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Highlights

- Assessment of Groundwater
- Synthesis of New Macrocycles
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- The Mechanism of Hail Formation

Discovering Thoughts, Inventing Future

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Assessment of Groundwater Quality around Two Major Active Dumpsites in Lagos, Nigeria

By Adeyi, A. A. & Majolagbe, A. O.
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Abstract- This paper presents the results of physico-chemical parameter data and water quality index used to assess the groundwater quality around Olusosun and Solus dumpsites in Lagos southwest, Nigeria. Twenty (20) water samples each were collected in wells and boreholes around these two dumpsites, once in two months consecutively for two years. The parameters determined include pH, acidity, alkalinity, conductivity (EC), total dissolved solids (TDS), total hardness (TH), Cl-, SO42-, NO3- and PO43- and heavy metals: Pb, Ni, Cd, Zn, Cu, Fe, Mg, Ca, Na and K using standard analytical methods. Descriptive statistics and correlation coefficient were carried out on the data generated. There is strong correlation at $p < 0.05$ in some of the parameters such as TDS versus EC ($r = 0.95$), TDS versus TS ($r = 0.90$), TH versus acidity ($r = 0.73$) in Olusosun while TDS versus EC ($r = 0.93$), TDS versus acidity ($r = 0.82$), TH versus alkalinity ($r = 0.77$) in Solus, suggesting common source. However, very weak chemical associations ($r < 0.30$) were observed in majority of the quality parameters particularly anions and trace metals, indicating multiple anthropogenic sources.

Keywords: physico-chemical, water quality index, correlation coeficiency, dumpsites, water quality parameters.

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ASSESSMENTOFGROUNDWATERQUALITYAROUNDTWOMAJORACTIVEDUMPSITESINLAGOSNIGERIA

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Assessment of Groundwater Quality around Two Major Active Dumpsites in Lagos, Nigeria

Adeyi, A. A. [α] & Majolagbe, A. O. [σ]

Abstract- This paper presents the results of physico-chemical parameter data and water quality index used to assess the groundwater quality around Olusosun and Solus dumpsites in Lagos southwest, Nigeria. Twenty (20) water samples each were collected in wells and boreholes around these two dumpsites, once in two months consecutively for two years. The parameters determined include pH, acidity, alkalinity, conductivity (EC), total dissolved solids (TDS), total hardness (TH), Cl-, $SO4^{2-}$, NO_{3-} and PO_4^{3-} and heavy metals: Pb, Ni, Cd, Zn, Cu, Fe, Mg, Ca, Na and K using standard analytical methods. Descriptive statistics and correlation coefficient were carried out on the data generated. There is strong correlation at $p < 0.05$ in some of the parameters such as TDS versus EC ($r = 0.95$), TDS versus TS ($r = 0.90$), TH versus acidity ($r = 0.73$) in Olusosun while TDS versus EC ($r = 0.93$), TDS versus acidity ($r = 0.82$), TH versus alkalinity ($r = 0.77$) in Solus, suggesting common source. However, very weak chemical associations ($r < 0.30$) were observed in majority of the quality parameters particularly anions and trace metals, indicating multiple anthropogenic sources. Water quality index (WQI) reveal minimal contamination on the groundwater, delineates Solus groundwater as excellent – good – poor and Olusosun groundwater as good – poor – very poor. However, the nitrate level in almost all the groundwater collected around the Olusosun dumpsite reveal is worrisome and portends a great health risk. Therefore, concerted efforts must be put in place to address the situation and ensure sustainable environment.

Keywords: physico-chemical, water quality index, correlation coeficiency, dumpsites, water quality parameters

I. Introduction

Groundwater remains a major source of water supply for drinking, recreation, agricultural and industrial purposes in most part of Nigeria. Lagos state provides about 220 million liters of water daily for domestic and industrial purposes for about 37 % of its population 1. About 8.3 million residents in Lagos (62.6% of the population) and some industries have no access to pipe borne water, thus depend solely on groundwater through privately dug bore holes and shallow wells. This trend could be as a result of either lack of improper planning by government for new residential areas that are emerging in the state, inadequate and erratic water supply by the government water works and or the population - water facility ratio, which is on the high side. Therefore, the ever increasing population will continue to rely on groundwater as a main source of water supply. However, the quality of groundwater assessed by the citizens is doubtful because groundwater resources particularly in Lagos state are under varying degree of threats 2. These threats include various sources of pollution, such as industrial effluents discharges, municipal solid wastes (MSW), residential effluents discharges, agricultural chemicals and fertilizers applied on farms, oil spills and leakages, as well as salt water intrusion and urban surface runoff [3,4].

Landfills have been identified as one of the major threats to groundwater resources [5,6,7]. The volume of wastes generated in Lagos is increasing with time and is presently at about 1000 tons per day apart from the medical wastes which is given separate and special treatment [8]. The quantity and quality of municipal solid waste (MSW) depends upon various factors such as population, life style, food habit, standard of living, industrial and commercial activities in the area9, cultural and tradition of inhabitants, as well as climate[6]. Of the 2.3 million tons of waste generated annually in Lagos since 2008, only about 50% are accounted for by Lagos State Waste Management Authority (LAWMA). The remaining is disposed illegally and haphazardly without regards for environmental consequences. In most open dumpsites across the cities, wastes collected are burnt in the open and ashes abandoned at the site .The practice of burning wastes, destroy the organic component, oxidize metals, thereby enriching the ashes left behind with metal[7]. Wastes in landfills or open dumps are subjected to either groundwater underflow interaction or infiltration from precipitation. The dumped solid wastes gradually release its initial interstitial water (leachate) and some of its decomposition by-products get into water moving through the waste deposit. This leachate accumulates at the bottom of the wastes and percolates through the soil[5] migrate downward and contaminate the groundwater. The leachate sometimes contains mainly organic carbon largely in the form of fulvic acids. It usually contains toxic substances, especially, when wastes are of industrial origin[4]. Such contamination of groundwater resource poses a substantial risk to local resource user and to the natural environment. Each year, about two million people die as a result of poor sanitation and contaminated water, of which ninety percent (90%) are children 10. Various

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studies on the impact of landfill on groundwater quality have been reported using different approaches11,12. These approaches include experimental determination (i.e. physicochemical assessment), estimation through mathematical modeling[13.14,] geophysical method[15,16,17] and the use of water quality indices [18].

Physicochemical assessment of water quality has been extensively used to evaluate the quality status of both surface and groundwater and to suggest possible usage of such water [19,2,20],. A major advantage of physicochemical assessment method of evaluating the quality of water bodies is that the water samples(s) are directly analyzed, thereby generating data that show the true quality or the actual values of the water quality parameters. A large data is usually the results which indicate comprehensive quality assessment. The largeness of the data also helps in revealing possible various trends and patterns that are inherent in the data obtained [21].

Water quality index (WQI) takes the complex scientific information of water quality variables and synthesizes them into a single number, thereby, also reducing the dimensionality of the water data. Various models used in calculating WQI include weighted arithmetic mean, weighted geometric mean, un-weighted harmonic square mean, use of fuzzy logic model and baseline comparative model22. By comparing the mean values of these parameters in the water samples with the maximum permissible limits by various known regulatory bodies such as World Health Organization (WHO), United State Environmental Protection Agency (USEPA) etc., it becomes possible to declare a water body contaminated, polluted or either safe or not for human consumption.

This study therefore, aimed at presenting the use of water quality index and physicochemical assessment of quality of groundwater around two major active refuse dumpsites in Lagos state, thereby evaluating the impact of these dumpsites on the quality of groundwater. The data generated will assist regulatory agencies in formulating policies to ensure sustainable environment and a baseline for further studies.

II. Material and Methods

a) *Description of the study areas*

The study area consists of Olusosun and Solus refuse dumpsites and their environ.

i. *Olusosun refuse dumpsite*

It is a controlled dumpsite located at Ojota, Lagos within longitude 03.372 E to 03.374 E and latitude 06.588 N to 06.595 N. It is the largest government owned dump facility in Nigeria. It is about 18 meters deep and covers close to 42 hectares of land. Olusosun refuse dump was established in 1988 with a life span of 35 years. The dump is surrounded by Oregun industrial layout, Olusosun residential compound, Shangisah residential areas and commercial neighborhood (Fig.1.0). It receives an average of 1.2 million tons of assorted wastes annually and is presently serving as a pilot project for biogas production in Nigeria.

ii. *Solus refuse dumpsite*

The Solus dumpsite is situated between longitude 03.255 E to 03.253 E and latitude 06.569 N to 06.574 N in Igando area of Lagos State. It started operations in 1996. It is of 9 m depth and covers about 3 hectares. Solus refuse dump receives 4000 tons of waste per day and has reached over 70% of its full capacity [23]. The dump is entirely surrounded by residential area (Fig. 2.0). An abattoir and a small fast shrinking Oba stream is located about 2.5 km westward of the dumpsite.

b) *Sampling*

Groundwater samples were collected from twenty (20) locations around each dumpsite determination for physico – chemical parameters and trace metal analyses. Sampling was done once in two months, for two years (January, 2009 -December, 2010). The samples collected around Olusosun dumpsite are represented as OSW 1 – OSW 20, while that of Solus are represented as SWS 1 – SWS 20. The samples were preserved accordingly based on standard methods (APHA/AWWA, 2005). Two samples (1.5 L and 0.75 L for physico –chemical parameters and trace metals respectively) were taken from each location. The 0.75 L water samples were preserved by acidifying with 1.5 ml of concentrated HNO_3 (Analar grade) per liter of water sample. Blank samples were collected (using double distilled water) and treated in the same way as the other water samples 24. The samples were stored in an ice box, transported to the laboratory where they were refrigerated until analysis using standard methods.

c) *Sample chemical analyses*

The pH, electrical conductivity (EC) and temperature of the groundwater samples were determined on sites. Temperature was measured using thermometer, while EC and pH were measured using conductivity meter (Mettler Toledo) and pH meter (pHep HANNA HI 98107) respectively. Alkalinity and acidity were determined titrimetrically [25], total hardness by complexomertry[25], Total suspended solids (TSS), dissolved solids (TDS) and total solids (TS) by gravimetry[25,26] chloride by silver nitrate method[25], sulphate by turbidimetric method [25], phosphate by colorimetric and nitrate by phenol disulphonic acid method [27,28]. Determination of cations Na+, and K+ and trace metals were done with flame photometer and Flame Atomic Absorption Spectrophotometry (Buck scientific 210V model) respectively after acid digestion

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of the samples [29]. Each sample was analyzed in duplicate, so as to ascertain the validity of the method.

d) Statistical Analysis

Descriptive analyses of data generated were carried out using Graph Pad Prism (version 5.00). Correlations coefficiency was performed in a pair wise fashion employing Pearson correlation coefficent.

e) Water Quality Index

Three steps are followed as described by Srinivas and Nageswararao 18, to calculate Water Quality Index (WQI). In the first step each of the parameters has been assigned a weight (w_i) according to its relative importance in the overall quality of the water for drinking purpose. A maximum weight of 5 has been assigned to nitrate due to its major importance in water quality assessment. In the second step, the relative weight is calculated from the following equation

$$W_i =$$

where W_i is the relative weight, w_i is the weight of each parameter and n is the number of parameters. Calculated W_i values of the parameter are given in Table 1.0. In the third step, a quality rating scale (q_i) for each parameter is assigned by dividing its concentration of each water sample by its respective standard according to the WHO guidelines and the result multiplied by 100. $q_i = (C_i - C_{io} / S_i - C_{io}) \times 100$ where Ci is the concentration of each chemical parameter in each water sample in mg/L, Cio is the ideal value of the parameter in pure water and Si is the Indian drinking water standard for each chemical parameter in mg/L according to the guidelines of the WHO. For pH, C_{io} is 7 and $q_i = (C_i - 7)/(S_i - 7) \times 100$. For the remaining parameters the ideal value is 0.

Table 1.0 : Relative Weight of the Chemical Parameters

Chemical parameters	WHO standards	Weight	Relative weight (Wi)
pH	6.5 – 8.5	4	0.1818
Total hardness	300	2	0.0909
Ca	75	2	0.0909
Mg	30		0.0909
Cl	250	3	0.1363
Total dissolved solids	500	4	0.1818
Nitrate	10	5	0.2272

To calculate the WQI, the sub index (SI) is first determined for each parameter, which is used to determine the WQI using the following equations.

$$SI_i = W_i \times q_i,$$

$$W_i =$$

Where SI_i is the sub index of the ith parameter. The calculated WQI values are classified into four types as shown in Table 2.0.

Table 2.0 : Water Quality Classification based on WQI value

WQI	Water quality
< 50	Excellent
50 – 100	Good
100 – 200	Poor
200 – 300	Very poor

III. Results and Discussion

a) Physicochemical Properties Groundwater

The results of physico-chemical analysis and trace metal load of water samples around the two major active controlled dumpsites in Lagos (Olusosun and Solus) are presented in Tables 3.0and 4.0 respectively. The correlation coeficiency between various physicochemical variables are presented in Tables 5.0 and 6.0 for Olusosun and Solus groundwater respectively. The mean and range of pH values for the Olusosun and Solus groundwater were 5.23 ± 0.97 (3.10-7.10) and 5.71 ± 0.75 (4.40-6.90), respectively. All the mean values were in the acidic range and lower than the allowable limits of 6.5 – 8.5 WHO (2006) and Nigerian Standard for Drinking Water Quality [30]. The acidic nature of Lagos water is probably as a result of high volume of CO_2 in atmosphere, an indication of high level of population and industrialisation. Longe et al.[4] however pointed out that acidic status of Lagos groundwater is also a charateristic of coastal water, whose pH is primarily controlled by its hydrological settings. 15% and 20% of Olusosun and Solus water samples collected have the mean value within the WHO

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allowable range. The neutral average pH value observed in samples OWS11and OWS12 could be as a result of the proximity of the location of the wells to a big canal that bound Ketu market, hence infiltration of waste water to the neighbouring water,which results in the pH obtained might be responsible for this. The pH of water controls its solubilty and the rate of reaction of the metal species that is invovlved in corrosion recaction [31].

The temprature of groundwater around Olusosun ranges from 24.8 °C and 27.4 °C with mean value of 25.6 ± 0.7 °C, while the mean for Solus was 25.9 ± 0.5 °C and range from 24.9 to 26.9 °C.

Temperature, if high can cause legionellosis, schistomiasis and other related health conditions whose causative organism thrives more in warm water and in the presence of nutrients. Temperature can affects the amount of dissolved oxygen of water.It also control fluoride content which causes dental coloration in childeren and skeletal damage[32]. The mean and range of total alkalinity value were 144 ± 91 (26.2 to 373) and 123 ± 106 (12.3 to 396) mg/L for water around Olusosun and Solus dumpsites respectively. Generally, most groundwater tends to be high in alkalinity and Oxygen rich except in high populated and industrialized areas. The larger percentage of the water samples collected around both Olusosun and Solus dumpsite had values lower than 100 mg/L. However, going by Ragunath classification of groundwater based on alkalinity [33], only 10% and 25% of the groundwater samples around Olusosun and Solus respectively can be said to be good for drinking purpose. Phenolphthalein alkalinity was zero in all water samples analyzed; therefore all alkalinity values were due only to bicarbonate.

The electrical conductivity (EC) is a quality parameter that describes the ability of water to allow passage of conducting electrons. It is an indication of dissolved inorganic in groundwater [34]. The importance of EC of water is its measure of salinity, which greatly affects the taste consequently; it has a significant impact on the users' acceptance of the water as potable [35]. Water around Olusosun dumpsite gave 0.9 mS /cm as the mean value of electrical conductivity. Highest conductivity value were observed from wells OWS5 and OWS6 that were closer (4 m) to the dumpsite. For Solus groundwater sample, values reported were lower, than that of Olusosun groundwater samples. This may be probably because of age of the Olusosun dumpsite, the geological formations of Olusosun area and the depth of wells found around Solus dumpsite. The direction of groundwater flow may also not be unconnected with this finding. About 5% and 10% of water sample collected around Solus in rain and dry season respectively show conductivity values higher than WHO maximum limit of 1.4 mS/cm for drinking water, while all the water collected around Olusosun dumpsite, have conductivity values within the acceptable limit. The EC value in Olusosun water maintains strong correlation with TDS at $p < 0.05$ ($r = 0.98$), TS ($r = 0.54$) and TSS ($r = 0.90$) (Table 8.0) while strong correlation was observed also between EC and TDS ($r = 0.94$) as well as TS ($r = 0.85$) in water collected around Solus dumpsite.

