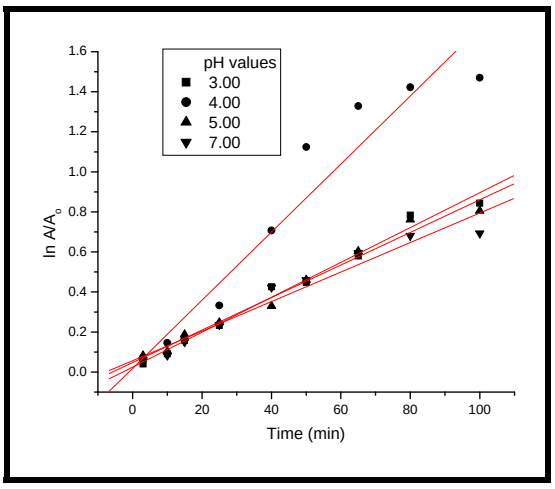


Fig. (5b): Kinetic of photodegradation of C.I. Mordant Orange 1 1.0 x 10^{-5} M at different initial concentrations of pH values in presence of 30.00 mM H_2O_2 Photo-Fenton process and Fe^{2+} 3.0 x 10^{-5} M

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The Efficacy of Community Based Intervention in Newborn Care Practices and Neonatal Illness Management in Morang District of Nepal

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Abstract: Background: In developing countries like Nepal, most of the births take place in the home, where high-risk care practices are common. This study is focused to find the efficacy of a community based intervention in newborn care practices and neonatal illness management in Morang district of Nepal. **Methods**: In Morang district of Nepal, intervention (base line & follow on) and non intervention (control) area were randomly selected. A community based program was launched in intervention area. The program mobilized the female community health volunteer (FCHV) to provide antenatal service (ANC), essential newborn care (ENC) and identify, assess and basic management of sick newborn. The survey included 624, 620 and 613 eligible married women of reproductive age (MWRA) in baseline (BSL), follow on (FON) and non intervention (NI) group respectively. During the survey, data regarding ANC services, clean and safe delivery, implementing ENC practices and managing ill babies were collected. The data, thus collected were analyzed using SPSS for windows. **Results**: The population characteristics of all the three groups; BSL, FON and NI were similar. The number of women receiving ANC service increased from 85.4% to 89% after intervention. The practice of home delivery was low in FON (64.8%) than BSL (69.6%) and NI (70.1%). In case of home delivery, presence of skilled and trained attendant increased to 60.6% with introduction of intervention program. The total illness rate in BSL, FON and NI groups were 41.2%, 38.2% & 29.7% respectively. The most commonly observed danger sign was respiratory problem 38.1%, 41.8% and 30.2% respectively in three groups. A significant improvement was seen in ENC practices of early breastfeeding, cord care, warming baby and delay in bathing practices in FON group (p<0.005). The fatality rate in FON group was low (3.2%) than BSL (14.1%) and NI (15.6%). **Conclusion**: Neonatal illness can be diagnosed and managed earlier if proper training is given to grass root level health worker. The intervention in Morang district showed the reduced neonatal fatality rate and this program can be extended in other rural areas of Nepal.

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Key words: Essential Newborn Care, Neonatal illness, Community Based Intervention

Abbreviations:
ANC – Antenatal Care
BSL – Baseline
CHDK- Clean Health Delivery Kit
Cotrim- Oral Co-trimoxazole P
ENC- Essential Newborn Care
FCHV- Female Community Health Volunteer
FON– Follow on
MDG- Millennium Development Goal
MINI- Morang Innovative Neonatal Intervention
MWRA-Married Women of Reproductive Age
NI- Non Intervention
PSBI- Possible Severe Bacterial Infections
TBA– Traditional Birth Attendant
VDC- Village Development Committee
VHW– Village Health Worker

Globally, each year around 10 million young children in low and middle income countries die and about 4 million children die within the first 28 days of life. Reduction of child mortality by two third between 1990 and 2015 is one of the goal of MDG. During this period (1990-2006), the children under 5 mortality rates in Nepal has been reduced from 117 to 82 and infant mortality rate from 82 to 48. [1] In the last 15 years, Nepal has observed a significant

decline in the under 5 mortality. Nepal is one of those six countries who are on the track to achieve the MDG 4 for child health. [2]

Nepal's estimated neonatal mortality rate is 33/per 1000 lives birth. [1] Over 90% of births occur at home unattended by skilled health professionals. [3] Therefore there should be an effective intervention program to create awareness in rural areas regarding new born care practices at home to reduce neonatal morbidity and mortality. A large proportion of the babies who die could be saved with low tech and low cost interventions. MINI is one of such project which launched a community based program giving emphasis on ENC along with identification and management of the illness in babies in Morang district of Eastern Terai region of Nepal. The Project has been approved by Government of Nepal and is supported by Saving Newborn Lives Program (SNL-1) through Save the Children/US with support from the Bill & Melinda Gates Foundation, NFHP/USAID.

This study is a part of the MINI intervention program to describe the efficacy of the intervention for essential newborn care practices and management of neonatal illness.

1 Material and Methods

1.1 Location and population

Nepal is a developing country with a population of 23.2 million. [4] Morang is densely populated district of Eastern Terai region of Nepal with a population of 914,000 living in 65 VDCs and one municipality, smallest administrative units.

1.2 Study design

The intervention area included 21 VDCs and NI areas included 44 VDCs and were selected by randomization. However, 8 VDCs were excluded from intervention area because of political instability. MINI conducted a BSL survey in 2004 and then intervention program (mentioned below in 1.3)was launched. After 30 months of intervention, a FON survey was conducted. At the same time, FON survey was conducted in NI area as control group. During the study, the information was collected from all eligible MWRA giving birth in the last one year. The information collected was about mothers' age, her first pregnancy age, literacy and no. of live child. Similarly, mothers were also interviewed about ANC service, delivery related practice, new born care practice and the problem seen in babies. A same proforma was developed to collect the information from all the three groups – BSL, FON and NI.

1.3 Intervention

A specific training was given to existing health workers to manage neonatal infections. FCHVs were specially trained to provide ANC counseling or health education to new mothers; weigh all newborns to identify those at higher-risk; use a simple clinical algorithm to assess sick newborns (based on an algorithm tested in other countries); manage local bacterial infections (ophthalmic, umbilical, skin); initiate treatment for PSBI with cotrim; facilitate treatment with injectable gentamicin by VHWs; follow up and record outcomes; and conduct simple birth and death recording.

1.4 Statistics

The data were analyzed using SPSS 16 for windows. The data were expressed in mean $\pm$ standard deviation or median and range. Chi square test was used for qualitative data and t- test was used for numerical variable. P value less than 0.05 was considered as significant.

2 Results

2.1 Population Characteristics

A total of 624, 620 and 613 of eligible mothers were interviewed in BSL, FON and NI group respectively. Population characteristics with maternal age, first pregnancy age, no of live child and mother's literacy are presented in table 1.

Table 1. Population Characteristics

	INT_BSL		INT_FON		NON_INT		Pvalue
	No.of MWRA	%	No.of MWRA	%	No.of MWRA	%	
No. of House hold visited	5449		4411		4309		
Mothers	624		620		613		
Mothers' Age (years)							**0.929#**
15~	124	19.9	120	19.4	106	17.3	
21~	322	51.6	324	52.3	334	54.5	
27~	114	18.3	120	19.4	125	20.4	
33~	45	7.2	39	6.3	37	6.0	
39~	11	1.8	17	2.7	11	1.8	

45~	2	0.3	0	0.0	0	0.0	
Don't know*	6	1.0	0	0.0	0	0.0	
Mean ± Std. deviation	25 ± 5		25 ±5		25±5	0.0	
Mothers' Literacy							**0.090 ^**
Literate (can read easily)	227	36.4	287	46.3	245	40.0	
Semi Literate (read with difficulty)	68	10.9	58	9.4	66	10.8	
Iliterate (can't read)	329	52.7	275	44.4	302	49.3	
First Pregnancy age (years)							**0.067#**
<15	4	0.6	8	1.3	13	2.1	
15~	412	66.0	431	69.5	448	73.1	
21~	186	29.8	161	26.0	137	22.3	
27~	12	1.9	17	2.7	12	2.0	
33~	3	0.5	2	0.3	2	0.3	
39~	0	0.0	0	0.0	1	0.2	
45~	0	0.0	0	0.0	0	0.0	
Don't know*	7	1.1	1	0.2	0	0.0	
Mean ± std. deviation	19.6±3		19.4±3		19.2±3.1		
No of live child							**0.239 ^**
1~2	427	68.4	450	72.5	436	71.1	
3~4	167	26.8	139	22.5	157	25.6	
>4	30	4.8	31	5.0	20	3.3	

* Without considering missing value (don't know)
^ chi square test, # ANOVA test

2.2 Antenatal care service and delivery

ANC practice, home delivery and delivery attendant are presented in table 2.

Table2. ANC and delivery

	INT_BSL		**INT_FON**		**NON_INT**			**P value**
	NO. of MWRA	**%**	**NO. of MWRA**	**%**	**NO. of MWRA**	**%**	χ^2	
ANC received	533	85.4	552	89.0	550	89.7	6.3	**0.043**
Median month	4		4		4			
First ANC visit at							25.1	**0.001**
1st Trimester	185	29.6	212	34.2	211	34.4		
2nd Trimester	275	44.1	305	49.2	305	49.8		
3rd Trimester	73	11.7	35	5.6	34	5.5		
Place of Delivery								
Home	434	69.6	402	64.8	430	70.1	19.7	**0.001**
Hospital	110	17.6	110	17.7	127	20.7		
Others	80	12.8	108	17.4	56	9.1		
Delivery assisted by							13.0	**0.001**
Skilled & trained Attendant	315	50.4	376	60.6	332	54.2		
Untrained Attendant	293	47.0	236	38.1	276	45.0		
No Attendant at all	16	2.6	8	1.3	5	0.8		

In BSL, 533 (85.4%) women received ANC service and 552 (89%) and 550(89.7%) in FON and NI group. Most of the women seek first ANC service in 2nd trimester 44.1%, 49.2% and 49.8% respectively in BSL, FON and NI group. In BSL 434 (69.6%) women delivered the baby at home. Similarly in FON and NI group home delivery were 402 (64.8%) and 430 (70.1%) respectively. The skilled & trained attendant conducted 50.4%, 60.6% & 54.2% of the

deliveries while untrained assisted in 47%, 38.1% and 45% cases respectively in the BSL, FON and NI group. In this study, birth attendant were grouped into skilled and trained attendant (doctor/Nurse/paramedics/Trained TBA) and untrained attendant (Family members, neighbours, relatives, untrained dais). There is a significant different in antenatal service and delivery practices among the groups. (ANC received $\chi^2 = 6.3$; $p<0.05$; home delivery $\chi^2 = 19.7$; $p<0.001$; delivery assistance by skilled $\chi^2 = 13.1$; $p<0.001$).

Table3. ENC Practices

	INT_BSL		INT_FON		NON_INT			
	No.of MWRA	%	No. of MWRA	%	No. of MWRA	%	χ^2	P value
Cord care	177	28.4	375	60.5	261	42.6	130.8	0.001
Breastfeeding (within 1st hour of birth)	181	29	270	43.5	264	43	35.8	0.001
First colostrums fed	473	75.8	545	87.9	508	82.8	31.4	0.001
Baby wiped	367	58.8	394	63.5	328	53.5	12.8	0.002
Baby wrapped	433	69.3	446	71.9	381	62.1	14.5	0.001
Baby bathing (>24 hrs)	109	17.5	261	42	215	35	69.8	0.001
Weighing of baby (within 24 hrs)	178	28.5	313	50.4	209	34	68.9	0.001

2.3 Essential Newborn Care Practices

There was a significant improvement in cord care among the groups ($\chi^2 = 130.8$, $p<0.001$). The practice of breastfeeding to babies within 1st hour of birth and feeding of colostrums was significant different among the groups ($\chi^2 = 35.8$, $p<0.001$ and $\chi^2 = 31.4$, $p<0.001$). Newborn was wiped immediately after birth. Babies were dried and wrapped in clothes. Both the practices were significantly improved after intervention ($\chi^2 = 12.8$; $p<0.005$ and $\chi^2 = 14.5$; $p<0.001$). The practice of delay to bath the babies were reported more after intervention. ($\chi^2 = 69.8$; $p<0.001$). The significant improvement in taking weight of babies after birth was seen ($\chi^2 = 68.9$; $p<0.001$) The newborn care practices are in table 3.

2.4 Status of Ill Babies

FCHVs had assessed ill babies in their villages using a standard algorithm. The danger signs of the newborn identified are listed in Table 4.

Table 4. Danger Signs Identified

	INT_BSL		INT_FON		NON_INT			
	No.of MWRA	%	No. of MWRA	%	No.of MWRA	%	χ^2	P value
Unable to suck milk	46	18	51	21.5	30	16.5	1.9	0.382
Difficulty in breathing	98	38.1	101	42.6	55	30.2	6.0	**0.050**
Lethargic	8	3	9	3.8	4	2.2	0.8	0.646
Redness around cord	5	2	18	7.6	5	2.7	11.1	**0.004**
Fever	164	64	157	66.2	123	67.6	0.7	0.697
Cold body	0	0	0	0.0	4	2.2	10.9	**0.004**
Watery + bloody stools	20	8	14	5.9	14	7.7	0.78	0.674
Yellow in eyes, skin	24	9	13	5.5	11	6.0	3.19	0.202
Failed to pass urine	3	1	3	1.3	4	2.2	0.89	0.641
Skin problem	9	4	32	13.5	15	8.2	16.2	**0.001**
Red eyes with discharge	14	5	11	4.6	8	4.4	0.29	0.861
Others	127	49	37	15.6	39	21.4	75.8	**0.001**
Total ill babies	257	41.2	237	38.2	182	29.7	18.9	0.001

In BSL, 257 babies were ill within 60 days of their life with illness rate of 41.2%. In FON, 237 were ill and illness rate was 38.2%. In NI group, 182 were ill with illness rate of 29.7%. The most commonly observed danger sign was respiratory problem 38.1%, 41.8% and 30.2% respectively. Other illness includes the danger sign like unable to feed,

bluish palm and sole, excess cry or no cry, no urine, convulsion, abnormal movement and other unclassified illness. Two or more danger signs were seen in 66.2% of babies in FON group. They were considered as possible severe bacterial infection. The symptoms of difficulty in breathing, redness around cord, skin problem were significantly different among the groups (p<0.005) where as non specific symptoms or danger sign had significantly decreased (p<0.001).

2.5 Outcome

A total of 14, 5 and 10 babies died in BSL, FON and NI groups respectively. Case fatality rate of PSBI was 14.1%, 3.2% and 15.6% in BSL, FON and NI group, respectively. The treatment and its outcome are in table 5.

Table 5. Effect Of Treatment In Neonatal Illness

	INT_BSL		INT_FON		NON_INT			
	NO. of MWRA	%	NO. of MWRA	%	NO. of MWRA	%	χ^2	P value
PSBI cases	99	38.5	157	66.2	64	35.2	52.8	0.001
Babies receiving cotrim	-	-	78	32.9	33	18.1	11.5	0.001
Babies receiving gentamicin	0	0	73	30.8	21	11.5	21.9	0.001
Death	14	5.4	5	2.1	10	5.5	215.6	0.001
Case Fatality Rate of PSBI		14.1		3.2		15.6		

The PSBI cases of 99(38.5%), 157 (66.2%) and 64 (35.2%) were seen in BSL, FON and NI groups. There is a significant difference in PSBI cases among the group ($\chi 2$ = 52.8; p<0.001). There was no any information about the treatment of ill babies in BSL. In FON group ill babies were treated by FCHV and VHW with cotrim and gentamicin. Cotrim was given to 78 (32.9%) ill babies and 73 (30.8%) of babies received gentamicin in FON group. In NI, babies treated with cotrim and gentamicin were 33 (18.1%) and 21 (11.5%) respectively. These were also found to be significantly different. ($\chi 2$ = 11.5; p<0.001and $\chi 2$ = 21.9; p<0.001). Total death of ill babies were 14 (5.4% of total ill babies), 5 (2.1%) and 10 (5.5%) in BSL, FON and NI group. The total case fatality rate of PSBI are high in BSL (14.1%) and NI group (15.6%) compared to FON (3.2%).

3 Discussion

The infants who are born at home are almost all exposed to substantial infectious challenge during their neonatal period. The study shows illness is associated with the care taking practices. [5] In our study, we had taken three groups – BSL, FON and NI, to find the efficacy of intervention in the essential new born care practices and management of neonatal illness in Morang district of Nepal. ANC receiving practice has been increased from BSL to FON but no difference in NI group. In NI group, MWRA received ANC from the regular government health services but intervention was not done. In all the three groups, it has been seen that pregnant women went for first antenatal service mostly in 2nd trimester only. However, the new mothers should be encouraged for first ANC within first trimester. The previous findings show that home delivery is a common practice in rural part of Nepal. [1, 6] After intervention, in the FON group, home delivery was low (64.8%). David O et al had reported 90% of home delivery in rural part of Nepal [3] In rural part of India also, home delivery is quite common. [7] Though the delivery attendant by untrained has been decreased, the skilled and trained health workers need to increase their coverage more during home delivery. In the study by David O et al, there were 11% of women who gave birth alone [3], we found a much lower rate of 1.3%.

Cord cutting practices have been identified as risk factors for neonatal infection. [8-12] During the delivery, in FON group, 60.5% of women had proper care of cord. This shows the increment in the use of clean and safe instruments. A.H. Baqui et al suggested a low coverage of clean cord care among home deliveries in South Asia. [3, 13, 14] The application of the material on the cord especially oil is a risk factor for the infection. [15-17] There is no any additional benefit of topical substances on the cord. [18,19] So MINI's recommendation of keeping cord dry as per World Health Organisation guidelines of ENC were followed widely in FON group. However, ointment/dettol/powder was commonly used in FON group and oil in NI group. Other case control studies suggested that the use of antiseptics may reduce the risk of neonatal sepsis. [20-22] In our study in FON group, the practice of applying substances on the cord has been decreased than that of BSL and NI group.

Breastfeeding is one of the most important contributors to neonatal health, growth and development. Several studies have demonstrated

effective reduction of mortality rate in neonate with early and exclusive breastfeeding. [23-29] However, there are few studies that have evaluated the breastfeeding in neonatal outcome. Huffman et all concluded that early & exclusive breastfeeding played an important role in reducing neonatal mortality. [30] The benefits are enhanced if breastfeeding starts within one hour after birth. Many neonatal health problems can be avoided or reduced by such a pattern of breastfeeding. However many women are not aware of the benefits of early breastfeeding. Our study shows that 29%, 43.5% & 43% of babies were breastfed within 1 hour of birth in BSL, FON and NI group respectively which is very lower compare to previous studies of 63%.[31] Though early breastfeeding was lower, all most all the women practiced breastfeeding (including breastfeeding within 1 hour and after 1 hour). Though the constant effort by the health workers to promote breastfeeding has resulted positive findings but it is not up to the mark. So health worker should promote for the early breastfeeding. In other studies [32,33], the first colostrum was commonly given to babies in our report. The breastfed babies have less risk to the infection. The practices of wiping, drying & wrapping babies were increased in FON group than BSL and NI group showing the positive effect of intervention.

As per WHO guidelines, bathing of neonate should be postpone [34], but there is a trend to bathe the babies soon after birth. After intervention, the practice of delay bathing (>24hrs.) increased. Birth weight is an important indicator of child survival but this is difficult in developing countries since most of the deliveries are conducted at home where adequate facilities to weigh a new born does not exist. [35] So in many rural part of Nepal, it is difficult to take the weight of babies immediately after birth mainly in home delivery. In our study, FCHV has weighed the baby using spring balance and identified the low weight baby during intervention. Early identification of low birth weight babies (<2500 gms) is vital in preventing neonatal deaths. Low birth weight is one of the risk factor for the illness of baby which is consistent with previous studies of community based new born care practices and might be improved through appropriate behaviour change intervention. [9, 13, 36]

Our study also focused in identification and management of neonatal illness by FCHV and its outcome. The term "illness" includes all the danger sign identified by other studies. [36] The danger sign includes no suckling, lethargic, cold or warm body, respiratory problem (grunting or chest drawing), skin pustules, red eyes with discharge, redness around cord, yellow in skin and eyes and others (blue palm & sole, no urine, weak/excess cry, abnormal movement, convulsion etc) The presence of more than two of these danger sign was considered as PSBI. Breathing problem was most frequently reported in FON group (42.6%). The incidence of breathing problem (pneumonia or ARIs) in community level is not known. However, significant proportion of newborn diagnosed with sepsis or severe infections may have associated with pneumonia. [37] For the treatment of this severe problem in developing countries in health care facility, WHO recommended administration of antibiotics. Majority of neonatal death are in rural part/in home and families are reluctant to seek care outside the home for neonatal illness. [36] Therefore, grass root health worker should be trained for the treatment of neonatal illness. MINI project focused on this and trained FCHV for the treatment and management of neonatal illness. Before intervention, danger sign of neonatal illness was not known to the community health worker. After intervention, FCHV has identified these danger sign as per the approved algorithm due to which danger signs were more identified in FON group compared to BSL and control groups. After identification of illness FCHV initiated for treatment after obtaining consent from family. If any of the sign of PSBI seen, FCHV initiated treatment with cotrim and facilitate referral to higher level of health worker for injectable gentamicin. The babies receiving cotrim and gentamicin in FON group were 33% & 30.8% respectively. It seems that the illness rate in babies in FON is higher than BSL and NI; however, the total outcome or the fatality rate is low in FON compared to BSL and NI which reveals the effectiveness in intervention. The case fatality rate for PSBI was 14.1% in BSL which reduced to 3.2% in FON while in NI it is still high (15.6%). In India (Gadchiroli) the trained health workers could identify sick newborns in their home and were able to treat with antibiotics (cotrim & gentamicin) reducing neonatal mortality. [38] Our study also confirms the findings that if health workers are trained to identify the danger sign then the infection in neonate will be reduced. Infection being one of the major cause of neonatal death [39,40], lives of newborn can be saved by preventing it on the home setting too for which health workers and mothers should also be trained to identify the danger sign.

4 Conclusion & Recommendation

Our study suggested that neonatal infection and death are preventable through interventions. The intervention should focus on training to grass root level health worker. The proper and effective training may lead to change in knowledge, attitude and practice in community. The trained health workers are need of rural areas in Nepal where hospitals are

not easily accessible. The intervention should focus on antenatal counseling, safe and hygienic delivery, implement essential newborn care practices, identify the danger sign, assess them and treat with prescribed antibiotics. Apart from these, it is also recommended to provide training to all mothers to identify the danger sign so that early treatment can be started.

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Sorption Energies Estimation Using Dubinin-Radushkevich and Temkin Adsorption Isotherms

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Abstract: In this study, we add to scholarly knowledge in simple terms, the forces or energy defining certain adsorption phenomenon, using isotherm models. GCMS measurement of equilibrium phase atrazine after adsorption onto Sheanut shells (SS) acid derived activated carbon were fitted into the D-R and Temkin isotherm relationships for energy data estimation. Sorption energy value (B_D), mean free energy (E_D) and heat of sorption (B). They were estimated as $0.7600 mol^2KJ^{-2}$, 0.8111 $kjmol^{-1}$ and $0.790 Jmol^{-1}$ respectively. The parameter predicting the type of adsorption was evaluated B_D , B < 20kJ/mol and E_D<8 which is an indication that physisorption (Non specific adsorption) dominates chemisorption and ion exchange. The D-R model with a higher correlation coefficient values, R^2 = 0.979 proves a better choice in explaining sorption energies. Generally, sheanut shells can be used as alternative precursors for activated carbon production via the two steps and acid treatment schemes.

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Key words: Dubinin-Radushkevich , Temkin, Adsorption, GCMS, Isotherm, Sorption energy

1. Introduction

It was reported that quality evaluation of activated carbon with adsorption capacity, intensity ,thermodynamics and kinetics parameters among others are as vital as the estimation of certain energy parameters namely mean free energy, sorption energy etc. An approximation from such energy parameter gives a clue as to the type of adsorption in question.

Previous studies showed that the herbicide, atrazine disrupt the production and functionality of human hormones and a higher incidence was reported for cases of cancer in humans and laboratory animals (Zhongren *et al*., 2006). The use of active carbon was prescribed by USEPA as the best available technology for the removal of atrazine from drinking water. Many studies have revealed adsorption of atrazine using conventional activated carbon granules and fibres.

A chemical activation using activating agents is a new generation of adsorption fibre development sorbents obtained with this method provides higher yield, high surface area, high mesopores volumes and some unusual pore surface chemistries (Zhongren *et al*., 2006; WHO, 1999; Hertrick *et al*., 2000).The sorption of herbicide aqueous phase by activated carbon has been reported (Agdi *et al*.,2000;WHO,1999; Zhongren *et al*., 2006). This present study reports atrazine sorption, not in a micro quantity but within range that could account for both the topical and systemic poisoning reportedly associated with atrazine (Raymond, 2003).

Basics of adsorption : Adsorption is actually a mechanism in which the forces of interaction between surface atoms and the adsorbate molecules are similar to Van der Waals forces that exist between all adjacent molecules .There are both attractive forces and repulsive forces with the net force depending on the distance between the surface of the adsorbent and the adsorbate molecule (Cooney,1999).

In other words,adsorption is a process in which a soluble chemical (the adsorbate) is removed from a fluid (liquid or gas) by contact with a solid surface (the adsorbent).It is the trapping of impurities by strong physical bond within the porous structure of Carbon. It is one of the many processes used to purify, concentrate, or separate component (Glenn,1993) and competes with other processes like evaporation, solvent extraction, distillation, sublimation, drying, sedimentation, filteration, screening, ion exchange, centrifugation, and absorption (Glenn,1993).It is used in industry for product separation and waste treatment. In general, adsorption is the process by which a component moves from one phase to another while crossing

some boundary. It was found that the observed effect of adsorption was achieved within porous solids and that adsorption was the result of interactive forces of physical attraction between the surface of porous solids and component molecules being removed from the bulk phase (Arun, 2002).

Improved Adsorption via Surface Activation: The surface of an adsorbent is typically composed of various surface functional groups (SFG). Adsorption of organic adsorbate is greatly dependent on the amount and nature of surface oxide groups (Cooney, 1999). Surface functional groups (carbon/oxygen) are created by oxidation occurring during the activation process of an adsorbent. Some of the common basic functional groups created are lactones, quinones, and carboxylates (Arun, 2002). Some of the common acidic functional groups created are phenolic, hydroxyl, carbonyl, and carboxylic acids (Arun, 2002). The presence of oxygen-containing basic groups such are a key factor in promoting irreversible adsorption (Vidic *et al.*, 1993).Strongly dissociated adsorbates are weakly adsorbed when compared to nondissociated adsorbates (Cooney, 1999). The more non polar an adsorbate, the higher the adsorption capacity. This is attributed to the fact these adsorbate molecules tend to prefer the adsorbent surface rather than being in the solution (Cooney, 1999). It has also been shown that an increase in the molecular weight of the adsorbate will generally enhance adsorption until the size of the adsorbate is larger than the pore size of the adsorbent. Typically, aromatic compounds are more adsorbable than aliphatic compounds of similar molecular size and branched-chain molecules are generally more adsorbable than straight-chain molecules (Cooney, 1999). In addition, solubility of the adsorbate is also an important factor. In general, the lower the solubility of the adsorbate, the higher the adsorption capacity since the forces of attraction between the adsorbate molecules and the adsorbent surface molecules will be greater than the forces of attraction between the adsorbate and the solvent molecules (Cooney, 1999).

Types of Adsorption based on Energy values

1. Chemisorption: This is a shorter way of writing chemical adsorption. It is also called specific adsorption and limited to monolayer coverage of the substrate. Here, a covalent bond is formed between the adsorbate and adsorbent. The enthalpy of chemisorption is within the range of 200kJ/Mol (Atkins, 1999)

2. Physisorption: This stands for physical adsorption. It is also called non specific adsorption which occur as a result of long range weak Van der Waals forces between adsorbates and adsorbents. The energy released when a particle is physisorbed is of the same magnitude as the enthalpy of condensation. The enthalpy of physisorption is measured by monitoring the rise in temperature of a sample of known heat capacity. Typical values of in the region of 20kJ /Mol (Atkins, 1999).

Chemisorption occurs at high temperatures with a significant activation energy, which involves strong bonds and is not reversible. The heat of adsorption is typically high in chemisorption and is similar to heat generated during a chemical reaction. There are several factors that impacts physical adsorption (LaGrega *et al.*, 1994; Cooney, 1999). The major factors which affect physical adsorption include the surface area of the adsorbent, pore structure of the adsorbent, surface chemistry of the adsorbent, nature of the adsorbate, pH of the solution, and the presence of competing adsorbates. It is due to these factors, physical adsorption is considered to be a complex phenomena. Surface area of the adsorbent is one of the most important factors on which adsorption greatly depends. The surface area is comprised of two types, the external surface area and the internal surface area (pore walls). When molecules are larger than the pore diameter, lesser adsorption would take place because of steric hindrances.

Adsorption isotherm: Isotherm are empirical relationship used to predict how much solute can be adsorbed by activated carbon (Steve and Erika, 1998). Chilton *et al.*,(2002) defined Adsorption isotherm as a graphical representation showing the relationship between the amount adsorbed by a Unit weight of adsorbent (eg activated carbon) and the amount of adsorbate remaining in a test medium at equilibrium. It maps the distribution of adsorbable solute between the liquid and solid phases at various equilibrium concentration (Chilton *et al.*, 2002).The adsorption isotherm is based on data that are specific for each system and the isotherm must be determined for every application. An adsorption isotherm beside providing a panorama of the course taken by the system under study in a concise form indicate how efficiently a carbon will allow an estimate of the economic feasibility of the carbons' commercial application for the specific solute (Chilton *et al.*, 2002).

Dubinin-Radushkevich isotherm: This isotherm model was chosen to estimate the characteristic porosity of the biomass and the apparent energy of adsorption. The model is represented by the equation 1 below:

$qe = q_D \exp(- B_D [RT \ln (1 + 1/Ce)$ (1)

Where, B_D is related to the free energy of sorption per mole of the sorbate as it migrates to the surface of the biomass from infinite distance in the solution and q_D is the Dubinin-Radushkevich isotherm constant related to the degree of sorbate sorption by the sorbent surface (Horsfall *et al.*, 2004;Itodo *et al*.,2009b). The linear form of equation is given as 2;

$\ln qe = \ln q_D - 2B_D RT \ln (1 + 1/Ce)$ (2)

A plot of ln qe against RT ln (1 + 1/Ce) for modified sorbents, yielded straight lines and indicates a good fit of the isotherm to the experimental data. The apparent energy (E_D) of adsorption from Dubinin-Radushkevich isotherm model can be computed using the relation given as 3 below (Horsfall *et al.*, 2004).

$E_D = \sqrt{1/2B_D}$ (3)

Temkin adsorption Isotherm: The Temkin was tested for equilibrium description at room temperature. The model was respectively represented by equations 4 and 5 below. Therefore, by plotting *q*e versus ln *C*e , enables the determination of the constants *A* and B.B is the Temkin constant related to heat of sorption (J/mol), *A* is the Temkin isotherm constant (L/g), *R* the gas constant (8.314 J/mol K), *b* is Temkin isotherm constant and *T is* the temperature (K). (Nunes *et al*., 2009; Hameed,2009).

$q_e = B \ln (A+C_e)$ (4)

Where $B = RT/b$ (5)

Aim of this work: In this present work, chemically activated carbon was formulated to adsorb atrazine traces from water. This research was based on an initial qualitative study based on atrazine sorption as earlier predicted by FTIR analysis (Itodo *et al*.,2009a). The specific objectives include; Generation of activated carbon thereby adding values to the wastes. Sorption energy studies, (Evaluation of the mean free and sorption energies as would be predicted by Temkin isotherm and R-D isotherm models.

2. MATERIALS AND METHODS

Choice of equipment: The gas chromatographic technique is at best a mediocre tool for qualitative analysis. It is best used with other technique to answer the question of what is present in a sample. Besides the simplicity of the instrument, ease of operation, GC also provides the answer to how much? It is an excellent quantitative analytical tool in quantifying micrograms in a litre or one volume in millions of volumes (Robert and Eugene, 2004).The sample herbicide (containing atrazine) is a multicomponent mixture containing atrazine (test sample) and other organochlorine moieties, which are very similar to atrazine. Secondly, the GC column has a very high efficiency which was claimed to be in excess of 400,000 theoretical plates. The column is about 100m long, a very dispersive type of stationary phase retaining the solute approximately in order of increasing boiling point. Helium carrier gas was selected since it can realize high efficiencies with reasonable analysis time (Raymond, 2003). Techniques of external standardization entails the preparation of standards at the same levels of concentration as the unknown in the same matrix with the known. These standards are then run chromatographically under ideal conditions as the sample. A direct relationship between the peak size and composition of the target component is established and the unknown was extrapolated graphically

Sample treatment and preparations: The method of sample treatment by Fan *et al*., (2003); Itodo *et al* .,(2009a&b) were adopted. The samples were washed with plenty of water to removes surface impurities and sundried, then, dried in an oven at $105^{O}C$ overnight (Omonhenle *et al.,* 2006). The samples were separately pounded/ grounded followed by sieving with a <2mm aperture sieve. The less than 2mm samples were stored in airtight containers. About 3g of each pretreated biosolid (< 2mm mesh size) were introduced into six (6) different clean and pre weighed crucibles. They were introduced into a furnace at $500^{O}c$ for 5 minutes after which they were poured from the crucible into a bath of ice block. The excess water was drained and the samples were sun dried. This process was repeated until a substantial amount of carbonized samples were obtained (Gimba *et al.,* 2004). The carbonized sample was washed, using 10% HCl to remove surface ash, followed by hot water wash and rinsing with distilled water to remove residual acid (Fan *et al*., 2003) the solids were then sun dried, then, dried in the oven at $100^{O}C$ for one hour . Accurately weighed 2g each of already carbonized samples were separately mixed with $2cm^3$ of each 1M activating agent (H_3PO_4 and $ZnCl_2$). The samples were introduced into a furnace, heated at $800^{O}c$ For 5 minutes. The activated samples were cooled with ice cold water. Excess water was drained and samples were allowed to dry at room temperature (Gimba *et al.,* 2004,). The above procedure was

repeated for different residual time (5min and 15 min).Washing of the above sample was done with 10% HCl to remove surface ash, followed by hot water and rinsing with distilled water to remove residual acid (Fan *et al*, 2003). Washing was completed when pH of the supernatant of 6-8 was ascertained (Ahmedna *et al.*, 2000). The sample were dried in an oven at 110^{O}C overnight and milled or grounded, followed by filtration to different mesh size and stored in air tight container.

Atrazine standard solution for equilibrium studies: For the sorption/equilibrium studies, several concentrations viz; 10, 20, 30, 40 and 50g/L Herbicide equivalent of 5, 10, 15, 20 and 25g/L Atrazine was prepared by respectively dissolving 0.25, 0.5, 0.75, 1.0 and 1.25g of herbicide into a conical flask, poured gently into a 25cm^3 volumetric flask, homogenized and made to the mark with chloroform (i.e. 5,000ppm – 25,000ppm atrazine).A three point calibration curved based on external standard method was prepared with the GC/MS run.

Batch equilibrium experiment: 5g of substrate was diluted to the mark of 100cm^3 volumetric flask. This concentration of 50g/L herbicide is equivalent to 25g/L or 25,000ppm atrazine stock. 10cm^3 of the atrazine solution was interacted with 0.1g of each sorbent and allowed to stand for 12hours. The mixture was filtered and the filtrate was analyzed with a gas chromatography (coupled with a mass spectrophotometer detector) for atrazine equilibrium phase concentration (Min and Yun, 2008; Agdi *et al.*, 2000). The amount of atrazine at equilibrium, q_e was calculated from the mass balance equation given in equation 6 by Hameed *et al.* ,(2006).

$$q_e = (C_O - C_e)\, V/W \qquad (6)$$

where C_o and C_e are the initial and final Dye concentrations (mg/L) respectively. V is the volume of dye solution and M is the mass of the acid catalyzed Poultry waste sorbent (g). while t is the equilibrium contact time, when q_e = qt, equation 6 will be expressed as equation 7 below:

$$q_t = (C_o - C_t)v/w \qquad (7)$$

where $q_e = q_t$ and C_t is the concentration at time,t. The percent dye removal (RE %) was calculated for each equilibration by the expression presented as equation 8

$$R\,E(\%) = (C_0 - C_e)/Co \times 100 \qquad (8)$$

Where RE (%) is the percent of dye adsorbed or removed. The % removal and adsorption capacities were used to optimize the activation condition. The test were done at a constant temperature of 27 ± 2^0C.(Rozada *et al.*,2002). The equilibrium concentration of atrazine (herbicide), q_e and Adsorption efficiency (% Removal) were estimated. The extent of atrazine removal (by difference) from chloroform spiked with 25g/L of atrazine was expressed as equation 3 (Hameed *et al.*, 2006).

3. Results

The chromatogram shown as Figure 1 stands for unadsorbed sorbate out of the 5g/dm^3 atrazine which was interacted with SS/A sorbent. The chromatogram was characterized by a baseline disturbance. This is caused by either hydrocarbon impurities or by impure carrier gas (Robert and Eugene, 2004).The former could be linked to the fact that the sorbate concentration (5g/dm^3) is too low for the 0.1g carbon dose. Unoccupied pore size could as well, lead to desorption of the sorbate with a resultant poor percentage removal (46.08%).

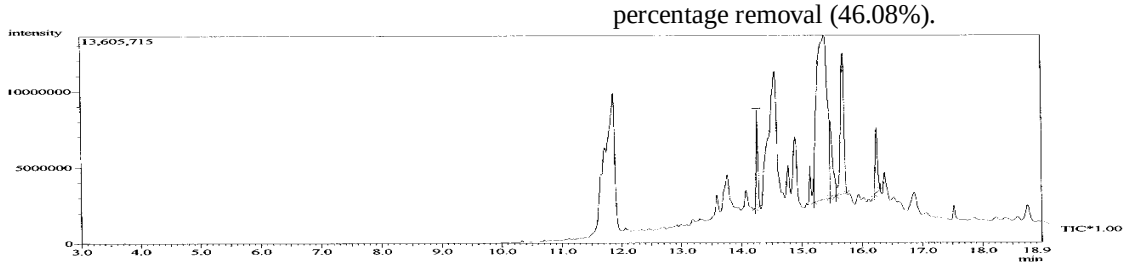

Figure 1: GC/MS chromatogram of equilibrium concentration atrazine after adsorption onto SS/A/5gL^{-1} sorbent (*Carrier gas-Helium 100.2kpa,Column temperature –60^{o}c,Injection temperature-250^{o}c,Injection volume—1µL,Flow rate-1.61mL/min, Injection method- split, Linear velocity- 43.6cm/sec.*)

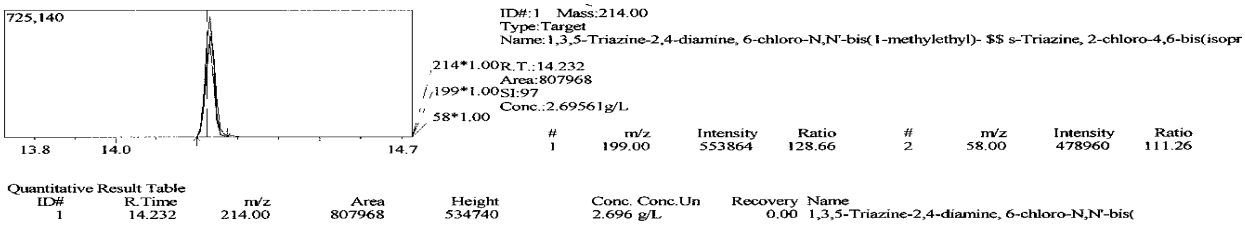

Figure 2: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/5gL^{-1} sorbent

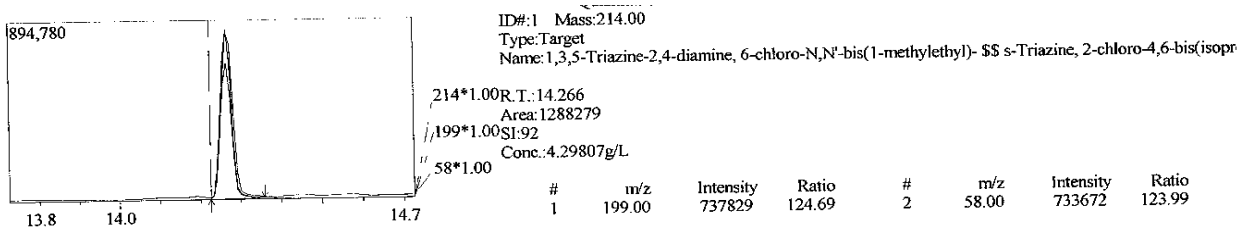

Figure 3: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/10gL^{-1} sorbent

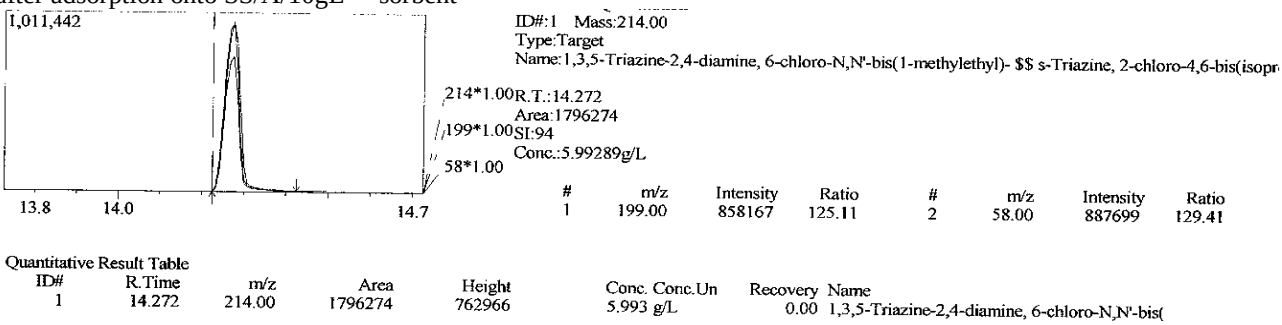

Figure 4: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/15gL^{-1} sorbent

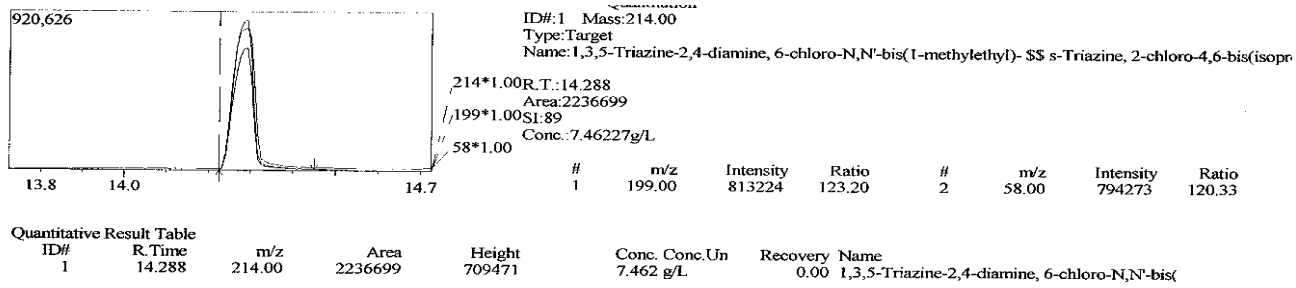

Figure 5: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/20gL^{-1} sorbent

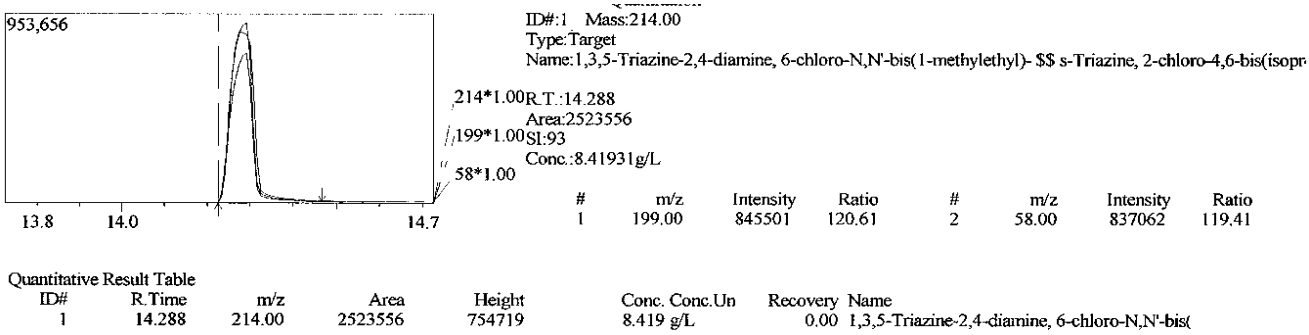

Figure 6: GC/MS chromatogram, quantitative measurement and spectral information of equilibrium phase atrazine after adsorption onto SS/A/25gL^{-1} sorbent

Equilibrium experimental data from Figures 2 to 6 were treated as Table 1

Table 1: Adsorption experimental data of atrazine uptake by fixed mass of SS-Sorbents at different initial sorbate concentration, using GC/MS

Sorbent	Co (g/dm^3)	Ce (g/dm^3)	Ca (g/dm^3)	% RE	Ads.m (mg.10^{-3})	q_e (mg/g x 10^{-3})	Kc	ΔG (kJ/mol)
SS/A/5	5	2.696	2.304	46.08	0.0230	0.230	0.854	+393.941
SS/A/10	10	4.298	5.702	57.02	0.0570	0.570	1.327	-705.381
SS/A/15	15	5.993	9.007	60.047	0.0901	0.901	1.503	-1016.670
SS/A/20	20	7.462	12.558	62.69	0.1254	1.254	1.680	-1294.989
SS/A/25	25	8.419	16.581	66.324	0.1658	1.658	1.969	-1691.330

SS/A/15 -Sheanut shells, treated with H_3PO_4,activated for 15 minute dwell time, **SS/A/25** -Sheanut shells, treated with H_3PO_4,activated for 25 minute dwell time

4. Discursion

Effect of initial atrazine concentration on removal efficiency

Table 1 presents the role played by initial sorbate concentration and its effect on sorption efficiency. The highest percentage atrazine removal was observed with the interaction of 0.1g atrazine with 10Cm^3 of a 25g/L atrazine solution. Hence, out of 25g/L, 20g/L, 15g/L, 10g/L and 5g/L initial atrazines concentration, a total of 16.581, 12.538, 9.007, 5.702 and 2.696g/L atrazine was attracted onto the sheanut shell (SS) bioadsorbent. These accounts for a 66.324, 62.69, 60.007, 57.020 and 46.080% removal efficiency respectively. Findings in this research showed that for the selected time (1hour interaction) and within the 0.1g sorbent dose on 10Cm^3 sorbate solution, (i) Adsorption efficiency increases with initial sorbate concentration.

(ii) Adsorption within the low sorbate concentration (5 – 10g/dm^3) range could possibly be followed by desorption. Hence, a less than 50% adsorption was investigated.