The average values of total dissolved solids observed in groundwater around Olusosun was almost twice of the value in water around Solus dumpsite. The mean and range of values of TDS for water collected around Olusosun and Solus were 412 ± 179 (122.0 - 849.0) mg/L and 393 ± 186 (112.0 -1294) mg/L respectively. The TDS of all water collected from Olusosun are within fresh water range and lower than the WHO a permissible limit of 1000 mg/L for drinking water. However, 5% of water around Solus had TDS within brackish water range. Total dissolved solid is a measure of amount of dissolved minerals that influences the usability of water. These mineral including inorganic salts (Ca, Mg, K, Na, HCO_3- and SO_4^2-) and small amount of organic matter, some of which are dangerous, if ingested at high concentration. TDS varies considerably in different geological regions owing to differences in the solubility of minerals. The palatability of water with TDS value less than 500 mg/L is generally considered good36, while water with TDS value above 1000 mg/L is objectionable to consumer because taste of such water is affected. The TDS in this study correlated with electrical conductivity ($r = 0.93$) and total solids ($r = 0.90$) in Solus groundwater while in Olusosun groundwater, TDS correlate strongly with electrical conductivity ($r = 0.98$), total solids ($r = 0.90$) and TSS($r = 0.55$) indicating a common source of the contaminant, while weak association were observed TDS/Cl, TDS/Alkalinity, TDS/Acidity and TDS/ pH.

The mean values of total hardness observed in Solus are almost twice of the TH values in water collected around Olusosun. Hardness is an important operational water quality parameter; it defines the suitability of usage of water. Hardness can be caused by Ca and or Mg, these usually prevents formation of lather thereby consuming more soap. Olusosun and Solus groundwater have mean and range of TH value 45.6 ± 36 (4.30 - 125) mg/L and 74.0 ± 61 (14.5 - 210) mg/L respectively. These values are higher than the result of similar study in Lagos 37 with 61.3 mg/L. TH as water quality parameter helps in control of corrosion.

Depending on the interaction with other factors such as pH and alkalinity, water with hardness value above 200 mg/L may carry scale of deposition in treatment work. A number of ecological studies have shown a great significant inverse relationship between hardness of drinking water and cardiovascular diseases.

This indicates that, soft water affect the functional behavior of human system. Hardness values in water around Olusosun dumpsite(Table 5.0) reveal strong correlation with acidity ($r = 0.73$) and moderate correlation with total suspended solids ($r = 0.52$), while

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it maintains weak correlations with conductivity (r = 0.26), total solid (r = 0.45), alkalinity (r = 0.38), and sulphate (r = 0.23), while water collected around Solus reveal strong correlation between total hardness and acidity (r = 0.81) and alkalinity (r = 0.77) as well as weak associations with pH, nitrate and sulphate as shown in Table 6.0.

The chloride values from Solus groundwater were twice higher than the corresponding chloride values in Olusosun groundwater. This could indicate that bulk of the solid waste received at Solus dumpsite are non industrial, as chloride is major anion from food waste. The mean and range of chloride value in water around Solus and Olusosun dumpsites 146 ± 112 (17.3- 354.0) and 68.2 ± 32 (19.9 – 184) mg/L. The highest chloride values were in wells SWS1, SWS2, SWS 3, SWS 10 and SWS 11, possibly due to their close proximity to the refuse dumpsite. 20% of water collected around Solus dumpsite showed concentration higher than the 250 mg/L permissible chloride limits for drinking water. This may be a pointer to chronic chloride toxicity from Solus groundwater. The values recorded in this study are lower than that reported by Laluraj 38 but higher than Niger delta study19 and Lagos groundwater study by Ikem [37].

The sources of salt water in these areas may be diversified. Tindal et al.39 suggested that it may be due to the retention of ions from salt trapped at the time of deposition, from solution of minerals and constituents or by infiltration of clay and recharge atmospheric precipitation containing ions. Chloride was observed to maintain weak correlations in Olusosun groundwater samples (Table 5.0) with nitrate, sulphate, acidity, alkalinity, EC and TDS with coefficient values ranging from 0.01 to 0.39, and a negative correlation with phosphate. However, in Solus groundwater (Table 6.0), chloride had weak correlation with sulphate, acidity, alkalinity and total hardness and negative correlations with phosphate, nitrate, EC and TDS.

The mean and range of sulphate value in groundwater collected around Olusosun and Solus were 22.4 ± 17 (9.27 - 69.5) mg/L and 12.9 ± 6.4 (3.24 - 25.3) mg/L respectively. Sulphate concentrations in groundwater collected around both dumpsites were generally lower than 250 mg/L limit stipulated by World Health Organization for drinking water. The level of SO_4= in groundwater under investigation could be traced to geological nature of the soil because interlocation of clays, sands and silt could encourage dissolution of sulphite such as pyrite from inter stratified matters percolating water to produce SO_4^{2-} water35. Vehicle emission can also contribute to the SO_4^{2-} concentration in an environment. High level of SO_4^{2-} can cause laxative effect to unaccustomed consumers 31.Excessive intake of SO_4^{2-} can lead to diarrhea, hydration and intestinal irritation, though no health based guideline has been fixed for SO_4^{2-}. Sulphate water collected around Solus dumpsite maintained weak correlation with pH, alkalinity, acidity, total hardness, TDS, TSS, conductivity and all the other anions with coefficient values (r) ranging from 0.004 to 0.48.This shows that the quality parameters are of different sources. However, in groundwater collected around Olusosun dumpsite sulphate maintained negative correlation with EC, TDS and TS, an indication that the higher the sulphate concentration ,the lower the quality parameters mentioned above. A weak chemical association was also observed for SO_4^{2-} versus pH, SO_4^{2-} versus acidity, and $SO_4$2- versus TSS indicating common origin of the paired parameters.

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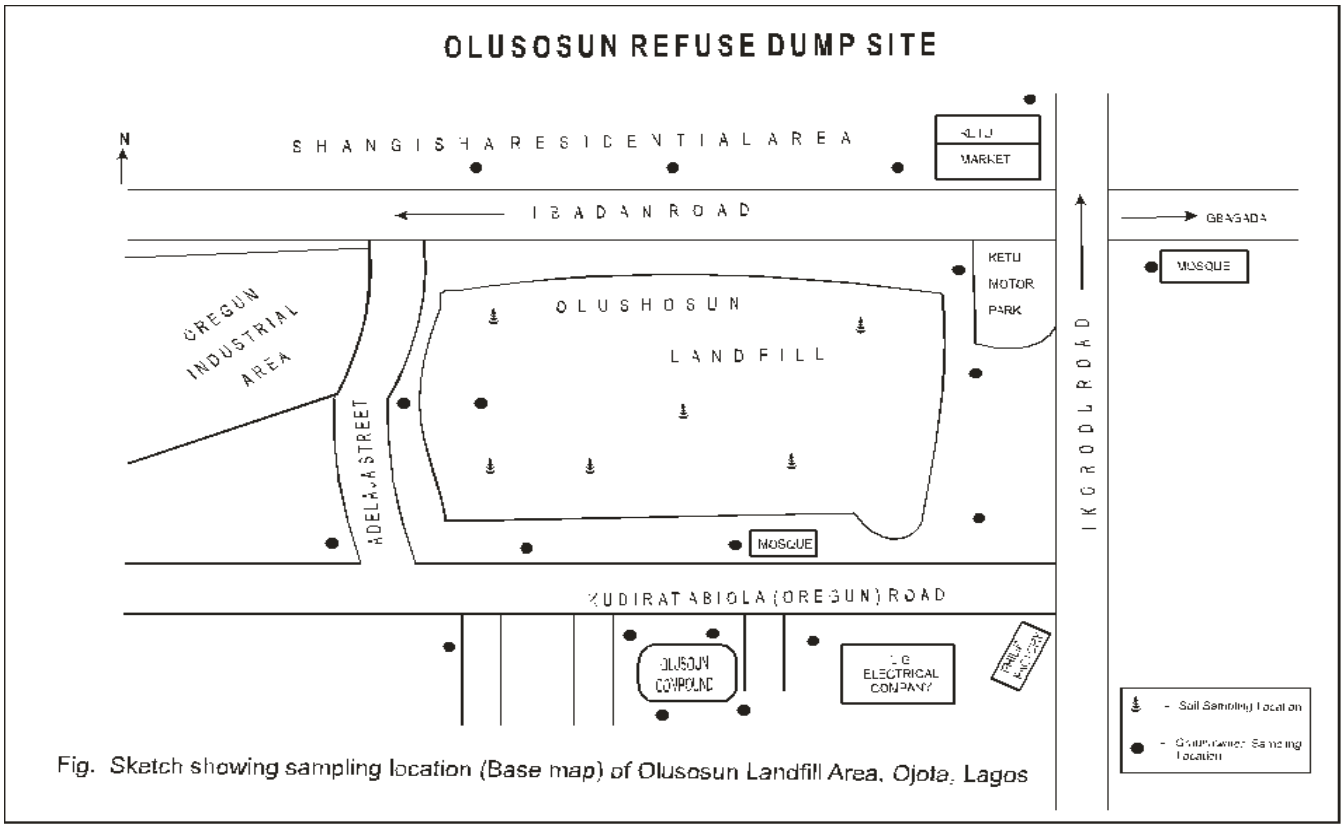

Fig. 1.0 : Sampling locations of Olusosun dumpsite area, Ojota, Lagos

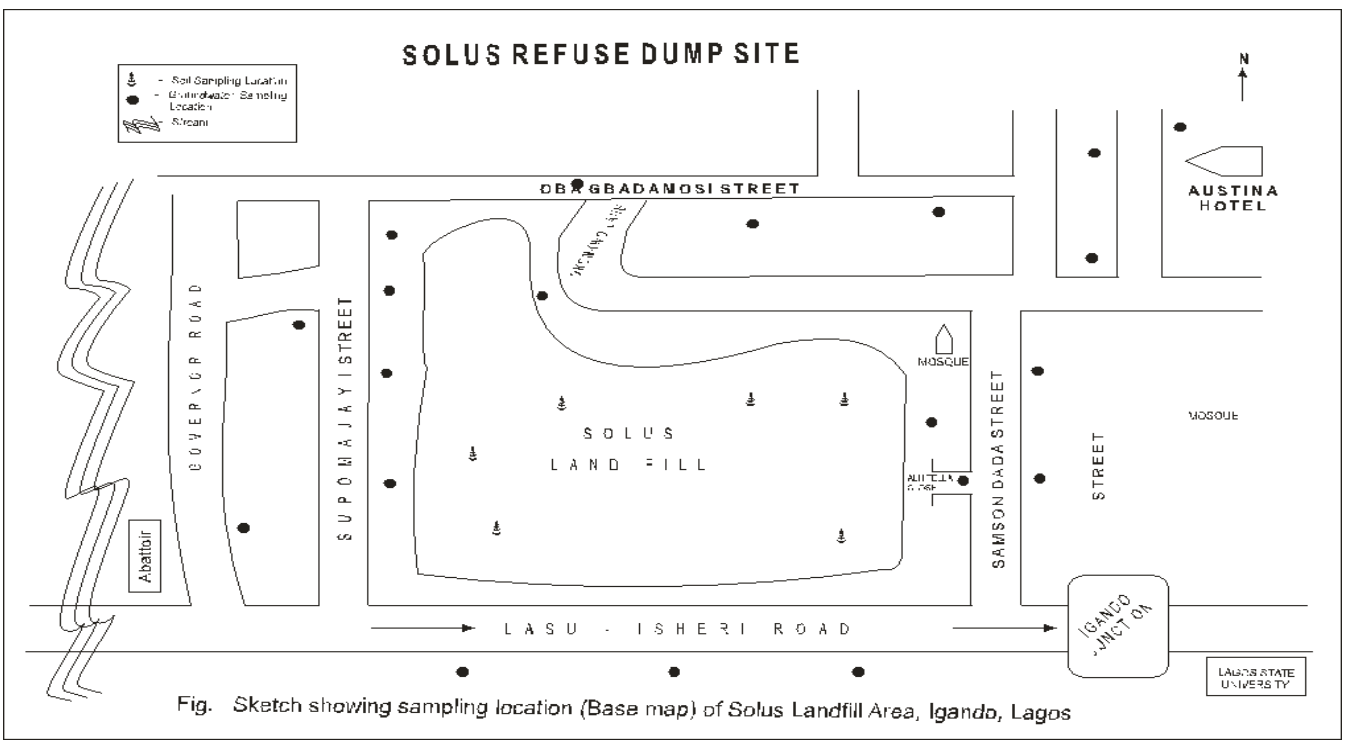

Fig. 2.0 : Sampling locations of Solus dumpsite area, Igando, Lagos

The average concentration of nitrate in groundwater around Olusosun was almost ten times that of nitrate value in Solus groundwater. The mean and range of nitrate values in water around Olusosun and Solus were 14.1 ± 6.4 (7.5 -35.3) mg/L and 1.47 ± 1.1 (7.4 - 35.3) mg/L respectively. The nitrate concentration

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in over 85% of the water from Olusosun area exceeded the 10 mg/L permissible limit by WHO[31] and Nigeria Standard drinking quality water[30]. This portends great danger. However, all samples analysed in Solus dumpsite area are safer from nitrate toxicity. The health effect of excess nitrate concentration beyond acceptable limit includes death of infant of about age six, while shortness of breath and blue-baby syndrome has also been identified. Generally, the sources of nitrate in groundwater include domestic sewage, runoff from urban on agricultural field and leachate from landfill site[34]. The leachate from the refuse dump is suspected to be responsible for the high nitrate level observed. The low value of NO_3- level observed in the Solus refuse dumpsite unlike most of the Olusosun samples investigated, could be as a result of age of Olusosun dumpsite, the nature and volume of the solid waste received and the level of clay soils in the Solus area. Tindas 39 also pointed out that low level of nitrate in an area indicate low level of nitrifying bacteria which convert nitrogen to nitrate under aerobic condition. Phosphate, total hardness and acidity were observed to have weak correlation with nitrate in Solus as shown in Table 6.0.

The mean and range of phosphate value water collected around Olusosun were 0.39 ± 0.27(0.07 - 0.84) mg/L while that of Solus groundwater were 0.44 ± 0.22(0.22 - 0.96). Generally, the phosphate concentration in Solus observed was relatively higher than that of Olusosun. The result is similar to Ibadan and Lagos studies by Ikem [37]. However, it is lower than the values reported in Lebanon Basins [40]. This indicates low level of detergent pollutant from the landfills. The mean and range of Cu value water around Olusosun and Solus were 0.12 ± 0.17(0.01 to 0.23) mg/L and 0.08 ± 0.03 (0.03 to 0.15) mg/L respectively. Copper is an essential nutrient as well as a toxic element, depending on the level of its concentration. It acts as aco-factor in specific cupro- enzymatic reaction. High concentration of copper can cause gastrointestinal irritation in man. The sources of copper in groundwater include leachate from landfills, runoff from agricultural field and geochemical composition of the soil/rock holding the water.

The mean and range of iron values in water around Olusosun and Solus dumpsites 12.9 ± 7.0(4.12 – 31.2) mg/L and 11.7 ± 7.7 (0.36 – 20.1) mg/L. The rusting of water pipes often resulted in unnecessary high level of iron "red water". Other major sources of iron include nature of rock and soil which house the groundwater particularly the igneous rock, leachate from refuse dumps, industrial waste, and seepage from septic tank in residential areas. Iron is an essential element in human nutrient; it helps in the formation of hemoglobin which is important during pregnancy and lactation. Zinc is an essential trace element found virtually in all food and potable water. It is necessary in minimal quantity for animal and plant metabolism. However, high levels of zinc often result in zinc toxicity, which may cause cancer in the body system. 0.59 ± 0.58(0.12 – 1.90) mg/L and 0.86± 0.78(0.18 – 2.24) mg/L were mean and range of zinc values in the groundwater collected around Olusosun and Solus dumpsites respectively.

Sodium, Calcium, Potassium and Magnesium are of nutritional importance to life, so they are referred to as essential metals. They help in various metabolic activities in the human body system. The mean of sodium (Na) Sodium, in water around Olusosun and Solus were 171 ± 128 mg/L and 143 ± 109 mg/L respectively; potassium, 24.3 ± 8.8 mg/L and 20.3 ± 11 mg/L respectively; calcium, 59.0 ± 41 mg/L and 57.9 ± 48 mg/L and magnesium,10.5 ± 8.6 mg/L and 13.9 ± 7.0 mg/L. These minerals (K, Na, Mg and Ca) are probably derived from chemical weathering of feldspars as micas which are some of the minerals characterizing the plain sands. The three cations Na+, Ca2+ and Mg2+ are among the species that are constantly involved in cations exchange process and interactions with the aquifer materials. The concentration of these quality parameters were mainly compared to (WHO) guidelines for drinking water.

The extents by which some of the parameters exceed the WHO guidelines are expressed as exceedance level. This level is calculated as ratio of mean concentration of each water quality variable to respective guideline values and expressed with no unit41. exceedance level of variables such as nitrate, lead, iron, cadmium and magnesium are presented in Table 7.0.