(iii) Adsorption of fairly high concentrated atrazine (15 – 25g/L) could be governed by a multilayer adsorption with resultant intraparticle attraction. In light of this, sorption efficiency or percentage sorbate uptake is greater than 60%.

Sorption isotherm modeling

The regression equation and R^2 values for Dubinin-Radushkevich model was observed that this isotherm also gave very good description of the sorption process, over the range of concentration studied. The apparent energy of adsorption and Dubinin-Radushkevich isotherm constants are shown on Table 2 and obtained from the plot of type in figure 7. The high values of q_D shows high sorption capacity. The values of the apparent energy of adsorption also depict physisorption process.

Table 2 - Temkin and R-D adsorption experimental GC/MS data for atrazine uptake by chemically modified SS-sorbent.

ISOTHERMS	Relationship (Y=)	R^2	Parameters (constant)	Values.
Temkin	0.790x _ 0.947	0.927	b (Unitless) B(J/mol) A(Lg^{-1})	3158.794 0.790 3.316
R-D	-9.466x-2.360	0.979	q_D (mgg^{-1}) B_D ($Mo1^2$ KJ^{-2}) E_D(kjmo1-2)	$4.37x10^{-3}$ 0.7600 0.8111

The two isotherms experimental data on Table 2 were used to estimate certain energy parameters. The Dubinin-Radushkevich, (R-D) isotherm model is more general than the Langmuir isotherm as its deviations is not based on ideal assumptions such as equipotential of sorption sites, absence of steric hindrances between sorbed and incoming particles and surface homogeneity on microscopic level (Monika *et al*,. 2009). The estimated constant, B_D, related to adsorption energy was presented as 0.7600 mol^2kJ^2. This constant gives an idea about the mean free energy which was valued as E_D= 0.811 $kJmol^{-1}$. E_D is a parameter used in predicting the type of adsorption. An E_D value < 8 $kJmol^{-1}$ is an indication of physisorption (Monika *et al*., 2009).

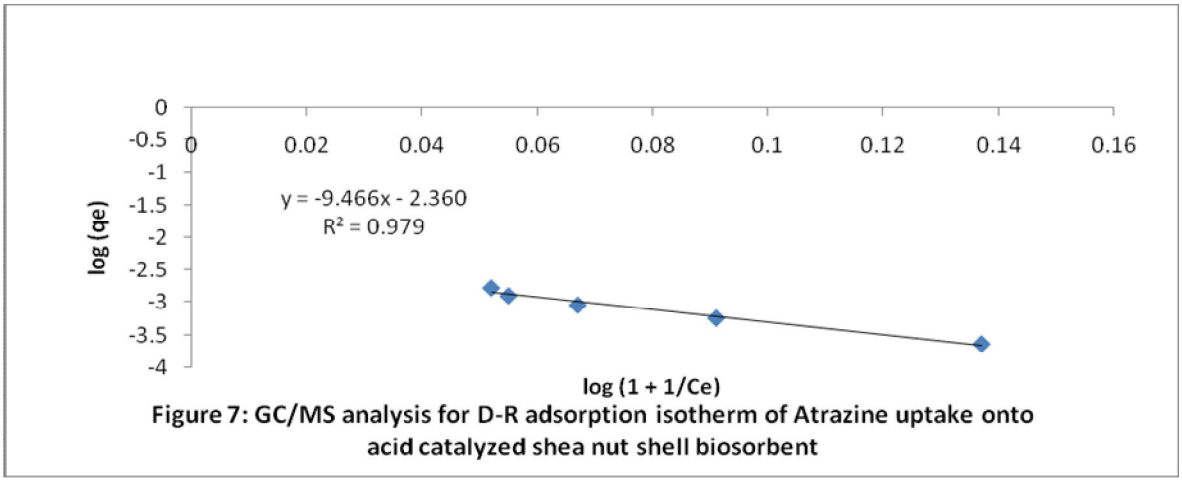

Figure 7: GC/MS analysis for D-R adsorption isotherm of Atrazine uptake onto acid catalyzed shea nut shell biosorbent

The R-D Theoretical saturation capacity, q_D and the Langmuir maximum adsorption capacity, q_m were both estimated as q_D = $4.37x10^{-3}mgg^{-1}$ and q_m = $0.772x10^{-3}$ mgg^{-1}. No known and proven reference could make us conclude that the theoretical saturation capacity, q_D is always higher than the maximum adsorption capacity as the case was made evidence in this research.

The Temkin constant related to heat of sorption, B was estimated as 0.790 J/mol. The unit less quantity, b (3158.794) and Temkin constant, A (3.3159 Lg^{-1}) were in good agreement with values presented by Hameed, (2009) on the Evaluation of papaya seed as a non conventional low cost adsorbent for removal of dye.

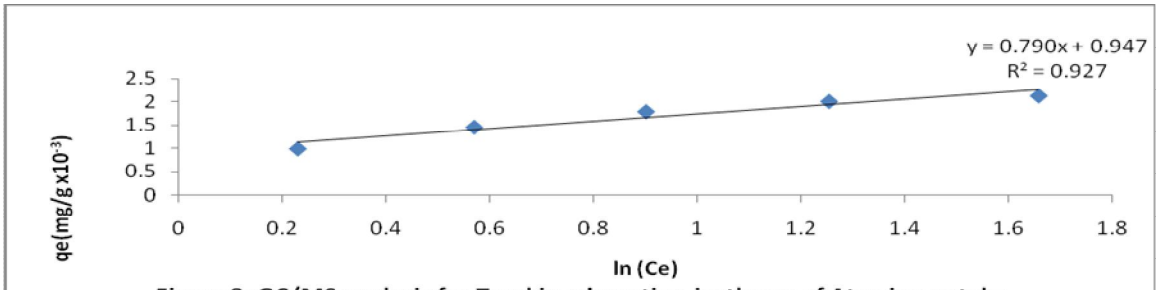

Figure 8: GC/MS analysis for Temkin adsorption isotherm of Atrazine uptake onto acid catalyzed shea nut shell biosorbent

In the energy parameter models, R-D and Temkin were used to present sorption energy value (B_D), mean free energy (E_D) and heat of sorption (B). They were estimated as $0.7600mol^2KJ^{-2}$, $0.8111\ kjmol^{-1}$ and $0.790Jmol^{-1}$ respectively, the parameter predicting the type of adsorption was evaluated as E_D which is an indication of physisorption dominates chemisorption, ion exchange etc. The R-D model with a higher correlation coefficient values, R^2 = 0.979 proves a better choice in explaining sorption energies. Besides the E_D validation test, The mean free and energy values and heat of sorption values (in kJ/mol) were lower than 20KJ/mol. This ,according to Atkins,(1999) is characteristics for physisorption. In their report, physisorption is also called non specific adsorption which occur as a result of long range weak Van der Waals forces between adsorbates and adsorbents. The energy released when a particle is physisorbed is of the same magnitude as the enthalpy of condensation. The enthalpy of physisorption was however measured by monitoring the rise in temperature of a sample of known heat capacity to give typical values of in the region of less than 20kJ /Mol (Atkins, 1999).He also argued that Chemisorption is a specific adsorption and limited to monolayer coverage of the substrate. Here, a covalent bond is formed between the adsorbate and adsorbent. The enthalpy of Chemisorption is within the range of 200kJ/Mol (Atkins, 1999).Reports (Unpublished) on surface coverage in this experiment fitted best into the Freundlich Isotherm (sorption on heterogeneous surface).Hence, adsorption is not restricted to monolayer coverage as purposed for Chemisorption.

Conclusion

Generally, sheanut shells can be used as alternative precursors for activated carbon production via the two steps and acid treatment method. On the same fashion, sorption quantification was feasibly observed for a multicomponent system (Herbicide) with a gas chromatography coupled with mass spectrophotometer as detector when the simulated concentration was established via the external standard method. A physorption type of adsorption was predicted for the process.

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Maize (*Zea mays*) Response to Phosphorus and Lime on Gas Flare Affected Soils.

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Abstract: Response of maize to phosphorus and lime was evaluated on two gas flare affected sites. The experimental design was a 2 x 2 x 4 factorial of 2 sites (S_1 and S_2), 2 P rates (0 and 30 kg P_20_5 ha^{-1}) and 4 lime rates (0, 1, 1.5 and 2.0 t ha^{-1}) in a CRD and replicated 3 times. Plant height, leaf area, dry matter yield, nutrient uptake (N and P) and residual soil properties (pH, Ca, Mg and P) increased with treatments up to 30 kg P_20_5 ha^{-1} and 1.5 t ha^{-1} lime combined rates in both sites. Maize performance and residual soil properties were better in S_2 than S_1 due its higher fertility status and distance (400 m) from the gas flare pit. Performance of all measured parameters were best using 30 kg P_20_5 ha^{-1} and 1.5 t ha^{-1} lime combined rates and hence could be the optimum rate for maize production in gas flare affected soils of the Niger Delta.
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Keywords: maize; phosphorus; lime; Niger Delta

Introduction

Acid rain due to gas flaring is a significant source of soil acidity in the Niger Delta region of Nigeria and this also, has serious environmental effects on corrugated iron sheets.. As people (farmers) living in rural areas take water from this source, they can be harmed adversely health wise. Its effects include nutrient deficiency, toxicity of Al, Fe and Mn ions, depressed microbial activities, poor soil organic matter and low soil pH (Walna et al 2001, Isirimah et al. 2004). This manifests as poor growth, low yield and high mortality especially in crops grown close to gas flare sites (Evoh 2002). Goigi and Baruah (2001) observed that the inhibitory effect of gas flare on rice was severe 45 m to a flare site with intensity of damage increasing below 30 m. This constitutes serious threat to food security in the region, dominated by enormous gas flares and where population is high due to opportunities in the oil and gas industries.

Addition of Lime, phosphorus, gypsum and organic matter can be used to ameliorate the soil acidity (Ernani et al. 2002, White et al. 2006), with combined use of inputs being more effective. Combined application of lime and organic matter has been reported with success in Southwestern Nigeria (Busari et al 2005). Though the potentials of integrating lime and phosphorus has been reported elsewhere (Lelei 1999) its use in the Niger delta could be effective since P is often very limiting under acid conditions of tropical soils (Isirimah 2004). Effects of lime include an increase in soil pH, nutrient concentration and organic matter decomposition while phosphorus increases immediate soil P concentration by precipitating aluminum into insoluble aluminum phosphate (Lelei 1999). Presently, information concerning the combined use of lime and phosphorus to control soil acidity in the Niger Delta is scanty. The main objective of this study was therefore to evaluate the response of maize to phosphorus and lime on gas flare affected soils.

Material and Methods

Soil sample collection and laboratory analysis

Soil samples were collected at three depths (0 -15, 15 -30 and 30 -60 cm) from two sites; 1. a Typic Tropaquent (S_1) and 2. a Haplic Dystrudept (S_2) soil survey staff (1999) located less than 30 m and 400 m respectively from a gas flare pit of the Shell Petroleum development Company (SPDC) gas flow station in Egbema, Imo state. The samples were air dried, sieved through 2 mm diameter aperture and sub samples subjected to laboratory analysis. Result is presented in Table 1. Particle size distribution (Gee and Bauder 1986), organic carbon (Olsen and Sommers 1982), total nitrogen (Bremner and Malveney 1982), pH in 1: 2.5 soil/H20 ratio using a Jenway 5130 model pH meter, Exchangeable cations (Na, Ca, Mg, K, Al and H) (Juo 1981), available phosphorus (Olsen and Sommers1982) and ECEC by summation of exchangeable cations.

Pot studies

Pot studies under green house condition were conducted at the School of Agriculture, Federal University of Technology, Owerri (Lat 5^0 $21^/$ and 7^0 $15^/$) in 2005, using fine earth surface (0 -15 cm)

samples from both sites. The experimental design was a 2 x 2 x 4 factorial in a CRD replicated 3 times. The factors were 2 sites; S_1 (located less than 30 m to the flare pit) and S_2 (located about 400 m to the flare pit), 2 P rates P_0 (control) and P_1 (30kg P_20_5 ha^{-1}) as SSP and 4 lime rates; L_0 (control), L_1 (1.0), L_2 (1.5) and L_3 (2.0 t ha^{-1}) as $CaC0_3$. 10 kg soil samples of each site was weighed into separate pots (height: 25 cm and diameter: 24 cm), lime and P treatments as above, in addition to basal doses of 120 kg N ha^{-I} as urea and 30 kg K_20 ha^{-1} as muriate of potash were incorporated into the pots. A total of 48 pots were used. The soils were watered and 2 maize seeds (var. Oba super) which were later thinned to one seedling per pot, one week after planting were planted (WAP). The soils were maintained moist by watering every 4 days and the plants grown for 8 weeks. During the growth period, plant height and leaf area were measured at weekly intervals, starting from the second week after planting (WAP). Plant height was determined using a steel tape from the base to the collar of the last leaf. Leaf area was estimated by multiplying leaf length and width with 0.75 (Odiete et al 2000). Dry matter yield was determined on a balance after harvest by thoroughly washing plant material in tap water and air drying for 3 days before oven drying to constant weight at 65^0c. Plant materials were milled and P content determined after di- acid mixture ($NH0_3$ and $HCl0_4$) digestion on a Spectronic 20 Colorimeter using vanadomybdo yellow method. Plant N was estimated after digestion with H_2S0_4 using micro – Kjeldahl distillation method. Plant N and P uptake were obtained by multiplying the dry matter weights with the plant N and P contents. Also P, Ca, Mg and pH of soils used for the experiment were analyzed using the methods for soil analysis as above. All data collected were subjected to statistical analysis using ANOVA and treatment means separated by Duncan Multiple Range Test at 5% probability.

Result and Discussions

Plant height and leaf area

Effects of treatments on plant height and leaf area are presented in Tables 2 and 3 respectively. Plant height differed significantly in the 5^{th}, 6^{th} and 7^{th} week after planting (WAP) with treatment interactions (soil/phosphorus/lime) compared to the control but not with those at the $2^{nd,}$ 3^{rd} and 4^{th} WAP. Interactions between site/phosphorus/lime significantly increased leaf area compared to the control except for S1 – 0 – 2.0, at $2^{nd,}$ 3^{rd} and 4^{th} and S1 – 30 – 0 at 2nd and 3^{rd} weeks after planting (WAP). Leaf area was generally high at all growth stages using 1.5 t ha^{-1} lime rates for the different P rates and sites. Largest leaf area occurred at S_2 irrespective of P and lime rates used. Differences in plant height and leaf area could be attributed to the effects of the treatments. Lime and phosphorus promote plant growth through improved soil conditions such as increased soil pH, nutrient availability, soil organic matter, soil solution P concentration and decreased aluminum toxicity and micronutrient accumulation (Lelei 1999, Ernani et al 2002, Busari et al 2005, White et al 2006). Superiority of S_2 over S_1 with respect to leaf area could be due to higher fertility status of the former than the later (Table 1). It could also be attributed to its distance from the flare pit. Inhibitory effect of gas flare on rice was severe 45 m and increased in intensity below 30 m to the flare site (Gogoi and Baruah 2001).

Table 1 Characterization of Gas flare affected Sites

Soil depth	Silt + Clay g/kg	TC -----	Na ----------	K Cmol/kg	Mg ----------	Ca	ECEC	TN g/kg	Avail P mg/kg	OC g/kg	pH H_20
A. S_1											
0 -15	140	LS	27.0	0.3	0.4	1.3	29.9	0.7	16.8	0.12	4.6
15 – 30	160	SL	21.7	0.3	0.2	1.0	25.7	0.6	8.1	0.12	4.4
30 – 60	220	SL	21.7	0.4	0.3	1.8	27.9	0.7	13.6	0.81	4.4
B. S_2											
0 – 15	150	LS	0.12	1.15	0.69	1.20	7.29	0.9	21.5	1.22	5.6
15 -30	190	SL	0.16	0.52	0.66	1.40	7.78	0.8	19.35	0.81	4.9
30 -60	240	SL	0.16	0.32	0.33	0.83	5.13	0.6	18.30	0.29	4.8

TN = Total nitrogen, Avail P = Available phosphorus, OC = Organic carbon,
TC = Textural class, LS = Loamy sand SL = Sandy loam

Table 2 Analysis of variance of the effect of treatments on plant height (g/pot)

Source of Variation	df	Weeks after planting					
		2	3	4	5	6	7
		F - Value					
A	1	61.06**	36.39**	90.06**	69.94**	137.87**	126.35**
B	1	35.20**	58. 51**	58.12**	67.80**	189.59**	194.36**
C	3	9.80**	9.76**	40.53**	63.32**	92.59**	97.13**
A x B	1	0.002ns	1.76ns	1.41ns	1.25ns	9.92**	9.98**
A x C	3	0.67ns	0.13ns	5.89**	3.38*	5.83**	6.67**
B x C	3	0.37ns	1.34ns	4.77**	10.03**	12.50**	13.26**
A x B x C	3	0.37ns	0.13ns	0.56ns	6.36**	3.41**	3.53**
CV (%)		8.79	12.89	10.99	10.43	10.79	10.56

** ---- Significant at 0.01, * ----- Significant at 0.05 and ns ---- Non significant.
A – Site, B --- Phosphorus and C --- Lime.

Table 3 Effect of treatment interactions on leaf area

Site	Treatments Phosphorus (kg ha^{-1})	Lime (t ha^{-1})	Weeks after planting 2	3	4	5	7
S_1	0	0	12.07k	13.80k	14.20j	14.80r	17.36n
S_1	0	1.0	14.02j	15.70j	17.35h	18.14p	22.40m
S_1	0	1.5	15.00gh	19.40gh	22.80ef	26.23k	32.56j
S_1	0	2.0	13.00k	14.13k	16.26hj	17.53q	21.33m
S_1	30	0	13.00k	15.53jk	18.27gh	22.53n	25.30l
S_1	30	1.0	14.67hj	16.90j	19.20g	23.73n	27.97kl
S_1	30	1.5	16.20g	18.87h	24.32e	30.43j	41.23h
S_1	30	2.0	19.07e	20.77g	21.70f	25.13l	30.57k
S_2	0	0	18.20ef	53.23f	70.02d	115.17h	174.13g
S_2	0	1.0	20.47d	63.03e	98.43b	131.04d	217.40d
S_2	0	1.5	29.50b	84.13b	101.56a	139.33b	218.97d
S_2	0	2.0	28.00b	82.37c	96.73b	118.63g	214.47e
S_2	30	0	21.67d	62.67e	77.47c	119.57f	181.30f
S_2	30	1.0	25.03c	71.30d	99.53ab	136.57e	221.60c
S_2	30	1.5	33.03a	86.90a	98.83b	141.30a	241.40a
S_2	30	2.0	26.73c	83.13bc	96.40b	126.57e	224.43b

Means in a column followed by similar letters are not significantly different using Duncan multiple range test at 5% probability

Dry matter yield and Nutrient uptake

Dry matter yields and nutrient (nitrogen and phosphorus) uptake at different treatment rates are shown in Table 4. Yields increased significantly with treatment interactions compared to the control. In both sites, best yields were obtained using P and lime rates of 30 kg P_20_5 ha^{-1} and 1.5 t ha^{-1} respectively with yields in S_2 being higher than that in S_1. Depressed yield however occurred beyond these rates. Nitrogen and phosphorus uptake also followed the same trend as dry matter yield except that best P uptake in S_2 occurred at the highest P (30 kg P_20_5 ha^{-1}) and lime (2.0 t ha^{-1}) rates. Increased yield and N and P uptake with lime and P fertilizers have been reported by other workers (Newton and Valdinei1997, Lelei 1999, Busari et al 2005). This has been attributed to better soil amelioration by the amendments (Miranda and Rowell 1987). Sharma et al (2000) observed increased root volume, root mass density and root length due to phosphorus which enhanced nutrient especially P uptake, thereby significantly promoting the productivity of wheat in terms of dry matter and grain. Yields. Depressed yields and nutrient uptake after rates of best dry matter yield has been attributed to nutrient imbalance and over liming (Kogbe and Adediran 2003, Busari et al 2005). Higher yields and nutrient uptake in S_2 than S_1, could be related to the

fertility soil status of the sites (Table1) and also to poor physico – chemical properties of S_1 over S_2. Poor physico – chemical properties have been reported on soils less than 100 meters to a gas flare site (Uzoho 2005).

Residual soil properties

Interaction of treatments on residual soil properties (Table 5) shows an increase in soil properties; soil pH, exchangeable Ca, Mg and available P with treatments compared to the control. For instance best pH in S_1 and S_2 were an increase of 3.4 and 1.9 units respectively over the control and occurred at the highest P and lime rates (30kg ha^{-1} P_20_5 and 2.0 tha^{-1} lime), just as soil P, Ca and Mg (S_2) Highest Mg in S_1 was at P and lime rates of 30kg P_20_5 ha^{-1} and 1.5 t ha^{-1} respectively. Increased soil pH, Ca, Mg and P due to lime and phosphorus has been reported by others and attributed to the effect of applied treatments (Ernani et al 2002, White et al. 2006). According to Lelei (1999), the raised soil pH after liming and application of phosphorus, enhanced the decomposition of organic matter and mineralization of N and P. Liming also encouraged the formation of calcium phosphates, increased mineralization of organic phosphate and consequent availability of phosphorus (Miranda and Rowell 1987). As expected the increase was better in S_2 than S_1.

Table 4 Mean dry matter yield, Nitrogen and phosphorus uptake as affected by treatment interactions.

Site	Treatments P ($t\ ha^{-1}$)	Lime g/pot	Dry matter yield g/kg	Nitrogen mg/kg		Phosphorus ($kg\ ha^{-1}$)
S_1	0	0	0.68m	0.005j		0.14j
S_1	0	1.0	1.26k	0.008j		0.37h
S_1	0	1.5	2.26g	0.022g	0.56g	
S_1	0	2.0	1.51h	0.013h	0.39h	
S_1	30	0	1.42j	0.037f		0.41gh
S_1	30	1.0	2.54f	0.098e	0.99f	
S_1	30	1.5	3.16d	0.158c	1.30f	
S_1	30	2.0	2.22g	0.153c	0.91f	
S_2	0	0	1.48h	0.015h	0.44gh	
S_2	0	1.0	2.62e	0.049f		1.06f
S_2	0	1.5	3.55b	0.116d	3.85e	
S_2	0	2.0	3.24c	0.097e	4.41cd	
S_2	30	0	1.13l	0.111d	4.26d	
S_2	30	1.0	3.21c	0.160c	4.83c	
S_2	30	1.5	6.27a	0.374a	12.98b	
S_2	30	2.0	3.24c	0.201b	13.45a	
LSD (0.05)			0.06	0.13		0.08

Means in a column followed by similar letters are not significantly different using Duncan multiple range test at 5% probability.

Table 5 Effect of treatment interactions on residual soil properties (pH, Ca, Mg and P)

Site	Treatments Phosphorus Kg P_20_5 ha^{-1}	Lime $t\ ha^{-1}$	pH H_20	Soil properties Ca ---- Cmol/kg ----	Mg mg/kg		P
S1	0	0	4.6n	0.5h	0.1e		9.41i
S1	0	1.0	4.7n	0.7g	0.2e		14.e
S1	0	1.5	5.9k	0.9fg	0.3e		16.4h
S1	0	2.0	6.6h	1.1ef	0.2e		18.7d
S1	30	0	6.8g	1.2def	0.3e		20.4c
S1	30	1.0	7.1f	1.3cde	0.5d	21.2ab	
S1	30	1.5	7.5d	1.3cde	1.8a	21.6ab	
S1	30	2.0	8.0b	1.5bcd	1.0c	22.2a	
S2	0	0	5.2m	0.7g	0.1e		13.7j

S2	0	1.0	5.4l	0.9fg		0.3e	14.4g
S2	0	1.5	6.4j	1.2def		0.3e	14.5g
S2	0	2.0	6.9g	1.4cde	0.3e	15.3gh	
S2	30	0	7.3e	1.5bcd	0.4de	16.4g	
S2	30	1.0	7.7c	1.6abc	0.4de	17.3f	
S2	30	1.5	8.1b	1.8ab		0.9c	18.2e
S2	30	2.0	8.3a	1.9a		1.4b	18.9d
LSD (0.05)			0.14	0.20		0.19	0.75

Means in a column followed by similar letters are not significantly different using Duncan multiple range test at 5% probability

Conclusion

Acidity in the soils is a serious health and environmental problem being higher in S_1 (less than 30 m to a flare pit than S_2 (about 400 m to flare pit). Integration of lime and phosphorus improved soil conditions through increase in plant height, leaf area, dry matter yield, nutrient uptake (N and P) and soil properties (pH, Ca, Mg and P). S_2 was generally better than S_1. In both sites (S_1 and S_2) combinations of 30 kg P_20_5 ha^{-1} and 1.5 t ha^{-1} lime rates gave best performance in all the measured parameters and could be optimum rate for maize production in the soils. Farmers living in this area should be given good sources of irrigation and drinking water.

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Study of the Influence of Environmental Tobacco Smoke To Trachea and Lung of the Animal Model

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Abstract: The environmental tobacco smoke (ETS) can influence the expression of androgen acceptor (AR) in organs of trachea and lung of animals of Wistar Rats. The rising of AR expression could be one of the mechanisms of smoking pathogenesis. Moreover, discontinuing ETS can not make the ascension of the AR back to normal level for the animals.
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Key Words: Environmental tobacco smoke (ETS), androgen acceptor (AR), pathogenesis

Introduction

World Health Organization's (WHO) indicated the population of smoker being approximately 13 hundred million in the world. The investigation demonstrated [1] male smoker is about 66% [2]. The environmental tobacco smoke (ETS) can produce 6000 different kinds of chemical substance [1, 2, 3]. Major parts of the substances can be harmful and being as carcinogen. ETS is acting direct or the indirect role at many kinds of disease as well as the developing process, such as respiratory disorders, lung cancer, chronic bronchitis and asthma. Recently, multi-aspects research seeing from the immunity function, cell apoptosis, and oxidized damage and so forth, have carried on the discussion to the smoking pathogenesis mechanism. It is well known that ETS can affect the shape and function of testis [4]. However, a pathogenesis mechanism proposed that AR consists of eight exons with coding nucleoprotein being composed of 918 amino acids [5] is related to ETS. Androgen can diffuse into both target and non-target organs. But, it only functions in target organs. Similar like steroid hormone, AR is also a transcriptional factor. AR, if excited by Androgen, can recognize the target factor in a specific segment in DNA and combine with it to adjust the gene transcription expressing a new protein as well as changing the function of the cell [5]. In this article, we use RT-PCR and immunostaining LAB-SA to exam the AR expressing in tracheal sac and lung of the mice.

Material and Method

36 Wistar healthy male mice with body weight 180-220g provided by the Henan Province experimental animal center were randomly divided into three groups, group A was for ETS exposure, group B for being as control group, and group C as natural ETS exposure group. Each group has two cages. Each cage was raised six mice. An ETS room, 1740mm×1100mm×1500mm was constructed by acrylic plate with a 2mm×3 mm air hole on top for exposing tobacco smoke and air. Group A was in ETS exposed 60 minutes, twice a day for the first 38 days and changed to being in exposure for once a day 60 minutes for another 38 days. In group A, the ETS was provided by burning “Hongxi Cigarette” in a bundle of five pieces of 84mm cigarettes in every fifteen minutes for four times. Basic components of a cigarette consist of 17mg tar, 1.1mg smoke alkali. The antibody of AR is taken anti-AR carboxyl group end multi-peptide fragment to affine purified multi-clone immune body from the rabbit which is the product of Santa Cruz Corporation. The SP series driving fluid reagent box and DAB reagent box were the products from Beijing Zhongshan Biological Technology Limited Company. In control group B, no ETS was provided. In natural group C, same ETS exposure was provided but the animals being sacrificed one month later after quitting ETS exposure. The animals in group A should be in surgical treatment on the day of 76th at abdominal cavity ketamine (3mg/kg) injection for taking organs of trachea and lung being fixed in 4% formaldehyde solution for 24 hours. For group C, similar sample collection procedures with group A were performed 30 days later after 76 days ETS exposure. Tissue sample in preparation: Using ethyl alcohol gradient for dehydration of tissue sample embedded with paraffin wax (low melting point) in 3~4 μm slice, and then, processed with the chrome alum gelatin on glass slide being ready for staining.

Method of immunohistochemistry staining (immunostaining) of AR: Taking PBS as negative group in contrary to be in comparison of the organ of testicle for lab-animal as positive group. Normal and the benign prostate gland proliferation of the sample in situ RT-PCR, AR mRNA signal can be with purple pellet in positive group mainly located in the nearness karyotheca cytoblastema. Report from Liang Lijian research [5] revealed AR could be possible found in both cytoblastema and nucleus. Obvious yellowish brown pellet appears in intranuclear area. We can take the cytoplasma or the cell as the positive expression to determine the AR positive cell with the HPIAS-1000 high resolution pathology chart article analysis system for averaging gradation and luminosity**.** SPSS 10 software was used for t-test analysis and depicted with average ± STD (x±s).

Results

Yellowish brown pellets can be observed on both pseudostratified cilium cylinder epithelium cells and chondrocytes in the organ of trachea of the rats. We can also observe the yellowish brown pellets in the trachea pseudostratified cilium cylinder epithelium cells in organ of lung of the rats, most of them are in kytoplasm and a few in cell nucleus. In contrary, we can not observe the yellowish brown pellets in control group (replacement of anti-staining by PBS).

1. The AR expression in the organ of trachea of male rats

In Table 1, the AR expressions are listed for all animal groups. In comparison with Figure 1 and Figure 2, AR expression in group A is higher than in group B ($P<0.01$). However, no obvious difference can be observed between group C and A ($P>0.05$).

2. The AR expression in the organ of lungs of male rats

AR can only expressed in the organ of trachea of the rats, however, no expression in the organ of lung of pulmonary alveoli. The AR expression are listed in Table 2. In comparison with the figure 4 (control), AR expression in group A is higher than in group B($P<0.05$ or $P<0.01$) but no difference with Group C ($P>0.05$). No positive cells were observed in Figure 7 and 8.

Table 1. The AR expression in the organ of trachea of the rats (x±s)

groups		n	mean ash density	mean optical density
Control	B	12	99.9±7.75	0.293±0.05
ETS	A	12	87.0±9.60**	0.358±0.06**
Natural	C	12	90.6±6.28**△	0.346±0.03**△

In comparison with control ** $P<0.01$; with group C **△ $P>0.05$

Table 2. The AR expression in the organ of lung of the rats (x±s)

groups	n	mean ash density	mean optical density
Control B	12	140.5±6.04	0.114±0.015
ETS A	12	134.4±5.92*	0.143±0.023**
Natural C	12	135.1±5.29*△	0.139±0.017**△

In comparison with control * $P<0.05$; ** $P<0.01$; with group C *△ and **△ $P>0.05$

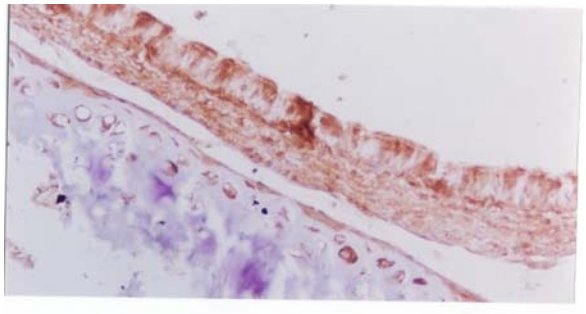

Fig 1. The AR expression in the organ of trachea of the rats in control group B, Immunostaining ×200

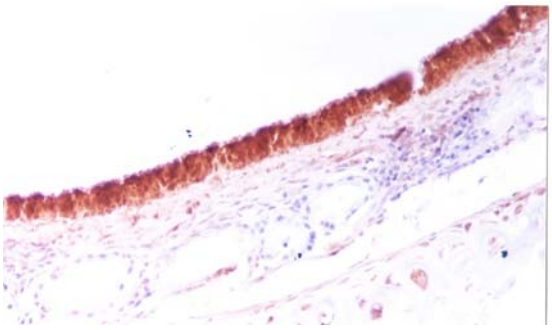

Fig 2. The AR expression in the organ of trachea of the rats in group A, Immunostaining ×200

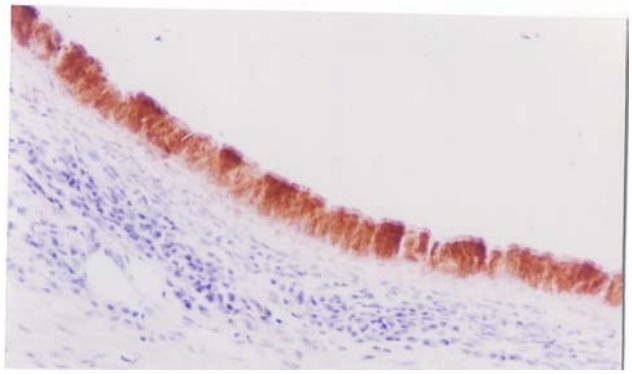

Fig 3. The AR expression in the organ of trachea of the rats in group C, Immunostaining ×200

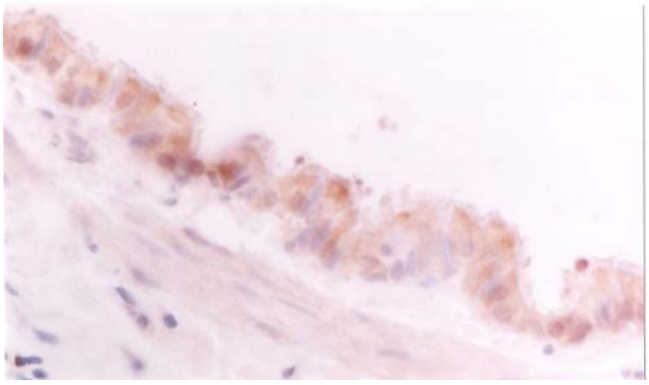

Fig 4. The AR expression in the normal organ of lung of the rats, Immunostaining ×400

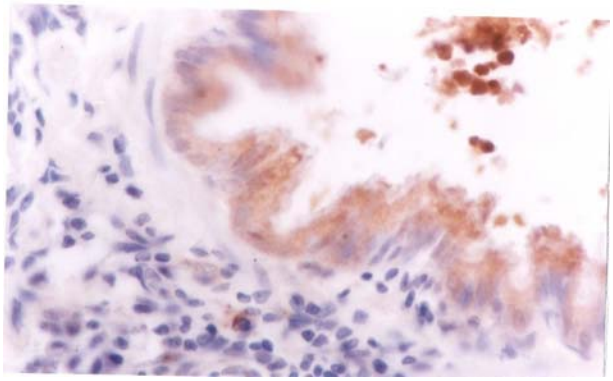

Fig 5. The AR expression in the organ of lung of the rats after ETS exposure, Immunostaining ×400

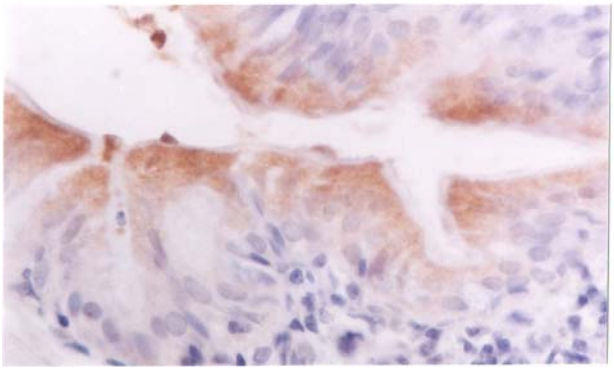

Fig 6. In Group C, AR expression in the organ of lung of the rats, Immunostaining ×400

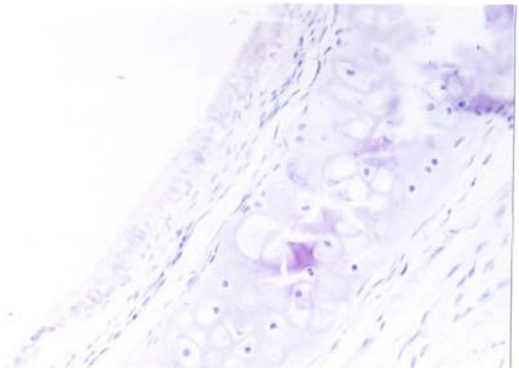

Fig 7. Negative Control Replace PBS to the antistaining in the organ of trachea of the rats of ETS, Immunostaining × 100

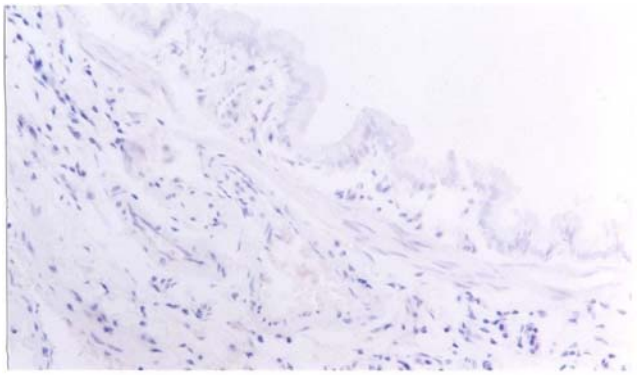

Fig 8. Negative Control Replace PBS to the antistaining in the organ of lung of the rats of ETS, Immunostaining × 100

Discussion and Conclusion

The experimental results have shown that the AR expression in the organ of lung and trachea of the rats upon ETS exposure is obvious stronger than in control. The report of Wei Sha Li et al. [6] demonstrated smoking may reduce the blood serum androgen standard. Our research revealed the similar result [7]. Extrapolating the low standard of the androgen may possibly cause AR in high expression because the androgen automatically makes the adjustment of AR expression [8]. Thus, ETS can reduce the blood serum androgen standard in the trachea and lung is very obvious. Liang Shu et al. [9] reported the AR expression for lung cancer patients can be high to 61.7%, also, AR expression of the adenocarcinoma of the lung is higher than lung cancer [10]. AR may participate the carcinogenesis in early stage. The natural did not show too much difference with the experimental group may result that quitting smoke can not help to reduce the expression of the AR in a short time period. However, the mechanism of the promotion of the AR expression and why the target should be the organs of lung and trachea would still be unknown and further research should be conducted.

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8/8/2009

Model for Calculating the Amount of Water Removable during Thermo-processing of Kaolin Based Bioceramic Material Designated for Production of Human Bone Replacement

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Abstract: Model for calculating the amount of water removable during thermo-processing of kaolin based bioceramic material (designated for production of human bone replacement) has been derived. The model; $\beta = \exp[(9.8405/T^{0.34}]$ shows that the quantity of evaporated water during the drying process is dependent on the drying temperature, the evaporating surface being constant. It was found that the validity of the model is rooted on the expression $\ln\beta = (A/T)^N$ where both sides of the expression are correspondingly approximately equal to 2. The maximum deviation of the model-predicted quantity of water removable from the corresponding experimental value was found to be less than 39.5% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model.
[C. I. Nwoye, K. Okeke, C. C. Nwakwuo, G. C. Obasi and S. U. Ofoegbu. Model for Calculating the Amount of Water Removable during Thermo-processing of Kaolin Based Bioceramic Material Designated for Production of Human Bone Replacement. Life Science Journal 2010;7(4):88-92]. (ISSN: 1097-8135).

Keywords: Model, Water Removed, Thermoproessing, Bioceramic Material.

1. Introduction

Ceramics have been found [1] to comprise various kinds of non-metallic and inorganic materials. These include naturally occurring clays (kaolin) which is mainly alumino-silicates ($Al_2O_3.2SiO_2.2H_2O$) and vary concentrations of carbides, nitrides, and oxides. Ceramic material can exist in the crystalline, amorphous, or glassy states.

Bioceramics and associated biomedical devices are used in so many parts of the human body. Because human life and well being often depend on these devices there are stringent controls and constraints placed upon the application of devices and materials that can be used. It has been found [2] that when a prosthetic device is placed into the body, two aspects must be taken into account:

Biofunctionality:

This concerns the effect of the physiological environment on the material (bioceramics)/device. The material must satisfy its design requirements in service. The varied functions of bioceramics include: load transmission and stress distribution; eg bone replacement, articulation to allow movement; eg artificial knee joint, and space filling; eg cosmetic surgery[2].

Biocompatibility

This is associated with the effect of the prosthetic device/material (and any degradation product) in the body. The material is not expected to degrade in its properties within the environment of the body and must not cause any adverse reactions within the host body.

Nature of the physiological environment:

Studies [2] carried out on the effect of the physiological environment on biomaterials/device show that NaCl aqueous solution (0.9M) containing organic acids, proteins, enzymes, biological macromolecules, electrolytes and dissolved oxygen, nitrogen compounds, and soluble carbonates are environments where biomaterials and devices can operate favourable. It was found [2] that pH ≈ 7.4 is normal for physiological extracellular fluid. It has been discovered that cells (eg. inflammatory cells and fibrotic cells) secrete several complex compounds that may significantly affect an implanted biomaterial. Applications of these biomaterials/devices have been found [2] to be also dependent on mechanical environment: static, dynamic, stress, strain and friction.

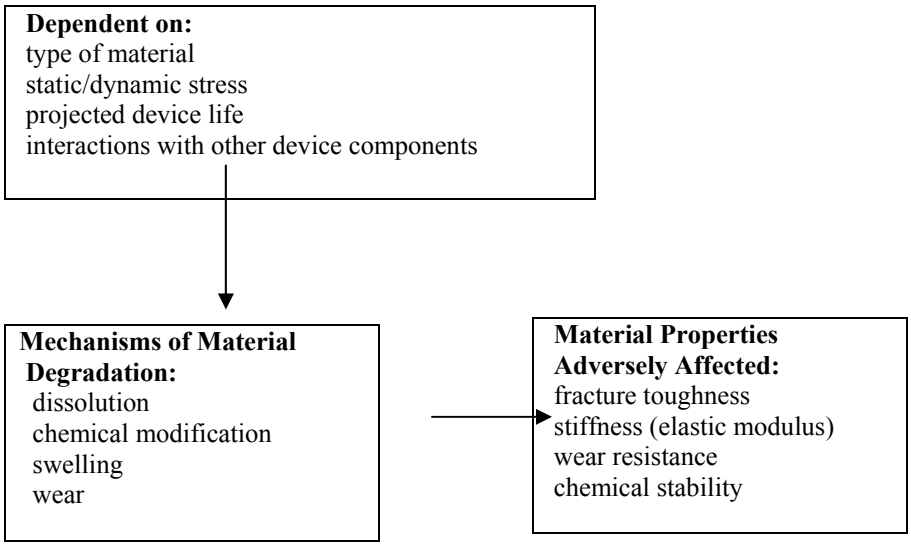

Fig. 1: The effect of the physiological environment on materials/devices [3]

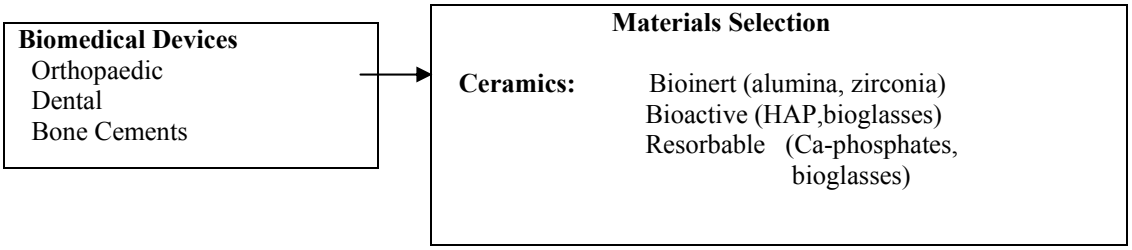

Fig. 2: Material selection for functional performance [3]

Bioceramic Materials (Functional Properties)

Ceramics are stiff, hard and chemically stable and are often used in situations where wear resistance is vital. Of the large number of ceramics known, only a few are suitably biocompatible. These ceramics can be grouped according to their relative reactivity in physiological environment. The main problem with ceramic component is that they are brittle and relatively difficult to process.

Studies [4] reveal three types of bioceramics; bioinert, bioactive and resorbable ceramics. Bioinert ceramics includes; alumina (Al_2O_3), partially stabilized zirconia (ZrO_2) and silicone nitride (Si_3N_4). For these materials, foreign body response equals encapsulation. They were found [4] to be extremely stable and elicit minimal response to host tissues. The functional properties of bioinert ceramics were found [3] to include; high compressive strength, excellent wear resistance and excellent bioinertness. Alumina has been found [3] to be a traditional bioinert material being chemically inert and highly stable oxide. It has been discovered that alumina has low fracture toughness and tensile strength implying that it can be used in compression only. Applications of alumina were found [3] to include; femoral head of total hip replacement (polycrystalline) and single crystal (sapphire) in dental implants. It has been found that zirconia combines with a metal oxide dopant (stabilizing oxide-MgO or Y_2O_3) to form a ceramic known as partially stabilized zirconia (PSZ). This ceramic exhibits excellent toughness compared to other ceramics. This was found to be as result of a process known as transformation toughening [5]. This involved an energy absorbing phase change at the front of propagating crack tip which slows down the advancement of cracks. PSZ has been found to be useful in hip joint prosthesis.

It has been found [5] that bioactive ceramics direct chemical bond with tissue and in particular, bone. They are surface-reactive but has low solubility, allows fixation of implants in the skeletal system. Investigations [4] carried out on the properties of bioactive ceramics reveal that they are hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and bioglasses, having low mechanical strength and fracture toughness. It was reported [3] that bioactive ceramics can find application in: coatings used on stainless steel, Ti and CoCr for tissue on-growth, bone filler for dental and maxillofacial reconstruction.

It has been found [4] that resorbable ceramics are chemically broken down by the body and resorbed. This type of ceramic was also found [3] to control dissolution rate by composition and surface area (density). Investigations [3] indicate that chemicals produced as the ceramic is resorbed, are processed through the normal metabolic pathways of the body without evoking any deleterious effects. Chemically resorbable ceramics is made up of calcium phosphate e.g., tri-calcium phosphate, $Ca_3(PO_4)_2$.This ceramic has found applications in bone repairs such as maxillofacial and periodontal defects, as well as in temporary scaffold or space-filler material [3].

It has been discovered that on drying clays through heating, water is given off. With time, a hard but porous piece forms. A swollen appearance might occur during the release of some gases, but overall shrinkage must occur when verifications set in leading to a strong dense piece [6].

The present work is to derive a model for calculating the amount of water removable during thermo-processing of kaolin based bioceramic material (mined at Otamiri (Nigeria)) designated for production of human bone replacement. Evaporation of water from the kaolinitic clay ($Al_2O_3.2SiO_2.2H_2O$) occurred in the course of drying the clay (in the oven) during the extraction of alumina (Al_2O_3). The need for this extraction resulted from the indispensable role played by alumina (in biomedical engineering) as bioinert ceramics for total hip replacement.

2. Model formulation

Experimental data obtained from research work [7] carried out at SynchroWell Research Laboratory, Enugu were used for this work. Results of the experiment used for the model formulation are as shown in Table 1.

Computational analysis of the experimental data [7] shown in Table 1, gave rise to Table 2 which indicate that;

$$\ln\beta = \left(\frac{A^N}{T}\right) \quad \text{(approximately)} \qquad (1)$$

$$\ln\beta = \left(\frac{A^N}{T^N}\right) \qquad (2)$$

Introducing the values of A and N into equation (2)

$$\ln\beta = \left(\frac{(833)^{0.34}}{T^{0.34}}\right) \qquad (3)$$

$$\ln\beta = \left(\frac{9.8405}{T^{0.29}}\right) \qquad (4)$$

$$\beta = \text{Exp}\left(\frac{9.8405}{T^{0.29}}\right) \qquad (5)$$

Where

(β) = Weight of water evaporated during the drying process (g)
A = Area of evaporating surface (mm^2)
N = 0.34(Collapsibility coefficient of binder-clay particle boundary at the drying temperature range ;80-110^{0}C) determined in the experiment[7].
(T) = Drying temperature (^{0}C).

Table 1: Variation of the amount of water removable with drying temperature. Variation of concentration of dissolved iron with weight input of iron oxide ore and final solution pH. (Nwoye; 2006) [7]

(T)	A	(β)
80	833	6.60
85	833	7.50
88	833	7.74
90	833	8.40
95	833	8.92
110	833	10.72

Table 2: Variation of lnβ with $(A/T)^N$

(A/T)	$(A/T)^N$	lnβ
10.4125	2.2180	1.8871
9.8000	2.1728	2.0149
9.4659	2.1473	2.0464
9.2556	2.1310	2.1282
8.7684	2.0922	2.1883
7.5727	1.9904	2.3702

3. Boundary and Initial Conditions

Consider a rectangular shaped clay product of length 49mm, width 17mm, and breadth 9mm exposed to drying in the furnace while it was in wet condition. Initially, atmospheric levels of oxygen are assumed. Atmospheric pressure was assumed to be acting on the clay samples during the drying process (since the furnace is not air-tight). The grain size of clay particles used is 425μm, weight of clay and binder (bentonite) used (for each rectangular product); 100g and 10g respectively, quantity of water used for mixing; 2% (of total weight), range of drying temperature used; 80-110^{0}C, area of evaporating surface;833mm^2 and drying time used;110mins.These and other process conditions are detailed in the experimental technique [7].

The boundary conditions are: Atmospheric levels of oxygen at the top and bottom of the clay samples since they are dried under the atmospheric condition. No external force due to compression or tension was applied to the drying clays. The sides of the particles and the rectangular shaped clay products are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of the model-predicted β values and those from the experiment for equality or near equality.

Analysis and comparison between these β values reveal deviations of model-predicted β from those of the experimental values. This is believed to be due to the fact that the surface properties of the clay and the physiochemical interactions between the clay and binder, which were found to have played vital role during the evaporation process [7] were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted β value to that of the corresponding experimental value (Table 3).

Deviation (Dv) (%) of model-predicted β values from the experimental β values is given by

$$Dv = \frac{DP - DE}{DE} \times 100 \quad (6)$$

Where DP = β values predicted by model

DE = β values obtained from experiment

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \quad (7)$$

Therefore

$$Cf = -100\left(\frac{DP - DE}{DE}\right) \quad (8)$$

Introduction of the value of Cf from equation (8) into the model gives exactly the corresponding experimental value of β [7].

5. Results and Discussion

The derived model is equation (5). A comparison of the values of β obtained from the experiment and those from the model shows little deviations, hence depicting the reliability and validity of the model (Table 3).The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 39.5% which is quite within the acceptable deviation range of experimental results [8], hence depicting the usefulness of the model. It was found that the validity of the model is rooted in equation (1) where both sides of the equation are correspondingly approximately equal to 2. Table 2 also agrees with equation (1) following the values of lnβ and $(A/T)^N$ evaluated from Table 1 as a result of corresponding computational analysis.

Table 3: Comparison between quantities of evaporated water as predicted by model and as obtained from experiment [7].

β_{exp}	β_M	Dv (%)	Cf (%)
6.60	9.1892	+39.23	-39.23
7.50	8.7826	+17.10	-17.10
7.74	8.5617	+10.62	-10.62
8.40	8.4229	+0.27	-0.27
8.92	8.1023	-.9.17	+9.17
10.72	7.3186	-31.60	+31.60

Where β_{exp} = β values obtained from experiment [7]
β_M = β values predicted by model.

6. Conclusion

The model calculates the amount of water removable during the thermoprocessing of kaolin based bioceramic material.. It was found that the validity of the model is rooted in the expression; $\ln\beta = (A/T)^N$ where both sides of the expression are correspondingly approximately equal to 2. The maximum deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 39.5% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model.

Further works should incorporate more process parameters into the model with the aim of reducing the deviations of the model-predicted β values from those of the experimental.

Acknowledgement

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Diarrhoea in Neonatal baraki kids-goats

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Abstract: A survey was carried out in 130 kids-goats aged from 2 days to 3 month from different private farms in El Mounofia and Kalubia Governorates. Out of these animals, 100 were suffering from diarrhoea. Bacteriological examination of the faecal samples revealed the presence of *E. coli* (58%), *Salmonella*, (27%), and *Shigella* (15%), as the main causative agents of diarrhoea. They were sensitive to common antibiotics and less sensitive to 10% garlic extract and 40% *Hibiscaus subdarifa.* Haematological studies revealed significant decrease in hemoglobin content (Hb), erthrocytic (RBCs) count. On contrary, haematocrit value (PCV %) showed significant increase in affected animals. A significant decrease was detected in the values of serum total proteins, albumin, iron, copper, and growth hormone. On the other hand, there was a significant increase in cortisol hormone, lactate dehydrogenase (LDH), and alkaline phosphatase enzymes. We emphasize that the demonstrated diarrhoea caused many harmful clincopathological effects, reduced growth hormone, and caused severe anaemia in kids-goat.
[Mona S. Zaki; Nagwa S. Ata; Shalaby, S. I. and Iman M. Zytoun. Diarrhoea in Neonatal baraki kids-goats. Life Science Journal 2010;7(4):93-97]. (ISSN: 1097-8135).

Keywords: Kids-goat - kids - diarrhoea - haemogram - Salmonella - *E. coli* -serum biochemistry - LDH - alkaline phosphatase - hormones - trace elements - garlic extract - *Hibiscous subdarfa*.

Introduction

With the increasing application of intensive husbandry methods the various causes of ill-thrift in sheep and goats have attracted increasing attention. The results of many investigations have shown that the greatest loss among these species occurs in the neonatal period (Snodgrass and Angus, 1983). Neonatal diarrhoea in kid-goat is a common problem with not very well understood cause (Snodgrass, et al., 1977). This syndrome has been ascribed to a variety of causes such as nutritional imbalance, faulty management and infectious agents (Durham et al., 1979). Infectious diarrhoea affecting kids-goat occurs mainly where intensive systems of breeding which use paddocks, pens and indoor kids-goat sheds are employed. Such systems unless very carefully managed, encourage the progressive build-up of infection (Allan and John, 1987; Aly, et al., 1996 and Angus, et al., 1982).

Aim of the present work to study the cause of diarrhoea, the clinicopathological changes in blood of infected animals and the suitable antibiotic for treatment.

Material and Methods

Animals:

One hundred and thirty kids-goat (100 diarrhoeic + 30 apparently healthy as a control group), aged from 2days to 3 months were used in this study. These kids-goats belonged to different localities in El-Mounifia and Kalubeia Governorates and under semi-intensive management system.

Sampling:

All animals were sampled once before administration of any treatment.

Bacteriological Studies:

Fecal samples:

Two faecal samples were taken directly from the rectum of all animal in the investigation. One sample was taken in a clean dry plastic packs for parasitological examination to detect gastrointestinal parasites (Coles, 1986) and the second using sterile swabs for further bacteriological analysis. These swabs were immediately inoculated on Carry and Blair's transport medium and were cultured on selective and differential culture media at 37^{o}C for 24 hours and the isolated colonies were then identified according to Carter (1984) sand Baily and Scott (1990) as follows: Isolated colonies from MacConky's agar plate were examined to be either Lactose fermenting or non-lactose fermenting. Lactose fermenting colonies appeared to be rose pink in color and non-lactose fermenting as pale yellow colonies. Isolated colonies were then examined by Gram staining. Colonies, which appeared as Gram negative bacilli were then described for further

identification of Gram negative isolates. These were then subjected to biochemical reactions such as indol production, methyl red Gobes Proskauer test (MR/VP), citrate utilization, hydrogen suiphide production, reaction of triple sugar iron agar (TSI), urease production and oxidase test.

Detection of K99 antigen was performed by slide agglutination test (SAT) according to Baily and Scott (1990), with specific antisera Cryptoporidia were examined in faecal smears on glass slides which were air dried, fixed in methanol and stained with Geimsa stain according to Abou-Zaid and Nasr (1995).

Haematological Studies:

Whole blood samples with EDTA were obtained form the jugular vein for determination of hemoglobin content, haematocrit (PCV%) value, erythrocytic (RBCs) count and total leukocytic (WBCs) count according to Coles (1986).

Biochemical and Hormonal Studies:

Serum samples were used for determination of copper and iron by atomic absorption according to Issac and Kerber (1971). Total proteins, albumin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) were determined b spectophotomoeter in the rang UV "240 nm". Cortisol hormone was measured according to Kuehn and Burvenich (1986). Growth hormone was measured by special kits according to the method described by Ronge and Blum (1988).

Sensitivity Test:

1. Sensitivity test using common antibiotics:

The following chemotherapeutic agents were used in testing the isolated micro-organisms:

Gentamycin (10mcg/disc), chloramphenicol (30mcg/disc), rifamycine (30mcg/disc), tetracycline (30mcg/disc), ampicillin (10mcg/disc), streptomycin (10mg/disc), nalidixic acid (30 mg/disc), and colistin (10 meg/disc).

2. Sensitivity test using Garlic aqueous solutions:

The isolates were incubated in about 1 0% garlic aqueous solution at 28°C till the colonial broth become evident. The degree of inhibition was compared to control.

3. Sensitivity test using dry *Hibiscus subdarifa* flowers:

The flowers were extracted with 75% ethyl alcohol using apparatus Soxhlet till complete exhaustion occurs. Alcohol was then evaporated to obtain a semisolid extract. Dilutions to 40% were obtained by dissolving the extract in distilled water. The resultant dilutions were used to test microorganisms were streaked with 0.4 mm loop on the extract into the gutter avoiding it over flow on the surface.

Statistical analysis:

All data were subjected to statistical analysis using T- test according to Gad and Well (1967).

Results:

Kids-goat were divided after, careful clinical examination and bacteriological examination of the faecal samples into three groups as shown in Table (1).

Clinical Signs:

Diseased kids-goats showed severe depression unable to stand or move and some of them showed sternal or lateral body recumbent. Soft to watery of faeces tinged with mucus or occult blood or both and having putrefied odour. Varying degree of dehydration and severe losses of skin elasticity. Contaminated skin of anal region, rough hairs, dry muzzle, increase of body temperature, pulse and respiratory rates.

Bacteriological Studies:

Bacteriological examination of the faecal samples of diarrhoeic kids revealed that 100 samples were positive for pathogenic bacteria. The distribution of thee indicated that enteropathogic E. Coil and Salmonella constituted the high incidence while Shigella recorded the very lowerest incidence. The increase in packed cell volume (PCV %) reflected the severity of dehydration occurred in diarrhoeic kids with bacterial enteritis in group 2 (infected with E. coli) and group 3 (infected with Salmonella) than in group 4 (infected with Shigella) and apparently healthy kids (group1). This reflects the severity of diarrhoea caused by enterotoxins produced by enterotoxigenic bacteria proliferation in the intestine which lead to toxaemia and that in turn aggravates the dehydrations. The most characteristic features in diarhoeic kids faeces was watery and contained mucus or occult blood or both and was having putrefied odour could explain the high incidence of isolated enteropathogenic E. coli and Salmonella. However the presence o other pathogenic bacteria was also suggested but their incidence was very low as Shigella

Concerning sensitivity test; the result indicate that E. coli and Salmonnela were highly sensitive to gentamycine, chioramphenicol, rifamycine, and tetracycline, less sensitive to ampicillin and nalidixic acid and resistant to streptomycin and colistine. Moreover, E. coli was

moderately sensitive to *Hibiscous subdarifa* and garlic solution (Table 2).

Results of haematology and biochemistry:

A significant decrease in haemoglobin content (Hb), erythrocytic (RBCs) count while, haematocrit values (PCV%) and the leukocytic (WBCs) count showed significant increase in affected animals with E. coli (group2) and Salmonelh (group3) than the control healthy animals (group 1) as shown in Table (3).

As shown in Table (4, 5), there were a significant decrease in total proteins, albumin growth hormone, iron, and copper. On the other hand, there was a high level of cortisol hrrnone, lactate dehydrogenase, an alkaline phosphatase in diarrhoeic kids-goat in comparison with the control one.

Table (1): Bacterial examination of faecal samples of diarrhoeic kids-goats

The organism	Number of isolates	% of isolates
E. Coli	58	68.84
Salmonella spp.	27	44.68
Shigellaspp.	15	1.05

Table (2): Results of sensitivity test against different chemotherapeutic agents

Chemotherapeutic agents	Disc concentration	E. coli	Salmonella
Gentamycin	10 mcg	+++	+++
Chlorampheni-col	30 mcg	+++	+++
Rifamycine	30 mcg	+++	+++
Tetracycline	30 mcg	+++	+++
Ampicillin	10 mcg	++	+
Streptomycin	l0mcg	+	+
Naildixic acid	30 mcg	++	+
Garlic aqueous solution 10%	l0%	++	++
Hibiscous extract 40%	40%	++	+
Colistine	10%	+	+

+++ 0.58mm ++ = 0.38mm + = 0.23mm

Table (3): Means ± SE of haemoglobin (Hb) haematocrit (PCV%) and erythrocytic (RBCs) count in both healthy and diarrhoeic kids-goat.

Animals groups	Number of animals	PCV%	RBCs (x106/p.i)	Rb (g/dl)
Group I (control)	30	24.25 ± 0.12	10.20± 0.23	9.80± 0.20
Group 2 (E. coli)	58	40.00 ± 0.02 **	8.24± 0.24**	8.00± 0.14**
Group3 (Salmonella)	27	34.00±0.10**	8.10±0.13**	8.23± 0.74**
Group 4 (Shigella)	15	23.24 ± 0.72**	9.42± 0.40	9.03± 0.72

** = Highly significant at P≤ 0.01 SE = Standard error.

Table (4) Means ± SE of iron, copper, cortisol and growth hormones in both healthy and diarrhoeic kids-goat.

Animals groups	Number of animals	Iron (mg/dl)	Copper (mg/dl)	Cortisol (ng/dl)	Grbwth Hormone (ng/dl)
Group 1 (control)	30	250 ± 2.30	185 ± 3.4	0.098 ± 0.73	11.0 ± 0.08
Group 2 (E. coli)	58	178 ± .54**	130 ± 47**	0.130 ± 0.28**	8.0 ± 0.11 **
Group3 (Salmonella)	27	180 ± 3.53**	134 ± 4.0	0.140 ± 0.30**	7.8 ± 0.20**
Group 4 (Shigella)	15	168 ± 4.01**	148 ± 2.0 **	0.150 ± 0.40**	7.1 ± 0.30**

** = Highly significant at P≤ 0.01 SE = Standard error.

Table (5): Means ± SE of total proteins, albumin, lactate Dehydrogenase (LDH). alkaline phosphates (ALP) changes in both healthy and diarrhoeic kids-goat.

Animals groups	Number of animals	Total proteins (g/dl)	Albumin (g/dl)	LDH (U/l)	ALP (U/l)
Group 1 (control)	30	9.3 ± 0.40	4.90 ± 0.27	252 ± 23	15.3 ± 0.80
Group 2 (E. coli)	58	8.2 ± 0.27**	3.80 ± 0.14**	263 ± 31 **	18.7 ± 0.50*
Group3 (Salmonella)	27	7.0±0.10**	3.40± 0.72**	270± 14**	19.1 ± 60 **
Group 4 (Shigella)	15	6.8 ±0.78**	2.86 ± 0.73**	260 ± 26**	19.0 ± 0.54**

** = Highly significant at P≤ 0.01 SE = Standard error.

Discussion

Infectious diarrhoea is a common condition affecting kids-goat specially those which are bred under intensive system of breeding in this study. Fecal samples screened the presence of the common enteropathogenic organisms E. Coli, Salmonella species and Shigella which causing diarrhoea. E. Coli seems to be the dominant enteropathogen which plays the major role among diarrhoeic kids goat (Tzipori, et. al., 1981; Angus, et. al., 1982; Carter, 1984; Farid, et. al., 1987 and Rodostits, 1992). Isolation of Salmonella species from diarrhoeic kids-goat confirmed the opinion that Salmonellosis is a sporadic cause of enteritis and cause loss in young kids-goat and buffaloe-calves (Bhullar and Tiawana, 1985). E. Coli and Salmonella were sensitive to garlic 10 %. *Hibiscou.s sabdorifa* flowers 40% sensitive to E. Coli but less sensitive to Salmonella.

The significant decrease in serum total proteins, albumin, iron and copper, in diarrhoeic kids-goat may be referred to the cause of diarrhoea. Where, there was significant increase in bacterial enteritis this could be explained by impaired absorption of these trace elements through the damaged intestinal epithelium resulting from enterotoxins produced by these bacteria in the small intestine (Kasari, 1990 and Aly el. al., 1996). Concerning serum protein and albumin, they showed significant decrease in diarrehoeic kids-goat than the cntrol group. Such drastic reduction may be attributed to diarrhoea, which lower the synthetic power of albumin in the liver due to microorganism. This opinion is supported by finding of Aly, et al. (1996). The significant increase in alkaline phosphatase, lactate dehydrogenase was observed in diarrhoeic kids-goat. Similar results were observed by Sadiek (1987).

A highly significant decrease in serum iron was noticed in diarrhoeic kids-goat, this result agreed with those obtained by Aly, et. al. (1996). The decrease of iron was accompanied by decrease of copper and this lead to anaemia (Radostitis, 1992).

Concerning cortisol hormone, an increase of this hormone can be considered as an expression of stress and helps the organism to counteract this stress, bacmatological, metallic and endocrine changes enhanced protein catabolism and gluconeogenesis during endotoxaemia (Dvorak, et al., 1974). Growth hormone concentrations tended to decrease in diarrhoeic kids-goat. An effect which was probably in part mediated by tumour necrotic factor (Walton and Cronin, 1989).

We can conclude that a substantial, bacteriological haematologlcal, biochemical, and hormonal changes occur in diarrhoeic kids-goat when the cause of diarrhoea is enterotoxigenic bacteria. This means that we must interfere quickly with therapeutic plan to put in consideration the decrease in damaged intestinal epithelium and supporting the body immune status during infection along side with the traditional electrolyte therapy.

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Endothelial Dysfunction in Systemic Lupus Erythematosus

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Abstract: Despite improved prognosis, patients with systemic Lupus, remain at increased risk of early vascular events due to premature atherosclerosis. We assessed the endothelial dysfunction in SLE as a marker of early atherosclerosis.
In thirty seven (37) female patient endothelial dependant vasodilatation (EDD) was assessed at the brachial artery in response to shear stress and glyceril trinitrate administration (NMD), intimae media thickness of the common carotid artery was also measured using high resolution B-Mode ultrasonography., anticardolipin antibodies (done only in 18 patients) Lipid profiles, ANA were also assessed.
No statistically significant difference between patients and control in basal FMD (D_1) (P=0.5) or percent change in flow mediated dilation (D_2) P = 0.3 and no change in NMD (P = 0.2).
There was weak but statistically significant correlation between FMD% and NMD% (r = 0.3, P = 0.05). Despite the disease activity according to SLEDAI (where 45.9% of patients were severely active) there was no correlation between either disease activity and FMD (r =0.03, P = 0.8), or disease duration (2.4±3.3 years) (r = 0.7, P = 0.8) Weak but statistically significant negative correlation between hypercholesterolemia and endothelial dysfunction (r = 0.3, P = 0.05).We tried to find differences between patients themselves dividing them into those with FMD <10% (n = 23 patient, 62.2%) FMD ≥ 10% (n = 14 patient, 37.8%) or FMD %/ NMD % < 0.7 (n = 23 patient, 62%), FMD % /NMD> 0.7 (n = 14 patient, 38%). However no significant differences between them as regard clinical and laboratory data.
In conclusion, FMD was not different between patients and control thus its use as a predictor of future cardiovascular events is questionable.
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1. Introduction

Patients with systemic lupus erythematosus are still at considerable risk for premature death due to accelerated atherosclerosis as traditional risk factors alone cannot explain the increased prevalence of atherosclerosis (1)

The natural history of the initial vascular complications in patients with SLE is mulifactorial auto antibodies, immune complexes and cytokines play a major role in favouring endothelial dysfunction (2)

Thus the possibility to detect early vascular damage for early prevention strategies, management and treatment of the disease will thus positively influence outcome.

Endothelial function can be assessed with a well validated non –invasive technique using ultrasound to detect the vasoreactivity of the brachial artery to shear stress (flow mediated dilation (FMD) or to nitroglycerine (Nitro-glycerine mediated dilatation) NMD.

Conflicting data have been reported concerning the correlation between endothelial dysfunction and FMD.

Aim of the present study was to evaluate the efficacy of FMD in early detection of endothelial dysfunction in patient with SLE.

2. Patients and Methods:

Thirty seven patients (Female/Male = 33/4) mean age (24.1±7.6 years) chosen from Kasr El Aini University Hospital, Internal Medicine Department and Rheumatology Clinic fulfilling at least 4 of the update revised criteria of the American College of Rheumatology for SLE diagnosis (3)

The study was conducted from January 2009 to May 2010. Compared to 10 healthy female volunteers with mean age (24.4±8.5). A verbal consent was obtained from all subjects participating in the study after explaining its nature. Disease activity was evaluated at the beginning of the study using SLE disease activity index (SLEDAI). (4)

All patients underwent detailed medical history, complete physical examination detailed history of drug intake, BMI. Patient with diabetes, BMI ≥ 30 and smokers were excluded.

All sera after 12-14 hour fast were tested for cholesterol, triglycerides , AST, ALT, albumin were done on automated analyzer Hitachi 917;commercial

kits were supplied by Roche diagnostics(Boehringer Mannheim,Germany)ESR was evaluated by westergren method.24 hour urinary protein,C_3,C_4 were measured by immunonephelometric method supplied by (Siemens,Deerfield,USA)Antinuclear Antibodies were determined using immunoflorescence testing on HEP2-cells.Anti Double stranded DNA antibodies ,Anti cardiolipin antibodies were detected by ELISA kits (Nunc,Denemark)and (Sumilon,Tokyo)respectively.

Assessment carotid IMT

Doppler ultrasound was done for all patients and control subjects to asses FMD and carotid intimae media thickness (CIMT).

Colour-coded carotid duplex sonography was performed in all subjects in the supine decubitus position, during gentle respiration. The study was carried out by the same operator using a high-resolution B-mode ultrasonography (Philips HDI 5000 SONOCT with a 7-12 MHz linear –array transducer) placed on the patient neck with the least possible pressure in order not to compress the overlying jugular vein and allow expansion of the carotid artery in all directions. The carotid view was achieved in longitudinal scan on the extra-cranial artery segment at 1 cm from the common carotid bulb (5).The IMT was defined as the distance between the leading of the luminal echo to that of the media/adventitia echo.IMT <0.8mm (0.4-0.7 mm) was defined as normal (6) and plaque was defined as a localized thickening of at least 1.2 mm that does not uniformly involve the artery (7)

Assessment of FMD:

Participants lay in a supine position and sphygmomanometer cuffs were applied on arm just above the level of elbow.

The right brachial artery was assessed using high-resolution B-mode ultrasound (Philips HID 5000 SONOCT linear broad band 7-12 MHZ transducer) after the published protocol (8)

1-Endothelium-dependent FMD:

Follow a 2-min baseline period, a frozen 3-cm longitudinal image of the vessel without colour flow was obtained and frozen for 5s.

A pneumatic tourniquet placed around the forearm proximal to the target artery (upper arm occlusion) was inflated after the baseline phase to a pressure of 50 mm Hg above the subject systolic blood pressure (or until no blood flow was noticed through the brachial artery by the Doppler probe), and this pressure was held for 5 min.Increased flow was then induced by sudden cuff deflation.

A continuous scan was performed at deflation, 60 and 90s after cuff deflation, with frozen and Doppler measurements recorded at similar intervals to the baseline phase.

2-Nitroglycerin (NTG)-induced (non-endothelium dependent) FMD:

NTG acts as a positive control by inducing vascular smooth muscle dilation independently of endothelial function.

Thirteen minutes after cuff deflation, a second 2-min baseline resting scan was recorded to confirm vessel recovery. After the administration of sublingual NTG tablet, scanning was performed continuously for 5 min.

3-Data analysis:

The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia (m line) at a fixed distance .The mean diameter was calculated from four cardiac cycles synchronized with the R-wave peaks on the electrocardiogram.

All measurements were made at end diastole to avoid possible errors resulting from variable arterial compliance.

FMD at 5min post-ischemia (100x diameter (5min after deflation of cuff)—Diameter (basal)/ Diameter (basal).

In addition, nitroglycerine-mediated dilation (100x Diameter (after nitroglycerine) Diameter (basal)/Diameter (basal) was used to represent endothelial independent vasodilatation (9)

The diameter percent change caused by endothelium-dependent flow-mediated vasodilatation (%FMD) and endothelium-independent percent change from baseline in NTG-mediated vasodilatation (%NTG) were expressed as the percent change relative to that at the initial resting scan. Significant endothelial dysfunction was defined as FMD<10% and NMD>10 %(10)

In order to increase the sensitivity and specificity of the technique FMD/NMD <0.7 defined endothelial dysfunction (11)

4. Statistical Methodology:

Statistical package for social science (SPSS) program version 9.0 was used for analysis of data. Data was summarized as mean, SD. Non parametric test (Mann Whitney U) was used for analysis of two quantitative data.

One way ANOVA was done for analysis of more than two variables followed by post Hock test for detection of significance.

Simple linear correlation (Pearsons correlation for quantitative data was done to detect the relation between D1D2x100 andD3D1x100 with all other dermographic and laboratory data.

3. Results:

Thirty seven SLE participants characteristics shown in table (1) Mean disease duration in years

2.4±3.3, sex distribution F/M=33/4. 10 control subjects were studied. (Mean age: 24.4±8.5 years)
Disease activity using (SLEDAI) inactive (n=6, 16.2%), mild activity (n=5, 13.5 %), moderate activity (n= 9, 24.3%) severe activity (n=17, 46%)
History of Reynaud's (n=4, 10.8%),cerebrovascular stroke (n=4, 10.8%) no deep vein thrombosis ,no history of vasculitis, pulmonary embolism or other thrombosies .Steroids used by all patients mean duration (2.4±3.3years),mean dose (21.9±22.5), chloroquine was used in (n=13, 35.1%) mean duration (2.6±21 year) mean dose 250 mg.Azathioprine was used in (n=4, 10.8%) ,mean duration (3.5±2.1 year) mean dose 100mg.
ANA (n=31, 16.3%) ADNA (n=24, 64.9%) Anticardiolipin IgM (n=6, 33.3%) IgG (n=4, 22.2%).

Table 1: Difference between SLE patients and controls as regards demographic, anthropometric, and clinical & laboratory findings:

Variables	Patients n=37		Control n=10		P-value
	Range	Mean ± SD	Range	Mean ± SD	
Age (yrs)	15.0-50.0	24.1 ±7.6	15.0-43.0	24.4 ±8. 5	0.9
SBP(mmHg)	100-140	117.2± 11.7	100-130	118.0 ±9.2	0.7
DBP(mmHg)	60 -90	74.5 ±8. 5	60 -90	77.5 ±8.6	0.3
Wt (Kg)	56.0-89.0	66.5 ±7. 5	56.0-73.0	62.2 ±5.6	0.08
Ht(m)	1.6-1.8	1.6 ±0.06	1.6-1.7	1.6 ±0.03	0.09
BMI (Kg/m^2)	19.5-29.2	24.9 ±2.2	21.8-26.8	24.3 ± 1.5	0.4
Dis.dur. (yrs)	0.02-18.0	2.4 ±3.3	-	-	-
RBS (mg/dl)	53.0-131.0	86.5 ±22.2	50-114	82.6 ±22.0	0.7
T. chol. (mg/dl)	100-343	199.1 ±69.5	105. -204	159.1 ±39.0	0.1
TG (mg/dl)	59-721	210.7±166.0	90-205	133.0 ±36.0	0.5
Hb (gm/dl)	4.5-15.9	9.2 ±2. 2	9.0-12.0	10.5 ± 1.1	0.03*
TLC (mm^3)	2.3-1.1	2.4 ±3.3	3.2-10.4	6.3 ±2.4	0.008*
Lymph, count	100-5000	1316 .2 ±1022.4	1400-3000	1915 ±493.3	0.01*
Platelet (mm^3)	61.0-565.0	255.5±160.2	155-350	230.0±64.4	0.7
AST (IU/ L)	11.0-290.0	49. 8 ±55.6	13-43	25.1 ± 10.5	0.1
ALT (IU/L)	10-175	39.2 ±36. 7	17.0-47.0	25. 6 ±8. 9	0.7
S.Albumin(gm%)	2.1 -4.7	3.3 ±0.8	3.3-4.4	3.9 ±0.3	0.02*
S.Creat. (mg/dl)	0.3-5.5	1.3 ± 1.2	0.3-1.1	0.8 ±0.3	0.1
U.ptn(gm/24hr)	O.١ -3.7	0.9 ± 1.1	-	-	-
ESRl(mm/hr)	10-140	101.1 ±40.2	5.0-11.0	6.8 ±2.3	0.0001*
ESR2(mm/hr)	25-147	91.2±36.2	7.0-18.0	11.9±3.5	0.001*

*p<0.05 significant

Table (2) show no statistically significant difference between SLE cases and control as regards FMD considered as basal dilatation (p=0.5) or percent dilatation (p=0.3) or percent NMD (p=0.2).No significant difference as regards IMT between patients and control.(fig 2)

Table 2: Comparison study of the Doppler examination data between patients and controls:

Variables	Patients		Control		P-value
	Range	Mean ± SD	Range	Mean ± SD	
Basal diameter [D1]	0.22-0.47	0.3 ±0.1	0.29-0.33	0.3 ±0.01	0.5
Absolute FMD [D2]	0.23-0.48	0.3 = 0.1	0.31 -0.38	0.3 ± 0.03	0.3
Percent **FMD** [D2-D1/D1X100]	2-23	9.0 ±5.1	3-18	10:7±4.5	
Absolute NMD [D3]	0.25-0.51	0.4 ±0.1	0.34-0.39	0.4±0.02	0.2
Percent NMD [D3-D1/D1X100]	4-29	15.6±6.1	12.0-24.0	17. 9 ±3.4	
CIMT	0.3-0.6	0.4 ±0.07	0.3-0.5	0.5 ±0.07	0.8

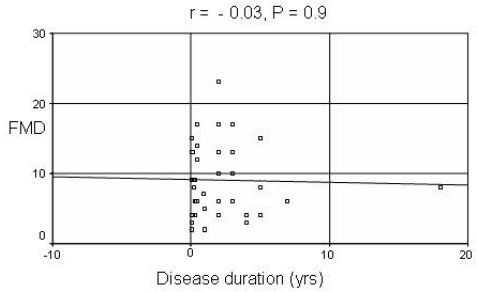
Table (3) show Comparative study between patients with FMD %<10 n=23, and FMD% ≥10 n=14, where there was no statistically significant difference between them as regards any clinical, laboratory data ,disease activity .

Table 3: Comparative study between patients with FMD %< 10 n=23, and FMD% ≥10 n=14

Variables	patients		P-value
	FMD<10 Mean ± SD N = 23	FMD > 10 Mean ± SD N= 14	
Age (yrs)	24.5 ± 8.3	23. 5 ±6.4	0.9
Disease duration. (yrs)	2. 7 ±4.0	2.0 ± 1.6	0.7
Ster. Duration (yrs)	2. 7 ±4.0	2.0 ± 1.6	0.7
Steroid dose	23.4 ±24.5	19.2 ± 19.2	0.9
SBP (mmHg)	115.9 ± 10.3	11 9.3 ±3. 8	0.5
DBF (mmHg)	73.3 ± 8.5	76.4 ±8.4	0.4
Wt(Kg)	65.3±6.1	68.3±9.3	0.4
Ht (m)	1.6±0.05	1.7 ±0.06	0.1
BMI (Kg/m^2)	25.0 ±2.3	24.8 ±2.0	0.6
SLEDAI score	10.2 ±7.0	9.9 ±7.7	0.8
RBS (mg/dl)	88.0 ±24.8	84.0 ± 17.8	0.7
T. chol (mg/dl)	209.6 ±69.9	IS 1.9 ±67.8	0.2
TG (mg/dl)	219.4± 188.8	196.4± 125.2	0.8
CIMT	0.05 ±0.008	0.05 ±0,005	0.8
Hb (gm/dl)	9.7 ±2.3	8.4 ± 1.9	0.2
TLC (mm^3)	2.1 ±3.1	2.8±3.6	0.9
Platelet (mm")	269.8 ± 149.1	221. 2 ± 198.7	0.5
AST (IU/ L)	49.7 ±58. 8	50.0 ±5 1.9	0.9
ALT (IU/L)	40.0 ±34.2	37.8 ±41.9	0.8
S.Alb.(mg/dl)	3. 2 ±0.7	3. 3 ±0.8	0.7
S.Creat(rng/dl)	1.3 ± 1.3	1.4 ±0.9	0.4
U.ptn(gm/24hr)	1 .0 ± 1 .2	0.8 ±0.8	0.7
ESR1	97.2 ±42.5	107.5 ±36.7	0.7
ESR2	79.1 ±26.8	106.7 ±42. 7	0.06

Figure (1) shows no significant correlation between FMD and disease duration p=0.9, r=-0.3

Fig-1: Correlation between FMD and disease Duration of SLE patients



To increase the specificity and sensitivity of the Doppler study Comparative study was done between patients with FMD/NMD <0.7 and those with FMD/NMD ≥0.7 as regards different clinical and

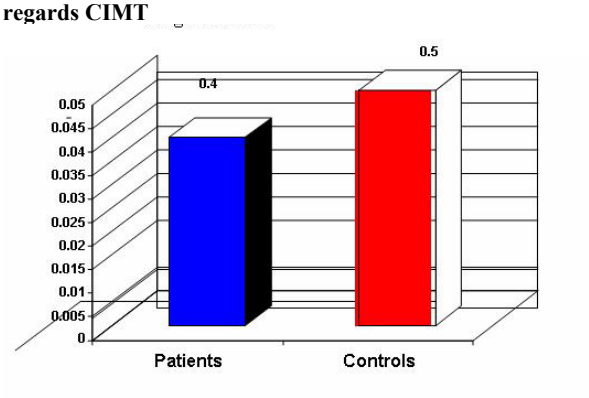
Laboratory data, however no statistically significant difference was found (Table-4).

Table 4: Comparative study between patients with FMD/NMD <0.7 and those with FMD/NMD≥0.7 as regards different clinical and Laboratory data.

	FMD / NMD< 0.7 N = 23		FMD / NMD≥ 0.7 N= 14		P-value
	N	%	N	%	
Sex: Males	2	8.7	2	14.3	0.5
Females	21	91.3	12	85.7	
Recurrent abortion	1	33.3	0	0	0.6
HTN:	1	4.3	1	7.1	0.5
Reynaud's:	2	8.7	2	14.3	0.3
Fever :	10	43.5	4	28.6	0.3
Oral ulcer :	10	43.5	4	28.6	0.3
Malar rash :	8	34.8	3	21.4	0.6
Photosensitivity:	6	26.1	4	28.6	0.3
Alopecia:	7	30.4	8	42.9	0.4
Discoid rash :	3	13	3	21.4	0.5
Puffy eve :	9	39.1	6	42.9	0.02*
Pallor :	4	17.4	8	57.1	0.5
Vasculitis :	2	8.7	2	14.3	0.3
CNS :	2	8.7	3	21.4	0.2
Arthritis :	7	30.4	2	14.3	0.6
Serositis :	1	4.3	1	7.1	0.5
Weight loss :	7	30.4	5	35.7	0.5
Lower limb edema:	9	39.1	6	42.9	0.7
DVT:	2	8.7	1	7.1	0.5
ANA : Positive	10	43.5	6	42.8	0.7
ADNA: Positive	9	39.1	5	35.7	0.6
ACL IgG: Positive	4	36.4	0	0	0.1
ACL IgM: Positive	5	45.5	1	14.3	0.2

Weak but statistically significant negative correlation between hypercholesterolemia and endothelial dysfunction(r=-0.3, p=0.05) was found.

Fig. 2 : Comparison between patients and control as regards CIMT



4. DISCUSSION

This study shows normal endothelial function as assessed by FMD either absolute or percent dilatation in a population with SLE.

This finding is in striking contrast to prior studies which have reported blunted FMD in S.LE. (11), (12), (13). (14)

The reason for this discrepancy may be due to application of the cuff of sphygmomanometer proximally where the more proximal arterial occlusion is known to produce a stronger shear stress stimulus which may be related to recruitment of more resistance vessels (8) leading to the production of significantly greater hyperaemic and vasodilatory responses (15).

Lima et al, Found reduced FMD in SLE patients even without coronary heart disease, but NMD was reduced only in anticardiolipin positive patients, Mean ±SD of FMD in SLE was 5.0±5% compared with12.0±6.0% in healthy control subject's .In that study, postmenopausal women and subjects with known CHD risk factors were excluded (Lima et al (14).

Also El- Magadmi, et al., found that SLE patients were significantly associated with impaired FMD (P=0.017) (12)

Kiss, et al., 2006, reported that the endothelium dependent vasodilatation (FMD) was significantly impaired in SLE patients as compared to controls. The absolute difference of vessel diameter after shear stress was 0.25±0.15 mm in patients vs.0.38±0.16 mm in controls (p=0.001) and as in percent of the rest diameter FMD % was 7.31±5.2% inpatients vs. 9.86±3.87% in controls (p=0.013) however NMD did not differ (16).

However in our study ,the absolute difference of vessel diameter after shear stress was 0.3 ±0.1% mm in patients vs. 0.3±0.03 mm in controls) and as in percent of the rest diameter (FMD%) was 9.0±5.1% in patients vs. 10.7±4.5% in controls (p=0.3).

Wright et al showed that FMD was significantly impaired in SLE patient compared to age and sex matched controls (p=0.001).They showed that, altered structure and function of the forearm microcirculation contribute to impaired FMD through a reduction in shear stress stimulus where there FMD was a strong correlation between FMD and diastolic shear stress (DSS) r=0.65, p=0.01 (17).

Piper et al., found that SLE patients showed significantly impaired endothelial function compared with healthy controls (p=0.001) however NMD did not differ between groups.(13)

Our results did not agree with *Palmiere .,et al, 2008,* who showed that SLE patients had lower FMD than controls (11).

Our results agree with *Aizer et al.,* where there was no statistically significant differences between SLE cases and controls in FMD considered as absolute dilation or as percent dilatation (P = 0.99). There was no statistically significant difference between patients or controls as regards NMD. *Aizer et al* results agreed with our results due to the proximal cuff application (18).

Our results regarding the relation between SLE and FMD agree with *Cypeine et al 2009* , who studied 30 SLE women (aged 37.45±9.22 years) and 66 control (aged 37.45±8.69).They showed that there was no statistically significant differences between patients and control as regards FMD (p =0.67) and that there are two other markers of arterial wall dysfunction , aortic AIX (augmentation index ;the parameter of systemic arterial stiffness) and to a less extent increased carotid –radial PWV (pulse wave velocity ;the indicator of diminished regional vessel flexibility),but not FMD and they found that both were increased in young SLE women with no history of cardiovascular disease and no severe organ damage when compared to healthy controls (p=0.004, 0.036,respectively).(19)

It may be assumed that arterial stiffness plays an independent pathogenic role in atherosclerosis and may be responsible for premature atherosclerosis in SLE and atherosclerosis lesions. (20)

In our study, there was no correlation between FMD and disease activity which was assessed in this study using SLEDAI (r= 0.03, p=0.9) or disease duration (r= 0.03, p= 0.9)

In contrast to our study ,Cypiene, et al., 2009 reported that FMD showed strong and significant relation with disease duration but their results come along with ours in that SLEDAI as a composite measure of disease activity had no impact on arterial wall function. Concomitantly, Raynaud phenomenon or anti-phospholipid syndrome had no impact on arterial wall endothelium as well .Moreover,TG and CRP ,which are commonly considered as predictor of cardiovascular risk in general population and were significantly higher in SLE group ,did not show any influence to FMD or AIX (Augmentation index) which was used in that study .Also they showed that endothelium-dependent dilatation was not related to anticardiolipin antibodies, Raynaud s phenomenon, SLE disease activity score (19)

Our results disagree with Wright, et al. who showed that there was a significant negative correlation between disease activity (as measured by SLAM-R) and FMD (r=0.67, p=0.01) and also a weaker negative association between CRP levels and FMD (r=0.41, p=0.05). (17)

In our study FMD showed weak but statistically significant negative association between endothelial dysfunction and hypercholesterolemia (r=-0.3, P= 0.05).

As we didn't find significant difference between patients and controls as regarding FMD%, we tried to find differences between patients themselves dividing them into those with FMD≥10% and those with FMD < 10% and we did not find significant difference .As *Kuvin, et al., (10),* palmieri et al., (11) stated that endothelial dysfunction is considered significant if FMD% <10 , FMD% ≥10 however in our study we didn't find any difference between groups.

In a study done by *Palmieri et al* where they selected SLE patients without clinically overt cerebrovascular events (evaluated by cardiac and vascular echo – Doppler techniques) stratified according to organ damage using systemic lupus International collaborating clinics (SLICC) damage index they defined endothelial dysfunction in his study as FMD/NMD < 0.7 to increase the sensitivity and specificity of the technique. (11)

In our study FMD/NMD < 0.7 (n = 23 case (62.2%) and those having FMN/MND >0.7 (38%) we found, no significant differences between them as regarding clinical, laboratory data, and SLEDAI.

As regards carotid intima – media thickness, there was no statistically significant difference between patients and controls (P = 0.8) and no correlation between IMT and FMD (r = 0.03). This may be contributed to the young age of the patients in the study mean age (24.1 $\pm$ 7.6 years) and to short disease duration (2.4 $\pm$ 3.3 years)

Unlike other studies which showed increased IMT with the progression of atherosclerosis process reaching highest level in patient with multiple cardiovascular complications. *(21) (22) (*23)

Our study agrees with *kiss* , (16) whose results did not show significant difference in IMT between lupus patients and control, however they found that IMT increased with progression of atherosclerosis process reaching the highest level in patients having multiple cardiovascular complications. However, in contrast with the publication of EL-Magadmi (12), who found negative correlation between IMT, FMD (r=-0.37, p=0.01). Kiss et al did not find this correlation which also agreed with our results.

5. Conclusion

Our study revealed that validation of FMD as a measure of endothelial dysfunction and predictor of future cardiovascular events in SLE is lacking despite data suggesting its predictive usefulness in other populations including patients with coronary artery disease and hypertension (24)

Thus we cannot rely on FMD as a solid evidence of the early detection of endothelial dysfunction as Doppler ultrasound is operator dependent and requires a trained sonographer to obtain accurate and reliable serial images of the brachial artery.

Also in the same time we cannot deny the presence of endothelial dysfunction in systemic lupus patients which could not be explained by traditional risk factors of atherosclerosis.

Long term close follow up is recommended, other modalities such as microcirculatory studies may be used.

Recommendation:

Further studies are required to evaluate endothelial dysfunction through measuring arterial wall stiffness. In particular, the pulse wave velocity (PWV) and augmentation index (AI) which determine the elasticity and other properties of the artery which correlate with arterial dispensability and stiffness and microcirculation.

Limitations of the study:

Our study has several limitations. First; the patients included in the study were with low age limit. Second, patients were with short mean duration of the disease. Third, small group of the study.

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10/13/2010

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Influence of resin-tags on shear-bond strength of butanol-based adhesives

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Abstract: Objectives: The aim of this in vitro study was to assess micro-shear bond strength (µSBS) of tertiary-butanol-based adhesive under moist and dry conditions and correlate the results to resin-tags surface-area. Methods: Thirty-extracted human molars were used. Flat dentin surfaces were prepared on buccal and occlusal surfaces ready for bonding. Specimens were randomly divided into three-groups; G1: Prime&BondNT, applied to moist dentin (control), G2: XPBond, applied to moist dentin, and G3: XPBond, applied to dry dentin. Etch&Rinse technique was used for both adhesives as per manufacturer's instructions. For G3, dentin was air-dried for 10s before XPBond application. Three-microcylinders of composite-resin (TPH A2 shade, Dentsply) were bonded to buccal dentin of each specimen for µSBS testing, while 2mm composite-resin was bonded to occlusal dentin for tags surface-area analysis. Curing was performed for 40s (LED, Bluephase, Ivoclar/Vivadent). All specimens were stored in distilled water at 37°C for 24h. µSBS testing was performed using testing machine (Model LRX-plus; Lloyd-Instruments Ltd., Fareham, UK) and data were recorded using software (Nexygen-MT Lloyd Instruments). Each specimen was then sectioned mesio-distally to expose resin–dentin interface, examined at 1500X using Environmental-Scanning-microscope, and tags surface-area were calculated. Data were analyzed by Pair-wise Newman-Keuls multiple comparison and regression-analysis ($P<0.05$). Results: G2 (29.06MPa) showed insignificantly higher µSBS than G1 (25.45MPa), while G3 (17.3MPa) showed significantly the lowest µSBS. G3 produced significantly highest tags surface-area (200.4µm²) compared to G1 (149.4 µm²) and G2 (94.54 µm²). Conclusion: - Butanol-based adhesive bonded to moist dentin, produced high µSBS and hybrid layer with short resin-tags that showed a perfectly infiltrated and sealed dentin-resin interface, - bonding to dry dentin showed lower µSBS, - there was significant correlation between tags surface-area and µSBS for G1&G2, - no correlation was found for G3. Acknowledgement: Dentsply/Caulk. The purpose of this in vitro study was to compare micro-shear bond strength (µSBS) of a tertiary-butanol-based adhesive to a 2-step etch and rinse one, under moist and dry conditions and correlate the results to resin-tags surface-area.

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Keywords: resin-tag; shear-bond; butanol-based adhesives; micro-shear bond strength (µSBS)

Introduction

The type of solvent used in dentin adhesives strongly influences their clinical application protocol. While acetone-based systems only work well on a moist dentin surface, acid-etched dentin with excess water shows detrimental effects, referred to as the "over-wet phenomenon"[1]. On the other hand, water-based systems are not so sensitive with regard to dentin moisture content, as they have inherent rewetting properties, but require a longer evaporation time. If the solvent is not completely evaporated before light-curing the adhesive, flaws can weaken the hybrid layer probably causing premature restoration failure[2]. A new type of solvent for adhesives, namely tertiary-butanol was claimed to be less sensitive to residual dentin moisture and allow full resin penetration under a wide range of dentin conditions. Manufacturers claim that these adhesives contain phosphate esters that may chemically bond with the mineral apatite component of dentin and enamel[3]. Together with the formation of a hybrid layer and chemicalbonding to dentin substrate,resin tags may become a key factor in the bonding oftert-butanoladhesives.Therefore, the purpose of this in vitro study was to compare micro-shear bond strength

(μSBS) of a tert-butanol-based adhesive to a 2-step etch and rinse one, under moist and dry conditions and correlate the results to resin-tags surface-area.