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Table 3.0 : Concentration of physicochemical parameters and trace metals in groundwater around Olusosun dumpsite

Sample Code	pH	Tem	Alk	Acid	TH	EC	TDS	TSS	TS	Cl^-	PO_4^{3-}	NO_3^-	SO_4^{2-}	Cu	Ni	Pb	Cd	Fe	Zn	Mg	Ca	Na	K
OWS1	4.1	25.4	120	45.4	20.4	0.49	240	215	455	47.5	0.93	10.5	21.4	0.08	0.07	ND	0.01	10.1	0.22	8.13	10.3	190	31.1
OWS2	4.9	25.6	102	18.5	37.5	0.42	201	258	459	30.2	0.62	13.7	13	0.08	0.04	ND	ND	0.61	0.3	3.53	9.8	200	31.1
OWS3	3.9	25.2	373	21.7	43.4	1.13	483	509	992	132	0.15	23.8	19	0.07	0.03	ND	ND	3.99	0.34	4.34	9.48	175	43.2
OWS4	5.1	25.5	60.2	22.9	15	0.82	400	128	523	76.2	0.33	21.2	9.6	0.08	0.05	ND	0.01	9.04	0.27	3.97	10.1	200	30.7
OWS5	6.3	25	98.6	65.3	20.7	1.77	849	426	1245	62.8	0.18	17.5	13.1	0.11	0.02	ND	ND	15	0.12	4.2	9.01	123	25.1
OWS6	6.6	25.1	80.3	62.2	32.5	1.59	758	475	1423	59.9	0.07	10.6	12.9	0.1	0.06	ND	0.01	0.36	0.13	6.19	26.9	11.7	33
OWS7	5.4	25	24.5	41.5	42.5	0.52	209	114	323	100	0.23	35.3	22.4	0.11	0.03	ND	0.01	19.7	0.25	10.8	101	38.1	31.4
OWS8	4.8	25.4	218	25.4	20.6	0.91	311	123	434	23.9	0.36	14.4	11.7	0.23	0.02	ND	ND	21.3	0.22	6.12	118	26.2	25.5
OWS9	5.1	26.5	126	15.4	6.7	0.42	213	155	368	19.9	0.35	12.6	12.4	0.03	0.07	ND	ND	2.76	0.15	7.31	231	134	17.6
OWS10	5.2	26.4	132	13.5	4.8	0.41	214	223	475	89	0.84	10.7	17.3	0.08	0.03	ND	0.02	2.01	0.21	31.2	130	22.2	25.3
OWS11	6.7	24.8	119	88.4	27.3	0.82	321	247	568	75.9	0.65	12.9	21.5	0.11	0.06	ND	0.01	1.11	0.22	28.2	141	376	10.8
OWS12	7.1	25.1	122	73.4	20.6	1.0	421	337	758	91.1	0.53	11.8	20.5	0.13	0.05	ND	0.01	17.1	0.28	30	176	429	10.2
OWS13	4.6	25	219	60.5	108	1.03	541	363	904	86.3	0.07	7.7	12.5	0.05	0.05	ND	ND	20	0.25	8.02	40.1	304	30.2
OWS14	4.0	25.7	240	152	125	1.21	529	446	989	105	0.74	7.4	10.7	0.06	0.01	ND	ND	20.1	1.9	4.11	71.5	426	32.1
OWS15	4.3	25.2	223	56.7	65.6	1.09	529	484	1103	118	0.15	9.6	17.6	0.08	0.01	ND	ND	18.2	1.41	9.17	49.5	152	20.8
OWS16	5.2	25.5	141	100	116	1.26	621	542	1164	32.9	0.16	14.5	21.4	0.08	0.02	ND	ND	15.2	1.24	9.03	6.03	20	22.2
OWS17	4.5	27.4	94.2	22.4	34.1	1.38	610	515	1125	31.8	0.64	12.6	25.7	0.08	0.01	ND	ND	10.9	1.74	9.84	7.99	170	21.1
OWS18	4.4	26.5	71.1	33.3	23.3	0.72	331	468	799	36.1	0.57	12.8	23.6	0.06	0.01	ND	ND	14.2	0.94	8.39	22.1	100	20.1
OWS19	6.1	26	302	99.5	79.6	0.72	327	417	744	67.2	0.24	12.4	69.5	0.04	0.02	ND	ND	15.2	1.2	8.91	12.1	157	13.8
OWS20	6.4	25	152	43.8	22.6	1.41	697	429	1275	66.2	0.428	18.3	18.5	0.01	0.01	ND	ND	17.1	0.35	8.28	8.02	166	11.4
Mean	5.23	25.6	144	55.8	45.7	0.9	412	349	775	68	0.38	14.1	22.4	0.12	0.03	0	0.01	11.7	0.59	10.5	59	171	24.3
SD	0.97	0.7	91	37	36.4	0.42	179	151	327	32.7	0.27	6.4	17	0.17	0.02	0.01	0.01	7.7	0.58	8.6		128	8.8
WHO	6.5-8.5		1000		500	1.4	1000			250		10	250	1.5	15	10	3	2.5	3	50		200	

N.B : All units are mg/L except EC (mS/cm) and Temperature (°C) and pH with no unit

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Table 4.0. Concentration of physicochemical parameters and trace metals in groundwater around Solus dumpsite

Sample Code	pH	Tem	Alka	Acid	TH	EC	TDS	TSS	TS	$^{-}$ Cl	PO_4^{3-}	NO_3^{-}	SO_4^{2-}	Cu (mg/L)	Ni	Pb	Cd	Fe	Zn	Mg	Ca	Na	K
SWS1	4.80	26.9	59.6	37.5	15.9	0.44	598	243	842	299	0.260	0.840	8.37	0.10	0.08	ND	ND	5.43	1.67	31.2	40.3	95.0	5.43
SWS2	5.40	25.6	195	43.4	14.5	0.41	261	242	517	261	0.760	0.690	6.08	0.08	0.02	ND	ND	4.11	1.21	22.5	10.2	125	4.11
SWS3	6.90	25.7	310	167	108	1.01	1294	521	1872	378	0.380	1.55	25.3	0.07	0.02	ND	ND	6.86	0.89	11.1	19.0	19.9	6.86
SWS4	4.60	25.7	41.8	26.6	30.4	0.71	120	139	256	25.2	0.240	1.57	14.1	0.09	0.03	ND	ND	9.00	0.18	23.0	16.1	23.9	9.00
SWS5	5.30	25.3	10.5	6.60	13.5	1.62	802	411	1213	354	0.280	0.29	5.95	0.08	0.01	ND	ND	15.0	0.18	10.2	21.0	210	15.0
SWS6	5.90	25.8	29.3	62.8	24.7	1.41	724	425	1235.	107	0.360	1.59	22.0	0.11	0.06	ND	ND	2.00	0.32	10.0	26.2	103	2.00
SWS7	6.80	25.6	110	98.5	90.6	0.38	209	140	347	158	0.340	2.85	12.9	0.12	0.03	ND	ND	27.0	0.22	10.1	102	38.1	27.0
SWS8	6.60	25.7	291	96.8	202	0.64	300	167	478	34.9	0.270	2.92	16.6	0.05	0.01	ND	ND	21.3	0.39	8.21	116	126	21.3
SWS9	6.10	24.9	221	110	181.8	0.39	209	184	417	241	0.220	2.64	24.1	0.03	0.07	ND	ND	31.3	0.09	11.0	14.0	152	31.3
SWS10	5.80	26.3	376	157	210.4	0.33	211	243	452	235	0.440	1.96	11.1	0.09	0.03	ND	0.01	21.9	0.20	11.2	85.6	203	21.9
SWS11	5.90	26.7	65.8	17.4	44.50	0.71	302	269	670	139	0.340	0.820	3.24	0.12	0.07	ND	0.01	10.2	0.01	21.1	55.0	347	10.2
SWS12	4.70	26.2	12.9	39.1	61.80	1.00	397	364	769	138	0.320	3.80	9.35	0.15	0.02	ND	0.01	16.1	2.23	22.0	24.0	420	16.1
SWS13	5.80	25.7	125	86.9	72.60	1.02	517	365	883	43.0	0.390	3.44	8.37	0.05	0.05	ND	0.01	21.1	0.25	12.0	41.0	304	21.1
SWS14	6.10	25.8	14.5	27.5	36.40	1.09	511	473	985	135	0.850	1.65	14.2	0.06	0.02	ND	ND	20.0	1.87	8.22	74.1	22.0	20.0
SWS15	6.30	25.6	21.3	13.4	31.30	1.22	501	452	982	21.2	0.960	0.760	20.2	0.06	0.01	ND	ND	18.1	1.32	12.0	50.4	152.	18.1
SWS16	6.90	25.7	124	72.6	94.70	1.13	609	598	1206	17.4	0.700	0.180	11.4	0.04	0.01	ND	ND	15.5	2.24	4.12	125	20.0	15.5
SWS17	5.70	25.8	65.4	27.6	57.98	1.14	581	583	1070	75.6	0.540	0.47	14.7	0.07	0.01	ND	ND	4.10	1.99	9.00	28.0	112	4.10
SWS18	4.70	25.9	126	65.7	71.70	0.13	317	423	738	67.4	0.520	0.390	3.54	0.07	0.01	ND	ND	2.29	0.34	6.13	177	120	2.29
SWS19	4.80	25.8	143	71.4	56.40	0.60	314	465	772	112	0.360	0.260	14.4	0.05	0.01	ND	ND	2.00	1.11	18.0	112	155	2.00
SWS20	5.25	26.4	113	73.2	61.60	0.23	112	549	669	81.1	0.250	0.760	12.4	0.08	0.02	ND	ND	4.21	0.30	16.9	98.0	115	4.21
Mean	5.71	25.9	123	65.1	74.04	0.78	393	362	763	146	0.440	1.47	12.9	0.08	0.03	0.0	0.01	12.9	0.86	13.9	57.9	143	12.9
SD	0.75	0.5	106	44	60.03	0.42	186	149	289	112	0.22	1.1	6.4	0.03	0.02	0.0	0.01	9.0	0.78	7.0	48	109	9.0
WHO	6.5-8.5		1000.0		500.0	1.4	1000			250		10	250	1.5	15	10	3	2.5	3.0	50		200	

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Table 5.0: Correlation coefficient between physicochemical parameters in groundwater of Olusosun area

Temp	-0.21718	1.0										
Alkalinity	-0.40342	0.066897	1.0									
Acidity	0.268037	-0.4445	0.159297	1.0								
TH	-0.21486	-0.30143	0.382054	**0.732725**	1.0							
EC	0.060558	-0.21612	0.189625	0.249704	0.245912	1.0						
TDS	0.03774	-0.20213	0.185418	0.240107	0.263872	**0.985504**	1.0					
TSS	-0.05724	0.019458	0.235771	0.452321	**0.524919**	**0.537175**	**0.554534**	1.0				
TS	-0.02186	-0.12031	0.241032	0.379682	0.45227	**0.90107**	**0.906597**	**0.834783**	1.0			
Cl	-0.05185	-0.34861	0.393472	0.255431	0.256245	0.137506	0.131044	0.176222	0.151613	1.0		
PO_4	-0.07713	0.178037	-0.18431	-0.05344	-0.32311	-0.37861	-0.41783	-0.32448	-0.44323	-0.1604	1.0	
NO_3	0.04809	-0.17466	-0.18086	-0.32171	-0.23698	-0.0617	-0.12299	-0.35315	-0.19063	0.199378	0.29723	1.0
SO4	0.300221	0.094885	0.015351	0.357699	0.227248	-0.33853	-0.34706	0.332921	-0.04984	0.013524	0.14355	0.08608	1.0

Table 6.0: Correlation coefficient between physicochemical parameters in groundwater of Solus area

pH	1.0												
Temp	-0.33669	1.0											
Alkalinity	0.344473	-0.10326	1.0										
Acidity	0.434588	-0.12313	**0.869089**	1.0									
TH	0.412146	-0.18854	**0.816777**	**0.770859**	1.0								
EC	0.247354	-0.28313	-0.46869	-0.36718	-0.36674	1.0							
TDS	0.147805	-0.34406	-0.4795	-0.37336	-0.3696	**0.93467**	1.0						
TSS	0.108837	0.024777	-0.23197	-0.10425	-0.28894	0.451343	0.482027	1.0					
TS	0.167952	-0.19255	-0.44806	-0.30514	-0.40398	**0.851505**	**0.901455**	**0.796885**	1.0				
Cl	0.01982	-0.0109	0.28214	0.262232	0.010354	-0.01232	-0.10794	-0.14883	-0.14586	1.0			
PO_4	0.294353	-0.15374	-0.15364	-0.2637	-0.24819	0.246632	0.241836	0.398825	0.35071	-0.23463	1.0		
NO_3	0.165828	-0.13376	0.196807	0.36781	0.453334	-0.06226	-0.17714	-0.48943	-0.35521	-0.05891	0.32869	1.0	
SO_4	0.4809	-0.4529	0.246798	0.421234	0.325313	0.220085	0.102509	0.109578	0.128819	0.028624	0.00475	0.213417	1.0

This approach according to Saroj42 has effectively visualized the levels. The order concentration levels of different water quality parameters in relation to the non-uniform increase in exceedance value in Olusosun groundwater sample was Mg > Fe > Cd > NO_3- -N >Pb, while in that of Solus groundwater, order concentration levels of different water quality parameters was Mg > Fe > Cd > Pb > NO_3- -N. Apart from the leachate as a major probable source of these pollutants in groundwater, there are other possible sources. The major source of magnesium (Mg) in the groundwater for instance, is due to ion exchange of minerals in rocks and soils by water[41].

Table 7.0: Exceedance levels of groundwater parameters with respect to WHO

Dumpsite	NO_3^-	Cd	Pb	Mg	Fe
Olusosun	1.57	1.8	1.0	21.1	11.5
Solus	-	1.85	1.0	20.7	13.4

b) Assessment of the water quality

The quality of groundwater under consideration in respect of drinking purpose has been established based on the WHO guidelines for drinking water. The calculated WQI values of these water samples put them water in excellent - good – poor class for Solus water and good - poor – very poor class for Olusosun using classification shown in Table 2.0. WQI vales ranged from

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39.1 to 126 mg /L and from 61.8 to 205 mg /L respectively for Solus and Olusosun water. The spatial variations revealed that about 10% of the samples collected around Solus are in excellent grade; 65%, good and 25%, poor for Solus water, while in Olusosun, it is 35% good, 55% poor and 5% very poor. Some sampling locations are critical to human health and need urgent attention. These locations include OWS 3, OWS 4, OWS 8, OWS 14, OWS 17 and OWS 18 around Olusosun area and SWS1 and SWS 12 around Solus area.

IV. Conclusion

The level of physico-chemical parameters in groundwater around Olusosun dumpsite was observed to be higher than the corresponding groundwater around Solus area. Age of Olusosun dumpsite, nature and volume of solid waste deposited as well as the percentage of clay content in the soil from the sampling area could be some of the factors responsible for the observation. All groundwater samples analyzed from the study area are acidic in nature. This might be as a results of high population density and industrialization in the area, consequently, the high level of CO2 in the atmosphere can result in acidic nature of water that percolates into aquifer. Very low pH in water is a form of health treats to human. Correlation coeficiency applied shows possible contribution of contaminant from diverging multiple sources. Water quality index (WQI) further revealed that the impact of the dumpsites is still minimal in groundwater around Solus dumpsite compared to Olusosun dumpsite. WQI delineate water samples around Solus dumpsite as excellent – good – poor and that of Olusosun dumpsite as good – poor – very poor. The level of Chloride in groundwater around solus dumpsite demonstrates a buildup of the anion in the aquifer and this may continue as the dumpsite matures. Almost all the groundwater collected around Olusosun dumpsite showed level of nitrate higher than that WHO permissible limit of 10 mg/L. This situation is of great health risk as nitrate pollution has been linked to mith and sometimes death. Some sampling locations in both dumpsites with very poor quality water samples require some treatment measures to make the water therein potable.

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Phytochemical and Therapeutic Studies of the Fruit Essential Oil of *Thuja orientalis* from Nigeria

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Abstract- The aim of this study was to establish correlations between the identified phytocompounds in the fruit essential oil of Thuja orientalis from Nigeria and their medicinal properties (free radical scavenging, antioxidant and antimicrobial). Forty-seven compounds were identified in the fruit essential oil of T. orientalis making up 97.5% of the total percentage composition. The essential oil was predominantly made up of monoterpenoids (62.2%). The most abundant components was 1R-**α**-pinene (15.2%) followed by **α**-pinene (9.6%), 1S-**α**-pinene (5.6%), cyclofenchene (5.6%), (+)-3- carene (4.5%), DL-pinene (4.5%) and trans-***β***-ocimene (4.0%). The high amount of terpenoids leads to more potent radical scavenging, antioxidant and antibiotic properties. The essential oil showed high potential as natural antioxidant and free radical inhibitor with IC50 value: 2.5µgml-1, the percentage inhibition of free radical ranged between 68-70%. Screening of the fruit essential oil for antimicrobial activities using the agar-well diffusion assay showed that the oil had high antibacterial properties against all bacteria isolates tested with zones of inhibition ranging from 10-30 mm.