Materials and Methods:

Flat bonding sites were prepared on the buccal and occlusal surfaces of thirty extracted human molars. Teeth were sectioned using a water cooled diamond disc and rotary instrument (Diamond instruments, DiaTessin,Switzerland), to expose the superficial buccal and occlusal dentin. Specimens were randomly divided into three-groups according to the adhesives and conditions of use (Table 1, 2).

Table 1: Materials used in this study

Material	Type	Composition	Composite	Manufacturer
Prime& Bond NT	2-step Etch& rinse, Acetone solvent	PENTA UDMA, resin5-62-1, resin-T, resin-D, bis-phenol A dimethacrylate, acetone, nano-scale filler cetylaminehydrofluoride	TPH resin composite (Microhybrid)	DentsplyDetrey, Konstanz, Germany
XPBond	2-step Etch& rinse, t-butanol solvent	PENTA, TCB resin, UDMA, TEGDMA, HEMA, nanofiller, camphorquinone, DMABE, butylatedbenzenediol, tertiary butanol	TPH resin composite (Microhybrid)	DentsplyDetrey, Konstanz, Germany

Table 2: Grouping, conditions of use, and material application

Grouping	Adhesive used and substrate condition	Application and Curing
G1 (control):	Prime&BondNT applied to moist dentin	Conditioner gel 36% is applied for 15 s, rinsed for 15 s and blot-dried with cotton. Application of P&B for 20 s, leave surface undisturbed for 20 s, gently air-dried for 5 s, and light cured for 10 s (LED, Bluephase, Ivoclar/Vivadent).
G2	XPBond, applied to moist dentin	Conditioner gel 36% applied for 15 s, rinsed for 15 s and blot-dried with cotton. Application of XP for 20 s, leave surface undisturbed for 20 s, gently air-dried for 5 s, and light cured for 10 s
G3	XPBond, applied to 10 s air-dried dentin	Conditioner gel 36% applied for 15 s, rinsed for 15 s and - dried for 10 s (to simulate over-drying). Application of XPBond for 20 s, leave undisturbed for 20 s, gently air-dried for 5 s, and light cured for 10 s

After application of the adhesive systems, three-microcylinders of composite resin were bonded to the buccal dentin bonding sites of each ground tooth for μSBS testing. Each tooth also, had a 2mm composite resin block bonded to the exposed bonding site on the occlusal dentin for resin tag surface-area evaluation. All specimens

were stored in distilled water at 37°C for 24h. Micro-shear bond testing was performed first using testing machine (Model LRX-plus; Lloyd-Instruments Ltd., Fareham, UK). Data were recorded using software (Nexygen-MT Lloyd Instruments). Each specimen was then sectioned mesio-distally to expose resin–dentin interface, soaked in 0.5 N HCl for 20 s followed by 5% NaOCl for 2 min to reveal the hybrid layer, then examined at 1500X using Environmental Scanning Electron Microscope (ESEM, Quanta200). Resin tags' surface-area was calculated using XT Document software (Netherland). Data were analyzed by Pair-wise Newman-Keuls multiple comparison and regression-analysis ($P<0.05$).

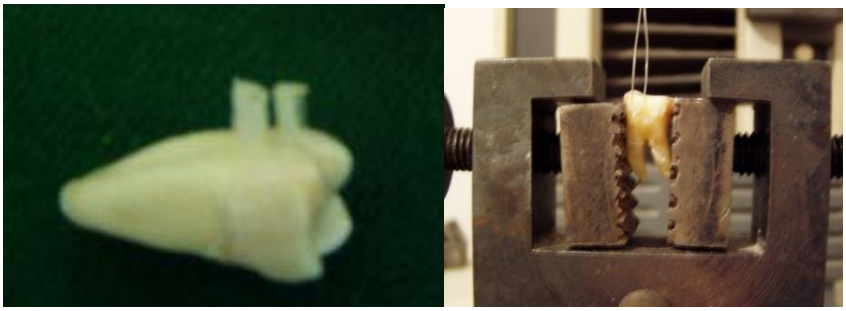

Figure 1: a, specimen with micro-cylenders bonded to buccal surface; b, µSBS compartments

Results:

1. µSBS results

   XP group with moist dentin attained statistically significant highest bond strength (29.06 MPa). Intermediate bond strength was obtained with P&B group (25.45 MPa), while XP group with dry dentin showed the statistically significant lowest bond strength (17.3 MPa (table 3).

Table (3) Descriptive statistics of µSBS in MPa for all groups ($p < 0.05$).

	P&B (G1)	XP moist dentin (G2)	XP dry dentin (G3)
Mean	25.45[a]	29.06[a]	17.31[b]
Std. Deviation	3.531	4.039	3.311

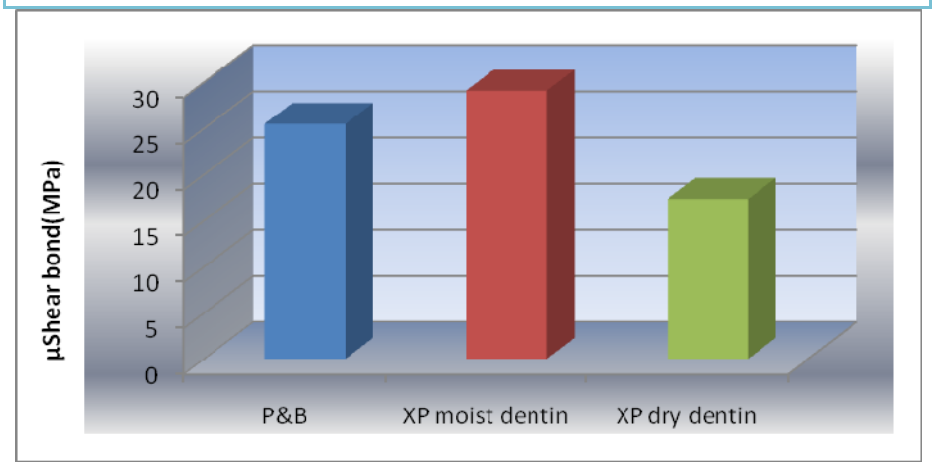

Figure (2) Column chart of µSBS mean values (MPa) for all groups

2. Resin tag surface area Results

XP group with dry dentin attained statistically significant highest tags area (200.4 µm²). Intermediate tags area was obtained with P&B group (149.4 µm²), while XP group with moist dentin showed the statistically significant lowest tags area (94.54 µm²).

Table (4) Descriptive statistics of tags area (µm²) for all groups

	P&B (G1)	XP moist dentin (G2)	XP dry dentin (G3)
Mean	149.4[b]	94.54[c]	200.4[a]
Std. Deviation	26.09	20.06	38.00

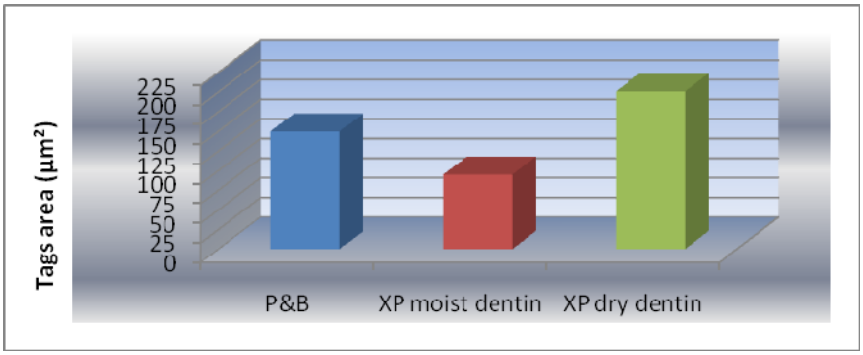

Figure (3) Column chart of tags area (µm²) mean value for all groups

3. Correlation results

A statistically significant correlation was found between tags area and bond strength for Prime & Bond and XP-Bond with moist dentin as revealed by regression statistics. There was no correlation between tags area and bond strength for XP-Bond with dry dentin as revealed by regression statistics.

Table (5) Correlation of tags area and bond strength for all groups

Regression Statistics	Correlation coefficient R	R Square	P-value
Prime & Bond	0.8585	0.7370	0.0286*
XP moist dentin	0.9653	0.9318	0.0018*
XP dry dentin	0.62480	0.3904	0.185 ns

*; significant ($p < 0.05$)

4. ESEM evaluation

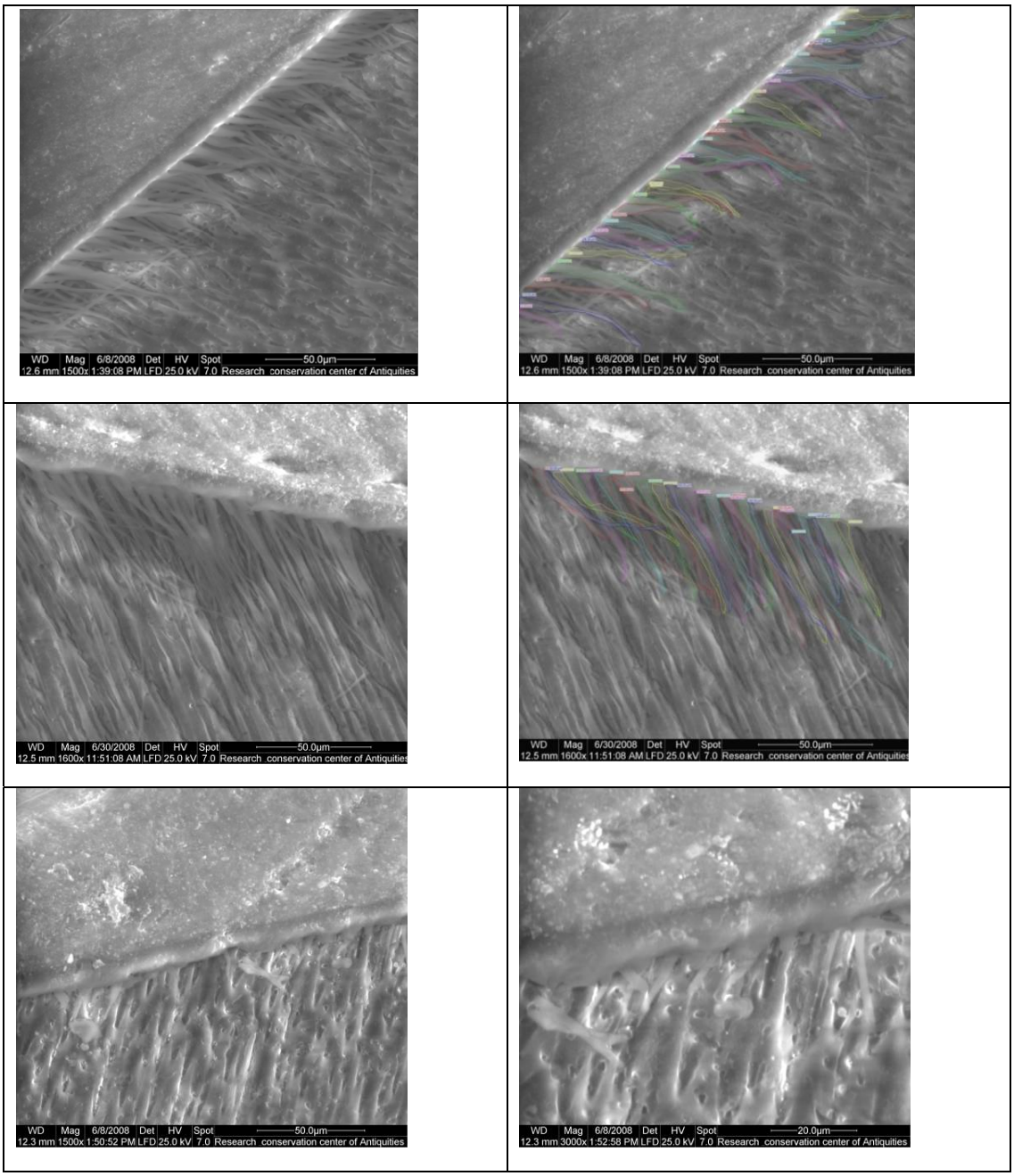

Figure 4: Prime&Bond NT with moist dentin (G1) a-e: ESEM micrographs showed a distinct adhesive layer, well and homogenously infiltrated hybrid layer (Magnification X1500). F: Dense, sealed hybrid layer (Magnification X3000).

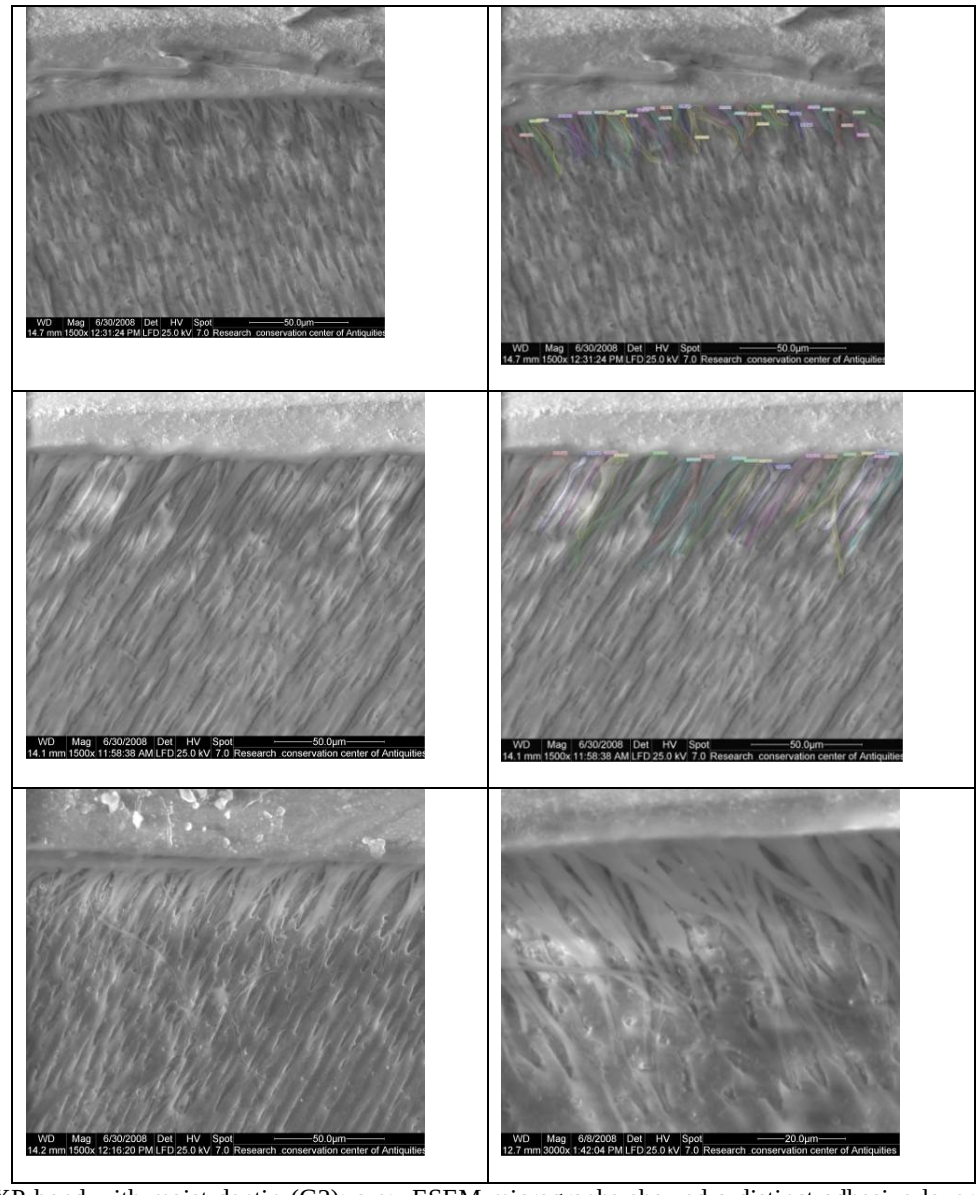

Figure 5: XP bond with moist dentin (G2): a-e: ESEM micrographs showed a distinct adhesive layer, a well and homogenously infiltrated hybrid layer with less resin tag area (Magnification X1500). f: Dense sealed hybrid layer (Magnification X3000).

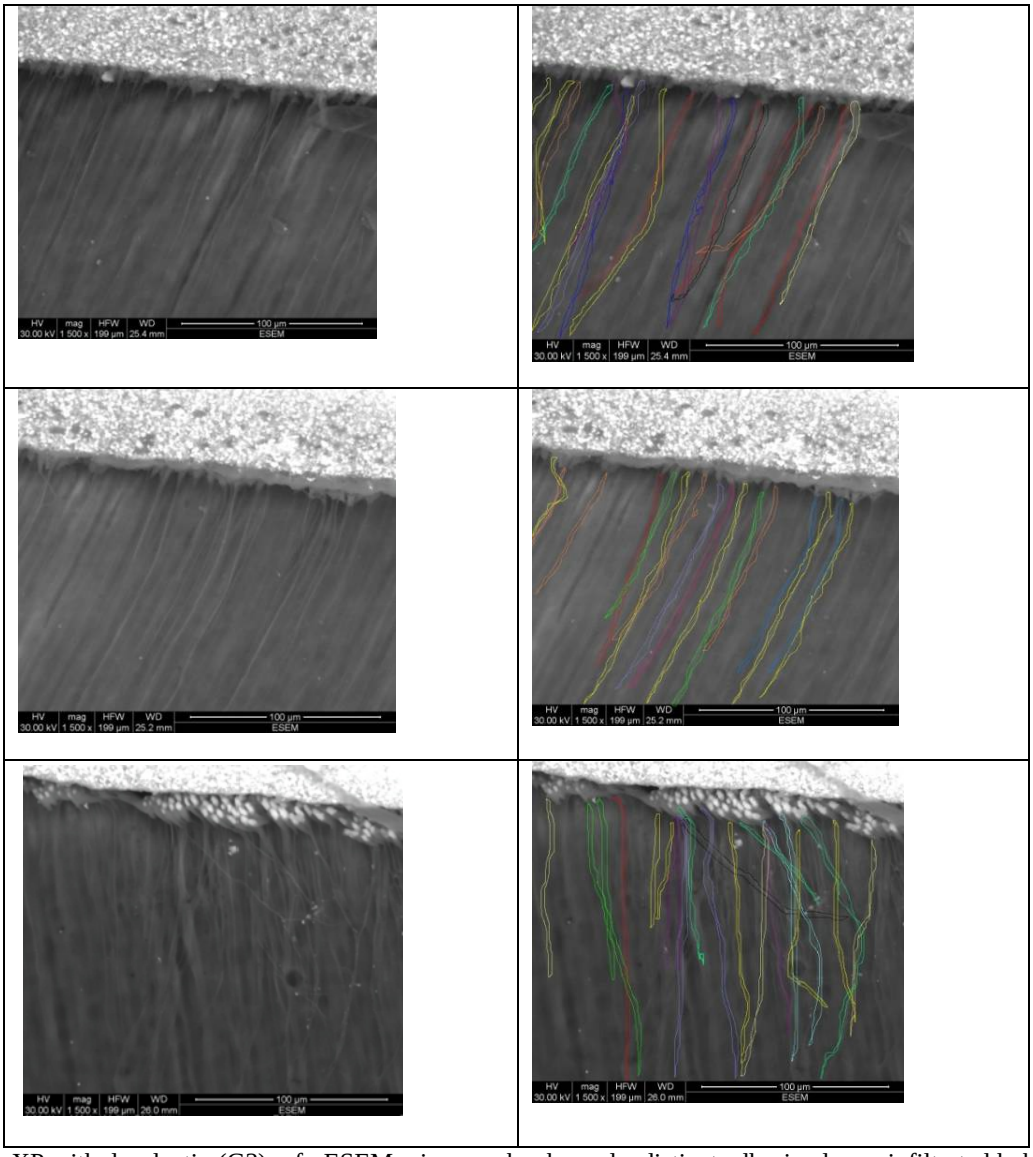

Figure 6: XP with dry dentin (G3) a-f: ESEM micrographs showed a distinct adhesive layer, infiltrated hybrid layer with areas of detachment from the underlying dentin. Long resin tags were observed. Hybrid layer seal was imperfect (Maginfication X1500).

Discussion

Micro-shear bond strength testing *(μSBS)*allows the measurements on small areas, making it possible to assess the adhesion strength of the resin composite to the clinically relevant dentin. The technique eliminates most of the cohesive resin or dentin fracture seen in more traditional shear strength test procedures that are due to nonuniform stress distributions.[4]Monomers of adhesive systems are carried by a solvent which is usually water, ethanol, acetone, or a combination of those[5]. Compared to Prime&Bond NT, in XP *BOND* acetone is replaced by tert-butanol. This solvent has a higher boiling point than acetone. Hence, tert-butanol is advantageous in daily practice by allowing the use of a dappen dish and the increase of the resin content.Moreover, it is totally miscible with both water and with the polymerisable resins[3]**.** It therefore helps the resin-containing adhesive to wet a moist tooth surface and produce dense, sealed hybrid layer thatwas recognizable in ESEM micrographs. Although Acetone has high vapor pressure, which is about four times as high as that of ethanol, it is highly

volatile which reduce its shelf life. Tert-butanol has similar vapor pressure as ethanol, but better stability towards chemical reaction with monomers[2].This may explain the high micro-shear bond strength of XP wet-bonded specimens.Tert-butanol is claimed to be less technique sensitive due to an improved ability to diffuse through partially collapsed demineralized dentin[6].This could be attributed to the H-bonding capacity of a solvent which has been shown to be important to re-expand the shrunken demineralized collagen network after dehydration[2]. Numerous publications have shown that collapsed air-dried dentin matrices do not always expand when bonding agents are applied[7, 8, and 9].Most monomers used in adhesive dentistry have h-values below those of dried dentin[10].Thus, such resins cannot expand dried, acid-etched dentin. This is why dry bonding to acid-etched dentin seldom gave shear bond strengths over 5 MPa[11]. In this study, shear bond strength of dry bonded dentin was significantly lower than wet bonded dentin specimens; however, micro-shear bond strength values were 17MPs. This could be credited to H-bonding capacity of tert-butanol solvent which breaks stabilizing H-bonds and other forces that keep the collagen in shrunken state. As seen in SEM micrographs of dry bonded dentin, the adhesive layer showed areas of detachment from underlying dentin which indicates incomplete resin infiltration or retention related to these areas. This may explain the lower micro-shear bond strength values. This was in contradiction to a previous investigation which stated that "The morphology of the hybrid layer when XP *BOND* was applied on dried dentin was not very distinct from the morphology corresponding to the application of the same adhesive on moist dentin."[12]The micro-shear bond strength of conventional etch and rinse systems has been theoretically modeled by Pashley et al as the sum of strength contributed by the resin tags, the hybrid layer and surface adhesion[13]. For etch and rinse adhesives, resin tag formation contribute quantitatively up to one-third of the total shear bond strength[14].Even though significantly lower tag area was reported with both adhesives in moist condition, there was significant correlation between resin tag area and micro-shear bond strength.This could be related to the quality of the hybrid layer as shown in SEM micrographs and not the quantity of resin tags. Although resin tag area was significantly highest with XP dry bonding,no correlation was found between resin tag area and shear bond strength which may confirm the same finding.

Conclusion:

1. The type of solvent strongly influences the clinical application protocol of etch-and-rinse adhesive systems.

2. Butanol-based adhesive bonded to moist dentin, produced high µSBS that was not considerably different from that of the P&BNT, with hybrid layer that showed a perfectly infiltrated, sealed dentin-resin interface with relatively short resin-tags.

3. Butanol-based adhesive bonded to dried dentin was able to infiltrate the demineralized collagen layer and produce long resin tags, however, hybrid layer was not perfectly sealed which may explain the lower bond strength values.

4. There was significant correlation between resin-tags surface-area and µSBS for moist substrate bonding for the two tested adhesives. However, no correlation was found for dry substrate bonded to tert-butanol based adhesive.

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10/14/2010

Effect of Palatal Surface Contouring Techniques on the Swallowing Function of Complete Denture Wearers.

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Abstract: This study is aimed to investigate the effect of two different surface palatal contouring techniques; namely arbitrary versus functional on swallowing activity of maxillary complete denture wearers. Ten completely edentulous healthy male patients with their ages ranged between 46-65 years were selected for this study according to definite inclusion-exclusion criteria. The swallowing function was evaluated pre- and postdenture state by using Videofluoroscopy. The patient was asked to swallow different bolus consistencies (thin and thick liquids, semisolid and solid) in small and large volumes. The swallowing measures selected for this study were: (1) Temporal measures of bolus and hyoid movements during swallowing; (2) Oropharyngeal residue; (3) Laryngeal penetration/aspiration observation and (4) Oropharyngeal Swallow Efficiency score. The results revealed a statistically significant increase in temporal measures of swallowing after denture insertion compared to predenture state regardless the palatal surface contouring technique. Functional contouring of maxillary denture palatal surface demonstrated a statistically significant general decrease in durations of bolus and hyoid movements (except at duration of hyoid maximum elevation) during swallowing, and led to a more efficient swallowing compared to dentures with arbitrary contoured palate. It can be concluded that the difficulty of swallowing with an artificial prosthesis should be addressed before the patient first makes use of the denture to eat and drink. Functional contouring of the palatal polished surface is recommended for completely edentulous patients. A further research may be useful to study the effect of functionally contoured palate in completely edentulous patients with oro-pharyngeal dysphagia due to neurological etiologies.
[Tamer Abou-Elsaad, Ahmad Habib, Mohamed Elkhodary, Abd-Allah Salem. Effect of palatal surface contouring techniques on the swallowing function of complete denture wearers. Life Science Journal 2010;7(4):114-118]. (ISSN: 1097-8135).

Keywords: surface palatal; arbitrary; maxillary; edentulous healthy; patient; dysphagia; etiology

1. Introduction:

Intra-oral presence of newly constructed denture may be associated with some initial difficulties in speech, mastication and swallowing. (Roessler, 2003). Contouring the facial, lingual, and palatal polished surfaces have been comparatively ignored during construction of conventional denture. These surfaces are usually carved into an "ideal" form without sufficient consideration for the position and function of cheeks, tongue, and lips which are always in contact with dentures. The arbitrary shaping of the polished surfaces may have an adverse effect on the success of the prosthesis. These prostheses are frequently in disharmony with the patient's anatomy and physiology of mandibular motion, resulting in impaired comfort and function (Jacob, 1998). The functionally constructed dentures are more comfortable and stable than conventionally constructed dentures (Lott and Levin, 1966). However, the precise effect of functional contouring of denture polished surfaces on the swallowing activity of completely edentulous patient was not clearly investigated. This prospective study was aimed to investigate the effect of different palatal contouring techniques of maxillary complete denture; namely arbitrary (conventional) versus functional palatal contouring on swallowing activity of complete denture wearers.

2. Subjects and Methods:

Ten completely edentulous healthy male patients were selected for this study with their ages ranged between 46-65 years (mean= 56 years) from Prosthodontic Department, Faculty of Dentistry, Mansoura University. All patients were completely edentulous for at least 6 months with no previous denture experience. The patients were selected free from any medical or surgical history of swallowing problem. All patients were of Angel's class I maxillomandibular relation with available inter-arch space. The residual alveolar ridges were free from severe undercuts.

I - Prosthetic Procedures:

For each patient, the following procedures were done:

1. After maxillary and mandibular final impression, the maxillary master cast was duplicated for each subject. A tentative maxillary and mandibular record blocks were constructed, and adjusted for contour, height and orientation respectively. The maxillary master cast was mounted on the articulator by using maxillary facebow.
2. Vertical and horizontal relation was recorded by using a standard method based on the physiologic technique developed by shanahan (1955) (fig. 1). Then the mandibular master cast was mounted on the articulator.
3. After arrangement of acrylic resin artificial teeth, waxing up was done according to Renner and Blakeslee (1978). According to the palatal polished surface contour; the dentures were grouped as follow:

1) Arbitrary dentures: where the palatal polished surface was arbitrary
2) contoured. The trial dentures were processed into heat cured acrylic resin,
3) finished and polished. Swallowing function was evaluated before and after two weeks of denture insertion (accommodation period).
4) Functional dentures: After 2 weeks of rest interval (without denture), the arbitrary contoured palatal polished surface was replaced by functionally contoured one constructed by swallowing technique using soft wax according to Lott and Levin (1966) and Shaffer and Kutz (1972) (fig. 2 and fig 3).

The waxed functional contoured palatal polished surface was then converted into auto-polymerized acrylic resin. Swallowing evaluation was then done after 2 weeks of denture insertion.

Iii- Swallowing Evaluation:

Evaluation of each patient's swallow was done in three states; predenture state, arbitrary (conventional) denture

state, and functional denture state. All patients were evaluated through a specially designed diagnostic protocol (Abou-Elsaad and Kotby, 2003). The protocol included: patient's interview, clinical examination, and videofluoroscopic evaluation of oro-pharyngeal swallowing. Each subject was asked to swallow three swallows of each of the following (Abou-Elsaad, 2003): (a) 3 and 10 ml thin liquid barium (20% barium sulfate [Prontobario H.D.®] and 80% water); (b) 3 and 10 ml thick liquid barium (50 % barium and 50% water); (c) 3 and 10 ml semisolid (pudding mixed with barium powder) and (d) ¼ of cookie (coated with pudding + barium powder). The patient was asked to hold the bolus in his mouth and then swallow the whole bolus once only. For cookie swallow the patient was asked to chew and swallow once ready. The videofluoroscopic images were then transferred to the computer by using a TV card (Life-View model) for later analysis. The images were analyzed using EO software program (Version 1.36, 2003) which place numbers (to the hundredth of a second) consecutively on each video frame for subsequent frame-by-frame analysis. The following temporal measures of swallowing events were recorded in accordance with bolus and hyoid movements:

A- Temporal measures of the bolus movement during swallowing:

(1) Oral transit duration (OTD):

From the initiation of posterior bolus movement to arrival of the bolus head at the ramus of the mandible (fig 4).

(2) **Oral clearance duration (OCD):**

From initiation of posterior bolus movement to arrival of bolus tail at ramus of mandible.

(3) **Pharyngeal transit duration (PTD):**

From arrival of bolus head at ramus of the mandible to bolus head enter upper esophageal sphincter (UES).

(4) **Pharyngeal clearance duration (PCD):**

From arrival of bolus head at ramus of the mandible to bolus tail through UES.

(5) **Total swallow duration (TSD)**:

By summing both oral transit and pharyngeal clearance durations.

(6) **Masticatory duration (MD):**

From once the patient masticate the bolus to beginning of posterior movement of the bolus.

B- Temporal measures of hyoid movement during swallowing:

(1) **Pharyngeal response duration (PRD):**

From beginning of maximum hyoid movement to hyoid return to rest.

(2) **Duration of hyoid maximum elevation (DOHME):**

From first frame showing maximum hyoid elevation to last frame showing maximum hyoid elevation (Fig. 5).

(3) **Duration of hyoid maximum anterior excursion (DOHMAE):**

From first frame showing maximum anterior hyoid movement to last frame showing maximum anterior hyoid movement (Fig. 6).

Three additional measurements were determined:

(1) **Oro-pharyngeal residue:**

A three-point scale (0= no residue, 1= coating, 2= pooling) was used to assess the amount of residue in the oropharynx.

(2) **Penetration/aspiration observation:**

Penetration means the bolus enters the airway down to the level of the vocal folds (Fig. 7). Aspiration means the bolus enters the airway below the level of the vocal folds.

(3) **Oro-pharyngeal Swallow Efficiency (OPSE) score (Rademaker et al., 1994)**:

Percent bolus swallowed (minus % oropharyngeal residue + % amount aspirated) on the first swallow attempt on a bolus divided by TSD. Normal OPSE score is 50 or better (100 % swallowed in 2 seconds or less).

Frequency, mean and standard deviation were used to describe data. To test for significance of change, the non-parametric Wilcoxon signed rank test was used to compare quantitative data in the same group. While the non-parametric McNemar test of change was used to compare qualitative data in the same group. P value was considered significant if less than 0.05.

3. Results:

I- Comparisons among pre-denture, arbitrary and functional contoured dentures in temporal measures of bolus and hyoid movements during swallowing: (tables 1, 2, 3, 4) Statistically significant decreases were detected in all temporal measures of bolus and hyoid movements during swallowing in predenture state when compared to arbitrary denture with different bolus consistencies and volumes ($p < 0.05$). Similarly, statistically significant decreases were detected in all temporal measures of bolus and hyoid movements during swallowing in pre-denture state when compared to functional denture ($p < 0.05$) except in pharyngeal response duration at large volume thick liquid, semisolid and solid bolus consistencies where there were statistically non-significant differences detected ($p > 0.05$). Statistically significant decreases were detected in all temporal measures of bolus and hyoid movements during swallowing in functional denture when compared to arbitrary denture dentures with different bolus consistencies and volumes ($p < 0.05$) except at duration of hyoid maximum elevation where statistically significant differences could not be detected in any bolus consistency and volume ($p > 0.05$).

II- Comparisons among pre-denture, arbitrary and functional contoured dentures in Oro-pharyngeal residue:

There were statistically non-significant differences in oropharyngeal residue between pre-denture state and after dentures insertions and also between arbitrary and functional dentures with different bolus consistencies and volumes ($p > 0.05$).

III- Comparisons among pre-denture, arbitrary and functional contoured dentures in penetration / aspiration observation**:** (table 5). There were statistically significant laryngeal penetrations in large volume thin and thick liquid bolus consistencies in pre-denture state when compared with both arbitrary and functional dentures ($p < 0.05$). On the other hand, no penetration/aspiration was observed in both arbitrary and functional dentures with different bolus consistencies and volumes.

IV- Comparisons among pre-denture, arbitrary and functional contoured dentures in Oro-pharyngeal Swallow Efficiency (OPSE) score: (table 6) There were statistically significant higher OPSE scores in predenture state when compared to both arbitrary and functional dentures ($p < 0.05$), and in functional denture when compared to arbitrary one ($p < 0.05$) with different bolus consistencies and volumes.

Table (1): Comparison of thin liquid bolus consistency durations in small and large volumes in all patients (n=10) before and after the two dentures insertions:

Bolus		Pre - denture Mean ± SD	Arbitrary denture Mean ± SD	Functional denture Mean ± SD	Z Values PA	PF	AF
OTD	3ml	0.33±0.07	0.55±0.12	0.40±0.08	-2.82*	-2.69*	-2.82*
	10ml	0.33±0.10	0.48±0.12	0.39±0.10	-2.83*	-2.71*	-2.84*
OCD	3ml	0.56±0.11	0.78±0.16	0.64±0.09	-2.81*	-2.68*	-2.83*
	10ml	0.56±0.10	0.77±0.11	0.64±0.11	-2.81*	-2.68*	-2.81*
PTD	3ml	0 .18±0.11	0.36±0.31	0.23±0.11	-2.91*	-2.76*	-2.88*
	10ml	0.18±0.10	0.27±0.12	0.21±0.01	-2.84*	-2.71*	-2.57*
PCD	3ml	0.50±0.14	0.76±0.31	0.57±0.12	-2.81*	-2.87*	-2.71*
	10ml	0.54±0.11	0.66±0.13	0.59±0.12	-2.83*	-2.75*	-2.70*
TSD	3ml	1.36±0.14	1.90±0.20	1.58±0.10	-2.81*	-2.81*	-2.81*
	10ml	1.44±0.11	1.86±0.12	1.60±0.14	-2.81*	-2.83*	-2.81*
PRD	3ml	1.16±0.16	1.36±0.19	1.26±0.15	-2.60*	-2.69*	-2.14*
	10ml	1.30±0.18	1.62±0.29	1.38±0.18	-2.70*	-2.68*	-2.40*
DOH ME	3ml	0.08±0.00	0.04±0.01	0.04±0.00	-3.00*	-3.16*	-1.00
	10ml	0.09±0.03	0.05±0.03	0.04±0.00	-2.50*	-3.05*	-1.34
DOH MAE	3ml	0.13±0.03	0.08±0.01	0.05±0.03	-2.75*	-2.87*	-2.83*
	10ml	0.12±0.02	0.08±0.01	0.05±0.02	-2.89*	-2.91*	-3.00*

Wilcoxon signed ranked test; *P < 0.05 (significant); PA= pre-denture versus arbitrary denture; PF= pre-denture versus functional denture; AF= arbitrary denture versus functional denture.

Table (3): Comparison of semi-solid bolus consistency durations in small and large volumes in all patients (n=10) before and after the two dentures insertions:

Bolus		Pre - denture Mean ± SD	Arbitrary denture Mean ± SD	Functional denture Mean ± SD	Z Values PA	PF	AF
OTD	3ml	0.41±0.13	0.73±0.13	0.51±0.08	-2.81*	-2.91*	-2.82*
	10ml	0.47±0.14	0.73±0.14	0.56+0.15	-2.81*	-2.68*	-2.81*
OCD	3ml	0.67±0.30	1.08±0.26	0.79±0.26	-2.81*	-2.87*	-2.81*
	10ml	0.76±0.32	1.07±0.35	0.89±0.29	-2.81*	-2.81*	-2.83*
PTD	3ml	0 .22±0.31	0.35±0.28	0.26±0.29	-2.82*	-2.64*	-2.75*
	10ml	0.20±0.23	0 .33±0.23	0.24±0.23	-2.82*	-2.81*	-2.84*
PCD	3ml	0.60±0.31	0.73±0.31	0.66±0.31	-2.82*	-2.91*	-2.72*
	10ml	0.62±0.25	0.76±0.27	0.70±0.26	-2.82*	-2.84*	-2.91*
TSD	3ml	1.63±0.24	2.22±0.27	1.83±0.22	-2.81*	-2.82*	-2.81*
	10ml	1.68±0.2	2.21±0.31	1.86±0.2	-2.80*	-2.81*	-2.81*
PRD	3ml	1.36±0.13	1.47±0.13	1.41±0.1	-2.19*	-2.21*	-2.00*
	10ml	1.37±0.14	1.52±0.21	1.39±0.17	-2.45*	-1.80	-2.81*
DOH ME	3ml	0.08±0.01	0.05±0.02	0.04±0.01	-3.00*	-3.05*	-1.41
	10ml	0.08±0.01	0.05±0.02	0.04±0.01	-2.83*	-3.16*	-1.41
DOH MAE	3ml	0.14±0.03	0.10±0.03	0.05±0.02	-2.46*	-2.87*	-2.81*
	10ml	0.13±0.03	0.10±0.03	0.06±0.02	-2.31*	-2.85*	-2.64*

Wilcoxon signed ranked test; *P < 0.05 (significant)

Table (5): Comparison of bolus penetration/aspiration in all patients (n=10) before and after the two dentures insertions:

Bolus		Pre-denture No P/A	P	A	Arbitrary denture No P/A	P	A	Functional denture No P/A	P	A
Thin liquid	3 ml	10 (100%)	0	0	10 (100%)	0	0	10 (100%)	0	0
	10 ml	1 (10%)	9 (90%)	0	10 (100%)	0	0	10 (100%)	0	0
Thick liquid	3 ml	10 (100%)	0	0	10 (100%)	0	0	10 (100%)	0	0
	10 ml	0	10 (100%) PA* PF*	0	10 (100%)	0	0	10 (100%)	0	0
Semi-solid	3 ml	10 (100%)	0	0	10 (100%)	0	0	10 (100%)	0	0
	10 ml	10 (100%)	0	0	10 (100%)	0	0	10 (100%)	0	0
Solid		10 (100%)	0	0	10 (100%)	0	0	10 (100%)	0	0

McNemar test;*p <0.05 (significant); P/A=Penetration/Aspiration. PA=predenture versus arbitrary denture; PF= pre-denture versus functional denture.

Table (2): Comparison of thick liquid bolus consistency durations in small and large volumes in all patients (n=10) before and after the two dentures insertions:

Bolus		Pre - denture Mean ± SD	Arbitrary denture Mean ± SD	Functional denture Mean ± SD	Z Values PA	PF	AF
OTD	3ml	0.36±0.08	0.60±0.16	0.44±0.12	-2.81*	-2.84*	-2.82*
	10ml	0.35±0.08	0.52±0.18	0.42±0.11	-2.82*	-2.72*	-2.84*
OCD	3ml	0.59±0.11	0.91±0.23	0.71±0.14	-2.81*	-2.84*	-2.82*
	10ml	0.62±0.10	0.97±0.30	0.71±0.10	-2.81*	-2.68*	-2.81*
PTD	3ml	0.16±0.08	0.27±0.10	0.22±0.01	-2.68*	-2.75*	-2.59*
	10ml	0.15±0.09	0.34±0.25	0.19±0.06	-2.55*	-2.97*	-1.71
PCD	3ml	0.58±0.14	0.69±0.14	0.65±0.14	-2.82*	-2.85*	-2.59*
	10ml	0.62±0.13	0.87±0.33	0.70±0.14	-2.81*	-2.70*	-2.38*
TSD	3ml	1.48±0.17	2.01±0.17	1.68±0.13	-2.81*	-2.83*	-2.81*
	10ml	1.52±0.13	2.09±0.24	1.71±0.16	-2.81*	-2.84*	-2.81*
PRD	3ml	1.24±0.18	1.41±0.2	1.31±0.15	-2.80*	-2.27*	-2.55*
	10ml	1.22±0.17	1.38±0.15	1.26±0.19	-2.81*	-1.79	-2.67*
DOH ME	3ml	0.09±0.02	0.06±0.02	0.04±0.01	-3.00*	-2.92*	-1.41
	10ml	0.08±0.01	0.05±0.02	0.04±0.01	-2.65*	-3.16*	-1.73
DOH MAE	3ml	0.12±0.02	0.08±0.02	0.05±0.02	-2.81*	-2.91*	-2.83*
	10ml	0.13±0.02	0.09±0.03	0.06±0.03	-2.64*	-2.85*	-2.71*

Wilcoxon signed ranked test; *P < 0.05 (significant)

Table (4): Comparison of solid bolus consistency durations in small and large volumes in all patients (n=10) before and after the two dentures insertions:

Bolus	Pre - denture Mean ± SD	Arbitrary denture Mean ± SD	Functional denture Mean ± SD	Z Values PA	PF	AF
OTD	0.38±0.13	0.68±0.10	0.55±0.07	-2.81*	-2.81*	-2.81*
OCD	0.59±0.13	0.92±0.24	0.74±0.15	-2.81*	-2.83*	-2.81*
PTD	0 .12±0.04	0.27±0.13	0.184±0.07	-2.68*	-2.64*	-2.55*
PCD	0.48±0.13	0.75±0.25	0.52±0.12	-2.81*	-2.97*	-2.53*
TSD	1.27±0.19	1.85±0.26	1.54±0.15	-2.80*	-2.81*	-2.81*
PRD	1.08±0.26	1.24±0.2	1.16±0.16	-1.99*	-1.88*	-1.99
DOHME	0.09±0.03	0.05±0.02	0.04±0.01	-2.43*	-2.76*	-1.41
DOHMAE	0.13±0.05	0.09±0.03	0.06±0.03	-2.16*	-2.57*	-2.45*
MD	-	10.5±3.95	6.56±2.3	-	-	-2.80*

Wilcoxon signed ranked test; *P < 0.05 (significant)

Table (6): Comparison of Oro-pharyngeal Swallow Efficiency scores in all patients (n=10) before and after the two dentures insertions:

Bolus		Pre - denture Mean ± SD	Arbitrary denture Mean ± SD	Functional denture Mean ± SD	Z Values PA	PF	AF
Thin liquid	3ml	66.93±9.27	44.09±6.36	56.39±3.38	-2.81*	-2.81*	-2.80*
	10ml	55.84±4.57	42.85±3.19	50.47±4.63	-2.81*	-2.81*	-2.81*
Thick liquid	3ml	54.55±6.77	39.18±3.57	47.35±4.77	-2.80*	-2.80*	-2.80*
	10ml	52.21±6.13	36.59±5.27	43.55±4.95	-2.81*	-2.81*	-2.81*
Semi-solid	3ml	49.51±4.76	34.86±3.64	42.32±5.70	-2.80*	-2.81*	-2.80*
	10ml	48.47±5.16	34.78±4.76	42.03±4.56	-2.80*	-2.81*	-2.80*
Solid		64.38±9.10	43.86±5.68	52.46±4.87	-2.80*	-2.81*	-2.80*

Wilcoxon signed ranked test; *P < 0.05 (significant)

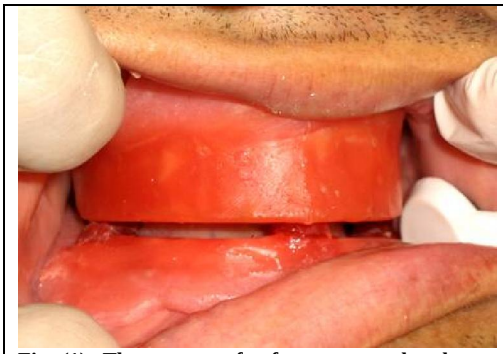

Fig. (1): Three cones of soft wax were placed over lower wax occlusal rim. The height of soft wax cones was reduced by asking the patient to swallow his

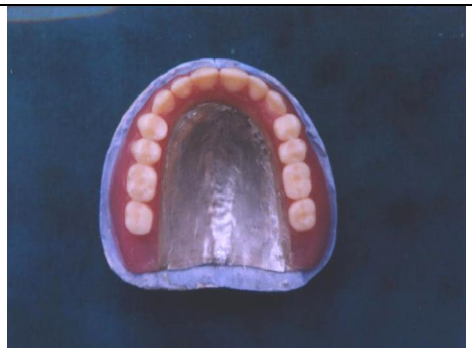

Fig. (2): Tinfoil was adapted on the palatal surface of the duplicate maxillary master cast and sealed to the denture palatal edges

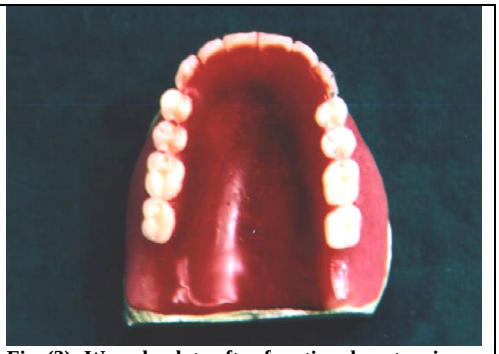

Fig. (3): Waxed palate after functional contouring by the swallowing technique.

saliva repeatedly.

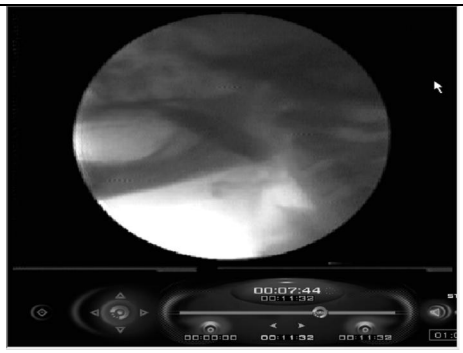

Fig. (4): Head of the bolus reach the angle of the mandible.

Fig. (5): Maximum hyoid elevation.

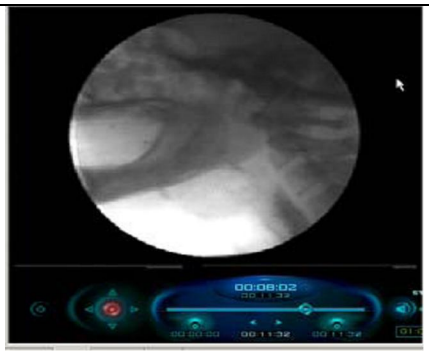

Fig. (6): Maximum anterior hyoid excursion.

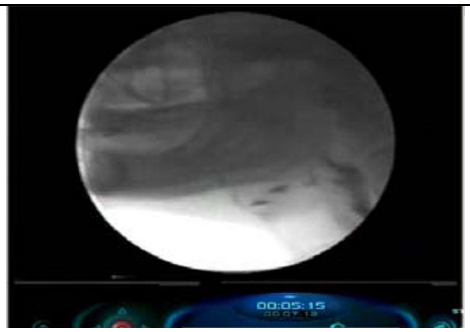

Fig. (7): Laryngeal penetration

4. Discussion:

The palatal mucosa together with the tongue area plays an important role in recognition of food (Oomi, 1959). Covering of the hard palatal mucosa by the maxillary complete denture might influence the movement of bolus from the oral cavity to the pharynx. Similarly, alteration in mandibular position by the presence of the denture can influence the tongue function (Lowe et al., 1977). Hence, the propulsive force for bolus transport is changed with oral denture. This could explain the prolonged oral and pharyngeal temporal measures (OTD, OCD, PTD and PCD) with different bolus consistencies and volumes after the two dentures insertions in relation to pre-denture state. This is in agreement with Kodaira et al., (2006) findings who observed a generalized prolongation of oral propulsion time when the palatal mucosa is covered. On the other hand, the oral and pharyngeal temporal measures (OTD, OCD, PTD, PCD and PRD) were decreased in case of functional denture when compared to arbitrary denture with different bolus consistencies and volumes. This could be explained that the technique of construction of the palatal polished surface in functional denture is based on tongue movement. Hence, the tongue has a more freedom to perform the kinetic function. Alternatively, construction of the arbitrary denture is based on the configuration of the hard palate with no respect to the tongue movement. Thus, arbitrary denture may decrease the tongue motor skills that might influence the lingual forces necessary for bolus formation and transport. This is in agreement with the study conducted by Koshino et al. (1997) who found that arbitrary denture decreases the motor skills of the tongue. They also found that the masticatory performance was highly correlated to tongue motor skills. This could explain the shorter masticatory duration in case of functional denture in our study when compared to arbitrary denture.

Normally, the start of the pharyngeal response is related to the onset of hyoid movement (Logemann, 1983). As stated before, the presence of complete denture influences the tongue movement, which is one component of the functional chain of the vocal tract that consists of tongue and hyoid bone (Kotby and Haugen, 1970). This could explain the delay in the onset of the hyoid movement by the presence of complete denture. The UES and the hyoid bone are anatomically and mechanically related to each other (Jacob et al., 1989). Thus, the delay in the onset of the hyoid bone may cause a delay in relaxation and opening of UES, thus delaying the bolus transport from the pharynx to the esophagus. This could explain the prolonged PRD after arbitrary denture insertion when compared to pre-denture state with different bolus consistencies and volumes. On the other hand, the increase in volume and consistency of the bolus, increase tongue forces and hence, leading to an increase in the velocity of bolus transport from the pharynx to the esophagus (Dantas et al., 1990).This may compensate the prolonged effect of complete denture insertion on PRD in functional denture and explaining the non significant differences between it and the pre-denture state in PRD at large volumes thick liquid, semisolid, and solid consistencies.

During edentulate swallowing the tongue is jammed between the alveolar ridges (Hattori, 2004). This might make the tongue to travel a longer distance, hence longer duration for the hyoid movement in edentulate swallowing than swallowing with denture. This could explain the shorter DOHME and DOHMAE with different bolus consistencies and volumes after denture insertions in relation to pre-denture state.

Similarly, the DOHMAE was decreased in case of functional denture when compared to arbitrary one. This may be due to the more natural tongue movement at the swallow onset after functional denture. On the other hand, the DOHME showed no significant differences between arbitrary and functional dentures. This may be due to hyoid maximum elevation may be more related to occlusal vertical dimension which is maintained after denture insertion rather than change in the tongue movement during swallowing. This is in agreement with Hattori (2004) who reported that differences of occlusal vertical dimension had strong relevance to displacement of hyoid bone without denture compared with

denture. Clearance of the oro-pharyngeal residue depends partly on the continued contact between the dorsum of the tongue and the hard palate during oral stage of swallowing and between the tongue base and the posterior pharyngeal wall during pharyngeal phase of swallowing (Logemann, 1983) which were not affected by the presence of maxillary complete denture in this study. This could explain the non significant differences in oro-pharyngeal residue between pre-denture state and after denture insertion and between the two types of dentures with different bolus consistencies and volumes. These findings are in agreement with Yoshikawa et al.'s, (2006) study who found no significant differences between pre-denture state and after denture insertion in the oropharyngeal residue. Laryngeal penetrations were consistent with large volume thin and thick liquid boluses and was absent at small volume liquid boluses in predenture state. This may be due to deterioration of coordinated swallowing movements; as elevation of the hyoid bone, that resulted from the loss of occlusal support in the pre-denture state. Further more, the large volume bolus requires an increase of the magnitude of structure movement, which if deteriorated may reveal abnormality of function (Dantas et al., 1990). These findings support those of Yoshikawa's et al (2006) study who found a significant laryngeal penetration with large volume liquid bolus in edentulous subjects. In this study, semisolid and solid boluses did not show any laryngeal penetration due to their cohesive nature; hence a less risk than liquid boluses to flow into the air way.

5. Conclusions:

Insertion of denture using different palatal contouring techniques demonstrated a general increase in durations of bolus movement and lower OPSE scores compared to the pre-denture state during swallowing of different bolus consistencies and volumes. This could account for the common clinical observation of the difficulty of swallowing after insertion of a new denture without any previous experience. On the other hand, functional denture demonstrated a general decrease in durations of bolus movement and higher OPSE scores compared to the arbitrary denture during swallowing of different bolus consistencies and volumes. This may be attributed to the physiologic nature of the technique, which produces palatal part more compatible with the tongue musculature, and neuromuscular memory of the patient.

Recommendations:

Functional contouring of the palatal polished surface is recommended for completely edentulous patients. However, the difficulty of swallowing with an artificial prosthesis should be addressed before the patient first makes use of the denture to eat and drink. A further research may be useful to study the effect of functionally contoured palate in completely edentulous patients with oro-pharyngeal dysphagia due to neurological etiologies.

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The value of blood brain natriuretic peptide for predicting clinical severity and prognosis in patients with acute coronary syndromes

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ABSTRACT: **Background:** B-type/brain natriuretic peptide (BNP) is a neurohormone synthesized predominantly in ventricular myocardium. Although the circulating level of this neurohormone has been shown to provide independent prognostic information in patients with heart failure, few data are available for Chinese patients with acute coronary syndromes (ACS). This study was designed to investigate the value of blood BNP for predicting clinical severity and prognosis in patients with ACS. **Methods:** Blood BNP concentration was measured in 106 ACS patients, 1-3 days after onset of ischemic symptoms. Patients were followed up for six months. The end-point were cardiac death, non-fetal myocardial infarction and readmission. **Results:** (1) The concentration of circulating BNP in patients with ACS were increased. (2) 1 month follow-up demonstrated that, levels of BNP in non- survivals were much higher than that in survivals (P<0.0005); step-wise logistic regression analysis demonstrated that ST segment deviation ≥ 1 mm and BNP ≥ 596 ng/L were independent predictors of short-term cardiac death in patients with ACS [OR=3.467, 95% confidence interval (CI) 1.336-32.836, P=0.002; OR=21.168, 95% CI 4.419-107.990, P<0.0005]. (3) area under the curve（AUC） of the receiver-operating-characteristic (ROC) of BNP to predict short-term cardiac death in patients with ACS was 0.878（95% CI 0.781-0.974, P<0.0005）. (4) Kaplan-Meier survival curve showed that the survival curve of patients with BNP above 596 ng/L was significantly lower than that of patients with BNP below 596 ng/L（Log-rank test, P<0.0005）. Cox proportional hazards regression models demonstrated that BNP and cardiac troponin I were risk factors which related to ACS prognosis (RR = 2.507, 95% CI 1.081-3.914, P =0.028; RR =2.208, 95% CI 1.609-3.874, P=0.030). **Conclusions:** (1) The circulating levels of BNP are significantly increased in patients with ACS. Myocardial ischemia and / or left ventricular systolic dysfunction are the main cause of stimulating BNP secretion. (2) BNP could provide important clinical value for predicting clinical severity and prognosis. It could be used for risk stratification in patients with ACS, especially when there is only ischemia without infarction and when blood is sampled very early after the onset of ischemia that would be missed by markers of myocyte necrosis.

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Keywords: B-type / brain natriuretic peptide; Acute coronary syndromes; Prognosis

1. Introduction

B-type/brain natriuretic peptide (BNP) is a neurohormone synthesized predominantly in ventricular myocardium. Although the circulating level of this neurohormone has been shown to provide independent prognostic information in patients with heart failure (Sun et al 2007), few data are available for Chinese patients with acute coronary syndromes (ACS). This study was designed to investigate the values of circulating BNP for predicting clinical severity and prognosis in Chinese patients with ACS.

2. Material and Methods

Patient selection

This study was approved by the institutional review board and consent was obtained from all participants. Between September 2003 and May 2004, total of 106 ACS patients (71 males, mean age 62±11 years, range 37-85) admitted to the coronary care unit and ordinary ward at the First Affiliated Hospital of Zhengzhou University were enrolled in the study.20 inpatients or outpatients (13 males, mean age 58±10 years, range 39-75) with stable angina pectoris were considered control group. Patients were followed up for up to six months after hospital

discharge. Events, including death, readmission to hospital with heart failure and non-fetal myocardial infarction were recorded.

The inclusion criteria were as follows: older than 18 years old; electrocardiographic changes (ST-segment depression or elevation of at least 0.5 mm, T-wave inversion of at least 3 mm in at least three leads, or left bundle-branch block), elevated levels of cardiac markers (CK-MB and troponin I), absence of immediate heart failure or cardiogenic shock, ischemic symptoms less than 72 hours, and survival for at least 24 hours after onset of ischemic symptoms. The exclusion criteria were as follows: chronic renal dysfunction (blood creatinine >133μmol/L); primary pulmonary hypertension and cor pulmonale; primary cardiomyopathy and severe valve disease.

Measurement of circulating BNP level

A point-of-care test of fluorescence immunoassay for the quantification of BNP was used (Biosite Diagnostics Inc, USA), 2ml of intravenous blood was collected at the early morning after 1-3days of admission and BNP was determined within 20 minutes. The range of measurement was 5ng/L-5000ng/L.

Echocardiogram examination

A GE VIVID-7 echocardiograph (GE company, USA) were used. All the enrolled patients accepted echocardiograph examination by the same echocardiographer. Left ventricular ejection fraction (LVEF) and left ventricular end-diastolic dimension (LVEDd) were measured from the apical four-chamber view using modified Simpson method.

Statistical analysis

Data were presented by mean±standard deviation (SD) and median. The difference of circulating BNP between the survival and the non-survival groups were compared using Wilcoxon signed rank test. Spearman rank correlation analysis was used to determine the factors that influence the circulating BNP level. Stepwise logistic forward regression analysis and Cox proportional hazards regression model were used to determine the factors related to prognosis. The accuracy of blood BNP in predicting cardiac death was assessed by a receiver-operator characteristic curve (ROC). $P<0.05$ was considered to be statistically significant. All data analysis was performed using the Statistical Package for Social Sciences (SPSS 11.0).

3. Results

After hospital admission, ST elevation myocardial infarction (STEMI) was diagnosed in 33, non- ST elevation myocardial infarction (NSTEMI) in 7 and unstable angina pectoris (UAP) in 66.

The changes of circulating BNP between ACS group and SAP group

1. The median of circulating BNP in ACS group and SAP group were 172ng/L and 18 ng/L, respectively. Wilcoxon signed rank test for two independent samples showed that the difference was statistically significant (U=136, P<0.0005). There were statistically significant difference between 2 groups about left ventricular ejection fraction (50.12 %±12.81% *vs.* 56.31%±9.60%, P＝0.031), leads number of ST segment deviation (2.75±3.43 *vs.* 0.53 ± 1.13, P＝0.015) and the degree of ST segment deviation (0.76±1.03 mm *vs.* 0.23±0.71mm, P＝0.042)".

2. Spearman rank correlation analysis showed that age, gender, blood pressure, smoking, drinking, history of diabetes mellitus, history of essential hypertension, serum C-reactive protein (CRP) were not related to blood BNP concentration. The BNP level were positively correlated with heart rate (r =0.229, P＝0.035), leads number of ST segment deviation (r =0.281, P＝0.009), the degree of ST segment deviation (r=0.284, P ＝ 0.008), left ventricular end-diastolic dimension (r=0.429, P<0.0005), history of heart failure (r=0.372, P<0.0005), history of myocardial infarction (r =0.220, P ＝ 0.043) and negatively correlated with left ventricular ejection fraction (r=－0.625, P <0.0005).

The relationship between circulating BNP levels and clinical condition and prognosis

After 1 month follow-up, 13 patients died, 2 patient experienced non-fatal myocardial infarction in ACS group. There were no end events in the SAP group at the end of 1 month follow-up. Among the 13 died patients, 12 patients' BNP levels were above 172ng/L (median) and 10 patients' BNP levels were above 596 ng/L (75% percentile). Those with a circulating BNP above 172 ng/L and 596 ng/L had a mortality of 23.6% (12/53) and 38.5% (10/26), respectively.

1. There were no significant differences in patient's age, gender, smoking or alcohol drinking, lipid profile, systolic blood pressure and the left ventricular ejection fraction （ 56.9±14.1% *vs.*56.9±14.1%, P=0.792）between the survival and non-survival groups.

The average and the median BNP levels in the non-survival group were significantly higher than that of the survival group (P<0.0005, Table 1). Univariate analysis indicated that the degree of ST segment deviation (r=0.289, P = 0.007), leads number of ST segment deviation (r =0.415, P<0.0005), BNP levels (r =0.469, P<0.0005) and Killip class (r =0.316, P =0.001) were risk factors of short-term prognosis. Step-wise logistic regression demonstrated that ST segment deviation ≥0.1 mV and BNP ≥596 ng/L were independent predictors of short-term death in patients with ACS (OR=3.467, 95% confidence interval 1.366-32.836, P=0.002; OR=21.168, 95% confidence interval 4.419-107.990, P<0.0005; [covariate were age, sex ,history of heart failure, history of myocardial infarction, diabetes mellitus, ST segment deviation ≥ 0.1mV, Killip class (Ⅰ/Ⅱ-Ⅳ), LVEF(≥50%/<50%), circulating BNP (≥596 ng/L/<596ng/L) and cTn I (positive/negative)]. Area under the curve of the receiver-operating-characteristic (ROC) of BNP to predict short-term death in patients with ACS was 0.878, 95% confidence interval 0.781-0.974, P<0.0005 (Figure 1). A circulating BNP cut-off value of 596 ng/L had a sensitivity of 76.9 percent, a specificity of 86.2 percent for predicting death at 1 month.

2. Kaplan-Meier survival curve showed that the survival curve of patients with BNP above 596 ng/L was significantly lower than that of patients with BNP below 596 ng/L (Log-rank test, P<0.0005, seen in Figure 2). Cox proportional hazards regression models demonstrated that BNP ≥ 596 ng/L and myocardial infarction were risk factors which related to ACS prognosis (RR=2.507, 95% confidence interval 1.081-3.914, P=0.028; RR =2.208, 95% confidence interval 1.069-3.874, P =0.030).

4. Discussions

This study indicates that the circulating BNP in patients with ACS are significantly higher , the degree of ST segment deviation are more severe, the number of ST segment deviation are more , but LVEF are lower than those in patients with SAP. These reveal that the increase of circulating BNP in patients with ACS are related to myocardial ischemia and left ventricular dysfunction, according with previous study (Wiviott 2004, Galvani 2004, de Lemos 2001, Morrow 2003, Sun 2006)

The release of BNP from myocardial cells is provoked by a variety of stimuli, including hypoxia, ischemia, increased wall stress, and dilation of ventricles. Early animal studies of experimental infarction in rats demonstrated rapid induction of ventricular BNP gene expression and BNP production not only in the infarct and the perinfarct regions but also in the nonischemic surrounding myocardium (Hama 1995). These changes in gene expression were seen as early as four hours postinfarction (Hama 1995). Clinically, there was transient increase in cardiac BNP secretion after percutaneous transluminal coronary angioplasty (Tateishi 2000). These results indicate that myocardial ischemia induces synthesis and secretion of BNP, even before myocyte necrosis and overt left ventricular dysfunction taking place. But the exact mechanism of stimulating BNP release in myocardial ischaemia is unclear (Talwar 2000). Cardiotrophin 1 is reased in myocardial ischaemia. It stimulates BNP production at a transcriptional level in vitro, suggesting that it may be involved in BNP secretion in vivo. This should be validated by basic and clinical studies (Talwar 2000).

De Lemos and his coworkers (de Lemos 2001) measured the blood BNP level of 2,525 patients with ACS and followed up for 10 months. They found that the base-line levels of BNP were correlated with the risk of death, heart failure, and myocardial infarction at 30 days and 10 months. After adjustment for independent predictors of the long-term risk of death, patients with blood BNP level in the second, third, and fourth quartiles had a risk of death in 10 months of 3.8 times, 4.0 times, 5.8 times higher than those with blood BNP level in the first quartile. Subgroups analysis showed similar results. Marrow （Morrow 2003） observed 1,676 patients with non-ST elevation ACS and found that the mortality of seven day and six month increased significantly in patients with elevated BNP (above 80 ng/L). Wang studied 110 AMI patients and founded that circulating BNP were related to cardiac events, heart failure and death （Wang 2005）. The present study demonstrated that the risk of death in patients with circulating BNP level above 596ng/Lwas 21 times higher than those with circulating BNP level under 596ng/L. A circulating BNP cut-off value of 596 ng/L had a sensitivity of 76.9 percent, a specificity of 86.2 percent for predicting death at 1 month.

The potential mechanisms of circulating BNP for evaluating prognosis in patients with ACS is unknown (Wiviott 2004, Galvani 2004, de Lemos 2001, Morrow 2003). Traditional biomarkers for the evaluation of myocardial infarction, such as CK-MB and cardiac troponins, are released when there is irreversible injury to cardiac myocytes. In contrast, BNP is released by intact cells, including those that are not ischemic. The functions of BNP, such as natriuresis , diuresis, vasodilatation, inhibition of the renin-angiotensin-aldosterone axis, and inhibition of sympathemic nerve activity, are beneficial to the heart. Accordingly, the magnitude of the increase in

BNP concentrations may reflect the size of the ischemic territory（Sun 2006, Talwar 2000）. Secondly, the present study and previous studies demonstrate that the increase of BNP are related to cardiac dysfunction （Sun 2006）. Thirdly, the increase BNP at the early time of MI can predict left ventricular remodling (Yoshitomi 1998). Finally, the increase of BNP is related to elder, renal dysfunction, heart failure, and arrhythmia. These also influence the prognosis of ACS (Sun 2006, Wiviott 2004, Galvani 2004, Sun 2006). In aggregate, we concluded that ischemia and cardiac dysfunction contributed to the adverse prognosis of ACS.

In conclusion, in patients with ACS, high levels of blood BNP levels are associated with a significantly increased risk of cardiac death in short term. BNP should be used, together with other biomedical markers for ischemia, as a prime factor for risk stratification in patients with ACS.

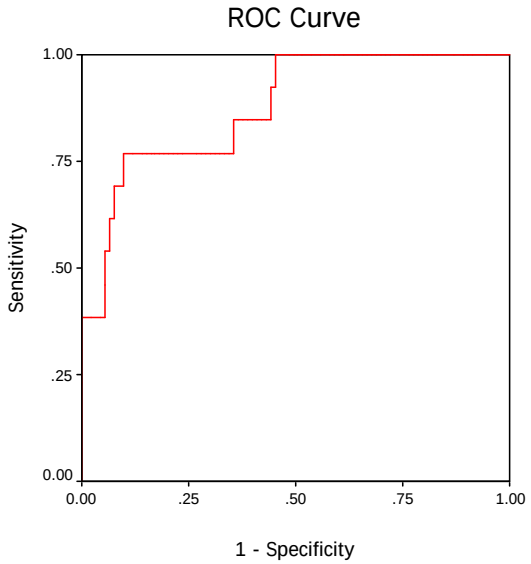

Fig 1. The ROC curve in assessing the predicting value of BNP for cardiac death

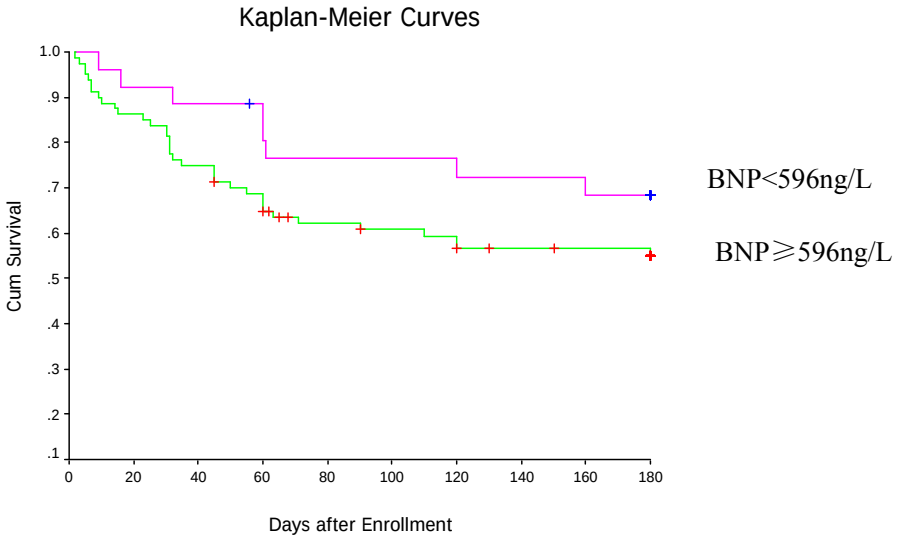

Fig 2. Kaplan-Meier survival curve

Table1: Comparison of BNP levels between survival group and non-survival group (ng/L)

group	BNP	LogBNP	Median
Survival (n=93)	320.87±425.54	2.10±0.68	116
Non-survival (n=13)	1762.31±1415.10	3.06±0.48	1132
Statistical value	*t* =-7.175	*t* =-4.879	*U*=149*
P value	<0.0005	<0.0005	<0.0005

*Wilcoxon signed rank test for 2 independent samples

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Bioformulations of Bacillus Spores for using as Biofertilizer

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Abstract: A maximum spore percentage of *Bacillus megatherium (B. megatherium)* (89 %) was recorded after 96 hours of inoculation into a modified nutrient medium containing a mixture of 500 ppm of MnSo4 , CaCl2, ZnSo4 and KCL. These spores were incorporated into 21 different talc, cellulose and clay based formulations and their viability were assessed over 6 months at room temperature. Of these bioformulations, Talc - glucose, Talc - yeast and Cellulose - clay based powder formulations were selected for additional in vivo testing because of their highest levels of viability . Field experiment was conducted to evaluate the efficiency of the treatment of bean seeds with selected powder bioformulations on the growth , yield parameters and root colonization ability of *B. megatherium* . The powder bioformulations as well as the free spore suspension effectively enhanced plant biomass ,increased the yield and accelerate the rhizosphere colonization by the bacterium under field condition . So, the commercially acceptable powder bioformulations of the *B. megatherium* which have a long storage life , aid product delivery, and promote the plant growth parameters were prepared to be used instead of the traditionally used free spore suspension.

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Key words: Formulations, Sporulation, *Bacillus megatherium*, Talc, Cellulose, Clay

1. Introduction:

Formulations generally composed of the active material which must be preserved or maintained in viable condition to produce its biological effect, the carrier material may or may not include the incorporation of enrichment materials or additives.

Generally, amendments can be grouped as either carriers (fillers, extenders) or amendments that improve the chemical, physical, or nutritional properties of the formulated biomass (Schisler *et al*., 2004). The active material is mixed with carrier materials such as water, clay, talc, oil or others to make the formulation safer to handle, easier to apply and better suited for storage. In some formulations, enrichment materials comprising of nutrient-rich medium such as, molasses, trehalose, maltose and sucrose are incorporated to further enhance the viability of core (active) materials (Brar *et al*., 2006; Tu and Randall, 2005**).**

The commercial use of plant growth-promoting rhizobacteria requires inoculum that retains a high cell viability and easily be transported and applied to seed. The aims of formulating viable cells are to ensure that adequate cell viability is sustained to increase the efficacy of the cells and to facilitate the delivery and handling processes (Filho *et al.,* 2001). For commercialization, a long shelf-life is an advantage for any inoculant (Fages 1990, 1992).

This can be achieved by producing granular formulations, powder or dust formulations, microcapsules, or oil-emulsion formulations (Brar *et al.,* 2006).

A formulated microbial product, for purpose of this paper, is defined as a powder product composed of biomass of a phosphate dissolving bacteria and ingredients to improve the survival and efficient of the product.

Most often, dry formulations are generally preferred over wet formulations because they provide extended shelf life and are easier to store and transport (Lumsden *et al.,* 1995).

In powder formulation, the active material is preferred to be in spore form to increase the shelf life and efficiency of active material. Gram-positive microorganism that produce heat- and desiccation-resistant spores that can be formulated into stable, dry-powder products offer a biological solution to the problem of biofertilizer agent formulation (Caesart and Burr 1991)

A crucial initial step toward preserving biomass viability during formulation is to optimize fermentation protocols for not only maximal total biomass but also for maximal spores production.

When producing biomass of *Bacillus spp.,* in most instances fermentation protocols should be designed to maximize the production of efficacious spores rather than vegetative cells (Driks, 2004).

Different factors can enhance the sporulation process of *Bacillus spp.*It is well known that endospore formation in *B. subtilis* can be promoted by a high cell density (Grossman and

Losick, 1988), nutrient limitation (Schaeffer *et al.* 1965), high mineral composition and transition metals, especially Zn and Cu (Kihm, *et al.,* 1988).

Several investigators have shown that there is considerable variation in spore production depending on the mineral composition of the medium (Krueger and Kolodziej, 1977, Mallidis and Scholefield, 1987)

The spore forming bacteria *B. megatherium* serve as phosphate dissolving bacteria for solubilizing inorganic phosphatic compounds into soluble forms which is available for plant. It is well known that seed inoculation of phosphorus solubilzing microbes enhance P uptake and yield of economic parts (Gharib, et al., 2004). There is increasing evidence that phosphorus solubilizing bacteria improve plant growth due to biosynthesis of plant growth substances rather than their action in releasing available phosphorous and the solubilization effect is generally due to the production of organic acids by these organisms. They are also known to produce amino acids, vitamins and growth promoting substances like indole acetic acid (IAA) and gibberellic acid (GA3) which help in better growth of plants. (Ponmurugan and Gopi 2006) .

In this study, heat-resistant endospores of *B. megatherium* were formulated with various combinations of organic carriers with and without the incorporation of enrichment and additive materials to develop a formulation which has potential for large-scale applications.

2. Materials and Methods:

*Bacillus megatherium (*Phosphate dissolving bacteria) was grown on nutrient, Pikovskaya (Pikovskaya, 1948) .and modified Bunt and Rovira media (Abd El- Hafez, 1966) for 24h, 48 h, 72 h, and 96 h of cultivation on an orbital shaker at 150 rpm at 30^0C. Both viable and heat - resistant spore counts were determined. For heat - resistant spore counts, cultures were heated at 80°C for 15 min to kill any vegetative cells present. Spores were then subsequently enumerated by plating aliquots of serial dilutions onto nutrient agar media which were incubated for 3 days at 30°C.

Enhancement of sporulation by addition of different metals:

Nutrient broth medium was used as the basal medium to which different metals at a concentration of 500 ppm were added, total viable count, heat - resistant spore count and the spore percentage were determined after 72 h of cultivation as described before. Duplicate flasks were set up for each experiment.

The metals tested were: KCL , $MgSo_4$, $MgCl_2$, $FeSo_4$, $CaCl_2$, Na_2So_4, $MnSo_4$, $CuSo_4$ and K_2So_4 .

Preparation of B. *megatherium* spore yield:

B. megatherium were grown on a modified nutrient medium supplemeted with a mixture of MnSo4, $CaCl_2$, $ZnSo_4$ and KCL at a concentration of 500 ppm for 3 days on an orbital shaker at 150 rpm at 30^0C till the maximum spore yield was produced, these were harvested and subsequently washed by repeated centrifugation at 5,000 × *g* for 20 min at 4°C /resuspension in sterile distilled water (Warriner and Waites, 1999). Finaly, the spore pellet was re-suspended in sterile distilled water and used as active material in different formulations .The final spore titer was $\geq 10^8$ CFU/ml .

Formulation of B. *megatherium* :

The inert carriers used in the formulations were talc, clay and cellulose. For each carrier type, 1% carboximethylcellulose (CMC) as binder, traces of sodium benzoate as stabilizer ,15% $CaCo_3$ as buffer and 0.25% of different enrichment materials were incorporated .The enrichment materials incorporated to be tested were : glucose , sucrose, mannitol , yeast and peptone . Also, combinations of the three carriers (talc-cellulose, talc-clay, and cellulose-clay) were developed to be evaluated.

The inert carriers, enrichment and additive materials were mixed and sterilized by autoclaving. Twenty ml of spore suspension were added into them, mixed well under aseptic conditions, then the mixtures were air dried in a laminar flow chamber for 48 hours. After drying, a 1-g sample was removed for initial population counts. Powder formulations were then placed in plastic petri plates, sealed with parafilm, stored at room temperature, and sampled for viability assessment.

Viability assessment:

In the viability assessment, population counts of bacteria among various formulations were determined by serial dilutions from formulations and plated in triplicate on nutrient agar, and the CFU per gram of formulation were enumerated at intervals of 1 to 6 months.

Seed coating with bioformulation:

One gram of the selected bacterial formulation (Talc-glucose, Talc-yeast and Clay-cellulose) was added to 100 g bean seeds wetted with 1 ml sterile distilled water in a sterile plastic bag. The mixture was shaken until the seeds were thoroughly coated with the formulation. Also, 100 g bean seeds were mixed with free-spore suspension of *Bacillus megatherium* ($\geq 10^8$spore/ml) using CMC (1%) as sticker (Amran, 2006). Three corn seeds coated with the formulation were taken randomly and placed

separately into test tubes containing 10 ml sterile distilled water. With a sterile pipette, 0.1 ml from a 10^{-2} dilution was placed onto nutrient plates and spread using a sterile glass rod hockey. The plates were sealed and incubated at 30^0C. After 48 hours, bacterial colonies were counted.

Field evaluation of selected bioformulation on growth and yield parameters of bean plant:

A field experiment was conducted in a complete randomized design with three replicates at Maryut Experimental Station of the Desert Research Center (DRC), Alexandria. A standard plot size of 5 x 4 m^2 was maintained for all treatments. Soil in all treatments was amended with recommended dose of super phosphate (15.5% P_2O_5) at a rate of 250 kg/fed, ammonium nitrate (33.3% N) at a rate of 300 kg/fed and K-sulphate (48% K_2O) at a rate of 200 kg/fed .

Seeds of faba bean (Vicia faba cv Giza 40) purchased from agriculture ministry, Giza, Egypt. were treated with selected bacterial formulations (Talc-Glucose , Talc- yeast and Clay –Cellulose) at the rate of 1% (powder formulation : seeds) to give a bacterial population of $\geq 10^7$CFU/seed of formulation , For the free-spore suspension treatment, seeds were moistened in CMC solution (1%) before application of inoculum to get a thin, uniform coating of inoculum on seeds. Inoculated seeds were dried in shade before sowing (Samasegaran *et al.,* 1982), an untreated control was maintained. .

Plant height, weight of 100seeds, seed and straw yield (kg/fed.) of bean plants were recorded at the time of harvest for all treatments

Chemical analysis of bean seeds and straw was carried out after harvest to determine total phosphorus, nitrogen and protein in both straw and seeds

The plant materials were dried in an oven at 70 °C until a constant mass was reached and then they were grounded for chemical analysis. Total nitrogen was determined according to **(**Bremner and Mulvaney, 1982). and phosphorus was determined spectrophotometrically according to by the ascorbic acid method at 650 nm according to Watanabe and Olsen (1965).

The degree of rhizosphere colonization was estimated as following: After 30 days from sowing, roots and attached soil were divided into primary roots by cutting just below the seed. Each primary root was cut into thirds according to length, and the top section of each root was placed into sterile distilled water. Bacterial colonization was significantly higher in the uppermost section of the root compared to that of lower sections (Oliver, *et al*, 2004(Root sections and attached soil were sonicated for 5 min. The resulting bacterial suspensions were vortexed , serially diluted and grown on selective modified Bunt and Rovira agar plates for counting of phosphate dissolving bacteria and on nutrient media for total count . Colonies were counted after 48 h of incubation at 30°C, and the CFU per cm of the root system was estimated.

Statistical analysis:

Data were subjected to statistical analysis using the method described by (Snedecor, 1966). The least significant difference (L.S.D) was used to differentiate means according to (Waller and Duncan, 1969).

3. Results and Discussion:

From table (1), the highest values of TVC and HRSC detected were 400×10 ^{8}CFU/ ml and 100 × 10^8 spore/ml after 72 h of cultivation, respectively. Whilst the highest spore percentage (35 %) was recorded after 96 h of incubation on nutrient media although both total viable count and heat-resistant spore count decreased.

The nutrient media increased both the total viable count and spore yield over 1 and 2.5 folds compared to Bunt and Rovira medium and over 2.5 and 3.3 folds compared to Pikovskaya medium.

As the nutrient media enhanced the sporulation process and increased the sporulating percentage of *B. megatherium, so* it was selected to be a basal medium for the production of spore yield.

Table (1): Effect of different media used on total viable cell counts, spore count and spore percentage;

Time	Media used								
	Nutrient			Bunt and Roveira			Pikovskaya		
	TVC×10^8 CFU/ml	HRSC×10^8 spore/ml	%	TVC×10^8 CFU/ml	HRSC×10^8 spore/ml	%	TVC×10^8 CFU/ml	HRSC×10^8 spore/ml	%
24 h	70	5	7	20	2	10	10	1	10
48 h	180	18	10	45	6	13	60	8	13
72 h	400	100	25	200	40	20	160	30	19
96 h	80	28	35	120	30	25	20	5	25

TVC: total viable count HRSC: Heat-resistant spore count

As shown from table (2), incorporation of metals to the basal sporulating media generally increase the sporulation process except for Cu, and this may be due to increasing of osmotic pressure of media which have appositive effect on sporulation process , this is supported by the fact that certain transition metals including iron (Fe) and manganese (Mn) in a complex sporulation medium stimulated spore formation in certain strains of *Clostridium botulinum*, but sporulation was drastically decreased by the addition of copper (Cu) to the medium (David, *et al*., 1990).

Medium containing 500 ppm of $MgSo_4$,$MnSo_4$, K_2So_4 or $ZnSo_4$ showed the highest values of both total viable count and heat-resistant spore count compared with other concentrations tested and this is agree with a fact that there is a correlation between growth rate and spore yield. (Osadchaya, *et al*., 997).

Addition of a mixture composed of 500 ppm of $MnSo_4$, $CaCl_2$, $ZnSo_4$ and KCL (the metals gave the spore percentage over 75%) to the basal nutrient medium enhanced the sporulation process ,increased the spore yield to about 160 $\times 10^8$CFU $ml^{-1}$and increased the spore percentage to 89% (over 64% compared to control) .

The specific functions of metal ions in sporulation are probably that they act as activators of the various enzyme systems necessary for sporulation (Bruno and Ralph, 1964).

Varying the metal concentration in the sporulation media is known to influence the thermal-resistance spores due to inducuction of genes coding for the two small acid soluble proteins earlier during sporulation in the media that contained higher metal concentrations (Oomes and Brul**,** 2004).

Table (2): Effect of different metals on the enhancement of sporulation process (total viable cell counts, spore count and spore percentage):

Metals added at 500 ppm	Total viable count $\times 10^8$CFU ml^{-1}	Heat-resistant spore count $\times 10^8$ spore ml^{-1}	Spore percentage %
Control	80	20	25
KCL	96	72	75
$MgSo_4$	160	88	55
$MgCl_2$,	80	32	40
$MnSo_4$	120	96	80
$CaCl_2$	90	74	82
$FeSo_4$	55	30	55
Na_2So4	34	22	65
K_2So_4	190	80	42
$CuSo_4$	0, 002	0.0004	20
$ZnSo_4$	110	86	78
*Mixture	180	160	89

*Amixture composed of each of the following: 500 ppm of $MnSo_4$, $CaCl_2$, $ZnSo_4$ and KCL.

In all formulations, bacterial populations declined steadily over time, the bacteria survived even up to 180 days of storage with different percentage although the population declined from 30 days of formulation as shown from table (3) .

The type of carriers used influenced the viability of bacterial cells, the bacterial populations for both cellulose and talc--based formulations were generally higher than that of clay-based formulation.. All cellulose-based formulations with and without enrichments were able to maintain the highest viable cell count throughout the storage period, with a mean cell count of 81.7 x 10^8CFU/g of formulation compared to talc -based formulations and clay-based formulations, with 80 and 9.3 x 10^8CFU/g , respectively after 180days of storage . Recent studies on beneficial rhizobacteria have investigated the efficacy of powder formulations in combination with methylcellulose and xanthan gum (Kloepper and Schroth, 1981, Suslow and Schroth , 1982).

Among the formulations, enrichment materials proved to be the most useful as highest number of viable cells were recovered.

These are in agreement with previous research which showed that high-molecular-weight (C6 to C12) compounds such as sucrose and trehalose enhanced survival of bacteria in dried biopolymers (Ilyina *et al.*, 2000). Among these enrichment materials, glucose and yeast were the most efficient ones in preserving bacterial populations at different formulations.

Also, the formula composed of clay- cellulose was the most effective one among other formulations in survival of bacterial populations. This is may be due to the advantages of both clay and cellulose as carriers.

This showed that clay materials benefited the cells by providing large surface areas which act as an effective survival unit for nutrient absorption and protection (Lunsdorf *et al.*, 2000, Ting *et al.*, 2010).

In addition to the catalytic and shielding properties, clay also has good cation exchange capacity which enhances the bacterial metabolic activity (Adamis *et al.*, 2005). High cation exchange capacity enhances bacterial metabolic activity leading to higher viability (Beveridge, 1988),

The great value of porosity and capacity of cellulose to absorbe the hydrophobic and hydrophilic liquids may be useful for application as carrier for microorganisms for agriculture objectives. These properties are important for distribution of microorganisms in the granules and absorption of substrates limiting the growth of microbial population (Ilyina *et al.*, 2000).

This indicates the possibility of developing simple powder formulations with the capacity to provide long-term survival of beneficial rhizobacterial strains at high populations.

Among these formulations, five formulations (T-glucose, T-yeast, Ce -glucose, Cellulose - yeast and Cellulose-clay) were selected for additional testing because they had higher levels of viability amongst bacterial cell populations.

Table (3): Effect of different carriers and amendments on survival of bacteria in powder formulations

Formulation	x10^{8}CFU/g of formulation							*Survival
	0day	30day	60day	90day	120day	150day	180day	%
Talc	200	190	80	60	500	40	40	20
T-glucose	220	180	180	160	160	145	150	68
T-sucrose	190	190	160	90	70	50	50	26
T-.manitol	210	200	90	90	60	60	45	21
T-yeast	200	200	200	190	180	170	150	75
T-pepton	180	180	120	80	70	45	45	25
Clay	200	110	30	10	6	5	2	1
C-glucose	190	180	90	50	40	20	20	11
C-sucrose	180	150	40	15	12	4	3	1.6
C-manitol	210	200	170	35	20	10	7	3
C-yeast	210	170	150	70	60	30	18	8.5
C-pepton	200	140	35	30	20	10	6	3
Cellulose	210	210	200	160	150	140	70	33
Ce-glucose	230	230	180	140	140	120	120	58
Ce-sucrose	180	160	160	140	100	100	70	40
Ce-.manitol	190	160	130	120	120	100	70	37
Ce-yeast	200	200	190	190	160	140	110	55
Ce-pepton	200	180	160	90	90	80	50	40
Talc-Cellulose	190	180	145	100	74	60	57	30
Talc-Clay	210	200	175	140	130	105	105	50
Cellulose-Clay	200	200	200	190	170	170	160	80

Percent survival of each formulation was determined as follows: [(CFU/g of formulation at sampling)/CFU/g of formulation at beginning of experiment)] x 100

Results from table (4) showed that all seeds were successfully coated with bacterial spores of *B. megatherium* with different ranges , the highest bacterial population (2.3x 10^7CFU /seed) was on seed coated with the cellulose - clay based-formulation followed by talc based-formulations and the lowest was on seed with the cellulose - based formulations (0.8 x 10^7 CFU /seed). It has been proposed that a uniform coating of approximately 10^7 CFU of bacteria per seed is necessary for successful bacterization (Suslow, 1982).

Generally, bacterial populations on seeds treated with free cell suspension and CMC was higher than that of powder based-formulations except for cellulose - clay based-formulation which gave the highest value.

The ability of any PGPB to colonize its target plant roots and to produce growth effects is an ultimate test (Bashanand, 1997; Glick and Bashan 1997).

The obtained data from the table (6) generally showed that application of biofertilizer in the form of free-spore suspension or powder formulations considerably stimulates both total microbial and phosphate dissolvers counts in the rhizosphere of bean plants. Maximum numbers of inoculated

bacteria were recovered from the rhizosphere of free-spore suspension and cellulose - clay formulation treated plants after 4 weeks of growth.

These indicate that the colonization capacity of *B. megatherium* in free-spore suspension was superior to that of other powder formulations except for cellulose -clay formulation which gave the same colonization capacity.

Table (4): Bacterial populations on seed surface treated with different formulations :

Formulation	x10^{7}CFU/seed	Formulation	x10^{7}CFU/seed
FSS	2	**Ce-glucose**	0.8
T- glucose	1.5	**Ce - yeast**	0.9
T- yeast	1.3	**Ce - clay**	2.3

FSS: free spore suspension

Table (5): The effect of selected formulations on the degree of rhizosphere colonization:

Treatments	Total count x10^{6}CFU/cm	PDB count x10^{4}CFU/cm
Control	9	8
Fss	20	16
T-glucose	18	10
T-yeast	17	11
Ce-clay	21	16

Generally, data represented in table (6) indicated that seed inoculation with all bioformulations were found to enhance the plant height, weight of 100 seeds ,seed and straw yield of the faba bean plant over the control . no significant differences among bioformulations treatments were detected .

For plant height, the highest remarkable increase was recorded with half free spore suspension application followed by Cellulose-clay based formulation relative to control.

Free spore suspension recorded the significance increase over the control and other powder formulations for straw yield,, while Cellulose-clay based formulation record the heights significance value for seed yield .

Biofertilization treatments caused an effective action on N and P uptake by different parts of faba bean plants (seed and straw) relative to the control. Concerned to N % , there is no significant differences among formulations of *B. megatherium* in case of straw although the free spore suspension gave the maximum N % in seeds followed by other powder formulations.

However, the overall mean of both seeds and straw P % as affected by *B. megatherium inoculation* indicated a relative increase by about 18 % and 25 %, respectively over the untreated one.

Higher accumulation of P in seeds were recorded with inoculation of free spore suspension followed by other powder formulations where there is no significant differences among them .For P % in straw, there is no significant differences among different biotreatments. Of these treatments, the free spore suspension and Cellulose-clay based powder formulation were more effective than the other formulations and the spore application without a carrier as compared with other treatments.

Table (6): Evaluation of selected formulations on the bean growth and yield parameters:

Treatments	Plant height (cm)	Weight of 100 seeds(gm)	Yield kg/fed			Nitrogen %		Phosphorus %	
			Total	Seed	Straw	Seed	Straw	Seed	Straw
Control	48 c	80 b	2592c	1160c	1432b	2.9 b	1.5 a	0.66 b	0.5 b
Fss	62 a	95 a	3100a	1288b	1812a	3.5a	1.9 a	0.88 a	0.79 a
T-glucose	52 bc	90 ab	2760bc	1300b	1460b	3.2ab	1.9a	0.81ab	0.73 a
T-yeast	55abc	90 ab	2800b	1364b	1436b	3.2ab	1.7 a	0.8 ab	0.73 a
Ce-clay	60ab	93 a	2800b	1500a	1300b	3.4ab	1.8 a	0.85ab	0.76 a
LSD(0.5%)	7.6	11.1	174.5	89.04	183.7	0.56	0.77	0.139	0.173

- FS: Free spore suspension
- Values with same letter are not significantly different (P = 0.05).

4. Conclusion

This study reports the results of investigations on the different formulation combinations in maintaining the efficacy and viability of the *B. megatherium* endospores.

When heat-resistant endospores of *B. megatherium produced* from modified nutrient medium were formulated with different inorganic carriers, the dormancy of the endospores was maintained for 6 months at room temperature depending on the carrier type, also the enrichment materials were effective in enhancing the cell viability especially glucose and yeast .Of these bioformulations, cellulose-clay and talc based powder formulations were more effective than other formulations tested.

From the field application, it is indicated that seed treatment with powder formulations especially Cellulose –clay based formulation is an effective delivery system that can provide a conducive environment for *B. megatherium* to solubilize phosphate ,enhance growth and yield parameters of plants and has the potential for utilization in commercial field application .The application of powder formulation of phosphate solubilizer *B. megatherium* spores undoubtedly shows their advantages over traditionally used free spore inoculation as they increase the efficacy of the spore , enhance cell viability , facilitate the delivery and handling processes and promote the growth parameters of the plant.

Finally, the powder bioformulations have a potential for large-scale applications instead of the traditionally used free spore inoculation**.**

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Eusyllinae, Anoplosyllinae, and Exogoninae (Polychaeta: Syllidae) for the Mediterranean Coasts of Egypt, Together the Description of One New Species

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Abstract: In this paper, 18 species of the subfamilies Exogoninae, Anoplosyllinae, and Eusyllinae (Syllidae, Polychaeta) are reported from the Mediterranean Egyptian coasts, 8 of them are new records for the area: *Odontosyllis fulgurans* (Audouin and Milne Edwards, 1833); *Syllides japonicus* Imajima, 1966; *Salvatoria clavata* (Clapare□de, 1863); *Salvatoria euritmica* (Sardá, 1984); *Sphaerosyllis glandulata* Perkins, 1981; *Parapionosyllis labornica* Cognetti, 1965; *Sphaerosyllis* sp.; and *Prosphaerosyllis* sp. Five species were reported previously in the area. Four species are new records for Mediterranean Sea: *Palposyllis prosostoma* Hartmann-Schröder, 1977; *Paraehlersia weissmaniodes* (Augener,1913); *Streptosyllis compoyi* Brito, Núñez and San Martín, 2000; and *Exogone africana* Hartmann-Schröder, 1974); *P. weissmaniodes* and *Exogone africana* are two widely distributed Indo-Pacific species, so they could be considered as Lessepsian migrants. Finally, one new species is described, *Parapionosyllis aegyptia*.