Keywords: Thuja orientalis, fruit essential oil, phytochemical, antioxidant, antibacterial.

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PHYTOCHEMICALANDTHERAPEUTICSTUDIESOFTHEFRUITESSENTIALOILOFTHUJAORIENTALISFROMNIGERIA

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Phytochemical and Therapeutic Studies of the Fruit Essential Oil of *Thuja orientalis* from Nigeria

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Keywords: Thuja orientalis, fruit essential oil, phytochemical, antioxidant, antibacterial.

I. Introduction

Essential oil is one of the plant based secondary metabolites that are used as the basis of many of the modern pharmaceuticals used today to treat some major diseases (Buchbauer, 2000). Essential oils are more environmentally friendly than synthetic products because they are biodegradable and have no residual effect. The impacts of essential oils and aroma-chemicals on the nervous system, gastrointestinal system, immune system, respiratory system, antimicrobial and antifungal activities have in recent years been the area of interest for researchers. Currently, there is global interest in finding new and safe antioxidants and antibiotics from natural sources, to prevent oxidative deterioration of foods and to minimize oxidative effect on living cells. The antioxidant and antimicrobial potentials of several essential oils extracted from odoriferous medicinal plants are very important to human because antioxidants inhibit free oxygen radicals and free radicals formed from the substrate by donating hydrogen atoms or electrons (Prior *et al*., 2005). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raise serious concern of health delivery and accessibility due to untreatable infections. There is therefore the needed urgency to the search for safe and more active antibiotics. Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents (Savithramma *et al*., 2011; Ibrahim *et al*., 2012).

Thuja orientalis (*Cupressaceae*) commonly known as arborvitae, is a medicinal plant whose leaves have been locally used to treat flu and cough, high blood pressure, bleeding arthralgia, cancer, haemostatic, gout, rheumatism, diarrhoea, and chronic tracheitis (Zhu *et al*., 2004). Homeopathic preparations of *T. orientalis* include pills, granules, oils, ointments and liquid dilutions. Due to its scent it has been traditionally used for clothing protection from moths and nowadays is added to pest repellant sprays and paints to protect against mites, moths, and rodents.

To the best of our knowledge, there is paucity of information on the phytochemicals, free radical scavenging, antioxidant and antimicrobial potentials of fruit of this plant from Nigeria. Therefore present study was undertaken for the first time with the aim at looking into these quantitative and qualitative parameters in the fruit of *T. orientalis grown in Nigeria*.

II. Material and Methods

a) *Identification, Collection of Plant Material and Isolation of the Essential Oil*

Fresh fruits of the plant were collected from its natural habitat in Ota, Nigeria and it was authenticated as *Thuja orientalis* (*Cupressaceae*). Fruit of the plant was subjected to hydro-distillation for 2 hours using a Clevenger-type apparatus and the essential oil extracted was stored at -4°C in a refrigerator (European Pharmacopoeia, 2004).

b) *GC and GC-MS Analyses*

Analysis of the fruit essential oil of *T. orientalis* was performed using multi-dimensional gas

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chromatograph coupled with Gas Chromatography-Mass Spectrophotometer (Shimadzu, Japan) equipped with non-polar and polar double capillary columns (25.0 m x 0.25 µm i.d., 0.25 µm df). High purity helium was used as the carrier gas at a constant flow rate of 0.99 ml/min. A total of 1 µl sample was injected (split ratio 100:1) into GC and GCMS using AOC20i auto injector for analysis. The initial temperature was set at 60°C, heated at a rate of 3℃/minutes to 280°C and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200°C, while the interface temperature was set at 250°C, solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40-700 a.m.u. at 0.50 scan/s. The constituents were identified by comparison of their retention indices with those of the literature. The retention indices were determined in relation to a homologous series of *n*-alkanes under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in National Institute for Standards and Technology (NIST) and with mass spectra from literature.

c) Free Radical Scavenging and Antioxidant Capacity

The free radical scavenging and antioxidant activities of the fruit essential oil against the stable free radical DPPH were measured using different concentrations (1000, 100 and 10 μgml^{-1}) of the essential oil in methanolic solution of DPPH. After 30 minutes of incubation at room temperature in the dark, the absorbance at 517nm was measured spectrophotometrically. Ascorbic acid was used as reference compound. The assay was carried out in triplicate. The percentage inhibition (I%) for each concentration was calculated by using the absorbance (A) values according to the following formula:

$$I\% = [(A_{blank} - A_{eo})/A_{blank}] \times 100$$

Where: A_{blank} is the absorbance of blank solution and A_{eo} is the absorbance of different concentrations of the essential oil. The dose-response curve was plotted and IC_{50} value for the essential oil and the standard were calculated (Formagio *et al*., 2011).

d) Antimicrobial Activity

Antibacterial activities of the fruit essential oil of *T. orientalis* were measured against Grampositive bacteria *(Streptococcus agalactiae, Streptococcus species and Staphylococcus aureus)*and Gram-negative bacteria *(Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Salmonella typhimurium)* using agar-well diffusion method. Briefly, Petri dishes containing 20 ml of nutrient agar medium were swabbed using cotton applicator with an overnight starter culture of the bacteria isolates which were prepared in dilution to match the turbidity intensity of the 0.5 McFarland standards. Wells (6mm diameter) were punched in the agar and filled with 10µl of different concentrations of the essential oil. The plates were incubated at 37°C for 24 hours. The quantification of microbial growth inhibition was determined by measuring the diameters (mm) of clear zones of microbial growth including the well itself. Gentamicin (GEN) and augmentin (AUG) were used as control (Pimentel *et al.,* 2013).

III. Results and Discussion

a) Essential Oil Composition

Hydrodistillation of the fruits of *T. orientalis gave* a crude essential oil with pleasant odour. Forty-seven compounds were identified from the GC and GC-MS analyses of the fruit essential oil amounted to 97.5% of the percentage total composition (Table 1). The most abundant components was 1R-α-pinene (15.2%) followed by α-pinene (9.6%), 1S-α-pinene (5.6%), cyclofenchene (5.6%), (+)-3-carene (4.5%), DL-pinene (4.5%) and trans-β-ocimene (4.0%). The principal classes of organic compounds in the fruit essential oil were monoterpenes (62.2%), sesquiterpenes (8.3%), sesterpenes (5.4%) and diterpenes (1.6%).

Comparatively, the chemical composition of this fruit essential oil was different from those reported in other studies such as foliage essential oils of four varieties of *Thuja* species from Poland which are *T. occidentalis 'globosa'*, *T. occidentalis 'aurea'*, *T. plicata* and *T. plicata 'gracialis'*. The study of the four samples resulted in the identification of thirty-one compounds in the essential oil of *T. occidentalis* "*globosa"* (96.92%) while in the oil of *T. occidentalis "aurea"* twenty-seven constituents have been identified (94.34%), major constituents in both were:α-thujone (50.14 and 51.60%, respectively), beyerene (8.54% and 11.28%, respectively), sabinene (4.55% and 3.43%respectively) and camphor (4.47 and 3.09 % respectively). The characteristic difference between them is that T. *occidentalis "globosa"* has a high content of the ketones (β-thujone and fenchone), while *T. occidentalis "aurea"* has high levels of the diterpene (rimuene). The chemical profiles of the oils of the two *T. plicata* were also comparable, as thirty-two compounds have been identified in *T. plicata* (94.75%) and thirty in the oil of *T. plicata* "*gracialis"* (96.36%) having also α-thujone (62.12% and 54.48%, respectively), β-thujone (7.06% and 6.39%), terpinen-4-ol (4.66% and 3.11%) and sabinene (6.00% and 2.94%) as the most abundant compounds. On the other hand, *T. plicata* shows higher content of the ketone (fenchone), while *T. plicata* "*gracialis"* has high levels of the diterpene (beyerene) (Tsiri *et al*., 2009), but fruit essential oil of *T. orientalis* was characterised with high hydrocarbon terpenes rather than ketone. The above results show that the fruit essential oil of *T. orientalis* grown in Nigeria could serve

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as good source of these pharmaceutical and industrial useful compounds.

Table 1: Chemical Composition of the Fruit Essential Oil of *T. orientalis*

Compounds	% Composition	RI
cyclofenchene	5.6	729
artemesia triene	1.0	896
(+)-sabinene	1.0	897
2-bornene	1.0	932
1R-α-pinene	15.2	937
α-pinene	9.6	938
1S-α-pinene	5.6	941
DL-pinene	4.5	943
(+)-2-carene	1.0	948
camphene	0.4	952
β-mycene	1.5	958
1S-camphene	0.4	964
β-pinene	1.5	970
trans-β-ocimene	4.0	976
(+)-3-carene	4.5	1003
4-methyl-3-(1-methylethylidene)-1-cyclohexene	1.0	1023
α-terpinolene	1.0	1052
camphene hydrochloride	0.4	1069
L-4-terpineol	1.0	1137
4-terpineol	2.0	1174
2-cyclopropylidene-1,7,7-trimethylbicyclo[2.2.1]heptane	0.2	1251
megastigma-7(*E*),9,13-triene	0.2	1278
α-terpineol acetate	1.0	1333
aromadendrene	0.5	1386
α-bergamotene	0.8	1430
γ-muurolene	0.2	1435
α-amorphene	0.2	1440
α-farnesene	0.8	1458
β-*cis*-caryophyllene	1.0	1477
β-*trans*-caryophyllene	1.0	1494
α-humulene	1.6	1497
germacrene	0.2	1515
L-globulol	1.0	1530
ledol	2.0	1565
Z,Z,Z-1,5,9,9-tetramethyl-1,4,7-cycloundecatriene	0.4	1579
epi-globulol	1.0	1582
globulol	1.0	1588
13-tetradecenal	2.8	1591

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9Z-9-tetradecenal	2.8	1609
α-bisabolol	0.4	1625
cis-9-hexadecenal	2.8	1808
dichloroacetic acid, undec-2-enyl ester	2.8	1834
n-pentadecanoic acid	1.6	1869
palmitic acid	3.0	1968
cis-9-octadecenal	2.8	2007
arachidic acid	1.6	2366
1-(+)-ascorbic acid 2,6-dihexadecanoate	1.6	4765
Percentage Total	**97.5**	

RI = Retention Index

b) Radical Scavenging and Antioxidant Activities

The free radical scavenging and antioxidant properties of the fruit essential oil of *T. orientalis* were examined using stable DPPH. The essential oil showed high potentials as a natural antioxidant and free radical inhibitor with IC_{50} value 2.5μgml^{-1}, the percentage inhibition of free radical was 68% at 10μgml^{-1}, 69% at 100μgml^{-1} and 70% at 1000μgml^{-1}. The results of antioxidant and free radical scavenging activities showed that the fruit essential oil of *T. orientalis* from Nigeria had better radical scavenging and antioxidant properties than the aerial (twigs) ethanolic extract of *Thuja occidentalis (Cupressaceae)* with DPPH IC_{50} values of 202.45µgml^{-1} and percentage radical scavenging values of 73% at concentration 300µgml^{-1}, 62.7% at 250μgml^{-1}, 50% at 200μgml^{-1}, 43.5% at 150μgml^{-1}, 16.8% at 100μgml^{-1} (Dubey and Batra, 2009) while the fruit methanolic extract of *T. occidentalis* had IC_{50} values of 150.98 μgml^{-1} (Das and Rani, 2013). Natural antioxidants are essential for helping the body maintain its natural health. They protect cells from free radicals, harmful oxygen molecules thought to damage cells that result in cancer, atherosclerosis and rheumatoid arthritis. Natural antioxidants also have many industrial uses, such as preservatives in food and to prevent the degradation of rubber and gasoline (Hamid *et al.,* 2010). The benefits of antioxidants are very important to good health, because if free radicals are left unchallenged, they can cause a wide range of illnesses and chronic diseases. The human body naturally produces free radicals and the antioxidants to counteract their damaging effects. However, in most cases, free radicals far outnumber the naturally occurring antioxidants. In order to maintain the balance, a continual supply of external sources of antioxidants is necessary in order to obtain the maximum benefits of antioxidants. Therefore, natural antioxidants benefit the body by neutralizing, removing the free radicals from the bloodstream, protects the cells against their toxic effects and contribute to disease prevention (Pham-*Huy et al*., 2008).

Table 2 : IC_{50} of the Antioxidant Properties of the Fruit Essential Oil of *T. orientalis*

Essential Oil and Reference Compound	DPPHIC_{50} μgml^{-1}
T. orientalis	2.5
Ascorbic acid	9.0

Data are presented as triplicate of the mean

c) Antibacterial Potentials

The fruit essential oil of *T. orientalis* was screened for the antibacterial activities against clinically isolated Gram-positive and Gram-negative bacteria with the following results as shown in table 3 below. The antibacterial activities obtained were categorized as follows: (a) - = no inhibition, (b) 6-9 mm = low inhibition, (c) 10-15 mm = moderate inhibition and (d) ≥ 15 mm = high inhibition. The antimicrobial properties of the fruit essential oil showed different selectivity for each organism. The results revealed that the fruit essential oil have high activities against all the tested organisms with the inhibition zones ranging from 10-30mm. The difference in susceptibility of the bacteria to the essential oil is thought to arise as a result of the differences in their cell membrane structure (Angienda *et al.,* 2010).The fruit essential oil of *T. orientalis* gave a comparable result with other related species in the *Cupressaceae* family such as *Thuja occidentalis*: *S. aureus* (17mm), *E. faecalis* (11mm), *E. coli* (11mm), *P. vulgaris* (-), *P. aeruginosa* (9mm), *Salmonella sp.* (11mm), *K. pneumoniae* (13mm). Moreover, *Thuja plicata* shows the following activities with the following organisms: *S. aureus* (30mm), *E. faecalis* (10mm), *E. coli* (11mm), *P. vulgaris* (7mm), *P. aeruginosa* (10mm), *Salmonella sp.* (11mm), *K. pneumoniae* (-) (Jirovetz et al., 2006), *Thuja koraiensis* extract gave the following zones of inhibition with *S. aureus* (17 mm), *B. subtilis* (13 mm), *E. coli* (15 mm) and *S. typhimurium* (12 mm) (Zhang *et al.,* 2014) while the fruit essential oil of *Juniperus excelas (Cupressaceae)* has no activity

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against *S. aureus, E. coli* and *P. aeruginosa* (Weli *et al.*, 2014).

Table 3 : Zones of Inhibition (mm) showing the Antimicrobial Properties of the Fruit Essential oil of *T. orientalis*

	Fruit Essential Oil			GEN	AUG
Conc. / Organism	1000	100	10	10µg	30µg
E. coli	14	-	-	22	-
K. pneumoniae	15	15	15	21	-
P. aeruginosa	12	12	-	20	11
P. mirabilis	10	10	10	20	-
S. agalactiae	15	13	10	-	-
S. aureus	13	12	11	-	-
S. typhimurium	14	14	12	21	-
S. species	30	30	30	-	-

Keynote:--- = Resistant, 6-9 mm = low inhibition, 10-14 mm = moderate inhibition and ≥ 15 mm = high inhibition.

IV. Conclusion

This study demonstrated that the fruit essential oil of *T. orientalis* produces a pronounced free radical scavenging, antioxidant and antimicrobial activities and could be further investigated for possibility of developing a cheap, acceptable and easy available therapeutic agent. The study therefore not only reveals the plant excellent natural antioxidants to be utilized nutritionally and pharmaceutically, but also provides good scientific justification for increased in traditional use of the plant.

Conflict of interest: We have no conflict of interest.

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The Mechanism of Hail Formation and a Method to Prevent it

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Abstract- The new hypothesis about the building mechanism of hail showers is made under atmosphere conditions. It is suggested, contrary to other famous theories that hail showers building is stipulated by the generation of high temperature in lightning strike in atmosphere. Quick water evaporation along and around the discharge channel leads to its rough freezing with the advent of hail showers of different sizes. The transition of zero-degree isotherm is not necessary for the building of hail showers; they are formed in the lower atmosphere. Storm is accompanied by hail showers. Hailstorm is observed only in case of severe thunderstorm.

In contrast to the previous well-known theory of hail showers, in this paper we propose an original and simple method to prevent hail with the use of a lightning rod. Lightning rod in turn, can prevent together with hail and lightning discharge.

Keywords: hailstone; zero temperature; evaporation; cold; lightning, storm.

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The Mechanism of Hail Formation and a Method to Prevent it

Sokhrab Ismailov

Abstract- The new hypothesis about the building mechanism of hail showers is made under atmosphere conditions. It is suggested, contrary to other famous theories that hail showers building is stipulated by the generation of high temperature in lightning strike in atmosphere. Quick water evaporation along and around the discharge channel leads to its rough freezing with the advent of hail showers of different sizes. The transition of zero-degree isotherm is not necessary for the building of hail showers; they are formed in the lower atmosphere. Storm is accompanied by hail showers. Hailstorm is observed only in case of severe thunderstorm.

In contrast to the previous well-known theory of hail showers, in this paper we propose an original and simple method to prevent hail with the use of a lightning rod. Lightning rod in turn, can prevent together with hail and lightning discharge.

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I. Introduction

People are often confronted with terrible natural phenomena of nature and constantly fight against them. Natural disasters and the consequences of catastrophic natural events (earthquakes, landslides, lightning, tsunamis, floods, volcanic eruptions, tornadoes, hurricanes, hail) attracted the attention of scientists from all over the world. It was not by chance, when UNESCO had established a Special Commission on account of UNDRO disaster (disaster response of the United Nations, the United Nations). Knowing the objective world and acting in accordance with it, the man subdues nature forces them to serve their purposes and turns from a slave to nature in the Lord of nature and ceases to be powerless before nature, becomes free. One of those scourges is hail.

The fall of the hail, first of all, destroys cultural combines of plants, kills livestock, as well as the man himself. The fact of the matter is that a sudden and large influx of offensive hail eliminates protection against it. Sometimes the surface is covered with minute read barrage with a thickness of 5-7 cm. In the area of Kislovodsk in the 1965 year was hail, covering the ground layer in 75 cm. Normally hail covers the 10-100 km distance. Let us remember some terrible events of the past.

In 1593, in one of the provinces of France due to the raging winds and lightning knocked out the sparkling hailstones with a huge weight of 18-20 pound! This has caused great damage to crops and destroyed many churches, castles, houses and other structures. The victims of this terrible event began and the people themselves. *(Here it is necessary to consider that in those days the pound as a unit of weight has several meanings).* It was a terrible natural disaster, one of the most disastrous hailstones that hit France. In the eastern part of the State of Colorado (United States) annually about six hailstones, each of them brings great losses. Hail most frequently occur in the North Caucasus, Azerbaijan, Georgia, Armenia, in the mountains of Central Asia. With 9 on June 10, 1939, in the city of Nalchik fell hail size about an egg, accompanied by strong rain. In the result has been destroyed more than 60 thousand hectares of wheat and about 4,000 hectares of other crops; killed 2,000 sheep. When it comes to hail, first of all, pay attention to the size of it. Hail, as usual, vary in size. It is interesting to know about absolutely fantastic hail. In India and China it was found falling from the sky ice blocks weighing 2-3 kg. Even say that in 1961 in North India heavy hailstones killed an elephant. 04.14.1984 In the small town of Gopalganj district of Bangladesh hail fell 1 kg, which killed 92 people and several dozen elephants. Even this hail entered the Guinness Book of Records. In 1988, 250 people in Bangladesh were victims hail. And in 1939, the hail was found with a weight of 3.5 kg. More recently (20/05/2014) in Sao Paulo, Brazil, large hailstones, many of which were recovered from the streets with the help of heavy machinery. All these data show that life with a barrage important than the nature of the unusual phenomenon. On this basis, a comprehensive study of the causes and finding his formation using modern physical and chemical methods of research, as well as the fight against this terrible natural phenomenon is the primary problem facing humanity around the world.

II. What is the Mechanism of Hail Formation

In advance, I note that there is still no proper and positive response to this question. Despite the creation of the first hypothesis about this back in the first half of the 17th century with Descartes, however, scientific theory hail processes and practices impact on Physics and meteorologists have developed only in the middle of the last century. It should be noted that even in the middle ages and in the first half of the 19th

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century there have been several assumptions of different researchers, such as, Bussengo, Swedes, Klossovskiy, Volta, Rayee, Ferrell, Gan, Faraday, Zonke, Reynold, etc. Unfortunately, their theory did not receive their confirmation. It should be noted that recent views thereon do not constitute scientific validity, and there is still no comprehensive views on the mechanism of hail formation. The presence of numerous experimental data and a set of literary materials, dedicated to this topic were given the opportunity to assume the next formation mechanism of hail, which was recognized by the World Meteorological Organization and continues to operate so far *(to avoid controversy, we literally give these arguments)* [1, 6, 21]:

"Rising from the Earth's surface on a hot summer day, the warm air cools with height, and the moisture is condensed, formed a cloud. Cooled liquid droplets in the clouds are found even when the temperature is –40 °C at a height of about 8-10 km). But these drops are very unstable. Raised from the Earth's surface the smallest particles of sand, salt, combustion products and even bacteria with super cooled drops disturb the fragile balance. Cooled liquid drops that came into contact with solid particles, turn into an ice embryo of hailstones.

Small hailstones are in the top half of almost every cumulonimbus clouds rain, but most often such hailstones when approaching the Earth's surface are melting. So, if the speed of the upward flow in a cumulonimbus cloud rain reaches 40 km/h, they cannot keep the hailstones formed, therefore, passing through a warm layer of air at a height of 2.4 to 3.6 km, they fall out of the clouds in the form of small soft hail or rain at all. Otherwise, the updrafts lift the small hailstones up to layers of air with temperature from-10° c to-40° c (height between 3 and 9 miles), a diameter of hailstones begins to grow, reaching sometimes several centimeters. It should be noted that in exceptional cases the speed of ascending and descending cloud flows can reach 300 km/h! And the higher the speed the upward flow in a rain cloud, cumulonimbus, the larger the hail.

For the formation of hailstones the size of a golf ball more than 10 billion needed super cooled water drops, and hailstone cloud must remain at least 5-10 minutes to reach such a large size. It should be noted that a single drop of rain is about a million such small super cooled droplets. Hailstones with a diameter of more than 5 cm in cumulonimbus clouds, which are very powerful rising air currents. It is thunderstorms produce tornadoes, heavy downpours and gusts.

Hail usually falls when severe thunderstorms in the warm season, when the temperature at the Earth's surface not is below 20 °C ".

It must be stressed that even in the middle of the last century, or rather, in 1962; p. By Ludlam also proposed a similar theory [16] provides the education prerequisite hailstones. They also discussed the formation of hailstones in the super cooled part of the cloud of small water droplets and ice crystals by coagulation. Last operation must be strong lifting and lowering of the hailstones several kilometers, passing a null isotherm. On the type and size of hailstones and modern scholars say that the hailstones during his life repeatedly to get up and down the strong convection currents. As a result of the collision with the super cooled drops of hailstones increase their size. The World Meteorological Organization in 1956 gave a definition of what a hail*: "Hail-precipitation in the form of spherical particles or pieces of ice (hailstones) with a diameter ranging from 5 to 50 mm, sometimes more, falling separately or in the form of incorrect complexes. Hailstones consist only of transparent ice or of a number of layers with a minimum thickness of 1 mm, alternating with translucent layers. Hail is observed usually in strong thunderstorms ".*

Almost all of the former and contemporary sources on the subject indicate that the hail is formed by a powerful concentrate the cloud at strong upward air flows. This is true. Unfortunately, most forgotten about lightning and thunderstorms. And the subsequent interpretation of the formation of hailstones, in our view, It is not logical and pointless.

Professor Klossovskij carefully reviewed the appearances of hailstones and discovered that they also have a number of spherical form other geometric forms of existence [8]. These data indicate the formation of hailstones in the troposphere on a mechanism. After reading all of these theoretical perspectives, caught our attention several intriguing questions:

1. Composition of the clouds, found at the top of the troposphere, where temperature is approximately — 40 °C operating system already contains a mixture of super cooled of water droplets, ice crystals and sand particles, salts, bacteria. Why not disturbed the fragile power balance?

2. With the recognized modern general theory [1, 6, 21], hailstone could emerge and no lightning or thunder storm. For the formation of hailstones with a larger size, small ice must climb a few kilometers up (at least 3-5 km), and fall down, passing a null isotherm. While this should be repeated until it was formed in a sufficiently large amount of hail. Still the same, the greater the upward flow velocity in the cloud should get, the bigger the hailstone (from 1 kg to several kg) and for the consolidation of it should remain in the air for 5-10 minutes. Interesting!

3. In general, it is difficult to imagine that in the upper atmosphere will focus as huge ice blocks weighing 2-3 kg? It turns out that the hailstones were still large in cumulonimbus cloud rain than seen on earth because of it melted when falling through the warm layer of the troposphere.

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4. Because meteorologists often confirm: ". ... hail usually falls when severe thunderstorms during summer, when the temperature at the Earth's surface is below 20 °C ", however, does not indicate the cause of the phenomenon. Of course, the question is: what is the effect of a lightning storm? Hail almost always falls to the shower or at the same time with him and never after. He falls for the most part in the summer and during the day. Hail at night - a very rare phenomenon. The average duration of hail - from 5 to 20 minutes. Hail as usual, going to the place where there is a strong bolt of lightning, and is always associated with a thunderstorm. *Without lightning hail does not happen!* Hence, the reason for the formation of hail, we need to look for in it. The main drawback of all existing mechanisms for the formation of hail, in our opinion, is the lack of recognition of the dominant role of the lightning discharge.

Studies of the distribution of hail and thunderstorms in Russia, produced A.V. Klossowski [8] confirm the existence of a particularly close link between these two phenomena: hail with thunderstorms usually happens in the south-eastern part of the cyclone; he often where most thunderstorms. North of Russia is poor cases of hail, in other words, hail, the cause of which is explained by the lack of strong lightning. And what role is played by lightning? No explanation several attempts to find a connection between the hail and the storm still were in the middle of the 18th century. [9]. Guyton de Morvo Chemist, rejecting all the existing ideas, offered his theory: electrified the cloud better conduct electricity [20]. But Nolle [23] put forward the idea that water evaporates faster when she electrified and reasoned that it must amplify the chill and the fantasized that couples can become the best conductor of heat, if you put electricity on them. Guyton criticized Jean Andre Monsey and wrote, [22]: it is correct that electricity increases evaporation, but electrified drops must mutually repel and not merge into large hailstones. The electrical theory of hail was offered another famous physicist Alexander Volta [25]. In his view, the electricity was used as the root causes of the cold, and to explain why hailstones remain suspended for so long, that manage to grow. Cold is the result of very rapid evaporation of clouds, which contributed to the strong sunlight, rarefied dry air, lightness of volatilization of bubbles, which are made of clouds, and the estimated effect of electricity, which helps evaporation. But as the hailstones are kept in the air for sufficient time? For this reason Volta to look only for electricity. Well, how?

Anyway, to the 20 years of the 19th century there was a general belief that the combination of hail and lightning simply means that both of these effects occur when the same weather conditions. This was clearly expressed in 1814; the view of von Bush [17] and in 1830 it was strongly asserted Olmsted of Denison Ielâ [24]. Since then, the theory of hailstones was mechanical and based more or less steadily on perceptions of ascending air flows. On the theory of Ferrell [18], each hailstone may repeatedly fall and rise. The number of layers in the hailstone, which are sometimes up to 13, Ferrell′ judged the speed of hailstone. Circulation occurs until the hailstones do not become very large. By his calculation, rising current speeds of 20 m/s is unable to hail in 1 cm in diameter, and the speed for tornadoes is quite moderate.

There are a number of relatively new scientific research [11, 13, 14], devoted to the issues of formation of hail. In particular, argue that the history of the hail is reflected in its structure: large hailstone, cut in half, is similar to the root: it is composed of multiple layers of ice. Sometimes hailstones like pie, where alternate ice and snow. And this is his explanation for such layers, you can calculate the number of times a piece of ice was on a journey from the rain clouds in the supercool layers of the atmosphere. It is hard to believe: Grad with a weight 1-2 kg could jump back up to distances of 2-3 km? The layering of ice (hailstones) can appear for a variety of reasons. For example, the difference between the ambient pressures will cause such a phenomenon. There have already talked about the snow. Where is the snow? Unclear.

In a recent site http://tornado2.webnode.ru/-obrazovanie-grada/YegorChemezov puts forward his idea and tries to explain the formation of hail and his ability to stay for a few minutes in the air with the advent of the "black hole" in the cloud. In his view: "The hailstone has a negative charge. The greater the negative charge of the object, the less the concentration of ether (physical vacuum) in the site!? And the less the concentration of air in the material object, the more anti-gravity he possesses. On Chemezov, the black hole is a good trap for hailstones. Once, lightning, is a negative charge, and start falling hailstones.

Analysis of the literature shows that in this area of science has a lot of flaws and often speculation.

On completion of the all-Union Conference in Minsk in 13.09.1989, devoted to the theme: "Synthesis and investigation of prostaglandins," we are in the middle of the night with the staff of the Institute were returning by air from Minsk in Leningrad. A flight attendant reported that our plane flies at an altitude of 9 km. We watched the monstrous spectacle. Below us from us in a distance of about 7-8 km (just above the surface of the Earth) that was a terrible war. These were powerful storm level. While the above our cloudy and shining star. And when we were over Leningrad, reported to us that an hour ago in the city the hail fell out with the rain. With this episode I want to emphasize, that the strong lightning often sparkles closer to the ground. For the occurrence of hail and lightning did not necessarily raise the flow of cumulonimbus at the height of 8-10 km. And there is no need to move the clouds above a zero isotherm. Huge ice blocks are formed in the warm layer of the troposphere. For such a process

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does not require freezing temperatures and high altitude. Everyone knows that without lightning hail does not happen. Apparently, for electrostatic fields don't have collision and friction for small and large crystals of solid ice, as often write, although the phenomenon of friction hot and cold enough clouds in the liquid state (convection). For the formation of thundercloud requires plenty of moisture. When the same relative humidity, warm air contains more moisture than cold air. Therefore, Thunder and lightning, usually occur during the warm seasons-spring, summer, autumn. The mechanism of electrostatic field in the clouds also remains an open question. There is much speculation on the subject [9]. In one recent reported [5] that the rising moist air flows along with not charged cores are always present, positively and negatively charged nucleus. Any of them could be condensation. Found that the condensation of moisture in the air, the first begins to negatively charged nuclei, than neutral or positively charged nuclei [10.12]. For this reason, at the bottom of the clouds accumulate negative particles, and the top is positive. Therefore, inside the clouds is created a huge electric field intensity which is 10^6-10^9 V and the current 10^5— 3 • 10^5 A. such a strong difference of potentials, in the end, leads to a powerful electric discharge. Lightning can last 10-6 (one millionth) of a second. When discharge lightning releases enormous thermal energy, and temperature reaches – 30 000 °K. This is about 5 times greater than the Sun's surface temperature. Of course, this huge energy area particle must exist in the form of plasma, which after recombination becomes neutral atoms or molecules.

III. What can Cause this Terrible Heat

Many know that when a strong lightning discharge neutral molecular oxygen air is easily converted into ozone, and felt his peculiar smell:

$$2O_2 + O_2 \rightarrow 2O_3\uparrow \quad (1)$$

In addition, it is established that in these harsh environments simultaneously reacts chemically inert nitrogen with oxygen to form mono-NO and nitrogen dioxide NO_2:

$$N_2 + O_2 \rightarrow 2NO + O_2 \rightarrow 2NO_2 \quad (2)$$

$$3NO_2 + H_2O \rightarrow 2HNO_3\downarrow + NO \quad (3)$$

Formed NO_2 nitrogen dioxide in turn, aligning him with water, becomes a nitric acid HNO_3, which in the sediment falls to the ground.

Previously thought that containing in cumulonimbus clouds of table salt (NaCl), carbonate (Na_2CO_3) alkaline and alkaline earth metals ($CaCO_3$) react with nitric acid and eventually is formed nitrates.

$$NaCl + HNO_3 = NaNO_3 + HCl \quad (4)$$

$$Na_2CO_3 + 2\,HNO_3 = 2\,NaNO_3 + H_2O + CO_2 \quad (5)$$

$$CaCO_3 + 2HNO_3 = Ca(NO_3)_2 + H_2O + CO_2 \quad (6)$$

Nitrate when mixed with water leads to cold. Adhering to this, Gassendi fantasized that the upper layers of air are cool, not because they are far away from the source of heat reflecting from the Earth, and because of the "corpuscles" nitrogen (nitrate) out there that are very numerous. In winter the less and they generate only snow, but in summer there are more, so there may be hail [19]. Subsequently, this hypothesis has also been subject to criticism by his contemporaries.

IV. What Might Happen to the Water in Such a Harsh Environment

In the literature, there is no information. Heating to a temperature of 2500 ° C [2], or the water passing through the non-alternating electric current at room temperature, [7], it is decomposed into its constituent parts and the heat of the reaction is shown in equation (7):

$$2H_2O\,(liq) \rightarrow 2H_2\uparrow(g) + O_2\uparrow(g) - 572\ kC \quad (7)$$

$$2H_2\uparrow(g) + O_2\uparrow(g) \rightarrow 2H_2O\,(liq) + 572\ kC \quad (8)$$

The decomposition reaction of water (7) is an endothermic process and energy to break the covalent bonds must be introduced externally. In this case, the energy comes from the system (in this case the electrostatic polarized – water). This system resembles an adiabatic process, after which there is no gas heat exchange with the environment, and such processes are very fast (lightning).