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Keywords: *Eusyllinae, Anoplosyllinae, Exogoninae, Taxonomy, Mediterranean, Egypt, New species.*

1. Introduction:

Syllidae represent one of the most diverse and systematically challenging families of Polychaeta (Glasby, 2000; Rouse & Pleijel, 2001; San Martín, 2003, 2005; San Martín & Hutchings, 2006; Aguado & San Martín, 2009). It is a widely distributed group, found from the intertidal zone to the abyssal plains all over the world (Glasby, 2000), but less common at depth, with some species symbiotic or parasitic on other marine invertebrates (Martín and Britayev, 1998).

This family is currently divided into 5 subfamilies (Aguado& San Martín 2009): Eusyllinae Malaquin, 1893; Exogoninae Langerhans, 1879; Autolytinae Langerhans, 1879; Syllinae Grube, 1850; and the recently erected Anoplosyllinae Aguado and San Martín , 2009.

To detect newly recorded or new species we will depend on accurate taxonomic identifications and the local biodiversity. The possible existence of complexes of species, whose identity is blurred under one common specific name are present (Aguado and San Martín, 2007).

Knowledge about Polychaetes in the Egyptian waters is still far from complete; as result of less taxonomical studies and less sufficient data about this group. This paper is the second report about Egyptian Syllids, collected from the Northwestern Coast of Egypt through Salsabeel cruise, Autumn 2008 and Spring 2009, also from Gamasa (Spring 2009), under the frame work organized by National Institute of Oceanography and fisheries branch Alexandria, and from Port Said Harbour (Spring 2008), to study the benthic invertebrates. While the first report about syllid and sabellid species in the Northwestern coast of Egypt, were done by Selim (2008a & b respectively), Abd- Elnaby (2009) also studied polychaetes in Gamasa.

Generally, scarce attention has been given to the polychaetes in Egyptian waters. Fauvel (1927) recorded 8 syllid species from the Suez Canal waters of which 6 belonging to genus *Syllis*. On his work on the polychaetes collected from the fishery grounds near Alexandria, Fauvel (1937) gave a checklist of polychaetes were recorded in this area.

Only 16 species of Syllidae were recorded in that paper. More recently, Selim (1978) reported two syllids species in the Eastern Harbour of Alexandria, namely *Syllis (Typosyllis) variegata* and *Trypanosyllis zebra*. Later, the same author (Selim,

1996) added 6 syllid species from Alexandria coast (*Branchiosyllis exilis, Syllis gracilis, S. hyalinae, S. mediterranea, S. prolifera and S. variegate*). Finally, Abd-Elnaby (1999) recorded 7 syllid species, and later (2005) 21 species from Alexandria coast.

The Syllidae of the neighbouring areas were studied by several authors; from Aegean Sea by Çinar & Ergen (2002); Çinar (2003); and Çinar (2005); from Israel and the Gulf of Elat by Ben-Eliahu (1977a & 1977b), and anteriorly Fauvel (1955, 1957), from Cyprus by Ben-Eliahu (1972), Çinar (2003a& b) and Çinar & Ergen (2003); Lebanon by Aguado & San Martín (2007), and from Turkey by Ergen (1976). A checklist, distribution, and ecological features of Syllidae and other polychaetes from Greece can be reported in Simboura & Nicolaidou (2001), also from Cyprus by many authors, the most recent one Musco et al. (2005), and the biogeographic revision on Syllidae from the Mediterranean Sea (East and West areas) was carried out by Musco & Giangrande (2005).

During the present study 18 species were recorded, 11 of which are new records for the Egyptian waters. Four species are considered as new species for Mediterranean Sea. Three species are considered as new species, although two of them are under process of description, and one species is described here as new for Science. In this paper, detailed description is given also of some interesting species

2. Materials and methods

Two cruises were carried out on the Northwestern Mediterranean coast of Egypt; on two stations (El Hammam, El Alamein), during Autumn 2008 and Spring 2009, and also one collection Spring 2008. The stations are; Port Said Harbour (station 1), in which samples were collected during Spring 2008, and Gamasa (station2, Spring 2009), depth ranging from 0 .25m to 20 m. (Fig. 1). Sediment samples were collected by a Van Veen grab; while, samples from Port Said Harbour were collected by knife and net used for collecting fauna. Sediment samples were washed up and sieved through o.3 µm sieve, then sorted under Stereomicroscope. Specimens of Syllidae were extracted and fixed in 10 % formaldehyde in sea water-solution. Examinations and identification were done by using compound microscope. Drawings were made by a camera lucida. The specimens were Preserved in the Marine Reference Collection Center of National Institute of Oceanography and Fisheries, Alexandria, under Code Number (N. Sp. 2/8/3).

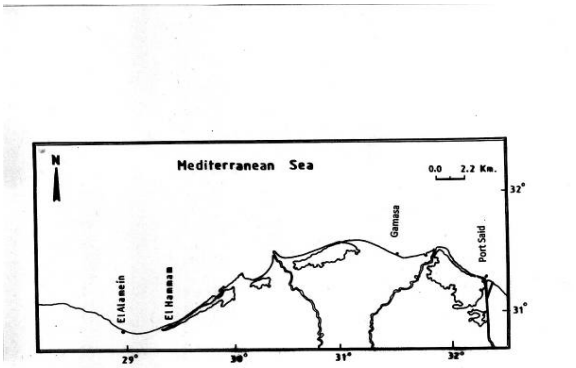

Fig. (1) Map showing the sampling sites, (North western Coast of Egypt, Gamasa and Port Said).

3. Results

In the present study, 18 species belonging to the subfamilies Exogoninae, Anoplosyllinae and Eusyllinae (Syllidae, Polychaeta) were recorded and identified from the Mediterranean Egyptian coasts, 8 of them considered new records for the Egyptian Mediterranean waters: *Odontosyllis fulgurans* (Audouin and Milne Edwards, 1833); *Syllides japonicus* Imajima, 1966; *Salvatoria clavata* (Clapare□de, 1863); *Salvatoria euritmica* (Sarda□, 1984); *Sphaerosyllis glandulata* Perkins, 1981; *Parapionosyllis labronica* Cognetti, 1965; *Sphaerosyllis* sp.; and *Prosphaerosyllis* sp.; the two later are new species in process of description, although a description of both without any specific name is given by San Martín (2003). Five species were reported previously from different places of Egypt. In addition, four species are considered as a new records for the Mediterranean Sea (*Palposyllis prosostoma* Hartmann-Schröder, 1977; *Paraehlersia weissmaniodes* (Augener,1913); *Streptosyllis compoyi* Brito, Núñez and San Martín, 2000; and *Exogone africana* Hartmann-Schröder, 1974). Finally one species *Parapionosyllis aegyptia* is described as new species. The locations, dates , depth, number of specimens and geographical distribution are presented in Table (1).

The most important species will be described in details.

Table(1): Showing the Polychaete species recorded in the present study, Location, Date, Depth, Bottom, Number of specimens, and Distribution.

Name of the species	Location	Depth (m)	Date	No.	Bottom	Distribution
****Palposyllis prosostoma* Hartmann-Schröder, 1977**	**Gamasa**	**13.7**	**Spring 2009**	**1**	**S-M**	**At**
***Paraehlersia weissmaniodes* (Augener,1913)**	Port Said	0.25	Spring 2008	3	F	At
*****Brevicirrosyllis weismanni* Langerhans, 1879**	El Alamein	20.0	Autumn 2008	1	C-S	At, Med
****Odontosyllis fulgurans* (Audouin and Milne Edwards, 1833)**	Port Said	0.25	Spring 2008	1	F	Cos
***Streptosyllis compoyi* Brito, Núñez and San Martín, 2000**	Elhammam	20.0	Autumn 2008	1	C-S	At
****Syllides japonicus* Imajima, 1966**	Elhammam	20.0	Spring 2009	2	C-S	At, Med, P
****Salvatoria clavata (Claparède, 1863).**	Elhammam	20.0	Spring 2009	1	C-S	Cos
****Salvatoria euritmica* (Sardá, 1984)**	Elhammam	8.0	Spring 2009	1	C-S	At, Med, P
*****Salvatoria vieitezi* San Martín 1984**	El Alamein	20.0	Autumn 2008	2	C-S	At, Med, P
****Sphaerosyllis glandulata* Perkins, 1981**	Gamasa	13.7	Spring 2009	1	S-M	At, Med
*****Sphaerosyllis taylori* Perkins, 1981**	Elhammam	20.0	Spring 2008	1	C-S	At, Med
****Sphaerosyllis* sp.**	El Alamein	20.0	Autumn 2008	1	C-S	At
****Prosphaerosyllis* sp.**	Elhammam	8.0	Spring 2009	1	C-S	At
***Exogone africana* Hartmann-Schröder,1974**	Port Said	0.25	Spring 2008	2	F	At
***Parapionosyllis aegyptia* n. sp.**	El Alamein	20.0	Autumn 2008	2	C-S	n. sp.
*****Parapionosyllis brevicirra* Day, 1954**	El Alamein	20.0	Autumn 2008	2	C-S	At, Med
*****Parapionosyllis elegans* (Pierantoni, 1903)**	El Alamein	20.0	Autumn 2008	2	C-S	At, Med
****Parapionosyllis labronica* Cognetti, 1965**	Gamasa	13.7	Spring 2009	1	S-M	At, Med

At= Atlantic Ocean, P= Pacific Ocean, Med= Mediterranean, Cos= Cosmopolitan, S-M= Sandy mud, C-S= Coarse Sand, F= Fouling

*= New species, **= New record for Mediterranean Sea, ***= New record for Egyptian waters, ****= Recorded before from Egyptian waters

Paraehlersia weissmaniodes (Augener, 1913)

(Fig. 2 A-J)

Ehlersia ferrugina non Langerhans. Böggemann & Westheide, 2004: 418, fig. 6. *Paraehlersia weissmanioides* San Martin & *Paraehlersia weissmanioides* San Martin & Hutchings, 2006: 312, figs. 43A-C, 47A-I, 48 A-F, 49 D-F.

Material examined. Port Said 0.25 m depth, Spring 2008, one specimen.

Description. Body broad anteriorly, tapered posteriorly, 11 mm long, 0.2 mm wide, with 41 chaetigers (fig. 2 A). Prostomium oval (75 µm), 4 eyes in trapezoidal arrangement, and 2 anterior eye-spots; lateral antenna 162.5 µm long, midian antenna 150 µm long. Palps broad (87.5 µm), basally fused. Dorsal tentacular cirri 147.5-162.5 µm long, ventral tentacular cirri about one third in length of dorsal tentacular cirri. Antennae, tentacular and anterior dorsal cirri; elongated, indistinctly articulated; articulation variable with short and long articles, up to 22 articles; dorsal cirri becoming progressively smoother posteriorly. Infracirral papillae not seen. Parapodia conical, slightly elongate. Ventral cirri digitiform, slightly longer than parapodial lobes. Parapodia with 12-15 falcigerous compound chaetae; blades strongly bidentate, with fine spines on margin (fig. 2 B), 2-3 distalmost ones longer than remaining (30-42.5 µm) (fig. 2 C). Most dorsal compound chaetae, spiniger-like, blades (75 µm long) on midbody, and about 93 µm on posterior parapodia with fine spines on margin (figs. 2 C, E), absent on most posterior parapodia; indistinctly bidentate. Compound falcigers becoming wider progressively along body, with stronger proximal tooth, slightly hooked (fig. 2G). Dorsal simple chaetae appear from chaetiger 19, truncate, bifid with short spines margin (fig. 2 F, H). Ventral simple chaetae on posterior parapodia, thick, with few long spines on margin, strongly bidentate, proximal tooth large, slightly hooked, and distal one shorter than proximal one (fig. 2 J).

Anterior parapodia with 2-3 slender aciculae, two of them distally rounded with small bending tip and one straight (fig. 2D); from proventricular segments onwards, acicula solitary, with oblique, short tip (fig. 2 I). Pharynx through 6 segments; pharyngeal tooth anteriorly located. Proventricle, rectangular through 4 segments with about 21 muscle cell rows.

Distribution: Australia, Seyhelles. New report for the Mediterranean Sea.

Remarks: The Egyptian specimen is similar to Australian ones; it is likely an Indo-Pacific migrant through Suez Canal.

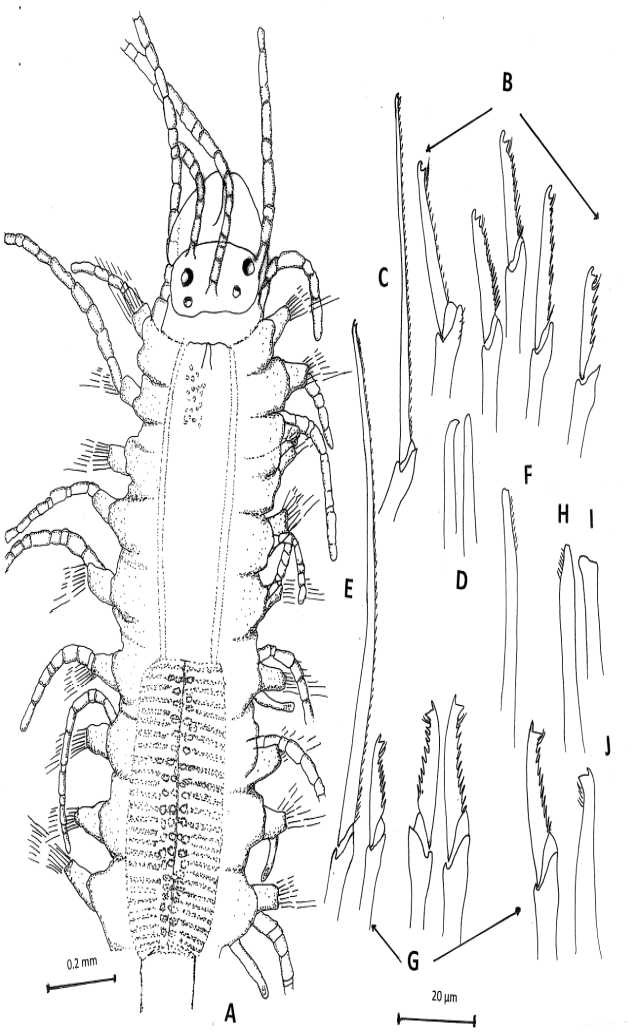

Figure 2.- *Paraehlersia weissmannioides* (Augener, 1913). A, anterior end, dorsal view. B, falcigers, anterior parapodium. C, Spiniger-like compound chaeta, anterior parapodium. D, anterior aciculae. E, Spiniger-like compound chaeta, posterior parapodium. F, dorsal simple chaeta, anterior parapodium. G, falcigers, posterior parapodium. H, dorsal simple chaeta, posterior parapodium. I, aciculum, posterior parapodium. J, ventral simple chaeta. Scale (A) 0.2 mm; (B-J) 20 µm.

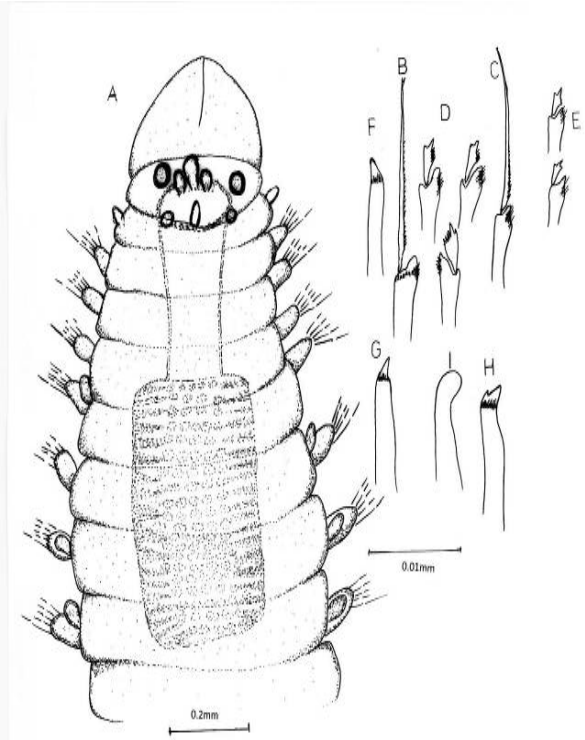

Fig. (3): *Exogone Africana* A: anterior part of body, B: anterior spiniger-like seta , C: posterior spiniger-like seta, D: anterior falcigers setae, E: posterior falcigers setae, F: anterior dorsal simple seta, G: posterior dorsal simple seta, H: ventral simple seta, I: acicula.

Exogone africana Hartmann- Schröder, 1974
(Fig. 3 A-I)

Exogone verugera africana Hartmann-Schröder,1974a:137, figs. 164-168; 1979; 108, figs.164-168.
Exogone africana San Martín, 2005: 143, fig. 90 a-f.

Material examined. Port Said, 0.25 m depth, on fouling, Spring 2008, 2 specimens.

Description. Body small, slender, relatively broad anteriorly, 3 mm long, 0.23 mm wide, 28 chaetigers. Prostomium oval (fig.3 A); 4 eyes in trapezoidal arrangement. Antennae short, oval, close to each other, inserted between anterior to eyes; median antenna slightly longer and thicker than lateral one.

Palps broad, longer than prostomium, totally fused, with a dorsal furrow (fig. 3A).

Peristomium shorter than subsequent segments; one pair of small, papilliform tentacular cirri. Dorsal cirri similar to antennae and tentacular cirri, slightly longer than lateral antennae, present on all segments. Compound chaetae of two types on all parapodia: 1-2 spiniger-like, with long blades 31 µm long (fig. 3 B) on anterior parapodia, slightly short on posterior one (25-27.5 µm), distally bifid, with short marginal spines (fig. 3 C), and 4 compound chaetae with short falcigerous blades about 7.5 µm, bidentate, subdistal tooth long and distal tooth short, moderate marginal spines (fig. 3 D); posterior falcigers smaller, three in number, blades about 5µm long (fig. 3 E). Dorsal simple chaetae from anterior segments, with rounded tips (fig. 3 F), subdistally serrated, thicker posteriorly with pointed tip (fig. 3 G). Ventral simple chaetae on posterior parapodia, sigmoid, thick, with some short spines on base of teeth, bidentate, subdistal tooth longer and thicker than distal tooth (fig. 3 H). Acicula solitary, slender, distally rounded (fig. 3 I). Pharynx long, through 4 segments; pharayngeal tooth located on anterior rim. Proventricle occupying 4 segments with 18 muscle cell rows. Pygidium with 2 long anal cirri.

Distribution: Circumtropical. First report to the Mediterranean Sea.

Parapionosyllis aegyptia n. sp.

(Fig. 4 A-G)

Material examined. El Alamein 20 m depth, Autumn 2008. Holotype and Paratype, coarse sand.

Description. Holotype 3.5 mm, 0.15mm wide 29 chaetigers (fig. 4 A). Prostomium ovate, wider than long; 2 pairs of eyes, anterior pair larger than posterior ones, arranged in trapezoidal arrangement, and 2 small anterior eye-spots. Antennae spindle-shaped to bowling-pin shaped, longer than prostomium; median antenna (77.6 µm) slightly longer than lateral ones (67.5 µm), arising between anterior eyes; lateral antennae arising on anterior margin of prostomium (right one missing on holotype). Palps basally fused, shorter than prostomium. Peristomium with 2 pairs of bowling-pin shaped tentacular cirri, smaller than antennae. Parapodia somewhat elongated (32.5 µm). Dorsal cirri bowling-pin shaped, from 36- 45µm in length anteriorly to 65µm on posterior parapodia. Ventral cirri digitiform, shorter than parapodial lobes.

Anterior parapodia with 7 compound falcigers, unidentate with hooked tips and serrated margin (fig. 4 B); about 10 µm long; shafts becoming posteriorly thick with long curved acute tip, blades with serrated margin on 2 most dorsal ones, and 4-5 unidentate curved, smooth (fig. 4 D). Superior dorsal simple chaetae thin with pointed tip, present in all parapodia, except first one (fig. 4 C), become thicker

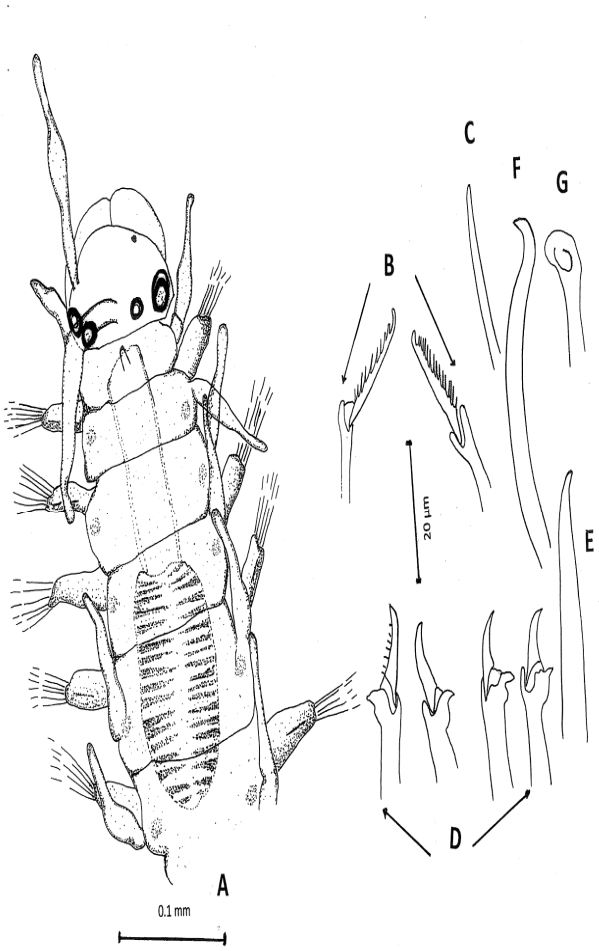

Figure 4.- *Parapionosyllis aegyptia*, n. sp. A, anterior end, dorsal view. B, compound chaetae, anterior parapodium. C, dorsal simple chaeta, anterior parapodium. D, compound chaetae, posterior parapodium. E, dorsal simple chaeta, posterior parapodium. F, ventral simple chaeta. G, aciculum. Scale (A) 0.1 mm; (B-G) 20 µm.

posteriorly (fig. 4 E). Ventral simple chaetae unidentate, sigmoid (fig. 4 F). Acicula solitary, bent with hollow rounded tip (fig. 4 G). Pharynx extending through 3.5 setigers; mid dorsal tooth on anterior edge. Proventricle extending through 2.5 segments, with 17 rows of muscle cells. Glands small, with granular material, pair on each segment, present from first chaetiger.

Remarks. About 16 species are recognized as *Parapionosyllis*, 6 of them recorded in the Mediterranean Sea. The most similar species is *P. labronica* also found in this collection; both species have posterior compound chaetae with thick shafts, distally curved, and short, unidentate blades, smooth or almost smooth. However, the anterior compound chaetae of *Parapionosyllis aegyptia* are more elongated and provided with somewhat longer spines on margin, and the dorsal simple chaetae are different, being smooth and unidentate in *P. aegyptia* and provided with a sub-distal, thick spine and others shorter, in *P. labronica*. The remaining Mediterranean species are clearly different of these two species, because they have longer compound chaetae (see San Martín, 2003); also, other species of other seas also have more elongated compound and different dorsal simple chaeta.

Etymology. The species is named after the country in which has been found, Egypt.

4. Discussion

The number of Syllids recorded on Egyptian waters reach about 60 species, a low number when compared with the 190 Syllid species were reported by Musco and Giangrande (2005) from the whole Mediterranean waters, which represents the 31.6 % of the total Mediterranean Syllidae. Also, many other species may possibly remain unreported because of the most coastal area of Egypt are unexplored and many studies are still needed.

Tovar- Hernández *et al*. (2002) referred to the dominance and diversity of syllid members in carbonate sediments, this observation was confirmed by Selim (2008), where El Hammam and El Alamein coasts contain carbonate bottom sediments.

Most of the studied species are well known, common and widely reported for Mediterranean and Atlantic Ocean. The analysis of samples resulted into 18 species,11 of them new record for the Egyptian Mediterranean waters. Four species were reported previously, (*Brevicirrosyllis weismanni* Langerhans, 1879; *Parapionosyllis elegans* (Pierantoni, 1903); *P.brevicirra* Day, 1954 and *Sphaerosyllis taylori* Perkins, 1981, from many Mediterranean coastal areas and one more (*Salvatoria vieitezi* San Martín 1984) from the Suez Canal. Two of the studied syllids are considered apparently cosmopolitan: *Salvatoria clavata,* and *Odontosyllis fulgurans.* In addition, 11 species were known before from Spainish coasts, only 4 species were previously recorded in Greece, 9 from North West Italian, 9 from Turkish Aegean and 6 from Cyprus. Also four species are considered as a new record for Mediterranean Sea: *Palposyllis prosostoma* Hartmann-Schröder, 1977; *Paraehlersia weissmaniodes* (Augener, 1913); *Streptosyllis compoyi* Brito, Núñez and San Martín, 2000; and *Exogone africana* Hartmann-Schröder, 1974.

According to geographic distribution through literature there are 9 species belong to Atlantic-Mediterranean category and one species (*Salvatoria vieitezi*) was recorded before from Suez Canal (Selim, 2009), and four species are new for Mediterranean, that means they are Lessepsian migrants; 3species are amphi-Atlantic, *Sphaerosyllis taylori*, *Shphaerosyllis glandulata* and *Salvatoria vieitezi.* Two species are considered cosmopolitan species, *Odontosyllis fulgurans* and *Salvatoria clavata* and five species are considered Atlantic-Pacific categories.

In spite of it, many new recorded species usually discovered by way in new researches, still more not recorded until now, more studies requisite to be done along the Mediterranean and Red Sea coasts of Egypt to cover this point.

The present study showed richness of Eusyllinae, Anoplosyllinae and Exogoninae species inhabiting Egyptian water benthic assemblage.

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Severe Anemia in Children Infected With Malaria in Taiz - Yemen and Its Relation to Age, Parasitaemia and Eosinophilia

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Abstract: In Yemen, about 12 million individuals live in endemic areas of malarias, out of them more than 90% were due to Plasmodium falciparum (WHO, 2001 a) Malaria continues to be a major health problem in Yemen. Severe anemia in malaric children occurs more frequently than cerebral malaria (Laurence et al, 1994). The aim of this work is to detect the relation of severe anemia in malaric patients < 5ys with the parasitaemia level of Plasmodium falciparum. This research was done in Alsewedi pediatric hospital in Taiz governorate from January to September 2008, for 100 admitted cases, results, of this study indicated the strong relation of severe anemia in falciparum malaric children to age < 5ys and there was no relation between the severe anemia in falciparum malaric children to the parasitaemia level .There was strong relation between younger ages and low level of Hb (P<0.05). These finding suggest that increase level of parasitaemia not related to severe anemia in P falciparum malaria. Also younger ages <5ys has strong relation to severe anemia Hb<5g per ul in falciparum malaria.
[AM.Elbadr, Amal. M. Abdo. Elmatary, N.A.Saif, E.Mahmoud and R. Osman. **Severe Anemia in Children Infected With Malaria in Taiz - Yemen and Its Relation to Age, Parasitaemia and Eosinophilia**. Life Science Journal 2010;7(4):140-143]. (ISSN: 1097-8135).

Keywords: severe malaria, parasitaemia, severe anemia.

1. Introduction

Plasmodium falciparum malaria causes 1-2 million deaths per year (WHO, 2001b), another half billion get infected but survive, most cases are found in sub-sahran Africa (Kahl, 2003).

Age and transmission intensity are known to influence the manifestations of severe falciparum malaria in African children (Idro, et al., 2006) .Malaria is one of the main causes why 3.5 millions low-birth weight infants are born each year in the region (Roger, 2000).

Reduction in severe disease and death from falciparum malaria in Africa requires new, more effective and inexpensive public health measures (Miller and Greenwood, 2002).

The burden of malaria in tropical world is estimated to involve 300-500 million episodes of acute illness and more than million deaths per year, mainly in African children. The emergence of Plasmodium falciparum resistance to widely used antimalarial drugs such as chloroquin has made malaria control and treatment much more difficult (D'AleTssandro and Butteins, 2001).

Resistance of Plasmodium falciparum to chloroquin was confirmed in most countries of Sub-Sahran Africa (Trape, 2001 & Carter and Mendis, 2002)

Highly complex and dynamic system (Craig et al., 2004). Malaria is mesoendemic and transmission is perennial, falciparum is the main species identified and A.arabiensis the main vector in Taiz (Daoud, 1988).

Malaria is one of the most common diseases in Yemen Arab Republic, Plasmodium falciparum represented > 90%, 20% of cases resistant to chloraquin (Al-Mawri, 2000).

2. Materials and Methods:

Study area:

Taiz Governorate in Yemen .It lies in the foothill and middle heights, which rang from 200-2000m elevation. The mean annual temperature between 20-3°c with little seasonal variation and relative humidity 40-60.The annual rainfall is 800-1200mm, and most of this fall in March-May and Augest-September.

In the present study used questionnaire for personal data, laboratory tests for each admitted malaric child and we get agree from each patient "consent"

The study was in Alsewedi - pediatric hospital in Taiz governorate / Yemen on 100 admitted positive Plasmodium falciparum malaria, age from 1 to 10 years regardless the sex .This study was from January to September 2008.

Peripheral blood smear (thick and thin) stained with Giemsa stain is used to identified the species, a sexual stage of parasite and the level of parasitemia (WHO, 1985, Shute, 1988 & Garcia, 2001). Parasitaemia may be expressed as a percentage of RBCs infected, or as number of

parasites present in lul of blood. Since lul of blood contains 5×10^6 RBCs,a 1% parasitaemia represents 50000 parasites per ul. (Moody, 2002).

Drabkins solution is used to determined hemoglobin level in g/dL(a colorimeter). Alonso et al (2002) defined severe anemia as Hb <5g per dl in patient with asexual form of P.falciparum in peripheral blood.

Counting eosinophil levelrEosinophil relative frequencies were determined by differential counting and for hospital study the absolute eosinophil count was obtained by multiplying the frequency with total leucocytes.

The eosinophil level were counted in non infected and infected children and the infected children divided as acute infection and asymptomatic infection.,the level of eosinophil also counted in infected children after cure.

The statistical analysis of data was carried out using statistical package for social science (Spss/ps) spss software.

3. Results:

The research aimed to detect the relation between severe anemia in malaric children to the age and level of parasitemia.

- According to study all the 100 positive cases were Plasmodium falciparum species.
- In present study, hemoglobin was classified into < 5g/dL and > 5-10g/dL according to (Alonso et al., 2000).

Table (1): shows the number of admitted patients according to age.

There were 45 positive cases their age < 5ys old, and 55 cases, their age from 5-10 ys , Total cases positive were 100.

Table (1): Number of malaric children according to age.

Age	< 5ys	5-10y
No	45	55
Total	100 cases	

<: less than. >: More than. g/dL : gram per deciliter. p/uL : parasite per micro liter. y: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Normal children (noninfected)

Table (2): Represented the percentage of Hb in malaric children in relation to their age:

In positive cases < 5ys old a bout 15/45 cases their Hb < 5g/dL, means those cases suffered from severe anemia, then was positive significant correlation between the level of Hb and the age of positive case (P< 0.05) .

Table (2): Hb precent in malaric children according to age.

Age	<	5ys	5-10y	
Hb%	<5g/dL	>5g/dL	<5g/dL	>5g/dL
Cases	15	30	10	45
P.value	0.045			

<: less than. >: More than. g/dL : gram per deciliter. p/uL : parasite per micro liter. y: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Level of Hb decreased with age, but level of Hb was > 5g/dL in about 30 positive cases their age > 5 ys .In positive cases their age from 5-10 ys about 10 cases their Hb was < 5 g/dL but 45 cases their Hb > 5 g/dL .This indicated that increase the age of patients , increasing the level of hemoglobin .At end of this table, the severe anemia was in malaric patients their age < 5 ys.

Table (3): Represented the % of Hb in relation to level of parasitaemia (PD) . The numbers of positive cases < 5 ys and their Hb < 5 g/dL, their (PD) was 100 - 250 (P/ul) . The numbers of positive cases < 5 ys and their Hb > 5 g/dL, their parasite density was 500 - 1000 p/ul.

This indicated that there was positive relation between the Hb in malaric cases and age (P < 0.05). , but no correlation between severe anemia in malaric cases and PD (P > 0.05) . This mean high level of parasitaemia not related to severe anemia in malaric patients . The numbers of positive cases > 5 ys ,thejr Hb level > 5g / dL and their PD was 500-1000 p/uL but the numbers of positive cases > 5 ys and their Hb > 5 g/dL and their PD was > 10,000 p/uL . Means again that no relation between severe anemia and PD .Decrease Hb not means high level of parasite in peripheral blood.

Table (3): Hb concentration level in relation to level of parasitaemia (PD) in malaric children.

Age	<	5ys	5-10y	
Hb%	<5g/dL	>5g/dL	<5g/dL	>5g/dL
No. of cases	15	30	10	45
PD (p/uL)	100-250	500-1000	500	1000
P. value	> 0.05			

<: less than. >: More than. g/dL : gram per deciliter. p/uL : parasite per micro liter. y: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Table (4): It represented the positive malaric cases with severe anemia who received blood transfusion < 5 ys . About 15 out of 45 positive cases received blood. In positive cases > 5 ys 10 out of 55 cases received blood.

Table (4): Number of positive malaric children with severe anemia and received blood transfusion

Hb%	Blood transfusion	
	Yes	No
<5g/dL	15	30
>5g/dL	10	45
Total	25	75

<: less than. >: More than. g/dL : gram per deciliter. p/uL : parasite per micro liter. y: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Eosinophilia in Plasmodium falciparum infected children and non infected children:

About the infected child they are investigated for eosinophilia as acute infection and asymptomatic parasitemia. A significant drop in eosinophils was observed during acute illness, returning to normal value after cure, the percentage of eosinophil in non infected child was 5-5.5%.and the percentage of eosinophil in infected children in acute stage was 2.5-5%. In contrast a significant increase in eosinophil frequency was observed in children with asymptomatic parasitemia at the times of infection eosinophil frequency was scientifically higher in asymptomatic children than in clinical malaria., the percentage of eosinophilia in this group was 5-8%.

4. Discussion

Malaria caused by *P.falciparum* remain the major life threatening parasitic infection in the world, (Durand et al., 2005).

From table 1: indicate that the age <5 yrs is more exposed to severe malaria and anemia.

Idro et al, 2006 reported a relation between manifestation of severe malaria in children < syrs and transmission intensity and anemia, which agree with our funding.

It is clear that there is a strong relation between age <5 ys and decreased Hb. (Tbale 2). Kwadwo et al., 2003, recorded that severe anemia was diagnosed in 30 individuals ranging form 3 months to 5 ys, but was primary noticed in children <24 months. These results coinced with Price et al., 2001 they stated that there was relation between decreased age and decrease Hb. They also added that children age <5ys were more likely than bolder to become anemic in Thailand.

It is clear that no relation between decreased Hb and parasitaemia level (Table 3). On the other view Kwadwo et al., 2003, showed that parasitaemia was strongly associated with lower Hb in children <2ys of age. But Al Serouri et al., 2002, not agreed with our result, they showed that parasitaemic group had a significantly lower Hb than non parasitaemic group.

And about the level of eosinophil in our study, our data indicate that *p. falciparum* infection induce eosinophilia. Our study led us hypothesis that *plasmodium falciparum* infection induces eosinophil production but that the excess production in clinical malaria is out balanced may be due to increase sequestration or destruction due to inflammatory process in the tissue, kurtzhals et al., (1998) agree with our study, they mentioned the increase in eosinophil activity in acute Plasmodium falciparum infection in association with cerebral malaria. Shanks and Wilairatanaporn (1992) said that eosinophilia in persons from malaria endemic area my represent a normal late response to malaria infection.

Jungwon et al., (2005) mentioned that hematologist should consider the possibly of pseudoeosinophilia as result hemozoin-containing WBCs and confirm the WBCs differential count by microscopy in cases of malaria.

The absolute eosinophil count was increase above the normal range in 7% of American servicemen with acute malaria eosinophilia was found in 6% before treatment and 30% after treatment of malaria (Reilly and Barrett, 1971).

In Gambian children with acute uncomplicated malaria there was reduction or absence of eosinophils in the peripheral blood at presentation and these returned to at normal numbers in all cases 3-7 days after treatment of malaria (Abdullah, 1988). No such changes were seen in patients with chronic malarial anemia. A study in Thailand found that eosinophil counts were elevated in 11% of patients with acute malaria at presentation and 93%had elevated eosinophil count by the day 7 after treatment there was then a marked reduction of eosinophil count by day -14 followed by another increase by 28 day (Camach et al., 1999).

Recommendation:

Malaria as a cause of severe anemia in our children should be given a wide attention and followed from the researchers, a cadmic staff, ancftnedical students.

Malaria is a dangerous disease we should become more a wariness to this problem.

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Physiological Formation of Mosquitocidal Toxin by a novel *Bacillus thuringiensis* isolate under Solid State Fermentation

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Abstract: Sixty eight cultures were isolated from soil of different locations in El-Sharkia governorate that were cultivated by cereals, rice, clover, cotton, and maize crops. Based on the LC_{50} and LC_{90} values, the Egyptian isolate No 4 was selected for further study due to its lower LC_{50} and LC_{90} than the international *Bacillus thuringiensis var.israelensis (Bti)* upon bioassay against second instars larvae *Culex pipiens*. The Egyptian isolate No 4 is defined morphologically and biochemically as *Bacillus thuringiensis*. Physiological factors affecting growth and toxin biosynthesis in *B. thuringiensis* isolate No 4 in comparison to *Bti* under solid state fermentation were studied. Talcum powder and silica gel were the best carriers yielding highest mosquitocidal activity for *Bacillus thuringiensis* No.4 and *Bacillus thuringiensis* var.*israelensis*, respectively.The highest mortality were obtained upon utilization of kidney beans (3%) and black eyed beans (6%) as base solid substrate for *B. thuringiensis* isolate No 4 and *B. thuringiensis* var.*israelensis*, respectively. Toxicity increased with extended the incubation period up to 9 days for both of tested organisms. Highest values for growth and toxicity were obtained in cultures with initial moisture content adjusted at 60% (w/w) for both organisms. Mosquitocidal toxin activity were fairly stable within a wide range of increasing aeration level up to a ratio of 10g culture/250ml conical flask and 10g culture/500ml for growth and toxicity of *Bacillus thuringiensis* var.*israelensis*, and *Bacillus thuringiensis* isolate No 4, respectively.

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Key Words: *Bacillus thuringiensis*, isolation, characterization, mosquitocidal toxin, physiology, solid state fermentation.

1. Introduction

The first realistic possibility for using *Bacillus* as a mosquito control agent was offered in 1977 with the isolation of *Bacillus thuringiensis* var. *israelensis* (*Bti*) Goldberg and margalit (1977).This strain possessed a high level of toxicity for mosquito and black fly larvae. Since 1980, the use of *Bacillus thuringiensis* var. *israelensis* formulation for mosquito control has greatly increased in California, Florida in the United States and various tropic countries Aronson *et al*. (1986).

According to Rawlins (1989), the advantages of *Bacillus thuringiensis* var. *israelensis* are its temperature tolerance, family specificity, handled easily and when stored at room temperature, formulations are stable for long periods. However, in the aquatic environment, larvicidal activity is short lived.

Bacillus thuringiensis and *Bacillus sphaericus* are being used widely as larvicidal bacteria for mosquito control, this microbial pesticides are eco-friendly and specific to the target organisms (Armengol *et al*., 2006).

Lonc *et al.* (2004) recommended a successful model of control strategy based on currently integrated methodologies with emphasis on preventive treatment of aquatic larvae with microbial insecticides was conducted. *Bacillus thuringiensis* var. *israelensis*-based formulation replaced chemicals to control *Culex pipiens*

According to Mudget (1986) solid-state fermentation, or alternatively called semi-solid fermentation, SSF may be briefly defined as that in which microbial growth and product formation take place on the surface of solid substrates. This type of fermentation, SSF, is distinguished from submerged fermentation by the fact that microbial growth occur at or near the surface of solid materials with low moisture content as contrasted to that taking place in continuous aqueous phase in case of submerged fermentation.

In spite of the extensive application of SSF technology in production of different microbial products, yet only very meagre information has so far been published on the possible use of SSF technology in the production of bioinsecticides e.g. *B. thuringiensis*. Foda *et al* (2002).

In the present investigation a series of experimental studies were carried out to investigate the possible use of SSF methodology for production mosquitocidal toxins from the local isolate of *B. thuringiensis* No.4 as well as from the International strain *B. thuringiensis* var.*israelensis* for comparative purposes.

2. Materials and Methods:

Microorganisms

The International strain *Bacillus thuringiensis* var. *israelensis*was kindly obtained from prof. F.G. Priest, School of Life Sciences, Heriot Watt University, UK A new *Bacillus thuringiensis* isolate namely *B.t.*No.4 was isolated from soils of El-Sharkia Governorate ,Egypt.

Media

1. Media used for isolation of *Bacillus thuringiensis* from soil

A) L- agar acetate medium (Morris et al., 1988): This medium was used as a selective medium for isolation of *Bacillus thuringiensis* after addition of 0.25 M acetate buffer. It consisted of (g/L); 10 peptone, 5 yeast extract, 10 sodium chloride, 20 Agar.

Acetate buffer: a) Sodium acetate 2.5 M, b) Acetic acid 2.5 M. Add (a) to (b) until pH 6.8, filter sterilized. Add 100 ml of acetate buffer to L-agar medium.

2. Synthetic media used for cultivation of the pure organisms and their activation prior to physiological studies

a) Nutrient Broth medium: (g/l): 5 peptone, 3 beef extract, for solidification 25 g agar were added.

b) Luria- Burtani(LB) medium: (g/l) peptone 10, yeast extract 5, sodium chloride 10.

c) NYSM broth medium: nutrient broth, yeast extract 0.5 g/l

Trace elements, (g/100ml): Manganese chloride 0.09, Calcium chloride 1.03, Magnesium chloride 2.o3.

1 ml of the filter sterilized trace elements solution was added to 100 ml of the medium.

3. Media used in solid state fermentation (SSF).These media were used for the study of potency of mosquitocidal toxin production, growth and sporulation of *B. thuringiensis* under SSF conditions. These types of media are mainly composed of three components namely an inert carrier, solid nutrient sources and an inorganic salt solution.

a) The inert carrier materials were either of organic nature e.g. Wheat bran and rice hull or some inorganic clay materials e.g. talcum powder, celite and silica gel

b)The mineral salt solution: The following composition were used (KH_2PO_4,0.5g/L,$MgSO_4.7H_2O$ 0.25g/L, $CaCl_2$ 0.1g/L, $FeSO_4.5H_2O$ 0.01 g/L) at appropriate concentration Foda *et al* (2002).

c) The main nutrient solid source: This was represented by some high protein content agroindustrial by-products that are locally available in Egypt. These include dried fodder yeast, feather meal and offal's meal which is a byproduct of chicken slaughter house residues being dried and thoroughly minced. The SSF were carried out in 250 ml conical flasks. The SSF medium composed of 10 ml mineral salt solution added to 10g of solid substrate-inert carrier mixture, thoroughly mixed and autoclaved Foda *et al* (2003).

Gel electrophoresis

Dissociating polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to a protocol proposed by Laemmli (1970).

Subunit molecular weight estimation by SDS-PAGE

The method of Weber and Osborne (1969) was used to determine the apparent (subunit) molecular weight of proteins dissolved or extracted in the presence of SDS. Electrophoretic mobilities were calculated relative to the mobility of the bromophenol blue marker band in 12% and 5% polyacrylamide slab gel. The following proteins were used as molecular weight standards: β-galactosidase (116 KDa), Bovine serum albumin (66.2 KDa), Ova albumin (45 KDa), Lactate dehydrogensae (35 KDa), β-lactoglobulin (18.4 KDa).

Bioassay of bacterial toxins against Mosquitoes larvae.

Bioassay of locally isolated *Bacillus thuringiensis* was carried out as described by Priest and Youstin (1991).Toxicity was determined with laboratory reared *Culex pipiens* serial dilutions in distilled water. The range of concentration of full grown whole culture (FWC) which killed 50% and 90% of the larvae were identified. Then further toxicity tests were done in the range recorded to evaluate precisely the LC_{50} and LC_{90} values for each highly promising bacterial culture. The corrected mortality was then plotted against culture dilution of cells/ml on log paper to determine LC_{50} andLC_{90} values for each highly promising bacterial cultures. The bacterial dilutions were placed in small cups in duplicates along with 10 second instar larvae. Appropriate controls were run simultaneously using distilled water instead of cultures. The cups kept at room temperature 27±2°C. The mortality percentage was recorded by counting the number of living larvae and corrected by using appropriate control and applying Abbott's formula (Abbott, 1925). The medium lethal concentrations LC_{50} of potent isolates

was computed through probit analysis within 95% confidence limits using propan program.
Abbott's formula:

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \% - Corrected}}{\text{100-Control mortality \%}} \times 100$$

3. Results:

1. Isolation of Mosquitocidal Toxin Producers from *Bacilli* isolated from the Egyptian environments

Sixty eight isolates were obtained from soils of five locations in El-Sharkia Governorate. Among these isolates, isolate No 4. obtained from location cultivated with wheat was the only isolate giving 100% mortality up to culture dilution 10^{-5}. Accordingly this isolate obtained from El-Sharkia Governorate was selected for further investigation.

2. Determination of LC_{50} and LC_{90} values of the Egyptian isolate No.4 obtained from El-Sharkia Governorate soils.

LC_{50} and LC_{90} of isolate No.4 and *Bacillus thuringiensis* var. *israelensis* are bioassayed against second instar larvae of *Culex. pipiens* revealed that the Egyptian isolate No.4 is highly toxic than the reference strain (Table 1).

3. Identification of the Egyptian isolate No.4 isolated from El-Sharkia Governorate

The morphological features of colonies exhibited a white colour and the colonies shape have a typical features of *Bacillus thuringiensis*. Microscopic examination of the cells indicated the presence of different types of crystals developed after 3 days of incubation. The formation of crystals inside the sporangium with oval spores as shown in Figures (1& 2), while Figures (3&4) describe the shape of crystals of *B. thuringiensis* var. *israelensis.*
Some biochemical tests were carried out for the identification of the Egyptian isolate No.4 obtained from El-Sharkia Governorate(`Table 2).
Molecular taxonomy of organism No 4:

In order to further identify the taxonomical position of the organism No 4 isolated from soils of EL-Sharkia governorate in Egypt, the crystalline protein formed by this culture were analyzed by SDS-PAGE technique. Thus the method of Weber and Osborne (1969) was used to determine the applied subunit molecular weight of proteins extracted in the presence of SDS. Standard bacterial cultures namely *Bacillus sphaericus* No(1), International strain *Bacillus sphaericus* 2362 No (2), and *Bacillus thuringiensis* var. *israelensis* No(3) were used as standard cultures in SDS-PAGE run. The standard cultures were obtained from Prof Dr Fergus Priest, Heriot- Watt University, Scotland. The obtained results showed clearly the presence of intimate similarities between SDS-PAGE pattern of the apparent (subunit) molecular weight of proteins detected in case of *Bacillus sphaericus* No(1)and the standard *Bacillus sphaericus* international strain 2362 rows r3, r4, r5, r11 (Table 3). The two *Bacillus sphaericus* are apparently belong to the same serotype 5A5B and having the same molecular weight of proteins subunits (Fig 5). On the other hand, SDS-PAGE chromatogram indicated the high degree of relatedness between proteins subunits of *Bacillus thuringiensis israelensis* and that of *Bacillus thuringiensis* organism No (4) isolated in the present studies. Further studies may be needed before arriving to decisive conclusion.