In short, in an adiabatic process (the decomposition of water into hydrogen and oxygen) (7) is internal energy and hence it starts to cool itself. Of course, when the balance shifts toward the right side to form a gases - hydrogen and oxygen by the action of the electric arc instantly snap ("explosive mixture") reverse reaction occurs between the hydrogen and oxygen (8). This reaction is performed in the laboratory. Despite a decline in volume of reactive components in this reaction, the result is a strong rumbling. The speed feedback based on Le Chatelier's principle affects favorably received by reaction (7) high pressure. The fact of the matter is that and direct reaction (7) should be strong with a roar, as liquid water modular condition instantly formed gases (most authors attribute this to intense heating and expansion within or around the channel of air created by a discharge of Lightning). It is possible that the sound of thunder, therefore, is not monotonous, that is, not to mention the sound of an explosive or weapon. First comes the decomposition of water (first thunder), followed by accession of hydrogen

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with oxygen (the second thunder). However, these processes are occurring so quickly, they discern not everyone.

V. As is Formed Hail

When the discharge of lightning happen because of the huge amount of heat, the water in lightning channel or around his intensely evaporates as soon as stop flashing lightning, water begins to freeze. In the well-known law of Physics: a strong evaporation leads to cooling. It is noteworthy that the heat during discharge of lightning is not imposed from the outside, on the contrary, it derives from the system itself (in this case system: polarized electrostatic water). The process of evaporation is the kinetic energy of the polarized water system. In this process, a strong and instant evaporation of water causes a sharp its solidification. The stronger the evaporation, the more intense is the process of solidification of water.

For such a process is not necessary that the ambient temperature was below zero. When lightning flashes produced the hailstone in different sizes. A dimension of hail depends on the power and intensity of lightning. The stronger and more intense lightning are, the larger the hailstone. Typically residue hailstones quickly stop as soon as will cease flashing lightning.

Similar processes operate in other areas of nature. Here are a few examples.

1. Refrigeration systems work on this principle. That is, the artificial cold (sub-zero temperature) in the evaporator is formed by boiling of liquid refrigerant that is fed back through the capillary tube. Due to the limited capacity of the capillary tube, the refrigerant enters the evaporator is relatively slow. Boiling refrigerant – usually about 30 ° C. Getting in warm evaporator, the refrigerant boils instantly, strongly cooling evaporator wall. The refrigerant vapor generated as a result of its boiling point, fall from the evaporator to the suction pipe of the compressor. Evacuating the gaseous refrigerant from the evaporator, the compressor pumps it under high pressure to the condenser. Gaseous refrigerant in the high pressure condenser cooled gradually condenses in the gas passing from the liquid state. Re-liquid refrigerant from the condenser is fed through the capillary tube into the evaporator and the cycle is repeated.

2. Chemists are well known to produce solid carbon dioxide (CO_2). Carbon dioxide is usually transported in steel cylinders in liquefied liquid aggregate phase. With slow passage of gas from the cylinder at room temperature passes to a gaseous state, if it produce intense, it immediately goes into the solid state, forming a "snow" or "dry ice" having a temperature of sublimation of –79 to –80 °C. Intensive evaporation leads to solidification of the carbon dioxide, passing the liquid phase. Obviously, the temperature inside the container above zero, however, this highlighted by solid carbon dioxide ("dry ice) has a sublimation temperature of about –80° C [15].

3. Another important example regarding this topic. Why does a person sweats? Everyone knows that in normal conditions or on exertion, as well as in nervous excitement person sweats. Pot - liquid secreted by the sweat glands and containing 97.5 - 99.5% water, small amounts of salts (chlorides, phosphates, sulphates), and several other compounds (organic compounds - urea, uric acid salts, creatine esters, sulfuric acid) [3]. However, excessive sweating may indicate the presence of serious diseases. May be several reasons: colds, tuberculosis, obesity, violation of the cardiovascular system and etc. However, more importantly, sweating regulates body temperature. Increased sweating under the hot and humid climate. We usually covered with sweat when we are hot. The higher the ambient temperature, the more we sweat. The body temperature of a healthy person is always equal to 36.6 ° C and one of the methods of maintaining a normal temperature - is sweating. Through the enlarged pores is an intensive evaporation of moisture from the body - a person sweats heavily. A moisture evaporation from any surface, as indicated above, it promotes cooling. When the body is in danger to the health of overheating, the brain triggers sweating and evaporating from our skin sweat cools the body surface. That's why people sweat in the heat.

4. Furthermore, water may also be converted into ice in a conventional glass laboratory setup (Figure 1) under reduced pressure without external cooling (at 20 ° C). You need only connect to this setting the deep vacuum pump and trap.

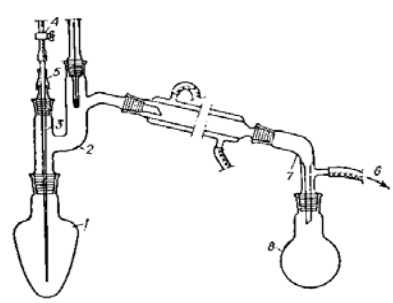

Figure 1 : Laboratory vacuum unit for distillation

Figure 2 : The amorphous form of ice inside a hailstone

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Figure 3 : Aggregation of hailstones

In conclusion, I want to address a very important question regarding the layering of hailstones (see Fig. 2-3). What causes turbidity in the structure of hailstones? The authors report that [5, 13, 14]: *"To be hailstone diameter of about 10 centimeters, the ascending air stream in the thundercloud must have a speed not less than 200 km / h, and thus it includes snowflakes and air bubbles. Such layer looks turbid. But if the temperature is higher, the ice freezes slowly and included snowflakes have time to melt, and the air escapes. Therefore, such a transparent layer of ice. The rings can be traced, in which layers of clouds visited-air before falling to the ground".* Of Fig. 2,3 clearly shows that the ice that makes up the hailstones, really, is not uniform. Almost every hailstone is pure and in the center of a muddy ice. The opacity of the ice may be caused by various reasons. In large hailstones sometimes alternating layers of transparent and opaque ice. In our opinion, the white layer is responsible for the amorphous and the transparent layer of ice - the crystalline form. In addition, the amorphous form of ice aggregate is obtained by extremely rapid cooling of liquid water (at a rate of about 10^{7o}K/sec), as well as the rapid increase in the ambient pressure, so that the molecules do not have time to form a crystal lattice [4]. In this case this is a discharge of lightning, which is fully in line with the favorable condition of formation of metastable amorphous ice. From Figure 2-3 shows clearly that the huge boulders were formed from clusters of relatively small hailstones. Both of these factors suggest that the formation of a transparent or opaque layers of hail, caused by exposure to extremely high pressures generated during lightning.

The hail is one of the most terrible natural disaster causing damage to mankind. In the United States the hail destroyed an average of 1 to 2% of crops. In some areas the figure is 6%. Damage is 1-1.5 billion United States Dollars [26]. Extremely great damage from hail in North America was registered in May 1995 in Texas (about $ 2 billion). May 14, 2001 in Stavropol region of Russia hail damaged 4,500 homes, killed the agricultural crops area of 1000 sq km and perennials on the area of 30 sq km, the damage reached $ 17 million USA. The Midwest United States hit by storm. 12 Tornadoes recorded in the states of Nebraska, Iowa, Wyoming, and Kansas. In Blair town suffered 4,500 cars dealer network Woodhouse Auto Family. The total value of the damaged property is $ 152 million [27]. About the formation of hail and its of negative consequences are given detailed information in the recently published articles [28-35].

It should be noted that, the reason for the formation of hail have been determined incorrectly, the false views and the theories put forward till today. That is why, these scientists have been unable to prevent or avoid it. The major trends in this research are not to prevent hail, but to diminish it from a large part of the mass [36-42]. Thus, to this end, were used the various chemical reagents (eg, CO_2, AgI, NaCl, tetraalkilamoniumun in freon solution, and so on) the rockets and the projectiles, as carriers. The aim of this difficult operation was: to create a large number of crystallization centers, with this way replaced hail with large mass with smaller particles and thus reduce the amount of damage caused.

In February 2010, the newspaper RIA "News" reported that the Moscow scientists have developed a new modified aircraft or so-called "multi-functional aviation complex" Nart ", designed to prevent catastrophic natural weather events, such as hail, torrential rain, tornadoes and typhoons, through active impacts on meteorological processes. According to the developers, the range influence of the complex is 300-350 kilometers and has passed several tests in the south of Russia. Nevertheless, the use of this device produced no tangible results; Moscow and its region again are showered of hail with the size of the Greek walnut.

In the review [42] emphasize that there is a relationship between the parameters of the number of days with hail and thunderstorm. The authors clearly recognize that the hail is almost always falls during a thunderstorms and it is interesting to compare the parameters of the number of days with hail and thunderstorms. The comparison shows that there is quite a distinct and strong correlation between these parameters. Found that it in the years before the defense was 2.5:26.4 = 0.095, and in the years protect 1.5:31 = 0.048. However, recognize that, despite the continuous improvement of technical equipment and technological schemes impact hail albeit reduced, but each year brought significant damage to the protected areas in all regions where the work was carried out. On this basis, it can be concluded that the current methods of influence on hail processes exhausted its possibilities. It should come up with a new type of protection hail.

On the formation of hail and its negative consequences described in detail in the recently published studies [28-35], and we strongly emphasize

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that in this context, the use of chemicals as a protective agent against the hail will not give positive results.

We believe:"When the lightning begins due to the tremendous amount of heat, the water in the channel of lightning or around his intensely evaporates as soon as stop flashing lightning, the water starts to freeze. In accordance with the known law of Physics: the strong evaporation causes cooling. It is noteworthy that the heat during discharge of lightning is not imposed from the outside, on the contrary, it derives from the system itself (in this case system: polarized electrostatic water). In this process, a strong and instant evaporation of water causes a sharp its solidification. The stronger the evaporation, the more intense is the process of water solidification. For such a process is not necessary that the ambient temperature was below zero. The lightning discharge generates a variety the size of the hailstones. The hailstones depend on the power and intensity of lightning. The stronger and more intense lightning are, the larger the hailstone. Usually the hail stops as soon as quickly stops flashing lightning."

VI. How to Prevent Damage by Hail

From the point of view of the authors of [42], developed in the sixties, scientists and specialists of several institutes and other academic institutions world missile way to impact on sedimentation processes of hailstorm voted the world's most efficient. According to them, the method is most appropriate to the modern concepts of physics and hail precipitation and management in order to prevent the growth of hail and increase rainfall. Its development has been invested efforts of large teams of scientists and much money. In addition, the implementation of this method requires an extremely high cash costs, to use of sophisticated equipment (radars, missiles, computers, etc.), the highest level of training and work organization. Nevertheless, even if all these conditions are realized often hail destroys crops and planting of large areas.

The above requirements it extremely difficult to implement. Need for new science-based, effective, but less complex and less expensive methods of process control in the clouds.

Methods are based on the influence on cloud processes with the aim of suppressing the growth processes of hail. Works on the effects of clouds with a view to their scattering attenuation hail, precipitation increase are conducted in many countries for over 50 years. Nevertheless, the problem is so complex that their level of efficiency so far is controversial according to theorists and experimentalists.

In our opinion, the only way ofhail prevention to overcome the lightning discharge or at least mitigate it. We suggest using the following construct for this purpose (Fig.4.):

It is known that the only way to protect against lightning first developed in 1750, the American scientist, Benjamin Franklin. He showed that electric charges or a bolt of lightning can be easily and safely escort to the ground through the metal wires. The latter is called "lightning rod" and is actively used today. Regarding to this, his invention helps prevention of hail.

In this sense, this invention can help in the prevention of hail. So, one end of the cable connects to the protected ground ("grounding") and the other end fastened balloon, filled with helium.Then the balloon is released into the air. The greater the distance between the balloon and the Earth (1-2 miles), the better the efficiency of the installation. In this case, the lightning rod closer to the cumulonimbus clouds and its effective conical effect will be even greater. Absolutely, electrical charges accumulated in the cloud will descend through a metal wire is easier than through the airspace or water layer. Here is the principle.

As for the engineering, construction and Assembly, this question gets their decision in other more specific professionals.

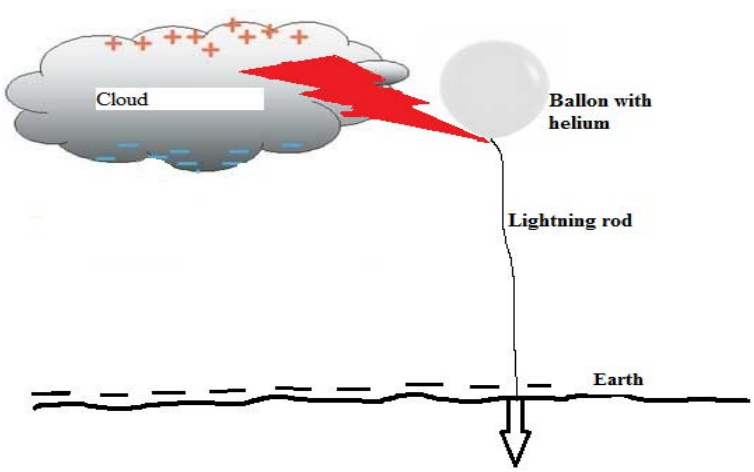

Figure 4 : Construction to prevent lightning and hailstone

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VII. Conclusions

- Without the lightning and strong thunderstorms not grad. A thunderstorm accompanied by hail.
- The cause of hail is instant and massive amounts of heat lightning discharge in the cumulonimbus clouds. The mighty heat produced leads to strong evaporation ofwater in the channel and around the lightning. Strong water evaporation is rapid cooling ice formation, respectively.
- The process does not require the need to shift the zero isotherm atmospheres, with negative temperatures, and can easily occur at low and warm troposphere.
- The process is essentially close to adiabatic process, since the thermal energy generated is not entered into the system from the outside, and it comes from the system itself.
- Powerful and intense lightning provides the condition for the formation of large hailstones.
- On the basis of our theory thelightning rodis original tool to prevent of the hail.

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Ocimum basilicum var. *purpureum* Floral Essential Oil: Phytochemicals, Phenolic Content, Antioxidant, Free Radical Scavenging and Antimicrobial Potentials

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Abstract- This study examined the phytochemicals and medicinal properties of the floral essential oil of *O. basilicum* var. *purpureum* from Nigeria. The GC and GC-MS analyses revealed the presence of twenty-five organic compounds making up 99.7% of the total percentage composition of the essential oil. The most abundant components was phenolic compound called methyleugenol (15.5%), followed by 2-phenyl-1-hexanol (14.0%), 1-(4,5-dimethyl-2-nitrophenyl)-1H-tetraazole (14.0%), 2-methyl-3,5-dodecadiyne (14.0%), *o*-nitrocumene (14.0%) and patchoulane (6.7%). The total phenolic content was quantitatively determined as 459 μgmg^{-1} gallic acid equivalent (GAE) confirming the presence of high amount of phenolic compounds in the floral essential oil. The DPPH IC_{50} value was 1.0 μgml^{-1}, the essential oil was capable of scavenging free radicals in a range of 73-86% and the antioxidant power of the essential oil increased with concentration.

Keywords: Ocimum basilicum var. purpureum, floral essential oil, phytochemical, phenolic content, pharmacological potentials.

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OCIMUMBASILICUMVARPURPUREUMFLORALESSENTIALOILPHYTOCHEMICALSPHENOLICCONTENTANTIOXIDANTFREERADICALSCAVENGINGANDANTIMICROBIALPOTENTIALS

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Ocimum basilicum var. *purpureum* Floral Essential Oil: Phytochemicals, Phenolic Content, Antioxidant, Free Radical Scavenging and Antimicrobial Potentials

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Keywords: Ocimum basilicum var. purpureum, floral essential oil, phytochemical, phenolic content, pharmacological potentials.

I. Introduction

Phytochemicals are huge varieties of organic substances which accumulated in plants. Plant essential oils are recognized as one of the most promising secondary metabolites for the development of cheap and safer drugs (Varma and Dubey, 2001). Essential oils are volatile, natural and complex compounds characterized by a strong odour and are produced from odoriferous medicinal plants. In addition to essential oils, odoriferous plants are also characterized by the presence of phenolic compounds that have been shown to possess multiple pharmacological activities. Essential oils, their fractions and isolated aroma chemicals are valuable ingredients of flavour foods, toiletries, fine chemicals and pharmaceutical industries, they are utilized as such or in diluted forms in therapy or by the aromatherapy sector (Daferera *et al.,* 2000; Mimica-Dukic and Bozin, 2008). According to world health organization (WHO), greater than 80% of the total world's population depends on natural products in order to satisfy their primary health care needs. Investigations of these secondary metabolites intensified when some commercial synthetic antioxidants were found to exhibit toxic, mutagenic and carcinogenic effects and other problems associated with their usage (Rajendran *et al.,* 2014). Knowledge of the chemical composition of medicinal plants is desirable because such information will be of value for the synthesis of complex chemical substances (Yadav and Agarwala, 2011).

The genus *Ocimum* comprises more than 150 species and is considered as one of the largest genera of the *Lamiaceae* family. *Ocimum basilicum* var. *purpureum* is an annual plant which grows well in Nigeria. The purple colour of the plant is due to the presence of anthocyanins mainly cyanidin-3-(di-*p*coumarylglucoside)-5-glucoside and small amount of peonidin compounds, therefore, this plant is considered a potential source of red pigments for the food industry (Janick *et al.,* 1999). The plant is widely used in food and oral care products. The plant is a good source of magnesium, which promotes cardiovascular health, also helps muscles and blood vessels to relax, thus improving blood flow and lessening the risk of irregular heart rhythms or a spasming of the heart muscle or a blood vessel. It is also an excellent source of vitamin K and manganese; a very good source of copper, vitamin A and vitamin C; a good source of calcium, iron, folate, and omega-3 fatty acids (Patil *et al*., 2011). The plant is also used as condiment, calmative and flavouring agents. Traditionally, it is commonly used in treatments

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of diuretic, constipation, intestine ache, galactogogue, headaches, coughs, diarrhoea, warts, worms, kidney, anti-inflammatory and antispasmodic agent (Khelifa *et al.,* 2012; Uyoh *et al.,* 2013).

To the best of our knowledge, there is paucity of information on the phytochemical, total phenolic content, free radical scavenging, antioxidant and antimicrobial potentials of this plant so far. Therefore, the present research was undertaken for the first time with the aim of looking into the composition and pharmacological properties in the floral essential oil of *O. basilicum* var. *purpureum* from Nigeria.

II. Material and Methods

a) Plant Materials and Isolation of the Essential Oil

The floral parts of the plant were collected from their natural habitat in Ota, Nigeria and were authenticated as *O. basilicum* var. *purpureum*. The floral parts of the plant were extracted by hydrodistillation using clevenger-type apparatus to give a neat essential oil which was preserved in a vial at low temperature to prevent evaporation (European pharmacopoeia, 2004).

b) GC and GC-MS Analyses

Analyses of the floral essential oil of *O. basilicum* var. *purpureum* were performed using multi-dimensional gas chromatograph coupled with Gas Chromatography-Mass Spectrophotometer (Shimadzu, Japan) equipped with non-polar and polar double capillary columns (25.0 m x 0.25 µm i.d., 0.25 µm df). High purity helium was used as the carrier gas at a constant flow rate of 0.99 ml/min. A total of 1 µl sample was injected (split ratio 100:1) into GC and GCMS using AOC20i auto injector for analyses. The initial temperature was set at 60°C, heated at a rate of 3°C/minutes to 280°C and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200°C, while the interface temperature was set at 250°C, solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40-700 a.m.u. at 0.50 scan/s. The constituents were identified by comparison of their retention indices with those of the literature. The retention indices were determined in relation to a homologous series of n-alkanes under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in National Institute for Standards and Technology (NIST) and with mass spectra from literature.

c) Determination of Total Phenolic Content

Total phenolic content of the floral essential oil of *O. basilicum* var. *purpureum* was determined using the Folin-Ciocalteau method. 1 ml aliquot solution of the essential oil was mixed with 46 ml distilled water and 1 ml of Folin Ciocalteau reagent, then 3 ml of (2% w/v) Na_2CO_3 solution was added after 3 minutes and the mixture was allowed to stand for 2 hours for incubation in dark with intermittent shaking, the absorbance of the reaction mixture was measured on a UV-Visible spectrophotometer at 760 nm against a blank (containing all reagents except the test sample). The total phenolic content was expressed as gallic acid equivalents (Govindappa *et al.,* 2011).

d) In vitro 2,2´-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging and Antioxidant Activities

The free radical scavenging and antioxidant activities of the floral essential oil against the stable free radical DPPH were measured. Briefly, three different concentrations (1000, 100 and 10 µgml^{-1}) of the essential oil were incubated with a methanolic solution of DPPH for 30 minutes of incubation at room temperature in the dark, then absorbance at 517 nm was measured spectrophotometrically. Ascorbic acid was used as reference compound. The assay was carried out in triplicate. The percentage inhibition (I%) for each concentration was calculated by using the absorbance values according to the following formula:

$$I\% = [(A_{blank} - A_{eo})/A_{blank}] \times 100$$

Where: A_{blank} is the absorbance of blank solution and A_{eo} is the absorbance of the essential oil. The dose-response curve was plotted and IC_{50} value for the essential oil and the standard were calculated (Adeniran *et al.,* 2013).

e) In vitro Antimicrobial Activities

The antibacterial potentials of the floral essential oil were evaluated by agar-well diffusion method against representative multi-drug resistance Gram-positive organisms (*Streptococcus agalactiae*, *Staphylococcus aureus* and *Streptococcus* species) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*). The bacteria isolates were first sub-cultured in Nutrient agar and incubated at 37°C for 24 hours. All the bacteria cultures were adjusted to 0.5 McFarland standards, 20 ml of sterilized Nutrient agar medium was poured into each Petri dish aseptically and plates were then swabbed with inocula of the test organisms, and kept for 15 minutes for absorption. Using sterile cork borer of 6 mm diameter wells were bored into the seeded agar plates, and these were loaded with 10 µl of different concentrations (1000, 100 and 10 µgml^{-1}) of the essential oil in dimethylsulfoxide (DMSO). The plates were allowed to stand in the refrigerator for 1 hour to allow proper diffusion of the essential oil into the medium and incubated at 37°C for 24 hours before visual assessment of the inhibition zones. The Antibacterial potential of the essential oil were evaluated by measuring the clear zones of growth inhibition against the test organisms. Gentamicin (GEN)

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and Cloxicillin (CXC) were used as control (Agu *etal.,* 2013).

III. Results and Discussion

a) *Chemical Constituents of the Essential Oil*

In this study, the floral essential oil of *O. basilicum* var. *purpureum* was investigated for its chemical constituents. The essential oil imparted pleasant aromatic odour. The GC and GC-MS analyses of the floral essential oil of *O. basilicum* var. *purpureum* showed the presence of 25 compounds making up 99.7% of the total percentage composition (Table 1). Compounds were listed in order of their retention indexes. The most abundant component was phenolic compound called methyleugenol (15.5%), the other major compounds present in the essential oil were 2-phenyl-1-hexanol (14.0%), 1-(4,5-dimethyl-2-nitrophenyl)-1H-tetraazole (14.0%), 2-methyl-3,5-dodecadiyne (14.0%), o-nitrocumene (14.0%) and patchoulane (6.7%). The principal classes of organic compounds in the floral essential oil were phenolic compounds (29.5%), sesquiterpenes (16.9%) and monoterpenes (1.4%). Comparatively, the chemical constituents of the investigated floral essential oil were different from those reported in other studies. The main constituents in the leaf essential oil of *O. gratissimum* were eugenol (68.8%), methyl eugenol (13.21%) and *cis*-ocimene (7.47%) (Matasyoh *et al.,* 2007) while linalool (65.38%, 74.22%, 38.60%), eugenol (5.26%, 3.47%, 10.20%) and *tau*-cadinol (8.18%, 3.47%, 10.20%) were the main components in *O. basilicum* var. *genovese*, *O. gratissimum* and *O. tenuiflorum* from Romania (Stefan *et al.,* 2013). Joshi (2013) also reported that the main composition of *O. gratissimum* and *O. sanctum* were eugenol (75.1%) and methyl eugenol (92.4%) respectively.

Table 1 : Chemical Composition of the Floral Essential Oil of *O. basilicum* var. *purpureum*

Compounds	% Composition	RI
2,3,4-trimethyl-1,4-pentadiene	0.4	687
2,3,3-trimethyl-1,4-pentadiene	0.7	689
1,3-dimethyl-1-cyclohexene	0.4	852
1,9-decadiyne	1.0	1011
3-[(1Z)-1-butenyl]-4-vinyl-1-cyclopentene	1.0	1100
1-(1-Ethylvinyl)-1-(2-methylene-3-butenyl)cyclopropane	1.0	1115
iso-borneol	0.4	1138
8-methylenedispiro[2.1.2.4]undecane	1.0	1215
copaene	0.3	1221
1-(4,5-dimethyl-2-nitrophenyl)-1H-tetraazole	14.0	1250
megastigma-7(*E*),9,13-triene	0.3	1278
2-methyl-3,5-dodecadiyne	14.0	1284
nopol	0.3	1290
trans-7-hydroxymethyl-3-cyclopropylbicyclo[4.1.0]heptane	1.8	1307
o-nitrocumene	14.0	1324
1-(2-nitro-2-propenyl)-1-cyclohexene	1.0	1339
α-cubebene	0.5	1344
methyleugenol	15.5	1361
aromadendrene	2.0	1386
2,4-diisopropenyl-1-methyl-1-vinylcyclohexane	1.8	1398
β-elemene	3.6	1403

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(5E,9E)-12-methyl-1,5,9,11-tridecatetraene	2.0	1404
2-phenyl-1-hexanol	14.0	1469
β-cis-caryophyllene	2.0	1494
patchoulane	6.7	1968
Percentage Total	99.7	

RI = Retention Index

b) Total Phenolic Content (TPC)

Total phenolic content analysis revealed the presence of high quantity phenolic compounds in the floral essential oil. This was found to be 459 μgmg^{-1} gallic acid equivalents. The essential oil gave a higher TPC when compared with the previous studies on the related species such as methanolic seed extracts of *O. gratissimum* (168 mgg^{-1}), *O. americanum* (123 mgg^{-1}), *O. minimum* (110 mgg^{-1}), *O. citriodorum* (96 mgg^{-1}), *O. kilimandscharicum* (82 mgg^{-1}), *O. grandiflorum* (61 mgg^{-1}), *O. lamiifolium* (54 mgg^{-1}), *and O. selloi* (42 mgg^{-1}) (Hakkim *et al*., 2008). The floral essential oil of *O. basilicum* var. *purpureum* exhibited the high TPC due to the presence of low molecular mass phenolic compounds like methyleugenol and 2-phenyl-1-hexanol. Phenolic compounds in the floral essential oil were oxidized by Folin-Ciocalteu reagent which reduced to a mixture of blue oxides of tungsten, W_8O_{23}, and molybdenum, Mo_8O_{23} after oxidation of the phenolic compounds (Walch *et al.,* 2011). Phytophenolic compounds are very important because their hydroxyl groups which are highly effective scavengers of most oxidizing molecules, including reactive oxygen species, and various free radicals implicated in several diseases. Plant phenolic compounds have been widely consumed for many years as dietary components with no side effect, they play important beneficial roles in mammalian systems, they are especially important in prevention of cancers, cardiovascular diseases, and other degenerative diseases. Methyleugenol and 2-phenyl-1-hexanol are natural phenolic compounds that recently received attention for their extensive pharmacological properties, including anti-tumor, antibacterial, cardio-protective and gastroprotective effects (Georgiev *et al*., 2014). Phenolic compounds play a key role in scavenging free radicals that cause oxidative stress because they have substantial antioxidant capacity against peroxyl radicals. In addition, they have been shown to possess potential antioxidant abilities, which helps them to scavenge electrophiles and active oxygen species, slow down nitrosation and chelate metal ions to limit auto-oxidation and increase the ability to adjust some enzyme actions (Mediani *et al.,* 2013).

c) In vitro Free Radical Scavenging and Antioxidant Potentials

The free radicals scavenging and antioxidant potentials of the floral essential oil of *O. basilicum* var. *purpureum* were evaluated by DPPH assay. The essential oil was able to inhibit the formation of DPPH radicals in a concentration dependent manner. The percentage inhibitions of the essential oil at various concentrations (1000, 100 and 10 μgml^{-1}) were 86±0.001, 78±0.001 and 73±0.000% respectively; while the IC_{50} value was found to be 1.0 μgml^{-1} in comparison to ascorbic acid which gave 96±0.000, 69±0.002 and 54±0.002% as the percentage inhibitions with IC_{50} value of 9.0 μgml^{-1}. The DPPH radical scavenging capacity of the floral essential oil of *O. basilicum* var. *purpureum* was higher than that of ascorbic acid. The free radical scavenging and antioxidant properties of the essential oil were found to be nine times more active than the synthetic antioxidant (ascorbic acid) as shown in Table 2 below. Moreover, the floral essential oil of *O. basilicum* var. *purpureum* inhibited the DPPH free radicals than extracts of other related species such as *O. americanum* which has lower percentage inhibitions ranging from 32.9-67.4% (IC_{50}: 290 μgml^{-1}) in ethanolic extract, 20.9-63.2% (IC_{50}: 350 μgml^{-1}) in chloroform extract, 37.2-59.8% (IC_{50}: 430 μgml^{-1}) in petroleum ether extract and 26.5-56.2% (IC_{50}: 510 μgml^{-1}) in aqueous extract at different concentrations between 100-500 μgml^{-1} (Sarma and Babu, 2011). The antioxidant activity has been related to the number and position of free hydroxyl groups in terpenoids and phenolic compounds, which could be as a result of their hydrogen donating abilities (Burda and Oleszek, 2001). The essential oil showed significantly higher inhibition percentage and positively correlated with the content of the secondary metabolites in the essential oil. As shown in the equation below DPPH involved hydrogen atom transfer reactions (HAT) and single electron transfer (SET). Natural antioxidants (AH) neutralize the free radicals (R•) by interfere with the oxidation process by reacting with free radicals, chelating, catalytic and reactive oxygen scavenging activities (Prior *et al.,* 2005).

Hydrogen Atom Transfer

$$R^{\bullet} + AH \rightarrow RH + A^{\bullet}$$

Single Electron Transfer

$$R^{\bullet} + AH \rightarrow R^{-} + AH^{\bullet +}$$

$$AH^{\bullet +} \xleftrightarrow{OH_2} A^{\bullet} + H_3O^{+}$$

$$R^{-} + H_3O^{+} \rightarrow RH + H_2O$$

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Table 2 : IC_{50} of the Antioxidant Property of the Floral Essential Oil of *O. basilicum* var. *purpureum* and Reference drug

Essential Oil and Reference Compound	DPPH IC_{50} μgml^{-1}
O. basilicum var. *purpureum*	1.0
Ascorbic acid	9.0

d) Antibacterial Potentials

The antimicrobial activities of the floral essential oil of *O. basilicum* var. *purpureum* against *E. coli, K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. typhimurium*, *S. aureus, S. agalactiae* and *S. species* were shown in Table 3. The essential oil showed variable activities against tested bacteria. The essential oil was highly effective on all the tested bacteria. The highest inhibitory effect of the floral essential oil of *O. basilicum* var. *purpureum* was observed against *E. coli* (30 mm) followed by *S. aureus* (25 mm), *K. pneumoniae* (20 mm), *S. species (20 mm), P. aeruginosa (20 mm), P. mirabilis (20 mm), S. agalactiae (18 mm) and S. typhimurium (18 mm).* The tested bacteria were found to be resistant to Cloxicillin (CXC) but some were sensitive to Gentamicin (GEN) synthetic antibiotics. The antibacterial properties of this essential oil were comparable to that of leaf essential oil of *Ocimum gratissimum* which gave zones of inhibition between 7.0-26.6 mm for the following Gram positive (*S. aureus*, *Bacillus* spp.) and Gram negative (*E. coli, P. aeruginosa*, *S. typhi, K. pneumoniae, P. mirabilis*) bacteria (Matasyoh *et al.,* 2007). The observed antibacterial effects of the plant correlate its folk uses. In this study the essential oil of the plant inhibited the growth of Gram positive and Gram negative bacteria to a high degree. The observed activities may be due to the presence of some secondary metabolites such as terpenoids and phenolic compounds which are known to possess various medicinal activities in different organisms (Egharevba *et al., 2010). The antimicrobial activities* may also be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacteria cell wall. It has also been reported that the antimicrobial properties of essential oil results from the combined effect of direct vapour absorption on organisms and indirect effect through the medium that absorbed the vapour (Wang *et al.,* 2012). The vapour absorption on microorganisms is determined by their membrane permeability. Gram negative bacteria are less susceptible to essential oils than Gram positive bacteria because they possess outer membrane surrounding the cell membrane which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Angienda *et al*., 2010). Therefore, higher cell damage is expected to occur from the floral essential oil on the tested bacteria (Tyagi and Malik, 2010). Methyl eugenol is a phenolic compound that has been reported to have antimicrobial, central nervous system depressant, anaesthetic, hypothermic, myorelaxant, anticonvulsant, insecticidal, anthelminthic and nematocidal properties (Matasyoh *et al*., 2007). This study showed that the floral essential oil of *O. basilicum* var. *purpureum* has greater potential as antibiotic against bacteria and that they can be used in the treatment of infectious diseases caused by resistant pathogenic organisms in human beings.