4. Comparative physiological studies on production of *Bacillus thuringiensis* (*Bt*) under conditions of solid substrate fermentation (SSF).

4.1 Comparison between different carrier materials.

Some selected natural materials of organic and inorganic nature were tested as supports and medium - containing agent when incorporated in the solid medium for growth, sporulation, and toxin production of the Egyptian isolate *Bacillus thuringiensis* No.4 in comparison with *B. thuringiensis* var. *israelensis*. The results in Tables (4&5) indicated that talcum powder and silica gel were the best carriers yielding highest mosquitocidal activity. Thus they were selected as the carriers of *Bacillus thuringiensis* No.4 and *Bacillus thuringiensis* var.*israelensis,* respectively.

4.2. Selection of suitable base solid substrate

A group of finely grinded agroindustrial by-products that are rich in proteins and locally available, as well as powdered leguminous seeds were used as solid substrates and as the main source of nutrients under SSF conditions. These included fodder yeast, feather meal, offal's meal and grinded seeds of soy beans, kidney beans, lentils and yellow splite pea. Each of these substrates was used at a concentration of 6% w/w. The results are summarized in Tables (6 &7) The highest mortality were obtained upon utilization of kidney beans and black eyed beans as base solid substrate for *B. thuringiensis* isolate No.4 and *B.thuringiensis* var. *israelensis*, respectively.

4.3. Effect of incubation period

The extent of growth and mortality were determined after 3,6,9,12 days of incubation at 30°C .The results are shown in Figure (6), toxicity

increased with extended the incubation period up to 9 days for both of tested organisms. No further increase was obtained upon extended incubation under the SSF conditions.

4.4. Effect of substrates (kidney beans and black eyed beans) concentration

Different concentrations ranging between 1.5 to 24 % w/w of kidney and black eyed beans used as a sole nutrient source with talcum powder and silica gel as an inert carriers as a growth media for *Bacillus thuringiensis* isolate No. 4 and *Bacillus thuringiensis* var.*israelensis*, respectively in the presence of moisture content 50% (w/w) under SSF conditions. The obtained results were shown in Figure (7).Progressive increase in the toxicity was obtained up to concentration 3% of kidney beans for growth of *B. thuringiensis* No.4 and concentration 6% of black eyed beans for growth of *Bacillus thuringiensis* var. *israelensis*.

4.5. Effect of initial moisture content of the medium

The percentage of moisture in the medium was varied between 20% to 80% (w/w) as final concentration. Results are in illustrated in Figure (8).The highest values for growth and toxicity were obtained in cultures with initial moisture content adjusted at 60% (w/w) for both organisms. At higher moisture contents the cultures exhibited fast and progressive decrease in growth parameters and toxicity(mortality%).

4.6. Effect of extent of aeration

The influence of aeration level was studied by using conical flasks with different sizes ranging between 50 ml to 1000 ml capacities The results are shown in Figure (9), the growth parameters and the level of mosquitocidal activity were fairly stable within a wide range of increasing air space available in the experimental flasks (up to a ratio of 10g culture/250ml conical flask and 10g culture/500ml for growth and toxicity of *Bacillus thuringiensis* var. *israelensis*, and *Bacillus thuringiensis* isolate No.4, respectively.

Table (1): LC_{50} and LC_{90} ($p \leq 0.05$) of *Bacillus thuringiensis* No.4 and *Bacillus thuringiensis* var. *israelensis* against 2 nd instar larvae of *Culex. pipiens.*

Isolate	LC_{50} by µl ($p \leq 0.05$)	LC_{90} by µl ($p \leq 0.05$)	Slope ± S.E.
Egyptian isolate No.4	142.4 (55.5 –220.7)	448.8 (290 .8– 1085. 2)	2.5 ± 0.7
Reference strain (*B. thuringiensis israelensis*)	220.4 (97.8-335.8)	825.9 (516.6-2697.7)	2.3± 0.6

Table(2): The biochemical tests for identification of the Egyptian isolate No.4 obtained from soil of El-Sharkia Governorate.

Biochemical tests	*B .thuringiensis* var. *israelensis*	Egyptian isolate *B. thuringiensis* No.4
Tolerance to NaCl 2%	+	+
5%	+	+
7%	-	-
10%	-	-
Degradation of adenine	+	+
Hydrolysis of urea	+	+
Hydrolysis of casein	+	+
Hydrolysis of Starch	+	+
Hydrolysis of gelatin	+	-
Utilization of citrate	-	-
Methyl red test	-	-
Vogesproskauer test	-	-
Catalase test	-	-
Nitrate reduction test	-	-

A B

Fig (1): Electron micrograph showing shapes of crystals of the Egyptian *B. thuringiensis* isolate No.4 isolated from El-Sharkia Governorate soil.
A. E.M. Showing two crystals after 3 days of incubation (X20.000).
B. E.M. Showing one crystal after 3 days of incubation (X20.000).

Fig (2): E.M. of the Egyptian isolate *B. thuringiensis* No.4 showing spore and crystals(X 5000).

Fig (3): E.M. showing two spores and crystal of *B. thuringiensis* var.*israelensis* (X10.000).

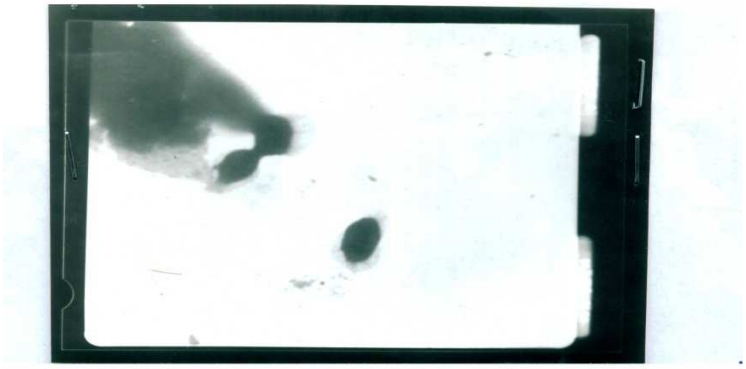


Fig (4): E.M. Showing crystal of *B. thuringiensis* var.*israelensis*(X5000).

Table (3): R_f values for different proteins subunits of SDS-PAGE of organism No (4) *Bacillus thuringiensis* in comparison with standard bacterial cultures.

Lanes:	2	1	M	3	4
Rows	(M.W.)	(M.W.)	(M.W)	(M.W)	(M.W)
r1			116		
r2			66.2	70.662	70.662
r3	63.203	63.203		63.203	63.203
r4	58.734	58.734			
r5	53.125	53.125		51.51	51.51
r6			45		
r7				40.492	40.493
r8			35		
r9				32.070	32.071
r10				23.916	23.916
r11	21.778	21.778			
r12			18.4		

Table (4): Effect of various carriers on growth parameters and mosquitocidal activity of *B. thuringiensis* No.4 under solid state fermentation conditions. The bioassay was carried out against second instar *Culex pipiens* larvae, using diluted culture (10^{-6}).

Carrier Used	Final pH at Harvest Time	(Mean ± S.D.) Plate Count		(Mean ± S.D.) Mortality % after
		CFU/g Product (× 10^{-6})	Spore/g Product (× 10^{-6})	48 hr
Orange Peels	4.5	3.50 ± 1.05	1.50 ± 1.52	10.00 ± 0.00
Beans Peels	6.4	1.33 ± 0.51	0.67 ± 0.8	8.33 ± 1.83
Wheat Bran	7.0	13.50 ± 2.17	8.17 ± 1.17	62.33 ± 6.89
Sugar-beet	7.3	1.50 ± 1.05	1.17 ± 1.17	14.17 ± 4.92
Corn Cobs	4.2	62.83 ± 2.04	51.67 ± 2.07	73.33 ± 4.08
Rice Husk	7.6	68.67 ± 5.75	64.50 ± 5.43	41.50 ± 5.96
Rice Hull	7.3	10.17 ± 0.60	10.00 ± 0.26	6.00 ± 8.94
Celite	4.8	70.67 ± 5.79	51.17 ± 3.49	80.29 ± 6.50
Talcum Powder	7.2	77.50 ± 5.01	63.50 ± 5.75	99.17 ± 10.68
Silica Gel	6.0	30.83 ± 3.37	19.67 ± 3.83	70.33 ± 3.27

Table(5): Effect of various carriers on growth parameters and mosquitocidal activity of standard *B.thuringiensis* var. *israelensis* .under solid state fermentation conditions. The bioassay was carried out against second instar larvae of *Culex pipiens* using diluted culture(10^{-6}).

Carrier Used	**Final PH at Harvest Time**	**(Mean ± S.D.) Plate Count**		**(Mean±S.D.) Mortality % after 48 hr**
		cfu/g Product (× 10^{-6})	**Spore/g Product (× 10^{-6})**	
Orange Peels	4.5	1.00 ± 1.26	1.17 ±1.60	6.67 ± 8.16
Beans Peels	6.3	1.67 ± 2.73	2.00 ±2.10	6.67 ± 7.53
Wheat Bran	6.1	4.67± 1.63	3.17 ±1.17	8.33 ± 7.53
Sugar-beet	4.8	3.67± 1.51	0.50 ±0.84	7.50 ± 7.58
Corn Cobs	7.3	2.50± 2.74	1.50 ±1.38	5.00 ± 5.48
Rice Husk	7.4	30.67±1.75	22.83±3.06	6.67 ± 8.16
Rice Hull	4.4	4.17 ± 1.47	3.33 ±1.37	25.83 ±5.64
Celite	8.5	70.50±4.64	21.00±4.05	78.00 ±4.47
Talcum Powder	7.0	7.50 ± 1.76	4.00 ± 2.10	67.83 ±9.91
Silica Gel	7.5	57.33±5.65	32.83±1.94	99.33 ±3.27

Table (6): Effect of nutrient substrate on growth and toxin production of *B. thuringiensis* No.4 under solid state fermentation conditions against 2nd instar larvae of *Culex pipiens*,using diluted culture 10^{-6}.

Nutrient.	Final PH at Harvest Time	(Mean ± S.D.) Plate count		(Mean ± S.D.) Mortality % after	
		Viable Count× 10^{-6}	Spore Count × 10^{-6}	24 hr	48 hr
Black eyed beens	7.6	33.17 ± 2.23	22.50 ± 3.89	63.67 ± 2.88	67.50 ± 5.24
Kidney beans	8.8	58.33 ± 1.97	47.00 ± 13.89	87.33 ± 4.08	99.17 ± 0.98
Soy beans	8.0	32.33 ± 1.63	25.67 ± 3.14	52.33 ± 2.16	61.83 ± 1.94
Lentils	8.2	62.67 ± 1.21	54.33 ± 3.39	89.83 ± 3.31	97.17 ± 4.02
Yellow splite pea	7.9	47.33 ± 1.51	31.67 ± 1.63	68.17 ± 4.79	78.00 ± 2.68
Sesame seed meal	7.8	41.50 ± 1.38	21.33 ± 1.21	83.67 ± 3.50	84.50 ± 3.02
Cotton seed meal	7.7	43.67 ± 3.08	35.00 ± 2.76	73.17 ± 1.94	74.00 ± 1.55
Offals meal	7.3	41.83 ± 1.60	34.33 ± 4.93	43.17 ± 2.48	52.83 ± 3.13
Father meal	7.8	81.00 ± 0.89	66.83 ± 15.80	71.67 ± 2.73	73.00 ± 2.45
Fodder yeast	7.9	51.83 ± 2.48	33.17 ± 2.64	55.83 ± 5.64	55.83 ± 5.85

Table (7): Effect of nutrient substrate on growth and toxin production of *B. thuringiensis* var.*israelensis* under solid state fermentation conditions against 2nd instar larvae of *Culex pipiens*, using diluted culture 10^{-6}.

Nutrient.	**Final PH at Harvest Time**	**(Mean ± S.D) Plate Count**		**(Mean ± S.D) Mortality % after**	
		Viable Count × 10^{-6}	**Spore Count× 10^{-6}**	**24 hr**	**48hr**
Black eyed been	8.0	27.83 ± 1.17	21.17 ± 0.75	88.33 ± 2.58	99.20 ± 0.84
Kidney bean	8.3	32.00 ± 1.10	26.67 ± 2.34	40.67 ± 5.50	51.83 ± 1.94
Soy bean	7.8	42.50 ± 1.38	31.50 ± 1.05	72.00 ± 2.10	79.17 ± 5.64
Lentils	7.5	20.83 ± 5.64	16.50 ± 3.62	17.00 ± 6.32	24.17 ± 3.43
Yellow splite pea	8.2	14.00 ± 2.83	11.50 ± 1.05	16.67 ± 5.16	24.33 ± 4.80
Sesame seed meal	8.0	21.33 ± 1.21	16.17 ± 2.04	30.67 ± 5.50	36.67 ± 6.06
Cotton seed meal	7.8	34.50 ± 1.76	21.67 ± 2.34	15.83 ± 3.76	27.83 ± 5.49
Offals meal	7.8	12.33 ± 2.58	10.33 ± 1.37	14.00 ± 3.22	20.00 ± 6.32
Father meal	8.2	20.67 ± 5.47	18.67 ± 2.16	29.17 ± 4.67	34.33 ± 3.50
Fodder yeast	8.0	26.83 ± 3.31	21.67 ± 1.03	77.83 ± 6.46	82.67 ± 6.86

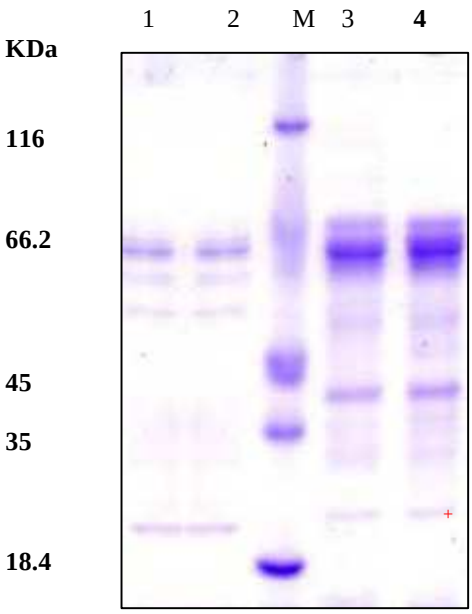
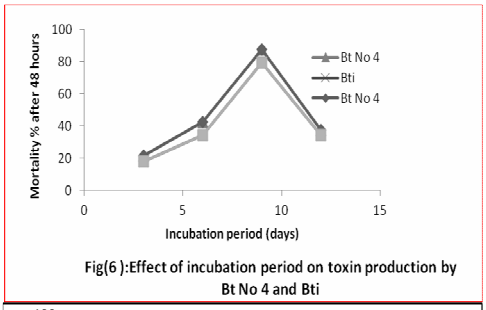


Fig (5): Mobility of protein subunits for standard bacterial cultures and *Bacillus thuringiensis* culture isolated from El-Sharkia Governorate of Egypt.



Fig(6):Effect of incubation period on toxin production by Bt No 4 and Bti

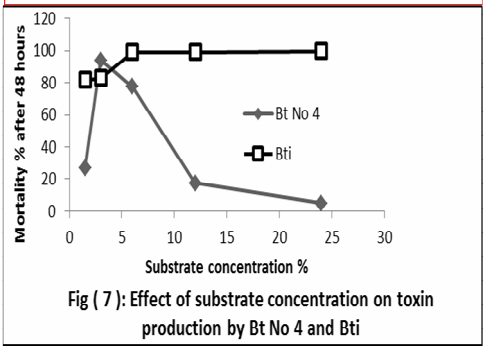


Fig (7): Effect of substrate concentration on toxin production by Bt No 4 and Bti

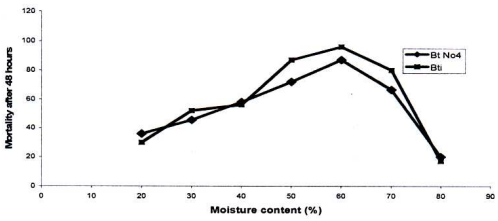


Fig (8): Effect of moisture content on toxin production by *Bt* No 4 and *Bti*

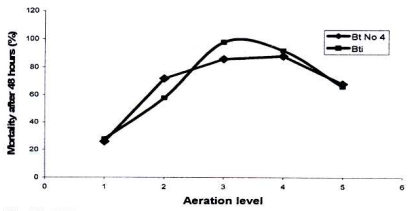


Fig (9): Effect of aeration level on toxin production by Bt No 4 and Bti

4. Discussion:

Sixty eight isolates were obtained from five locations of El-Sharkia Governorate using the enrichment approach reported by Morris *et al.* (1988), only one isolate No.4 was selected for its highest toxicity among the other isolates screened. It has different types of crystals and it was identified as a typical isolate of *B. thuringiensis.*

In the present work, special attention was given to search for new media that are suitable with low-priced and locally available in Egypt for *B. thuringiensis* production on a large scale. The goal stemmed from the fact that the feasibility of economic production of spores and crystals of *B. thuringiensis* is dependant to a large extent on production costs and availability of raw materials.

Some of Egyptian isolates of *B. thuringiensis* were grown on economic media containing 4% of fodder yeast on tap water, and incubated under shaking conditions for four days. (Zayed and Bream. 2004).The biological activities of these isolates against *Culex pipiens* were tested

to determine their effectiveness against field and laboratory strains of its 3rd larval instar. All isolates of *Bt* were more pathogenic to laboratory strains, causing up to 84% larval mortality. The insecticidal activities of these isolates were extended to the pupae stage causing a significant effect on pupae mortality in both strains tested. A pronounced effect on adult emergence was noticed with remarkable adult malformations. The reproductive performance of females was affected significantly by all isolates applied. (Zayed and Bream. 2004).

Growth, sporulation, synthesis of delta-endotoxins, and toxicity against the larvae of *Aedes aegypti* and *Culex pipiens* were studied during fermentation of *Bacillus thuringiensis* H14 in a 20-L fermentor (Sarrafzadeh *et al*. 2005).

Solid state fermentation technology was selected for toxin production to increase the production feasibility under the Egyptian conditions as well as the reduction of the production costs as compared to the submerged fermentation. Capalbo *et al*. (1994) reported the development of novel bioreactores for SSF purposes the production of bacterial insecticides. They devised two column bioreactores namely an aerated fixed bed and a fluidized bed fermentors for SSF of *Bt*. They intended to carry out a comparative study on those two types of bioreactores including parameters and variable to solve the questions addressed and encountered in SSF methodology including heat and mass transfer, aeration extent, sterility level as well as yield and productively of this approaches. Capalbo (1995) reviewed the aspects of the fermentation process and risk assessment of *Bt* production in developing countries. He concluded that several factor make the local production of *Bt* highly appropriate for pest control in developing nations. He reported that *Bt* can be cheaply produced on a wide variety of low cost, organic substrates. Foda *et al*. (2002) devised a novel approach for production of *B. thuringiensis subsp. aizawai* H-133 through SSF technology using ground soybean seeds as a substrate in the presence of talcum powder as an inert carrier material. They studied in detail factors affecting growth and sporulation of the organism under SSF conditions to evaluate the possible use of this biotechnology for bioinsecticides production at lower costs to combat major insect pests in Egypt.

Finally, it is of interest to note that the present work represent the first report that apply solid state fermentation as a promising biotechnology for the production of safe mosquitocidal toxins from the novel *B. thuringiensis* isolate No 4 and *B.thuringiensis* var. *israelensis.* This novel fermentation approach is expected to be highly feasible, cost- effective and inexpensive for production of biopesticides in developing countries.

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Evaluation of Human Telomerase Activity as a Novel Tumor Marker for Hepatocellular Carcinoma

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Abstract: Objectives: Liver cancer is the most common neoplasm and the most common cause of cancer death within the world. Early detection of hepatocellular carcinoma (HCC) will increase the potential for curative treatment and improves survival. Telomerase is reactivated in various types of malignant tumors and may contribute to the development of HCC. To explore its clinical implications for early diagnosis of HCC, we analyzed its activity in peripheral blood mononuclear cells (PBMC).The diagnostic accuracy of telomerase activity and other conventional tumor markers such as serum α-fetoprotein (AFP) and prothrombin induced by vitamin K antagonist (PIVKA-II) were evaluated to select the most reliable diagnostic and prognostic markers in HCC. This study was conducted on 25 healthy controls, 25 cirrhotic patients and 30 patients with HCC. All patients had been diagnosed with HCV-associated chronic liver disease. Methods: Serum PIVKA-II and AFP were measured by enzyme linked immunosorbent assay (ELISA),while telomerase activity in peripheral blood was estimated by polymerase chain reaction- enzyme- linked immunosorbent assay (PCR- ELISA method). Results: Mean telomerase activity, PIVKA-II and AFP levels were significantly higher in HCC patients as compared to both cirrhotic patients and controls, also a significant elevation in cirrhotic patients were found as compared to controls. Positive correlation was found between telomerase activity and size of hepatic focal lesions. Also, a positive correlation was found between both telomerase activity and PIVIKA-II and between the pathological grades of HCC. In HCC the sensitivity/specificity (88.2/79.6) of telomerase activity was much higher than both PIVIKA-II (80.5/69.3) and AFP (72.6/61.5). Conclusion: The usefulness of telomerase activity assay in HCC diagnosis and it's superiority to other tumor marker were recorded. Therefore, telomerase activity is a novel, available detector and prognostic marker for HCC diagnosis.

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Key words: hepatocellular carcinoma; telomerase; PIVKA-II; molecular diagnostic marker; telomerase PCR ELISA.

Introduction:

Hepatocellular carcinoma (HCC) is a major challenge in contemporary medicine. The incidence of HCC is increasing and it is becoming more and more significant both clinically and epidemiologically. Now HCC represents the fifth most common cancer in the world and the third most frequent cause of mortality amongst oncological patients[1]. It is responsible for more than 500 000 deaths with over 600 000 new cases yearly worldwide[2].More than 95% of HCC patients present underlying hepatopathy in particular of viral etiology. The majority of the cases (>85%) have liver cirrhosis, which masks symptoms of cancer progression. The clinical course of HCC is mostly asymptomatic. Suspected focal liver changes are often detected incidentally while monitoring the patient' s condition during ultrasound (US) examination, and often are too large and too advanced for the tumor to be subjected to potentially effective and radical therapy[3]. So far, it is necessary to concentrate on the earliest possible diagnosis, particularly sensitive detection of resectable focal liver changes preferably when tumors are less than 2 cm in diameter[4]. The first serological assay for detection and clinical follow up of patients with HCC was alpha fetoprotein (AFP) [5]. Numerous data have proved that significantly higher AFP serum levels accompany various liver diseases (viral hepatitis, liver cirrhosis, liver tumors: primarily HCC and hepatoblastoma, also metastasis in 5% -10% cases), which diminish its specificity as a golden standard serum marker for HCC[6]. Since the first description by Liebman et al. in 1984,prothrombin induced by vitamin K absence or antagonist -II (PIVKA –II), also referred to as des-gamma-carboxy prothrombin (DCP), has been found to be another useful tumor marker for HCC[7]. Recent study demonstrated that the diagnostic accuracy of PIVKA-II seemed to be higher compared to AFP[8]. It has been proved that significant concentrations of serum PIVKA-II in 50%--60% of all HCC patients, but in only 15%-30% of early HCC cases[9]. Clinical utility of PIVKA-II was further

investigated in view of correlation between several factors such as tumor size, intrahepatic metastases, and histological activity of tumor tissue.[10, 11] These two markers (AFP and PIVKA-II) which are supposed to be produced independently by HCC may serve complementarily in the diagnosis of HCC.[12,13,] Although the modalities such as ultrasonography and conventional tumor markers are important for detection of HCC, they are still not sensitive enough to detect HCC at the early stage.[14]

Hepatoma tissues synthesize and secrete valuable molecular markers such as AFP-beta1-mRNA, IGF-II-mRNA, telomerase, etc into blood. The analyses of these circulating hepatoma-specific biomarkers are useful to early diagnosis of HCC or monitoring metastasis or postoperative recurrence of HCC.[15, 16] Telomerase is a ribonucleoprotein enzyme composed of two key components: a catalytic component (human telomerase reverse transcriptase, hTERT) and a RNA template. It helps to stabilize telomere length in human stem cells, reproductive cells and cancer cells by adding TTAGGG repeats onto the telomeres using its intrinsic RNA as a template for reverse transcription, and it is a limiting component in telomerase activity.[17,18] During the early stages of carcinogenesis, cells undergo extensive proliferation until telomere length becomes critically shortened.[19,20] Telomere shortening is an early event in multistep hepatocarinogenesis, occurring in preneoplastic lesions of dysplastic nodules.[21] Shortened telomeres have been reported to induce chromosomal instability in hepatocytes, especially important in viral-related hepatocarcinogenesis.[22,23] It has been suggested that detection of cancer-related gene expressions in serum is very useful for diagnosis and follow-up of cancer patients. hTERT mRNA in serum was detected in breast cancer but not in benign diseases, suggesting that hTERT is available for cancer diagnosis.[24] Overexpression of telomerase is associated with HCC development, and its abnormality in liver tissues or in peripheral blood could be a useful marker for diagnosis and prognosis of HCC .[25] In the present study, we mainly focused on the comparison of telomerase activity in peripheral blood with serum AFP, and PIVKA-II to select the most reliable early diagnostic and prognostic markers in HCC. A correlation between AFP, PIVKA-II and telomerase activity and tumor size and histopathological grades was performed.

Patients and Methods:

This study was conducted on 55 patients; 30 patients with biopsy proven hepatocellular carcinoma and 25 patients with biopsy proven liver cirrhosis. They were selected from attendants of the out and inpatients clinic of Theodor Bilharz Research Institute. Twenty five healthy age and sex matched controls were enrolled in this study.

All casses were subjected to the following:

I. Full history taking with special stress on jaundice, blood transfusion and bilharziasis.

II. General and abdominal clinical examination.

III. Ultrasonographic examination of the liver was done to assess its size and echo pattern in order to expose any pathology such as liver cirrhosis and to detect focal masses. The site and size of any detected mass were recorded. The portal tract as well as the portal and splenic veins were also measured.

IV. Percutaneous liver needle biopsy was done for all patients. Patients not fit for biopsy were excluded from the study. The specimens were processed and embedded into paraffin blocks, cut at 5 um thick sections and stained with Hx and Eosin.

V. Laboratory tests:

A) Routine laboratory tests including:

1- Complete blood picture.

2-Liver function tests (serum albumin, bilirubin, AST, ALT, Alkaline phosphatase and prothrombin time) were measured by conventional methods.

3- Serological markers for hepatitis B (HbsAg and HbcAb) by ELISA of Boehringer Mannheim and hepatitis C (HCV Ab) by Murex version111.

B) Specific laboratory tests:

1. Determination of AFP level: by ELISA method (Chieregatti, 1990).[26]

2. Measurement of PIVKA-II level (Amiral etal, 1991)[27]: by Asserachro PIVKA-II ELISA Kit (Diagnostica Stago).

3. Assessment of telomerase activity: The protein extracts (2µl) from peripheral blood mononuclear cells (PBMCs), were analyzed by using Telomerase PCR ELISA (Telomerase PCR ELISA, Boehringer Mannheim).[28,29] In brief, 50 µl containing 25 µl reaction buffer, 2 µl of protein extract and 2 µl of primers telomeric repeats .The PCR condition was as follows: incubation for 30 min at 25°C for primer extension. The mixture was then incubated at 94°C for 5 min to induce telomerase inactivation. The reaction mixture was then subjected to 30 PCR cycles at 94°C

for 40 sec, 50 °C for 40 sec, and 72°C for 90 sec (72°C, 10 min for the final step). An aliquot of the PCR product was denatured, hybridized to a digoxigenin (DIG)-labelled telomeric repeat-specific probe and bound to a streptavidin-coated 96 well plate. Finally, the immobilized PCR product was detected with an anti-DIG-peroxidase antibody and visualized by tetramethyl benzidine substrate (colour reagent). The absorbance (A) of telomerase was measured at a wavelength of 450 nm (reference wavelength 620 nm) within 30 minutes of addition of the stop reagent. The cell extract was heated to 65˚C for 10 minutes as a negative control.

Sampling procedure:

Under aseptic conditions, fasting venous blood sample (12 ml) was withdrawn by clean venipuncture from the antecubital vein: 2 ml on EDTA for blood picture; 3 ml to separate serum for routine laboratory tests on the collection day and aliquot of serum was stored at -20 °C for AFP level measurement; 2 ml on citrate to separate citrated plasma for PIVKA-II level measurement; 5 ml on heparin (for anticoagulant) and 2.5 ml of ficoll to be centrifuged at 2000 /min for 20 minutes at 4 °C, peripheral blood mononuclear cells (PBMCs) were collected from the ficoll/plasma interface. The cells were then washed three times in normal saline and pelleted by using low-speed centrifugation for 10 minutes. The samples were immediately aliquoted and stored at -80 °C for determination of telomerase activity.

Statistical Analysis:

Data were processed using SPSS version 17 software program. Means and standard deviations were computed for clinical data. T-test analysis was done between means of two groups. Analysis of variance was done between measured of variables more than two groups and receiver-operator characteristic (ROC) curve used. *P* value equal to or less than 0.05 was considered the threshold for significant.

Results:

Results were summarized, statistically analyzed and tabulated in tables (1-5), and graphically presented in figures (1-5). Thirty hepatocellular carcinoma patients, 25 cirrhotic patients and 25 control cases were included in this study. The clinical data of all patients were illustrated in table 1. Mean telomerase activity, PIVKA-II and AFP levels were significantly higher in HCC patients as compared to both cirrhotic patients and controls with a significant elevation in cirrhotic patients as compared to controls (table 2, fig.1). The mean telomerase activity and PIVKA-II values were found to be significantly higher in poorly differentiated grade III HCC compared to moderately differentiated grade II HCC and well differentiated grade I HCC ((table 4, fig. 5 & 3). As regards the size of the focal lesions, it was found that telomerase activity was significantly higher in patients with lesions 5-10 cm than in those with lesions below 5 cm. By using correlative studies, statistical significant correlation could be found between the mean telomerase activity in relation to the size of the hepatic focal lesions (table 5). No correlation was found between telomerase activity, PIVKA-II and AFP in the studied groups. In HCC, the sensitivity/specificity (88.2/79.6) of telomerase activity was much higher than both PIVIKA-II (80.5/69.3) and AFP (72.6/61.5). Combination of AFP and PIVKA-II yielded increasing sensitivity/specificity 82.6%/77.6% and accuracy 87.8%, while both AFP and telomerase increasing sensitivity (90.4%), specificity (82.3%) and accuracy (89.9%) (Fig. 4 &Table 3).

Discussion:

Hepatocellular carcinoma (HCC) is the most common form of primary hepatic malignancy. Although recent advances in imaging diagnosis such as real time ultrasonography, computed tomography and magnetic resonance imaging have changed the diagnostic strategy for early diagnosis of HCC, determination of tumor markers for HCC at regular intervals is still a common practice. Nowadays it is believed that early HCC diagnosis is presently considered feasible in 30%-60% of the cases in the developed countries. Tumors smaller than 2 cm in diameter represented < 5% of cases in 1990s, whereas now they represent up to 30% of cases in Japan.[4] Significantly more effective surveillance strategies lead to earlier HCC detection and earlier qualification for effectively curative radical surgery, with very good postoperative survival rates.[9]

The current study enrolled 30 patients with biopsy proven hepatocellular carcinoma and 25 patients with biopsy proven liver cirrhosis and 25 healthy age and sex matched controls. We mainly focused on the comparison of telomerase activity in peripheral blood with serum AFP, and PIVKA-II to select the most reliable early diagnostic and prognostic markers in HCC. A correlation between AFP, PIVKA-II and telomerase activity and tumor size and histopathological grades was performed.

It has been confirmed on numerous occasions that AFP serum concentration increases in parallel with HCC tumor size. For this reason AFP has to be considered 'the golden standard' for HCC serum

markers. However, the usefulness of AFP testing for the population at risk should be seriously questioned. AFP diagnostic values for this assay are undoubtedly poor. AFP specificity varies from about 76% to 96% and increases with elevated cut-off value. Simultaneous sensitivity decrease much more from about 25% for potentially respectable of less than 3 cm in diameter to about 50% for lesions of >3 cm in diameter.[30, 31] 2o%-30% AFP sensitivity coincides with cut- off >100 µg/L, which means that 70%-80% of liver tumors, normally respectable are non-detectable and unfortunately do not undergo treatment or are subjected to it too late.[32] Therefore new biomarkers with better sensitivity and specificity than AFP to complement the imaging diagnosis are needed.[33]

Since the first description by Liebman et al. in 1984,prothrombin induced by vitamin K absence or antagonist -II (PIVKA –II), also referred to as des-gamma-carboxy prothrombin (DCP), has been found to be another useful tumor marker for HCC. It was demonstrated that there is excessive synthesis of prothrombin precursors by human HCC tissue and that it might be a contributing factor to the production of DCP by HCC.[7] Several case control studies have shown sensitivities of PIVKA-II of 28% to 89% and specificities of 87% to 96% in the diagnosis of HCC.[34-36, 37, 38] In some studies, PIVKA-II was more sensitive than AFP,[36,37,38] whereas, in other studies, AFP was more sensitive.[34,35] A recent study of 1377 HCC patients and 355 non- HCC controls with chronic hepatitis or cirrhosis showed that the accuracy of PIVKA-II was inferior to AFP, particularly for small tumors.[39] Various factors may influence the performance of HCC biomarkers, including ethnicity, cutoff value, patient demographics, cause of underlying liver disease, presence of cirrhosis, tumor stage and tumor biology and so on.[40,41]

In the present study, both AFP and PIVKA-II showed statistically significant higher levels in HCC patients compared to cirrhotic and control groups. . In HCC, the sensitivity/specificity of PIVKA-II (80.5/69.3) and accuracy (70.3%) were higher than AFP (72.6/61.5, 69.8%).Combination of both AFP and PIVKA-II yielded increasing sensitivity/specificity 82.6%/77.6% and accuracy 87.8%. In agreement with the current study, has been postulated that PIVKA-II is a useful marker for detecting HCC, especially in small HCC and may have correlations with known staging systems, especially in combination with AFP.[42, 43] In contrary to our findings, recent study reported that AFP was more sensitive than PIVKA-II and AFP-L3% for the diagnosis of early stage HCC at a new cutoff of 10.9 ng/ml [44] while others concluded that PIVKA-II was not superior to AFP in the early detection of HCC and that neither AFP alone, PIVKA alone, nor the combination of both was sufficiently accurate to be used for early HCC diagnosis and monitoring recurrence after curative resection.[45]

Hepatoma tissues synthesize and secrete valuable molecular markers such as AFP-beta1-mRNA, IGF-II-mRNA, Telomerase, etc into blood. The analyses of these circulating hepatoma-specific biomarkers are useful to early diagnosis and monitoring metastasis or postoperative recurrence of HCC.[15, 16] Telomerase is a ribonucleoprotein enzyme composed of two key components: a catalytic component (human telomerase reverse transcriptase, hTERT) and an RNA template.[25] In this assay, telomerase activity showed statistically significant higher levels in HCC patients compared to cirrhotic and control groups. . In HCC, the sensitivity/specificity (88.2/79.6) was much higher than both PIVIKA-II (80.5/69.3) and AFP (72.6/61.5). Combination of both AFP and telomerase is increasing sensitivity (90.4%), specificity (82.3%) and accuracy (89.9%). The mean telomerase activity was found to be significantly higher in poorly differentiated HCC compared to moderately differentiated HCC and well differentiated HCC. As regards the size of the focal lesions, it was found that telomerase activity was significantly higher in patients with lesions 5-10 cm in diameter than in those with lesions below 5 cm. By using correlative studies, statistical significant correlation could be found between the mean telomerase activity in relation to the size of the hepatic focal lesions .No correlation was found between telomerase activity, PIVKA-II and AFP in the studied groups. To some extent, these results are consistent with a previous studies which concluded that although the measurement of telomerase activity in peripheral blood is a useful and potential approach to establish a practical diagnostic/predictive marker of HCC, it would be imprudent to state that telomerase activity is a specific molecular marker for circulating malignant hepatoma cells.[46, 47] Other recent studies reported that in HCC, serum hTERT mRNA showed higher sensitivity/specificity values than AFP, PIVKA-II and AFP mRNA .There was a positive correlation of hTERT mRNA between tumor tissue and serum and this proved that serum hTERT mRNA is derived from tumor hepatoma cells.[48] In a study done by Bong et al., on 49 HCC patients showed long telomere, high telomerase activity and positive correlation with hTERT mRNA and more advanced tumor stage.[49]

In conclusion, in HCC patient's telomerase activity, is more specific marker and is related more closely to the degree of histologic differentiation and tumor size of HCC than AFP and PIVKA-II. Therefore, telomerase activity might be a useful tumor

marker in early diagnosis of HCC and the monitoring of recurrence after curative resection. We recommend testing of AFP with telomerase, as in combination they showed higher overall accuracy.

Table (1): Clinical , sonographic, hispathological data of cirrhosis and HCC groups.

	Cirrhosis	HCC
Number	25	30
Age (years)	47.7±8.95	52.3±7.63
Mean±SD	20/5	26/4
Gender, M/F		
Viral serological markers		
HBsAg positive	2	1
HBcAb positive	5	4
HCVAb positive	14	19
HBsAg, HCVAb positive	6	7
Sonographic findings		
Liver pattern		
Size		
normal	6	7
enlarged	0	21
shrunken	14	2
Focal lesion		
Number:		
single		19
multiple		11
Size < 2 cm		6
2-5 cm		11
5-10 cm		13
Ascites	3	7
Histopathological Differentiation		
		10
Grade I		12
Grade II		8
GradeIII		

Table (2): Tumor markers: AFP, PIVKA-II and Telomerase in control, cirrhosis and HCC groups

	AFP (ng/ml)	PIVKA-II (ng/ml)	Telomerase Optical density (units)
	mean±SD	mean±SD	mean±SD
Control (n=25)	3.05±1.2	1.26±0.5	0.39±0.14
Cirrhosis (n=25)	13.5±5.9	1.44±1.o7	0.43±0.26
HCC (n=30)	98.8±95.42[ab]	2.29±1.0 3[ab]	2.08±0.98[ab]

[a] Significant difference from control P < 0.0 1

[b] significant difference from Cirrhosis P < 0.01

Table (3): Accuracy of AFP, PIVKA, Telomerase and combined markers in HCC group

	AFP	PIVKA-II	Telomerase	AFP+ PVIKA-II	AFP+ Telomerase
Cut off value for HCC	>10ng/ml	>1.3 ng/ml	>0.7 unit	Combined	Combined
Sensitivity	72.6%	80.5%	88.2%	82.6%	90.4%
Specificity	61.5%	69.3%	79.6%	77.6%	82.3%
PPV	67.4%	77.4%	85.7%	73.7%	85.3%
NPV	62.3%	66.7%	58.7%	55.9%	70.1%
False negative	23.6%	41.7%	49.2%	14.9%	11.6%
Over all accuracy	69.8%	70.3%	73.5%	87.8%	90.1%

PPV = Positive predictive value NPV=Negative predictive value

Table (4): Tumor markers in patients with different grades of HCC

Variables	Grade I N=10	Grade II N=12	Grade III N=8
AFP mean±SD (ng/ml)	83.55±8.69	95.25±10.50	116.80±25.6
PIVKA-II mean±SD (ng/ml)	1.68±0.09	2.19±0.06[a]	2.68±0.08[ab]
Tolomerase mean±SD unit of activity	1.08±0.059	1.65±0.086[a]	2.38±0.095[ab]

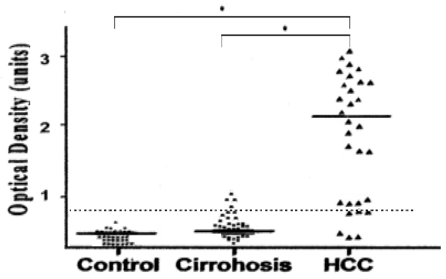


Fig. 1: AFP, PIVKA-II and Teolmerase in control, cirrhosis and HCC groups

[a] $p < 0.01$ grade I versus grade II &III

[b] $p < 0.01$ grade II versus grade III

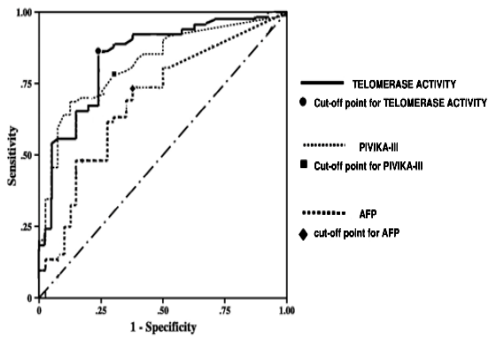


Fig.(2): Comparison of telomerase activity in peripheral blood from HCC, cirrhosis and normal controls. Arbitrary cut-off level = 0.7 units

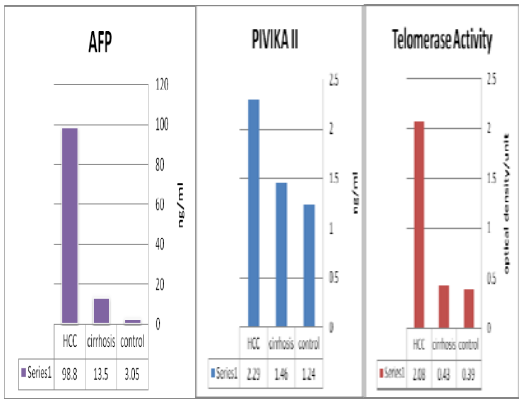


Fig.(3): Relationship between the histopathological staging of hepatocellular carcinoma (HCC) and peripheral telomerase activity.

[a] $P < 0.01$ grade I versus grade II &III

(5): Tumor markers in patients with HCC in relation to size of tumor.

Variables	< 2 cm N= 6	2-5 cm N=11	5-10 cm N=13
AFP mean±SD ng/ml	89.73±106.28	96.37±79.46	110±88.50
PIVKA-II mean±SD ng/ml	2.o7±0.74	2.45±0.89	2.16±0.62
Telomerase mean±SD unit of activity	1.69±0.32	2.07±0.56 [a]	2..52±0.54 [ab]

[a] Significant difference from tumor size (<2cm) P<0.01

[b] Significant difference from tumor size (2-5) P<0.01

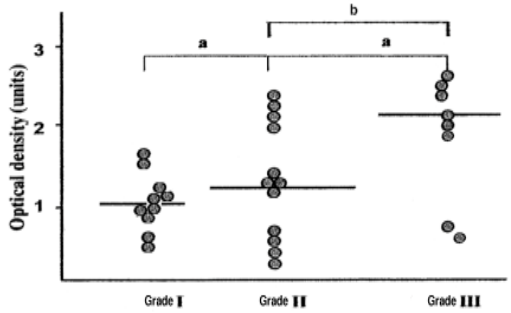
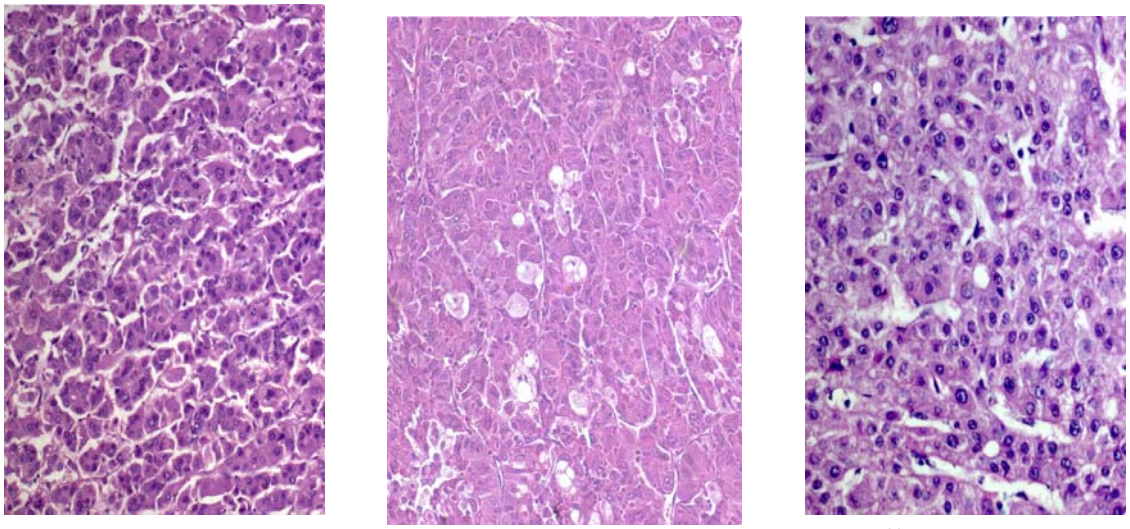


Fig.(4): Receiver-operator characteristic curve analysis curves were representing, AFP, PIVIKA–III and telomerase respectively obtained by importing quantified raw data and the sensitivity/specificity values were calculated. Each line has a cutoff point for a marker.



Poor differentiated HCC Moderate differentiated HCC Well differentiated HCC

Fig. 5: Histopathological stages of HCC, well differentiated, moderate differentiated and poor differentiated (hemotoxlin and eosin, original magnification x400).

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Role of Selenium in Attenuating Cardiac and Hepatic Damages Induced By the Antitumor Agent, Doxorubicin

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Abstract: Background and Objectives: The clinical use of doxorubicin, one of the most effective antitumor agents, soon proved to be hampered by such serious problems as the development of cardiomyopathy and liver damage. The current study aims at evaluating the role of trace element, selenium, in attenuating cardiac and hepatic damages induced by the antitumor agent, doxorubicin. **Materials and Methods:** Animals were divided into normal control group and doxorubicin -treated group injecting doxorubicin i.p. as 6 equal doses of 2.5 mg/kg, twice weekly/ 3 weeks. The doxorubicin - treated animals were divided into 2 groups, one kept without further treatment (doxorubicin -group), second group, (doxorubicin + selenium) received selenium (Na Selenite) 0.5 mg/kg orally, 3 times/week/4 weeks including one week before the doxorubicin 1st dose. Serum creatine phosphokinase, lactate dehydrogenase, as cardiac damage markers, and alanine aminotransferase, as indicator of hepatic damage, were measured. Malondialdehyde and nitric oxide levels, as cardiac oxidative status indices, cardiac glutathione content, glutathione peroxidase, glutathione-S–transferase and superoxide dismutase activities, as measures for cardiac antioxidant capacity, were also investigated. Histopathological changes in cardiac and liver tissues were examined. The results were analyzed statistically by one-way analysis of variance with subsequent multiple comparisons using Tukey test. **Results:** doxorubicin induced significant increase in serum lactate dehydrogenase; creatine phosphokinase; alanine aminotransferase activities, cardiac nitric oxide, malondialdehyde levels, superoxide dismutase, glutathione peroxidase, glutathione-S–transferase activities, and reduction in glutathione content. Selenium co-administration caused significant decrease in serum lactate dehydrogenase and creatine phosphokinase levels; normalization of serum alanine aminotransferase; significant decrease in cardiac malondialdehyde, nitric oxide levels, glutathione peroxidase, glutathione-S–transferase, superoxide dismutase activities and significant elevation in cardiac glutathione content, compared to doxorubicin -treated group values. Histopathological examination of cardiac and liver tissues supported the previous biochemical results. **Conclusions:** Chronic doxorubicin administration caused cardiomyopathy and hepatic damage. Selenium co-administration produced partial, but significant, protection against cardiomyocyte damage; however, it alleviated hepatic damage-induced by the antitumor agent, doxorubicin.
[Safinaz S. Ibrahim, Maged A. Barakat, Hebatalla M. Helmy. Role of Selenium in Attenuating Cardiac and Hepatic Damages Induced By the Antitumor Agent, Doxorubicin. Life Science Journal 2010;7(4):162-172]. (ISSN: 1097-8135).

Key words: Doxorubicin, cardiomyopathy, liver damage, selenium

Introduction:

Anthracyclines rank among the most effective anticancer drugs ever developed [1]. The first anthracycline was isolated early in the 1960s from the pigment producing Streptomyces Peucetius, a species of actinobacteria [2] and was named doxorubicin (Dox). Doxorubicin is an essential component of treatment of breast cancer [3], soft tissue sarcomas [4] and many other cancers [5]. Because Dox has been shown to produce free radicals, it was suggested earlier that free radical injury might be a mechanism of Dox antitumor activity [6]. There now appears to be general agreement that oxidative stress is unlikely to be a significant contributor to the antitumor activity of Dox [7]. Liver is the main site of Dox metabolism, reduction of side chain carbonyl group by NADPH-Cyto P450 yields a more polar and toxic metabolite, doxorubicinol. Such metabolite accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity of Dox [8]. The enormous value of Dox in treating a variety of solid and hematologic malignant conditions is unquestioned. However, as with any other anticancer agent, the clinical use of Dox soon proved to be hampered by such serious problems as the development of resistance in tumor cells [9] or toxicity in healthy tissues, most notably in the form of chronic cardiomyopathy and congestive heart failure[1]. These adverse effects of the drug can preclude its use in some patients and limit the duration of its use in many others [10].

Selenium (Se) plays an important biological role in living organisms, mostly through its

incorporation in a family of proteins, selenoproteins. The main biological form of Se is selenocysteine, a cysteine analog that is synthesized from a serine bound to tRNA. The biological roles ascribed to Se include the prevention of cardiovascular disease [11] and cancer [12]. In the heart, Se supplementation caused increase in the cardiomyocyte glutathione peroxidase (GPx) activity, the total antioxidant activity, glutathione (GSH) concentration and catalase activity, leading to decreased generation of reactive oxygen species (ROS)[13]. The present study aims at evaluating the attenuating effect of Se, as an adjuvant therapy, on Dox- induced cardiac and hepatic damages.

Materials and Methods:

A- Animals:

Total numbers of 32 male albino rats of the Wister strain, weighing 170-200 g, were used in the present study. The animals were obtained from the central animal facility at the Faculty of Pharmacy, Cairo University, Cairo, Egypt. All rats were housed in a room with a controlled environment, at a constant temperature of 23 ± 1^0C, humidity of 60% ± 10%, and a 12 hrs light/dark cycle. The animals were housed in groups and kept at constant nutritional conditions throughout the experimental period. The experimental protocols were approved by the Ethical Committee of Cairo University.

B- Drugs and chemicals:

Doxorubicin HCL was obtained from Pharmacia & Upjohn, Milan, Italy. Sodium selenite was obtained from Sigma Chemical Company, USA. Other chemicals in the experiments were of analytical pure grade and supplied by British Drug House (BDH, UK), Merk (Germany) and Sigma Chemical Company (USA).

C- Experimental design:

Animals were divided into a normal control group (10 rats), receiving the appropriate volume of saline i.p, and Dox-treated group. Doxorubicin was dissolved in saline and injected i.p., as a total cumulative dose equal to 15 mg/kg, divided into 6 equal doses, 2.5 mg/kg each. They were injected twice weekly/ 3 weeks [14]. Dox-treated animals were divided into tow groups, one kept without further treatment (Dox-group), and a second group (Dox + Se) received Se, as sodium selenite, 0.5 mg/kg, orally, 3 times/week/4 weeks including one week before the 1^{st} Dox dose[15].

D- Serum and Tissue sampling:

24 hours following the last Dox injection, rates were sacrificed by decapitation. Blood sample of each animal was collected into a dry centrifuge tube. Serum was separated by centrifugation at 3000 r.p.m. /15 minutes and used to determine creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and alanine aminotransferase (ALT). Serum CPK activity was determined using a kit provided by STANBIO, USA. CPK catalyses the transphosphorylation of ADP to ATP through a series of coupled enzymatic reactions. NADH is provided at a rate directly proportional to CPK activity. The method determines NADH absorbance increase per minute at 340 nm [16]. Serum LDH activity was determined using a kit provided, also, by STANBIO, USA. LDH specifically catalyzes the oxidation of lactate into pyruvate with subsequent reduction of NAD to NADH. Rate of NADH formation is proportional to LDH activity. The method described determines NADH absorbance increase per minute at 340 nm [17]. Serum ALT activity was determined, using a kit provided by Quimica Clinica Aplicada, Spain[18].

Histopathological study:

The hearts and livers were removed by dissection, washed by ice-cold isotonic saline and blotted between two filter papers. Autopsy samples were taken from heart and liver in different groups of rats and fixed in 10% formol saline for 24 hrs. Washing was done, then, serial dilutions of alcohol were used for dehydration. Paraffin bees wax tissue blocks were prepared for sectioning the studied tissues. The obtained sections were stained by hematoxylin and eosin stains [19] for histopathological examinations through the light microscope.

Biochemical parameters:

10% w/v homogenate was prepared in ice-cold deionized water for the remainder of the heart tissues of the different groups.

Measurement of cardiac oxidative status indices:

A portion of the homogenate was mixed with ice-cold 2.3% KCL (in ratio of 1:1) and centrifuged at 3000 r.p.m./15 minutes. Thiobarbituric acid (TBA) – reactive substance, malodialdehyde (MDA), content was determined in the supernatant [20], depending on measuring the coloured complex formed between TBA and MDA in acidic medium. Another aliquot of homogenate is centrifuged at 17.000 r.p.m./4ºC/20 minutes. The resulted supernatant was used for the determination of nitric oxide (NO), as nitrite ($N0_2^-$) and nitrate ($N0_3^-$) concentrations [21], using Griess reagent after the enzymatic reduction of nitrate to nitrite. The Griess reaction involves the reaction of nitrite with sulfanilamide in an acidic solution to yield a diazonium salt, followed by coupling with N-(1-

naphthyl) ethylenediamine to yield a colored azo dye that can be measured colourimetrically at 540 nm.

Measurement of some cardiac antioxidant systems:

A portion of homogenate was mixed with ice-cold 7.5% sulfosalicylic acid (in a ratio 1:1) and centrifuged at 3000 r.p.m/ 15 minutes. The resulted supernatant was used for determination of GSH [22]. Another part of homogenate was mixed with equal volume of ice-cold Tris- EDTA buffer (pH =7.6), centrifuged at 39.000 r.p.m./ 4º C/ 20 minutes. The supernatant was used for determination of superoxide dismutase SOD; glutathione peroxidise (GPx) and glutathione S-transferase (GST). Determination of GST activity [23, 24] depends on the ability of GST to catalyze the formation of glutathione adduct with 1-chloro,2,4 dinitrobenzene(CDNB). This adduct was measured by noting the net increase in absorbance at 340nm. Determination of GPx [25] depends on measuring the rate of oxidized GSH formation, by following up the decrease in absorbance of the reaction at 340 nm as NADPH was converted to NADP. Superoxide dismutase activity was determined [26], depending on the fact that the spontaneous autoxidation of pyrogallol, at alkaline pH less than 9.5, produces superoxide anion, which in turn enhances further oxidation of pyrogallol with a resultant increase in absorbance at 420 nm. The presence of SOD in the reaction medium retards pyrogallol autoxidation by scavenging the formed superoxide anion.

Statistical analysis:

The results were analyzed statistically by one-way analysis of variance (ANOVA test) with subsequent multiple comparisons using Tukey test. Differences were considered statistically significant at p less than 0.05. The results were presented as the mean ± standard error of the mean (SEM). Data obtained were submitted to a computerized statistical treatment using SPSS statistical package, version 17. Graphs were represented by Harvard graphics version 4 computer program.

Results:

Results revealed that Dox caused significant increase in serum levels of LDH and CPK, amounting to 182.4% and 183.6% respectively, as compared to the normal values (Fig. 1). Selenium co-administration caused significant decrease in the activities of LDH and CPK reaching to 139.5% and 153.6%, respectively of the control values. Figure (2) illustrated that, Dox caused a significant increase in cardiac MDA and NO contents, amounting to 183.36% and 177.7%, respectively, compared to the control values. Concomitant administration with Se caused significant decrease in MDA and NO levels reaching to 126% and 120%, compared to the control values.

As shown in figure (3), Dox administration caused a significant decrease in cardiac GSH level reaching to 64% of the normal values. Co-administration of Se significantly elevated GSH content to about 77.9% of the control values. Figure (4) showed significant increases in cardiac activities of GPx and GST in the Dox-treated rats, amounting to 410% and 184% respectively, compared to the normal values. Meanwhile, the co-administration of Se caused significant decrease in the levels of GPx and GST to about 166% and 136% of the normal values. Figure (5) showed significant increases in cardiac activity of SOD in the Dox-treated rats amounting to 225% compared to the normal value. Co-administration of Se caused significant decrease in the level of SOD to about 172% of the normal values.

Results of figure (6) revealed that Dox administration caused significant elevation in the serum ALT level to reach 118% of the normal control level. Selenium co-administration caused normalization of the elevated ALT level.

Cardiac histopathological results showed that; in the control sections, the cardiac muscle fibers were grouped in bundles with connective tissue in between. The single muscle fiber had acidophilic cytoplasm and a central nucleus (Figure 7). In the cardiac sections obtained from rats administrated Dox, hyalinization was observed in the myocardial bundles associated with either inflammatory cells infiltration only or inflammatory cells and edema in focal manner in between the bundles. Edema was also noticed in the subendocardial layer. The subendocardial adipose tissue was infiltrated by inflammatory cells (Figures 8, 9, 10). In the cardiac sections obtained from rats administrated Dox + Se, there was mild hyalinization in the myocardial muscle bundles (Figure 11).

Examination of liver sections of the different groups illustrated that: Liver tissue of the normal group showed hepatic lobules with normal architecture (Figure 12). In case of liver sections of rats administrated Dox, congestion was observed in the central vein, in addition to kupffer cells proliferation in diffuse manner between the fatty degenerated hepatocytes (Figures 13). In case of liver sections of rats administrated Dox + Se, least liver damage was shown, just kupffer cells proliferation was observed in between hepatocytes (Figure 14).

Discussion:

Doxorubicin-induced cardiomyopathy has long been a serious side effect in treating human cancers, which limits the clinical dosage of Dox [27]**.** The

mechanism of Dox-induced cardiotoxicity is attributed to the formation of ROS and subsequent changes of membrane fluidity and integrity. Oxidative stress is generally held as the mediating mechanism in the multiple biological processes leading to Dox cardiotoxicity [28]**.** Nutritional strategies designed to augment cellular defense systems have been identified as a promising approach to combat oxidative stress-associated disease conditions. In this respect, dietary supplementation with Se, potentially adjusting antioxidant enzymatic status, could offer protection in preventing free radical-induced cardiac injury. In the present study, role of trace element, selenium, in attenuating cardiac and hepatic damages induced by antitumor agent, doxorubicin was studied.

Results of the present study revealed that 15 mg/kg total cumulative dose of Dox induced cardiac and hepatic damages, manifested biochemically by significant increase in serum activities of LDH; CPK and ALT. Additionally, Dox caused elevation in cardiac NO, MDA levels, SOD, GPx, GST activities, and reduction in GSH content. Histopathological examination of heart and liver sections of Dox-treated animals supported these biochemical results. Selenium administration, concomitant to Dox therapy, caused significant decrease in the serum activities of LDH and CPK; cardiac MDA, NO levels, GPx, GST and SOD activities and significant elevation in cardiac GSH content, compared to Dox-treated group values, as well as normalization of serum ALT level.

The present results showed significant increase in serum levels of LDH and CPK in Dox-treated group. These enzymes are considered important markers of cardiac injury. Many previous studies have demonstrated similar results in rats following Dox administration [29, 30]**.** Different types of Dox cardiotoxicity can be recognized [31]**:** "Acute" cardiotoxicity occurs during Dox administration**, h**owever, these effects are never of major concern because these are generally reversible and/or clinically manageable. "Early chronic" cardiotoxicity develops later in the Dox treatment course and characterized by dilated cardiomyopathy, with subsequent development of congestive heart failure [32]**.** It is now well established that Dox cardiotoxicity may manifest even decades after the completion of anticancer treatment [33]**.** Co-administration of Se with Dox therapy resulted in decrease in the elevated activities of serum LDH and CPK. This finding is in harmony with this stated by Simoni *et al*.[34], who reported that the elevation in serum LDH activity, as a result of hemoglobin cardiotoxicity, is significantly decreased by Se dietary supplementation. Our biochemical results are supported by the histopathological examination of the cardiac tissue, since, the marked morphological changes shown in the hearts of Dox-treated animals have been partially preserved by Se administration.

Dox therapy caused significant increase in MDA level. Previous studies reported similar results [35, 36]**.** This elevation might be attributed to Dox mediated oxidative stress. Heart tissue is rich in mitochondria, which occupy about forty percent of the total intracellular volume of cardiomyocytes [37]. Dox has high affinity for cardiolipin, a negatively charged phospholipid abundant in the mitochondrial inner membrane, leading to mitochondrial accumulation of Dox [38]. Under clinically relevant plasma Dox concentrations, the heart becomes a site of redox reactivity. The quinone functionality of Dox is transformed, in the presence of NADH, into a semiquinone via one-electron reduction by complex I of the electron transport chain [39]. The semiquinone form reacts with O_2 to produce a superoxide radical ($O^{\bullet -}$), whereby Dox returns to the quinone form. The cycling of Dox between quinone and semiquinone generates large amounts of O_2, which further give rise to a variety of ROS/RNS species [40]. ROS can damage membrane lipids and other cellular components and consequently lead to cardiomyocyte apoptosis or death [41]**.** Our results showed that lipid peroxidation induced by Dox is significantly decreased in the pretreatment of Se, as manifested by significant reduction in the elevated level of cardiac MDA, which is consistent with previous studies [34, 42]. Previously, it was reported that Se supplementation can protect against free radical damages by increasing myocardial Se content and improving the expression and activity of GPx [43].

The present study revealed a marked increase in cardiac NO level in those Dox-treated rats. This finding is in agreement with the results reported by Saad *et al* [36] who used a model of doxorubicin chronic cardiotoxicity similar to that used in our study. The increase in NO level can be explained on the basis of the ability of Dox to mediate the induction of NOS expression and NO release in heart [44]. Several reports indicate that exposure of endothelial cells to H_2O_2 promotes eNOS expression [45]. Previous studies also suggested that stimulation of endothelial cells with calcium-mobilizing agents could activate eNOS [46]. Because Dox-induced toxicity is mediated by intracellular H_2O_2 as well as the calcium influx, Dox treatment causes an increase in eNOS transcription and protein activity in aortic endothelial cells and thus NO synthesis. On the same line, recent study provides evidence of upregulation of iNOS gene and protein expressions in Dox-induced cardiomyopathy[47]. The concomitant overproduction of NO and ROS is known to yield highly reactive nitrogen species, peroxinitrite, which may attack and destroy important cellular biomolecules [48].

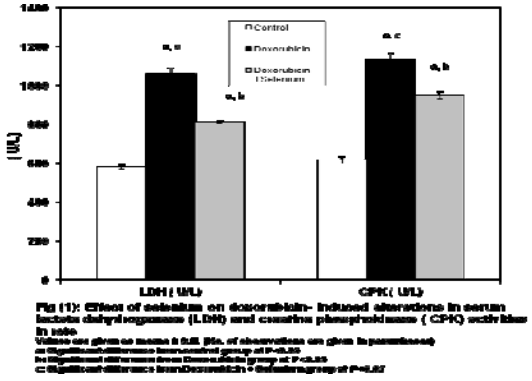
Selenium, in the present study, caused significant decrease in the elevated cardiac NO level shown in the Dox-treated group, which is in agreement with that reported by Ayaz and Turan [49]. The exact mechanism by which Se influence cardiac NO synthases expression is unknown. Of interest in this context is the report that treatment of nuclear extracts of lipopolysaccharide-activated human T cells with relatively high concentrations of selenite inhibited nuclear factor -κB binding and thus decreased NO production [49]. This is because nuclear factor -κB is a transcription factor that regulates a number of cellular genes, such as those encoding iNOS [50].

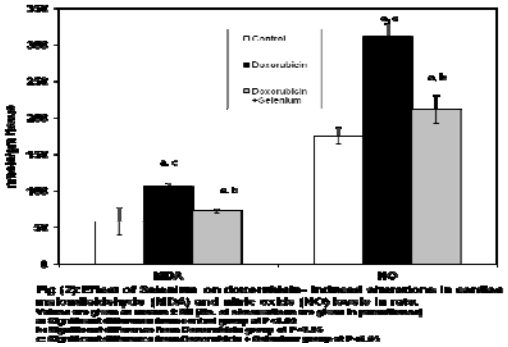
Doxorubicin administration, as shown in our results, caused a significant decrease in cardiac GSH content, which is quiet compatible with previous studies [35]**.** The overproduction of ROS, caused by Dox administration, can account for this decrease in GSH content, as these species are detoxified by endogenous antioxidants mainly GSH causing their cellular stores to be depleted [51]. The decrease of cardiac GSH content may also be attributed to the enhanced activities of GSH metabolizing enzymes by Dox administration, as shown in the present study. One is GPx which reduces H_2O_2 and various peroxides using GSH as reducing agent.The other is GST which consumes GSH in the conjugation of Dox toxic metabolites [52]. The present study showed that cardiac GSH concentration is higher in (Se+ Dox) -treated rats rather than those administered Dox alone, which is in agreement with recent results [53]. Such effect might be attributed to the antioxidant properties of Se and its ability to reduce Dox- induced oxidative stress. Selenium reduces the consumption of GSH by ROS [53]**.** Because GSH is one of the essential compounds for maintaining cell integrity [54] and the GSH redox cycle is one of the most important intracellular antioxidant systems, the increase in GSH content could be one of the mechanisms for cardiac protection by Se supplementation [53]**.** In addition, we assumed that the observed increase in cardiac GSH content might be related to the decreased activities of GSH-utilizing enzymes, GPx and GST, shown in the (Dox + Se) -treated group, leading to preservation of their substrate, GSH.

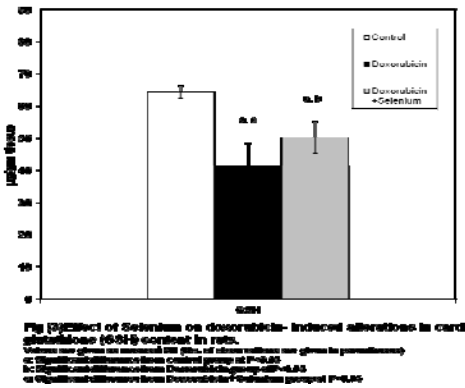
Our results showed significant increase in cardiac activity of SOD in the Dox-treated rats, which is consistent with some studies [35, 55]. The increase in SOD activity can be explained on the basis that the redox cycling of Dox between quinone and semiquinone forms generates large amounts of O_2 [40**]**, which in turn stimulate SOD as an adaptive response to counteract oxidative stress. The increased activity of SOD could lead to overproduction of hydroperoxides, in consequence, GPx might be stimulated in response to the accumulated peroxides**.** This assumption was supported by our results, which showed a significant enhancement in cardiac GPx activity in the Dox-treated group, and also by some authors [36]. On the same line, GPx have been reported to be over expressed in Dox-treated cells, especially those tumor resistant ones [56]. The current study revealed that Se administration caused significant decrease in Dox-induced elevation in SOD cardiac activity, which still higher than that of the normal group value. This result is in harmony with several previous studies. Selenium has been reported to decrease the elevated activity of SOD in heart as a result to cadmium toxicity [57] and hemoglobin mediated cardiotoxicity [34], which used Se dose similar to that used in our study. It is now well established that Se, through its incorporation in selenoproteins, could actively protect against free radicals generation, and hence, ROS- induced damage [58]. As a result of this protective effect, Se consumption could attenuate superoxide radical production, and consequently, decreased the activity of such antioxidant enzyme. Also, our study showed that pretreatment with Se relieved Dox induced hyperactivity of cardiac GPx, which is in harmony with Ayaz and Turan [49]. Glutathione peroxidase is one of the most active antioxidant enzymes in the myocardium [59]**,** and selenium, present in its active site, is essential for its activity. One of the major roles of this essential trace element in the body is to act as cofactor of this key antioxidant enzyme in which it contributes to both catalytic activity and spatial conformation [60]. Therefore, any significant modification of Se status would lead to changes in the activity of GPx and have important consequences on the susceptibly of the tissues to oxidative stress [13, 61].Selenium has prophylactic action, when it is administered before doxorubicin, it increases myocardial selenium content and improves both expression and activity of GPx. This may account for the increased cardiac GPx activity in (Se+ Dox)-treated group, compared to the normal control value, as shown in our results. Upon pretreatment with Se, myocardial tissues became already protected, and therefore, when exposed to Dox, we assume that there is no need for farther dramatic increase in cardiac GPx activity as an adaptive mechanism. This might be an explanation for the decrease in GPx cardiac activity in (Se+Dox) group value, compared to that result shown in the group treated with Dox alone. Additionally, the obtained biochemical results were supported by each other, since, as we mentioned later, Se supplementation caused decrease in the SOD activity, which consequently, leads to decreased production of H_2O_2 and hence, decreased activity of GPx enzyme.

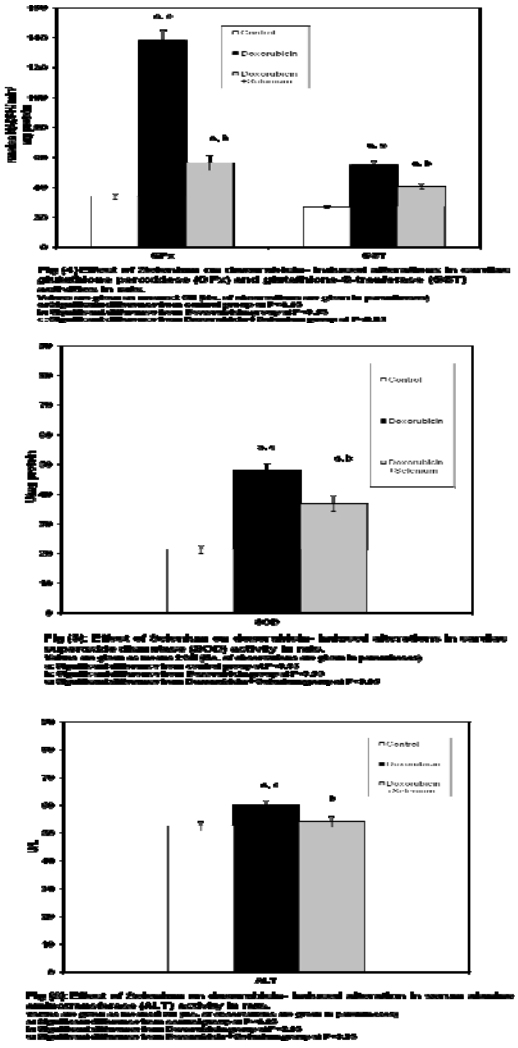
Results of the present study revealed significant increase in cardiac GST activity in rats treated with Dox, which is in agreement with many studies [62]. GSTs are family of dimeric proteins that posses a multitude of functions including the enzymatic conjugation of GSH to electrophilic xenobiotics [63]. It has been reported that cellular exposure to xenobiotics and antioxidants leads to coordinated induction of a battery of genes encoding detoxifying enzymes including GST [64]. Indeed, GSTs belong to phase II enzymes that in contrast to phase I, who can participate in both metabolic activation and inactivation, predominately participate in the detoxification of xenobiotics [65]. It has been known that Dox is metabolized via alkedoreductases yielding C13 hydroxyl derivative, doxorubicinol. This metabolite is actually more polar and toxic than Dox itself. Doxorubicinol accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity induced by Dox [8]. It has been reported that Dox toxic metabolites are efficiently conjugated with reduced GSH, a reaction that is catalyzed by GST [52]. Moreover, GST, due to its peroxidase activity, can serve to reduce Dox-induced peroxides [52]. In brief, GST has showed elevation after Dox injection to detoxify Dox and its metabolites and to attenuate the elevated oxidative stress [66]. The current study revealed that Se caused significant decrease in Dox-induced elevation in GST cardiac activity, which still higher than that of the normal group value. This result is in agreement with previous studies stated that Se afforded reduction in cadmium-induced elevation in GST cardiac activity [57]. The protective effects of Se are mainly related to its physiological antioxidant properties, and hence, decreased generation of ROS and RNS. Thus, Se supplementation could decrease the activity of GST enzyme responsible for the detoxification of such free radicals. Our results showed an elevation in serum ALT upon Dox administration which agrees with many previous studies [67, 68] and supported by the present histopathological examinations. Doxorubicin - induced hepatotoxicity might be less severe than its cardiotoxicity, which can be related to the fact that liver mitochondria, unlike cardiac mitochondria, lack the NADH-related pathway of reducing equivalents from the cytosol to the respiratory chain. As a result, liver mitochondria do not generate significant amounts of Dox semiquinones [69]. Selenium co-administration was shown to decrease the elevated serum ALT when administered with Dox, as reported previously [34]. Selenium supplementation could reduce hepatotoxicity by rendering hepatic tissues less susceptible to lipoperoxidative attack by the drug [70]. Selenium prevents hepatocyte oxidative damage and thus leakage of liver enzymes into serum as in the cases of cadmium hepatotoxicity [71]. This biochemical result is supported by the histopathological examination of liver sections of the different groups which illustrated that, in the liver sections of rats administrated Dox, congestion and kupffer cells proliferation were observed, while sections from rats administered Dox+Se showed least liver damage.

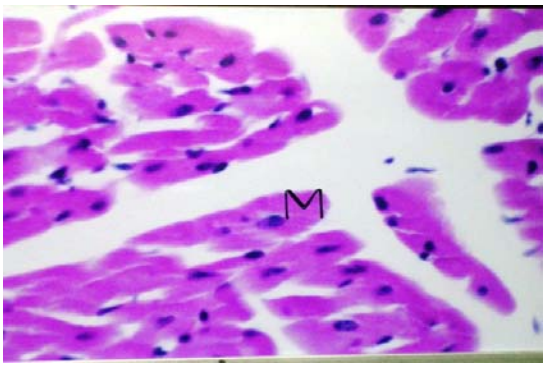
In conclusion, Se supplementation produced partial, but significant, protection against Dox-induced cardiomyocyte damage; however, such trace element could alleviate the Dox- induced hepatic damage, as evidenced by the biochemical measurements and histopathological examinations of the cardiac and hepatic tissues.



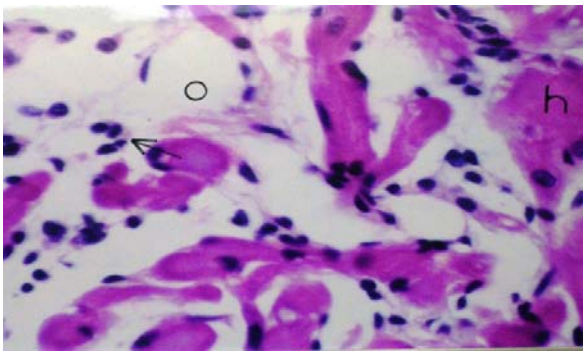




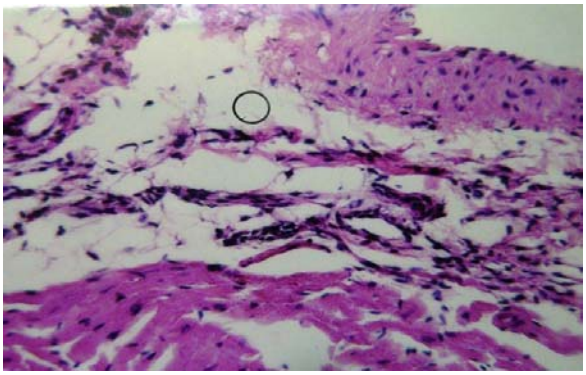




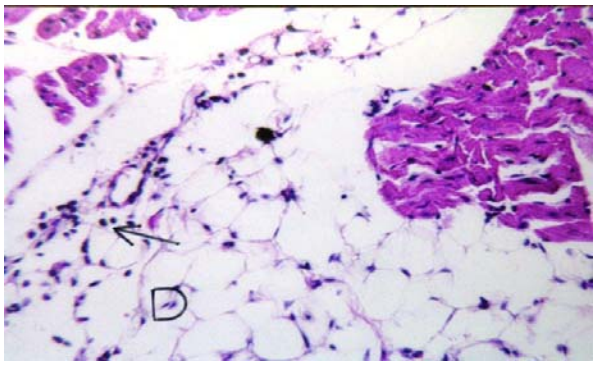
Figure(7): A photomicrograph of cardiac muscle fibers of control group showing normal histological strucrure of myocardium(M) (H&E x160)



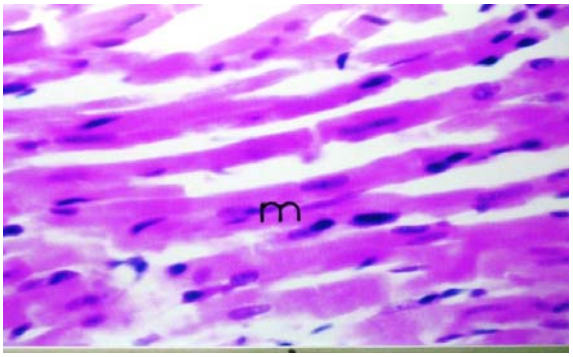
Figure(8): A photomicrograph of cardiac muscle fibers of Dxo group showing Oedema(o) with inflammatory cells infiltration(arrow) in focal manner between the myocardial bundles. (H&E x160)



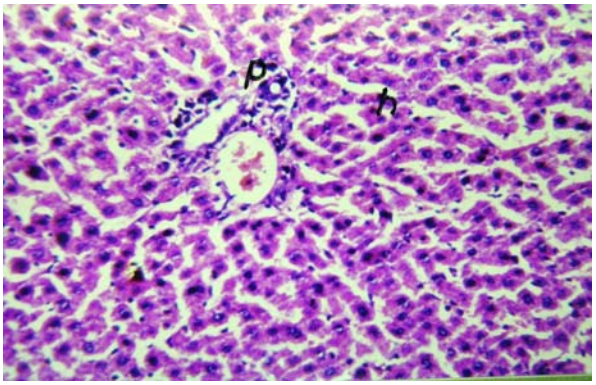
Figure(9): A photomicrograph of cardiac muscle fibers of Dox group showing Subendocardial oedema(o). (H&E x64)



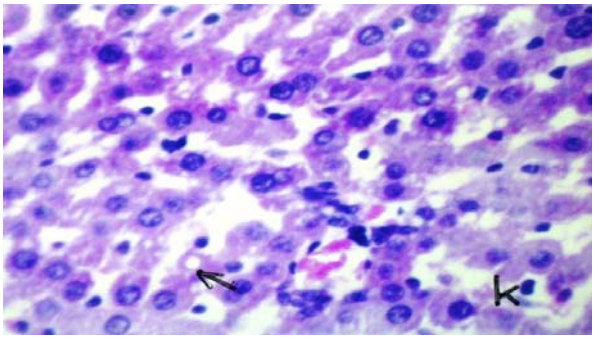
Figure(10): A photomicrograph of cardiac muscle fibers of Dox group showing inflammatory cells infiltration(arrow) in the subendocardial adipose tissue(D) (H&E x64)



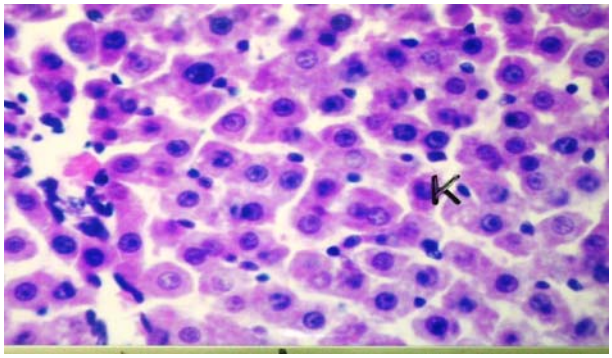
Figure(11): A photomicrograph of cardiac muscle fibers of Dox+Se group showing mild hyalinization in myocardial bundles(m) (H&E x160)



Figure(12): Photomicrograph of liver of normal group showed hepatic lobules (h) and portal vein (p) with normal architecture *(H&E x64*



Figure(13): Photomicrograph of liver of Dox group showing diffuse kuffer cells proliferation(k) inbetween the fatty degenrated hepatocytes (arrow) (H&E x160)



Figure(14): Photomicrograph of liver of Dox + Se group showing diffuse kuffer cells proliferation(k) inbetween the fatty hepatocytes (H&E x160)

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Opacification of a CeeON 911A Silicone Intraocular Lens Caused by Deposits on the Optic without Asteroid Hyalosis

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Abstract: This study reports a case of calcific opacification on an implanted silicone intraocular lens (IOL) without asteroid hyalosis. A 72-year-old female was referred for blurred vision in her left eye. Her history showed she had undergone uneventful phacoemulsification with in the bag implantation of a silicone foldable hydrophobic IOL (CeeON 911A, Pharmacia Corporation) 39 months earlier. Under slit-lamp examination, multiple diffuse granular –appearing opacification in the optic were noted. The surgery for IOL explanation and replacement was performed because of significant visual disturbance. Crystals of calcium phosphate in brush form were found under light microscopy. It looked like the feather beside the brush. The scanning electron microscopy revealed a morphous crust-like layer on the curved posterior optic surface of the lens. The transmission detecting systems showed that the transmission rate of the opacified silicon IOL was near 0%. It may have decreased the visual acuity of the patient and limited her daily-activities and stereo-acuity. To our knowledge, this is the first report of surface calcification of a silicone IOL in the absence of asteroid hyalosis. Although the mechanism of calcification has not been determined, careful clinical follow-up of patients with implanted silicone lenses is necessary to determine if this phenomenon is rare and sporadic or if it is more widespread.
[Feng-Chi Lin, Han-Yin Sun, Ming-Liang Tsai, Jeng-Shuan Shiang, Shang-Tao Chien, Chi-Ting Horng. **Opacification of a CeeON 911A Silicone Intraocular Lens Caused by Deposits on the Optic without Asteroid Hyalosis.** Life Science Journal 2010;7(4):173-176]. (ISSN: 1097-8135).

KEY WORDS: silicon intraocular lens, opacified intraocular, asteroid hyalosis

I. INTRODUCTION

LONG- term biocompatibility of intraocular lenses (IOLs) is a major factor in successful cataract surgery. The post-operative complication of IOL opacification raises concerns about the biocompatibility of these lenses. Opacification were seen in PMMA lens [1], hydrophilic acrylic IOLs [2,3], and several silicone IOLs in eyes with asteroid hyalosis [4], which has been noted in previous surveys. Now we describe opacification of a silicone IOL 39 months after uneventful cataract surgery. To our knowledge, this is the first report of posterior surface calcification of a silicone IOL without asteroid hyalosis.

Case Report

A 72-year-old female was referred for blurred vision in her left eye. Her history showed she had undergone uneventful phacoemulsification within the bag implantation of a silicone foldable hydrophobic IOL (CeeON 911A, Pharmacia Corporation) 39 months earlier (June 2007). Over the next 3 years, the opacification increased. On examination, the best-corrected visual acuity was 6/60 in left eye. Her biocular stereopsis was only 800msec tested by Stereotest-Circles (Stereo Optical Co., Inc. USA). Slit-lamp examination revealed diffuse multiple granular deposits on the posterior surface of the IOL optic (Figure 1). Neither asteroid hyalosis nor any other vitreous condition was noted in the vitreous cavity. Under the impression of after-catarat, we performed the laser capsulotomy to polish the posterior capsule but failed. The irregular deposits also still persisted and our treatment did not improve her vision (Figure 2). Then we arranged to remove the opacified IOL through superior approach, and a PMMA lens with scleral fixation in the sulus position was performed at the same time. Two month later, the bare visual acuity of her left eye had improved to 20/25.

Results and Discussions

1. The explanted IOL was stored in a dry, sterile vial without fixatives. Care was taken not to manipulate the surface of the IOL optic with instrument before analysis.

The lens was then bi-sected. One half was analyzed by light microscopy and scanning electron microscopy. Photographs were also taken. Half of the calcified IOL was checked to determine the transmission rate (%) by transmission detecting system (Figure 3).

2. The optical surface of the opacified IOL was covered by multiple irregular granular black deposits (Figure 4).

3. Light photomicrographys (Nikon,Japan) showed the crystals of calcium phosphate in brush form beneath the IOL. We checked the shape of the feather beside the brush, and the morphology looked like monomers of calcium phosphate in brush form Figure 5.

4. The opacified IOL was analyzed under the low magnification scanning by electron microscopy (Hitachi,Japan). It revealed small round deposits on the curved shape surface of silicon IOL(Figure 6).

5. We found the irregular deposits forming amorphous crust-like layer on the posterior optic surface of the lens under high-magnification scanning electron microscopy (Hitachi,Japan)(Figure7).

6. We used the detecting system to compare the transmission spectrum of various types of IOL. Four types of IOL inclunding Akreos (Adapt acrylic lens, Baush & Lomb), SoFlex(Silicon fordable IOL, Baush & Lomb)、AMO- DL65T (PMMA IOL, Advanced medical Optics, Inc.)、CeeON 911A (Silicone foldable IOL, Pharmacia Corporation) were analyzed by transmission rate (%).

It presented the transmission spectrum of the IOL covered from 320 nm to 750 nm. The transmission intensities are weak when the wavelength of light is less than 400 nm for these four types of IOLs. We found the higher transmission rate (almost 50%) of AMO- DL65T PMMA IOL and the lower transmission rate (nearly 0%) of opaficication of IOL CeeON 911A in the wavelength of visible light spectrum (Figure 8). It indicated that the lower transmission rate may induce the decreased visual acuity of this patient and the poor binocular steropsis may affect her daily activities.

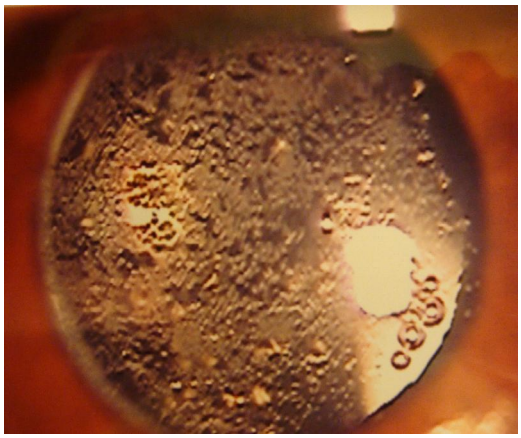

Figure 1. Opacification of Intraocular Lens under Slit-lamp Examination

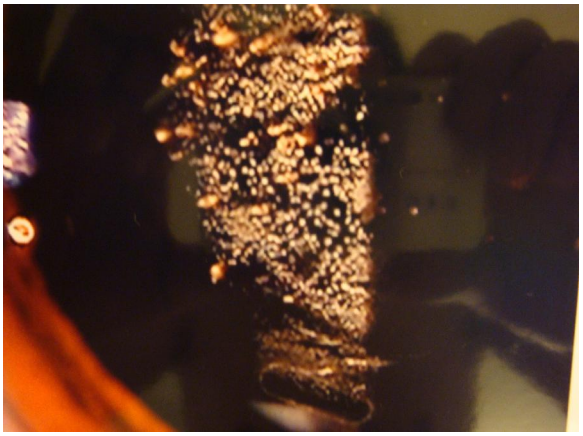

Figure 2. The Deposits of IOL Can Not Be Polished by Neodymium:yttrium-aluminum-garnet (Nd:YAG) Laser Treatment

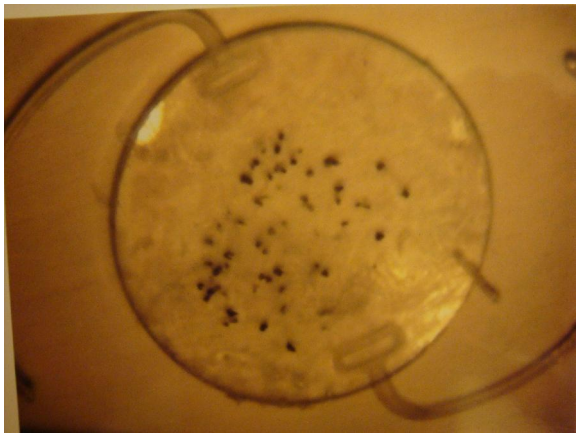

Figure 3. Multiple mutton-like black deposits on the posterior surface of the IOL after remove by surgery

This phenomenon of calcified silicon IOL was first reported by Foot [5] and Wackernagel et al. [6] since 2004. It often resulted in a clinically significant visual decrease long after the implantation, sometimes to a severity that required IOL explanation or exchange. Several types of late postoperative IOL were mentioned. Apple et al. reported a case of late postoperative degeneration of polymethyl methacrylate (PMMA) lenses [1]. Recent articles described some cases of late postoperative complications with hydrophilic IOLs caused by calcification [2-3]. However, intraocular lens calcification is not a common problem with silicone lenses. Since 2004, only a few cases of calcified silicone IOLs in eyes with asteroid hyalosis requiring explanation have been described in the literature [5-8].

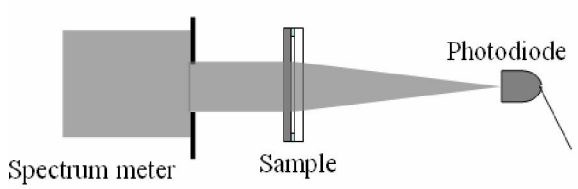

Figure 4. The measurement of transmission spectrum system

In 2010, Stringham et al [4] had a series of studies about the association between calcification of silicones lenes and asteroid hyalosis. In their study, there are 22 cases of calcification of silicone lenses involving 8 desings manufactured from different silicone materials described in the literature. There were noteworthy remarks in some eyes including vitreous hemorrhage, silicon oil retention with a small amount in the anterior chamber, low-grade postoperative endophthalmitis, SF6 gas retention in the anterior chamber to reattach a Descemet's stripping automated endothelial keratoplasty flap. However, they suggested the strong association between opacified silicon IOL and asteroid hyalosis.

Asteroid hyalosis is a vitreous disease chacterized by brilliant reflecting particles (so called asteroid bodies) floatering in an apparently normal vitreous body. Bergren et al. demonstrated a significant association between asteroid hyalosis with diabetes, systemic arterail hypertension, and atherosclerotic vascular disease [9]. Its prevalence was approximately 1%, and usually appearing unilateral without predispostion to gender or race. Their presence dose not cause any impairment to vision [9,10]. Topilow et al. reported that asteroid hyalosis enmeshed within normal vitreous collagen fibrils and that some were attended by macrophages or multinucleated epithelioid cells. Transmission electron microscopy disclosed irregular calcified material and complex lipids within the asteroid bodies [11]. Miller et al. found that x-ray micro-analysis confirmed the presence of calcilum and phosphorus in the asteroid bodies [12]. Recently, an electron spectroscopic imaging study conducted by Winkler et al. confirmed a homogeneous distribution of calcium, phosphorus, and oxygen within the asteroid bodies [13]. Therefore, they may represent a continuous supple of calcium or be an indicator of a continuous supply of this mineral to the vitreous.

Figure 5. The type of crystals of calcium phosphate in brush form was found under light microscopy. It looked like the feather beside the brush.

When the barrier function of the posterior capsule was damaged by laser capsulotomy, the calcium and phosphate originated from the vitreous in eyes with asteroid hyalosis may have cross the posterior capsule and precipate on the posterior IOL surface [14].It seems that direct contact between the posterior IOL surface and the vitreous, promoted by the laser posterior capsulotomies, accelerated the process of calcium precipitation [4,7].

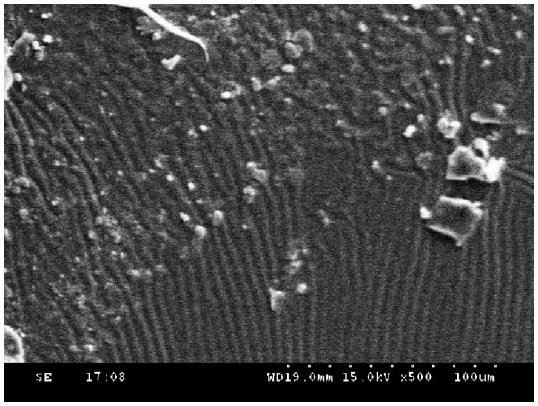

Figure 6. Low-magnification scanning electron photomicrography revealed small deposits on the curved-shape surface of silicon IOL

There is, therefore, increasing evidence that the material opacifying silicone IOLs is derived from the asteroid bodies because its composition was similar to that of hydroxyapatite (calcium and phosphate). It is, however, still unclear why only a few cases have been observed since silicone IOLs of various designs have probably been implanted in many patients with asteroid hyalosis. The small number of cases makes it difficult to prove a correlation.

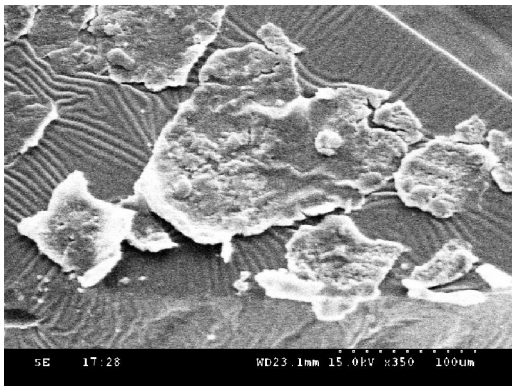

Figure 7. The deposits forming amorphous crustlike layer on theposterior optic surface of the lens under high-magnification scanning electron photography.

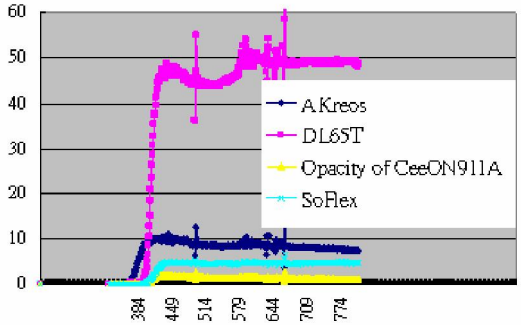

Figure 8. The transmission spectrum of IOL covered from 320 nm to 750 nm. The transmission intensities are weak when the wavelength of light is less than 400 nm for these four types of IOL.

In 2005, Werner et al. [7] described a patient with bilateral asteroid hyalosis. A 3-piece silicon IOL in one eye was explanted because of calcification. It is interesting to note that the hydrophobic acrylic lens implanted in the contralateral eye showed no opacities. The authors suggested that IOL in asteroid hyalosis is not associated with acrylic lenses.

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