Table 3 : Zones of Inhibition (mm) showing the Antimicrobial Properties of the Floral Essential oil of *O. basilicum* var. *purpureum*

	Floral Essential Oil			GEN	CXC
Conc. / Organism	1000	100	10	10µg	5µg
E. coli	30	30	30	22	-
K. pneumoniae	20	18	15	21	-
P. aeruginosa	18	18	18	20	-
P. mirabilis	20	20	20	20	-
S. agalactiae	18	18	18	-	-
S. aureus	25	25	25	-	-
S. typhimurium	18	18	18	21	-
S. species	20	18	18	-	-

Keynote:--- = Resistant, 6-9 mm = low inhibition, 10-14 mm = moderate inhibition and ≥ 15 mm = high inhibition.

IV. Conclusion

The results of the free radical scavenging, antioxidant and antimicrobial potentials of the part of the plant investigated in this study were basically due to the synergic effects of the phytochemical constituents in the floral essential oil. Natural antioxidants are helpful in assisting the body to neutralize free radicals in healthy individuals. Therefore, phytochemicals in the floral essential oil of this plant which are good antioxidants would help to reduce the harmful effects of oxidative stress and could be used to handle health problems caused by reactive oxygen species. Moreover, the ability of the floral essential oil to inhibit the growth of the bacteria in this study at low concentrations is an indication of its broad spectrum antimicrobial and great therapeutic potentials of this species. Plant having antimicrobial compounds have enormous therapeutic potentials as they can act without any side effect as often found with synthetic antimicrobial products.

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Synthesis of New Macrocycles of Ortho-Methoxy Salicylaldehyde using Claisen-Schmidt Condensation

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Abstract- In this research prepared some new families of macrocycles by reaction of ortho-methoxy salicylaldehyde sequently with ketone by claisen-Schmidt condensation. Claisen-Schmidt condensation proceed under base or acid catalysis, at moderate temperature, in high atom economy and with water as the sole by product. Macrocycle were characterized by IR, NMR and Mass spectroscopie.

Keywords: macrocyclic compounds, claisen-schmidt condensation, ortho-methoxy salicylaldehyde.

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SYNTHESISOFNEWMACROCYCLESOFORTHO-METHOXYSALICYLALDEHYDEUSINGCLAISEN-SCHMIDTCONDENSATION

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Synthesis of New Macrocycles of Ortho-Methoxy Salicylaldehyde using Claisen-Schmidt Condensation

Shana Balchi [α] & Alireza Banaei [σ]

Abstract- In this research prepared some new families of macrocycles by reaction of ortho-methoxy salicylaldehyde sequently with ketone by claisen-Schmidt condensation. Claisen-Schmidt condensation proceed under base or acid catalysis, at moderate temperature, in high atom economy and with water as the sole by product. Macrocycle were characterized by IR, NMR and Mass spectroscopie.

Keywords: macrocyclic compounds, claisen-schmidt condensation, ortho-methoxy salicylaldehyde.

I. Introduction

Calixarenes, resorcinarenes, cyclotriveratrylenes, cucurbiturils, and crown ethers, exemplify families of macro cyclic compounds that found extensive use in supra molecular chemistry . Pedersen in 1967 he published two works that are now considered classics [1]. He describes the methods of synthesizing crown ether (cyclic polyethers). Discovered a simple method of synthesizing crown ether when he was trying to prepare a complexing agent for divalent cations. The donut-shaped molecules were the first in a series of extraordinary compounds that form stable structures with alkali metal ions and a hetero cyclic compound is a cyclic compound which has atoms of at least two different elements as members of its ring(s). Although heterocyclic compounds may be inorganic, most contain at least one carbon atom, and one or more atoms of elements other than carbon within the ring structure, such as sulfur, oxygen or nitrogen recently chemists try to produce new macro cycles by Claisen-Schmidt condensation. The reactions between a ketone and an aldehyde (crossed aldol condensation) or between two aldehydes also go by the name Claisen-Schmidt condensation [2]. Claisen-Schmidt condensations proceed under either acid or base catalysis, at moderate temperature, In high atom economy and with water as the sole byproduct. Chemo selectivity is also high. It was first reported in 1925 that arylaldehydes and cycloalkanones afford predominantly α , ά bisarylidene cycloalkanones instead of α-mono arylidene cycloalkanones. Subsequent workers have found that this applies even when the ratio of starting ketone to aldehyde is greater than 1:1the orientation of exocyclic bonds generated is exclusively trans with respect to the aryl ring and the carbonyl group of the cycloalkanone [3].

II. Results and Discussion

In this research prepared some new families of macro cycles by reaction of ortho-methoxy salicylaldehyde. These aspects of the Claisen-Schmidt condensation and green chemistry have been exploited in this work, to produce members of a new family of macro cycles in a single step as shown in Scheme above. Starting dialdehydes were prepared (step1), in each case by linking two ortho-methoxysalicylaldehyde molecules with bromoalkans, their derivatives or analogues, with flexible linear tethers. To such compoundsemployed Williamson etherification conditions that required long reaction times (100 hr athightemperatures). In the presence of ketone and base, Cyclization occurred through sequential Claisen-Schmidt condensations, at α position to the carbonyl group and macrocycles are obtaining meanto moderate yields.Workersfoundin 2004 during the investigation of α , ά bisary lidenecycloalkanones the tethering unit contained fewer than seven atoms, cyclization isdimeric macrocycleresulting from the condensation of two cyclohexanone molecules and two dialdehyde linkers. Against them we produce the monomer macrocycles.

Reaction of ortho-methoxy salicylaldehyde sequently with 1,2 dibromoetane , 1,3 dibromopropane and 1,4 dibroboetane under base catalysis(step1) and output compound (I–III).Consequently inter- and intra molecular cyclization of one flexible connector with one type of ketones, through two sequential Claisen-Schmidt condensations, (step2) and output compound (IV - X).

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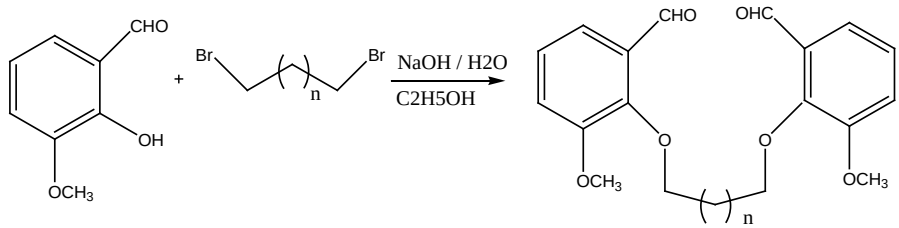
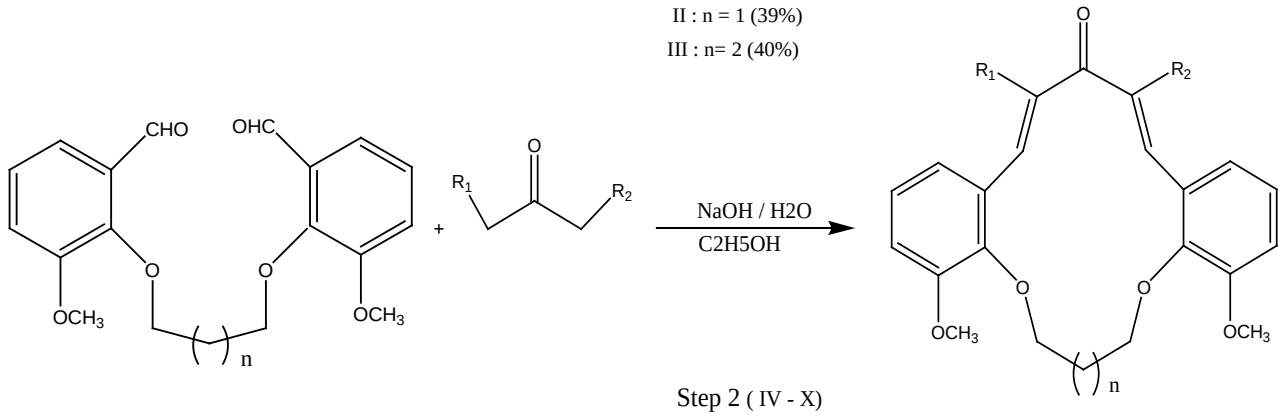


Step 1 (I- III)

I : n= 0 (36%)

II : n = 1 (39%)

III : n= 2 (40%)



Step 2 (IV - X)

I : n= 0, { IV = R1 = R2 = CH3 ; VII = R1 = CH3, R2 = H }

II : n = 1, { V = R1 = R2 = CH3 ; VIII = R1 = CH3, R2 = H ; X = R1 = H, R2 = H }

III : n= 2, VI = R1 = R2 = CH3

III. Experimental

a) Synthesis

The Synthesis of compound (I – III), to ortho-methoxy salicylaldehyde (24.4 g, 0.2 mol) in alcol (20 ml) was added sodium hydroxide (8.0g .02 mol) in water (400 ml) in three bollon then the mixture was warmed andin every of the bollonsequently1,2 dibromoetane1,3 dibromopropane and 1,4 dibroboetane (0.1 mol) was added. Sufficient alcohol (300 ml) to produce a homogeneous solution. The solution was refluxed under nitrogen for 100 hr and then cooled and let stand at 0°C The cream-colored crystals produced were washed with water of first two bollon and letter bollonproducedtan-colored crystals [3-4].

i. *Yield (36%) , Mp: 117.8- 118.3°C*

IR$\nu_{max(neat)/cm^{-1}}$: 2937.56 ,05/2785(CHO),1664.57(C=O), 785.03, 746.45

^{1}H-NMR ($CDCl_3$,TMS,300MHz) δ ppm : 3.905 (s,3H), 4.526 (s,2H), 7.183 (m,2H), 7.292-7.471 (m,1H), 10.547 (s,1H)

^{13}C-NMR($CDCl_3$,TMS,75MHz)δppm : 55.97,

117.94, 119.18, 124.33, 129.99, 151.11, 152.81, 190.57

MS m/z :[M =330],179,151,135,108,119

ii. *Yield (39%), Mp: 64.4 –66.7°C*

IR$\nu_{max(neat)/cm^{-1}}$: 2846.93, 2744.71(CHO), 1691.57(C=O), 734.88, 761.88

^{1}H-NMR ($CDCl_3$,TMS,300MHz) δ ppm : 2.309-2.317 (m,2H), 3.911(s,3H),4.400(t,2H), 7.318-7.190 (m,2H), 7.432-7.455 (m,1H) 10.489 (s,1H)

^{13}C-NMR (CDCl3,TMS,75MHz) δ ppm: 30.91, 56.04, 71.46, 118.04, 119.20, 124.19, 129.88, 151.62, 152.99, 190.27

MS m/z :[M = 344],151,165,193,122,137,108

iii. *Yield(40%),Mp: 111.3- 117.9°C*

IR$\nu_{max(neat)/cm^{-1}}$:2846.93,2765.74(CHO), 1672.14(C=O), 723.31, 765.74

^{1}H-NMR ($CDCl_3$,TMS,300MHz) δ ppm : 2.041(br.s,2H), 3.893(s,3H),4.218 (br.s,2H), 7.109- 7.147 (m,2H), 7.412-7.428 (m,1H), 10.465(s,1H)

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^{13}C-NMR ($CDCl_3$,TMS,75MHz) δ ppm: 26.69, 56.03, 74.46, 118.08, 119.08, 124.11, 129.94, 151.80, 153.05, 190.25

MS m/z:[M = 358],207,165,151,55

b) Synthesis

The Synthesis of compound (IV - X),to an ethanolic solution (95%, 1.83 L) of compound I (1.02g, 3.10 mmol) and diethyl keone (0.49 ml, 4.60 mmol) was added aqueous NaOH (1.87g in 3.75 ml H2O, 4.6 mmol). After stirring at rt for 6 d, abright yellow solution heterogeneous produce of the reaction. then it washed with 1.0 M HCL and water. and dried, then recrystallisation in etanol and produce cream- powder, compound (IV). The Synthesis of compound compound of (V – X) similar manner refered.

iv. *Yield (31.1%), Mp:128-127.7 °C*

IR$\nu_{max(neat)/cm}^{-1}$: 1622.62(C=O), 765.10,744.52

^{1}H-NMR (CDCl3,TMS,300MHz) δ ppm : 2.057 (s,3H) 3.913(s,3H) 4.477 (s,2H), 6.943- 6.972(m,2H), 7.091-7.146(m,1H), 7.867(s,1H)

^{13}C-NMR ($CDCl_3$,TMS,75MHz) δ ppm: 13.84, 55.78, 72.40, 112.42, 121.30, 123.54, 130.61, 130.66, 137.50, 145.93, 152.62, 202.58

MS m/z :[M = 380],335,203,191,175,91,83,43,57

v. *Yield (46.6%), Mp:140-138 °C*

IR$\nu_{max(neat)/cm}$-1: 1662.31(C=O), 758.02, 742.57

1H-NMR ($CDCl_3$,TMS,300MHz) δ ppm : 2.248 (s,3H), 2.307 (m,1H), 3.921(s,3H), 4.221 (t,2H), 6.973(d,1H), 7.070 (d,H), 7.117- 7.157(t,1H), 7.580 (s,1H)

13C-NMR ($CDCl_3$,TMS,75MHz) δ ppm: 15.08, 31.34, 55.58, 68023, 112.39, 121.95, 123.99, 130.91, 133.52, 137.33, 146.51, 153.51, 202.47

MS m/z :[M =394],336,321,91,43,57,191,103,131,380

vi. *Yield (35.1), Mp:142-140 °C*

IR$\nu_{max(neat)/cm}^{-1}$:1692.85(C=O), 798.53, 746.45

^{1}H-NMR ($CDCl_3$,TMS,300MHz) δ ppm : 1.968 (br,s,2H), 2.078 (s,3H), 3.891(s,3H),4.069 (br,s,2H), 6.937 (d,1H), 7.012 (d,1H), 7.077- 7.116 (t,1H), 7.504 (s,1H)

^{13}C-NMR ($CDCl_3$,TMS,75MHz) δ ppm:15.20, 26.56, 55.85, 72.89, 112.40, 121.90, 123.62, 130.57, 134.28, 138.14, 146.43, 152.97, 201.88

MS m/z :[M = 408],91,55,148,175,333

vii. *Yield (54%), Mp:140-138 °C*

IR$\nu_{max(neat)/cm}^{-1}$: 1635.46 (C=O), 732.95, 785.03

1H-NMR ($CDCl_3$,TMS,300MHz) δ ppm : 2.0721 (s,3H), 3.917 (s,6H), 4.403 (m,2H), 4.582 (m,2H), 6.651 (d,1H), 6.977 (s,3H), 7.075- 7.156 (m,2H), 7.697(s,H), 8.512 (d,H)

^{13}C-NMR ($CDCl_3$,TMS,75MHz) δ ppm:13.62, 55.86, 55.94, 72.14, 73.85, 112.88, 113.69, 117.93, 121.25, 123.08, 124.47, 126.91, 129.71, 129.78, 135.70, 137.16, 142.80, 146.21, 146.93, 151.94, 153.07, 200.83

MS m/z :[M=366],321,336,188,91,161,17

viii. *Yield (26.3%), Mp:169 -171°C*

IR$\nu_{max(neat)/cm}$-1: 1622.13(C=O), 767.67,731.02

^{1}H-NMR ($CDCl_3$,TMS,300MHz) δ ppm :

2.193(s,3H), 2.317 (m,2H), 3.915 (s,6H), 4.231 (t,4H), 6.777-6.820 (d,1H), 6.953-7.007(m,2H), 7.055 (d,1H), 7.122-7.169 (m,2H), 7.291- 7.334 (t,1H), 7.584 (s,1H), 8.104 (d,1H)

^{13}C-NMR ($CDCl_3$,TMS,75MHz) δ ppm: 14.85, 31.49, 50.84, 55.86, 68.79, 68.95, 68.79, 68.95, 112.30, 113.76, 118.01, 121.84, 124.05, 124.74, 127.30, 129.45, 130.78, 133.37, 137.00, 139.22, 146.56, 146.88, 152.85, 153.45, 199.94

MS m/z :[M=380],149,307,322,91,161,175,350

ix. *Yield (53%) , Mp: 131-129°C*

IR$\nu_{max(neat)/cm}^{-1}$: 28/1618 (C=O),792.74,67/740

^{1}H-NMR ($CDCl_3$,TMS,300MHz) δ ppm :2.342-2.392 (m,2H), 3.931)s,3H), 4.237 (t,2H), 6.750 (d,1H), 7-7.301 (m,3H), 8.135-8.092 (d,1H)

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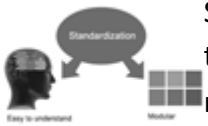

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- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

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30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

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	A-B	**C-D**	**E-F**
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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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